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5′-Epimeric 3′-deoxy-3′,4′-didehydronucleoside-5′-*C*-phosphonates: synthesis and structural assignment by NMR and X-ray analyses

Magdalena Petrová, Miloš Buděšínský, Blanka Klepetářová, Ivan Rosenberg *

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Flemingovo 2, 166 10 Prague 6, Czech Republic

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ABSTRACT

Epimeric 5′-(*RS*) dialkyl 3′-deoxy-3′,4′-didehydro-5′-*C*-phosphonates were prepared by nucleophilic addition of various dialkyl phosphites to 3′-deoxy-3′,4′-didehydronucleoside-5′-aldehydes. Whereas direct NMR configuration assignment for the C5′ atom bearing the phosphoryl and hydroxy groups using the *J* (P,H4′) and *J* (H5′,H4′) coupling constants is impossible due to the absence of the H4′ atom, successful separation, crystallisation and X-ray crystallographic analysis of a pair of epimeric 5′-*C*-phosphonates, followed by correlation with a series of NMR parameters, led to efficacious configuration assignment of individual epimers in the mixtures.

1. Introduction

Nucleoside phosphonic acids containing a *P*–*C* bridging bond instead of the ester *P*–*O* linkage represent a significant class of compounds, among which efficient antiviral agents have been found.[1–12] A number of structurally diverse nucleoside phosphonic acids have been synthesised and evaluated in our laboratory, e.g., compounds **1a**–**e**, which differ in the number of bridging atoms of the phosphoester mimic[13–26] (Fig. 1). Some of these compounds have interesting biological properties.[27–29]

The recently reported straightforward, high-yielding synthesis of 3′-deoxy-3′,4′-didehydronucleoside-5′-aldehydes[35] **6** prompted us to synthesise 5′-epimeric 3′,4′-didehydronucleoside-5′-hydroxy phosphonates **8** (Scheme 2). These compounds combine in their scaffold both an enzymatically stable hydroxy phosphonate moiety with bridging P–C bond mimicking the phosphoester group and a 3′,4′-unsaturated[36] nucleoside motif with the 2′-hydroxy group. Its presence in the molecule allows to consider the compounds **8** as ribonucleoside-5′-phosphate analogues, similarly as the 3′-deoxynucleotides, which are recognized by RNA-polymerases as substrates, the chain terminators. Due to the absence of the H4′ atom in compounds **8**, the previously reported direct NMR assignment of both the C5′-*R*/*S* configuration and conformation around the C4′–C5′ linkage,[20] exploiting the characteristic values of the *J* (P,H4′) and *J* (H5′,H4′) coupling constants, was inapplicable, and thus, the C5′-*R*/*S* assignment appeared to be a challenging problem.

Abramov nucleophilic addition of dialkyl phosphites to carbonyl compounds is a straightforward, high-yielding reaction for the

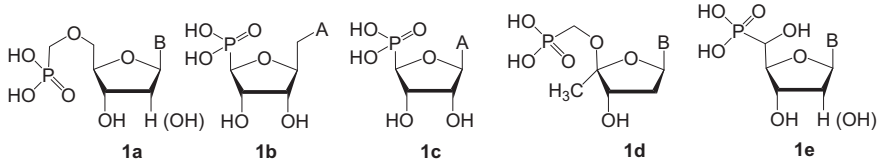

Fig. 1. Examples of isopolar nucleoside 5′-phosphonic acids.

Geminal 5′-hydroxy phosphonates **1e**, described by Wiemer[30–32] and our group,[20,21,33,34] are isopolar, non-isosteric 5′-nucleotide analogues possessing chirality at the C5′ atom. These compounds have not been fully biologically evaluated, although some biochemical experiments with arabinocytosine 5′-hydroxy phosphonate have been performed.[32]

* Corresponding author. E-mail address: rosenberg@uochb.cas.cz (I. Rosenberg).

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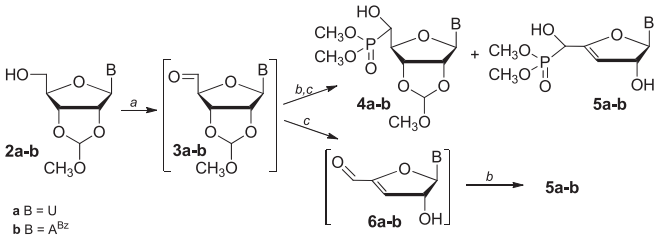
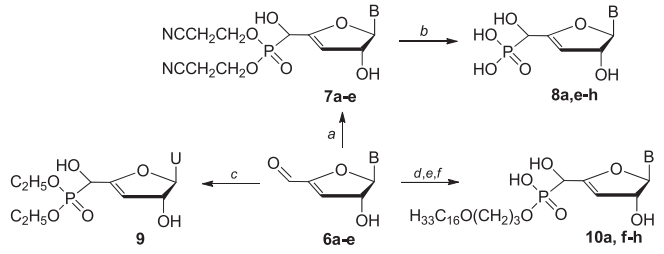
preparation of geminal hydroxy phosphonates.[37] To produce nucleoside 5′-hydroxy phosphonates,[20,34] 2′,3′-*O*-protected ribonucleosides were oxidised to 5′-aldehydes using a modified Moffat procedure (DMSO/DCC in the presence of TFA and pyridine), and these compounds were subjected to the Et_3N-mediated nucleophilic addition of dialkyl phosphites. As we have already reported,[35] the 2′,3′-*O*-methoxymethylidene ribonucleoside-5′-aldehydes **3**, prepared in situ from the respective 2′,3′-*O*-methoxymethylidene ribonucleosides **2**, underwent the elimination of the orthoester moiety upon a basic treatment, resulting in the formation of 3′-deoxy-3′,4′-didehydro-5′-aldehydes **6** (Scheme 1).



a) DMSO, DCC, pyridine, TFA; *b)* $HP(O)(OCH_3)_2$; *c)* Et_3N;

Scheme 1. Synthesis of phosphonates **4** and **5** from 2′,3′-*O*-orthoester **2**.

Accordingly, when $HP(O)(OMe)_2$ was added to the in situ prepared 2′,3′-*O*-methoxymethylidene ribonucleoside-5′-aldehyde **3**, prior to Et_3N, in addition to 2′,3′-*O*-methoxymethylidene-5′-*C*-phosphonates **4**, 3′,4′-didehydro derivatives **5** were also formed. On the contrary, the treatment of the oxidation mixture with Et_3N followed by the addition of $HP(O)(OMe)_2$ after a period of 15 min lead to exclusive formation of dimethyl 3′-deoxy-3′,4′-didehydro-5′*C*-phosphono derivatives **5**. For practical reasons, the synthesis of 3′,4′-unsaturated phosphono derivatives **7**–**10** was started from 3′-deoxy-3′,4′-didehydronucleoside-5′-aldehyde[35] **6** (Scheme 2).



a) $HP(O)(OCH_2CH_2CN)_2$-Et_3N, DMF; *b)* $MeNH_2$, EtOH, 50 °C; *c)* $HP(O)(OC_2H_5)_2$-Et_3N, H_2O; *d)* $H(O)P(OCH_3)(OC_3H_6OC_{16}H_{33})$-$Et_3N$, DMF; *e)* 60% aq. Py, 50 °C; *f)* NH_3 in MeOH or $MeNH_2$ in EtOH

B	U a	A^{Bz} b	C^{Bz} c	G^{iBu} d	T e	A f	C g	G h
6 → 7	70	85	90	74	74	-	-	-
7 → 8	85	-	-	-	90	88	84	87
6 → 9	60	-	-	-	-	-	-	-
6 → 10	38	-	-	-	-	58	62	21

Scheme 2. Synthesis and isolated yields (%) of phosphono derivatives **7**–**10**.

