Preparation of nucleoside 5′-deoxy-5′-methylenephosphonates as building blocks for the synthesis of methylenephosphonate analogues

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Efficient synthesis of suitably protected 2′-deoxycytidine, 2′-deoxyadenosine, and 2′-deoxyguanosine derivatives bearing the 5′-methylenephosphonate moiety with the 4-methoxy-1-oxido-2-picolyl function as an intramolecular nucleophile catalytic group is described.

Introduction

Although naturally occurring oligonucleotides with 3′,5′-internucleoside phosphodiester bonds can modulate gene expression[1,2] *via* formation of stable complexes with DNA or RNA targets, they do not meet the criteria for potential antisense or antigene agents; in the first place, due to their high susceptibility to nuclease degradation *in vivo*. To overcome this and other problems connected with antisense therapy a plethora of oligonucleotide analogues have been designed,[3] but a modification (or combinations of modifications) that can provide satisfactory chemical, enzymic and pharmaco-dynamic properties to a potential oligonucleotide drug has yet to be found.[4]

Since the presence of a P–C bond confers nuclease resistance to biologically active phosphates, the most well studied class of DNA analogues are oligonucleoside methylphosphonates. However, due to chirality of the phosphorus centre in these compounds, their chemical synthesis usually leads to a pool of diastereomeric products with different physicochemical properties; *e.g.*, varying ability to form stable complexes with a target DNA or RNA. In this respect, oligonucleoside methylenephosphonate analogues, bearing the P–C bond in a bridging position of the internucleosidic linkage (the 3′ or 5′ position), may offer some advantages. Due to the presence of a P–C bond, they retain resistance to nucleases similar to that of methylphosphonate analogues, but the achiral $\succ$P(=O)O$^-$ functionality (a high resemblance to natural oligonucleotides) alleviates problems of diastereomeric inhomogeneity of the produced oligonucleotide analogues.

Apart from these, ^{1}H NMR studies revealed that in nucleosides with a 5′-deoxy-5′-*C*-(phosphonomethyl) moiety, the most populated rotamers around the C4′–C5′ bond are γ^t and γ^-.[5] In the light of the observed correlation between biological activity and the rotamers' distribution around the C4′–C5′ bond in some nucleoside analogues,[6,7] it is possible that the structural motif present in the 5′-methylenephosphonate analogues, namely the P–C–C function, can be of biological importance for modulation of their interactions with DNA-synthesizing enzymes.

Recently, we have reported on a new method for the formation of phosphonate esters that makes use of intramolecular nucleophilic catalysis exerted by a 4-methoxy-1-oxido-2-picolyl † group attached to the phosphonate centre.[8] The efficacy of this method in solid-phase synthesis of oligonucleotide analogues was demonstrated by preparing several 5′-deoxy-5′-methylphosphonate-linked thymidine oligonucleotides with a chain length of up to 20 nucleotidic units.[9] As expected, the produced oligonucleotide analogues in which the 5′-oxygen atom was replaced by a methylene group bound to the phosphorus were resistant to bovine spleen phosphodiesterase (BSPDE) digestion.[9]

In this paper we report on the preparation of protected 4-methoxy-1-oxido-2-picolyl 5′-deoxy-5′-methylenephosphonate derivatives of deoxycytidine, deoxyadenosine, and deoxyguanosine, suitable for incorporation into oligonucleotides or other biologically active phosphorus-containing natural products, *e.g.* phosphorylated sugars, phospholipids.

