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Geminal hydroxy phosphonate derivatives of nucleosides: A novel class of nucleoside 5′-monophosphate analogues

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Abstract

A novel type of phosphonate-based, isopolar, nonisosteric 5′-nucleotide analogue, the nucleoside 5′-hydroxy phosphonates, was prepared by Abramov nucleophilic addition of various phosphorous acid esters to nucleoside 5′-aldehydes. The newly formed compounds are distinguished by chirality of the 5′-carbon atom. The configuration of the 5′-epimers was assigned from NMR spectra. © 2000 Elsevier Science Ltd. All rights reserved.

The investigation of structurally diverse phosphonate-based nucleotides containing a *P–C* bond instead of the ester *P–O* linkage originated from the necessity to search for enzymatically stable nucleotide analogues usable as efficient antiviral/anticancer agents. Various nucleotide analogues containing the *O*-phosphonomethyl moiety have been investigated in our laboratory,[1] and recently we reported the synthesis of a novel class of conformationally restricted nucleotide analogues, the 2′,3′- and 3′,5′-*O*-phosphonoalkylidene derivatives of nucleosides[2] related to compounds studied earlier.[1]

Herein we report the synthesis of a novel type of isopolar 5′-nucleotide analogues **4** and **5** containing a geminal hydroxy phosphonate moiety on the 5′-carbon of the 2′-deoxyribonucleoside and ribonucleoside pentofuranose ring, respectively. These analogues possess strong structural similarity to nucleoside 5′-monophosphates and are related to compounds prepared earlier, namely, nucleoside-5′-phosphonates **1**,[3] 6′-deoxy-homonucleoside-6′-hydroxy phosphonates **2**,[4] and geminal nucleoside 3′-hydroxy phosphonates **3**.[5]

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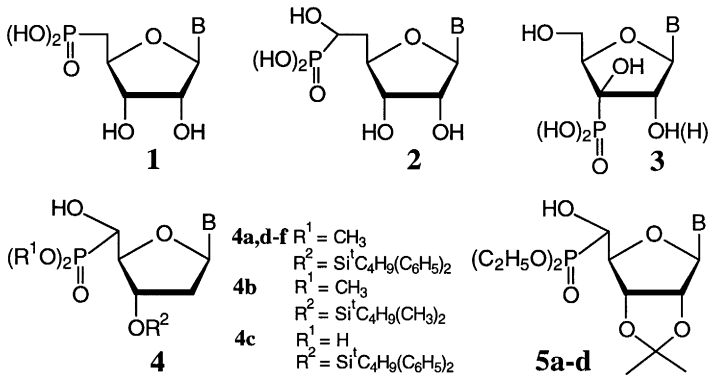

Although the general procedure for the synthesis of dialkyl α-hydroxy phosphonates via nucleophilic addition of phosphites to carbonyl compounds has been known for a long time,[6,7] the first application of this reaction to nucleoside/nucleotide chemistry leading to compounds **3** was only recently published.[5] We have found that various dialkyl phosphites, dialkyl trimethylsilyl phosphites and tris-trimethylsilyl phosphite react smoothly with nucleoside 5′-aldehydes to afford, in good yields, 5′-*C*-phosphono derivatives of nucleosides[8] **4** and **5**, as pairs of epimers, resolvable by RP-HPLC (for a complete set of dialkyl 5′-hydroxy phosphonates **4** and **5** see Table 1). The starting nucleoside 5′-aldehydes were mostly prepared by Swern oxidation (DMSO/$(COCl)_2$)[9] of suitably protected nucleosides. This procedure has been found to be the method of choice for the 3′-*O*-*tert*-butyldiphenylsilyl derivatives of all four 2′-deoxynucleosides and also, surprisingly, for the 2′,3′-*O*-isopropylidene derivatives of ribonucleosides except for the adenosine derivative (for unknown reasons). The 6-*N*-benzoyl-2,3-*O*-isopropylideneadenosine-5′-aldehyde was prepared by the Pfitzner–Moffatt oxidation procedure.[10] In the case of Swern oxidation of 3′-*O*-benzoyl and 3′-*O*-trimethylacetyl derivatives of 2′-deoxynucleosides we did not detect any expected nucleoside-5′-aldehyde. It seems that under Swern oxidation conditions, 3′-*O*-acyl protecting groups of nucleosides are unstable and this instability leads to oxidative destruction of the sugar part of the nucleoside.

