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Study of chemical stability of antivirally active 5-azacytosine acyclic nucleoside phosphonates using NMR spectroscopy

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ABSTRACT

Hydrolytic decomposition of four 5-azacytosine acyclic nucleoside phosphonates was studied. Products of the decomposition are carbamoylguanidine derivatives. Stability and decomposition products of HPMP-5-azaC (a 5-azacytosine derivative with strong antiviral activity) differ from the other derivatives. The reaction pathway of HPMP-5-azaC involves a formyl derivative formed by intramolecular transformylation reaction.

1. Introduction

Acyclic nucleoside phosphonates (ANPs), a special class of nucleotide analogues, are compounds of great interest due to their broad-spectrum of biological activities (e.g., antiviral, cytostatic, antiparasitic, immunomodulatory) resulting in their utilization in clinical practice (antivirals: cidofovir, adefovir and tenofovir).[1] Preclinical and/or clinical investigation of several other ones is currently under way. Recently, we have synthesized a series of acyclic nucleoside phosphonates with 5-azacytosine base moiety.[2] This work follows up on a long-term investigation of 5-azacytosine compounds in our Institute, especially nucleosides with antileukemic activity. At present time, two of them are used as therapeutics: 5-azacytidine[3] (azacytidine, Vidaza™) and 2′-deoxy-5-azacytidine[4] (decitabine, Dacogen™). Both compounds were approved for the therapy of all subtypes of myelodysplastic syndrome (MDS), disorders belonging to a group of bone marrow stem cell hyperplasias and dysplasias that result in ineffective hematopoiesis.[5] Myelodysplastic disorders and transformed leukemia have poor prognosis and minimal response to chemotherapy. The great attention is also paid to clinical investigation of azacytidine and decitabine for the treatment of solid tumors, especially metastatic lung cancer[6] and hormone-independent prostate cancer.[7] The efficacy of 5-azacytosine nucleosides is bound to their inhibitory activity toward DNA methylations: hypermethylation of DNA was found to be a characteristic property of tumor cells that causes silencing of tumor suppressor genes, and hence tumor progression.[8–11]

Regarding these facts, introduction of a 5-azacytosine component to the chemistry of ANPs resulted as consequent and very promising step in our search for new biologically active compounds. Three types of phosphonomethoxyalkyl side chains were selected for attachment to N-1 position of 5-azacytosine: 2-(phosphonomethoxy)ethyl (PME-5-azaC, **1**), 3-hydroxy-2-(phosphonomethoxy)propyl (HPMP-5-azaC, **2**), and 1-[(*R*)-2-(phosphonomethoxy)propyl]-5-azacytosine (PMP-5-azaC). Contrary to expectation, none of the compounds exhibited cytostatic efficacy in vitro; therapeutic potential of 5-azacytosine acyclic nucleoside phosphonates was shown to be bound to their antiviral effects. While activities of PME derivative **1**, (*R*) enantiomer of HPMP-5-azaC and 5,6-dihydro form of (*S*)-HPMP-5-azaC were marginal only and (*R*)-PMP derivative completely inactive, compound **2**, 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine exerted exceptionally strong activity against practically all types of DNA viruses: adenoviruses, poxviruses (vaccinia virus, cowpox virus, orf virus), herpes simplex (type 1 and 2) virus, varicella-zoster virus (VZV), and human cytomegalovirus (HCMV). From the structural point of view, compound **2** represents a *sym*-triazine analogue of cidofovir, that is, 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine, a broad-spectrum antiviral agent approved for intravenous treatment of cytomegalovirus retinitis in AIDS patients (and topically used also for various other indications caused by DNA viruses). The drawback of cidofovir (and all other ANPs) is its nephrotoxicity and low bioavailability disallowing its oral application. Comparison of cidofovir and its 5-aza analogue