To obtain free phosphonic acids **8**, the dimethyl esters **5a**,**b** were treated with bromotrimethyl silane in acetonitrile in the presence of 2,6-lutidine. However, during this procedure, substantial cleavage of the nucleoside bond was observed. Therefore, we used bis(2-cyanoethyl) phosphite[38] as the phosphonylating agent and conducted the reaction with 3′-deoxy-3′,4′-didehydronucleoside-5′-aldehydes **6a**–**e** in DMF in the presence of Et_3N. Thus, bis(2-cyanoethyl) esters **7a**–**e** were obtained as 5′*R*/*S* epimeric mixtures in excellent yields (70–90%; Scheme 2). The only drawback of this procedure is the susceptibility of one 2-cyanoethyl group to rapid β-elimination, which, in some cases, decreased the overall yields of diesters. The obtained diesters **7a**–**e** were smoothly deprotected via β-elimination in 8 M methylamine in ethanol at 50 °C (removal of both 2-cyanoethyl groups and the *N*-protecting groups), and free acids were converted into sodium salts of **8a**,**e**–**h** on Dowex 50 (Na^+ form) in high yields (84–90%; Scheme 2). Interestingly, the reaction of 3′-deoxy-3′,4′-didehydronucleoside-5′-aldehydes **6** with dialkyl phosphites under Et_3N catalysis also proceeded very well in aqueous solution. In this case, the crystalline 5′*R*/*S* diethyl phosphonate **9** was obtained in 60% yield (Scheme 2).

To improve the cellular uptake of phosphonic acids and thus, facilitate drug delivery, phospholipase *C*-cleavable, lipophilic monoesters were introduced.[39] In our case, 3-hexadecyloxypropyl methyl phosphite[38] was selected as the most convenient phosphonylating agent. Upon its reaction with 3′-deoxy-3′,4′-didehydronucleoside-5′-aldehydes **6** in DMF in the presence of Et_3N and subsequent treatment with 60% aqueous pyridine at 50 °C (removal of the methyl ester group), followed by treatment with methanolic ammonia or 8 M methylamine in EtOH to remove *N*-acyl groups, we obtained 3-hexadecyloxypropyl 3′-deoxy-3′,4′-didehydro-5′-*C*-phosphonates **10a**,**f**–**h** (21–62% yields with respect to aldehydes **6a**–**d**; Scheme 2). Monoesters **10f**–**h** were converted into Na^+ salts in excess of saturated sodium bicarbonate solution upon heating, followed by desalting on a C18 column in a water/methanol mixture. Monoester **10a** was isolated in the form of an Et_3NH^+ salt.

Since the synthesised 3′-deoxy-3′,4′-didehydronucleoside-5′-*C*-phosphonates **5a**,**b**, **7a**–**e**, **8a**,**e**–**h**, **9** and **10a**,**f**–**h** were obtained as 5′-*R* and 5′-*S* epimeric mixtures, a methodology for their separation and identification is highly desirable. In most cases, according to HPLC and NMR analyses, both epimers were formed in a nearly equal ratio. Whereas only partial separation of the 5′-*R* and 5′-*S* epimers of the bis(2-cyanoethyl) phosphonates of purine nucleosides **7b** and **7d** was achieved by HPLC on a C18 column in water/acetonitrile, complete separation of epimers of uracil diethyl phosphonate **9** was accomplished in water/methanol. Epimers (5′*S*)-**9** and (5′*R*)-**9** were successfully crystallised from ethanol and subjected to X-ray analysis. The crystal structures of both the 5′-*R* and 5′-*S* epimers of **9** were very similar providing us by important findings. Thus, dihydrofuran ring is nearly planar, the uracil base adopts the anti orientation and, surprisingly, the phosphorus moiety in both 5′-*R* and 5′-*S* epimers is oriented cis to the uracil nucleobase. The torsion angle γ around the C4′–C5′ linkage is approximately 105°. The 5′-*R* and 5′-*S* epimers differ only in the orientation of the 5′–OH group and the H5′ proton (see Fig. 2 and Table 1). The found cis position of the phosphorus moiety and nucleobase underlines the structural similarity of compounds **8** with natural nucleotides, and thus, the synthesis and biological evaluation of these compounds as nucleotide analogues seem to be justify.

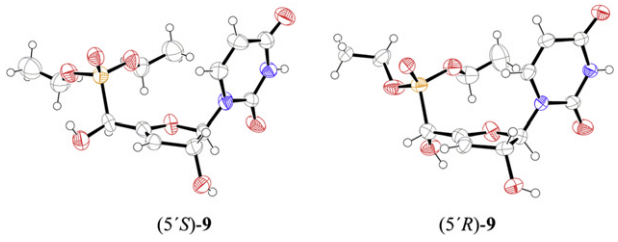


Fig. 2. Crystal structures of the (5′*S*)-**9** (HPLC-slower) and (5′*R*)-**9** (HPLC-faster) epimers (thermal ellipsoids at the 50% probability level). For clarity reasons, only the more occupied parts of the disordered groups are shown.

The NMR spectra of 5′-*R* and 5′-*S* epimer of **9** in both solvents (DMSO and D_2O) were extremely similar. The very small differences in the chemical shifts and coupling constants (see Table 3 and Table 4) and the nearly identical NOE contacts observed do not allow

Table 1
Comparison of the selected torsion angles of the 5′-*S* and 5′-*R* epimers of **9** in the crystals

Torsion angle	5′*S*	5′*R*[a]	5′*R*[a]
<χ(C2−N1−C1′−O4′)	−100.7	−102.1	−101.8
<ν_0(C4′−O4′−C1′−C2′)	5.7	5.4	2.8
<ν_1(O4′−C1′−C2′−C3′)	−5.4	−5.7	−3.9
<ν_2(C1′−C2′−C3′−C4′)	3.5	4.1	3.7
<ν_3(C2′−C3′−C4′−O4′)	−0.2	−1.0	−2.3
<ν_4(C3′−C4′−O4′−C1′)	−3.7	−2.9	−0.4
<γ(C3′−C4′−C5′−P)	105.1	102.8	102.8
<(C3′−C4′−C5′−H5′)	−136.0	−15.4	−19.9
<(C3′−C4′−C5′−O5′)	−20.4	−133.8	−139.5
<(C4′−C5′−P−O)	−63.1	−67.4	−67.0

[a] The asymmetric unit contains two slightly different independent molecules of (5′*R*)-**9**.

independent direct assignment of the C5′ configuration from NMR spectra. Therefore, we attempted to use the NMR spectra of the 5′-*R* and 5′-*S* epimers of **9** to find some correlations of their NMR parameters with those observed in NMR spectra of epimer mixtures **5a**,**b**, **7a**–**e**, **8a**,**e**–**h** and **10a**,**f**–**h**. As we have already mentioned, the X-ray structures of the 5′-*R* and 5′-*S* epimers differ only in the orientation of the 5′−OH group and H5′ proton. This difference should be most sensitively reflected by the H3′ proton. The multiplet patterns of H3′ in the spectra of the 5′-*R* and 5′-*S* epimers show nearly identical values of *J* (3′,2′) ~2.5 Hz (in accordance with nearly identical conformation of the dihydrofuran ring in solution), but somewhat larger allylic couplings *J* (3′,5′) and *J* (3′,P) for the 5′-*S* epimer (1.2 and 4.2 Hz) than for the 5′-*R* epimer (0.8 and 3.4 Hz), which could be explained by the larger angle between the planes containing interacting atoms in the 5′-*S* epimer (~45°) in comparison with the 5′-*R* epimer (4° and 12° found in two conformers in the crystal structures), leading to larger allylic couplings in the 5′-*S* epimer due to a higher π-contribution to the allylic coupling.[40] Detailed analysis of the ^{1}H NMR spectra revealed similar differences in the H3′ multiplet patterns in the whole series of compounds **5a**,**b**, **7a**–**e**, **8a**,**e**–**h** and **10a**,**f**–**h**; this pattern has been used for the assignment of the configuration at the C5′ carbon atom (see examples of H3′ multiplet patterns in Fig. 3 and *J*-values in Table 2). The allylic couplings *J* (3′,P) reflect differences between the phosphonate diesters (**5a**,**b**, **9**, **7a**–**e**), monoesters (**10a**,**f**–**h**) and free phosphonates (**8a**,**e**–**h**).

Given the similar preferred conformations of the studied compounds in crystals and in solution (supported by geometry optimisation calculation using DFT B3LYP 6-311G**), we used the correlation of chemical shifts of C3′ carbon. Applying the observation in compound **9**, in which the C3′ carbon of the 5′-*S* epimer is shifted upfield (probably as the result of the γ-*gauche* interaction of 5′-OH with C3′—see Fig. 4) relative to the whole series of prepared compounds, led to the same assignment of the configuration at C5′ as that derived from the allylic couplings (compare Table 2).

