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Oxidative cleavage of ribofuranose 5-(α-hydroxyphosphonates): a route to erythrofuranose-based nucleoside phosphonic acids

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Abstract—We report here an oxidative cleavage of (5*R*)- and (5*S*)-ribofuranosyl-5-*C*-phosphonate derivatives with periodate anion under both strong acidic and neutral conditions. In both cases, only (5*R*)-configured compound underwent the expected oxidation reaction and afforded the desired (4*R*)-erythrofuranosylphosphonate, whereas the second epimer, (5*S*)-ribofuranosyl-5-*C*-phosphonate did not provide the corresponding (4*S*)-erythrofuranosylphosphonate derivative. This different behavior of epimers toward oxidative cleavage is an important phenomenon. The obtained (4*R*)-erythrofuranosylphosphonate was used for the preparation of phosphonate mimic of adenosine 5′-phosphate via classical nucleosidation reaction. Condensation of the protected shortened AMP analogue with adenosine derivatives, however, provided only the 2′,5′-linked ApA analogue. Study on hybridization of the modified 2′-5′ ApA with polyU revealed its ability to form stable triplex-like complex, similar to natural 2′-5′ r(ApA) and 3′-5′ r(ApA). NMR spectroscopy study showed that the erythrofuranose part of the phosphonate nucleotide unit of modified 2′-5′ ApA was predominantly in the C2′-*endo* conformation, which is characteristic for B-DNA.

1. Introduction

Structurally diverse isopolar phosphonate nucleotide analogues containing a bridging *P–C* bond have attracted our attention for many years.[1–7] This class of compounds, known as nucleoside phosphonic acids, represents a pool of potential antimetabolites exhibiting absolute stability against phosphomonoesterase and nucleotidase cleavage.[8] Some of these compounds have already found clinical use as potent antivirals.[9–11] The search for novel nucleotide analogues can reveal new biologically active compounds capable of discriminating between cellular and viral or tumor enzymes of nucleic acid metabolism, which could be used as antiviral and/or anticancer agents. In addition, novel phosphonate nucleotide analogues can be used for the construction of isopolar chimeric oligonucleotides containing nuclease-stable internucleotide linkages,[7] for instance, similar to already prepared phosphonate 2′,5′-oligoadenylates, which are potent, enzyme stable RNase L activators[12] intended for RNase L-mediated cleavage of pathogenic RNA.[13]

Here we present the synthesis of the isopolar, nonisosteric AMP analogue **13**, a representative of a new class of modified nucleoside 5′-phosphates, the structure of which resembles the features of the earlier reported nucleoside phosphonic acids **1**[14,15] and **2**[16,17] (Fig. 1), and the *ribo* ApA dimer **18a** with the isopolar, shortened phosphonate internucleotide linkage.

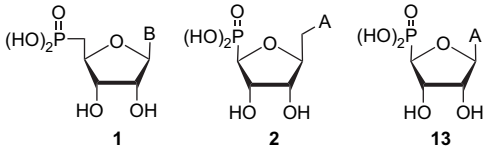

Figure 1.

2. Results and discussion

The strategy for the synthesis of **13** originated from the suitably protected ribofuranosyl-5-*C*-phosphonate derivative **7a**, the key compound obtained by a four-step synthesis from 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**3**).[18] Benzoylation of **3** afforded compound **4**, which was selectively deprotected in 60% aqueous acetic acid at 50 °C to give **5**. Oxidation of the vicinal diol of **5** with sodium periodate in aqueous acetone resulted in the aldehyde **6**. Subsequent addition of diethyl phosphite to aldehyde **6** in the presence of triethylamine provided a 1:1 epimeric mixture of (*R*)- and (*S*)-5-*C*-phosphonates **7a** and **7b**, respectively, in an overall 54% yield (Scheme 1). The epimers were

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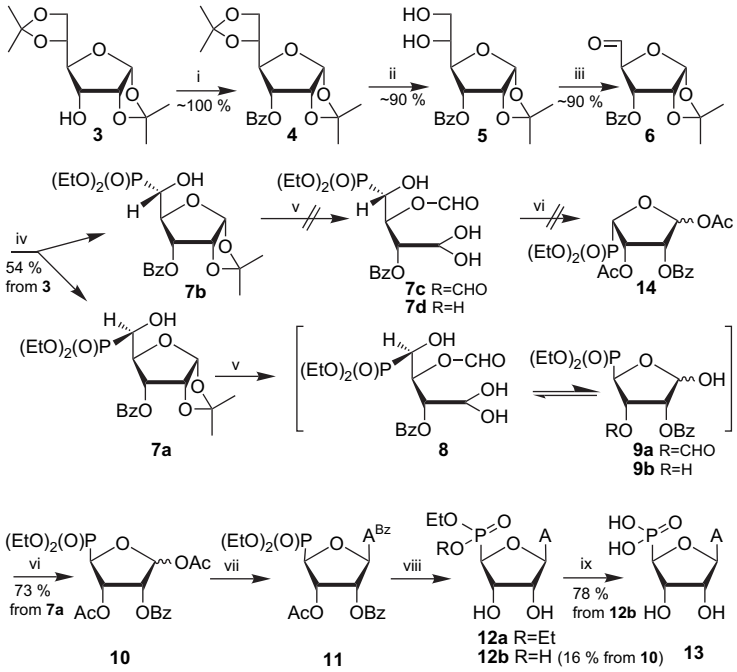


Scheme 1. (i) Benzoylcyanide, CH_3CN, TEA; (ii) 60% aq acetic acid, 50 °C, 3 h; (iii) $NaIO_4$, 70% aq acetone, 0 °C, 8 h; (iv) $HP(O)(OEt)_2$, TEA, DCM, 80 °C, 24 h; (v) (a) HIO_4, 50% aq dioxane, 60 °C, 3 days; (b) 0.1 M TEAB; (vi) Ac_2O, DMAP, pyridine, 16 h, rt; (vii) silylated 6-*N*-benzoyladenine, $SnCl_4$, CH_3CN, 24 h , rt; (viii) 1 M NaOH, 48 h, rt; (ix) Me_3SiBr, CH_3CN, 24 h, rt. Yields of compounds **4**, **5**, and **6** were estimated from TLC (compounds were used for further reaction step without purification).

separated by silica gel chromatography and subjected to subsequent oxidative cleavage separately.

2.1. Oxidative cleavage of 7a (Scheme 1)

Hydrolysis of the 1,2-*O*-isopropylidene group of **7a** and subsequent oxidative cleavage of the C1–C2 bond, performed in one step by aqueous periodic acid, led to an equilibrium mixture of **8** and **9a** in which the 3-*O*-formyl group protects the acyclic form **8** (if present) from further oxidative cleavage of the C4–C5 bond. We attempted to verify the presence of the 3-*O*-formyl group in the product (**8** or **9a**) but neither treatment with 0.1 M TEAB in aqueous dioxane[19] nor heating in 80% aqueous pyridine[20] changed the mobility on TLC or RP HPLC. It did not seem that the 3-*O*-formyl group in **8** could survive in strongly acidic conditions during several days' treatment with aqueous periodic acid. Thus, we concluded that the nascent acyclic form **8** has to undergo a very fast cyclization reaction to furanose **9a**. Then both the formyl derivative **9a** and the deformylated compound **9b** are resistant against oxidative cleavage by periodic acid. Since the subsequent acetylation resulted in the expected acetyl derivative **10** in good yield, the final product of oxidative cleavage of **7a** with periodic acid must have been predominantly the furanose **9b**.