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2 showed that antiviral activity data of **2** [based on 50% effective concentration (EC_{50} values)] are similar or in some cases higher, and the antiviral selectivity index [ratio of 50% cytotoxic concentration (CC_{50}) to EC_{50}] was 2- to 16-fold higher than that of cidofovir.[2] To improve bioavailability of **2**, several structurally diverse ester prodrugs were synthesised.[12] Esterification was carried out on the level of its cyclic form **3** using reaction of its tetrabutylammonium salt with corresponding alkyl bromides or acyloxymethyl chloride. All these compounds exerted an extraordinary activity against all types of DNA viruses; the most active compound was found to be hexadecyloxyethyl ester with. EC_{50} values in the range of 0.003–0.008 μg/ml for HSV, ⩽0.0008 to ⩽0.0014 μg/ml for VZV, ⩽0.00014 to ⩽0.00038 μg/ml (HCMV), 0.008 to 0.037 μg/ml for HHV-6, and 0.037 μg/ml for vaccinia virus. The activity of ester prodrugs was decreasing in the order: 2-(hexadecyloxy)ethyl > pivaloyloxymethyl ∼ octadecyl > erucyl (compounds **4a**–**4d**, Fig. 1).[12] Regarding exceptional activities of **2** and its ester prodrugs, detailed studies directed to efficacy against particular viruses (camelpox virus,[13] polyomavirus[14]) in different cell culture models as well as some in vivo studies[15] were carried out. Progression in investigation of HPMP-5-azaC and its derivatives is still under way.

In contrast to cidofovir, HPMP-5-azaC has more complicated metabolic profile due to its chemical (and also enzymatic) instability. Concerning chemical instability, in aqueous solutions ring opening between C-6 and N-1 of the triazine moiety occurs and HPMP-5-azaC is successively degraded to 2-{[(2*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]carbamoylguanidine (**9**). This ring-opening reaction is a general property of all 5-azacytosine nucleosides (e.g., riboside, 2′-deoxyriboside, arabinoside) as well as other derivatives with hydroxyl containing side-chain. The chemical stability of 5-azacytidine was first studied by Pithova et al. who proved that triazine ring in 5-azacytosine opens between C-6 and N-1 to form *N*-(formylamidino)-*N*′-β-D-ribofuranosylurea in a reversible reaction followed by irreversible loss of a formyl group to form 1-β-D-ribofuranosyl-3-guanylurea.[16,17] The first stage of this hydrolysis consists in nucleophilic attack of hydroxyl ion in position 6 of 5-azacytosine which electron density is much lower compared to that of cytosine which results from quantum chemical calculations.[16] Similar course of alkaline hydrolysis was described for 5-aza-2′-deoxycytidine (decitabine) while in neutral conditions this compound was decomposed not only to expected 2′-deoxy-*N*-(formylamidino)-*N*′-β-D-ribofuranosylurea but also to small amount of several other products as resulted from HPLC analysis.[18] The complete characterization of decomposition products of decitabine in water and human plasma was carried out recently using a new liquid chromatography/tandem mass spectrometry quantification method. This study revealed that these previously unknown compounds are partially an isomer of decitabine and partially two various hydrated open-ring form isomers, that is, 2′-deoxy-*N*-(formylamidino)-*N*′-β-D-ribofuranosylureas.[19]

The aim of this work was investigation of hydrolytic destruction of both new 5-azacytosine antivirals (HPMP-5-azaC and its cyclic form) targeted to identification of decomposition products for the purpose of their antiviral and toxicity studies and investigation of kinetic parameters and mechanism of these reactions.

2. Results and discussion

To approximate conditions in plasma or amino acids and/or phosphate-containing buffers generally used as media for biochemical assays we monitored the course of decomposition of our compounds in 1 M glycine or PBS buffer (both as D_2O solutions) by NMR methodology. This method was selected as the most suitable for identification and characterization of all reaction products in any moment of the decomposition process. Thus, quantification of products is not bound to their UV absorption or other physiochemical properties which can differ for single products or intermediates. Glycine was selected due to its single structure not disturbing NMR spectrum of reaction mixture. Its 1 M solution (pH 5.75) enables to study decomposition process in acidic medium and eventually possible interactions with amino acids from plasma; PBS buffer (pH 7.4, a phosphate buffer with NaCl) was used to study this process under slightly basic (physiological) conditions. The following compounds were submitted to the stability study: 1-[2-(phosphonomethoxy)ethyl]-5-azacytosine (**1**), 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine (HPMP-5-azaC, **2**), its cyclic form, that is, 1-{[(5*S*)-2-hydroxy-2-oxido-1,4,2-dioxaphosphinan-5-yl]methyl}-5-azacytosine (**3**), and (2*S*)-[(2,3-dihydroxy)propyl]-5-azacytosine (**5**); the latter one to compare decomposition process of **2** with an analogous compound lacking phosphonomethyl group.

