Bioorganic & Medicinal Chemistry 18 (2010) 387–395

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Acyclic nucleoside phosphonates with 5-azacytosine base moiety substituted in C-6 position

Marcela Krečmerová *, Milena Masojídková, Antonín Holý

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Gilead Sciences & IOCB Research Centre, Flemingovo nám. 2, CZ-166 10, Prague 6, Czech Republic

ARTICLE INFO

Article history:
Received 20 August 2009
Revised 22 October 2009
Accepted 24 October 2009
Available online 29 October 2009

Keywords:
5-Azacytosine
Acyclic nucleoside phosphonates
Antivirals
Orthoesters
Condensation
Carbamoylguanidine

ABSTRACT

Two methods for preparation of 6-substituted derivatives of anti DNA-viral agent 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine (HPMP-5-azaC) were developed: (1) ammonia mediated ring-opening reaction of diisopropyl esters of HPMP-5-azaC (**4**) to carbamoylguanidine derivatives followed by ring-closure reaction with orthoesters and (2) condensation reaction of 6-substituted 5-azacytosines with diisopropyl (1*S*)-[2-hydroxy-1-tosyloxymethyl)ethoxy]methylphosphonate (**15**). Deprotection of diisopropyl esters to free phosphonic acids was performed with bromotrimethylsilane in acetonitrile followed by hydrolysis. In contrast to parent compound HPMP-5-azaC, a substantial decrease of antiviral activity in case of 6-substituted analogues occurred. Surprisingly, N-3 isomer of 6-methyl-HPMP-5-azaC in the form of isopropyl ester revealed activity against RNA viruses (Sindbis virus).

1. Introduction

Triazine compounds, especially derivatives of 5-azacytosine play an important role in medicinal chemistry. In particular, 5-azacytosine nucleosides are known for their antileukemic activity. Two of them, 5-azacytidine[1] (azacitidine, Vidaza™) and 2′-deoxy-5-azacytidine[2] (decitabine, Dacogen™), compounds developed originally in our Institute in early 1960s were approved recently in clinical practice for the therapy of myelodysplastic syndrome.[3] Clinical investigation of both compounds for the treatment of solid tumors, especially metastatic lung cancer[4] and hormone-independent prostate cancer[5] is currently under way. In principle, the efficacy of 5-azacytosine nucleosides is related to their inhibitory activity towards DNA methylations.[6–10] Systematic investigation of new biologically active compounds resulted among others also in introduction of a *sym*-triazine component to the chemistry of acyclic nucleoside phosphonates (ANPs). These compounds have been developed in our Institute for many years and exerted a broad spectrum of diverse biological activities (mostly antiviral, but also cytostatic, antiparasital and immunomodulatory).[11] At present, three of them are utilized in clinical practice (adefovir, tenofovir, cidofovir) while several others undergo intensive preclinical or clinical investigation. Recently, we have described a new class of acyclic nucleoside phosphonates containing as a base moiety 5-azacytosine; among them 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine (HPMP-5-azaC, **1**, Fig. 1) its cyclic form **2** and ester prodrugs **3a–d** exerted remarkable activity against all types of DNA viruses.[12,13] From the structural point of view, HPMP-5-azaC is a 5-azacytosine analog of cidofovir, 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (HPMPC, Fig. 1). This compound with exceptional activity against DNA viruses was approved in clinical practice for the treatment of cytomegalovirus retinitis in AIDS patients[14] but it is also used off label, either systemically or topically, for the treatment of papillomatosis (anogenital or laryngeal)[15] infections, progressive multifocal leukoencephalopathy (caused by JC polyomavirus),[16] adenovirus[17] infections and some severe infections caused by poxviruses[18] (e.g., vaccinia, orf and molluscum contagiosum). Comparison of cidofovir and its 5-aza analog **1** showed that antiviral activity data of **1** (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 2–16-fold higher than cidofovir.[12] To improve bioavailability of **1**, several types of lipophilic ester prodrugs were synthesised. Esterification process was carried out on the level of the cyclic phosphonate **2**. The most active compound was found 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), 0.008–0.037 μg/ml for HHV-6 and 0.037 μg/ml for vaccinia virus. The activity of ester prodrugs was decreasing in the order: 2-

* Corresponding author. Tel.: +420 220183475; fax: +420 220183560.
E-mail addresses: marcela@uochb.cas.cz (M. Krečmerová), masojid@uochb.cas.cz (M. Masojídková), holy@uochb.cas.cz (A. Holý).

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doi:10.1016/j.bmc.2009.10.044

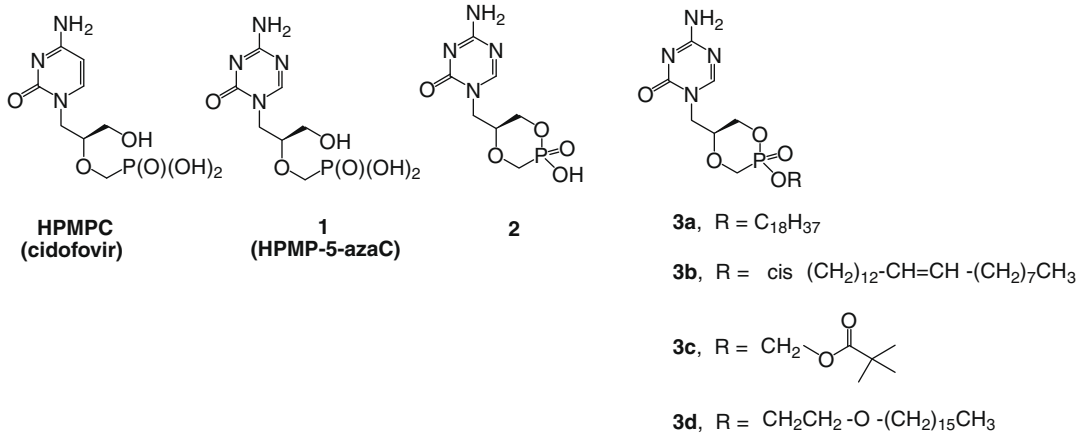


Figure 1. Structures of cidofovir and its 5-azacytosine analogues: HPMP-5-azaC (**1**), cyclic HPMP-5-azaC (**2**) and ester prodrugs of cyclic HPMP-5-azaC (**3a**–**3d**).

(hexadecyloxy)ethyl > pivaloyloxymethyl ~ octadecyl > erucyl (compounds **3a–d**, Fig. 1).[13] Progression in investigation of HPMP-5-azaC and its derivatives is currently still under way.

In contrast to cidofovir, HPMP-5-azaC has more complicated metabolic profile due to its chemical (and also enzymatic) instability. Concerning to 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.[12,19] Recently, we carried out a detailed study of this process under different conditions using NMR methodology for identification and quantification of all reaction products and intermediates.[19] The ring-opening reaction is a general property of all 5-azacytosine compounds; the first study of this type was carried out by Pithova on 5-azacytidine.[20] It was 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 a formyl group to form 1-β-D-ribofuranosyl-3-guanylurea.[20] The reaction is initiated by nucleophilic attack of hydroxyl ion in position 6 of 5-azacytosine whose electron density is lower compared to cytosine.

The aim of this work was finding appropriate modification of 5-azacytosine moiety in HPMP-5-azaC (and related compounds) in order to increase its stability, especially in aqueous solutions. Regarding above described facts, we focused to preparation of analogs modified in C-6 position with various alkyl or aryl substituents. Electron donating effect of these substituents causing decrease of electrophilicity of C-6 position as well as steric hindrance of C-6 position are predicted to have a positive influence to overall stability of 5-azacytosine ANPs.

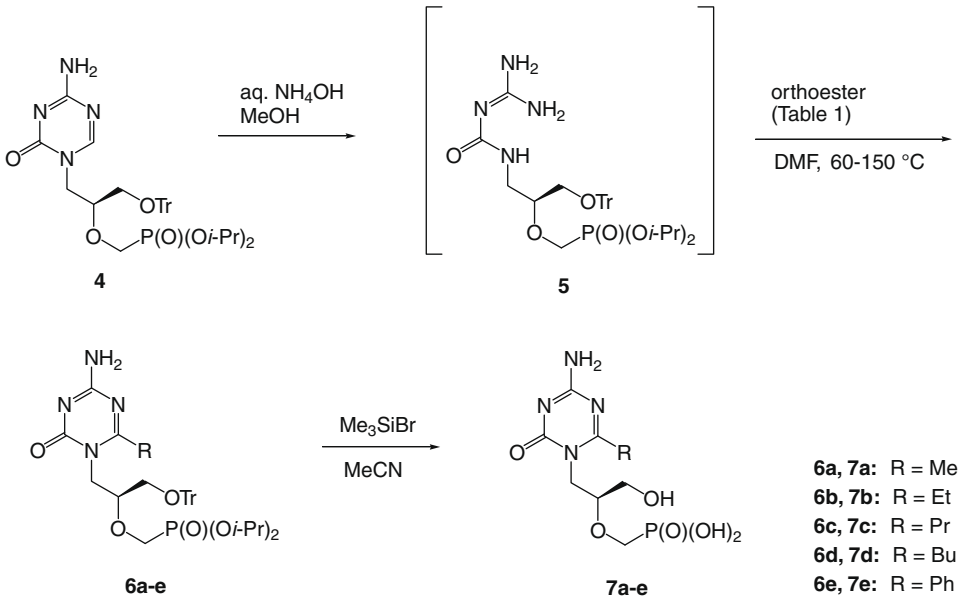
2. Results and discussion

It is known that *sym*-triazine ring (5-azacytosine and its 6-substituted derivatives) can be formed by condensation of guanylurea with orthoesters. This methodology was described by Piskala in one of his pioneer works on 5-azacytosine compounds.[21] 3-Guanylureas substituted with a sugar residue or with an aliphatic chain are formed as final products of alkali catalyzed ring-opening degradation of 5-azacytosine compounds. In a series of 5-azacytosine acyclic nucleoside phosphonates, we proposed that such degradation products could be appropriate starting material for synthesis of their stabilized 6-substituted derivatives. In this effort we focused on antiviral agent HPMP-5-azaC and utilization of its triazine ring degradation product for reaction with diverse types of orthoesters. Such reactions require both elevated temperature and performance in dipolar aprotic solvents, usually dimethylformamide. Unfortunately, in such solvents free phosphonic acids are completely insoluble. Therefore, we avoided the use of HPMP-5-azaC and its open-ring form as free phosphonic acids, selecting instead the protected diisopropyl ester of HPMP-5-azaC (compound **4**, Scheme 1) as the starting compound for our synthesis. This compound is an intermediate in preparation of HPMP-5-azaC (**1**) starting from (2*S*)-2-[(trityloxy]methyl]oxirane.[12]

