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A new class of phosphanucleosides containing a 3-hydroxy-1-hydroxymethylphospholane 1-oxide ring

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ABSTRACT

Novel phosphanucleosides containing a 3-hydroxy-1-hydroxymethylphospholane 1-oxide ring were synthesized as compounds with potential biological activity. A double Arbuzov reaction was employed to prepare a phospholene precursor, which was then converted into an epoxide and subsequently into racemic phosphanucleoside via nucleobase construction.

1. Introduction

Sugar-modified nucleosides and nucleotides represent an important group of antimetabolites, which exhibit a variety of biological properties. Carba-,[1,2] aza-,[3,4] and thianucleosides,[5] in which the furanose oxygen is replaced by a carbon, nitrogen, or sulfur atom, respectively, were found to exhibit very interesting antibacterial, antiviral, and antitumor properties.[6,7] However, the rapid evolution of drug resistance has recently become a serious issue; therefore, the search for new compounds with various chemical, biological, and pharmacological properties is becoming increasingly required.

In the field of phosphasugars, several classes of furanose and pyranose analogues whose hemiacetal ring oxygen was replaced by phosphorus atom have been prepared.[8–10] Among these analogues, several compounds with anticancer activity have recently been discovered.[11–13] Surprisingly, phosphanucleosides have not received significant attention, and only a few types of these compounds have been reported in the literature[8,14–16] (Fig. 1). Compounds **1**–**3** were prepared from the appropriate 2-aminophospholane or 2-azidophospholane 1-oxide derivatives using nucleobase construction or 1,3-dipolar cycloaddition.[14–16]

Given the potential of phosphanucleosides and phosphanucleotides as novel therapeutic agents, the preparation of these compounds is an attractive area of medicinal research. Moreover, due to their novelty, the synthesis of phosphanucleosides is of interest to the field of organic synthesis. Therefore, we report herein the synthesis of a novel set of phosphanucleoside analogues **4** containing a 3-hydroxy-1-hydroxymethylphospholane 1-oxide ring (Fig. 1). The aimed compounds **4** exhibit the structural similarity with 3′-deoxynucleosides.

2. Results and discussion

Our strategy for the synthesis of phosphanucleosides **4** consisted of a multistep synthesis of a suitable phospholane precursor and subsequent introduction of a nucleobase. There are three general routes to introduce a nucleobase: (i) alkylation of a nucleobase with a protected reactive compound, such as a mesylate,[17–19] (ii) nucleobase construction using an amino compound[20–24] and (iii) Mitsunobu reaction of a hydroxy compound with a purine nucleobase.[17–19]

Considering our previous syntheses of pyrrolidine and piperidine derivatives of nucleobases[17–19,21,23] and the known tendency of 2-*O*-alkyl/arylsulfonyl- and 2-haloethylphosphonates to undergo elimination reactions, we decided to employ a phospholane 3,4-epoxide as a starting synthon for the synthesis of phosphanucleosides. We expected this compound to be more stable towards elimination than the corresponding 3-*O*-mesyl- or

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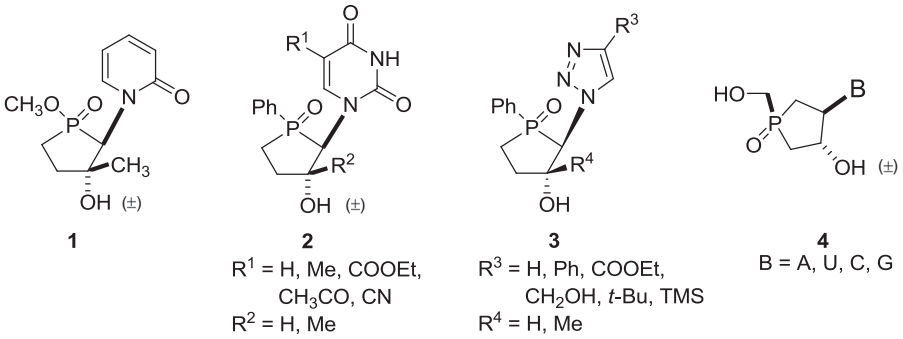


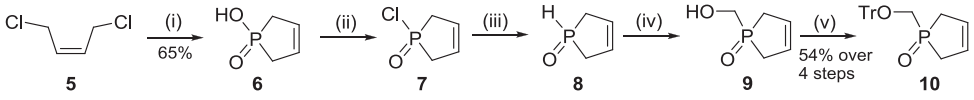
Fig. 1. Known (**1**–**3**) and novel (**4**) phosphanucleosides.

3-halophospholane derivatives. This synthetic protocol afforded the target compounds as racemic mixtures.

It is well established that phosphines and phosphites react with alkyl halides or epoxides to form P–C bonds. Therefore, we employed a double Arbuzov reaction to prepare the phosphol-3-ene 1-oxide ring.[25] Reaction of *cis*-1,4-dichlorobut-2-ene **5** with bis(trimethylsilyl) hypophosphite afforded phosphinic acid **6**. Our improved protocol, which uses *N*,*O*-bis(trimethylsilyl)acetamide (BSA) instead of hexamethyldisilazane, provided the desired phosphinic acid **6** in 65% yield (Scheme 1). We subsequently followed a previously established protocol for the preparation of 2,5-diphenyl-1-hydroxyphospholane 1-oxides,[26] which was based on the transformation of phosphinic acids to H-phosphinates. Finally, the reaction of H-phosphinate **8** with formaldehyde followed by protection of the primary hydroxyl provided the desired starting synthon **10** in 35% overall yield (Scheme 1).

and in 79% yield (Scheme 2). This mixture of diastereomers was easily separated by chromatography on silica gel. The faster eluting isomer was identified as racemate **12**, wherein the hydroxymethyl group and bromine atom are in a trans configuration. The slower eluting isomer was identified as racemate **11**, wherein the hydroxymethyl group and bromine atom are in a *cis* configuration. The structures of diastereomers **11** and **12** were assigned using 2D-H and H-ROESY spectra. Phosphanucleosides derived from the *cis* series exhibit structural similarity to naturally occurring β-nucleosides, while phosphanucleosides derived from the trans series resemble α-nucleosides.

Epoxides **13** and **16** were prepared by the reactions of bromohydrins **11** and **12** with potassium carbonate. Next, we attempted to prepare the desired phosphanucleosides by the reactions of epoxides **13** and **16** with a nucleobase in the presence of sodium hydride or cesium carbonate, or with a persilylated nucleobase in the pre-

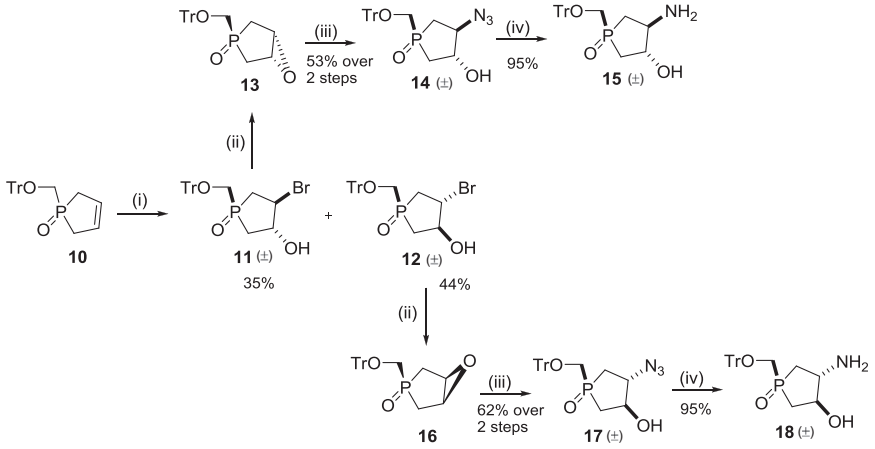


Scheme 1. (i) $NH_4H_2PO_2$, BSA, 110 °C 72 h (ii) $(COCl)_2$, DCM, rt 16 h (iii) DIBAL, DCM, −78 °C 2 h (iv) CH_2O, TEA, MeOH, rt 16 h (v) Tr–Cl, pyridine, 70 °C 36 h.

The epoxidation of phosphol-3-ene derivative **10** using MCPBA afforded in agreement with the literature data[27] diastereomer **16** as a major product in 90% yield. Because we were interested in the synthesis of both diastereomers **13** and **16**, we decided to prepare the desired epoxide precursors using a known two-step protocol. The reaction of **10** with NBS in acetone/water afforded a mixture of two racemic bromohydrins, **11** and **12**, in an approximately 1:1 ratio

sence of a Lewis acid ($SnCl_4$, $BF_3 \cdot Et_2O$).[17–19,28] Unfortunately, these alkylations all afforded the corresponding 3-hydroxyphosphol-2-ene derivatives, i.e., the products of elimination. Meanwhile, the reactions with a Lewis acid led to the decomposition of the starting compounds.