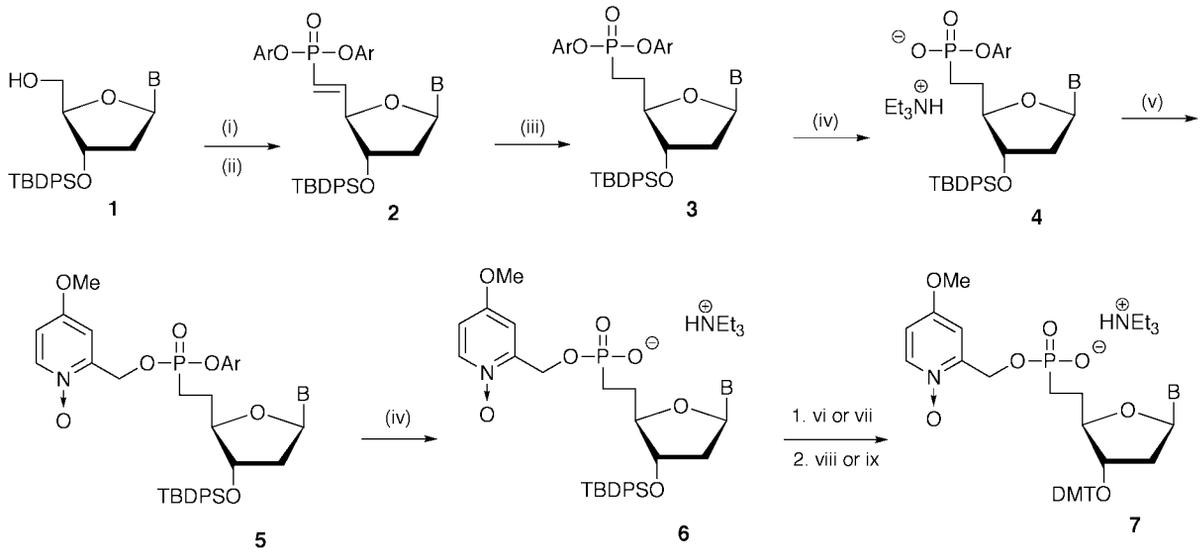
Results and discussion

We considered that the most convenient combination of protecting groups in nucleoside 5′-deoxy-5′-methylenephosphonate building blocks would consist of the acid-labile dimethoxytrityl group for protection of the 3′-OH function, base-labile acyl groups for protection of amino functions in the heterocyclic bases, and the 4-methoxy-1-oxido-2-picolyl group, as a phosphonate-protecting group. The last group should also simultaneously act as an intramolecular nucleophile catalytic group to facilitate coupling of the 5′-methylenephosphonate building blocks to a suitable acceptor molecule, *e.g.* protected oligonucleotide, carbohydrates, lipids, *etc*.

The general synthetic scheme for the preparation of building blocks **7** (Scheme 1) followed that developed by us previously for the thymidine unit,[9] and used as starting materials easily accessible N-protected nucleosides **1** bearing a 3′-*O*-*tert*-butyldiphenylsilyl (TBDPS) group. This rather stable 3′-OH protection group can at the end of synthesis be replaced by the 4,4′-dimethoxytrityl (DMT) group, which is more suitable for further applications.

The 5′-phosphonomethyl group was introduced into nucleosides **1** using a modification of the procedure previously developed by Jones and Moffatt.[10] This commenced with a one-pot oxidation of the 5′-hydroxy function of **1** using DCC–DMSO, followed by reaction of the produced aldehyde with a mixed phosphorane–phosphonate Wittig reagent[11] to give the appropriate vinylphosphonate **2** [almost exclusively in the (*E*)-form, ^{1}H NMR]. For the deoxyguanosine **1b** and deoxyadenosine **1c** derivatives, the introduction of the phosphonate function was effected by diphenyl triphenylphosphoranyl-

† 2-Picolyl = pyridin-2-ylmethyl.