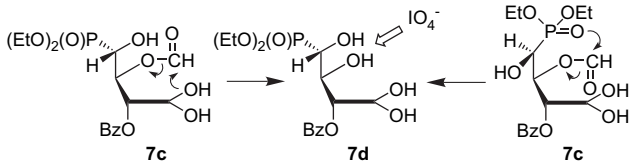
Note: the transformation of compound **9b** to **10**, which we performed several times has resulted in several cases in pure β-anomer of **10** (3-*O*-acetyl-2-*O*-benzoyl) but in several cases also in an anomeric mixture of both 3-*O*-acetyl-2-*O*-benzoyl and 2-*O*-acetyl-3-*O*-benzoyl regioisomers. We believe that each of these isomers afforded, after nucleosidation and deprotection, the identical product. The formation of the regioisomers (migration of the benzoyl group) seems to depend on the time of oxidation of **7a**, workup of **9a**, and conditions for acetylation of **9a** to **10**.

2.2. Oxidative cleavage of 7b (Scheme 1)

On the other hand, treatment of **7b** with periodic acid to obtain **14** caused a total decomposition of compound **7b** with the formation of a very complex mixture of derivatives. Application of a two-step procedure[20] using 90% aqueous TFA for hydrolysis of 1,2-*O*-isopropylidene group in **7b** in the first step, and then, after removal of TFA, sodium periodate oxidative cleavage in the second step also led to a total decomposition of **7b**. Under the same conditions, the epimer **7a** reacted as smoothly as with periodic acid alone and following acetylation provided a comparable yield of product **10**.

The nature of the very different reactivities of **7a** and **7b** toward periodic acid (or TFA–sodium periodate[20]) is not quite clear. The explanation for this phenomenon could perhaps be seen in the equilibrium between acyclic structure **7c** and its furanose form, which can be shifted in favor of the acyclic compound **7c**. Under such conditions, hydrolysis of the 3-*O*-formyl group in the acyclic structure **7c** leads to **7d** and this compound, bearing a C3–C4 vicinal diol, is immediately cleaved by periodic acid (or sodium periodate) (Scheme 2). It can be speculated that the cleavage of the *O*-formyl group could proceed in an oxidative manner via the extremely labile carbonic acid monoester (R-OCOOH) but, more likely, groupings like P═O or hydrated C1 aldehyde $CH(OH)_2$ in **7c** could facilitate the formyl group hydrolysis via intramolecular participation due to a favorable conformation (Scheme 2). Similarly, the different reactivity of epimers **7a** and **7b** toward acetolysis was described recently; whereas the epimer **7a** provided the expected

product, the second epimer **7b** afforded a mixture of compounds.[21]



Scheme 2. Proposed mechanisms of periodate degradation of (*S*)-configured 5-hydroxyphosphonate **7c**.

Concerning the different reactivity of **7a** and **7b** toward periodate oxidation, the separation of epimers **7a** and **7b** does not seem to be, from a practical point of view, too advantageous since the yield of **10**, when the reaction was carried out with an epimeric mixture, varied in the range of 38–51% whereas with pure epimer **7a**, it was 65–73%.

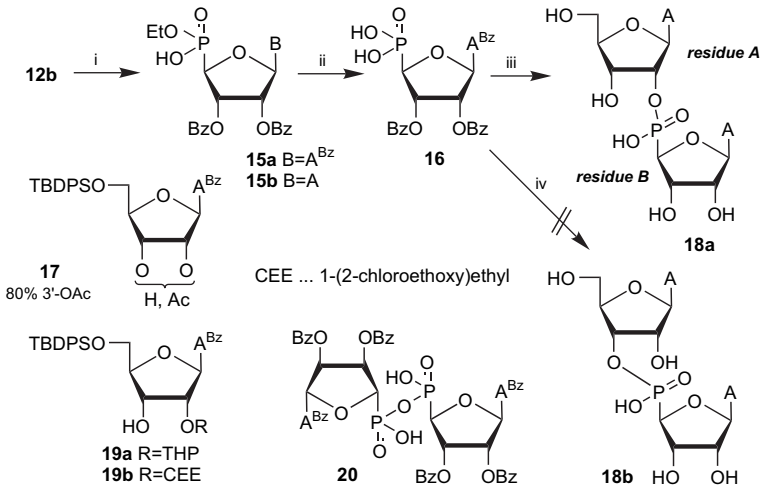
The nucleosidation reaction of the sugar phosphonate **10** with silylated 6-*N*-benzoyladenine in the presence of $SnCl_4$ provided a mixture of products in which the expected derivative **11** was not recognized. Therefore, the mixture was treated with concentrated aqueous ammonia to remove acyl-protecting groups, and the adenine-containing compounds were desalted on Dowex 50. A mixture of diethyl- and monoethyl ester **12a** and **12b** was obtained as the only products in a low yield of 13–20%. Diester **12a** was converted to the monoester **12b** by treatment with dilute sodium hydroxide and compound **12b** was isolated on Dowex 1 by a gradient elution with acetic acid in water. With respect to the low yield, the nucleosidation reaction, a weak point of the synthesis, still remains open for improvement. Replacement of tin tetrachloride by trimethylsilyl triflate in the nucleosidation reaction did not provide any of the expected products **12a** and **12b**. Compound **12b** was transformed into the free phosphonic acid **13** by treatment with bromotrimethylsilane in the presence of 2,6-lutidine to prevent potential furanose ring cleavage. Ion exchange chromatography on Dowex 1 afforded the pure free phosphonic acid **13**.

Since compound **13** is a 'shortened' nucleotide analogue, its incorporation into an oligonucleotide chain was considered to be very interesting with respect to hybridization properties of the modified chain. Shortening of the internucleotide linkage renders the chain with reduced number of degrees of freedom, which could positively influence hybridization ability as it was found recently for another type of shortened linkage.[22] For the study of changes induced by the presence of a modified internucleotide linkage, the dimers, as the shortest oligonucleotides capable of base-stacking and base-pairing, have been employed as model compounds.[23,24] For the synthesis of modified ApA dimers **18a** and **18b**, the monoester **12b** was benzoylated by *N*-benzoyltetrazole[25] to yield a mixture of fully and partially benzoylated phosphonates **15a** and **15b**, which was resolved by silica gel chromatography. Derivative **15a** was converted into the protected phosphonic acid **16** by bromotrimethylsilane treatment, and this compound was condensed with a mixture of 2′(3′)-*O*-acetyl derivatives **17** in pyridine using DCC. Deprotection in concentrated ammonia and chromatography on Dowex 1 in acetate form afforded only the 2′-5′ regioisomer **18a** in a low yield (15%). No 3′-5′ regioisomer **18b** was isolated. Concerning the ratio of the 2′-*O*- and 3′-*O*-acetyl derivatives of **17**, we found by NMR for regioisomers at the beginning of the condensation and after 48 h of standing in pyridine solution practically identical ratios (31:69 and 29:71, respectively). Since we still reasoned that 3′-5′ dimer **18b** was not obtained due to both low yield of condensation and relatively low content of 2′-*O*-acetyl regioisomer in the mixture of acetyl derivatives **17**, we prepared compounds **19a** and **19b** in which the 2′-hydroxyl was protected with nonmigrating, alkali-stable tetrahydropyranyl and 1-(2-chloroethoxy)ethyl groups, respectively, and condensed them with **16**. Surprisingly, also in this case we did not obtain the expected 3′-5′ isomer **18b**. Neither higher concentration of the reactants nor the presence of DMAP gave rise to the product **18b**. Concerning the formation of the phosphonate anhydride **20** as the primary product in the DCC-induced condensation, and its further activation by DCC, it can be concluded that the DCC-activated anhydride **20** cannot be attacked, due to a steric hindrance, by the 3′-hydroxyl of compounds **19a** and **19b**. We did not find any other reasonable explanation for this phenomenon.