Instead, we converted the epoxides **13** and **16** to phospholane azides **14** and **17**, respectively, via treatment with sodium azide in



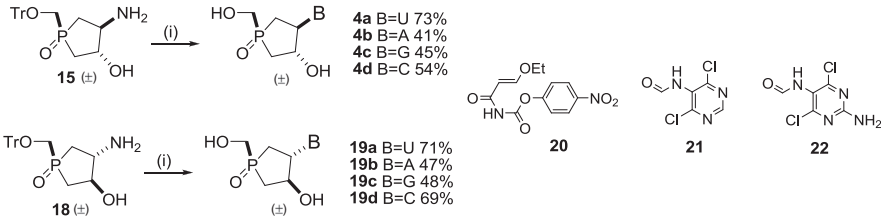
Scheme 2. (i) NBS, H_2O, acetone, rt 16 h (ii) K_2CO_3, acetone, rt 16 h (iii) NaN_3, NH_4Cl, $EtOH/H_2O$, 80 °C 16 h (iv) H_2, Pd/C, EtOH, rt 16 h.

the presence of ammonium chloride. These reactions proceeded in good yield (**11** to **14**: 53% overall yield, **12** to **17**: 62% overall yield) (Scheme 2). Azides **14** and **17** were hydrogenated at atmospheric pressure in the presence of catalytic Pd/C to provide the corresponding amines as starting compounds for nucleobase construction. Under these reaction conditions, no cleavage of the trityl group was observed.

To build the uracil nucleobase, we utilized 4-nitrophenyl-3-ethoxyacryloylcarbamate **20**, a reagent previously invented in our laboratory (Scheme 3).[21] The reaction of phospholane amines **15** and **18** with **20** provided acyclic products, which were immediately cyclized by heating with Dowex H^+ to form uracil derivatives **4a** and **19a** in a one-pot reaction sequence.

The prepared compounds were tested for their cytostatic (HepG2, HL60, HeLa S3, CCRF-CEM) and antimicrobial (*Escherichia coli* CCM 3954, *Enterococcus faecalis* CCM 4224, *Pseudomonas aeruginosa* CCM 3955, *Staphylococcus aureus* CCM 4223, *Bacillus subtilis*, *Streptococcus agalactiae*, *Candida albicans*, and *Candida krusei*) activities, but no significant effects were observed.

The structures of phosphanucleosides **4a**–**d** and **19a**–**d** were confirmed by ^1H, ^{13}C, and ^{31}P NMR spectra in DMSO and D_2O. These data are summarized in Tables 2–4 in the Experimental section. The numbering scheme for the atoms in these five-membered rings is shown in Fig. 2. The standard atom numbering of nucleobases is used.



Scheme 3. (i) B=U (1) **20**, dioxane, rt 2 h (2) DOWEX H^+, 90 °C 4 h; B=A (1) **21**, DIPEA, *n*-BuOH, 130 °C 16 h (2) aq NH_3, dioxane, rt 48 h (3) 80% AcOH, 60 °C 16 h; B=G (1) **22**, DIPEA, *n*-BuOH, 130 °C 16 h (2) 1 M HCl, MeOH 60 °C 16 h; B=C (1) **4a**, **19a**, BzCN, TEA, acetonitrile, rt 16 h (2) TIPS-Cl, TEA, DMAP, acetonitrile, rt 16 h (3) aq NH_3, acetonitrile, rt 16 h.

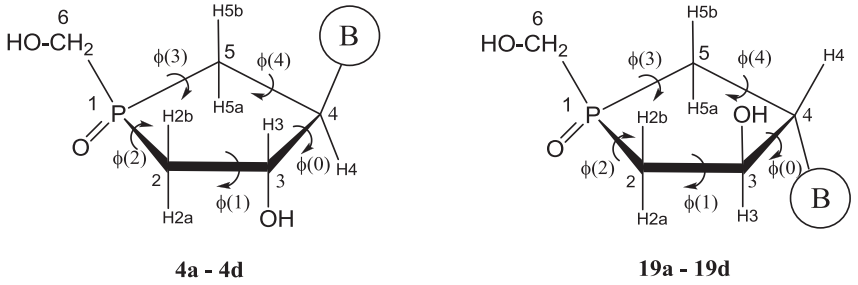


Fig. 2. Numbering of atoms and torsion angles in the five-membered rings of phosphanucleosides **4a**–**d**, **19a**–**d**.

Cytosine derivatives **4d** and **19d** were prepared from the appropriate uracil derivatives. Compounds **4a** and **19a** were protected with benzoyl groups, and they were then converted to 4-*O*-TIPS derivatives. These compounds were then treated with aqueous ammonia to afford cytosine derivatives **4d** and **19d**.

For the syntheses of the adenine (**4b**, **19b**) and guanine (**4c**, **19c**) phosphanucleosides we employed 4,6-dichloro-5-formamidopyrimidine **19** and 2-amino-4,6-dichloro-5-formamidopyrimidine **20**, respectively (Scheme 3).[22,24] These reagents showed several advantages over the commonly used 5-amino-4,6-dichloro- and 2,5-amino-4,6-dichloropyrimidine.[23] First, the purine rings were formed in one-pot reactions under conditions of heating at 130 °C. The addition of a ring-closing reagent, such as diethoxymethyl acetate or triethyl orthoformate, was not required.[22–24] Second, the reactions were complete in less time (16 h compared to 40 h using 5-amino reagents). Third, the reactions using these reagents provided the desired products in approximately 45% yield compared to approximately 20% yield using the corresponding 5-amino reagents.

The 6-chloropurine derivatives were converted into adenine phosphanucleotides **4b** and **19b** by treatment with aqueous ammonia followed by treatment with acetic acid to remove the trityl group. The 2-amino-6-chloropurine derivatives were treated with hydrochloric acid in methanol to afford guanine phosphanucleotides **4c** and **19c**.

Table 3 in Experimental part highlights the very similar values of the vicinal coupling constants and, therefore, indicates that these compounds have very similar conformations in both DMSO and D_2O. Additionally, the effect of the type of nucleobase in both series **4a**–**d** and **19a**–**d** seems to be negligible. Large values (~10 Hz) of $^3J(3,4)$ indicate the pseudoaxial positions of H-3 and H-4 and therefore the pseudoequatorial positions of the 3-OH and nucleobase. Quantum chemical calculations[29] (Gaussian 09, DFT B3LYP 6-31Gd in vacuo) were used to scan the energy profile of phosphanucleoside **19a** during pseudorotation of its five-membered ring. For the two maximum puckers (ϕ_{max}=40° and 50°) the phase angles (*P*) were changed in 18° steps with fixed torsion angles $\phi(0)$ and $\phi(3)$ (defined by *P* and ϕ_{max}) and the others allowed to optimize. This calculation showed two energy minima around 0° and 180° with a slightly lower energy for ϕ_{max}=50°. Geometry optimizations for *P*=0° and 180° and ϕ_{max}=50° were then performed for all compounds **4a**–**d** and **19a**–**d**. Surprisingly, the conformers with their 3-OH group and nucleobase in pseudoaxial positions provided somewhat lower energy than those with these groups in pseuoequatorial positions, in contrast with the observed vicinal *J*(H,H). The inspection of models suggested that H-bonding between the 3-OH and the nucleobase, possible only in their pseudoequatorial position, could decrease the energy and stabilize such a conformation. This hypothesis was confirmed by a new set of calculations where the starting orientations of the OH and

nucleobase were turned to allow an H-bond between the 3-OH and the C(2)=O in the pyrimidine phosphanucleosides (**4a**, **4d**, **19a**, **19d**) or N(3) in the purine phosphanucleosides (**4b**, **4c**, **19b**, **19c**). Calculations of geometry-optimized conformers with H-bonds provided markedly lower energy (2–6 kcal/mol) and confirmed the very high preference (>95%) of the forms with pseudoequatorial substituents, which is in agreement with preliminary estimations from the vicinal *J*(H,H) values.

The quantum chemical calculations of *J*(H,H) (again with DFT B3LYP 6-31Gd) for these H-bonded conformers showed satisfactory agreement with the observed vicinal *J*(H,H)s (average difference 1.0 Hz). Similar levels of agreement (average difference 0.9 Hz) were obtained when ^{3}J(H,H) were estimated using calculated proton torsion angles and a generalized Karplus-type relation.[30] A comparison of the calculated and observed ^{3}J(H,H) values is shown in Table 1. The preferred conformation of phosphanucleosides **4a**–**d** and **19a**–**d** can be described as $^{3}T_4$ and $_3T^4$ (Fig. 3) containing the nucleobase and hydroxyl groups in pseudoequatorial positions. The values of the endocyclic torsion angles have nearly the same absolute values, but they differ in sign (Table 1). Maximum pucker in both conformations is close to 50°. The calculated lowest energy conformations with the H-bond of phosphanucleosides **4a**, **4b**, **19a**, and **19b** are shown in Fig. 4.