Ring-opening reaction of **4** was performed using highly concentrated aqueous-alcoholic solution of ammonia. Regarding the chemical instability of 5-azacytosine nucleosides[22] or free ANPs (Ref. 19) in alkaline or even neutral solutions, we expected very rapid decomposition. In fact, the degradation of triazine ring in **4** was surprisingly slow: its complete degradation with 7 M-NH_4OH at 25 °C required 6 days. The formed carbamoylguanidine intermediate **5** was subjected to ring-closure reaction with a series of diverse orthoesters to give 6-substituted azacytosine phosphonate esters **6** (Table 1). The final deprotection of trityl group and isopropyl ester groups was performed in one step by reaction with bromotrimethylsilane followed by hydrolysis giving free 6-alkyl/aryl-HPMP-5-azaC (**7**) (Scheme 1).

In our previous paper we studied a course of chemical decomposition of several ANPs with 5-azacytosine base in diverse buffers.[19] The most labile phosphonate was found HPMP-5-azaC (**1**) with a half-life time 10.3 days at pH 10.0 (phosphate buffer). In contrast to other studied compounds of the series, HPMP-5-azaC was the only one having a free hydroxyl group in 3′ position of an aliphatic chain. The reaction rate of HPMP-5-azaC was approximately four times higher than the decomposition rates of the other compounds and the formyl derivative **9** was formed as an intermediate. This formyl derivative **9** was then hydrolyzed to form carbamoylguanidine derivative **10** and formic acid. Formation of the formiate **9** was not observed in the decomposition reaction of other 5-azacytosine ANPs. The explanation for this different reaction pathway is the following: the first intermediary formed formylamido derivative **8** (this structural type was previously shown to be an intermediate in the 5-azacytosine nucleoside decomposition[16]) can react in a transformylation reaction with a spatially close hydroxy group to form the formiate **9** (see Scheme 2). 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.

We believe that the surprisingly slow decomposition rate of **4** is caused by protection of hydroxyl group in the 3′ position, making the transformylation reaction impossible. Another factor is esterification of phosphonomethyl group in 5-azacytosine ANPs. Numerous observations made during a long time revealed that cyclic phosphonates, esters of cyclic phosphonates and phosphonate



Scheme 1. Transformation of tritylated ester of 1-(*S*)-[3-hydroxy-2(phosphonomethoxy)propyl]-5-azacytosine (**4**) to C-6 substituted HPMP-5-azaC (**7a–e**).

Table 1
Reaction of the 'open-ring' intermediate **5** with orthoesters

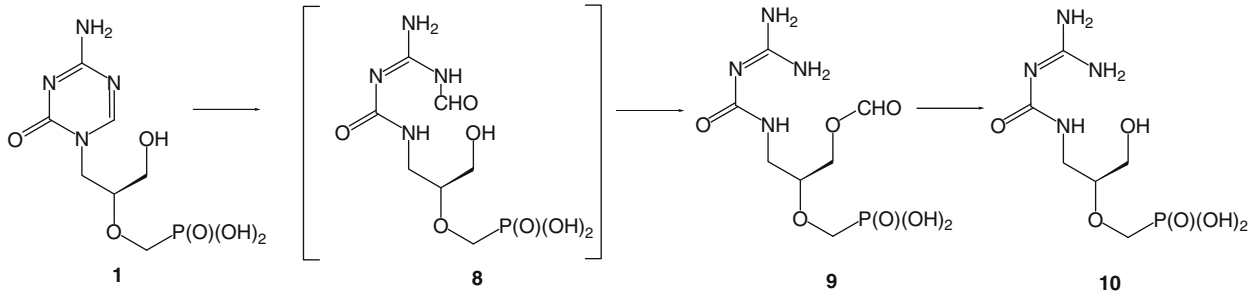
Orthoester	Product	Conditions	Yield (%) of **6**[a]
$CH_3C(OEt)_3$	**6a**	150 °C, 3 h	67
$CH_3CH_2C(OEt)_3$	**6b**	60 °C, 2 h	47
$CH_3(CH_2)_2C(OMe)_3$	**6c**	120 °C, 2 h	62
$CH_3(CH_2)_3C(OMe)_3$	**6d**	110 °C, 1.5 h	73
$C_6H_5C(OMe)_3$	**6e**	150 °C, 2 h	64

[a] Overall yield from **4**.

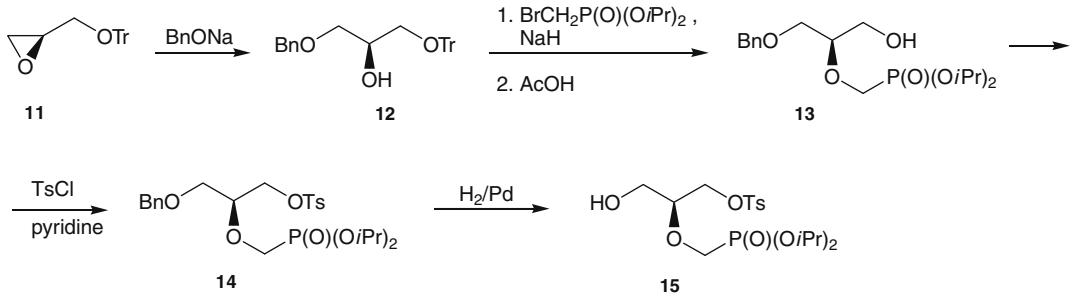
diesters in 5-azacytosine ANPs are generally more stable compared to free phosphonic acids. Besides the stabilizing effect, esterification of a phosphonate function in HPMP-5-azaC (and ANPs generally), has also a beneficial effect to increase of their antiviral activity. Regarding these facts, it is preferable their further pharmacological investigation in a form of ester prodrugs.[13]

The second approach to 6-substituted 5-azacytosine ANPs is alkylation of 6-alkyl 5-azacytosines with appropriate synthon, that is, an aliphatic part activated with a suitable leaving group, usually tosyl or halogen. 6-Alkyl-5-azacytosines are accessible by condensation reaction of guanylurea with orthoesters.[21] Preparation of '(*S*)-HPMP synthon', diisopropyl (1*S*)-[2-benzyloxy-1-(tosyloxymethyl) ethoxy] methylphosphonate (**14**) followed the protocol originally developed for its racemic form.[23] The starting (2*S*)-1-benzyloxy-3-trityloxypropan-2-ol (**12**) accessible from (2*S*)-2-[(trityloxy]methyl] oxirane (**11**) was treated with diisopropyl bromomethanephosphonate, the trityl group removed and the intermediary formed **13** tosylated by standard procedure (Scheme 3). In contrast to other nucleobases, 5-azacytosine is sensitive towards catalytic hydrogenation and 5,6-dihydro derivatives would be formed during hydrogenolytic removal of benzyl group. Therefore, removal of benzyl group was performed first, still in a stage of the synthon and thus formed diisopropyl (1*S*)-[2-hydroxy-1-tosyloxymethyl)ethoxy]methylphosphonate (**15**) was used for condensation with a sodium salt of 5-azacytosine and/or its 6-substituted analogues.

Interestingly, there is a substantial difference in regioselectivity of condensation of **15** with 6-substituted and usubstituted 5-azacytosines. Condensation with a sodium salt of 5-azacytosine proceeds exclusively to form N-1 isomer, diisopropyl ester of HPMP-5-azaC (**16**), accompanied by N-3 and O-isomers (**17** and **18**) in small amount only. This reaction thus revealed as advantageous approach to antivirally active HPMP-5-azaC, especially for its larger-scale syntheses. Its synthesis from **16** utilizes a standard procedure with bromotrimethylsilane followed by hydrolysis. The same procedure performed with **17** afforded antivirally inactive N-3 isomer of HPMP-5-azaC while in case of O-isomer **18**, its treatment with bromotrimethylsilane lead to a complete degradation of the compound. Contrary to unsubstituted 5-azacytosine, reaction of a sodium salt of 6-methyl-5-azacytosine with **15** affords N-3 isomer **19** as a main product accompanied by N-1 isomer **20** as by-product in small amount. It can be explained by electron donating effect of C-6 methyl group: in neighborhood of N-1 position it probably partially compensates its nucleophilic character



Scheme 2. The course of hydrolytic triazine-ring opening in HPMP-5-azaC (**1**).