Scheme 1 *Reagents and conditions*: (i) DCC, pyridine, TFA, DMSO; (ii) $Ph_3P{=}CHP(O)(OPh)_2$ or $Ph_3P{=}CHP(O)(OC_6H_4Cl\text{-}o)_2$; (iii) PADA, AcOH, pyridine; (iv) pyridine-2-aldoxime, TMG, dioxane–H_2O (3 : 1 v/v); (v) 4-methoxy-1-oxidopyridine-2-methanol, 4-methoxypyridine 1-oxide, 2-chloro-5,5-dimethyl-2-oxo-2λ^5-1,3,2-dioxaphosphinane, pyridine; (vi) Bu_4NF, THF; (vii) TAF, THF; (viii) DMT-Cl, DMAP, pyridine, 70 °C; (ix) DMT-Cl, $AgNO_3$, THF–DMF (1 : 1 v/v). **1a**–**7a**, B = N^4-isobutyrylcytosin-1-yl; Ar = *o*-chlorophenyl. **1b**–**7b**, B = N^2-isobutyrylguanin-9-yl; Ar = phenyl. **1c**–**7c**, B = N^6-benzoyladenin-9-yl; Ar = phenyl. *Abbreviations*: DMT—4,4′-dimethoxytrityl; DCC—1,3-dicyclohexylcarbodiimide; DMAP—4-dimethylaminopyridine; DMSO—dimethyl sulfoxide; PADA—potassium azodicarboxylate; TAF—triethylamine trishydrofluoride; TBDPS—*tert*-butyldiphenylsilyl; TFA—trifluoroacetic acid; TMG—1,1,3,3-tetramethylguanidine.

idenemethylphosphonate,[11] while for the deoxycytidine **1a** the di-(*o*-chlorophenyl) derivative was used instead. In this instance, the more labile *o*-chlorophenyl phosphonate protecting group was required to achieve a better compatibility in some of the later reaction steps (*vide infra*).

Catalytic hydrogenation that worked well for the vinylphosphonate derived from thymidine[9] was found to be inefficient for vinylphosphonates **3a**–**c**. Very sluggish catalytic hydrogenation of an adenosine vinylphosphonate was also reported by Jones and Moffatt,[10] who proposed using diimide as reducing agent in this instance. We found that potassium azodicarboxylate (PADA) in the presence of acetic acid and pyridine[12] efficiently reduced vinylphosphonates **2b** and **2c** to produce the appropriate phosphonates **3**. For the deoxycytidine derivative **2a**, a partial loss of the base protection was observed during this reduction. It was, however, possible to restore the N-protection through a reaction with isobutyric anhydride before the purification and thus achieve a satisfactory yield of the phosphonate **3a**.

The selective removal of one aryl group from **3** to produce phosphonate monoesters **4** was effected by treatment with pyridine-2-aldoximate.[13] To secure stability of the N^4-isobutyryl group in the deoxycytidine derivatives under the reaction conditions, it was necessary to use an *o*-chlorophenyl instead of a phenyl group for protection of the phosphonate function in **3a**. This allowed a significant shortening of the deprotection time of **3a** with pyridinealdoximate (from several hours to 30 min) and thus eliminated the occasionally observed losses of the N^4-isobutyryl group.

The intramolecular catalytic group, 4-methoxy-1-oxido-2-picolyl, was introduced analogously as for the thymidine derivative,[9] *i.e.*, *via* condensation of the phosphonate monoesters **4** with 4-methoxy-1-oxidopyridine-2-methanol in the presence of 2-chloro-5,5-dimethyl-2-oxo-2λ^5-1,3,2-dioxaphosphinane (NEP-Cl) and 4-methoxypyridine *N*-oxide as a nucleophilic catalyst. From the produced phosphonate diesters **5**, the aryl protecting groups were removed *via* the oximate treatment (*vide supra*) to produce the phosphonate monoesters **6**.

The conversion of **6a** and **6b** into the final products **7** was achieved *via* removal of the 3′-*O*-TBDPS groups with tetrabutylammonium fluoride followed by dimethoxytritylation with DMTCl in pyridine in the presence of DMAP. In the case of **6b**, some depurination during the removal of the silyl group was observed, which may explain the rather moderate yield of the isolated **7b** (41%). For deoxyadenosine derivative **6c**, which was expected to be even more prone to depurination, these reaction conditions failed to produce **7c**. However, using triethylamine trishydrofluoride as desilylating reagent, followed by precipitation of the crude product before the dimethoxytritylation, produced the desired product **7c** in good yield. In this instance, changes in the tritylation conditions (RT, DMF, $AgNO_3$)[14] had a beneficial effect on the total yield of the conversion **6c** to **7c**.