Table 1
Observed values of vicinal *J*(H,H), theoretically calculated (DFT B3LYP 6-31Gd) torsion angles and *J*(H,H)

Coupled nuclei	4a	4b	4c	4d	19a	19b	19c	19d
Observed vicinal J(H,H) in D_2O								
2a, **3**	10.6	10.4	10.6	10.5	7.6	7.5	7.5	7.6
2b, **3**	7.3	6.7	7.2	7.2	10.6	10.3	10.6	10.4
3, **4**	10.3	10.0	10.2	10.3	10.2	10.4	9.9	10.3
4, **5a**	8.5	8.3	8.1	8.5	12.0	11.7	11.5	11.9
4, **5b**	12.2	12.0	12.1	11.9	7.9	7.4	7.9	7.6
DFT calculated endocyclic torsion angles in H-bonded structures								
$\varphi(0)$	52	46	48	52	−51	−44	−47	−51
$\varphi(1)$	−38	−37	−37	−38	36	32	36	35
$\varphi(2)$	13	16	14	13	−12	−11	−13	−11
$\varphi(3)$	15	9	12	15	−16	−14	−14	−17
$\varphi(4)$	−40	−33	−36	−40	41	35	37	41
P	−1.4	4.4	1.2	−1.4	176.7	177.5	179.2	175.6
φ(max)	52	46	48	52	−51	−44	−47	−51
Conf. type	$^{3}T_4$				$_3T^4$			
DFT calculated torsion angles of hydrogen atoms in H-bonded structures								
2a, **3**	−166	−165	−165	−166	42	39	42	42
2b, **3**	−46	−46	−46	−47	162	157	161	161
3, **4**	179	171	173	179	−179	−169	−173	−178
4, **5a**	−44	−37	−40	−44	171	165	166	172
4, **5b**	−167	−158	−162	−168	47	42	44	48
DFT calculated vicinal J(H,H) in H-bonded structures								
2a, **3**	9.3	10.1	10.2	9.2	7.5	8.2	7.8	7.6
2b, **3**	6.7	6.9	7.0	6.7	8.8	9.0	9.6	8.6
3, **4**	9.5	7.4	7.9	9.3	9.4	7.0	7.7	9.2
4, **5a**	7.3	8.8	8.3	7.0	12.8	10.5	10.6	13.0
4, **5b**	12.7	9.8	10.3	12.8	6.3	7.3	7.2	6.1
Calculated vicinal J(H,H) using torsion angles and generalized Karplus relation								
2a, **3**	11.0	10.9	10.9	11.1	7.0	7.5	7.0	7.0
2b, **3**	6.3	6.2	6.3	6.1	10.5	9.8	10.3	10.4
3, **4**	9.3	8.8	8.9	9.3	9.3	8.6	8.9	9.2
4, **5a**	6.1	7.3	6.8	6.1	12.1	11.5	11.6	12.2
4, **5b**	11.8	10.7	11.1	11.8	5.5	6.4	6.1	5.4

The comparison of preferred conformations of five-membered rings of compounds **4a**–**d** and **19a**–**d** with standard nucleosides required renumbering of atoms and torsion angles as shown in Fig. 5.

Schematic graphical representation of the conformation ranges of standard nucleosides is shown in Fig. 6. The X-ray data on β-D-

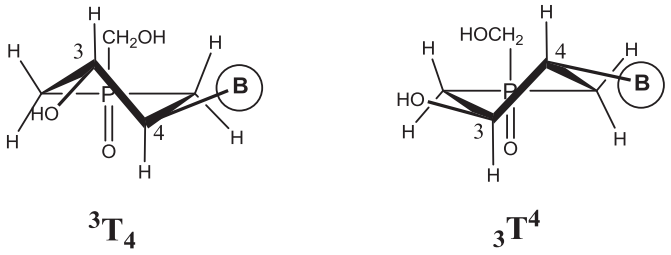


Fig. 3. Schematic representation of the highly preferred conformations $^{3}T_4$ (compounds **4a**–**d**) and $_3T^4$ (compounds **19a**–**d**).

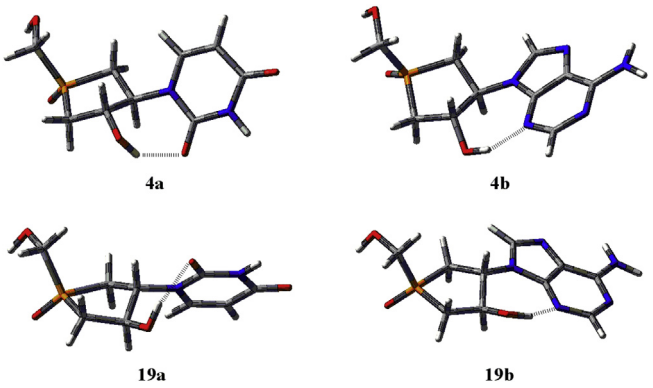


Fig. 4. Calculated conformations of phosphanucleosides **4a**, **19a**, **4b**, and **19b** (hydrogen bonds are indicated with dotted lines).

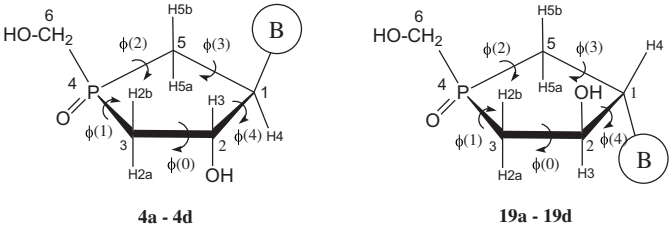


Fig. 5. Numbering of atoms and torsion angles in compounds **4a**–**d** and **19a**–**d** used for the comparison with standard nucleosides.

ribonucleosides and 2′-deoxyribo-β-D-nucleosides provided cluster of 'north' conformers ($-1°<P_N<34°$) and 'south' conformers ($137°<P_S<194°$) with $30°<\phi_{max}<46°$.[31] The limited amount of the X-ray structures of α-nucleosides showed somewhat shifted

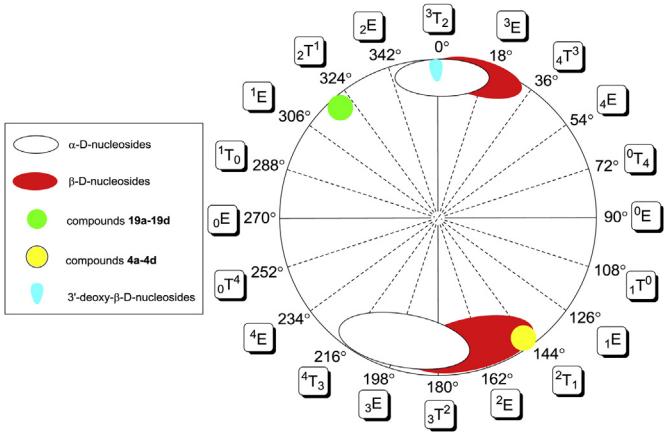


Fig. 6. Schematic representation of the conformation ranges of different types of nucleosides.

clusters of 'north' conformers ($-18°<P_N<19°$) and 'south' conformers ($168°<P_S<224°$) with $\phi_{max}<49°$.[31] The vicinal J(H, H) values of 3′-deoxy-β-D-adenosine indicated preferred 'north' form ($P_N\sim-4°$),[32] whereas applicable data on 3′-deoxy-α-D-adenosine were not found in the literature. For more details on the conformation of five-membered rings of various nucleosides see Supplementary data.

The projection of phosphanucleosides into the pseudorotation wheel (Fig. 6) showed that the compounds **4a**–**d** preferred 'south' $^2T_1$ conformation ($141°<P_S<148°$ and $44°<\phi_{max}<49°$), whereas the compounds **19a**–**d** 'north' $_2T^1$ conformation ($-42°<P_N<-37°$ and $42°<\phi_{max}<48°$). The counterclockwise shift of the positions of the compounds **4a**–**d** and **19a**–**d** was probably due to presence of >P(=O)CH_2OH group and H-bond interactions between the hydroxyl and a nucleobase (shown in Fig. 4).

3. Conclusions

In summary, we have developed a synthetic route leading to a new class of nucleoside analogues, the phosphanucleosides containing 3-hydroxy-1-hydroxymethylphospholane 1-oxide rings. We employed nucleobase construction to prepare phosphanucleosides bearing all four nucleobases in good yields. Up to now, we did not find any significant biological activity of these compounds. Nevertheless, thorough studies on the antiviral, antibacterial, and antitumor activities of these phosphanucleosides are in progress. Moreover, phosphanucleosides provide possibilities for the transformation into the nucleotide analogues via phosphorylation or phosphonylation of the primary hydroxyl of 1-hydroxymethylphospholane ring. The preparation of series of phosphanucleosides related to 2′-deoxynucleosides and ribonucleosides is also of our interest.