Scheme 3. Preparation of (*S*)-HPMP synthons **14** and **15** from (2*S*)-2-[(trityloxy]methyl]oxirane.

and an alternative formation of anion at more remote N-3 position in creation of salt can be thus preferable. Deprotection of esters to free phosphonic acids was performed by a standard procedure with bromotrimethylsilane. In case of N-3 isomer, the final product **21** was accompanied by a large amount of monoester **22** due to its increased tendency to crystallization and lower solubility in reaction mixture. Both compounds can be separated by a reverse phase HPLC technique (see Scheme 4).

3. Biological activity

Free phosphonic acids (**7a–e**, **21**, **23**) and the isopropyl ester **22** were tested for activity against DNA viruses [in human embryonic lung (HEL) cells], retroviruses (HIV-1, HIV-2) in human T-lymphocyte (CEM) cells and against RNA viruses (in Vero cells). Concerning DNA viruses, none of the tested compounds was active against herpes viruses HSV-1 and HSV-2. Activity of 6-substituted derivatives **7a–e** was decreased against poxviruses (vaccinia virus, cowpox virus, orf virus) and varicella zoster virus (50–100-fold compared to parent HPMP-5-azaC) and 1000–1500-fold against cytomegalovirus. All prepared compounds were inactive against HIV-1 and HIV-2. Surprisingly, in case of N-3 isomers we found anti RNA-viral activity at isopropyl ester **22** (20 μg/ml for Sindbis virus). Since the corresponding free phosphonic acid **21** was antivirally inactive, we suppose that esterification of these structures (N-3 isomers) is necessary to allow passage through the cell membrane to achieve antiviral activity.

The activity of **22** against RNA virus is rather atypical; acyclic nucleoside phosphonates of HPMP structural type are typically active against DNA viruses. One of the rare exceptions is recently described activity of alkoxyalkyl esters of HPMPA against HCV (but HPMPA as free phosphonic acid has no anti-HCV activity).[24]

All prepared compounds are non-toxic (CC_{50} >100 μg/ml) and none of the compounds revealed cytostatic activity.

4. Conclusions

A new series of acyclic nucleoside phosphonates with 6-substituted 5-azacytosine base moiety was prepared using two different synthetic procedures. Synthesis of enantiomerically pure modified '(*S*)-HPMP synthon', that is, diisopropyl (1*S*)-[2-hydroxy-1-tosyloxymethyl)ethoxy]methylphosphonate (**15**) has a general importance for preparations of HPMP derivatives whose base moiety is sensitive to catalytic hydrogenation. Especially in case of antivirally active HPMP-5-azaC, synthesis based on utilization of this synthon is easier and gives higher yields compared to the originally described procedure.[12] The described methodology is applicable especially for large-scale syntheses of HPMP-5-azaC. Unfortunately, compared to HPMP-5-azaC, introduction of an alkyl (aryl) group to C-6 position of a base moiety leads to a substantial decrease of antiviral activity. On the other hand, anti RNA-viral activity of isopropyl ester of N-3 isomer **22** gives an impetus for preparation of other (especially lipophilic) esters of this type of phosphonates and their subjection to detailed biological investigation.

5. Experimental

5.1. General

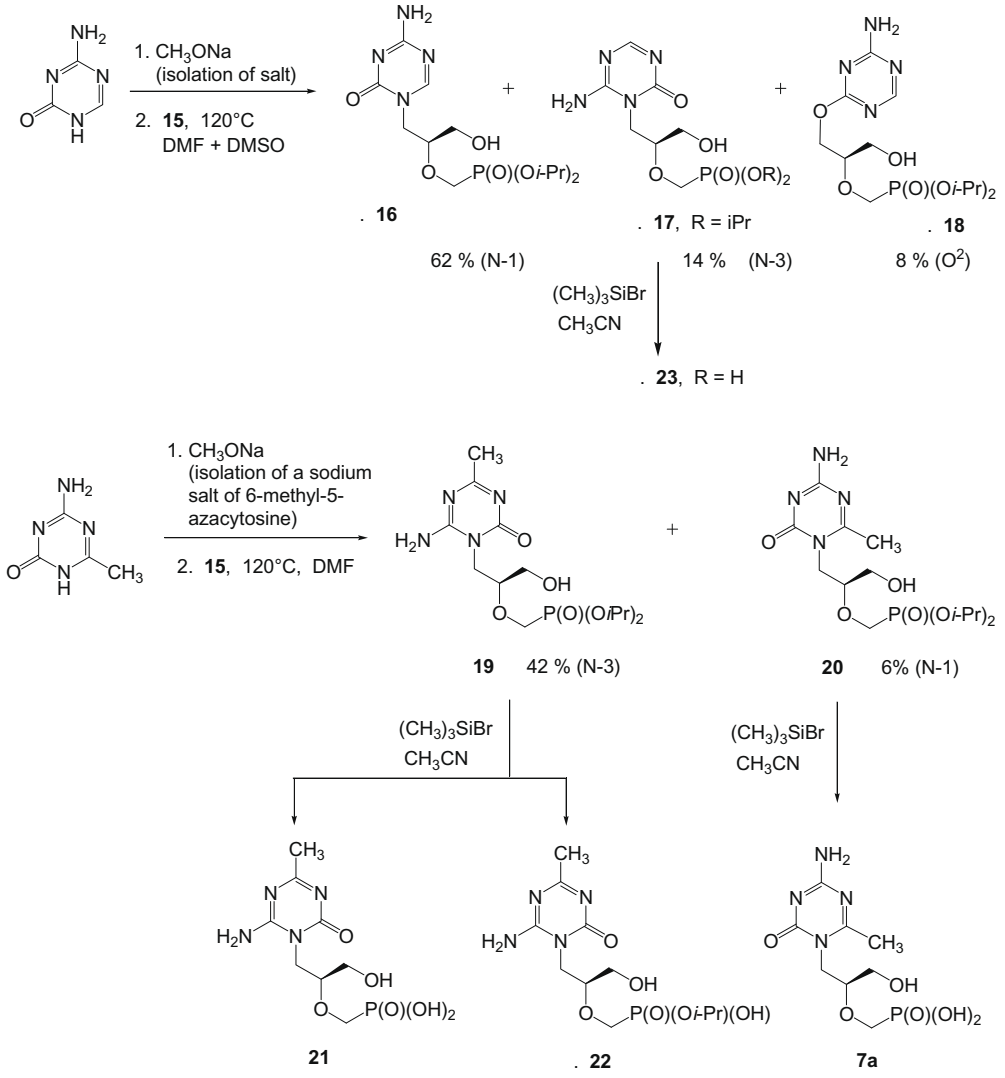
Unless stated otherwise, solvents were evaporated at 40 °C/2 kPa and compounds were dried at 13 Pa. Melting points were determined on a Kofler block and are uncorrected. Analytical TLC was performed on Silica Gel 60 F_{254} plates (Merck KGaA, Darmstadt, Germany); chromatographic systems are described in text. Column chromatography was performed on silica gel 60 μm (Fluka). Preparative reverse phase HPLC separations were performed on a Waters Delta 600 instrument with a Waters 2487 Dual λ Absorbance Detector using a column Luna Phenomenex® C-18 (10 μm, 21 × 250 mm), flow 12 ml/min. ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance 500 spectrometer (^{1}H at 500 MHz, ^{13}C at 125.7 MHz) in D_2O or DMSO-d_6 solutions (referenced to sodium 3-(trimethylsilyl)propane-1-sulfonic acid (DSS) or residual solvent signal). The numbering system for assignment of NMR signals is outlined in Figure 2. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionization with xenon, accelerating voltage 8 kV, glycerol matrix) or by ESI technique. Optical rotations were measured on Autopol IV polarimeter (Rudolph Research Analytical, USA) at 20 °C, $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Most of chemicals and ion-exchange resins (Dowex 1 × 2-400) were purchased from Sigma–Aldrich (Czech Republic). Compound **4** was prepared according to previously described procedure.[12]

5.2. C-6. Substituted 5-azacytosine phosphonate esters: general procedure

25% Aqueous ammonia (30 mL) was added to a solution of **4** (950 mg, 1.57 mmol) in methanol (30 mL), the mixture was stirred for 6 days at rt and then evaporated under reduced pressure. The residue (intermediate **5**) was coevaporated with absolute ethanol (2 × 50 ml), and after drying in vacuo at 50 °C, dissolved in DMF (7 mL). An appropriate orthoester (10 mmol) was added and the mixture was heated under conditions given in Table 1. Then, the solution was evaporated, the residue coevaporated with xylene (20 mL) and subjected to column chromatography on silica gel (150 mL) in system described below.

5.2.1. 1-(2*S*)-2-[(Diisopropoxyphosphoryl)methoxy-3-(triphenylmethoxy)propyl-6-methyl-5-azacytosine (6a)

Chromatographed in system EtOAc–acetone–EtOH–water (18:3:1:1), R_f = 0.24. White foam. $[\alpha]_D$ −38.7 (*c* 0.626, $CHCl_3$). ^{1}H NMR (DMSO-d_6, ppm) δ: 1.13, 1.18, 1.19 and 1.20 (4 × d, 4 × 3H,



Scheme 4. Alkylation of (*S*)-HPMP synthon **15** with sodium salts of 5-azacytosine and 6-methyl-5-azacytosine.