The phosphorus-31 chemical shifts of the studied compounds clearly distinguish between phosphonate diesters **5a**,**b**, **9**, **7a**–**e** (δ 20.41–23.74), monoesters **10a**,**f**–**h** (δ 12.54–14.05) and free phosphonic acids **8a**,**e**–**h** (δ 12.32–12.57). However, the chemical shift differences between the signals of 5′-*R* and 5′-*S* epimer are either very small (e.g., 0.05 ppm in C-5′ epimers of compound **9**) or even zero (see Table 4), and therefore, these differences are not usable for correlation with the configuration at the C5′ carbon atom.

2. Conclusion

Novel 5′-epimeric 3′-deoxy-3′,4′-didehydronucleoside-5′-*C*-phosphonates **8** and their 3-hexadecyloxypropyl esters **10** as prodrugs were prepared by Et_3N-mediated addition of the corresponding dialkyl phosphites to 3′-deoxy-3′,4′-didehydronucleoside-5′-aldehydes **6**. X-ray analysis of the crystals of both the 5′-*R* and 5′-*S* epimers of diester **9** showed very similar structures—the nearly planar dihydrofuran ring, the *anti* orientation of the uracil base and the cis orientation of the phosphorus moiety and nucleobase in both epimers. Only the orientation of the 5′-hydroxy group and the H5′ proton was different. The found cis orientation of phosphorus atom towards nucleobase underlines, together with 2′-hydroxyl the structural similarity of these compounds with natural ribonucleotides. On the base of X-ray structure of pure 5′-*R* and 5′-*S* epimers of **9** we succeeded in the correlation of their NMR parameters with those observed in NMR spectra of epimer mixtures. Neither free phosphonic acids **8a**,**e**–**h** nor their prodrugs **10a**,**f**–**h** exhibited activity against HCV in a replicon system. Further biochemical evaluation of phosphonates **8** in the form of their diphosphoryl derivatives (NTP analogues) in RNA polymerase assay is underway, as well as the inhibition study on nucleoside phosphorylases, and pyrimidine specific 5′-nucleotidases.

Table 2
Coupling constants of H3′ and chemical shifts of C3′ in compounds **5a**, **5b**, **7a**–**e**, **8a**,**e**–**h**, **9** and **10a**,**f**–**h** and configuration assignment at carbon atom C5′

Compd.	Solv.	*S*/*R*	H3′	*J* (3′, P)	*J* (3′, 2′)	*J* (3′, 5′)	H3′	*J* (3′, P)	*J* (3′, 2′)	*J* (3′, 5′)	C3′		
		Ratio	*S*-epimer				*R*-epimer				*S*-epi	*R*-epi	Δδ (*S*/*R*)
5a	D_2O	61:39	5.584	4.0	2.8	1.0	5.574	3.7	2.6	0.8	105.64	106.20	**−0.56**
5b	DMSO	50:50	5.497	4.0	2.6	1.2	5.488	3.5	2.6	0.9	102.60	103.36	**−0.76**
7a	DMSO	52:48	5.379	4.3	2.6	1.2	5.372	3.6	2.5	0.9	103.07	104.01	**−0.94**
7b	DMSO	56:44	5.549	4.2	2.6	1.2	5.523	3.8	2.5	0.9	103.18	103.68	**−0.50**
7c	DMSO	44:56	5.426	4.3	2.6	1.1	5.411	3.4	2.6	0.8	103.40	104.13	**−0.73**
7d	DMSO	40:60	5.494	4.3	2.6	1.2	5.479	3.7	2.6	0.9	102.84	103.65	**−0.81**
7e	DMSO	50:50	5.384	4.4	2.6	1.2	5.373	3.5	2.5	0.8	103.05	104.17	**−1.12**
8a	D_2O	50:50	5.466	2.8	2.8	1.2	5.445	2.8	2.8	0.9	101.64	102.39	**−0.75**
8e	D_2O	46:54	5.470	2.8	2.8	1.2	5.450	2.7	2.7	0.8	101.61	102.92	**−1.31**
8f	D_2O	50:50	5.505	2.8	2.8	1.2	5.485	2.8	2.8	0.7	101.36	102.50	**−1.14**
8g	D_2O	53:47	5.334	2.8	2.8	1.1	5.304	2.8	2.8	0.9	101.38	102.02	**−0.64**
8h	D_2O	59:41	5.443	2.8	2.8	1.2	5.458	~2.5[b]	~2.5[b]	<<1[b]	100.79	102.56	**−1.77**
9	DMSO	[a]	5.309	4.2	2.6	1.2	5.317	3.4	2.5	0.8	102.35	103.41	**−1.06**
10a	DMSO	58:42	5.100	3.7	2.5	1.2	5.145	2.6	2.6	0.8	99.53	101.23	**−1.70**
10f	DMSO	64:36	5.108	3.5	2.6	1.2	5.150	2.6	2.6	0.8	97.95	99.66	**−1.71**
10g	DMSO	52:48	4.994	3.5	2.6	1.3	5.031	~2.5	~2.5	<<1	98.42	100.37	**−1.95**
10h	DMSO	49:51	5.097	3.3	2.6	1.1	5.128	2.7	2.7	1.0	98.05	99.23	**−1.18**

[a] Data for separated C5′ epimers.
[b] Line broadening prevented the determination of more accurate data values.

Table 3
Proton NMR data of compounds **5a**,**b**, **7a**–**e**, **8a**,**e**–**h**, **9**, and **10a**,**f**–**h** at 27 °C. Coupling constants are given in brackets