4. Experimental

4.1. General

Unless stated otherwise, all used solvents were anhydrous. Reagent **18** was prepared according to Ref. 21. Reagent **19** was prepared according to Ref. 24. Reagent **20** was purchased from TCI Co., Ltd., Japan. Mass spectra were recorded on a ZAB-EQ (VG Analytical) instrument using FAB (ionization with Xe, accelerating voltage 8 kV; glycerol and thioglycerol as matrices) and on LTQ Orbitrap XL (Thermo Fisher Scientific) using ESI ionization. NMR spectra were measured on Bruker AVANCE 400 (^{1}H at 400 MHz, ^{13}C at 100.6 MHz), Bruker AVANCE 500 (^{1}H at 500 MHz, ^{13}C at 125.7 MHz, ^{31}P at 202.3 MHz) and Bruker AVANCE 600 (^{1}H at 600.1 MHz, ^{13}C at 150.9 MHz) spectrometers. The complete assignment of protons and carbons was performed by the analysis of correlated homonuclear 2D-COSY and heteronuclear ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC spectra. Relative configuration was checked using the DPFGSE-NOE and 2D-ROESY techniques. IR spectra were recorded using KBr pellets on Nicolet 6700 (Thermo Electron Corp.).

4.2. 1-Hydroxyphosphol-3-ene 1-oxide 6

N,*O*-Bis(trimethylsilyl)acetamide (146.7 mL; 0.6 mol) was added under nitrogen to ammonium hypophosphite (16.6 g; 0.2 mol) and the mixture was left to dissolve under 25 °C (30 min with occasional cooling in an ice bath to avoid overheating). After that, *cis*-1,4-dichlorobut-2-ene (23.1 mL; 0.22 mol) was added and the reaction mixture was heated for 72 h at 110 °C under nitrogen. The reaction mixture was quenched by the addition of 1% HCl in water (500 mL) and extracted with ethyl acetate (3×300 mL). The water layer was treated with Dowex H^+ (0.5 kg), filtered, and evaporated. The crude product was purified on Dowex OH^- (1 kg). After being washed with water (3×500 mL) and methanol (3×500 mL), the product was eluted with 3% HCl in water. In the end, the phosphinic acid **6** was treated with charcoal in refluxed methanol. Charcoal was filtered off and solvent evaporated to afford phosphinic acid **6** as yellow oil. Yield 15.3 g (65%). HRMS (FAB) calcd for $C_4H_6O_2P$ $(M-H)^-$ 117.0111, found 117.0108. ^{1}H NMR (600.1 MHz, $CDCl_3$): δ=10.99 (b, 1H, P–OH); 5.92 (dt, J(P,H)=33.4 Hz, J(H,H)=1.0 Hz, 2H, H-3 and H-4); 2.50 (dd, J(P,H)=12.9 Hz, J(H,H)=1.0 Hz, 4H, H-2 and H-5). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=126.84 (d, J(P,C)=15.9 Hz, C-3 and C-4); 30.16 (d, J(P,C)=93.2 Hz, C-2 and C-5). ^{31}P NMR (202.3 MHz, $CDCl_3$): δ=76.78.

Caution: Bis(trimethylsilyl)hypophosphite is potentially pyrophoric compound.

4.3. 1-Trityloxymethylphosphol-3-ene 1-oxide (10)

Oxalyl chloride (22.0 mL; 0.26 mol) was added at 0 °C to a solution of 1-hydroxyphosphol-3-ene 1-oxide **6** (15.3 g; 0.13 mol) in DCM (1 L). The reaction mixture was left to warm up gradually to rt, and then stirred for 16 h at rt. After that, the mixture was evaporated and coevaporated with toluene (3×300 mL). The crude 1-chlorophosphol-3-ene 1-oxide **7** was used without further purification. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ=83.5.

DIBAL (130 mL of 1 M solution in hexane; 0.13 mol) was added under nitrogen at −78 °C to a solution of 1-chlorophosphol-3-ene 1-oxide **7** in DCM (1 L). The reaction mixture was stirred for 2 h under nitrogen at −78 °C. The reaction mixture was quenched at −78 °C by the addition of methanol (15 mL) and extracted with 10% acetic acid in water (500 mL). Water layer was extracted with chloroform (6×200 mL). Organic layers were collected, dried over anhydrous sodium sulfate and evaporated. The crude phosphol-3-ene 1-oxide **8** was used without further purification. ^{1}H NMR (600.1 MHz, $CDCl_3$): δ=7.47 (dttt, J(P,H)=467.3 Hz, J(H,H)=4.9, 2.8, 1.2 Hz, 1H, P–H); 5.92 (dm, J(P,H)=28.1 Hz, ΣJ(H,H)=6.5 Hz, 2H, H-3 and H-4); 2.68 (m, 2H, H-2a and H-5a); 2.60 (m, 2H, H-2b and H-5b). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=126.27 (d, J(P,C)=12.8 Hz, C-3 and C-4); 29.93 (d, J(P,C)=64.6 Hz, C-2 and C-5). ^{31}P NMR (202.3 MHz, $CDCl_3$): δ=44.4.

Formaldehyde (19.4 mL of 36–38% solution in water; 0.26 mol) and triethylamine (18.1 mL; 0.13 mol) were added to a solution of phosphol-3-ene 1-oxide **8** in methanol (1 L). The reaction mixture was stirred for 16 h at rt and evaporated. The crude mixture was treated with chloroform (1 L) and anhydrous sodium sulfate was added. The solution was filtered, evaporated and coevaporated with pyridine (3×300 mL). The crude 1-hydroxymethylphosphol-3-ene 1-oxide **9** was used without further purification. ^{1}H NMR (600.1 MHz, $CDCl_3$): δ=5.86 (dm, J(P,H)=27.9 Hz, ΣJ(H,H)=5.3 Hz, 2H, H-3 and H-4); 4.01 (d, J(P,H)=1.8 Hz, 2H, P–CH_2–O); 2.65 (m, 2H, H-2a and H-5a); 2.48 (m, 2H, H-2b and H-5b). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=127.14 (d, J(P,C)=11.2 Hz, C-3 and C-4); 59.51 (d, J(P,C)=75.0 Hz, P–CH_2–O); 28.52 (d, J=63.2 Hz, C-2 and C-5). ^{31}P NMR (202.3 MHz, $CDCl_3$): δ=70.64.

Trityl chloride (43.5 g; 0.156 mol) was added to a solution of 1-hydroxymethylphosphol-3-ene 1-oxide **9** in pyridine (1 L). The reaction mixture was stirred for 36 h at 70 °C, quenched by the addition of methanol (20 mL) and evaporated. The residue was dissolved in chloroform (1 L) and extracted with saturated solution of sodium hydrogencarbonate (3×200 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. 1-Trityloxymethyl-phosphol-3-ene 1-oxide **10** was purified by chromatography on silica gel (elution with gradient of 0–50% acetone in toluene) to yield 26.3 g (54%) of white foam. HRMS (FAB) calcd for $C_{24}H_{24}O_2P$ $(M+H)^+$ 375.1514, found 375.1518. ^{1}H NMR (600.1 MHz, $CDCl_3$): δ=7.39 (m, 6H, *ortho*-ArH); 7.31 (m, 6H, *meta*-ArH); 7.26 (m, 3H, *para*-ArH); 5.93 (dm, J(P,H)=28.6 Hz, ΣJ(H,H)=5.3 Hz, 2H, H-3 and H-4); 3.64 (d, J(P,H)=8.2 Hz, 2H, P–CH_2–O); 2.65 (bdd, J(H,H)=17.7 Hz, J(P,H)=6.4 Hz, 2H, H-2a and H-5a); 2.52 (bdd, J(H,H)=

17.7 Hz, *J*(P,H)=15.0 Hz, 2H, H-2b and H-5b). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=142.58 (3C, *ipso*-ArC); 128.57 (6C, *ortho*-ArC); 128.03 (6C, *meta*-ArC); 127.44 (3C, *para*-ArC); 127.36 (d, *J*(P,C)=11.0 Hz, C-3 and C-4); 84.45 (d, *J*(P,C)=12.3 Hz, >C<); 59.86 (d, *J*(P,C)=81.4 Hz, P–CH_2–O); 30.13 (d, *J*(P,C)=65.1 Hz, C-2 and C-5). ^{31}P NMR (202.3 MHz, $CDCl_3$): δ=66.90.