Decomposition products and reaction courses were similar for compounds **1**, **3** and **5**. In all these three cases a carbamoylguanidine derivative (**6a**–**6c**, Fig. 2) and formic acid were the only observed products of the decomposition in both 1 M glycine solution and PBS buffer. The decomposition of all three compounds (**1**, **3**, **5**) is a first order reaction. This can be clearly seen from the dependence of logarithm of reactant concentration on time (Fig. 3). The rate constants and half-life times are given in Table 1. The reaction is in all cases slower in PBS buffer than in 1 M glycine solution. The structure of all products was determined by ^1H,

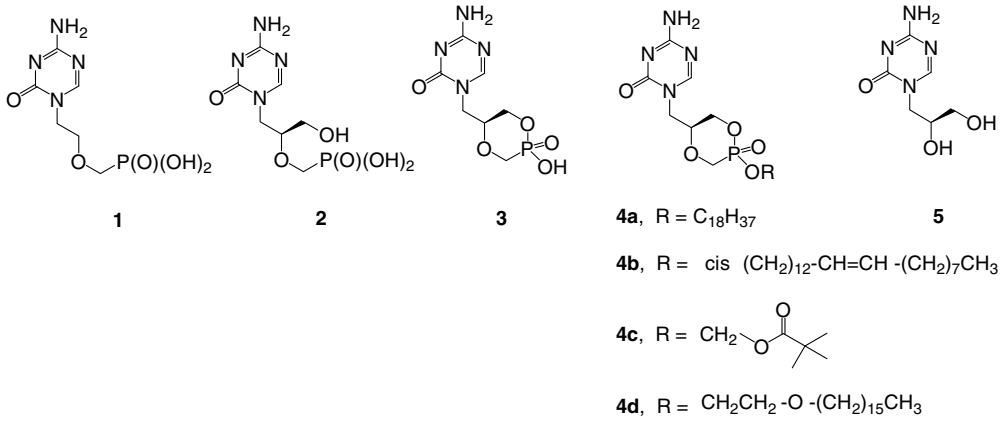


Figure 1. Structures of 5-azacytosine acyclic nucleoside phosphonates and other analogues: PME-5-azaC (**1**), HPMP-5-azaC (**2**), cyclic HPMP-5-azaC (**3**), ester prodrugs of cyclic HPMP-5-azaC (**4a–4d**) and (2*S*)-(2,3-dihydroxy)propyl]-5-azacytosine (**5**).

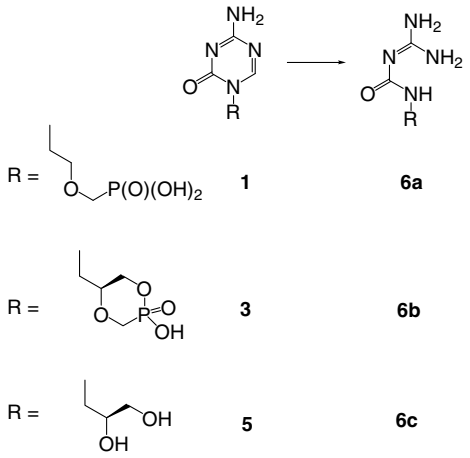


Figure 2. Decomposition products of compounds **1**, **3** and **5**.