Compd. (Solvent)	Config. (popul.)	H-1′	H-2′	H-3′	H-5′	H-2	H-5	H-6	H-8[c]
5a	5′*S*	6.349d (2.1)	5.084m	5.584 ddd	5.01 dt	—	5.871 d (8.1)	7.454 d (8.1)	—
(D_2O)	(~61%)			(4.0, 2.7, 1.0)	(16.0; ~1; ~1)				
	5′*R*	6.336 d (2.3)	5.102m	5.574 ddd	5.03 dt	—	5.876 d (8.1)	7.452 d (8.1)	—
	(~39%)			(3.7; 2.6; 0.8)	(15.0; ~1; ~1)				
5b	5′*S*	6.45 d (2.6)	5.33m	5.497 ddd	4.69 ddt	8.797 s	—	—	8.47 s
(DMSO)	(~50%)			(4.0; 2.6; 1.2)	(15.2; 6.9; 1.1; 1.1)				
	5′*R*	6.465 d (2.0)	5.25m	5.488 ddd (3.5; 2.6; 0.9)	4.65 br dd	8.801 s	—	—	8.525 s
	(~50)				(16.0; 7.0; 0.9; <1)				
7a	5′*S*	6.19 d	4.81m	5.379 ddd	4.71 dddd	—	5.665 dd (8.1; 2.1)	7.275 d (8.1);	—
(DMSO)	(~52%)	(2.4)	(5.7; 4.0; 2.4; 0.6)	(4.3; 2.6; 1.2)	(15.6; 6.5; 1.2; 0.6)				
	5′*R*	6.17 d	4.84m	5.372 ddd	4.775 dddd	—	5.67 dd (8.1; 2.0)	7.30 d (8.1)	—
	(~48%)	(2.7)	(5.8; 4.3; 2.7; 1.2)	(3.6; 2.5; 0.9)	(15.0; 6.9; 1.2; 0.8)				
7b	5′*S*	6.47 d	5.28m	5.549 ddd	4.74 ddd	8.79 s	—	—	8.47 s
(DMSO)	(~56%)	(2.2)	(5.5; 4.3; 2.2)	(4.2; 2.6; 1.2)	(15.9; 6.8; 1.0; <1)				
	5′*R*	6.455 d	5.365m	5.523 ddd	4.78 ddd	8.79 s	—	—	8.52 s
	(~44%)	(2.8)	(5.4; 3.7; 2.8)	(3.8; 2.5; 0.9)	(15.3; 6.9; 0.8)				
7c	5′*S*	6.283 d	4.803m	5.426 ddd	4.816 br dd	—	7.350 br d	7.964br d (~7.5)	—
(DMSO)	(~44%)	(2.0)		(4.3; 2.6; 1.1)	(16.0; 6.6)		(~7.5)		
	5′*R*	6.264 d	4.774m	5.411 ddd	4.884 br dd	—	7.359 br d	7.936br d (~7.5)	—
	(~56%)	(2.2)		(3.4; 2.6; 0.8)	(15.1; 6.9)		(~7.5)		
7d	5′*S*	6.173 d	5.08 dddd	5.494 ddd	4.705 dddd	—	—	—	7.94 s
(DMSO)	(~40%)	(2.7)	(5.8; 4.1; 2.7; 0.5)	(4.3; 2.6; 1.2)	(15.8; 6.8; 1.2; 0.5)				
	5′*R*	6.187 d	5.15 dddd	5.479 ddd	4.78 ddt	—	—	—	7.99 s
	(~60%)	(2.2)	(5.7; 3.6; 2.2; 1.0)	(3.7; 2.5; 0.9)	(15.8; 6.8; 1.0; 1.0)				
7e	5′*S*	6.18 d	4.83m	5.384 ddd	4.80m	—	—	7.16 q (1.2)	—
(DMSO)	(~50%)	(2.8)	(5.9; 2.8; 2.6)	(4.4; 2.6; 1.2)	(15.4; 6.9; 1.2)				
	5′*R*	6.20 d	4.78m	5.373 ddd	4.72m	—	—	7.15 q (1.2)	—
	(~50%)	(2.5)	(5.8; 2.5; 2.5)	(3.5; 2.5; 0.8)	(15.6; 6.7; 0.7)				
8a	5′*S*	6.373	4.985m	5.466 td	4.43 dt		5.944 d (8.1)	7.70 d (8.1)	—
(D_2O)	(~50%)	(1.7)	(2.8; 2.2; 1.7; 1.5)	(2.8; 2.8; 1.2)	(14.9; 1.5; 1.2)				
	5′*R*	6.367 d	4.965 ddd	5.445 td	4.47 br d	—	5.940 d (8.1)	7.82 d (8.1)	—
	(~50%)	(1.6)	(2.8; 2.0; 1.6)	(2.8; 2.8; 0.9)	(14.9; 0.9; <0.5)				
8e	5′*S*	6.40 d	4.99m	5.47 td	4.40 dt	—	—	7.54 q (1.2)	—
(D_2O)	(~46%)	(1.9)		(2.8; 2.8; 1.2)	(14.4; 1.4; 1.4)				
	5′*R*	6.39 d	4.96m	5.45 td	4.47 br d	—	—	7.44 q (1.2);	—
	(~54%)	(1.8)		(2.7; 2.7; 0.8)	(14.8; <1)				
8f	5′*S*	6.460	5.15m	5.505 td	4.34 dt	8.16 s	—	—	8.21 s
(D_2O)	(~50%)	(1.7)	(2.8; 1.7; 1.4)	(2.8; 2.8; 1.2)	(14.7; 1.4; 1.2)				
	5′*R*	6.467	5.11 dt	5.485 td	4.45 br d	8.18 s	—	—	8.25 s
	(~50%)	(1.6)	(2.8; 1.6; 1.5)	(2.8; 2.8; 0.7)	(15.1; 0.8)				
8g	5′*S*	6.27 d	4.79 ddd	5.334 td	4.32 dt	—	5.998 d (7.6)	7.73 d (7.6)	—
(D_2O)	(~47%)	(1.7)	(4.0; 2.5; 1.7)	(2.8; 2.8; 1.1)	(14.3; 1.4; 1.2)				
	5′*R*	6.26 d	4.77 td	5.304 td	4.35 dd	—	6.001 d (7.5)	7.59 d (7.5)	—
	(~53%)	(1.6)	(2.4; 2.2; 1.6)	(2.8; 2.8; 0.9)	(14.8; 1.0)				
8h	5′*S*	6.27 d	5.08m	5.443 td	4.33 br d	—	—	—	7.84 s
(D_2O)	(~59%)	(1.8)		(2.8; 2.8; 1.2)	(14.5)				
	5′*R*	6.26 d	5.05m	5.458 br t	4.43 br d	—	—	—	7.82 s
	(~41%)	(1.6)		(2.5; 2.5; <1)	(14.2)				
9	5′*S*	6.17 d	4.805m	5.309 ddd	4.52 br d	—	5.62 d (8.1)	7.29 d (8.1)	—
(DMSO)		(2.3)		(4.2; 2.6; 1.2)	(16.0)				
	5′*R*	6.145 d	4.83m	5.317 ddd	4.58 br dd	—	5.64 d (8.1)	7.32 d (8.1)	—
		(2.6)		(3.4; 2.5; 0.8)	(15.0; ~5.0; 0.8)				
(D_2O)	5′*S*	6.34 d	5.08m	5.572 ddd	4.93 ddd	—	5.875 d (8.1)	7.47 d (8.1)	—
		(2.0)	(3.9; 2.8; 2.0; 0.6)	(4.2; 2.7; 1.1)	(16.0; 1.1; 0.6)				
	5′*R*	6.33 d	5.10m	5.565 ddd	4.96 ddd	—	5.87 d (8.1)	7.46 d (8.1)	—
		(2.3)	(4.2; 2.6; 2.3; 1.0)	(3.6; 2.7; 0.8)	(15.0; 1.0; 0.8)				
10a	5′*S*	6.09 d	4.59m	5.100 ddd	4.075 dd	—	5.49 dd (8.0; 2.1)	7.85 d (8.0)	—
(DMSO)	(~58%)	(2.2)		(3.7; 2.5; 1.2)	(15.3; 1.0)				
	5′*R*	6.10 d	4.655m	5.145 td	4.155 br d	—	5.55 dd (8.0; 2.1)	7.57 d (8.0)	—
	(~42%)	(1.9)		(2.7; 2.6; 0.7)	(14.6; <1)				
10f[a]	5′*S*	6.25 d	4.64m	5.108 ddd	4.02 ddd	8.14 s	—	—	8.655 s
(DMSO)	(~64%)	(1.5)		(3.5; 2.5; 1.2)	(14.9; 5.2; 1.2)				
	5′*R*	6.235 d	4.83m	5.150 td	4.04 ddd	8.155 s	—	—	8.40 s
	(~36%)	(2.0)		(2.6; 2.6; 0.8)	(14.3; 5.7; 0.8)				
10g[a]	5′*S*	6.115	4.40m	4.994 ddd	3.98 d	—	5.67 d (7.5)	7.69 d (7.5)	—
(DMSO)	(~52%)	(1.7)		(3.5; 2.6; 1.3)	(15.0)				
	5′*R*	6.155	4.46m	5.031 br t	4.06 d	—	5.62 d (7.5)	8.02 d (7.5)	—
	(~48%)	(1.9)		(2.5; 2.5; <1)	(14.3)				
10h[a,b]	5′*S*	6.031 d	4.655m	5.097 ddd	3.985 br t	—	—	—	8.04 s
(DMSO)	(~49%)	(1.9)		(3.3; 2.5; 1.1)					
	5′*R*	5.996 d	4.923m	5.128 td	4.01 br t	—	—	—	7.76 s
	(~51%)	(2.6)		(2.7; 2.7; 1.0)					

[a] The signals of long aliphatic chain could not be structurally assigned.
[b] Measured at 40 °C.
[c] Data for other protons see Table 1S in Supplementary data.

Table 4
^{31}P and ^{13}C NMR data of compounds **5a**,**b**, **7a**–**e**, **8a**,**e**–**h**, **9**, and **10a**,**f**–**h** at 27 °C. Coupling constants *J* (C,P) are given in brackets