*4.3.1. (3S,4R,1S and 3R,4S,1R) 3-Bromo-4-hydroxy-1-trityloxymethylphospholane 1-oxide (**11**), (3R,4S,1S and 3S,4R,1R) 3-bromo-4-hydroxy-1-trityloxymethylphospholane 1-oxide (**12**).* NBS (13.8 g; 77.3 mmol) in acetone (150 mL) was added to a solution of 1-trityloxymethyl-phosphol-3-ene 1-oxide **10** (26.3 g; 70.3 mmol) in acetone/water 2/1 (450 mL). The reaction mixture was stirred for 8 h at rt and then evaporated to a volume of about 200 mL. Chloroform (1 L) was added and the mixture was extracted with saturated solution of sodium hydrogencarbonate (3×200 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The diastereoisomers were separated by chromatography on silica gel (elution with gradient of 0–10% ethanol in chloroform) as faster eluting racemate **12** (14.6 g; 44%; white foam) and slower eluting racemate **11** (11.6 g; 35%; white foam). HRMS (FAB) calcd for $C_{24}H_{25}(^{79}Br)O_3P$ $(M+H)^+$ 471.0725; found (**11**) 471.0729, (**12**) 471.0718.

11: ^{1}H NMR (600.1 MHz, $CDCl_3$): δ=7.42 (m, 6H, *ortho*-ArH); 7.34 (m, 6H, *meta*-ArH); 7.28 (m, 3H, *para*-ArH); 4.37–4.42 (m, 2H, H-3 and H-4); 3.67 (dd, *J*(P,H)=7.7, *J*(H,H)=12.7 Hz, 1H, P-CHa-O); 3.64 (dd, *J*(P,H)=7.9, *J*(H,H)=12.7 Hz, 1H, P-CHb-O); 2.69 (ddd, *J*(P,H)=15.8 Hz, *J*(H,H)=15.6 and 6.6 Hz, 1H, H-5a); 2.46 (dt, *J*(P,H)=7.8 Hz, *J*(H,H)=15.6 and 7.8 Hz, 1H, H-5b); 2.35 (ddd, *J*(P,H)=6.3 Hz, *J*(H,H)=15.5 and 5.7 Hz, 1H, H-5a); 2.06 (ddd, *J*(P,H)=16.3 Hz, *J*(H,H)=15.5 and 7.0 Hz, 1H, H-5a); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=142.29 (3C, *ipso*-ArC); 128.59 (6C, *ortho*-ArC); 128.20 (6C, *meta*-ArC); 127.64 (3C, *para*-ArC); 89.09 (d, *J*(P,C)=12.4 Hz, >C<); 76.50 (d, *J*(P,C)=9.0 Hz, C-4); 61.22 (d, *J*(P,C)=83.8 Hz, P–CH_2–O); 51.36 (d, *J*(P,C)=10.1 Hz, C-3); 33.90 (d, *J*(P,C)=56.4 Hz, C-2); 32.44 (d, *J*(P,C)=62.0 Hz, C-5). ^{31}P NMR (202.3 MHz, $CDCl_3$): δ=55.71.

12: ^{1}H NMR (600.1 MHz, $CDCl_3$): δ=7.39 (m, 6H, *ortho*-ArH); 7.34 (m, 6H, *meta*-ArH); 7.29 (m, 3H, *para*-ArH); 4.50 (dddd, *J*(P,H)=10.0 Hz, *J*(H,H)=8.0, 7.2 and 7.0 Hz, 1H, H-4); 4.11 (tdd, *J*(P,H)=9.6 Hz, *J*(H,H)=9.6, 8.9 and 7.2 Hz, 1H, H-3); 3.69 (m, 2H, P–CH_2–O); 2.64 (dt, *J*(P,H)=7.0 Hz, *J*(H,H)=15.6 and 7.1 Hz, 1H, H-2a); 2.43 (td, *J*(P,H)=15.4 Hz, *J*(H,H)=15.6 and 7.0 Hz, 1H, H-5a); 2.30 (ddd, *J*(P,H)=17.4 Hz, *J*(H,H)=15.6 and 8.9 Hz, 1H, H-2b); 2.07 (dt, *J*(P,H)=8.0 Hz, *J*(H,H)=15.6 and 8.0 Hz, 1H, H-5b). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=142.04 (3C, *ipso*-ArC); 128.51 (6C, *ortho*-ArC); 128.29 (6C, *meta*-ArC); 127.78 (3C, *para*-ArC); 89.60 (d, *J*(P,C)=12.1 Hz, >C<); 76.01 (d, *J*(P,C)=10.9 Hz, C-4); 59.86 (d, *J*(P,C)=83.5 Hz, P–CH_2–O); 51.03 (d, *J*(P,C)=9.6 Hz, C-3); 35.07 (d, *J*(P,C)=55.5 Hz, C-2); 31.48 (d, *J*(P,C)=61.6 Hz, C-5). ^{31}P NMR (202.3 MHz, $CDCl_3$): δ=55.94.

*4.3.2. (4S,3S,1R and 4R,3R,1S) 4-Azido-3-hydroxy-1-trityloxymethylphospholane 1-oxide (**14**).* Potassium carbonate (3.7 g; 27.1 mmol) was added to a solution of racemic bromohydrin **11** (11.6 g; 24.6 mmol) in acetone (500 mL). The reaction mixture was stirred for 16 h at rt and then evaporated. The residue was dissolved in chloroform (1 L) and extracted with saturated solution of sodium chloride (3×200 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude epoxide **13** was used without further purification.

Sodium azide (8.0 g; 123 mmol) and ammonium chloride (2.6 g; 49.2 mmol) were added to a solution of epoxide **13** in ethanol/water 8/2 (500 mL). The reaction mixture was stirred for 16 h at 80 °C and then evaporated. The residue was dissolved in chloroform (500 mL) and extracted with saturated solution of sodium hydrogencarbonate (3×200 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The product was purified by chromatography on silica gel (elution with gradient of 0–10% ethanol in chloroform) to yield 5.7 g (53%) of white foam.

HRMS (FAB) calcd for $C_{24}H_{25}N_3O_3P$ $(M+H)^+$ 434.1634, found 434.1631. ^{1}H NMR (600.1 MHz, DMSO-d_6): δ=7.39 (m, 12H, *ortho*-ArH and *meta*-ArH); 7.31 (m, 3H, *para*-ArH); 5.78 (d, *J*(H,H)=5.1 Hz, OH); 4.06 (m, 1H, H-4); 3.89 (m, 1H, H-3); 3.40 (dd, *J*(P,H)=7.4 Hz, *J*(H,H)=12.5 Hz, 1H, P-CHa-O); 3.36 (dd, *J*(P,H)=7.4 Hz, *J*(H,H)=12.5 Hz, 1H, P-CHb-O); 2.22 (dt, *J*(P,H)=6.7 Hz, *J*(H,H)=15.0 and 6.7 Hz, 1H, H-2a); 2.15 (ddd, *J*(P,H)=15.5 Hz, *J*(H,H)=15.2 and 7.6 Hz, 1H, H-5a); 1.83 (dt, *J*(P,H)=8.9 Hz, *J*(H,H)=15.2 and 8.8 Hz, 1H, H-5b); 1.68 (ddd, *J*(P,H)=17.8 Hz, *J*(H,H)=15.0 and 8.1 Hz, 1H, H-2b). ^{13}C NMR (150.9 MHz, DMSO-d_6): δ=142.83 (3C, *ipso*-ArC); 128.50 (6C, *ortho*-ArC); 128.37 (6C, *meta*-ArC); 127.65 (3C, *para*-ArC); 88.20 (d, *J*(P,C)=11.9 Hz, >C<); 72.99 (d, *J*(P,C)=10.1 Hz, C-3); 64.61 (d, *J*(P,C)=10.6 Hz, C-4); 61.79 (d, *J*(P,C)=81.4 Hz, P–CH_2–O); 33.48 (d, *J*(P,C)=59.0 Hz, C-2); 28.65 (d, *J*(P,C)=59.2 Hz, C-5). ^{31}P NMR (202.3 MHz, DMSO-d_6): δ=51.44.

*4.3.3. (4R,3R,1R and 4S,3S,1S) 4-Azido-3-hydroxy-1-trityloxymethylphospholane 1-oxide (**17**).* Potassium carbonate (4.7 g; 34.1 mmol) was added to a solution of racemic bromohydrin **12** (14.6 g; 31.00 mmol) in acetone (500 mL). The reaction mixture was stirred for 16 h at rt and then evaporated. The residue was dissolved in chloroform (1 L) and extracted with saturated solution of sodium chloride (3×200 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude epoxide **16** was used without further purification.

Sodium azide (10.1 g; 155 mmol) and ammonium chloride (3.3 g; 62 mmol) were added to a solution of epoxide **16** in ethanol/water 8/2 (500 mL). The reaction mixture was stirred for 16 h at 80 °C and then evaporated. The residue was dissolved in chloroform (500 mL) and extracted with saturated solution of sodium hydrogencarbonate (3×200 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The product was purified by chromatography on silica gel (elution with gradient of 0–10% ethanol in chloroform) to yield 8.3 g (62%) of white foam.