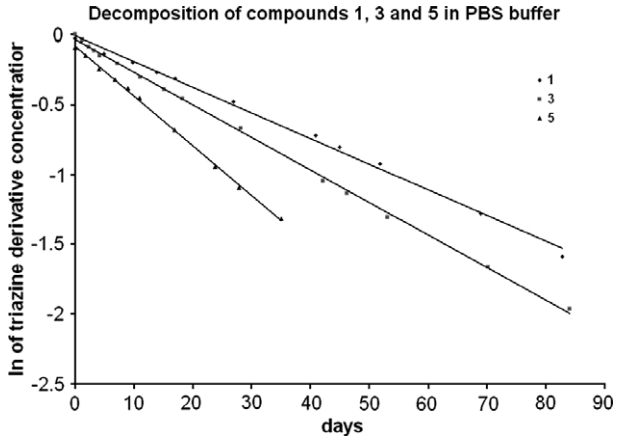


Figure 3. Plot of logarithm of concentration of compounds **1**, **3** and **5** versus time.

^{13}C and 2D-H,C-HSQC, 2D-H,C-HMBC and 2D-H,H-COSY NMR spectra. The biggest change in NMR shifts are experienced by the signals of hydrogens in position 1′. In the triazine derivatives their resonance is near 4 ppm while in the carbamoylguanidine products their resonance is shifted upfield to 3.4 ppm.

The decomposition of compound **2** differs from the other reactions. The reaction rate is higher (approximately four times) than the decomposition rates of the other compounds, and intermediate formyl derivative **8** is formed. The formyl derivative **8** is then hydrolyzed to form carbamoylguanidine derivative **9** and formic acid (Fig. 4). Compound **5** differs only slightly from HPMP derivative **2**; it does not have the phosphonomethylene group, which is quite far from the triazine ring of compound **2**. However, quite surprisingly, no intermediate formyl derivative was observed during its decomposition.

We considered two possible reasons for different behavior of compounds **2** and **5** in aqueous solutions. First, the different reaction path could be caused by protonation of triazine ring of compound **2**. Triazine derivative **2** can form a zwitterion with negative charge on the phosphonate oxygens and protonated triazine ring. To confirm or disconfirm this possibility we observed the decomposition of compound **5** also in two 50 mM phosphate buffers with pH 4 and 10. In the acidic buffer we expected protonation of the triazine moiety but the reaction rate (see Table 2) differed only slightly from the PBS buffer, and no formyl derivative was observed in the reaction mixture. In the basic buffer the reaction was faster by a factor of two when compared with the PBS buffer. The hydrolytic decomposition is probably catalyzed by both acids and bases, but no qualitative change in the mechanism was observed for the protonated triazine derivative.

Table 1
Rate constants and half-life times of decomposition reaction of compounds **1**–**3** and **5**

	1 M glycine			PBS		
	k [d^{-1}]	k [s^{-1} × 10^{-7}]	$t_{1/2}$ [d]	k [d^{-1}]	k [s^{-1} × 10^{-7}]	$t_{1/2}$ [d]
1 → **6a**	0.027	3.13	26.2	0.018	2.08	39.3
2 → **8**	0.149	17.2	4.75	0.142	16.4	4.98
3 → **6b**	0.048	5.56	14.7	0.023	2.70	29.7
5 → **6c**	0.044	5.09	16.1	0.036	4.17	19.6

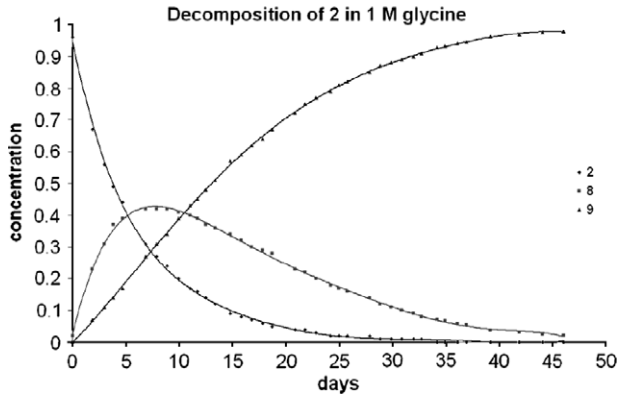


Figure 4. Plot of concentration of HPMP-5-azaC (**2**) and its decomposition products versus time.