Compd.(Solv.)	Config.(popul.)	*P*[a]	C-1′	C-2′	C-3′	C-4′	C-5′	C-2	C-4	C-5	C-6	C-8[d]
5a	5′*S*	23.68	95.83	80.52	105.64	160.09	65.80	153.95	168.86	105.43	144.07	—
(D_2O)	(~61%)			(<1)	(9.4)	(3.8)	(166.3)					
	5′*R*	23.74	95.88	80.63	106.20	159.25	65.53	153.95	168.83	105.54	144.22	—
	(~39%)			(1.1)	(9.3)	(3.3)	(166.9)					
5b	5′*S*	22.57 and 22.71	91.54	77.46	102.60	158.13	63.88	152.31	152.06	125.39	150.69	142.35
(DMSO)	(~50)				(8.1)	(~0)	(163.4)					
	5′*R*		91.49	77.42	103.36	157.08	63.47	152.27	152.00	125.49	150.75	142.02
	(~50%)				(8.3)	(~0)	(163.7)					
7a	5′*S*	21.10	92.50	77.80	103.07	158.12	64.04	150.42	163.25	102.91	140.05	—
(DMSO)	(~52%)				(8.8)	(1.4)	(163.9)					
	5′*R*		92.48	77.80	104.01	156.90	63.69	150.44	163.25	102.99	140.28	—
	(~48%)				(9.2)	(~0)	(164.3)					
7b	5′*S*	20.90	91.49	77.37	103.18	157.43	64.06	152.30	152.03	125.39	150.72	142.06
(DMSO)	(~56%)				(8.7)	(~0)	(164.0)					
	5′*R*	21.02	91.65	77.33	103.68	156.50	63.76	152.30	152.08	125.34	150.76	142.90
	(~44%)				(8.8)	(~0)	(164.0)					
7c	5′*S*	21.03	94.02	78.37	103.40	157.75	64.00	154.40	163.66	96.68	144.55	—
(DMSO)	(~44%)				(8.3)	(~0)	(163.7)					
	5′*S*	21.15	93.99	78.40	104.13	156.68	63.60	154.40	163.66	96.80	144.76	—
	(~56%)				(8.1)	(~0)	(164.2)					
7d	5′*S*	21.04	90.96	77.69	102.84	156.47	64.07	157.53	148.49	119.93	148.68	136.64
(DMSO)	(~40%)				(9.1)	(~0)	(163.9)					
	5′*R*	21.03	90.93	77.65	103.65	155.02	63.72	157.53	148.57	120.04	148.69	136.84
	(~60%)				(8.6)	(~0)	(163.7)					
7e	5′*S*	21.28 and 21.24	92.26	77.83	103.05	156.75	63.74	150.47	163.93	110.74	135.59	—
(DMSO)	(~50%)				(8.9)	(~0)	(163.5)					
	5′*R*		92.34	77.83	104.17	158.18	64.14	150.42	163.93	110.59	135.72	—
	(~50%)				(9.1)	(1.4)	(163.5)					
8a	5′*S*	12.57	95.34	81.55	101.64	165.60	69.90	154.18	169.28	104.96	144.31	—
(D_2O)	(~50%)				(6.0)	(~0)	(139.8)					
	5′*R*		95.34	81.55	102.39	165.34	69.78	154.18	169.23	104.74	144.46	—
	(~50%)				(6.8)	(2.8)	(139.2)					
8e	5′*S*	12.52	95.31	81.89	101.61	165.52	70.27	154.58	169.77	114.52	140.09	—
(D_2O)	(~46%)				(5.5)	(2.4)	(138.9)					
	5′*R*	12.50	95.31	81.89	102.92	166.34	70.27	154.59	169.82	114.79	140.24	—
	(~54%)				(6.1)	(~0)	(138.9)					
8f	5′*S*	12.39 and 12.36	93.67	81.15	101.36	165.24	69.68	155.37	150.96	120.96	158.09	142.10
(D_2O)	(~50%)						(141.8)					
	5′*R*		93.70	81.15	102.50	164.56	69.63	155.45	150.86	121.02	158.16	142.13
	(~50%)						(142.6)					
8g	5′*S*	12.35	95.99	81.91	101.38	165.68	70.02	160.08	169.05	98.85	143.32	—
(D_2O)	(~47%)				(5.7)	(1.1)	(137.7)					
	5′*R*	12.32	95.99	81.90	102.02	165.52	70.22	160.10	169.06	98.65	144.13	—
	(~53%)			(0.8)	(6.6)	(2.8)	(138.1)					
8h	5′*S*	12.51	93.09	81.15	100.79	165.69	70.06	161.57	153.68	118.30	156.79	139.20
(D_2O)	(~59%)				(5.7)	(1.6)	(138.0)					
	5′*R*	12.49	92.63	81.11	102.56	165.70	69.78	161.43	153.45	118.02	156.70	139.00
	(~41%)				(7.5)	(1.5)	(~142)					
9	5′*S*	20.36	92.34	77.66	102.35	159.05	64.11	150.50	163.31	102.64	140.17	—
(DMSO)					(8.5)	(1.4)	(164.0)					
	5′*R*	20.41	92.23	77.75	103.41	157.69	63.68	150.43	163.19	102.80	140.32	—
					(8.0)	(~0)	(164.5)					
(D_2O)	5′*S*	20.63	95.72	80.45	105.32	160.51	66.21	153.98	168.86	105.39	144.11	—
					(9.1)	(3.8)	(166.5)					
	5′*R*	20.74	95.73	80.60	106.17	159.43	65.87	153.98	168.84	105.50	144.22	—
					(7.2)	(3.6)	(167.1)					
10a	5′*S*	14.05	92.41	78.87	99.53	162.81	65.74	150.44	163.40	101.80	140.79	—
(DMSO)	(~58%)				(6.5)		(145.2)					
	5′*R*		91.86	78.60	101.23	160.99	65.13	150.45	163.31	102.40	141.26	—
	(~42%)				(6.9)		(148.4)					
10f[b]	5′*S*	12.94	91.73	79.27	97.95	163.67	66.31	152.73	149.78	118.79	156.08	139.28
(DMSO)	(~64%)				(6.2)	(1.9)	(136.6)					
	5′*R*	12.77	91.14	78.82	99.66	161.88	65.73	152.93	149.02	118.67	156.18	138.86
	(~36%)				(6.7)	(~0)	(139.4)					
10g[b]	5′*S*	13.43	93.36	79.50	98.42	163.44	66.16	155.01	165.83	94.39	141.54	—
(DMSO)	(~52.5%)						(137.9)					
	5′*R*	13.28	92.72	79.24	100.37	161.65	65.46	155.07	165.90	93.85	142.17	—
	(~47.5%)						(141.9)					
10h[b,c]	5′*S*	12.81	91.02	78.15	98.05	163.18	66.18	153.99	150.49	116.30	156.91	135.34
(DMSO)	(~49%)						(137.2)					
	5′*R*	12.54	90.56	79.13	99.23	161.57	65.85	154.32	150.88	116.29	156.88	134.74
	(~51%)						(139.5)					

[a] The phosphorus signals were structurally assigned to C-5′ epimers only in case of their different intensities and criteria based on the multiplicity pattern of H-3′ difference between C-3′ chemical shifts.
[b] The signals of long aliphatic chain could not be structurally assigned.
[c] Measured at 40 °C.
[d] Data for other carbons see Table 2S in Supplementary data.

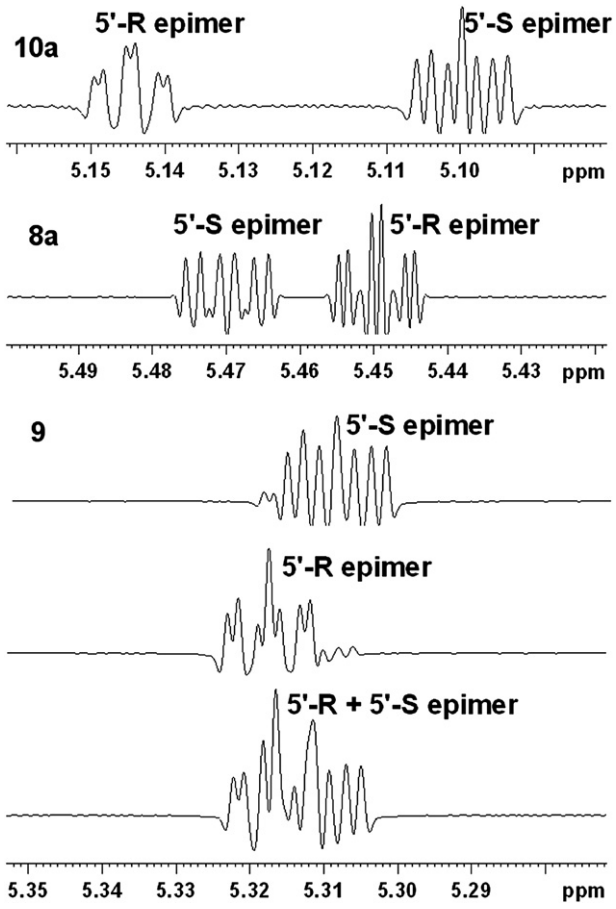


Fig. 3. Multiplet pattern (resolution enhanced) of H3′ in phosphonate diester **9** (pure 5′-*S*, pure 5′-*R* and their mixture) and 5′-epimeric mixtures of free phosphonate **8a** and phosphonate monoester **10a**.