HRMS (FAB) calcd for $C_{24}H_{25}N_3O_3P$ $(M+H)^+$ 434.1634, found 434.1639. ^{1}H NMR (600.1 MHz, DMSO-d_6): δ=7.41 (m, 6H, *ortho*-ArH); 7.37 (m, 6H, *meta*-ArH); 7.30 (m, 3H, *para*-ArH); 5.73 (d, *J*(H,H)=4.9 Hz, OH); 4.12 (ddtd, *J*(P,H)=10.6 Hz, *J*(H,H)=7.9, 6.9, 6.8 and 4.9 Hz, 1H, H-3); 3.79 (ddt, *J*(P,H)=9.8 Hz, *J*(H,H)=8.1, 6.9 and 6.9 Hz, 1H, H-4); 3.43 (dd, *J*(P,H)=7.0 Hz, *J*(H,H)=12.3 Hz, 1H, P-CHa-O); 3.41 (dd, *J*(P,H)=6.7 Hz, *J*(H,H)=12.3 Hz, 1H, P-CHb-O); 2.31 (ddd, *J*(P,H)=7.8 Hz, *J*(H,H)=15.3 and 6.9 Hz, 1H, H-5a); 2.05 (td, *J*(P,H)=15.4 Hz, *J*(H,H)=15.2 and 6.8 Hz, 1H, H-2a); 1.90 (dt, *J*(P,H)=7.9 Hz, *J*(H,H)=15.2 and 7.9 Hz, 1H, H-2b); 1.68 (ddd, *J*(P,H)=16.8 Hz, *J*(H,H)=15.3 and 8.1 Hz, 1H, H-5b). ^{13}C NMR (150.9 MHz, DMSO-d_6): δ=142.92 (3C, *ipso*-ArC); 128.56 (6C, *ortho*-ArC); 128.33 (6C, *meta*-ArC); 127.61 (3C, *para*-ArC); 88.12 (d, *J*(P,C)=11.9 Hz, >C<); 72.75 (d, *J*(P,C)=9.3 Hz, C-3); 64.83 (d, *J*(P,C)=10.6 Hz, C-4); 62.64 (d, *J*(P,C)=81.0 Hz, P–CH_2–O); 32.11 (d, *J*(P,C)=59.3 Hz, C-2); 29.62 (d, *J*(P,C)=59.3 Hz, C-5). ^{31}P NMR (202.3 MHz, DMSO-d_6): δ=52.64.

*4.3.4. (4S,3S,1R and 4R,3R,1S) 4-Amino-3-hydroxy-1-trityloxymethylphospholane 1-oxide (**15**).* Azido derivative **14** (5.7 g; 13.1 mmol) and 10% Pd/C catalyst (0.3 g) were suspended in ethanol (200 mL). The mixture was hydrogenated at atmospheric pressure for 16 h at rt. The reaction mixture was filtered through a layer of Celite and the solvent was evaporated. The crude amino derivative **15** was used without further purification (yellow oil). ESI-MS calcd for $C_{24}H_{27}NO_3P$ $(M+H)^+$ 408.2, found 408.3.

*4.3.5. (4R,3R,1R and 4S,3S,1S) 4-Amino-3-hydroxy-1-trityloxymethylphospholane 1-oxide (**18**).* Azido derivative **17** (8.3 g; 19.1 mmol) and 10% Pd/C catalyst (0.4 g) were suspended in ethanol (200 mL). The mixture was hydrogenated at atmospheric pressure

for 16 h at rt. The reaction mixture was filtered through a layer of Celite and the solvent was evaporated. The crude amino derivative **18** was used without further purification (yellow oil). ESI-MS calcd for $C_{24}H_{27}NO_3P$ $(M+H)^+$ 408.2, found 408.0.

*4.3.6. (4S,3S,1R and 4R,3R,1S) 4-(Uracil-1-yl)-3-hydroxy-1-hydroxymethylphospholane 1-oxide (**4a**).* 4-Nitrophenyl-3-ethoxyacryloylcarbamate **20** (0.8 g; 2.9 mmol) was added to a solution of 4-amino-3-hydroxy-1-trityloxymethylphospholane 1-oxide **15** (1.0 g; 2.5 mmol) in dioxane (25 mL). The reaction mixture was stirred for 4 h at rt. After that, 5 g of Dowex H^+ was added and the suspension was stirred for 2 h at 90 °C. The resin was filtered off and washed with dioxane (100 mL). The product was washed off of the resin with water (100 mL), evaporated, purified by preparative HPLC (isocratically water) and freeze-dried from water to yield 0.47 g (73%) of white lyofilisate. HRMS (FAB) calcd for $C_9H_{14}N_2O_5P$ $(M+H)^+$ 261.0640, found 261.0643. IR ν_{max} (KBr) 3424, 3349, 3192, 3156, 3100, 3050, 1709, 1674, 1621, 1467, 1422, 1414, 1398, 1388, 1263, 1170, 1084, 1049, 1043, 767, 718. NMR data—see Tables 2–4.

*4.3.7. (4S,3S,1R and 4R,3R,1S) 4-(Adenin-9-yl)-3-hydroxy-1-hydroxymethylphospholane 1-oxide (**4b**).* 4,6-Dichloro-5-formamidopyrimidine (0.7 g; 3.8 mmol) and DIPEA (1.7 mL; 10.0 mmol) were added to a solution of 4-amino-3-hydroxy-1-trityloxymethylphospholane 1-oxide **15** (1.0 g; 2.5 mmol) in n-butanol (25 mL). The reaction mixture was stirred for 16 h at 130 °C and evaporated. The residue was treated 5 days at rt with aqueous ammonia/dioxane 1/1 (25 mL) and evaporated. The crude adenine derivative was treated for 16 h at 60 °C with 80% acetic acid in water (100 mL), diluted with water (100 mL) and treated with Dowex H^+ (10 g). The resin was filtered off and washed with methanol (100 mL) and water (100 mL). The product was washed off of the resin with 5% aqueous ammonia (100 mL), evaporated, purified by preparative HPLC (elution with gradient of 0–20% methanol in water) and freeze-dried from water to yield 0.29 g (41%) of white lyofilisate. HRMS (FAB) calcd for $C_{10}H_{15}N_5O_3P$ $(M+H)^+$ 284.0913, found 284.0910. IR ν_{max} (KBr) 3328, 3266, 3178, 1648, 1603, 1572, 1507, 1485, 1419, 1331, 1309, 1218, 1157, 1072, 1050, 795, 726, 644. NMR data—see Tables 2–4.

*4.3.8. (4S,3S,1R and 4R,3R,1S) 4-(Guanin-9-yl)-3-hydroxy-1-hydroxymethylphospholane 1-oxide (**4c**).* 2-Amino-4,6-dichloro-5-formamidopyrimidine (0.8 g; 3.8 mmol) and DIPEA (1.7 mL; 10.0 mmol) were added to a solution of 4-amino-3-hydroxy-1-trityloxymethylphospholane 1-oxide **15** (1.0 g; 2.5 mmol) in *n*-butanol (25 mL). The reaction mixture was stirred for 16 h at 130 °C and evaporated. The residue was treated 16 h at 60 °C with concentrated hydrochloric acid/methanol 1/10 (25 mL), diluted with water (100 mL) and treated with Dowex H^+ (10 g). The resin was filtered off and washed with methanol (100 mL) and water (100 mL). The product was washed off of the resin with 5% aqueous ammonia (100 mL), evaporated, purified by preparative HPLC (isocratically water) and freeze-dried from water to yield 0.34 g (45%) of white lyofilisate. HRMS (FAB) calcd for $C_{10}H_{15}N_5O_4P$ $(M+H)^+$ 300.0862, found 300.0865. IR ν_{max} (KBr) 3403, 3322, 3200, 3161, 3113, 1736,