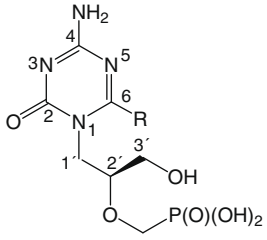


Figure 2. General numbering scheme for assignment of NMR signals.

$J_{CH3,CH}$ = 6.2, CH_3), 2.43 (s, 3H, CH_3), 3.00 (dd, 1H, $J_{3'a,2'}$ = 3.8, J_{gem} = 10.4, H-3′a), 3.27 (dd, 1H, $J_{3'b,2'}$ = 3.4, H-3′b), 3.55 (dd, 1H, $J_{P,CHa}$ = 10.1, J_{gem} = 13.7, PCH_a), 3.72 (dd, 1H, $J_{P,CHb}$ = 8.4, PCH_b), 3.86 (m, 1H, H-2′), 3.94 (m, 2H, H-1′), 4.51 (m, 2H, P–OC*H*), 7.23 and 7.24 (2 × br s, 2 × 1H, NH_2), 7.28 (t, 3H, H-arom.), 7.36 (t, 6H, H-arom.), 7.41 (d, 6H, H-arom.). ^{13}C NMR (DMSO-d_6, ppm) δ: 22.41 (CH_3), 23.63 and 23.82 (2 × d, $J_{P,C}$ = 4.4, 2 × CH_3), 23.85 and 23.88 (2 × d, $J_{P,C}$ = 3.4, 2 × CH_3), 46.44 (C-1′), 62.52 (C-3′), 63.95 (d, $J_{P,C}$ = 165.0, P–C), 70.15 and 70.31 (2 × d, $J_{P,C}$ = 6.3, P–OCH), 78.85 (d, $J_{P,C}$ = 12.7, C-2′), 86.36 (Tr), 127.34 (3C, Tr), 128.17 (6C, Tr), 128.41 (6C, Tr), 143.63 (3C, Tr), 155.19 (C-2), 165.36 (C-4), 168.19 (C-6). FAB-MS, *m/z*: 621 $[M+H]^+$, 379 $[M+2H-Tr]^+$, 243 [Tr], 165. Anal. Calcd for $C_{33}H_{41}N_4O_6P$: C, 63.86; H, 6.66; N, 9.03; P, 4.99. Found: C, 63.53; H, 6.51; N, 8.74; P, 4.76.

5.2.2. 1-(2*S*)-2-[(Diisopropoxyphosphoryl)methoxy-3-(triphenylmethoxy)propyl-6-ethyl-5-azacytosine (6b)

Chromatographed in system chloroform–MeOH (9:1), R_f = 0.21. White foam. $[\alpha]_D$ −50.6 (*c* 0.419, $CHCl_3$). ^{1}H NMR (DMSO-d_6, ppm) δ: 1.13 (t, 3H, *J* = 7.3, CH_3CH_2), 1.13, 1.18, 1.19 and 1.205 (4 × d, 4 × 3H, $J_{CH3,CH}$ = 6.2, $(CH_3)_2CH$), 2.81 (m, 2H, CH_2), 2.99 (dd, 1H, $J_{3'a,2'}$ = 3.7, J_{gem} = 10.6, H-3′a), 3.29 (dd, 1H, $J_{3'b,2'}$ = 3.4, H-3′b), 3.54 (dd, 1H, $J_{P,CHa}$ = 10.2, J_{gem} = 13.8, PCH_a), 3.71 (dd, 1H, $J_{P,CHb}$ = 8.4, PCH_b), 3.86 (m, 1H, H-2′), 3.93 (dd, 1H, $J_{1'a,2'}$ = 4.2, J_{gem} = 14.3, H-1′a), 3.97 (dd, 1H, $J_{1'b,2'}$ = 8.3, H-1′b), 4.51 (m, 2H, P–OC*H*), 7.21 and 7.23 (2 × br s, 2 × 1H, NH_2), 7.27 (t, 3H, H-arom.), 7.30 (t, 6H, H-arom.), 7.37 (d, 6H, H-arom.). ^{13}C NMR (DMSO-d_6, ppm) δ: 10.14 (CH_3CH_2), 23.59 (d, $J_{P,C}$ = 4.4, CH_3), 23.83 (d, 2C, $J_{P,C}$ = 3.9, CH_3), 23.87 (d, $J_{P,C}$ = 3.9, CH_3), 26.66 (CH_2), 45.35 (C-1′), 62.36 (C-3′), 63.98 (d, $J_{P,C}$ = 165.2, P–C), 70.14 and 70.29 (2 × d, $J_{P,C}$ = 3.9, P–OCH), 78.66 (d, $J_{P,C}$ = 13.0, C-2′), 86.31 (Tr), 127.33 (3C, Tr), 128.15 (6C, Tr), 128.39 (6C, Tr), 143.61 (3C, Tr), (155.32 (C-2), 165.40 (C-4), 170.99 (C-6). +ESI-MS, *m/z:* 1291 $[2\ M+Na]^+$, 1269 $[2\ M+H]^+$, 657 $[M+Na]^+$, 243 [Tr]. Anal. Calcd for

$C_{34}H_{43}N_4O_6P$: C, 64.34; H, 6.83; N, 8.83, P, 4.88. Found: C, 63.96; H, 7.01; N, 8.64; P, 5.11.

5.2.3. 1-(2*S*)-2-[(Diisopropoxyphosphoryl)methoxy-3-(triphenylmethoxy)propyl-6-propyl-5-azacytosine (6c)

Chromatographed in system chloroform–MeOH (9:1), R_f = 0.22. White foam. $[\alpha]_D$ −46.6 (*c* 0.222, $CHCl_3$). ^{1}H NMR (DMSO-d_6, ppm) δ: 0.97 (t, 3H, $J_{CH3,CH2}$ = 7.4, CH_3), 1.05 (m, 2H, CH_2), 1.13, 1.19, 1.20 and 1.21 (4 × d, 4 × 3H, $J_{CH3,CH}$ = 6.2, CH_3), 2.70 (dd, 1H, $J_{3'a,2'}$ = 6.3, J_{gem} = 10.6, H-3′a), 2.97 (dd, 1H, $J_{3'b,2'}$ = 3.5, H-3′b), 2.79 and 3.31 (2 × m, 2 × 1H, CH_2), 3.52 (dd, 1H, $J_{P,CHa}$ = 10.2, J_{gem} = 13.6, PCH_a), 3.69 (dd, 1H, $J_{P,CHb}$ = 8.6, PCH_b), 3.85 (m, 1H, H-2′), 3.92 (dd, 1H, $J_{1'a,2'}$ = 3.5, J_{gem} = 14.2, H-1′a), 3.99 (dd, 1H, $J_{1'b,2'}$ = 9.0, H-1′b), 4.52 (m, 2H, P–OCH), 7.21 and 7.22 (2 × br s, 2 × 1H, NH_2), 7.28 (t, 3H, H-arom.), 7.36 (t, 6H, H-arom.), 7.41 (d, 6H, H-arom.). ^{13}C NMR (DMSO-d_6, ppm) δ: 13.85 (CH_3), 19.14 (CH_2), 23.60 (d, $J_{P,C}$ = 4.2, CH_3), 23.83 (d, $J_{P,C}$ = 3.9, CH_3), 23.86 (d, $J_{P,C}$ = 4.6, CH_3), 23.90 (d, $J_{P,C}$ = 3.9, CH_3), 35.49 (CH_2), 45.43 (C-1′), 61.99 (C-3′), 63.87 (d, $J_{P,C}$ = 165.5, P–C), 70.14 and 70.29 (2 × d, $J_{P,C}$ = 6.2, P–OCH), 78.47 (d, $J_{P,C}$ = 13.4, C-2′); 80.26 (Tr), 127.35 (3C, Tr), 128.14 (6C, Tr), 128.38 (6C, Tr), 143.61 (3C, Tr), 155.28 (C-2), 165.38 (C-4), 170.18 (C-6). FAB-MS, *m/z*: 671 $[M+Na]^+$, 243 [Tr], 165. Anal. Calcd for $C_{35}H_{45}N_4O_6P$: C, 64.80; H, 6.99; N, 8.64; P, 4.77. Found: C, 64.44; H, 6.97; N, 8.46; P, 4.60.