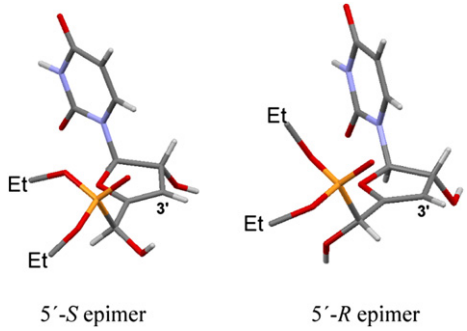


Fig. 4. Crystal structures of diester **9** showing the different orientations of the 5′-OH group to C3′. The γ-*gauche* interaction of the 5′-OH with C3′ in the 5′-*S* epimer could be responsible for the upfield position of its carbon C3′.

3. Experimental

3.1. General

Solvents were evaporated at 40 °C and 2 kPa, and products were dried over phosphorus pentoxide at 13 Pa. The course of the reactions was checked by TLC (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). For flash column chromatography, silica gel 40–60 μm (Fluka) was used. 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 triethylammonium acetate. 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. High resolution FAB mass spectra were recorded on a ZAB-EQ (VG Analytical) instrument with glycerol and thioglycerol as matrices. The NMR spectra of compounds **5a**,**b**, **7a**–**e**, **8a**,**e**–**h**, **9** and **10a**,**f**–**h** were measured on Bruker Avance-600 instrument (^{1}H at 600.13 MHz and ^{13}C at 150.9 MHz) and Bruker Avance-500 instrument (^{31}P at 202.3 MHz) in DMSO or D_2O. ^{1}H and ^{13}C chemical shifts in D_2O are referenced to added dioxane (using δ(H)=3.75 and δ(C)=69.3) and in DMSO to the solvent peak (using δ(H)=2.50 and δ(C)=39.7). Phosphorus chemical shifts are referenced to H_3PO_4 as the external standard. Structural assignment of proton and carbon signals was done using the 2D-H,H-COSY, 2D-H,C-HSQC and 2D-H,C-HMBC spectra. The ^{1}H, ^{13}C and ^{31}P NMR data are presented in Tables 1S and 2S of Supplementary data. The X-ray crystallographic analysis of single crystals of (5′*R*)-**9** (colourless, 0.04×0.15×0.30 mm) and (5′*S*)-**9** (colourless, 0.06×0.14×0.26 mm) was performed with Xcalibur X-ray diffractometer with Cu Kα (λ=1.54180 Å), data collected at 150 K. Both structures were solved by direct methods with SIR92[41] and refined by full-matrix least-squares on F with CRYSTALS.[42] All hydrogen atoms were located in a difference map but those attached to carbon atoms were later repositioned geometrically and then refined with riding constraints, while all other atoms were refined anisotropically in both cases.

*3.1.1. Dimethyl (5′RS)-3′-deoxy-3′,4′-didehydrouridine-5′-C-phosphonate (**5a**).* 2′,3′-Di-*O*-methoxymethylidene uridine[43] **2a** (1.43 g, 5.00 mmol) was co-distilled twice with dry DMF and dissolved in DMF (35 mL) at room temperature under the inert atmosphere of argon. To the stirred solution, DMSO (2.13 mL, 30.0 mmol) and DCC (3.10 g, 15.0 mmol) were added, followed by pre-mixed pyridine (404 μL, 5.00 mmol) and TFA (191 μL, 2.50 mmol) in DMF (5 mL). After completion of oxidation (checked by TLC in 10% EtOH in $CHCl_3$; within 3 h), triethylamine (2.80 mL, 20.0 mmol) was added. After elimination was completed (TLC in 10% EtOH in $CHCl_3$; within 5 min), dimethyl phosphite (1.83 mL, 20.0 mmol) was added and the mixture was left for 5 min. After TLC (10% EtOH in $CHCl_3$) revealed the complete conversion, the excess of oxidation agent was decomposed by oxalic acid (1.26 g, 10.0 mmol). The mixture was concentrated in vacuo, MeOH was added to the residue and the mixture was filtered through Celite, concentrated in vacuo and purified by chromatography on silica gel (elution with gradient of 20% MeOH in $CHCl_3$), on reverse phase (linear gradient of MeOH in water) and subsequently freeze-dried from water. Yield 0.73 g, 44% (off-white lyophylisate).

HRMS (ESI): for $C_{11}H_{15}N_2NaO_8P$ $(M+Na)^+$ calcd: 357.0458, found: 357.0462; 0.9816 ppm.

ν_{max} (KBr) 2959m, 1186m (P–OMe); 1715s, 1690vs, 1633m, 1462m, 1422m (base); 1660s (C=C); 1105m, 1082m (C–OH); 1256m (P=O); 1040s, 755m, 548m (POC).

^{1}H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

*3.1.2. Dimethyl (5′RS)-6-N-benzoyl-3′-deoxy-3′,4′-didehydroadenosine-5′-C-phosphonate (**5b**).* 2′,3′-Di-*O*-methoxymethylidene-6-*N*-benzoyladenosine[44] **2b** (1.57 g, 3.80 mmol) was co-distilled twice with dry toluene and dissolved in DMSO (11 mL) at room temperature under the inert atmosphere of argon. To the stirred solution, DCC (2.35 g, 11.4 mmol), followed by pre-mixed pyridine (307 μL, 3.80 mmol) and TFA (145 μL, 1.90 mmol) in DMSO (4 mL) were added. After completion of oxidation (checked by TLC in 10% EtOH in $CHCl_3$; within 2 h),

triethylamine (2.10 mL, 15.0 mmol) was added and after 15 min (TLC in 10% EtOH in $CHCl_3$), dimethyl phosphite (1.38 mL, 15.0 mmol) was added and the mixture was stirred for further 15 min (TLC in 10% EtOH in $CHCl_3$). The reaction mixture was freeze-dried to remove DMSO, the residue was dissolved in MeOH/water mixture (1:1) and filtered through Celite. The filtrate was co-distilled with ethanol. The crude product was purified by chromatography on silica gel (elution with gradient of 0–20% MeOH in $CHCl_3$) and subsequent chromatography on reverse phase (elution with linear gradient of MeOH in water) and subsequently freeze-dried from water. Yield 1.23 g 70% (white lyophylisate).

HRMS (ESI): for $C_{19}H_{20}N_5NaO_7P$ $(M+Na)^+$ calcd: 484.0993, found: 484.0988; −0.8620 ppm.

ν_{max} (KBr) 3418vs, 3272m, 3110m (OH, NH); 2957w, 1177m, 1048s, 1038s, 753w, 555m (P–OMe); 1700m, 1512m, 1073s, 711m (NHBz); 1661w (C=C); 1612s, 1583m, 1487m, 1337m, 1285m, 1222s, 797w, 642m (base); 1249s (P=O).

^1H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

3.2. Preparation of bis(cyanoethyl) 3′-deoxy-3′,4′-didehydronucleoside-5′-*C*-phosphonates 7a–e

To a solution of aldehyde[35] **6a–e** (1.00 mmol) in dried DMF (8 mL), freshly prepared bis(2-cyanoethyl) phosphite[38] (0.75 g, 4.00 mmol) was added at room temperature, followed by Et_3N (up to 0.06 mL, 0.45 mmol). The reaction was completed usually within 15–30 min (checked by TLC in 20% MeOH in $CHCl_3$). The mixture was concentrated in vacuo and the residue was purified by chromatography on silica gel (elution with gradient of 20% MeOH in $CHCl_3$).

*3.2.1. Bis-(2-cyanoethyl) (5′RS)-3′-deoxy-3′,4′-didehydrouridine-5′-C-phosphonate (**7a**).* Prepared from 5′-aldehyde **6a** (Et_3N 21.0 μL, 0.15 mmol); yield 130 mg, 70% (off-white foam).

HRMS (ESI): for $C_{15}H_{17}N_4NaO_8P$ $(M+Na)^+$ calcd: 435.0676, found: 435.0676; −0.1018 ppm.

ν_{max} ($CHCl_3$) 3386m, 3276m (OH, NH); 2256w (CN); 1720s, 1687vs, 1474w, 1412w, 1389w (base); 1261m (P=O); 1081s, 1050s, 1003m (POCC).