Table 2
Proton chemical shifts of phosphanucleosides in DMSO and D_2O

Comp.	H-2a	H-2b	H-3	H-4	H-5a	H-5b	H-6a	H-6b	H-2[a]	H-5[a]	H-6[a]	H-8[a]
In DMSO												
4a	1.66 ddd	2.59 dt	4.16 m	4.69 ddd	2.03 td	2.20 ddd	3.82 (2H) m		—	5.64 d	7.59 d	—
4b	1.75 ddd	~2.69 m	4.50 m	4.81 ddd	2.25 btd	~2.69 m	3.88 (2H) bm		8.12 s	—	—	8.15 s
4c	1.71 ddd	2.67 dt	4.37 m	4.62 ddd	2.22 dt	2.38 ddd	3.87 (2H) m		—	—	—	7.74 s
4d	1.66 ddd	2.58 dt	4.24 tt	4.64 ddd	1.99 dt	2.20 ddd	3.81 (2H) m		—	5.70 d	7.51 d	—
19a	2.06 m	1.94 dt	4.55 m	4.42 ddd	2.10 m	2.45 dt	3.83 dt	3.79 dt	—	5.57 d	7.82 d	—
19b	2.15 ddd	2.04 dt	4.85 m	4.52 m	2.68 dt	2.63 ddd	3.88 dt	3.84 dt	8.13 s	—	—	8.25 s
19c	2.11 ddd	1.99 dt	4.76 m	4.33 ddd	2.48 ddd	2.61 dt	3.86 dt	3.82 dt	—	—	—	7.83 s
19d	2.04 ddd	1.92 dt	4.60 m	4.36 ddd	2.10 ddd	2.43 dt	3.81 dt	3.77 ddd	—	5.67 d	7.66 d	—
In D_2O												
4a	2.05 dddt	2.90 dtd	4.54 tdd	4.87 dddd	2.42 dddd	2.59 dddd	4.18 dd	4.14 ddd	—	5.89 d	7.65 d	—
4b	2.17 ddd	3.00 dt	4.74 m	5.06 m	2.66 ddd	2.83 ddd	4.26 dd	4.23 dd	8.16 s	—	—	8.28 s
4c	2.11 bddd	2.95 bdt	4.68 tdd	4.90 m	2.59 m	2.83 dddd	4.23 dd	4.20 dd	—	—	—	7.89 s
4d	2.04 ddd	2.89 m	4.58 m	~4.81 m	2.40 m	2.60 m	4.17 dd	4.14 dd	—	6.03 d	7.59 d	—
19a	2.50 dddd	2.17 dddd	4.84 tdd	4.59 dddd	2.36 dddd	2.82 m	4.19 dd	4.16 ddd	—	5.91	7.75	—
19b	2.58 dddd	2.30 dtd	5.07 dddd	4.75 m	2.67 ddd	3.05 dt	4.25 dd	4.22 dd	8.22 s	—	—	8.29 s
19c	2.54 m	2.24 m	5.02 dddd	4.57 dddd	2.69 ddd	2.97 dt	4.22 dd	4.19 dd	—	—	—	7.94 s
19d	2.49 dddd	2.18 dtd	4.86 m	4.60 m	2.35 bddd	2.80 dt	4.19 dd	4.15 dd	—	6.06 d	7.69 d	—

[a] Nucleobase proton.

Table 3
Proton coupling constants *J*(H,H) and *J*(P,H) in phosphanucleosides

Coupled nuclei	**4a**	**4b**	**4c**	**4d**	**19a**	**19b**	**19c**	**19d**
In DMSO								
H-2a, H-2b	14.6	14.8	14.7	14.5	14.7	14.9	14.9	14.7
H-2a, H-3	10.3	10.4	10.4	10.4	7.7	7.6	7.7	7.6
H-2, H-3	7.2	*a*	6.1	7.0	10.4	10.4	10.4	10.2
H-3, H-4	10.3	10.0	10.2	10.4	10.3	10.1	9.8	10.2
H-4, H-5a	8.1	7.7	7.8	8.2	12.1	11.6	12.0	11.9
H-4, H-5b	12.5	12.4	12.4	12.2	7.4	7.6	7.4	7.5
H-5a, H-5b	14.7	~14.2	14.8	14.6	14.3	14.6	14.6	14.4
H-6a, H-6b	*a*	*a*	*a*	*a*	13.9	13.8	13.9	13.9
P, H-2a	19.2	18.9	19.2	19.2	13.0	12.6	12.7	12.9
P, H-2b	6.8	*a*	7.2	6.7	10.4	10.4	10.4	10.2
P, H-3	*a*	*a*	*a*	*a*	*a*	*a*	1.3	~0
P, H-4	*a*	*a*	*a*	*a*	*a*	*a*	<1	~0
P, H-5a	13.6	~14.2	13.4	14.0	19.1	18.2	18.8	18.9
P, H-5b	10.5	*a*	9.8	10.4	7.5	7.8	7.3	7.4
P, H-6a	*a*	*a*	*a*	*a*	5.6	5.6	5.3	~5.3
P, H-6b	*a*	*a*	*a*	*a*	4.6	5.1	4.9	~4.5
In D_2O								
H-2a, H-2b	15.4	15.4	15.4	15.2	15.5	15.6	15.4	15.4
H-2a, H-3	10.6	10.4	10.6	10.5	7.6	7.5	7.5	7.6
H-2b, H-3	7.3	6.7	7.2	7.2	10.6	10.3	10.6	10.4
H-3, H-4	10.3	10.0	10.2	10.3	10.2	10.4	9.9	10.3
H-4, H-5a	8.5	8.3	8.1	8.5	12.0	11.7	11.5	11.9
H-4, H-5b	12.2	12.0	12.1	11.9	7.9	7.4	7.9	7.6
H-5a, H-5b	15.8	15.8	15.8	15.6	15.4	15.7	15.6	15.4
H-6a, H-6b	14.6	14.5	14.6	14.6	14.6	14.5	14.5	14.6
P, H-2a	20.1	19.8	19.7	20.0	19.6	19.0	15.4	13.6
P, H-2b	6.7	6.7	6.4	6.8	15.5	7.4	10.2	10.4
P, H-3	0.8	*a*	*a*	~0.6	*a*	*a*	2.0	1.8
P, H-4	0.8	*a*	*a*	*a*	1.6	2.1	1.5	0.8
P, H-5a	14.3	14.1	14.2	14.4	13.7	13.6	13.4	19.4
P, H-5b	10.8	9.8	10.1	10.4	10.3	10.3	7.0	7.4
P, H-6a	4.4	4.4	4.5	4.3	4.8	4.6	4.6	4.7
P, H-6b	4.1	4.1	4.1	3.8	4.2	4.2	4.4	4.4

a—The *J* value was not determined.

2.4 mmol) was added to a mixture of 4-(uracil-1-yl)-3-hydroxy-1-hydroxymethylphospholane 1-oxide **4a** (0.26 g; 1.0 mmol) and TEA (0.33 mL; 2.4 mmol) in acetonitrile (10 mL). The reaction mixture was stirred for 16 h at rt, quenched by the addition of methanol (1 mL) and evaporated. The residue was dissolved in chloroform (100 mL) and extracted with saturated solution of sodium hydrogencarbonate (3×20 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude dibenzoyl derivative was used without further purification. ESI-MS calcd for $C_{23}H_{22}N_2O_7P$ $(M+H)^+$ 469.1, found 469.5.

2,4,6-Triisopropylbenzenesulfonyl chloride (0.91 g; 3.0 mmol) was added to a mixture of dibenzoyl derivative, TEA (0.84 mL; 6 mmol) and DMAP (0.12 g; 1.0 mmol) in acetonitrile (10 mL). The reaction mixture was stirred for 16 h at rt. After that, 5 mL of aqueous ammonia was added. The reaction mixture was stirred for 16 h at rt and evaporated. The crude cytosine derivative was dissolved in 50% methanol in water (100 mL) and treated with Dowex H^+ (10 g). The resin was filtered off and washed with methanol (100 mL) and water (100 mL). The product was washed off of the resin with 5% aqueous ammonia (100 mL), evaporated, purified by preparative HPLC (isocratically water) and freeze-dried from water to yield 0.14 g (54%) of white lyofilisate. HRMS (FAB) calcd for $C_9H_{15}N_3O_4P$ $(M+H)^+$ 260.0800, found 260.0804. IR ν_{max} (KBr) 3336, 3194, 1647, 1607, 1527, 1487, 1398, 1296, 1153, 1071, 1045, 786. NMR data—see Tables 2–4.

*4.3.10. (4R,3R,1R and 4S,3S,1S) 4-(Uracil-1-yl)-3-hydroxy-1-hydroxymethylphospholane 1-oxide (**19a**).* 4-Nitrophenyl-3-ethoxyacryloylcarbamate **20** (0.8 g; 2.9 mmol) was added to a solution of 4-amino-3-hydroxy-1-trityloxymethylphospholane 1-oxide **18** (1.0 g; 2.5 mmol) in dioxane (25 mL). The reaction mixture was stirred for 4 h at rt. After that, 5 g of Dowex H^+ was added and the

Table 4
^{13}C and ^{31}P chemical shifts in phosphanucleosides (coupling constants *J*(P,C) are given in brackets)