In conclusion, we have developed a reliable protocol for the preparation of protected nucleoside 5′-deoxy-5′-methylenephosphonates that can serve as building blocks for incorporation of the methylenephosphonate function into various biomolecules. By introducing appropriate changes into the presented method, 5′-methylenephosphonate building blocks with other combinations of protecting groups can also be prepared.

Experimental

Materials and methods

^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a JEOL GSX-270 FT or a Varian 300 MHz spectrometer. Chemical shifts are given in ppm relative to tetramethylsilane (^{1}H and ^{13}C NMR, $CDCl_3$, 25 °C) or 2% H_3PO_4 in D_2O (^{31}P NMR, CH_3CN, 25 °C). The assignment of proton and carbon resonances was done on the basis of known or expected chemical shifts in conjunction with ^{1}H–^{1}H and ^{1}H–^{13}C correlated NMR spectroscopy. Resonances marked with a superscript # refer to nuclei of the picolyl moiety in the corresponding compounds. The multiple number of some C and H resonances is due to the presence of diastereomers (compounds of type **5**) or to the diastereotopic character of certain substituents. High-resolution FAB mass spectra were recorded on a JEOL SX-102 instrument.

Pyridine was dried by refluxing with CaH_2 overnight followed by distillation, re-distillation from toluene-*p*-sulfonyl chloride and storage over molecular sieves (4 Å). 1,4-Dioxane was dried by distillation from $LiAlH_4$ and stored over Na-wire. Tetrahydrofuran was dried by distillation from $LiAlH_4$ directly

before use. Chloroform was passed through basic Al_2O_3 prior to use. DMF and DMSO were made anhydrous by distillation from CaH_2 at reduced pressure (≈10 mmHg) and stored over molecular sieves (4 Å).

NEP-Cl,[15] 4-methoxy-1-oxidopyridine-2-methanol[16–18] and diphenyl (triphenylphosphoranylidenemethyl)phosphonate,[9] were prepared by the published procedures. 3′-*O*-*tert*-Butyldiphenylsilylated nucleosides **1a**–**c** were synthesized analogously as described for thymidine[5] and di-(*o*-chlorophenyl) (triphenylphosphoranylidenemethyl)phosphonate, analogously to its diphenyl ester.[9] PADA was synthesised starting from azodicarboxamide in a one-step reaction according to Thiele's procedure.[19] Pyridine-2-aldoxime, tetrabutylammonium fluoride trihydrate, and triethylamine trishydrofluoride were commercial grades (Aldrich). 4-Methoxypyridine 1-oxide hydrate (Aldrich) was dried over P_2O_5 overnight at 70 °C at 0.2 mmHg, and 1,3-dicyclohexylcarbodiimide (Aldrich) and 1,1,3,3-tetramethylguanidine (Aldrich) were vacuum distilled. 1 M Triethylammonium bicarbonate buffer (pH ≈ 7) (TEAB) was prepared by passing carbon dioxide through an aqueous solution containing the appropriate amount of triethylamine. For column chromatography, silica gel (35–70 μm) from Amicon Europe was used, and the columns were run in the flash mode. All evaporations were carried out under reduced pressure using a rotary evaporator, unless stated otherwise.

Bis(*o*-chlorophenyl)[1-(3′-*O*-*tert*-butyldiphenylsilyl-2′,5′,6′-trideoxy-β-D-*erythro*-hex-5′-enofuranosyl)-4-*N*-isobutyrylcytosin-6′-yl]phosphonate 2a

To a solution of 3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxycytidine **1a** (3.54 g, 6.6 mmol) and 1,3-dicyclohexylcarbodiimide (4.09 g, 19.8 mmol) in dimethyl sulfoxide (25 mL) containing pyridine (0.53 mL, 6.6 mmol) was added trifluoroacetic acid (0.25 mL, 3.3 mmol) and the mixture was stirred for 20 h. After that time, bis(*o*-chlorophenyl) (triphenylphosphoranylidenemethyl)phosphonate (5.72 g, 9.9 mmol) was added. After 6 h the reaction was quenched by the careful addition of a solution of oxalic acid dihydrate (1.66 g, 13.2 mmol) in methanol (5 mL). The urea formed was removed *via* filtration and washed with toluene (150 mL). The combined filtrate and washings were washed with water (4 × 75 mL), dried over Na_2SO_4 and evaporated. The residue was purified using silica gel column chromatography with toluene–ethyl acetate (2:1) to afford the title compound as a white foam (3.87 g, 70%).