2.3. Conformational features of dimer 18a

Strong NOE contacts between H-8 of the adenine, and H-2′ and H-3′ of the furanose ring in both nucleoside residues indicate the preferred *anti* conformation of both adenine moieties. The only interresidual NOE contact was observed between H-2′and H-8 that is commonly used for sequential assignment in oligonucleotides. From the vicinal proton couplings in the furanose rings (Table 2), a generalized Karplus–Haasnoot equation,[26] and the two-state pseudorotation model we could establish for dimer **18a** (Scheme 3) in aqueous solution a similar population of C3′-*endo* and C2′-*endo* form (ratio ≈58:42) for 'upper' furanose ring (residue A), whilst for the 'lower' furanose ring (residue B) the C2′-*endo* form significantly prevails (ratio C3′-*endo*:C2′-*endo* ≈ 14:86). For comparison, in natural *ribo* ApA dimers (for NMR data see Tables 1–3) we estimated the ratios of C3′-*endo*:C2′-*endo* form to be approximately 45:55 for 'upper' ring and 55:45 for 'lower' ring in the case of 2′-5′ r(ApA), and ≈58:42 for both rings in 3′-5′ r(ApA). The dimer **18a** differs from natural *ribo* ApA dimers mainly in the preference for the C2′-*endo* conformation in the 'lower' ring with a phosphonate group in position C4′.

Concerning the conformational features of the prepared dimer **18a**, we have subjected it to a study of hybridization properties with polyU to compare them with those of natural 2′-5′ r(ApA) and 3′-5′ r(ApA) isomers. The hypochromic effect of the complexes [**18a***polyU] at various ratios of **18a** and polyU was measured at 260 nm in the presence of Mg^{2+} ions at 3 °C, and at total base concentration of 0.15 mM. The obtained mixing curve exhibited a local minimum at 1A:2U ratio, indicating triplex-like complex formation (curve not shown). The same stoichiometry was found for both natural ApA dimers. The thermal characteristics of the complex [polyU***18a***polyU] exhibited only single transition profile suggesting direct dissociation of pseudo triplex structure into individual strands. Shortening of the internucleotide linkage in ApA analogue **18a** does not influence the complex stability in comparison with natural phosphodiester linkage. Under experimental conditions, the T_m value of the complex is 15 °C and lies very close



Scheme 3. (i) *N*-Benzoyltetrazole, DMAP, DMF, 55 °C; (ii) Me_3SiBr, CH_3CN, 24 h, rt; (iii) (a) **16**, DCC, pyridine, 5 days, rt; (b) 1 M TBAF in THF, 16 h, rt; (c) concd aq ammonia, 48 h, rt; (iv) (a) **16** and **17a** or **17b**, DCC, pyridine, 5 days, rt; (b) 1 M TBAF in THF, 16 h, rt; (c) concd aq ammonia, 48 h, rt; (d) aq 0.1 M HCl, 16 h, rt.

Table 1. Proton NMR chemical shifts

Compd	Solv.	H-1′	H-2′	H-3′	H-4′	H-5′	H-5″	H-2	H-8	Other protons
13	D_2O+NaOD	5.81	4.75	4.33	4.08	—	—	8.67	8.22	—
7a	DMSO	5.91	4.88	5.20	4.63	4.16	—	—	—	5′-OH: 6.10; 2×OEt: 3.95 (4H), 1.15 (3H), 1.09 (3H); *i*-Pr: 1.41 (3H), 1.26 (3H); Bz: 8.01 (2H), 7.65 (1H), 7.54 (1H)
7b	DMSO	5.90	4.90	4.96	4.43	3.99	—	—	—	5′-OH: 5.85; 2×OEt: 4.05 (4H), 1.22 (3H), 1.21 (3H); *i*-Pr: 1.42 (3H), 1.26 (3H); Bz: 8.02 (2H), 7.70 (1H), 7.56 (1H)
10	DMSO	6.27	5.53	5.69	4.57	—	—	—	—	2×OAc: 2.10 (3H), 1.96 (3H); Bz: 8.03 (2H), 7.71 (1H), 7.57 (2H); 2×OEt: 4.09 (4H), 1.25 (3H), 1.245 (3H)
	$CDCl_3$	6.34	5.64	5.90	4.44	—	—	—	—	2×OAc: 2.15 (3H), 2.01 (3H); Bz: 8.04 (2H), 7.62 (1H), 7.48 (2H); 2×OEt: 4.20 (4H), 1.35 (6H)
12b	DMSO	5.99	4.62	4.34	4.15	—	—	8.50	8.24	NH_2: 7.83; 2×OH: 5.69 and 5.61; OEt: 3.93 (2H), 1.16 (3H)
	D_2O	6.11	4.79	4.60	4.30	—	—	8.53	8.18	OEt: 3.98 (2H), 1.24 (3H)
18a residue A	D_2O	6.17	5.16	4.61	4.24	3.90	3.77	7.72	8.06	—
18a residue B		5.60	4.41	4.49	4.26	—	—	8.11	8.21	—
2′-5′ ApA residue A	D_2O	5.79	4.62	4.60	4.31	3.85	3.7	7.86	8.19	—
2′-5′ ApA residue B		5.92	4.53	4.46	4.32	4.32	4.14	8.01	8.13	—

Table 2. Coupling constants of protons

Compd	Solv.	Proton–proton						Proton–phosphorus				
		1′,2′	2′,3′	3′,4′	4′,5′	4′,5″	5′,5″	1′,P	2′,P	3′,P	4′,P	5′,P
13	D_2O	7.4	5.3	2.2	—	—	—			7.2	6.2	
7a	DMSO	3.9	5.6	7.6	2.4	—	—				3.9	13.0
7b	DMSO	3.8	5.1	8.5	2.7	—	—				2.7	13.0
10	DMSO	1.2	4.6	7.6	—	—	—			13.8	1.0	
	$CDCl_3$	1.4	4.7	8.0	—	—	—			14.2	0.9	
12b	DMSO	7.6	4.6	1.6	—	—	—	2.0		6.1	3.5	
	D_2O	6.7	5.1	3.1	—	—	—	1.2		7.8	4.3	
18a residue A	D_2O	4.1	5.3	5.5	2.2	3.2	13.1		9.3			
18a residue B	D_2O	6.9	4.8	2.4	—	—	—			7.4	5.4	
2′-5′ ApA residue A	D_2O	3.5	5.0	5.0	2.5	3.6	13.1			9.0		
2′-5′ ApA residue B	D_2O	4.1	5.1	5.4	2.5	3.8	12.2					3.8

Table 3. Carbon-13 NMR data

Compd	Solv.	Chemical shift (coupling constants J(C,P))										
		C-1′	C-2′	C-3′	C-4′	C-5′	P-OEt	C-2	C-4	C-5	C-6	C-8
7a[a]	DMSO	104.79	78.00	79.52 (12.7)	71.30	66.32 (165.0)	62.48 (6.8)	—	—	—	—	—
							62.21 (6.8)					
7b[b]	DMSO	104.79	77.15	78.20 (2.4)	72.24 (9.8)	65.70 (165.0)	62.68 (6.8)	—	—	—	—	—
							62.10 (6.8)					
10[c]	$CDCl_3$	98.31	74.11	70.51	75.41	—	63.41 (5.9)	—	—	—	—	—
							64.57 (5.9)					
12b	DMSO	87.32	74.53	71.15 (6.8)	81.38 (155.3)	—	61.22 (6.3)	156.48	151.32	119.25	156.48	140.26
12b	D_2O	89.27 (4.4)	77.88 (3.4)	73.93 (5.4)	83.97 (158.2)	—	64.57 (5.9)	155.57	152.01	121.28	158.30	142.85
18a residue A	D_2O	91.32 (6.4)	81.16 (6.3)	79.07 (2.9)	87.61	63.77	—	155.60	151.06	120.86	158.12	143.64
18a residue B	D_2O	88.76 (3.9)	73.27	73.56 (7.3)	84.88 (157.2)	—	—	154.66	150.33	120.77	157.47	141.91
2′-5′ ApA residue A	D_2O	87.23	72.55	73.09	83.24	60.23	—	151.55	147.76	118.26	154.58	138.51
2′-5′ ApA residue B	D_2O	88.77	73.88	68.98	82.37	64.04	—	152.12	147.13	117.78	154.45	139.47