5.2.4. 6-Butyl-1-(2*S*)-2-[(diisopropoxyphosphoryl)methoxy-3-(triphenylmethoxy)propyl-5-azacytosine (6d)

Chromatographed in system EtOAc–acetone–EtOH–water (18:3:1:1), R_f = 0.40. Yellowish syrup. $[\alpha]_D$ −38.2 (*c* 0.169, $CHCl_3$). ^{1}H NMR (DMSO-d_6, ppm) δ: 0.92 (t, 3H, $J_{CH3,CH2}$ = 7.3, CH_3), 1.12, 1.18, 1.19 and 1.20 (4 × d, 4 × 3H, $J_{CH3,CH}$ = 6.1, CH_3), 1.24, 1.38 and 1.62 (3 × m, 3 × 2H, CH_2), 2.66 (dd, 1H, $J_{3'a,2'}$ = 5.4, J_{gem} = 10.5, H-3′a), 2.95 (dd, 1H, $J_{3'b,2'}$ = 2.9, H-3′b), 3.51 (dd, 1H, $J_{P,CHa}$ = 10.3, J_{gem} = 13.7, PCH_a), 3.68 (dd, 1H, $J_{P,CHb}$ = 8.4, PCH_b), 3.85 (m, 1H, H-2′), 3.91 (dd, 1H, $J_{1'a,2'}$ = 2.7, J_{gem} = 14.1, H-1′a), 3.99 (dd, 1H, $J_{1'b,2'}$ = 9.5, H-1′b), 4.51 (m, 2H, P–OCH), 7.25 and 7.35 (2 × br s, 2 × 1H, NH_2), 7.29 (t, 3H, H-arom.), 7.36 (t, 6H, H-arom.), 7.42 (d, 6H, H-arom.). ^{13}C NMR (DMSO-d_6, ppm) δ: 13.99 (CH_3), 22.13 (CH_2), 23.75 (2 × d, 2C, $J_{P,C}$ = 4.9, CH_3), 23.89 (2 × d, 2C, $J_{P,C}$ = 3.9, CH_3), 27.99 (CH_2), 33.45 (CH_2), 46.80 (C-1′), 61.94 (C-3′), 63.85 (d, $J_{P,C}$ = 165.5, P–C), 70.15 and 70.31 (2 × d, 2C, $J_{P,C}$ = 6.3, P–OCH), 78.54 (d, $J_{P,C}$ = 13.7, C-2′), 86.25 (Tr), 127.36 (3C, Tr), 128.15 (6C, Tr), 128.40 (6C, Tr), 143.60 (3C, Tr), 155.295 (C-2), 165.40 (C-4), 170.41 (C-6). FAB-MS, *m/z*: 685 $[M+Na]^+$, 243 [Tr], 165. Anal. Calcd for $C_{36}H_{47}N_4O_6P$: C, 65.24; H, 7.15; N, 8.45; P, 4.67. Found: C, 65.37; H, 6.97; N, 8.24; P, 4.42.

5.2.5. 1-(2*S*)-2-[(Diisopropoxyphosphoryl)methoxy-3-(triphenylmethoxy)propyl-6-phenyl-5-azacytosine (6e)

Chromatographed in system EtOAc–acetone–EtOH–water (18:3:1:1), R_f = 0.42. White foam. $[\alpha]_D$ −24.3 (*c* 0.323, $CHCl_3$). ^{1}H NMR (DMSO-d_6, ppm) δ: 1.20, 1.22 and 1.25 (3 × d, 12H, $J_{CH3,CH}$ = 6.2, CH_3), 3.19 (dd, 1H, $J_{3'a,2'}$ = 2.1, J_{gem} = 13.2, H-3′a), 3.44 (dd, 1H, $J_{P,CHa}$ = 10.1, J_{gem} = 13.6, PCH_a), 3.61 (dd, 1H, $J_{P,CHb}$ = 8.9, PCH_b), 3.71 (dd, 1H, $J_{1'a,2'}$ = 2.0, J_{gem} = 13.6, H-1′a), 3.91 (dd, 1H, $J_{3'b,2'}$ = 7.6, H-3′b), 4.02 (dd, 1H, $J_{1'b,2'}$ = 9.5, H-1′b), 4.54 (m, 2H, P–OCH), 4.60 (m, 1H, H-2′), 7.23 (t, 3H, H-arom.), 7.25 (br s, 1H, NH_2), 7.30 (t, 6H, H-arom.), 7.40 (br s, 1H, NH_2), 7.42 (d, 6H, H-arom.), 7.51 (t, 2H, H-arom.), 7.60 (t, 1H, H-arom.), 7.69 (d, 2H, H-arom.). ^{13}C NMR (DMSO-d_6, ppm) *δ: δ* 23.89 (d, 2C, $J_{P,C}$ = 4.9, CH_3), 23.93 (d, 2C, $J_{P,C}$ = 3.9, CH_3), 47.67 (C-1′), 61.50 (C-3′), 63.82 (d, $J_{P,C}$ = 166.2, P–C), 70.21 and 70.44 (2 × d, 2C, $J_{P,C}$ = 6.3, P–OCH), 77.45 (d, $J_{P,C}$ = 12.9, C-2′), 85.92 (Tr), 127.24 (3C, Tr), 128.08 (6C, Tr), 128.27 (8C, Tr, Ph), 128.55 (2C, Ph), 130.17 and 133.87 (Ph), 143.53 (3C, Tr). FAB-MS, *m/z*: 683 $[M+H]^+$, 441 [M+2H−Tr], 243 [Tr]. Anal. Calcd for $C_{38}H_{43}N_4O_6P$: C, 66.85; H, 6.35; N, 8.21; P, 4.54. Found: C, 66.80; H, 6.64; N, 7.95; P, 4.83.

5.3. Cleavage of the phosphonate esters: general procedure

Bromotrimethylsilane (1 mL, 7.5 mmol) was added to a solution of appropriate ester **6a–e** (0.7 mmol) in acetonitrile (8 mL), the mixture was set aside in the dark for 48 h at rt and evaporated. The residue was coevaporated with toluene (2 × 20 ml). 90% Aqueous methanol (50 mL) was added, the solution was neutralized drop wise with 0.2 M triethylammonium hydrogencarbonate to pH 7 and evaporated. Water (10 mL) was added and the mixture applied onto a column of Dowex 1 (AcO^- form, 25 mL). The column was eluted with methanol till disappearance of UV absorption (removal of TrOH), followed by water (300 mL), a linear gradient of acetic acid (0.5–1.0 M, 500 mL) and 1 M formic acid. UV absorbing formic acid eluate was evaporated, the residue coevaporated with water (4 × 30 mL) and lyophilized from water or crystallized.

5.3.1. 1-(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]-6-methyl-5-azacytosine (7a)

Yield: 165 mg, 80%. White powder. Mp: 137–140 °C (MeOH–EtOH). $[\alpha]_D$ −78.2 (*c* 0.253, H_2O). ^{1}H NMR (D_2O, ppm) δ: 2.68 (s, 3H, CH_3), 3.67 (dd, 1H, $J_{P,CHa}$ = 8.7, J_{gem} = 14.4, PCH_a), 3.96 (dd, 1H, $J_{P,CHb}$ = 7.7, PCH_b), 3.89 (m, 2H, H-3′), 4.16 (m, 1H, H-2′), 4.19 (m, 2H, H-1′). ^{13}C NMR (D_2O, ppm) δ: 20.00 (CH_3); 44.57 (C-1′), 57.22 (C-3′), 65.08 (d, $J_{P,C}$ = 159.5, P–C), 76.58 (d, $J_{P,C}$ = 8.8, C-2′), 145.57 (C-2), 154.67 (C-4), 171.40 (C-6). FAB-MS, *m/z*: 589 [2 M+H]$^+$, 295 $[M+H]^+$. FAB-HRMS calcd for $C_8H_{16}N_4O_6P$ 295.0807, found 295.0809 $[M+H^+]$.

5.3.2. 6-Ethyl-1-(S)-3-hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine (7b)

Yield: 149 mg, 69%, white amorphous solid. $[\alpha]_D$ −89.1 (*c* 0.191, H_2O). ^{1}H NMR (D_2O, ppm) δ: 1.22 (s, 3H, CH_3), 3.49 (dd, 1H, $J_{P,CHa}$ = 9.1, J_{gem} = 13.3, PCH_a), 3.65 (dd, 1H, $J_{3'a,2'}$ = 3.3, J_{gem} = 12.5, H-3′a), 3.77 (dd, 1H, $J_{P,CHb}$ = 8.8, PCH_b), 3.82 (m, 1H, H-2′), 3.89 (dd, 1H, $J_{3'b,2'}$ = 3.8, H-3′b), 4.14 (d, 2H, $J_{1',2'}$ = 5.6, H-1′). ^{13}C NMR (D_2O, ppm) δ: 8.90 (CH_3); 45.78 (C-1′), 59.53 (C-3′), 65.72 (d, $J_{P,C}$ = 157.1, P–C), 78.75 (d, $J_{P,C}$ = 10.6, C-2′), 150.57 (C-2); 159.15 (C-4), 175.94 (C-6). FAB-MS, *m/z*: 617 $[2M+H]^+$, 309 $[M+H]^+$. FAB-HRMS calcd for $C_9H_{18}N_4O_6P$ 309.0964, found 309.0959 $[M+H^+]$.

5.3.3. 1-(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]-6-propyl-5-azacytosine (7c)

Yield: 111 mg, 49%, white amorphous solid. $[\alpha]_D$ −90.0 (*c* 0.236, H_2O). ^{1}H NMR (D_2O, ppm) δ: 0.91 (t, 3H, $J_{CH3,CH2}$ = 7.3, CH_3), 1.64 (m, 2H, CH_2), 2.83 (m, 2H, CH_2), 3.40 (dd, 1H, $J_{3'a,2'}$ = 9.0, J_{gem} = 12.6, H-3′a), 3.56 (dd, 1H, $J_{3'b,2'}$ = 3.3, H-3′b), 3.67 (dd, 1H, $J_{1'a,2'}$ = 8.8, J_{gem} = 12.4, H-1′a), 3.73 (m, 1H, H-2′), 3.79 (dd, 1H, $J_{1'b,2'}$ = 2.5, H-1′b), 4.05 (d, 2H, $J_{P,CH}$ = 8.6, PCH_2). ^{13}C NMR (D_2O, ppm) δ: 12.48 (CH_3), 18.72 (CH_2), 35.90 (CH_2), 45.83 (C-1′), 59.55 (C-3′), 65.75 (d, $J_{P,C}$ = 159.0, P–C), 78.72 (d, $J_{P,C}$ = 10.2, C-2′), 151.10 (C-2), 159.42 (C-4), 174.95 (C-6). FAB-MS, *m/z*: 323) $[M+H]^+$. FAB-HRMS calcd for $C_{10}H_{20}N_4O_6P$ 323.1120, found 323.1113 $[M+H^+]$.