Table 1
The epimeric ratio and yields of the nucleoside 5′-hydroxy phosphonates **4** and **5**

Compound	**4a**	**4b**	**4c** [c]	**4d**	**4e**	**4f**	**5a**	**5b**	**5c**	**5d**
B[a]	T	T	T	C^{Bz}	A^{Bz}	G^{Bz}	U^{MEM}	C^{Bz}	A^{Bz}	G^{Bz}
(*R*):(*S*) [b]	79:21	80:20	63:37	85:15	75:25	66:34	70:30	57:43	67:33	52:48
Yield [%]	78	71	85	84	69	82	31	57	32	61

[a] T ... thymin-1-yl, C^{Bz} ... 4-*N*-benzoylcytosin-1-yl, A^{Bz} ... 6-*N*-benzoyladenin-9-yl, G^{Bz} ... 2-*N*-benzoylguanin-9-yl, U^{MEM} ... 3-*N*-methoxymethyluracil-1-yl; [b]epimeric ratio determined from ^{1}H NMR spectra; [c]prepared by the addition of *tris*-trimethylsilyl phosphite to 3'-*O*-*tert*-butyldiphenylsilylthymidine-5'-aldehyde and isolated after hydrolysis in aqueous methanol as free phosphonic acid

Since the addition of phosphites to nucleoside 5′-aldehydes, under standard reaction conditions,[8] did not proceed with significant stereoselectivity (Table 1), we examined several factors (solvent, nucleophilic catalyst, type of phosphite and temperature) to influence the reaction in favour of a single epimer. The solvent does not have any influence on the stereoselectivity of the phosphite addition to nucleoside-5′-aldehydes. Dichloromethane provided better yields of 5′-hydroxy phosphonates **4a** than tetrahydrofuran or acetonitrile. Also, increasing the amount of triethylamine (from 1 to 5 equivalents)[8]

did not influence the ratio of epimers. The use of DBU instead of triethylamine had a destructive effect on the nucleoside-5′-aldehydes. On the other hand, little change in the epimeric ratios was found when dialkyl phosphites with bulkier ester groups (dimethyl to diethyl to diisopropyl) were used in the presence of triethylamine. Surprisingly, neither diethyl trimethylsilyl phosphite nor the bulky tris-trimethylsilyl phosphite exhibited higher stereoselectivity in their respective additions to nucleoside 5′-aldehydes. No change in the ratio of the epimeric 5′-hydroxy phosphonates formed in the reaction of diethyl phosphite with 3′-*O*-*tert*-butyldiphenylsilylthymidine-5′-aldehyde in the presence of either lithium bis(trimethylsilyl)amide or *tert*-butylmagnesium chloride in tetrahydrofuran at −78°C was found. Under these conditions, a low yield of the 3′-*O*-*tert*-butyldiphenylsilyl-5′-*C*-diethylphosphonothymidine was obtained.

During our experiments, we succeeded in crystallizing the minor epimer of 3′-*O*-*tert*-butyldimethylsilyl-5′-*C*-dimethylphosphonothymidine (**4b**) from the epimeric mixture, and also in the separation of epimers of 2-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-5′-*C*-dimethylphosphonoguanosine (**4f**) by RP-HPLC (the *A-epimer* was the faster). These single epimers **4f** were subjected to NMR analysis to determine the configuration of the 5′-carbon. The structural assignment was carried out using characteristic chemical shifts (protons and carbons of the base and substituents), 2D-COSY and 2D-ROESY spectra (deoxyribose protons) and heterocorrelated 2D-HMQC spectra (deoxyribose carbons). Selected NMR parameters of the deoxyribose moieties are given in Table 2. Vicinal *J*(H,H)s showed a high preference for the C2′-*endo* form of the deoxyribofuranose ring in both epimers [~85% in **4f** (*A-epimer*) and ~95% in **4f** (*B-epimer*)]. The preferred *syn*-orientation of the base was determined by 2D-ROESY spectra. Determination of the configuration at C5′ is closely connected to the conformation around the C4′–C5′ bond. The high value of *J*(P,C3′)=14.6 Hz together with the low value of *J*(P,H4′)=2.3 Hz in **4f** (*A-epimer*) indicate the *trans*-arrangement of P and C3′ which, together with a *gauche*-relationship of H4′/H5′ [as indicated by *J*(H4′,H5′)=2.3 Hz], establishes the (*S*)-configuration at C5′ (Fig. 1). The configuration of the second epimer **4f** (the *B-epimer*) is therefore 5′-(*R*) and the set of its vicinal couplings (*J*(P,C3′)=8.8 Hz, *J*(P,H4′)=5.4 Hz and *J*(H4′,H5′)=6.4 Hz) indicate comparable populations of two conformers with P/C3′ *trans*- and *gauche*-arrangements, respectively (Fig. 1).