[a] Additional signals, OBz: 165.22, 133.82, 129.70 (2), 129.57, 128.97 (2); >$C(CH_3)_2$: 112.57, 27.19, 26.94.
[b] Additional signals, OBz: 165.48, 134.16, 129.82 (2), 129.35, 129.25 (2); >$C(CH_3)_2$: 112.68, 27.06, 26.91.
[c] Additional signals, 2×OAc: 169.21, 169.06, 20.91, 20.33; OBz: 164.89, 133.72, 129.79 (2), 128.71, 128.58 (2).

to the T_m values of both 2′-5′ and 3′-5′ natural *ribo* (ApA) dimers, which are 13 and 16 °C, respectively.[23]

3. Conclusion

Only (*R*)-epimer of the erythrofuranosyl-4-*C*-phosphonate has been prepared via periodic acid-mediated oxidative cleavage of an epimeric mixture of the pentofuranosyl-5-*C*-phosphonate derivatives. We proposed a mechanism for explaining different reactivities of starting (5*R*)- and (5*S*)-pentofuranosylphosphonates toward oxidation. The usefulness of the prepared sugar phosphonate derivative for the synthesis of novel nucleoside phosphonic acids was proved at the nucleosidation reaction with protected adenine giving an isopolar, nonisosteric AMP analogue. Besides, the corresponding 2′-5′-linked diadenosine monophosphate analogue was prepared and its hybridization properties were evaluated. NMR spectroscopy study of the dimer showed that the sugar part of the *ribo*-configured phosphonate nucleotide unit was predominantly in the C2′-*endo* conformation, which is characteristic for B-DNA and not for RNA. The obtained results suggest that a thorough study on the synthesis of nucleoside phosphonic acids with various nucleobases, their 2′- and 3′-deoxy derivatives, and also their incorporation into longer oligonucleotides seems to be fully justified and highly desirable.

4. Experimental

4.1. General

Unless stated otherwise, the solvents were concentrated at 40 °C using a rotary evaporator. The products were dried over phosphorus pentoxide at 40–50 °C and 13 Pa. The course of the reactions was followed by TLC on silica gel Merck and Fluka UV 254 foils and the products were visualized by UV monitoring. Preparative column chromatography (PLC) was performed on silica gel (40–60 μm, Fluka) whereby the amount of sorbent used was 20–40 times the weight of the mixture separated. Elution was performed at the linear flow rate of 2–4 cm min^{-1}. For TLC and PLC runs, the following solvent systems were used (v/v): toluene–ethyl acetate 1:1 (T), chloroform–ethanol 9:1 (C), ethyl acetate–acetone–ethanol–water 4:1:1:1 (H1) and 12:2:2:1 (H3), 2-propanol–concentrated aqueous ammonia–water 7:1:2 (IPAW). The HPLC analyses were performed on a LC 5000 liquid chromatograph (INGOS, Czech Republic) using Luna C18 5 μm reverse phase column (4.6×150 mm; Phenomenex), by gradient of methanol in 0.1 M triethylammonium acetate buffer. Mass spectra (*m*/*z*) were recorded on ZAB-EQ (VG Analytical) instrument, using FAB in both positive and negative modes (ionization by Xe, accelerating voltage 8 kV) with glycerol–thioglycerol (3:1) and 2-hydroxyethyldisulfide as matrices. ^1H and ^{13}C NMR spectra were measured on Varian Unity 500 instrument (^1H at 500 MHz, ^{13}C at 125.7 MHz) in DMSO (referenced to the solvent signals δ_H=2.50 and δ_C=39.7), $CDCl_3$ (referenced to TMS), and D_2O (referenced to DSS). Signals in the ^1H NMR spectra were assigned to protons on the basis of chemical shifts, observed multiplicities, and homonuclear 2D-COSY experiments. Chemical shifts and interaction constants were obtained from a first-order spectra analysis as

read from expanded records. The exchange of hydroxyl protons for deuterium was carried out using several drops of tetradeuterioacetic acid. Assignments of signals in ^{13}C NMR spectra were accomplished on the basis of J-modulated spectra (APT) enabling to discriminate between C, CH, CH_2, and CH_3 signals, and in some cases confirmed by ^{1}H, ^{13}C-correlated HMQC spectra. The ^{1}H and ^{13}C NMR data are summarized in Tables 1–3.

4.2. (5*RS*)-3-*O*-Benzoyl-1,2-*O*-isopropylidene-D-ribofuranos-5-*C*-ylphosphonate (7a, 7b)

The 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose[18] **3** (13.01 g, 50 mmol) was treated with benzoylcyanide (7.21 g, 55 mmol) and triethylamine (0.695 mL, 5 mmol) in acetonitrile (60 mL) at 0 °C under exclusion of moisture (TLC in T-1) overnight. The reaction mixture was quenched by addition of methanol (5 mL) and the solvent was evaporated in vacuo. The residue was dissolved in chloroform and the organic layer was washed several times with water. After evaporation of solvent, the benzoyl derivative **4** was treated with 60% aqueous acetic acid (600 mL) at 50 °C for 3 h (TLC in T-1 and C-1). The acid was evaporated in vacuo, the residue was taken into chloroform (300 mL), and the organic layer was washed with saturated aqueous solution of NaCl (250 mL). The layers were separated by centrifugation, the organic layer was washed with water (4×250 mL), dried over anhydrous Na_2SO_4, and evaporated. The residue was dissolved in 70% aqueous acetone (650 mL) and to this solution, saturated aqueous solution of sodium periodate (12.84 g, 60 mmol) was added at 0 °C. Resulting solution was stirred for 8 h at rt (TLC in C-1) and, after cooling the mixture in an ice bath, the suspension was filtered through Celite. Filtration cake was washed with acetone, and the combined filtrates were evaporated. The residue was re-dissolved in acetone (200 mL) and the rest of sodium iodate was filtered off. Crude aldehyde **6** was co-distilled with dry toluene, dissolved in dichloromethane (50 mL), and treated with diethyl phosphite (7.74 mL, 60 mmol) and triethylamine (2.78 mL, 20 mmol) at 80 °C for 24 h (TLC in T-1 and C-1). The solvent was evaporated in vacuo and the crude epimeric phosphonates **7a** and **7b** were purified on silica gel using elution with a linear gradient of ethyl acetate in toluene. Yield: 1.27 g (5%) of **7a**, 1.82 g (7%) of **7b**, and 10.72 g (42%) of the mixture of **7a** and **7b**. HR-FAB calcd for $C_{19}H_{28}O_9P$ $(M+H)^+$: 431.1471; found: 431.1470. Compound **7a**: ν_{max} (KBr) 3430 (w, br, OH), 3243 (m, OH), 3072 (w, C–H), 2986 (m, CH_3), 2876 (w, CH_3), 1251 (s, P═O), 1219 (m), 1206 (m), 1131 (s, COCOC), 1032 (vs, POC), 1480 (w, OCH_2CH_3), 1602 (w), 1584 (w), 1492 (w), 1452 (w), 1731 (m, C═O), 1724 (s, C═O), 1275 (s, C═O), 1217 (s, $C(CH_3)_2$), 1098 (m, COCOC), 1002 (m), 688 (w) cm^{-1}. Compound **7b**: ν_{max} (KBr) 3506 (w, br, OH), 3272 (m, OH), 3066 (w, C–H), 2992 (m, CH_3), 2871 (w, CH_3), 1256 (s, P═O), 1236 (s, P═O), 1219 (m), 1206 (m), 1152 (s, COCOC), 1033 (vs, POC), 1601 (w), 1583 (w), 1492 (w), 1452 (w), 1731 (m, C═O), 1720 (s, C═O), 1711 (s, C═O), 1282 (s, C═O), 1100 (s, COCOC), 999 (m), 692 (w) cm^{-1}. Compound **7a**: δ_H (500 MHz, DMSO) 8.01 (2H, m, *ortho*-Ar-H), 7.65 (1H, m, *para*-Ar-H), 7.54 (2H, *meta*-Ar-H), 6.10 (1H, br s, OH), 5.91 (1H, d, *J*=3.9 Hz, H-1′), 5.20 (1H, dd, *J*=7.6, 5.6 Hz, H-3′), 4.88 (1H, dd, *J*=5.6, 3.9 Hz, H-2′), 4.63 (1H, ddd, *J*=7.6, 3.9, 2.4 Hz, H-4′), 4.16 (1H, *J*=13.0, 2.4 Hz, H-5′), 3.95 (4H, q, *J*=7.0 Hz, 2×OCH_2), 1.41 (3H, s, CH_3), 1.26 (3H, s, CH_3), 1.15 (3H, t, *J*=7.0 Hz, OCH_2CH_3), 1.09 (3H, t, *J*=7.0 Hz, OCH_2CH_3); δ_C (125.7 MHz, DMSO) 165.22, 133.82, 129.70 (2), 129.57, 128.97 (2), 112.57, 104.79, 79.52 (d, *J*=12.7 Hz), 78.00, 71.30, 66.32 (d, *J*=165.0 Hz), 62.48 (d, *J*=6.8 Hz), 62.21 (d, *J*=6.8 Hz), 27.19, 26.94, 16.44 (d, *J*=5.9 Hz), 16.38 (d, *J*=4.4 Hz). Compound **7b**: δ_H (500 MHz, DMSO) 8.02 (2H, m, *ortho*-Ar-H), 7.70 (1H, m, *para*-Ar-H), 7.56 (2H, *meta*-Ar-H), 5.85 (1H, br s, OH), 5.90 (1H, d, *J*=3.8 Hz, H-1′), 4.96 (1H, dd, *J*=8.5, 5.1 Hz, H-3′), 4.90 (1H, dd, *J*=5.1, 3.8 Hz, H-2′), 4.43 (1H, dt, *J*=8.5, 2.7, 2.7 Hz, H-4′), 3.99 (1H, *J*=13.0, 2.7 Hz, H-5′), 4.05 (4H, q, *J*=7.0 Hz, 2×OCH_2), 1.42 (3H, s, CH_3), 1.26 (3H, s, CH_3), 1.22 (3H, t, *J*=7.0 Hz, OCH_2CH_3), 1.21 (3H, t, *J*=7.0 Hz, OCH_2CH_3); δ_C (125.7 MHz, DMSO) 165.48, 134.16, 129.82 (2), 129.35, 129.25 (2), 112.68, 104.79, 78.20 (d, *J*=2.4 Hz), 77.15, 72.24 (d, *J*=9.8 Hz), 68.68 (d, *J*=6.8 Hz), 62.10 (d, *J*=6.8 Hz), 27.06, 26.91, 16.69 (d, *J*=5.9 Hz), 16.62 (d, *J*=5.9 Hz). For ^{1}H and ^{13}C NMR data see Tables 1–3.