Table 2
Rate constants and half-life times of decomposition reaction of compound **5** in different buffers

Buffer	pH	k [d^{-1}]	k [s^{-1} × 10^{-7}]	$t_{1/2}$ [d]
Phosphate	4.0	0.039	4.51	17.8
1 M glycine	5.8	0.044	5.09	15.8
PBS	7.4	0.036	4.17	19.6
Phosphate	10.0	0.067	7.75	10.3

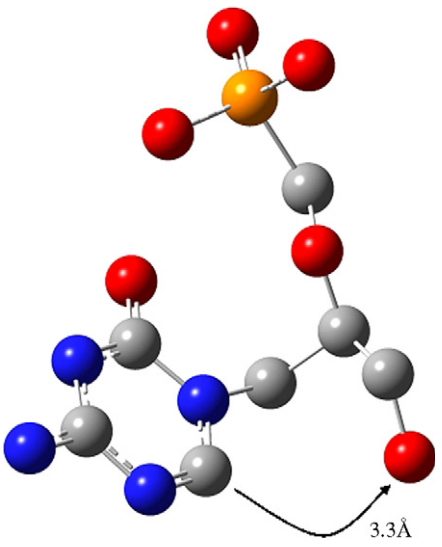


Figure 5. Global minimum energy conformation of compound **2** (hydrogens are omitted for clarity).

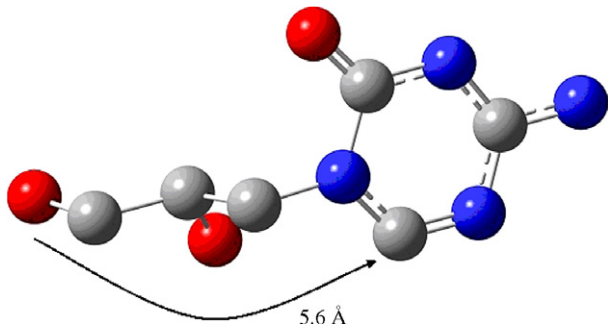


Figure 6. Global minimum energy conformation of compound **5** (hydrogens are omitted for clarity).

Second possible explanation of the different decomposition of compound **2** was that its steric arrangement differs significantly from that of compound **5**. To answer the question whether the conformation of the studied triazine derivatives could play a role in different reaction paths of **2** and **5** we performed a global energy minimum conformational search using a DFT method with polarizable continuum model of water solvation. We systematically changed torsion angles C(2)–N(1)–C(1′)–C(2′), N(1)–C(1′)–C(2′)–C(3′), and C(1′)–C(2′)–O–C(4′). The optimization started from 18 different conformations of compound **2** and 6 different conformations of compound **5**. We found out that in the global energy minimum conformation of compound **2** (see Fig. 5), the free hydroxy group in position 3′ is very close (3.3 Å) to carbon 6 of the triazine ring. The conformation is stabilized by formation of intramolecular hydrogen bond between the phosphonate hydroxy group and carbonyl oxygen of the azacytosine part of the molecule. The minimum energy conformation of compound **5** (see Fig. 6) differs significantly. Here, the distance between triazine C-6 and the hydroxyl group in position 3′ is much bigger (5.6 Å).

3. Conclusions

We have shown that 5-azacytosine acyclic nucleoside phosphonates are instable and they are decomposed to carbamoylguanidine derivatives. Decomposition of HPMP-5-azaC (**2**) involves formation of formiate **8** which was not observed in the decomposition reaction of the other derivatives. The explanation for this different reaction pathway (supported by ab initio calculations) is that intermediate formylamido derivative (**7**), which was previously shown to be intermediate in the 5-azacytidine decomposition,[16] can react in a transformylation reaction with a spatially close hydroxy group to form the formiate **8** (see Scheme 1). As a result, we can say that triazine can serve as a formylating agent when a free hydroxyl group is in a suitable spatial arrangement.