Table 2
Selected NMR parameters of deoxyribose parts of (*S*)- and (*R*)-hydroxy phosphonates **4f** in $(CD_3)_2SO$

Epimer	**Proton chemical shifts**							**Carbon-13 chemical shifts**				
	H1'	**H2'**	**H2''**	**H3'**	**H4'**	**H5'**	**OH**	**C1'**	**C2'**	**C3'**	**C4'**	**C5'**
***(S)*-4f**	6.45	2.51	2.46	4.60	4.17	3.50	6.08	83.98	41.18	75.74	87.44	66.74
***(R)*-4f**	6.45	2.77	2.19	4.77	4.30	3.97	6.18	83.27	38.61	74.97	87.54	67.11
	***J*(H,H)**							***J*(P,H)**		***J*(P,C)**		
	1',2'	***1',2''***	***2',3'***	***2'',3'***	***3',4'***	***4',5'***	***5',OH***	***P,H4'***	***P,H5'***	***P,C3'***	***P,C4'***	***P,C5'***
***(S)*-4f**	8.6	6.0	5.0	1.8	1.4	2.3	7.2	2.3	12.4	14.6	~ 0	163.1
***(R)*-4f**	10.0	5.5	4.6	<1	<1	6.4	7.2	5.4	8.8	8.8	6.8	160.2

These configurational assignments are supported by 2D-ROESY spectra where NOE contacts H5′/H2′, H4′/OH5′ and H5′/H8 are observed only for the second epimer in accordance with its 5′-(*R*)-configuration (Fig. 1). Detailed discussion of these configurational assignments and conformational features of the 5′-epimers will be presented in a full paper.

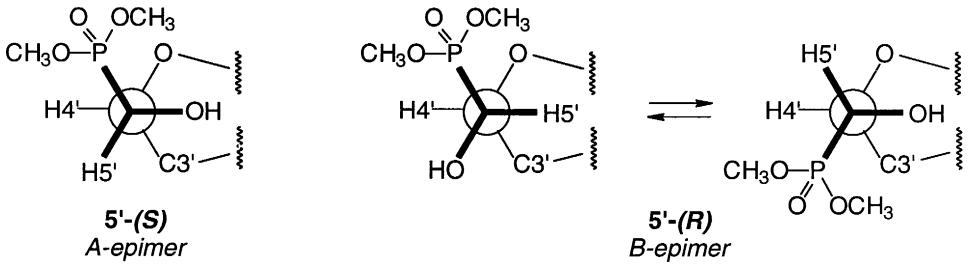

Fig. 1. The preferred C4′–C5′ rotamers of epimeric 5′-hydroxy phosphonates **4f**

Acknowledgements

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8. Typical procedure for the preparation of compound **4b**: Dimethylsulfoxide (0.213 ml, 3 mmol) was added with stirring to a solution of oxalyl chloride (0.131 ml, 1.5 mmol) in dichloromethane (3.5 ml) at −78°C under an argon atmosphere. After 10 min a solution of 3′-*O*-*tert*-butyldiphenylsilylthymidine (484 mg, 1 mmol) in dichloromethane (7 ml) was added dropwise and the reaction mixture was stirred for a further 30 min. Then the reaction was quenched by addition of triethylamine (0.7 ml, 5 mmol), the resulting suspension was stirred at low temperature for a further 5 min and, after allowing to warm to room temperature, dimethyl phosphite (0.183 ml, 2 mmol) was added. The reaction mixture was either set aside overnight at room temperature or heated at 40°C for several hours. The course of the reaction was checked by TLC on silica gel plates in chloroform:ethanol (9:1) and the product was detected both by UV monitoring and by spraying with 1% ethanolic solution of 4-(4-nitrobenzyl)pyridine [after a short heating and exposing to ammonia vapours the product (dimethyl ester) afforded an intense blue spot]. The reaction mixture was diluted with chloroform, extracted with water and dried over anhydrous sodium sulfate. Chromatography of the crude product on a silica gel column in chloroform–ethanol mixture afforded 459 mg (78%) of the expected 3′-*O*-*tert*-butyldiphenylsilyl-5′-*C*-dimethylphosphonothymidine (**4a**).
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