4.3. (4*R*)-Diethyl-[1,3-di-*O*-acetyl-2-*O*-benzoyl-D-erythrofuranos-4-yl]phosphonate (10)

Periodic acid dihydrate (0.99 g, 4.35 mmol) was added to a solution of phosphonate **7a** (1.25 g, 2.9 mmol) in 50% aqueous dioxane (20 mL), and the reaction mixture was heated at 60 °C in the dark for 1–3 days (TLC in C-1). The solution was treated with Dowex 1×2 in acetate form (10 mL) under stirring for 10 min to remove periodate and iodate anions, the resin was filtered off, washed with 50% dioxane (30 mL), and the combined filtrates were evaporated. The residue was co-distilled several times with water to remove acetic acid, and finally treated with 0.1 M TEAB (30 mL) for 20 min. The aqueous solution was evaporated to dryness, the crude **9b** was co-distilled with methanol (3×50 mL), dried with pyridine (3×10 mL), and treated with acetic anhydride (1.21 mL, 12.8 mmol) and DMAP (20 mg) in pyridine (12 mL) overnight (TLC in C-1). The reaction mixture was quenched by addition of water (1 mL) at 0 °C, the solvent was evaporated in vacuo, and the crude product **10** was purified on silica gel by elution with a linear gradient of acetone in toluene. Yield: 940 mg (73%, yellow oil) of **10** (β-anomer). HR-FAB calcd for $C_{19}H_{26}O_{10}P$ $(M+H)^+$: 445.1264; found: 445.1273. δ_H (500 MHz, $CDCl_3$) 8.04 (2H, m, *ortho*-Ar-H), 7.62 (1H, m, *para*-Ar-H), 7.48 (2H, *meta*-Ar-H), 6.34 (1H, d, *J*=1.4 Hz, H-1′), 5.90 (1H, ddd, *J*=14.2, 8.0, 4.7 Hz, H-3′), 5.64 (1H, dd, *J*=4.7, 1.4 Hz, H-2′), 4.44 (1H, dt, *J*=8.0, 0.9 Hz, H-4′), 4.20 (4H, q, *J*=7.0 Hz, 2×OCH_2), 2.15 (3H, s, OAc), 2.01 (3H, s, OAc), 1.35 (6H, t, *J*=7.0 Hz, 2×OCH_2CH_3); δ_C (125.7 MHz, $CDCl_3$) 169.21, 169.06, 164.89, 133.72, 129.79 (2), 128.71, 128.58 (2), 98.31, 75.41, 74.11, 70.51, 64.57 (d, *J*=5.9 Hz), 63.41 (d, *J*=5.9 Hz), 16.40 (2C, d, *J*=5.9 Hz), 20.91, 20.33. For ^{1}H and ^{13}C NMR data see Tables 1–3.

4.4. (4*R*)-Ethyl-[1-(adenin-9-yl)-1-deoxy-β-D-erythrofuranos-4-yl]phosphonate (12b)

6-*N*-Benzoyladenine (598 mg, 2.5 mmol) in hexamethyldisilazane (HMDS) (25 mL, 118 mmol) was refluxed in the presence of chlorotrimethylsilane (2.5 mL, 20 mmol) under

stirring and exclusion of moisture for 10 h. Volatiles were removed under reduced pressure and the resulting oil was co-distilled with xylene (2×15 mL) and acetonitrile (2×15 mL) to remove traces of HMDS and xylene, respectively. A solution of diethyl phosphonate **10** (940 mg, 2.1 mmol) (dried by co-distillation with toluene and acetonitrile) in acetonitrile (6 mL) was added via septum under argon to the silylated adenine derivative followed by the addition of tin tetrachloride (0.24 mL, 2 mmol). The mixture was kept at rt for 24 h (TLC in C-1, H-1). The reaction mixture was then diluted with pyridine (1 mL) and toluene (20 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. The residue was treated with aqueous 1 M NaOH (50 mL) at rt for 48 h to remove the acyl-protecting groups and one ethyl ester group from **12a** (TLC in C-1, H-1). The solution was neutralized by addition of Dowex 50 (H^+), the monoethyl ester **12b** was eluted with 2.5% aqueous ammonia, and the crude product **12b** was purified on Dowex 1×2 (CH_3COO^-) column (elution with a linear gradient of 0–0.5 M aqueous acetic acid). Yield: 160 mg (16%, related to **7a**) of ethyl phosphonate **12b**. HR-FAB calcd for $C_{11}H_{17}N_5O_6P$ $(M+H)^+$: 346.0917; found: 346.0913. δ_H (500 MHz, DMSO) 8.50 (1H, s, H-2), 8.24 (1H, s, H-8), 5.69 (1H, br s, OH), 5.61 (1H, s, OH), 5.99 (1H, dd, J=7.6, 2.0 Hz, H-1′), 4.62 (1H, dd, J=7.6, 4.6 Hz, H-2′), 4.34 (1H, ddd, J=6.1, 4.6, 1.6 Hz, H-3′), 4.15 (1H, dd, J=3.5, 1.6 Hz, H-4′), 3.93 (2H, q, J=7.0, 2×OCH_2), 1.16 (3H, t, J=7.0 Hz, OCH_2CH_3); δ_C (125.7 MHz, DMSO) 156.48 (2), 151.32, 140.26, 119.25, 87.32, 81.38 (d, J=155.3 Hz), 74.53, 71.15 (d, J=6.8 Hz), 61.22 (d, J=6.3 Hz), 16.71 (d, J=4.9 Hz). For ^{1}H and ^{13}C NMR data see Tables 1–3.