Some diagnostic spectral data: ^{31}P NMR, δ 12.2; ^{1}H NMR, δ 5.89 (1H, ddd, J 1.8, 17.2 and 22.0 Hz, H-6′), 6.41 (1H, t, J 6.6 Hz, H-1′), 6.62 (1H, ddd, J 4.8, 17.2 and 24.2 Hz, H-5′); ^{13}C NMR, δ 26.8 (3 × $CH_3^{t\text{-Bu}}$), 87.4 (C-1′).

Bis(*o*-chlorophenyl)[1-(3′-*O*-*tert*-butyldiphenylsilyl-2′,5′,6′-trideoxy-β-D-*erythro*-hexofuranosyl)-4-*N*-isobutyrylcytosin-6′-yl]phosphonate 3a

To vinylphosphonate **2a** (3.75 g, 4.5 mmol), coevaporated with and dissolved in pyridine (45 mL), was added potassium azodicarboxylate (3.50 g, 18.0 mmol), followed by a slow addition (90 min) of a mixture of acetic acid (5.15 mL, 90.0 mmol) in pyridine (5.2 mL). After stirring of the mixture overnight, another portion of acetic acid (2.57 mL, 45.0 mmol) in pyridine (2.6 mL) was added during 90 min. When the reaction was complete (TLC analysis) the formed precipitate was removed, the filtrate concentrated, and the residue partitioned between 0.5 M aq. $NaHCO_3$ (150 mL) and chloroform (2 × 150 mL). The combined organic phase was dried over Na_2SO_4, and evaporated with added toluene. The residue was coevaporated with pyridine, dissolved in *N*,*N*-dimethylformamide (25 mL) and, after addition of isobutyric anhydride (0.83 mL, 4.95 mmol), stirred for 3 h. The reaction mixture was taken up in toluene (250 mL) and washed with water (3 × 100 mL). The organic phase was dried over Na_2SO_4, evaporated and the residue was purified *via* silica gel column chromatography using a stepwise gradient of methanol (0–1%) in chloroform (2.81 g, 75%).

Some diagnostic spectral data: ^{31}P NMR, δ 26.7; ^{1}H NMR, δ 1.72–2.27 (5H, m, H-2′, H_2-5′, and H_2-6′), 6.28 (1H, t, J 6.4 Hz, H-1′); ^{13}C NMR, δ 23.2 (C-6′, d, J_{PC} 143.0 Hz), 87.0 (C-1′).

o-Chlorophenyl [1-(3′-*O*-*tert*-butyldiphenylsilyl-2′,5′,6′-trideoxy-β-D-*erythro*-hexofuranosyl)-4-*N*-isobutyrylcytosin-6′-yl]phosphonate, triethylammonium salt 4a

Phosphonate diester **3a** (0.77 g, 0.92 mmol) was dissolved in 1,4-dioxane–water (3:1 v/v; 10 mL) and pyridine-2-aldoxime (0.17 g, 1.4 mmol) and 1,1,3,3-tetramethylguanidine (0.17 mL, 1.4 mmol) were added. The reaction mixture was stirred for 5 min and then partitioned between 1 M aq. TEAB (40 mL) and chloroform (2 × 40 mL). The combined organic phase was evaporated and the residue was purified by silica gel column chromatography using a stepwise gradient of methanol (0–10%) in chloroform containing triethylamine (0.5%) (0.62 g, 82%).