Decomposition products of both antivirals HPMP-5azaC and its cyclic form were subjected to cytotoxicity assays performed in human embryonic lung (HEL) cells. Cytotoxicity measurements were based on the inhibition of cell growth (CC_{50} values, that is, the compound concentration required to reduce cell growth by 50%) or determination of MCC values, that is, the minimum cytotoxic concentration of the compound that causes a microscopically detectable alteration of cell morphology. Syntheses of pure formylamido and carbamoylguanidine derivatives (**7** and **9**) were described previously.[2] Compound **6b** was obtained from the cyclic HPMP-5azaC after its 60 days lasting treatment with 1 M glycine. None of these decomposition products exhibited cytotoxicity in vitro: in all cases CC_{50} and MCC values were >100 μg/ml.

4. Experimental

NMR spectra were measured on a Bruker Avance-500 and/or Bruker Avance-600 instruments and referenced to internal standard dioxane (δ 3.75 and 67.19, respectively). The kinetics of decomposition was studied at 22 °C. The samples (30 mg) were dissolved in 1 mM solution of glycine in D_2O or in a buffer. The PBS buffer and the 50 mM phosphate buffers (pH 4.0 and 10.0) were prepared in H_2O, then lyophilized and dissolved in the same volume of D_2O. ^{1}H NMR spectra were measured periodically and signals from the low-field region were chosen for integration and kinetic measurements. A singlet of hydrogen H-6 of the starting azacytosine derivative, singlet of CHO of intermediate formiate **8**, and singlet of formic acid (which is the final product of the reactions) appear in this region of ^{1}H NMR spectra. NMR data in the following list are taken from the spectra in 1 M glycine.

All calculations were carried out using the Gaussian 03 software package.[20] DFT calculations were performed using the Becke3-LYP[21,22] functional with 6-31G(d) basis set. Full geometry optimizations were carried out using polarizable continuum solvent model[23] (PCM).

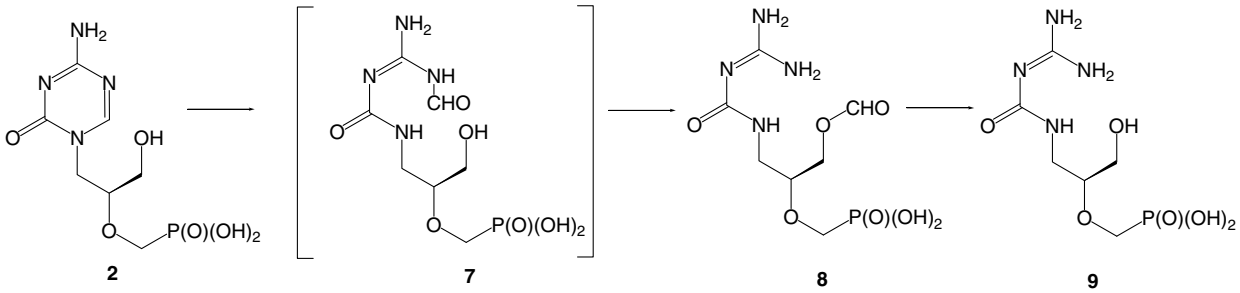
Description of synthesis and full characterization of studied compounds (elemental analyses, spectral data, physical constants) are available in our previous papers: see Ref. 2 for compounds **1**, **2**, **5**, **7**, and **9**, or Ref. 12 for compound **3**. Decomposition products of biologically inactive compounds **6a**, **6b**, and **6c** were characterized by NMR spectra only, their isolation from buffer solutions was not performed.

4.1. 1-[2-(Phosphonomethoxy)ethyl]-5-azacytosine (1)

^{13}C NMR: 165.91 (C-4); 161.41 (C-6); 156.02 (C-2); 70.04 d, J(2′,P) = 12.0 (C-2′); 67.59 d, J(CH_2P,P) = 156.8 (CH_2P); 47.78 (C-1′). ^{1}H NMR: 8.33 s, 1H (H-6); 4.04 t, 2H, J(1′,2′) = 4.9 (H-1′); 3.81 t, 2H, J(2′,1′) = 4.9 (H-2′); 3.65 d, 2H, J(CH_2P,P) = 8.9 (CH_2P).