4.5. (4*R*)-[1-(Adenin-9-yl)-1-deoxy-β-D-erythrofuranos-4-yl]phosphonic acid (13)

Carefully dried phosphonate **12b** (142 mg, 0.41 mmol) was treated with bromotrimethylsilane (0.32 mL, 2.46 mmol) and 2,6-lutidine (0.29 mL, 2.46 mmol) in acetonitrile (5 mL) at rt for 20 h (TLC in C-1, H-1). The reaction mixture was concentrated in vacuo, 2 M TEAB (2 mL) was added, and the resulting solution was evaporated to dryness. The crude phosphonic acid **13** was purified on Dowex 1×2 (CH_3COO^- form) column (elution with a linear gradient of 0–0.5 M aqueous acetic acid). Conversion into the sodium salt on Dowex 50×2 (Na^+) and freeze-drying from water afforded 101 mg (78%) of phosphonic acid **13**. HR-FAB calcd for $C_9H_{11}N_5O_6P$ $(M-H)^-$: 316.0447; found: 316.0443; ν_{max} (KBr) 3407 (s, vbr, OH), 3184 (s, vbr, OH), 2800–2200 (m–w, P-OH), 1691 (vs, NH_3^+), 1647 (m, NH_2), 1506 (w), 1419 (m), 1330 (w), 1222 (m, br), 1161 (m, br), 1128 (s, br), 785 (w), 721 (w), 643 (w) cm^{-1}. Compound **13**: δ_H (500 MHz, D_2O+NaOD) 8.67 (1H, s, H-2), 8.22 (1H, s, H-8), 5.81 (1H, d, J=7.4 Hz, H-1′), 4.75 (1H, dd, J=7.4, 5.3 Hz, H-2′), 4.33 (1H, ddd, J=7.2, 5.3, 2.2 Hz, H-3′), 4.08 (1H, dd, J=6.2, 2.2 Hz, H-4′). For ^{1}H NMR data see Tables 1 and 2.

4.6. 2′(3′)-*O*-Acetyl-6-*N*-benzoyl-5′-*O*-*tert*-butyldiphenylsilyladenosine (17)

6-*N*-Benzoyl-5′-*O*-*tert*-butyldiphenylsilyladenosine[1] (3 g, 4.92 mmol) was treated at rt with trimethyl orthoacetate (1 mL, 7.86 mmol) in DCM (50 mL) in the presence of toluenesulfonic acid hydrate (0.19 g, 1 mmol) at rt for 2 h. The resulting clear solution was washed with saturated aqueous solution of sodium hydrogen carbonate, the organic layer was dried over sodium sulfate, and evaporated. The residue was treated with 80% aqueous acetic acid (50 mL) for 30 min, the solution was concentrated in vacuo, and crude **17** was purified on a silica gel column in chloroform–10% ethanol. Yield: 2 g (62%; white foam) of **17** (a mixture of 2′-*O*-Ac and 3′-*O*-Ac regioisomers in a ratio 3:7 assigned from NMR). HR-FAB calcd for $C_{35}H_{38}N_5O_6Si$ $(M+H)^+$: 652.2591; found: 652.2581.

4.6.1. ^{1}H NMR (pyridine-d_5). Compound **17**, minor regioisomer (2′-*O*-Ac): 8.87 (1H, s, H-8), 8.77 (1H, s, H-2); 8.23 (2H, m), 7.75 (1H, m), 7.35 (2H, m, CO-C_6H_5), 7.75 (4H, m), 7.30–7.40 (6H, m, Si(C_6H_5)$_2$), 6.725 (1H, d, J(1′,2′)=4.1 Hz, H-1′), 6.32 (1H, dd, J(2′,1′)=4.1 Hz, J(2′,3′)=5.5 Hz, H-2′), 5.35 (1H, dd, J(3′,2′)=5.5 Hz, J(3′,4′)=5.0 Hz, H-3′), 4.56 (1H, ddd, J(4′,3′)=5.0 Hz, J(4′,5′)=3.2 Hz, J(4′,5″)=4.4 Hz, H-4′), 4.25 (1H, dd, J(5′,5″)=11.6 Hz, J(5′,4′)=3.2 Hz, H-5′), 4.11 (1H, dd, J(5″,5′)=11.6 Hz, J(5″,4′)=4.4 Hz, H-5″), 1.98 (3H, s, 2′-OAc), 0.99 (9H, s, C(CH_3)$_3$).

Compound **17**, major regioisomer (3′-*O*-Ac): 8.83 (1H, s, H-8), 8.78 (1H, s, H-2), 8.24 (2H, m), 7.75 (1H, m), 7.35 (2H, m, CO-C_6H_5), 7.75 (4H, m), 7.30–7.40 (6H, m, Si(C_6H_5)$_2$), 6.625 (1H, d, J(1′,2′)=6.0 Hz, H-1′), 5.58 (1H, dd, J(2′,1′)=6.0 Hz, J(2′,3′)=5.5 Hz, H-2′), 5.94 (1H, dd, J(3′,2′)=5.5 Hz, J(3′,4′)=3.9 Hz, H-3′), 4.59 (1H, q, J(4′,3′)=3.9 Hz, J(4′,5′)=4.0 Hz, J(4′,5″)=4.5 Hz, H-4′), 4.18 (1H, dd, J(5′,5″)=11.5 Hz, J(5′,4′)=4.0 Hz, H-5′), 4.06 (1H, dd, J(5″,5′)=11.5 Hz, J(5″,4′)=4.5 Hz, H-5″), 2.06 (3H, s, 2′-OAc), 1.02 (9H, s, C(CH_3)$_3$).

4.6.2. ^{13}C NMR (pyridine-d_5). Compound **17**, minor regioisomer (2′-*O*-Ac): 169.72 (CO(Ac)), 167.40 (N-CO), 151.95 (C-2), 151.67 (C-4), 151.17 (C-6), 142.35 (C-8), 125.52 (C-5), 137.40–127.40 (3×C_6H_5), 86.78 (C-1′), 85.11 (C-4′); 76.05 (C-2′), 68.74 (C-3′), 63.53 (C-5′), 20.10 (CH_3(Ac)), 18.76 and 26.32 (Si-C(CH_3)$_3$).

Compound **17**, major regioisomer (3′-*O*-Ac): 169.86 (CO(Ac)), 167.40 (N-CO), 152.47 (C-4), 151.95 (C-2), 151.17 (C-6), 142.02 (C-8), 125.52 (C-5), 137.40–127.40 (3×C_6H_5), 88.66 (C-1′), 82.78 (C-4′), 72.51 (C-2′), 72.88 (C-3′), 63.85 (C-5′), 20.27 (CH_3(Ac)), 18.76 and 26.36 (Si-C(CH_3)$_3$).