Some diagnostic spectral data: ^{31}P NMR, δ 21.2; ^{1}H NMR, δ 3.00 (6H, q, J 7.3 Hz, 3 × CH_2^{TEAH}), 6.30 (1H, t, J 6.4 Hz, H-1′); ^{13}C NMR, δ 45.2 (3 × CH_2^{TEAH}), 87.6 (C-1′).

4-Methoxy-1-oxido-2-picolyl *o*-chlorophenyl [1-(3′-*O*-*tert*-butyldiphenylsilyl-2′,5′,6′-trideoxy-β-D-*erythro*-hexofuranosyl)-4-*N*-isobutyrylcytidin-6′-yl]phosphonate 5a

Phosphonate monoester **4a** (2.06 g, 2.5 mmol), 4-methoxy-1-oxidopyridine-2-methanol (0.43 g, 2.8 mmol) and 4-methoxypyridine 1-oxide (1.07 g, 7.5 mmol) were rendered anhydrous by evaporation of added pyridine and then dissolved in pyridine (25 mL). 2-Chloro-5,5-dimethyl-2-oxo-2λ^5-1,3,2-dioxaphosphinane (1.38 g, 7.5 mmol) was added and the reaction mixture was stirred for 1 h. The reaction mixture was concentrated, partitioned between 0.5 M aq. $NaHCO_3$ (75 mL) and chloroform (2 × 75 mL), and the combined organic layer was dried over Na_2SO_4. After evaporation under vacuum, the residue was subjected to column chromatography using a stepwise gradient of methanol (0–6%) in chloroform (1.65 g, 77%).

Some diagnostic spectral data: ^{31}P NMR, δ 31.4 and 31.3; ^{1}H NMR, δ 5.37 (2H, m, $CH_2^{\#}$), 6.30 (1H, m, H-1′); ^{13}C NMR, δ 62.4 ($CH_2^{\#}$), 86.7 and 87.0 (C-1′).

4-Methoxy-1-oxido-2-picolyl [1-(3′-*O*-*tert*-butyldiphenylsilyl-2′,5′,6′-trideoxy-β-D-*erythro*-hexofuranosyl)-4-*N*-isobutyrylcytosin-6′-yl]phosphonate, triethylammonium salt 6a

Phosphonate diester **5a** (1.55 g, 1.8 mmol) was dissolved in 1,4-dioxane–water (3:1 v/v; 20 mL) and then pyridine-2-aldoxime (0.33 g, 2.7 mmol) and 1,1,3,3-tetramethylguanidine (0.34 mL, 2.7 mmol) were added. After 1 h the reaction mixture was partitioned between 1 M aq. TEAB (75 mL) and chloroform (2 × 100 mL), and the combined organic phase was evaporated. The residue was purified by silica gel column chromatography using a stepwise gradient of methanol (0–20%) in chloroform containing triethylamine (0.5%) (1.11 g, 72%).

Some diagnostic spectral data: ^{31}P NMR, δ 23.4; ^{1}H NMR, δ 5.08 (2H, d, J_{PH} 7.3 Hz, $CH_2^{\#}$), 6.31 (1H, t, J 6.4 Hz, H-1′); ^{13}C NMR, δ 60.6 ($CH_2^{\#}$), 87.5 (C-1′).

4-Methoxy-1-oxido-2-picolyl {1-[2′,5′,6′-trideoxy-3′-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-hexofuranosyl]-4-*N*-isobutyrylcytosin-6′-yl}phosphonate, triethylammonium salt 7a