5.3.4. 6-Butyl-1-(S)-3-hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine (7d)

Yield: 179 mg, 76%, white amorphous solid. $[\alpha]_D$ −51.5 (*c* 0.253, H_2O). ^{1}H NMR (D_2O, ppm) δ: 0.92 (t, 3H, $J_{CH3,CH2}$ = 6.4, CH_3), 1.43 (m, 2H, CH_2), 1.69 (q, 2H, *J* = 7.6, CH_2), 2.95 (m, 2H, CH_2), 3.57 (dd, 1H, $J_{P,CHa}$ = 8.5, J_{gem} = 13.8, PCH_a), 3.66 (dd, 1H, $J_{3'a,2'}$ = 2.7, J_{gem} = 12.2, H-3′a), 3.85 (dd, 1H, $J_{P,CHb}$ = 8.2, PCH_b), 3.87 (m, 1H, H-2′), 3.89 (dd, 1H, $J_{3'b,2'}$ = 4.0, H-3′b), 4.17 (d, 2H, $J_{1',2'}$ = 5.6, H-1′). ^{13}C NMR (D_2O, ppm) δ: 10.44 (CH_3), 19.02 (CH_2), 24.81 (CH_2),

30.0 (br, CH_2), 43.72 (C-1′), 57.20 (C-3′), 62.85 (d, $J_{P,C}$ = 157.9, P–C), 76.38 (d, $J_{P,C}$ = 8.9, C-2′), 146.23 (C-2), 155.11 (C-4), 173.77 (C-6). FAB-MS, *m/z*: 337 $[M+H]^+$. FAB-HRMS calcd for $C_{11}H_{22}N_4O_6P$ 337.1277, found 337.1273 $[M+H^+]$.

5.3.5. 1-(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]-6-phenyl-5-azacytosine (7e)

Yield: 153 mg, 49%, white amorphous solid. $[\alpha]_D$ −36.7 (*c* 0.185, H_2O). ^1H NMR (D_2O, ppm) δ: 3.41 (dd, 1H, $J_{P,CHa}$ = 9.2, J_{gem} = 13.5, PCH_a), 3.42 (dd, 1H, $J_{3'a,2'}$ = 3.6, J_{gem} = 12.6, H-3′a), 3.61 (dd, 1H, $J_{P,CHb}$ = 8.7, PCH_b), 3.65 (dd, 1H, $J_{3'b,2'}$ = 3.9, H-3′b), 3.74 (m, 1H, H-2′), 4.15 (m, 2H, H-1′), 7.61 (m, 2H, H-3″-Ph), 7.68 (m, 1H, H-4″-Ph), 7.23 (m, 2H, H-2″-Ph). ^{13}C NMR (D_2O, ppm) δ: 48.19 (C-1′), 60.30 (C-3′), 66.18 (d, $J_{P,C}$ = 157.8, P–C), 79.02 (d, $J_{P,C}$ = 10.3, C-2′), 128.66 (C-2″-Ph), 129.59 (C-3″-Ph), 131.85 (C-1″-Ph), 132.74 (C-4″-Ph), 151.62 (C-2), 159.99 (C-4), 171.86 (C-6). FAB-MS, *m/z*: 357 $[M+H]^+$. FAB-HRMS calcd for $C_{13}H_{18}N_4O_6P$ 357.0964, found 357.0956 $[M+H^+]$.

5.3.6. 3-(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]-6-methyl-5-azacytosine (21)

The compound was eluted from Dowex 1 with 0.3 M acetic acid (together with **22**). Both compounds were separated by reverse phase HPLC with a linear gradient of MeOH (0–50%): **21** was eluted first at 1–5% MeOH followed by **22** (eluted with 10% MeOH). Yield of **21**: 72 mg (35%) of a white amorphous solid after lyophilization from water. $[\alpha]_D$ −13.3 (*c* 0.318, H_2O). ^1H NMR (D_2O, ppm) δ: 2.43 (s, 3H, CH_3), 3.60 (dd, 1H, $J_{P,CHa}$ = 8.8, J_{gem} = 13.2, PCH_a), 3.68 (dd, 1H, $J_{3'a,2'}$ = 4.5, J_{gem} = 13.9, H-3′ a), 3.82 (dd, 1H, $J_{P,CHb}$ = 9.3, J_{gem} = 13.2, PCH_b), 3.90 (dd, 1H, $J_{3'b,2'}$ = 3.9, J_{gem} = 13.9, H-3′b), 3.91 (m, 1H, H-2′), 4.12 (dd, 1H, $J_{1'a,2'}$ = 7.8, J_{gem} = 15.4, H-1′a), 4.25 (dd, 1H, $J_{1'b,2'}$ = 3.3, J_{gem} = 15.4, H-1′b). Proton-coupled ^{13}C NMR (D_2O, ppm) δ: 21.61 (CH_3), 45.83 (C-1′), 59.70 (C-3′), 66.02 (d, $J_{P,C}$ = 156.7, P–C), 79.12 (d, $J_{P,C}$ = 10.7, C-2′), 148.75 (t, *J* = 3.9, C-2), 159.91 (t, *J* = 3.9, C-4), 170.32 (q, *J* = 5.8, C-6). FAB-MS, *m/z*: 295 $[M+H]^+$. FAB-HRMS calcd for $C_8H_{16}N_4O_6P$ 295.0807, found 295.0801 $[M+H^+]$.

5.3.7. 3-[(2*S*)-2-(Isopropoxyphosphoryl)methoxy-3-hydroxypropyl]-5-azacytosine (22)

Obtained as a by-product in preparation of **21**. Yield: 47 mg (20 as a white amorphous solid after lyophilization from water. ^1H NMR (D_2O, ppm) δ: 0.17 (s, 6H, $J_{CH3,CH}$ = 6.2, CH_3), 2.43 (s, 3H, CH_3), 3.58 (dd, 1H, $J_{P,CHa}$ = 9.0, J_{gem} = 13.2, PCH_a), 3.63 (dd, 1H, $J_{3'a,2'}$ = 3.3, J_{gem} = 11.4, H-3′a), 3.74 (pent, 1H, $J_{CH3,CH}$ = 6.2, P–OCH), 3.83 (dd, 1H, $J_{3'\ b,2'}$ = 4.5, J_{gem} = 11.4, H-3′b), 3.84 (dd, 1H, $J_{P,CHb}$ = 8.8, J_{gem} = 13.2, PCH_b), 3.98 (m, 1H, H-2′), 4.16 (dd, 1H, $J_{1'a,2'}$ = 7.7, J_{gem} = 15.4, H-1′a), 4.26 (dd, 1H, $J_{1'b,2'}$ = 3.5, J_{gem} = 15.4, H-1′b). ^{13}C NMR (D_2O, ppm) δ: 20.75 (2C, CH_3), 21.00 (CH_3), 46.20 (C-1′), 65.90 (C-3′), 65.96 (d, $J_{P,C}$ = 156.2, P–C), 77.67 (d, $J_{P,C}$ = 10.7, C-2′), 73.36 (P–O–C), 148.73 (C-2), 159.91 (C-4), 170.28 (C-6).

5.3.8. 3-(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine (23)

Yield 78 mg (40%), white foam, $[\alpha]_D$ +14.3 (*c* 0.224, H_2O). ^1H NMR (D_2O, ppm) δ: 3.60 (dd, 1H, $J_{P,CH}$ = 8.8, J_{gem} = 13.2, PCH_a), 3.69 (dd, 1H, $J_{3'a,2'}$ = 3.2, J_{gem} = 11.6, H-3′a), 3.84 (dd, 1H, $J_{P,CHb}$ = 9.2, J_{gem} = 13.2, PCH_b), 3.91 (dd, 1H, $J_{3'b,2'}$ = 3.9, J_{gem} = 11.6, H-3′b), 3.92 (m, 1H, H-2′), 4.14 (dd, 1H, $J_{1'a,2'}$ = 7.8, J_{gem} = 14.9, H-1′a), 4.27 (dd, 1H, $J_{1'b,2'}$ = 3.2, J_{gem} = 14.9, H-1′b), 8.32 (s, 1H, H-6). ^{13}C NMR (D_2O, ppm) δ: 46.05 (C-1′), 59.70 (C-3′), 65.94 d, *J*(P,C) = 156.7 (PCH_2), 79.06 d, *J*(P,C) = 10.7 (C-2′), 148.47 (C-2), 158.56 (C-6), 160.83 (C-4). FAB-MS, *m/z*: 281.1 $[M+H]^+$. FAB-HRMS calcd for $C_7H_{14}N_4O_6P$ 281.0651, found: 281.0652 $[M+H^+]$.