Atom	**4a**	**4b**	**4c**	**4d**	**19a**	**19b**	**19c**	**19d**
In DMSO								
C-2	33.61 (54.8)	28.28 (55.6)	34.21 (54.9)	33.88 (54.9)	30.79 (55.8)	31.21 (55.8)	31.22 (55.7)	31.17 (55.8)
C-3	69.77 (9.1)	70.95 (9.2)	71.04 (9.2)	69.55 (9.7)	69.73 (8.4)	71.05 (8.8)	71.10 (8.7)	69.67 (8.6)
C-4	60.66 (10.6	59.77 (10.4)	58.54 (10.1)	62.04 (10.4)	60.71 (9.6)	59.92 (10.4)	59.31 (10.4)	62.28 (6.3)
C-5	26.86 (55.2)	33.94 (54.8)	28.97 (55.1)	27.24 (55.3)	28.90 (55.2)	30.29 (55.5)	30.47 (55.4)	29.11 (55.3)
C-6	59.89 (79.8)	59.78 (80.0)	59.82 (80.0)	59.98 (79.3)	59.40 (81.0)	59.41 (81.0)	59.40 (81.0)	59.60 (80.4)
C-2[a]	151.40	152.32	153.55	165.53	151.41	152.27	153.38	165.49
C-4[a]	163.37	149.76	151.63	156.05	163.36	149.77	151.49	156.06
C-5[a]	101.77	119.48	116.88	93.99	101.68	119.48	117.26	93.98
C-6[a]	142.67	156.20	157.00	143.67	143.06	156.23	156.99	144.00
C-8[a]	—	140.33	135.86	—	—	140.48	136.97	—
P-1	47.79	48.76	49.73	48.46	48.80	49.48	49.63	49.73
In D_2O								
C-2	34.40 (56.3)	34.70 (56.3)	34.59 (56.5)	34.44 (54.9)	32.16 (57.2)	32.40 (57.2)	32.30 (57.4)	32.24 (57.3)
C-3	72.64 (11.2)	74.27 (10.7)	74.14 (9.1)	72.67 (11.7)	72.55 (9.5)	74.08 (9.7)	73.97 (10.0)	72.70 (9.6)
C-4	64.84 (11.5)	62.05 (10.7)	61.73 (10.8)	65.86 (10.3)	64.23 (12.5)	62.11 (9.6)	61.94 (11.4)	64.89 (10.4)
C-5	27.87 (7.6)	30.04 (57.7)	29.62 (57.8)	28.21 (57.5)	29.96 (57.1)	31.81 (57.0)	31.46 (57.4)	30.37 (57.0)
C-6	61.04 (81.8)	61.08 (81.8)	60.92 (81.6)	61.19 (81.2)	60.88 (81.6)	61.08 (81.5)	60.92 (81.9)	60.94 (81.6)
C-2[a]	154.83	155.19	156.26	168.67	154.91	155.28	156.32	168.52
C-4[a]	168.91	151.86	154.74	160.82	168.87	151.94	154.74	160.82
C-5[a]	105.13	121.57	119.10	99.14	105.22	121.61	119.16	99.27
C-6[a]	146.96	158.24	161.72	147.00	146.48	158.36	161.84	146.24
C-8[a]	—	143.54	141.08	—	—	143.44	141.11	—
P-1	58.49	59.07	59.05	58.81	58.57	58.90	59.15	58.87

[a] Nucleobase carbon.

1689, 1633, 1610, 1572, 1539, 1484, 1408, 1390, 1324, 1218, 1152, 1073, 1054, 784, 777, 729, 639. NMR data—see Tables 2–4.

*4.3.9. (4S,3S,1R and 4R,3R,1S) 4-(Cytosin-1-yl)-3-hydroxy-1-hydroxymethylphospholane 1-oxide (**4d**).* Benzoyl cyanide (0.31 g;

suspension was stirred for 2 h at 90 °C. The resin was filtered off and washed with dioxane (100 mL). The product was washed off of the resin with water (100 mL), evaporated, purified by preparative HPLC (isocratically water) and freeze-dried from water to yield 0.46 g (71%) of white lyofilisate. HRMS (FAB) calcd for $C_9H_{14}N_2O_5P$ $(M+H)^+$

261.0640, found 261.0638. IR ν_{max} (KBr) 3376, 3262, 1688, 1622, 1467, 1425, 1409, 1384, 1261, 1149, 1065, 1052, 765, 720. NMR data—see Tables 2–4.

*4.3.11. (4R,3R,1R and 4S,3S,1S) 4-(Adenin-9-yl)-3-hydroxy-1-hydroxymethylphospholane 1-oxide (**19b**).* 4,6-Dichloro-5-formamidopyrimidine (0.7 g; 3.8 mmol) and DIPEA (1.7 mL; 10.0 mmol) were added to a solution of 4-amino-3-hydroxy-1-trityloxymethylphospholane 1-oxide **18** (1.0 g; 2.5 mmol) in *n*-butanol (25 mL). The reaction mixture was stirred for 16 h at 130 °C and evaporated. The residue was treated 5 days at rt with aqueous ammonia/dioxane 1/1 (25 mL) and evaporated. The crude adenine derivative was treated 16 h at 60 °C with 80% acetic acid in water (100 mL), diluted with water (100 mL) and treated with Dowex H^+ (10 g). The resin was filtered off and washed with methanol (100 mL) and water (100 mL). The product was washed off of the resin with 5% aqueous ammonia (100 mL), evaporated, purified by preparative HPLC (elution with gradient of 0–20% methanol in water) and freeze-dried from water to yield 0.33 g (47%) of white lyofilisate. HRMS (FAB) calcd for $C_{10}H_{15}N_5O_3P$ $(M+H)^+$ 284.0913, found 284.0914. IR ν_{max} (KBr) 3419, 3331, 3265, 3214, 3133, 1659, 1606, 1573, 1515, 1483, 1423, 1405, 1396, 1335, 1303, 1295, 1226, 1221, 1156, 1071, 1041, 795, 713, 653. NMR data—see Tables 2–4.

*4.3.12. (4R,3R,1R and 4S,3S,1S) 4-(Guanin-9-yl)-3-hydroxy-1-hydroxymethylphospholane 1-oxide (**19c**).* 2-Amino-4,6-dichloro-5-formamidopyrimidine (0.8 g; 3.8 mmol) and DIPEA (1.7 mL; 10.0 mmol) were added to a solution of 4-amino-3-hydroxy-1-trityloxymethylphospholane 1-oxide **18** (1.0 g; 2.5 mmol) in *n*-butanol (25 mL). The reaction mixture was stirred for 16 h at 130 °C and evaporated. The residue was treated 16 h at 60 °C with concentrated hydrochloric acid/methanol 1/10 (25 mL), diluted with water (100 mL) and treated with Dowex H^+ (10 g). The resin was filtered off and washed with methanol (100 mL) and water (100 mL). The product was washed off of the resin with 5% aqueous ammonia (100 mL), evaporated, purified by preparative HPLC (isocratically water) and freeze-dried from water to yield 0.36 g (48%) of white lyofilisate. HRMS (FAB) calcd for $C_{10}H_{15}N_5O_4P$ $(M+H)^+$ 300.0862, found 300.0861. IR ν_{max} (KBr) 3480, 3438, 3318, 3215, 3138, 3119, 2732, 1747, 1688, 1658, 1641, 1628, 1589, 1578, 1544, 1480, 1421, 1400, 1386, 1324, 1224, 1166, 1062, 1050, 1036, 778, 734, 634. NMR data—see Tables 2–4.

*4.3.13. (4R,3R,1R and 4S,3S,1S) 4-(Cytosin-1-yl)-3-hydroxy-1-hydroxymethylphospholane 1-oxide (**19d**).* Benzoyl cyanide (0.31 g; 2.4 mmol) was added to a mixture of 4-(uracil-1-yl)-3-hydroxy-1-hydroxymethylphospholane 1-oxide **19a** (0.26 g; 1.0 mmol) and TEA (0.33 mL; 2.4 mmol) in acetonitrile (10 mL). The reaction mixture was stirred for 16 h at rt, quenched by the addition of methanol (1 mL) and evaporated. The residue was dissolved in chloroform (100 mL) and extracted with saturated solution of sodium hydrogencarbonate (3×20 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude dibenzoyl derivative was used without further purification. ESI-MS calcd for $C_{23}H_{22}N_2O_7P$ $(M+H)^+$ 469.1, found 469.5.

2,4,6-Triisopropylbenzenesulfonyl chloride (0.91 g; 3.0 mmol) was added to a mixture of dibenzoyl derivative, TEA (0.84 mL; 6 mmol) and DMAP (0.12 g; 1.0 mmol) in acetonitrile (10 mL). The reaction mixture was stirred for 16 h at rt. After that, 5 mL of aqueous ammonia was added. The reaction mixture was stirred for 16 h at rt and evaporated. The crude cytosine derivative was dissolved in 50% methanol in water (100 mL) and treated with Dowex H^+ (10 g). The resin was filtered off and washed with methanol (100 mL) and water (100 mL). The product was washed off of the resin with 5% aqueous ammonia (100 mL), evaporated, purified by preparative HPLC (isocratically water) and freeze-dried from water to yield 0.18 g (69%) of white lyofilisate. HRMS (FAB) calcd for $C_9H_{15}N_3O_4P$ $(M+H)^+$ 260.0800, found 260.0802. IR ν_{max} (KBr) 3444, 3414, 3292, 3201, 1658, 1645, 1623, 1610, 1532, 1492, 1416, 1394, 1300, 1291, 1145, 1066, 1048, 782. NMR data—see Tables 2–4.

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Supplementary data

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