4.2. 1-(*S*)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine (2)

^{13}C NMR: 166.19 (C-4); 161.48 (C-6); 156.50 (C-2); 79.71 d, J(2′,P) = 12.1 (C-2′); 66.67 d, J(CH_2P,P) = 157.3 (CH_2P); 60.57



Scheme 1. Hydrolytic decomposition of HPMP-5-azaC (**2**).

(C-3′); 48.79 (C-1′). ^{1}H NMR: 8.30 s, 1H (H-6); 4.14 dd, 1H, J_{gem} = 14.4, J(1′a,2′) = 3.2 (H-1′a); 3.84 dd, 1H, J_{gem} = 12.6, J(3′a,2′) = 3.8 (H-3′a); 3.83 dd, 1H, J_{gem} = 14.3, J(1′b,2′) = 8.4 (H-1′b); 3.77 dd, 1H, J_{gem} = 13.0, J(CH_2Pa,P) = 9.2 (CH_2Pa); 3.73 m, 1H (H-2′); 3.62 dd, 1H, J_{gem} = 12.6, J(3′b,2′) = 4.0 (H-3′b); 3.55 dd, 1H, J_{gem} = 13.0, J(CH_2Pb,P) = 9.8 (CH_2Pb).

4.3. 1-{[(5*S*)-2-Hydroxy-2-oxido-1,4,2-dioxaphosphinan-5-yl]methyl}-5-azacytosine (3)

^{13}C NMR: 166.04 (C-4); 161.35 (C-6); 156.11 (C-2); 74.01 d, J(2′,P) = 3.5 (C-2′); 70.29 d, J(3′,P) = 6.4 (C-3′); 65.82 d, J(CH_2P,P) = 143.3 (CH_2P); 47.51 (C-1′). ^{1}H NMR: 8.27 s, 1H (H-6); 4.22 m, 2H (H-3′); 4.07 dd, 1 H, J_{gem} = 14.5, J(1′a,2′) = 3.0 (H-1′a); 3.99 m, 1H (H-2′); 3.95 dd, 1H, J_{gem} = 14.0, J(CH_2Pa,P) = 8.9 (CH_2Pa); 3.76 dd, 1H, J_{gem} = 14.6, J(1′b,2′) = 8.3 (H-1′b); 3.72 dd, 1H, J_{gem} = 14.1, J(CH_2Pb,P) = 1.9 (CH_2Pb).

4.4. (2*S*)-[(2,3-Dihydroxy)propyl]-5-azacytosine (5)

^{13}C NMR: 166.79 (C-4); 161.16 (C-6); 157.25 (C-2); 69.38 (C-2′); 63.56 (C-3′); 50.83 (C-1′). ^{1}H NMR: 8.23 s, 1H (H-6); 4.09 dd, 1H, J_{gem} = 14.1, J(1′a,2′) = 3.3 (H-1′a); 3.98 m, 1H (H-2′); 3.68 dd, 1H, J_{gem} = 11.9, J(3′a,2′) = 4.4 (H-3′a); 3.66 dd, 1H, J_{gem} = 14.1, J(1′b,2′) = 8.9 (H-1′b); 3.59 dd, 1H, J_{gem} = 11.9, J(3′b,2′) = 5.9 (H-3′b).

4.5. 2-[2-(Phosphonomethoxy)ethyl]carbamoylguanidine (6a)

^{13}C NMR: 155.81 (C-4); 155.04 (C-2); 71.57 d, J(2′,P) = 11.4 (C-2′); 67.42 d, J(CH_2P,P) = 156.5 (CH_2P); 39.77 (C-1′). ^{1}H NMR: 3.69 t, 2H, J(2′,1′) = 5.2 (H-2′); 3.67 d, 2H, J(CH_2P,P) = 9.7 (CH_2P); 3.41 t, 2H, J(1′,2′) = 5.2 (H-1′).