4.7. Adenosin-2′-yl-(4*R*)-[1-(adenin-9-yl)-1-deoxy-β-D-erythrofuranos-4-yl]phosphonate (18a)

A mixture of phosphonate **12b** (391 mg, 1.13 mmol), aqueous 1.89 M tetraethylammonium hydroxide (0.598 mL, 1.13 mmol), and ethanol (10 mL) was evaporated to dryness, and the residue was dried by co-distillation with ethanol (2×15 mL) and DMF (2×10 mL). Resulting tetraethylammonium salt of the phosphonate **12b** was treated with benzoyltetrazole (1.18 g, 6.8 mmol) in DMF (10 mL) in the presence of DMAP (0.83 g, 6.8 mmol) at 55 °C under exclusion of moisture until the starting compound disappeared (ca. 24 h, TLC in H-1). The reaction mixture was quenched

by addition of methanol (0.5 mL) and the solvent was evaporated in vacuo. The residue was treated with 60% aqueous pyridine overnight to destroy the mixed anhydride. The solvent was evaporated in vacuo, and the residue was partitioned between chloroform (150 mL) and 1 M TEAB (3×100 mL). The organic layer was dried over anhydrous Na_2SO_4 and the crude product **15a** was purified on silica gel (elution with a linear gradient of H-3 in ethyl acetate followed by H-1 in H-3). Yield: 299 mg (40%) of **15a** and 141 mg (24%) of **15b**. HR-FAB calcd for $C_{32}H_{29}N_5O_9P$ $(M+H)^+$: 658.1703; found: 658.1704.

4.7.1. Preparation of protected phosphonic acid 16. The carefully dried phosphonate **15b** (265 mg, 0.4 mmol) was treated with bromotrimethylsilane (0.26 mL, 2.0 mmol) and 2,6-lutidine (0.23 mL, 2.0 mmol) in acetonitrile (4 mL) at rt for 48 h (TLC in H-1). The reaction mixture was concentrated in vacuo, 2 M TEAB (2 mL) was added, the solution was evaporated, and the product **16** was purified by reversed-phase chromatography (elution with a linear gradient of methanol in water). Yield: 190 mg (75%) of triethylammonium salt of **16**. HR-FAB calcd for $C_{30}H_{25}N_5O_9P$ $(M+H)^+$: 630.1390; found: 630.1383.

4.7.2. Preparation of dimer 18a. A mixture of triethylammonium salt of the phosphonic acid **16** (190 mg, 0.3 mmol) and 2′(3′)-*O*-acetyl-6-*N*-benzoyl-5′-*O*-*tert*-butyldiphenylsilyladenosine (**17**) (254 mg, 0.4 mmol) was treated with DCC (619 mg, 3 mmol) in pyridine (4 mL) at rt overnight and then concentrated to a thick oil that was set aside at rt for 5 days (disappearance of the starting phosphonic acid **16**, TLC in H-1). Reaction mixture was diluted with 60% aqueous pyridine (10 mL) and, after 2 h of standing, concentrated in vacuo. The residue was co-distilled with ethanol (2×20 mL) and toluene (2×10 mL), and finally treated, under stirring and exclusion of moisture, with 0.5 M TBAF in tetrahydrofuran (5 mL, 2.5 mmol) at rt for 16 h (TLC in H-1). The reaction mixture was diluted with concentrated aqueous ammonia (50 mL), the suspension was stirred at rt for 3 days, and then evaporated to dryness. The residue in 50% aqueous ethanol (20 mL) was deionized (removal of *tetra*-*n*-butylammonium cations) on Dowex 50×2 (Et_3NH^+), the resin was filtered off, washed with aqueous ethanol, and the combined filtrates were concentrated. The residue was suspended in 3% aqueous ammonia (50 mL) under sonication, dicyclohexylurea was filtered off, the filtrate was evaporated, and the crude dimer **18a** was purified on Dowex 1×2 (CH_3COO^-) column (elution with a linear gradient of aqueous 0–0.5 M acetic acid). Conversion into the sodium salt on Dowex 50×2 (Na^+) and freeze-drying from water afforded 26 mg (15%) of 2′,5′-isomer **18a**. HR-FAB calcd for $C_{19}H_{24}N_{10}O_9P$ $(M+H)^+$: 567.1466; found: 567.1469. ν_{max} (KBr) 3401 (s, br, OH), 3192 (br, OH), 1646 (vs, NH_2), 1602 (m), 1578 (m), 1507 (w), 1420 (w), 1333 (m), 1216 (m, br, P═O), 1068 (s, br, C-OH), 797 (w), 725 (w), 648 (w), 550 (w, br, POC) cm^{-1}. Compound **18a**: δ_H (500 MHz, D_2O) 8.21 (1H, s, H-8 (res. B)), 8.11 (1H, s, H-2 (res. B)), 8.06 (1H, s, H-8 (res.A)), 7.72 (1H, s, H-2 (res.A)), 6.17 (1H, d, *J*=4.1 Hz, H-1′ (res. A)), 5.60 (1H, d, *J*=6.9 Hz, H-1′ (res. B)), 5.16 (1H, ddd, *J*=9.3, 5.3, 4.1 Hz, H-2′ (res. A)), 4.61 (1H, dd, *J*=5.5, 5.3 Hz, H-3′ (res. A)), 4.49 (1H, ddd, *J*=7.4, 4.8, 2.4 Hz, H-3′ (res. B)), 4.41 (1H, dd, *J*=6.9, 4.8 Hz, H-2′ (res. B)), 4.26 (1H, dd, *J*=5.4, 2.4 Hz, H-4′ (res. B)), 4.24 (1H, ddd, *J*=5.5, 3.2, 2.2 Hz, H-4′ (res. A)), 3.90 (1H, dd, *J*=13.1, 2.2 Hz, H-5′ (res. A)), 3.77 (1H, dd, *J*=13.1, 3.2 Hz, H-5″ (res. A)); δ_C (125.7 MHz, D_2O) 158.12, 157.47, 155.60, 154.66, 151.06, 150.33, 143.64, 141.91, 120.86, 120.77, 91.32 (d, *J*=6.4 Hz), 88.76 (d, *J*=3.9 Hz), 87.61, 84.88 (d, *J*=157.2 Hz), 81.16 (d, *J*=6.3 Hz), 79.07 (d, *J*=2.9 Hz), 73.56 (d, *J*=7.3 Hz), 73.27, 63.77. For ^1H and ^{13}C NMR data see Tables 1–3.

4.8. 6-*N*-Benzoyl-5′-*O*-*tert*-butyldiphenylsilyl-2′-*O*-(tetrahydropyran-2-yl)adenosine (19a)

6-*N*-Benzoyl-2′-*O*-(tetrahydropyran-2-yl)adenosine[27] (0.456 g, 1 mmol) was treated with *tert*-butylchlorodiphenylsilane (0.3 mL, 1.15 mmol) in pyridine (3 mL) at rt for 48 h (TLC in C-1). The reaction mixture was quenched by addition of methanol (0.2 mL) followed by triethylamine (0.2 mL, 1.4 mmol), the resulting suspension was diluted with ethyl acetate (20 mL), filtered, the precipitate was washed with ethyl acetate, and the combined filtrates were concentrated in vacuo. The residue was applied on a silica gel column in chloroform and the compound was eluted with a linear gradient of ethanol in chloroform (0→10%). Yield: 0.645 g (93%, white foam) of **19a** (a mixture of diastereoisomers). HR-FAB calcd for $C_{38}H_{44}N_5O_6Si$ $(M+H)^+$: 694.3061; found: 694.3052. Compound **19a**: δ_H (500 MHz, DMSO) mixture of two diastereoisomers in the ratio∼1:1; most of the signals are doubled: 11.20 (2H, br s, NH), 8.655 (1H, s), 8.65 (1H), 8.60 (1H, s), 8.59 (1H, s, H-2 and H-8), 8.05 (4H, m), 7.70–7.50 (13H, m), 7.50–7.30 (13H, m, 2×C_6H_5 (TBDPS) and C_6H_5 (N-Bz)), 6.235 (1H, d, *J*=5.6 Hz), 6.23 (1H, d, *J*=6.0 Hz, H-1′), 5.41 (1H, d, *J*=5.5 Hz), 5.26 (1H, d, *J*=5.8 Hz, 3′-OH), 4.96 (1H, t, *J*=5.5 Hz), 4.95 (1H, t, *J*=5.1 Hz, H-2′), 4.76 (1H, dd, *J*=3.7, 2.7 Hz), 4.66 (1H, t, *J*=3.5 Hz), 3.40 (2H, m), 3.28 (1H, m), 3.13 (1H, m), 1.90–1.20 (12H, m, OTHP), 4.52 (1H, m), 4.51 (1H, m, H-3′), 4.12 (1H, m), 4.115 (1H, m, H-4′), 3.96 (1H, dd), 3.955 (1H, dd, *J*=11.2, 3.5 Hz, H-5′a), 3.82 (1H, dd), 3.815 (1H, dd, *J*=11.2, 4.5 Hz, H-5′b), 0.98 (18H, s, *t*-Bu).