^1H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

*3.2.2. Bis-(2-cyanoethyl) (5′RS)-6-N-benzoyl-3′-deoxy-3′,4′-didehydroadenosine-5′-C-phosphonate (**7b**).* Prepared from 5′-aldehyde **6b** (Et_3N 21.0 μL, 0.15 mmol); yield 128 mg, 85% (white foam).

HRMS (ESI): for $C_{23}H_{21}N_7O_7P$ $(M-H)^-$ calcd: 538.1246, found: 538.1243; −0.5431 ppm.

ν_{max} ($CHCl_3$) 3425m, 3321m, 3126m (OH, NH); 2253w (CN); 1717s, 1703m, 1688m, 1527m, 1511m, 712m, 704m (NHBz); 1666m (C=C); 1614vs, 1591m, 1481m, 1412m, 1334m, 797m (base); 1250vs (P=O); 1080vs, 1059s, 1050vs, 1034vs, 1005s (POCC).

^1H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

*3.2.3. Bis-(2-cyanoethyl) (5′RS)-4-N-benzoyl-3′-deoxy-3′,4′-didehydrocytidine-5′-C-phosphonate (**7c**).* Prepared from 5′-aldehyde **6c** (Et_3N 63.0 μL, 0.45 mmol); yield 140 mg, 90% (white foam).

HRMS (ESI): for $C_{22}H_{22}N_5NaO_8P$ $(M+Na)^+$ calcd: 538.1098, found: 538.1097; −0.3008 ppm.

ν_{max} (KBr) 3364m, 3212m (NH, OH); 2256w (CN); 1697s, 1681s, 1563m, 1603m, 1582w, 1501m, 1074s, 687m (NHBz); 1681s, 1666s, 1650m, 1619s, 1487vs, 1374m, 781m (base); 1254vs (P=O); 1085s, 1055s, 1037s, 1006s (POCC).

^1H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

*3.2.4. Bis-(2-cyanoethyl) (5′RS)-3′-deoxy-3′,4′-didehydro-2-N-isobutyrylguanosine-5′-C-phosphonate (**7d**).* Prepared from 5′-aldehyde **6d** (Et_3N 63.0 μL, 0.45 mmol); yield 121 mg, 74% (white foam).

HRMS (ESI): for $C_{20}H_{24}N_7NaO_8P$ $(M+Na)^+$ calcd: 544.1316, found: 544.1313; −0.6550 ppm.

ν_{max} (KBr) 3211m (OH, NH); 2256w (CN); 1715s, 1566s, 1376w (NH-*i*Bu); 1683vs, 1608s, 1534w, 1476m, 1408m, 785m, 721w (base); 1254m (P=O); 1080s, 1050s, 1036s, 1004m (POCC).

^1H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

*3.2.5. Bis-(2-cyanoethyl) (5′RS)-3′-deoxy-3′,4′-didehydro-5-methyluridine-5′-C-phosphonate (**7e**).* Prepared from 5′-aldehyde **6e** (Et_3N 36.0 μL, 0.26 mmol); yield 133 mg, 74% (white foam).

HRMS (ESI): for $C_{16}H_{19}N_4NaO_8P$ $(M+Na)^+$ calcd: 449.08327, found: 449.08326; −0.01753 ppm.

ν_{max} (KBr) 3270m (OH, NH); 2256w (CN); 1697s, 1654m, 1635m, 1377w, 784w (base); 1260w, 1228w (P=O); 1079m, 1054m (C–OH); 1035m, 1003m (POCC).

^1H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

3.3. Preparation of 3′-deoxy-3′,4′-didehydronucleoside-5′-*C*-phosphonic acids 8a,e–h

To bis-(2-cyanoethyl) phosphonate **7a–e** (1 mmol), 8 M $MeNH_2$ in absolute EtOH (40 mL) was added, and the mixture was heated to 50 °C overnight. TLC (H1: ethyl acetate/acetone/EtOH/water 4:1:1:1; and IPAW: 2-propanol/concentrated aqueous ammonia/water 7:1:2) revealed formation of a free phosphonic acid. The mixture was concentrated in vacuo, co-distilled three times with EtOH and purified on reverse phase (linear gradient of MeOH in water). The product was transformed to its Na salt on Dowex 50 (Na+) and freeze-dried from water.

*3.3.1. (5′RS) 3′-Deoxy-3′,4′-didehydrouridine-5′-C-phosphonic acid (**8a**).* Prepared from bis-(2-cyanoethyl) phosphonate **7a**; yield 100 mg, 85% (white lyophylisate).

HRMS (ESI): for $C_9H_{10}N_2O_8P$ $(M+H)^+$ calcd: 305.0180, found: 305.0184; 1.0731 ppm.

ν_{max} (KBr) 3423s, 3255s, 3110m, 2813w (OH, NH); 1697s, 1637m, 1473w, 1271m, 766w (base); 1091s, 973s, 583m, 486m (PO_3^{2-}).

^1H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

*3.3.2. (5′RS) 3′-Deoxy-3′,4′-didehydro-5-methyluridine-5′-C-phosphonic acid (**8e**).* Prepared from bis-(2-cyanoethyl) phosphonate **7e**; yield 340 mg, 90% (white lyophylisate).

HRMS (ESI): for $C_{10}H_{12}N_2O_8P$ $(M+H)^+$ calcd: 319.0337, found: 319.0342; 1.4914 ppm.

ν_{max} (KBr) 3422s, 3260m, 2756m (OH, NH); 1699vs, 1656m, 1519w, 1476m, 1391w, 1271m, 790w, 767w, 723w (base); 1088s, 971s, 581m, 484w (PO_3^{2-}).

^1H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

*3.3.3. (5′RS) 3′-Deoxy-3′,4′-didehydroadenosine-5′-C-phosphonic acid (**8f**).* Prepared from bis-(2-cyanoethyl) phosphonate **7b**; yield 147 mg, 88% (white lyophylisate).

HRMS (ESI): for $C_{10}H_{11}N_5O_6P$ $(M+H)^+$ calcd: 328.0452, found: 328.0445; −2.3776 ppm.

ν_{max} (KBr) 3364vs, 3222s (OH, NH); 1654s, 1648s, 1605m, 1578m, 1476m, 1421m, 1335m, 1209m, 797m, 645m (base); 1088vs, 1050 versus, 970m, 580m, 487m (PO_3^{2-}).

^1H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

*3.3.4. (5′RS) 3′-Deoxy-3′,4′-didehydrocytidine-5′-C-phosphonic acid (**8g**).* Prepared from bis-(2-cyanoethyl) phosphonate **7c**; yield 108 mg, 84% (white lyophylisate).

HRMS (ESI): for $C_9H_{11}N_3O_7P$ $(M+H)^+$ calcd: 304.0340, found: 304.0339; −0.2770 ppm.

ν_{max} (KBr) 3330s, 3187s, 3095s (OH, NH); 1655vs, 1610m, 1524m, 1492m, 1399m, 1289m, 784m, 600w (base); 1086s, 968s, 582m, 486w (PO_3^{2-}).

^{1}H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

*3.3.5. (5′RS) 3′-Deoxy-3′,4′-didehydroguanosine-5′-C-phosphonic acid (**8h**).* Prepared from bis-(2-cyanoethyl) phosphonate **7d**; yield 127 mg, 87% (white lyophylisate).

HRMS (ESI): for $C_{10}H_{11}N_5O_7P$ $(M+H)^+$ calcd: 344.0402, found: 344.0405; 1.0396 ppm.

ν_{max} (KBr) 3385m, 3122s, 2756m (OH, NH); 1698vs, 1635m, 1609m, 1578w, 1533m, 1483w, 1376w, 779w, 729w, 640w (base); 1088s, 972m, 578m, 487w (PO_3^{2-}).

^{1}H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

*3.3.6. Diethyl (5′RS)-3′-deoxy-3′,4′-didehydrouridine-5′-C-phosphonate (**9**).* To a solution of 5′-aldehyde[35] **6a** (225 mg, 1.00 mmol) in water (8 mL), diethyl phosphite (0.52 mL, 4.02 mmol) and Et_3N (10.0 μL, 0.07 mmol) were added at room temperature. After TLC (20% MeOH in $CHCl_3$) revealed total conversion (20 min), the mixture was concentrated in vacuo and co-distilled with EtOH. The crystal residue gave 220 mg (60%) of the phosphonate **9**, mp 130 °C decomp.; $[\alpha]_D^{20}$ −167.3 (*c* 0.518, H_2O).