4.6. 2-{[(5*S*)-2-Hydroxy-2-oxido-1,4,2-dioxaphosphinan-5-yl]methyl}carbamoylguanidine (6b)

^{13}C NMR: 155.79 (C-4); 155.05 (C-2); 75.33 d, J(2′,P) = 3.8 (C-2′); 70.59 d, J(3′,P) = 6.5 (C-3′); 65.65 d, J(CH_2P,P) = 143.2 (CH_2P); 39.84 (C-1′). ^{1}H NMR: 4.18 m, 2H (H-3′); 3.96 dd, 1H, J_{gem} = 14.1, J(CH_2Pa,P) = 8.9 (CH_2Pa); 3.86 m, 1H (H-2′); 3.77 dd, 1H, J_{gem} = 14.0, J(CH_2Pb,P) = 2.3 (CH_2Pb); 3.36 dd, 1H, J_{gem} = 14.6, J(1′a,2′) = 4.5 (H-1′a); 3.31 dd, 1H, J_{gem} = 14.6, J(1′b,2′) = 7.9 (H-1′a).

4.7. 2-[(2*S*)-2,3-dihydroxypropyl]carbamoylguanidine (6c)

^{13}C NMR: 156.18 (C-4); 155.84 (C-2); 70.84 (C-2′); 63.72 (C-3′); 42.53 (C-1′). ^{1}H NMR: 8.45 s, 1H (H-6); 3.82 m, 1H (H-2′); 3.62 dd, 1H, J_{gem} = 11.8, J(3′a,2′) = 4.3 (H-3′a); 3.54 dd, 1H, J_{gem} = 11.8, J(3′b,2′) = 6.2 (H-3′b); 3.37 dd, 1H, J_{gem} = 14.2, J(1′a,2′) = 4.5 (H-1′a); 3.24 dd, 1H, J_{gem} = 14.2, J(1′b,2′) = 7.4 (H-1′b).

4.8. 2-[(2*S*)-3-Formyloxy-2-(phosphonomethoxy)propyl]carbamoylguanidine (8)

^{13}C NMR: 164.28 (CO-formiate); 155.81 (C-4); 155.07 (C-2); 78.32 d, J(2′,P) = 11.1 (C-2′); 66.70 d, J(CH_2P,P) = 157.1 (CH_2P); 63.53 (C-3′); 40.42 (C-1′). ^{1}H NMR: 8.19 s, 1H (formiate); 4.40 dd, 1H, J_{gem} = 12.1, J(3′a,2) = 4.1 (H-3′a); 4.26 dd, 1H, J_{gem} = 12.1, J(3′b,2′) = 4.9 (H-3′b); 3.87 m, 1H (H-2′); 3.80 m, 1H (CH_2Pa); 3.71 m, 1H (CH_2Pb); 3.51 dd, 1H, J_{gem} = 14.4, J(1′a,2′) = 4.3 (H-1′a); 3.37 dd, 1H, J_{gem} = 14.4, J(1′b,2′) = 6.9 (H-1′b).

4.9. 2-[(2*S*)-3-Hydroxy-2-(phosphonomethoxy)propyl]carbamoylguanidine (9)

^{13}C NMR: 155.81 (C-4); 155.07 (C-2); 81.14 d, J(2′,P) = 11.3 (C-2′); 66.66 d, J(CH_2P,P) = 156.9 (CH_2P); 61.31 (C-3′); 40.37 (C-1′). ^{1}H NMR: 3.78 dd, 1H, J_{gem} = 13.1, J(CH_2Pa,P) = 9.2 (CH_2Pa); 3.74 dd, 1H, J_{gem} = 12.0, J(3′a,2′) = 3.7 (H-3′a); 3.72 dd, 1H, J_{gem} = 13.1, J(CH_2Pb,P) = 9.5 (CH_2Pb); 3.63 m, 1H (H-2′); 3.61 dd, 1H, J_{gem} = 11.8, J(3′b,2′) = 5.3 (H-3′b); 3.47 dd, 1H, J_{gem} = 14.3, J(1′a,2′) = 4.0 (H-1′a); 3.31 dd, 1H, J_{gem} = 14.3, J(1′b,2′) = 6.6 (H-1′b).

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