5.4. Diisopropyl (1*S*)-[2-benzyloxy-1-(tosyloxymethyl)ethoxy]-methylphosphonate (14)

The compound was prepared from (2*S*)-2-[(trityloxy]methyl]oxirane (**11**) according to a protocol described for the racemic form.[23] $[\alpha]_D$ +13.7 (*c* 0.246, $CHCl_3$). NMR data are in agreement with the literature.[23]

5.5. Diisopropyl (1*S*)-[2-hydroxy-1-tosyloxymethyl)ethoxy]methylphosphonate (15)

10% Palladium on carbon (1.05 g) was added to a solution of **14** (11.5 g, 22.4 mmol) in methanol (150 mL) and the mixture was hydrogenated at room temperature under atmospheric pressure for 24 h. The solid was filtered off through Celite, washed with methanol (2 × 100 mL) and combined filtrates were concentrated in vacuo. The syrupy residue was chromatographed on a column of silica gel (400 mL) in ethyl acetate. Product containing fractions were evaporated to give 7.7 g (81%) of compound **8** as a colourless syrup, $[\alpha]_D$ +21.4 (*c* 0.337, $CHCl_3$). ^1H NMR (DMSO-d_6, ppm) δ: 1.20 (d, 3H, $J_{CH3,CH}$ = 6.2, CH_3), 1.205 (d, 3H, CH_3), 1.225 (d, 6H, CH_3), 2.42 (s, 3H, CH_3), 3.37 (dt, 1H, $J_{CH2,OH}$ = $J_{CH2,CH}$ = 6.0, J_{gem} = 11.2, OCH_2), 3.45 (dt, 1H, $J_{CH2,OH}$ = $J_{CH2,CH}$ = 5.0, J_{gem} = 11.2), 3.62 (m, 1H, OCH), 3.71 (dd, 1H, $J_{P,CH}$ = 8.8, J_{gem} = 13.9, PCH_2), 3.81 (dd, 1H, PCH_2), 3.98 (dd, 1H, $J_{CH2,CH}$ = 6.0, J_{gem} = 10.6, OCH_2), 4.15 (dd, 1H, $J_{CH2,CH}$ = 3.1, J_{gem} = 10.6, OCH_2), 4.56 (m, 2H, P–OCH), 4.82 (t, 1H, $J_{OH,CH2}$ = 5.5, OH), 7.49 (d, 2H, H-arom.), 7.79 (d, 2H, H-arom.). ^{13}C NMR (DMSO-d_6, ppm) δ: 21.26 (CH_3), 23.81 (d, $J_{P,C}$ = 4.4, CH_3), 23.97 (d, $J_{P,C}$ = 4.4, CH_3), 59.13 (OCH_2), 69.73 (OCH_2), 70.39 d and 70.41 (2 × d, $J_{P,C}$ = 6.3, P–OCH), 79.41 (d, $J_{P,C}$ = 11.2, OCH); 127.80 (2C, arom.), 130.34, (2C, arom.), 132.32 and 145.12 (arom.). FAB-MS, *m/z*: 425 $[M+H]^+$. Anal. Calcd for $C_{17}H_{29}O_8SP$: C, 48.11; H, 6.89; S, 7.55; P, 7.30. Found: C, 47.71; H, 6.99; S, 7.80; P, 7.48.

5.6. Condensation reactions of 15 with 5-azacytosine and 6-methyl-5-azacytosine

1 M Sodium methoxide in methanol (10 mL) was added to a suspension of appropriate base (10 mmol) in absolute methanol (100 mL) and the mixture refluxed for 2 h. The mixture was stirred for 12 h at 25 °C and evaporated to 1/4 of an original volume. Dry ether (100 mL) was added, the precipitated sodium salt filtered by suction, washed with ether and dried in vacuo.

A mixture of thus obtained sodium salt of 5-azacytosine or 6-methyl-5-azacytosine and synthon **15** (4.2 g, 9.9 mmol) in dimethylformamide (30 mL) was heated to 90 °C with exclusion of moister for 5 h. Additional portion of **15** (1.2 g, 2.8 mmol) was added together with a catalytic amount of cesium carbonate (10 mg) and the heating was continued at 120 °C for 2 h. During that time, a complete dissolution occurred. The reaction mixture was taken down, the residue coevaporated with toluene (2 × 50 mL) and xylene (50 mL) and applied onto a column of silica gel (400 mL) in system chloroform–methanol (95:5). A column was eluted first with this system, after elution of approx. 1.5 L volume, the polarity of system was increased to the ratio chloroform–methanol (85:15). Three types of products were obtained in the following elution sequence: 2-O-isomer **18**, N^3-isomer (**17** or **19**) and finally, the N-1 isomer (**16** or **20**). The product containing fractions were taken down and coevaporated with absolute ethanol.

5.6.1. 1-[(2*S*)-2-(Diisopropoxyphosphoryl)methoxy-3-hydroxypropyl] -5-azacytosine (16)

Yield: 2.4 g (62%) as colourless oil crystallizing after several days standing, $[\alpha]_D$ −43.0 (*c* 0.49, ethanol). ^1H NMR (DMSO-d_6, ppm) δ: 1.19, 1.21, 1.22 and 1.23 (4 × d, 12H, $J_{CH3,CH}$ = 6.2, CH_3), 3.45 (ddd, 1H, $J_{3'b,2'}$ = 4.4, $J_{3'b,OH}$ = 5.8, J_{gem} = 12.0, H-3′b), 3.51 (tt, 1H,

$J_{3'a,OH}$ = $J_{3'a,2'}$ = 4.8, J_{gem} = 12.0, H-3′a), 3.59 (dd, 1H $J_{P,CHa}$ = 8.2, J_{gem} = 14.0, PCH_a), 3.63 (m, 1H, H-2′), 3.70 (dd, 1H, $J_{P,CHb}$ = 9.4, J_{gem} = 14.0, PCH_b), 3.89 (dd, 1H, $J_{1'a,2'}$ = 8.1, J_{gem} = 14.1, H-1′a), 3.96 (dd, 1H, $J_{1'b,\ 2'}$ = 2.4, J_{gem} = 14.1, H-1′b), 4.54 (m, 2H, P–OCH), 4.83 (t, 1H, $J_{OH,3'}$ = 5.5, OH), 7.38 (br s, 2H, NH_2), 8.06 (s, 1H, H-6). Proton-coupled ^{13}C NMR (DMSO-d_6, ppm) δ: 23.78 and 23.87 (2 × d, $J_{P,C}$ = 4.4, CH_3), 23.91 and 23.95 (2 × d, $J_{P,C}$ = 4.9, CH_3), 47.70 (C-1′), 60.34 (C-3′), 63.61 (d, $J_{P,C}$ = 165.0, P–C), 70.33 (d, 2C, $J_{P,C}$ = 6.4, P–O–C), 79.28 (d, $J_{P,C}$ = 10.7, C-2′), 154.29 (dt, $J_{C-2,H-6}$ = 4.9, $J_{C-2,H-1'a}$ = $J_{C-2,H-1'b}$ = 2.9, C-2), 166.63 d, $J_{C-4,H-6}$ = 12.7, C-4), 159.785 (dt, ^1J = 204.1, $J_{C-6,H-1'a}$ = $J_{C-6,H-1'b}$ =3.9, C-6). FAB-MS, *m/z*: 365 $[M+H]^+$, 281 [free phosphonic acid + H]$^+$, 113 [5-azacytosine + H]$^+$. Anal. Calcd for $C_{13}H_{25}N_4O_6P{\cdot}1/2C_2H_5OH$: C, 43.40; H, 7.29; N, 14.46; P, 8.00. Found: C, 43.58; H, 6.99; N, 14.45; P, 8.22.

5.6.2. 3-[(2*S*)-2-(Diisopropoxyphosphoryl)methoxy-3-hydroxypropyl] -5-azacytosine (17)

Yield 0.5 g (14%), amorphous solid, $[\alpha]_D$ −13.7 (*c* 0.352, $CHCl_3$). ^{1}H NMR (DMSO-d_6, ppm) δ: 1.20 (d, 3H, $J_{CH3,CH}$ = 6.1, CH_3), 1.22 (d, 6H, CH_3), 1.23 (d, 3H, CH_3), 3.50 (dt, 1H, $J_{3'a,2'} \approx J_{3'a,OH}$ = 5.2, J_{gem} = 11.8, H-3′a), 3.55 (dt, 1H, $J_{3'b,2'} \approx J_{3'b,OH}$ = 4.6, J_{gem} = 11.8, H-3′b), 3.75 (dd, 1H, $J_{P,CHa}$ = 8.5, J_{gem} = 14.2, PCH_a), 3.78 (m, 1H, H-2′), 3.90 (dd, 1H, $J_{1'a,2'}$ = 4.7, J_{gem} = 14.5, H-1′a), 3.91 (dd, 1H, $J_{P,CHb}$ = 8.0, J_{gem} = 14.2, PCH_b), 3.95 (dd, 1H, $J_{1'b,2'}$ = 7.6, J_{gem} = 14.5, H-1′b), 4.55 (m, 2H, P–OCH), 4.94 (t, 1H, $J_{OH,3'}$ = 5.2, OH), 7.50 (br s, 1H, NH_2), 8.30 (br s, 1H, NH_2), 7.88 (s, 1H, H-6). Proton-coupled ^{13}C NMR (DMSO-d_6, ppm) δ: 23.81 (d, $J_{P,C}$ = 4.4, CH_3), 23.89 (d, $J_{P,C}$ = 4.9, CH_3), 23.94 and 23.97 (2 × d, $J_{P,C}$ = 3.9, CH_3), 43.76 (C-1′), 60.95 (C-3′), 64.20 (d, $J_{P,C}$ = 164.6, P–C), 70.42 and 70.47 (2 × d, $J_{P,C}$ = 6.3, P–OCH), 79.32 (d, $J_{P,C}$ = 9.8, C-2′), 155.59 (dt, $J_{C-2,H-6}$ = 12.7, $J_{C-2,H-1'a}$ = $J_{C-2,H-1'b}$ = 3.9, C-2), 160.96 (dt, $J_{C-4,H-6}$ = 12.7, $J_{C-4,H-1'a}$ = $J_{C-4,H-1'b}$ = 3.9, C-4), 166.46 (d, ^1J = 195.3, C-6). FAB-MS, *m/z*: 365 $[M+H]^+$. Anal. Calcd for $C_{13}H_{25}N_4O_6P$: C, 42.86; H, 6.92; N, 15.38; P, 8.50. Found: C, 42.42; H, 7.05; N, 15.54; P, 8.68.