HRMS (ESI): for $C_{13}H_{19}N_2NaO_8P$ $(M+Na)^+$ calcd: 385.0771, found: 385.0770; −0.2976 ppm.

ν_{max} (KBr) 3422m, 3364m, 3316m, 3163m (OH, NH); 1721vs, 1699vs, 1687vs, 1672vs, 1455m, 1420m, 1382m, 1262s, 829m, 757w (base); 1246m, 1211s (P=O); 1076s, 1051vs (C–OH); 1162s, 1108m (P–OEt); 1051vs, 1018vs, 970s, 561m (POCC).

^{1}H and ^{13}C NMR data see Tables 3 and 4.

5′-*R* and 5′-*S* epimers were separated by preparative HPLC using gradient of MeOH in water and crystallised from EtOH.

*3.3.7. Diethyl (5′R)-3′-deoxy-3′,4′-didehydrouridine-5′-C-phosphonate (5′R-**9**).* Colourless crystals, mp 130 °C decomp.; $[\alpha]_D^{20}$ −152.3 (*c* 0.237, H_2O).

HRMS (ESI): (1) for $C_{13}H_{20}O_8N_2P$ $(M+H)^+$ calcd: 363.09518, found: 363.09503; −0.41193 ppm; (2) for $C_{13}H_{19}O_8N_2NaP$ $(M+Na)^+$ calcd: 385.07712, found: 385.07678; −0.90350 ppm.

Crystal data: $C_{13}H_{19}N_2O_8P$, triclinic, space group *P*1, *a*=9.4279 (18) Å, *b*=9.4286 (15) Å, *c*=10.8221 (15) Å, α=79.198 (12)°, β=81.553 (14)°, γ=61.700 (18)°, *V*=830.1 (3) Å^3, *Z*=2, *M*=362.27, 27,324 reflections measured, 6402 independent reflections. Final *R*=0.0685, *wR*=0.1129, GoF=0.11 for 4784 reflections with $I>2\sigma(I)$ and 452 parameters. The asymmetric unit contains two independent molecules of 5′*R*-**9**, which are not significantly different. In both of these molecules, the terminal carbon of one of the ethyl groups is disordered over two sites with their site occupation factors being 0.56 and 0.44 (Fig. 2: only one of the two sites is represented). CCDC 773860.

^{1}H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

*3.3.8. Diethyl (5′S)-3′-deoxy-3′,4′-didehydrouridine-5′-C-phosphonate (5′S-**9**).* Colourless crystals, mp 130 °C decomp.; $[\alpha]_D^{20}$ −172.5 (*c* 0.251, H_2O).

HRMS (ESI): for $C_{13}H_{20}O_8N_2P$ $(M+H)^+$ calcd: 363.09518, found: 363.09496; −0.60026 ppm.

Crystal data: $C_{13}H_{19}N_2O_8P$, monoclinic, space group C2, *a*=15.958 (3) Å, *b*=9.5771 (11) Å, *c*=10.9485 (18) Å, β=104.436 (18)°, *V*=1620.4 (5) Å^3, *Z*=4, *M*=1620.4 (5), 20,741 reflections measured, 3349 independent reflections. Final *R*=0.1016, *wR*=0.1226, GoF=1.14 for 2432 reflections with $I>2\sigma(I)$ and 254 parameters. Both ethyl groups are disordered over two sites with their site occupation factors being 0.55 and 0.45 (Fig. 2: only one of the two sites is represented). Several restrains were used to regularise the thermal motions of these groups. CCDC 773861.

^{1}H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

3.4. Preparation of 3-hexadecyloxypropyl 3′-deoxy-3′,4′-dideh ydronucleoside-5′-*C*-phosphonates 10a,f–h

To a solution of aldehyde[35] **6a–d** (1 mmol) in dried DMF (8 mL), freshly prepared methyl 3-hexadecyloxypropyl phosphite[38] (0.45 g, 1.20 mmol) was added, followed by Et_3N (0.14 mL, 1.00 mmol), and the mixture was left aside at room temperature overnight. The course of the reaction was checked by TLC in 10% EtOH in $CHCl_3$. The mixture was concentrated in vacuo and the residue was purified by chromatography on silica gel (elution with gradient of EtOH in ethyl acetate). The methyl group was removed upon heating in 60% aqueous pyridine (20 mL) at 50 °C overnight (TLC H1: ethyl acetate/acetone/EtOH/water 4:1:1:1), then the mixture was concentrated in vacuo and co-distilled three times with EtOH. Removal of *N*-benzoyl groups for adenine and cytosine compounds was achieved by concentrated methanolic ammonia (40 mL); the *N*-isobutyryl group from guanine compound was removed by 8 M $MeNH_2$ in absolute EtOH (20 mL). The course of the reaction was checked by TLC (H1). The solvent was then removed in vacuo and the resulting monoester was purified by chromatography on silica gel (elution with gradient of H-1 in ethyl acetate). Treatment with saturated sodium bicarbonate solution upon heating, provided sodium salt of the desired monoester, which was purified on reverse phase (linear gradient of MeOH in water), and subsequently freeze-dried from water.

*3.4.1. 3-Hexadecyloxypropyl (5′RS)-3′-deoxy-3′,4′-didehydrouridine-5′-C-phosphonate (**10a**).* Prepared from 5′-aldehyde **6a** as Et_3NH^+ salt; yield 222 mg, 38% (white lyophylisate).

HRMS (ESI): for $C_{28}H_{48}N_2O_9P$ $(M+H)^+$ calcd: 587.3103, found: 587.3106; 0.5904 ppm.

ν_{max} (KBr) 3404m, 3226m (OH, NH); 2920vs, 2851s (CH_2); 2679w, 2491w, 1434w, 1183m, 1161m, 836w, 819w (Et_3NH^+); 1713s, 1693vs, 1633w, 1467m, 1256m, 763w, 722w (base); 1209m, 1081m, 1055s, 991w, 971w (PO_2^-).

^{1}H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

*3.4.2. 3-Hexadecyloxypropyl (5′RS)-3′-deoxy-3′,4′-didehydroadenosine-5′-C-phosphonate (**10f**).* Prepared from 5′-aldehyde **6b**; yield 353 mg, 58% (white lyophylisate).

HRMS (ESI): for $C_{29}H_{51}N_5O_7P$ $(M+H)^+$ calcd: 612.3521, found: 612.3519; −0.2173 ppm.

ν_{max} (KBr) 3331m, 3264m, 3186m, 3120m (OH, NH); 2924vs, 2853s (CH_2); 1645s, 1601m, 1579w, 1469m, 1420w, 1334w, 1294w, 798w, 721w, 647w (base); 1218s, 1087s, 1058s, 993w, 967w (PO_2-).

^{1}H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

*3.4.3. 3-Hexadecyloxypropyl (5′RS)-3′-deoxy-3′,4′-didehydrocytidine-5′-C-phosphonate (**10g**).* Prepared from 5′-aldehyde **6c**; yield 364 mg, 62% (white lyophylisate).

HRMS (ESI): for $C_{28}H_{51}N_3O_8P$ $(M+H)^+$ calcd: 588.3408, found: 588.3408; −0.0024 ppm.

ν_{max} (KBr) 3400m, 3341m, 3198m (OH, NH); 2924vs, 2853s, 146m (CH_2); 1651vs, 1613m, 1525w, 1493m, 1398w, 1287w, 787w, 721w, 600w (base); 1378w (CH_3); 1219m, 1087m, 1058s, 1000w, 965w (PO_2^-).

^{1}H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

*3.4.4. 3-Hexadecyloxypropyl (5′RS)-3′-deoxy-3′,4′-didehydroguanosine-5′-C-phosphonate (**10h**).* Prepared from 5′-aldehyde **6d**; yield 129 mg, 21% (white lyophylisate).

HRMS (ESI): for $C_{29}H_{49}N_5O_8P$ $(M+H)^+$ calcd: 626.3324, found: 626.3332; 1.1931 ppm.

ν_{max} (KBr) 3487m, 3413m, 3125m (OH, NH); 2924vs, 2853s, 1468w (CH_2); 1693vs, 1632m, 1610m, 1532w, 1483w, 1412w, 1179m,

782w, 722w, 641w (base); 1376m (CH_3); 1220m, 1085m, 1057s, 1027m, 992w, 970w (PO_2^-).

^1H, ^{13}C and ^{31}P NMR data see Tables 3 and 4.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.04.037.

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