4.9. 6-*N*-Benzoyl-5′-*O*-*tert*-butyldiphenylsilyl-2′-*O*-[1-(2-chloroethoxy)ethyl]adenosine (19b)

6-*N*-Benzoyl-2′-*O*-[1-(2-chloroethoxy)ethyl]adenosine[28] (0.487 g, 1 mmol) was treated with *tert*-butylchlorodiphenylsilane (0.3 mL, 1.15 mmol) in pyridine (3 mL) at rt for 48 h (TLC in C-1). The workup of the reaction mixture was performed as described for **19a**. Yield: 0.609 g (85%, white foam) of **19b** (a mixture of diastereoisomers). HR-FAB calcd for $C_{37}H_{43}ClN_5O_6Si$ $(M+H)^+$: 716.2671; found: 716.2679. Compound **19b**: δ_H (500 MHz, DMSO) mixture of two diastereoisomers in the ratio ∼1.7:1. Major diastereoisomer: 11.25 (1H, br s, NH), 8.68 (1H, s), 8.62 (1H, H-2 and H-8), 8.05 (2H, m), 7.70–7.30 (13H, m, 2×C_6H_5 (TBDPS) and C_6H_5 (N-Bz)), 6.193 (1H, d, *J*=5.6 Hz, H-1′), 5.38 (1H, d, *J*=5.6 Hz, 3′-OH), 4.935 (1H, br t, *J*=5.2 Hz, H-2′), 4.88 (1H, q, *J*=5.4 Hz), 3.70–3.40 (4H, m), 1.24 (3H, d, *J*=5.4 Hz, 2′-O-CH(CH_3)-CH_2CH_2Cl), 4.47 (1H, br q, H-3′), 4.12 (1H, br q, H-4′), 3.987 (dd, 1H, *J*=11.5, 4.1 Hz, H-5′a), 3.847 (1H, dd, *J*=11.5, 4.9 Hz, H-5′b), 0.97 (9H, s, *t*-Bu). Minor diastereoisomer: 11.25 (1H, br s,

NH), 8.64 (1H, s), 8.62 (1H, H-2 and H-8), 8.05 (2H, m), 7.70–7.30 (13H, m, $2\times C_6H_5$ (TBDPS) and C_6H_5 (N-Bz)), 6.186 (1H, d, J=5.1 Hz, H-1′), 5.44 (1H, d, J=6.0 Hz, 3′-OH), 4.949 (1H, br t, J=5.1 Hz, H-2′), 4.55 (1H, br q, H-3′), 4.24 (1H, q, J=5.4 Hz), 3.70–3.40 (4H, m), and 1.19 (3H, d, J=5.4 Hz, (2′-O-CH(CH_3)-CH_2CH_2Cl)), 4.10 (1H, br q, H-4′), 3.989 (1H, dd, J=11.5, 3.9 Hz, H-5′a), 3.837 (1H, dd, J=11.5, 5.1 Hz, H-5′b), 0.99 (18H, s, *t*-Bu).

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References and notes

1. Endová, M.; Masojídková, M.; Buděšínský, M.; Rosenberg, I. *Tetrahedron* **1998**, *54*, 11151–11186.
2. Endová, M.; Masojídková, M.; Buděšínský, M.; Rosenberg, I. *Tetrahedron* **1998**, *54*, 11187–11208.
3. Králíková, Š.; Buděšínský, M.; Masojídková, M.; Rosenberg, I. *Nucleosides Nucleotides Nucleic Acids* **2000**, *19*, 1159–1183.
4. Králíková, Š.; Buděšínský, M.; Masojídková, M.; Rosenberg, I. *Tetrahedron Lett.* **2000**, *41*, 955–958.
5. Rejman, D.; Masojídková, M.; De Clercq, E.; Rosenberg, I. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1497–1522.
6. Králíková, Š.; Masojídková, M.; Buděšínský, M.; Rosenberg, I. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 329–347.
7. Rosenberg, I. *Frontiers in Nucleosides and Nucleic Acids*; Schinazi, R. F., Liotta, D. C., Eds.; IHL: Tucker, GA, 2005; pp 519–548.
8. Scheit, K. H. *Nucleotide Analogues. Synthesis and Biological Function*; Wiley-Interscience: New York, NY, 1980.
9. De Clercq, E.; Holý, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. *Nature* **1986**, *323*, 464–467.
10. Holý, A.; Rosenberg, I. *Collect. Czech. Chem. Commun.* **1987**, *52*, 2801–2809.
11. Holý, A.; Gunter, J.; Dvořáková, H.; Masojídková, M.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1999**, *42*, 2064–2068.
12. Páv, O.; Protivínská, E.; Pressová, M.; Collinsová, M.; Jiráček, J.; Snášel, J.; Masojídková, M.; Buděšínský, M.; Rosenberg, I. *J. Med. Chem.* **2006**, *49*, 3955–3962.
13. Xu, Z.; Kuang, M.; Okicki, J. R.; Cramer, H.; Chaudhary, N. *Antiviral Res.* **2004**, *61*, 195–206.
14. Parikh, J. R.; Wolf, M. E.; Burger, A. *J. Am. Chem. Soc.* **1957**, *79*, 2778–2781.
15. Raju, N.; Smee, D. F.; Robins, R. K.; Vaghefi, M. M. *J. Med. Chem.* **1989**, *32*, 1307–1313.
16. Otmar, M.; Rosenberg, I.; Masojídková, M.; Holý, A. *Collect. Czech. Chem. Commun.* **1993**, *58*, 2159–2179.
17. Otmar, M.; Rosenberg, I.; Masojídková, M.; Holý, A. *Collect. Czech. Chem. Commun.* **1993**, *58*, 2180–2196.
18. Specha, M. *Helv. Chim. Acta* **1993**, *76*, 1832–1846.
19. Smrt, J. *Collect. Czech. Chem. Commun.* **1982**, *47*, 2157–2169.
20. Kofoed, T.; Pedersen, E. B. *Nucleosides Nucleotides* **1995**, *14*, 345.
21. Králíková, Š.; Buděšínský, M.; Masojídková, M.; Rosenberg, I. *Tetrahedron* **2006**, *62*, 4917–4932.
22. Králíková, Š.; Buděšínský, M.; Novotná, M.; Páv, O.; Liboska, R.; Rosenberg, I. 16th International Round Table on Nucleosides, Nucleotides and Nucleic Acids, Minneapolis, Minnesota, USA. September 12–16. In *Abstract Book*; 2004; p 229.
23. Pressová, M.; Endová, M.; Točík, Z.; Liboska, R.; Rosenberg, I. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1225–1230.
24. Hanuš, J.; Barvík, I.; Ruszová-Chmelová, K.; Štěpánek, J.; Turpin, P.-Y.; Bok, J.; Rosenberg, I.; Petrová-Endová, M. *Nucleic Acids Res.* **2001**, *29*, 5182–5194.
25. Bhat, B.; Sanghvi, Y. S. *Tetrahedron Lett.* **1997**, *38*, 8811–8814.
26. Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; de Leeuw, H. P. M.; Altona, C. *Org. Magn. Reson.* **1981**, *15*, 43–52.
27. Markiewicz, W. T.; Biala, E.; Kierzek, R. *Bull. Pol. Acad. Sci., Chem.* **1984**, *32*, 433–451.
28. Sakatsume, O.; Yamaguchi, T.; Ishikawa, M.; Hirao, I.; Miura, K.; Takaku, H. *Tetrahedron* **1991**, *47*, 8717–8728.