5.6.3. Diisopropyl {[(1*R*)-2-[(4-amino-1,3,5-triazin-2-yl)oxy]-1-(hydroxymethyl)ethoxy]methyl}-phosphonate) (18)

Yield 0.3 g (8%), colourless oil, $[\alpha]_D$ + 2.3 (*c* 0.502, ethanol). ^{1}H NMR (DMSO-d_6, ppm) δ: 1.20, 1.21, 1.22 and 1.23 (4 × d, 12H, $J_{CH3,CH}$ = 6.1, CH_3), 3.53 (t, 2H, $J_{3',2'}$ = $J_{3',OH}$ = 5.5, H-3′), 3.72 (m, 1H, H-2′), 3.87 and 3.91 (2 × dd, 2H $J_{P,CH}$ = 8.7, J_{gem} = 13.8, PCH_2), 4.25 (dd, 1H, $J_{1'a,2'}$ = 5.9, J_{gem} = 11.6, H-1′a), 4.40 (dd, 1H, $J_{1'b,2'}$ = 3.7, J_{gem} = 11.6, H-1′b), 4.59 (m, 2H, P–OCH), 4.82 (t, 1H, $J_{OH,3'}$ = 5.5, OH), 7.45 and 7.51 (2 × br s, 2H, NH_2), 8.25 (s, 1H, H-6). Proton-coupled ^{13}C NMR (DMSO-d_6, ppm) δ: 23.85 (d, 2C, $J_{P,C}$ = 4.4, CH_3), 24.02 (d, 2C, $J_{P,C}$ = 3.9, CH_3), 59.98 (C-3′), 64.05 (d, $J_{P,C}$ = 165.0, P–C), 65.94 (C-1′), 70.36 (d, $J_{P,C}$ = 6.4, P–OCH), 70.43 (d, $J_{P,C}$ = 6.4, P–OCH), 80.09 (d, $J_{C,P}$ = 11.7, C-2′), 167.815 (d, ^1J = 200.2, C-6), 168.05 (d, $J_{C-4,H-6}$ = 10.7, C-4), 170.065 (dt, $J_{C-2,H-6}$ = 10.7, $J_{C-2,H-1'}$ = 2.0, C-2). FAB-MS, *m/z*: 365 $[M+H]^+$.

5.6.4. 3-[(2*S*)-2-(Diisopropoxyphosphoryl)methoxy-3-hydroxypropyl]-6-methyl-5-azacytosine (19)

Yield: 1.6 g (42%) as white crystals, Mp: 124 °C (EtOAc), $[\alpha]_D$ −24.9 (*c* 0.700, $CHCl_3$). ^{1}H NMR (DMSO-d_6, ppm) δ: 1.19, 1.21, 1.215 and 1.225 (4 × d, 12H, $J_{CH3,CH}$ = 6.1, CH_3), 2.06 (s, 3H, CH_3), 3.48 (dt, 1H, $J_{3'a,2'}$ = $J_{3'b,OH}$ = 5.0, J_{gem} = 12.0, H-3′a), 3.53 (dt, 1H, $J_{3'b,2'}$ = $J_{3'a,OH}$ = 4.2, J_{gem} = 12.0, H-3′b), 3.75 (m, 1H, H-2′), 3.75 (dd, 1H, $J_{P,CHa}$ = 8.6, J_{gem} = 14.2, PCH_a), 3.81 (dd, 1H, $J_{1'a,2'}$ = 5.1, J_{gem} = 14.6, H-1′a), 3.90 (dd, $J_{P,CHb}$ = 8.1, J_{gem} = 14.2, PCH_b), 3.93 (dd, 1H, $J_{1'b,2'}$ = 7.6, J_{gem} = 14.6, H-1′b), 4.55 (m, 2H, P–OCH), 4.93 (t, 1H, $J_{OH,3'}$ = 5.0, OH), 7.35 and 8.10 (2 × br s, 2H, NH_2). Proton-coupled ^{13}C NMR (DMSO-d_6, ppm) δ: 23.78 and 23.80 (2 × d, $J_{P,C}$ = 4.4, CH_3), 23.91 and 23.23.96 (2 × d, $J_{P,C}$) = 3.9, CH_3), 25.575 (CH_3), 43.36 (C-1′), 60.94 (C-3′), 64.195 (d, $J_{P,C}$ = 163.6, P–C), 70.42 and 70.47 (d, $J_{P,C}$ = 6.3, P–OCH), 79.44 (d, $J_{P,C}$ = 10.3, C-2′), 155.70 (t, *J* = 2.9, C-2), 160.16 (t, *J* = 2.9, C-4), 175.775 (q, *J* = 5.8, C-6). FAB-MS, *m/z*: 379.1 $[M+H]^+$. Anal. Calcd for $C_{14}H_{27}N_4O_6P{\cdot}1/2H_2O$: C, 43.41; H, 7.29; N, 14.46; P, 8.00. Found: C, 43.37; H, 7.36; N, 14.55; P, 7.72.

5.6.5. 1-[(2*S*)-2-(Diisopropoxyphosphoryl)methoxy-3-hydroxypropyl]-6-methyl-5-azacytosine (20)

Yield: 240 mg (7%) of a white foam, $[\alpha]_D$ −68.5 (*c* 0.263, $CHCl_3$). ^{1}H NMR (DMSO-d_6, ppm) δ: 1.15, 1.19, 1.20 and 1.215 (4 × d, 12H, $J_{CH3,CH}$ = 6.2, CH_3), 2.40 (s, 3H, CH_3), 3.46 (ddd, 1H, $J_{3'a,2'}$ = 3.9, $J_{3'a,OH}$ = 5.9, J_{gem} = 12.0, H-3′a), 3.59 (dd, 1H, $J_{P,CHa}$ = 10.3, J_{gem} = 13.9, PCH_a), 3.61 (ddd, 1H, $J_{3''b,2'}$ = 3.8, $J_{3'b,OH}$ = 5.0, J_{gem} = 12.0, H-3′b), 3.66 (m, 1H, H-2′), 3.76 (dd, 1H, $J_{1'a,2'}$ = 9.2, J_{gem} = 14.2, H-1′a), 3.92 (dd, 1H, $J_{P,CHb}$ = 7.7, J_{gem} = 13.9, PCH_b), 3.98 (dd, 1H, $J_{1'b,2'}$ = 2.8, J_{gem} 14.2, H-1′b), 4.51 (m, 2H, P–OCH), 4.84 (t, 1H, $J_{OH,3'}$ = 5.5, OH), 7.18 and 7.21 (2 × br s, 2H, NH_2). Proton-coupled ^{13}C NMR (DMSO-d_6, ppm) δ: 22.47 (CH_3), 23.67 (d, $J_{P,C}$ = 3.9, CH_3), 23.83 and 23.85 (2 × d, $J_{P,C}$ = 4.4, CH_3), 23.92 (d, $J_{P,C}$ = 3.9, CH_3), 46.265 (C-1′), 60.31 (C-3′), 63.71 (d, $J_{P,C}$ = 164.5, P–C), 70.12 and 70.23 (2 × d, $J_{P,C}$ = 6.3, P–OCH), 80.23 (d, $J_{P,C}$ = 11.7, C-2′), 155.32 (t, *J* = 2.9, C-2), 165.36 (C-4), 168.35 (qt, *J* = 2,9 and 5.9, C-6). FAB-MS, *m/z*: 379.1 $[M+H]^+$. Anal. Calcd for $C_{14}H_{27}N_4O_6P$: C, 44.44; H, 7.19; N, 14.81; P, 8.19. Found: C, 44.37; H, 7.26; N, 14.55; P, 7.85.

Acknowledgements

This work is a part of the research project of the Institute Z40550506. It was supported by the Centre of New Antivirals and Antineoplastics 1M0508 by the Ministry of Education, Youth and Sports of the Czech Republic, the Program of targeted projects of Academy of Sciences of the Czech Republic 1QS400550501 and by Gilead Sciences, Inc. (Foster City, CA, USA). The authors′ thanks are due to Professor Jan Balzarini and his group in the Rega Institute for Medical Research, Catholic University Leuven (Belgium) for antiviral and cytotoxicity assays of described compounds, to Dr. P. Fiedler from this Institute for a measurement of IR spectra, the staff of the mass spectrometry (Dr. J. Cvačka, Head) and analytical departments of the Institute (Dr. S. Matějková, Head). An excellent technical assistance of Mrs. Julie Křelinová is also acknowledged.

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