Phosphonate **6a** (1.02 g, 1.2 mmol) was dissolved in freshly distilled tetrahydrofuran (12 mL) and tetrabutylammonium fluoride trihydrate (1.51 g, 4.8 mmol) was added. After 2.5 h water (2 mL) was added, the reaction mixture was concentrated, and the residue partitioned between diethyl ether (40 mL) and water (40 mL). The aqueous layer was concentrated, the residue dissolved in water (20 mL) and the tetrabutyl-

ammonium ions were removed by passing the solution through an ion-exchange column (Dowex 50W, pyridinium form). The fractions containing the product were combined and lyophilized to afford the crude desilylated product. This material was made anhydrous by evaporation of added pyridine and dissolved in the same solvent (12 mL). 4-(Dimethylamino)pyridine (15 mg, 0.12 mmol) and 4,4′-dimethoxytrityl chloride (0.45 g, 1.3 mmol) were added and the reaction mixture was stirred at 70 °C for 6 h. After cooling of the product to room temperature, methanol (2 mL) was added and the reaction mixture was concentrated to near dryness. The residue was partitioned between 1 M aq. TEAB (50 mL) and chloroform (2 × 50 mL) and the combined organic phase was evaporated. The residue was purified using silica gel column chromatography with a stepwise gradient of methanol (0–25%) in chloroform containing triethylamine (0.5%) (0.58 g, 53%).

^{31}P NMR, δ 24.4; ^{1}H NMR, δ 1.17 (6H, d, J 6.4 Hz, 2 × CH_3^{ibu}), 1.27 (9H, t, J 7.3 Hz, 3 × CH_3^{TEAH}), 1.48–1.77 (5H, m, H-2′, H_2-5′ and H_2-6′), 2.20 (1H, m, H-2″), 2.58 (1H, septet, J 6.4 Hz, CH^{ibu}), 3.03 (6H, q, J 7.3 Hz, 3 × CH_2^{TEAH}), 3.75–3.81 (10H, m, 3 × CH_3O and H-4′), 4.00 (1H, m, H-3′), 5.10 (2H, d, J 7.0 Hz, $CH_2^{\#}$), 6.28 (1H, dd, J 5.9 and 8.2 Hz, H-1′), 6.73 (1H, dd, J 2.9 and 7.0 Hz, H-5$^{\#}$), 6.78–6.81 (4H, m, 4 × ArH), 7.15–7.43 (11H, m, H-3$^{\#}$, H-5 and 9 × ArH), 7.85 (1H, d, J 7.6 Hz, H-6), 8.14 (1H, d, J 7.6 Hz, H-6$^{\#}$), 8.61 (1H, br, NH), 12.88 (1H, br, TEAH); ^{13}C NMR, δ 8.7 (3 × CH_3^{TEAH}), 19.4 and 19.3 (2 × CH_3^{ibu}), 24.2 (C-6′, d, J_{PC} 138.1 Hz), 28.9 (C-5′), 36.2 (CH^{ibu}), 39.8 (C-2′), 45.5 (3 × CH_2^{TEAH}), 55.4 (2 × CH_3O), 56.4 (CH_3O), 60.8 ($CH_2^{\#}$), 77.2 (C-3′), 87.1 (C-4′, d, J_{PC} 21.3 Hz), 87.3 (C^{DMT}), 87.6 (C-1′), 97.0 (C-5), 108.9 (C-3$^{\#}$), 110.5 (C-5$^{\#}$), 140.2 (C-6$^{\#}$), 143.8 (C-6), 151.1 (C-2$^{\#}$, d, J 7.5 Hz), 155.4 (C-2), 159.1 (C-4$^{\#}$), 162.9 (C-4), 178.2 (C=O^{ibu}), 113.4, 127.2, 128.0, 128.4, 130.3, 130.4 (methine Cs of DMT), 136.3, 136.4, 145.2, 158.7, 158.8 (tertiary Cs of DMT); HRMS [M + Na]$^+$, Found: 837.2922. $C_{42}H_{47}N_4NaO_{11}P$ requires *m*/*z*, 837.2877.

Diphenyl [9-(3′-*O*-*tert*-butyldiphenylsilyl-2′,5′,6′-trideoxy-β-D-*erythro*-hex-5′-enofuranosyl)-2-*N*-isobutyrylguanin-6′-yl]-phosphonate 2b

3′-Silylated nucleoside **1b** (2.02 g, 3.5 mmol) was rendered anhydrous by evaporation of added pyridine and dissolved in dimethyl sulfoxide (15 mL). To this were added pyridine (0.28 mL, 3.5 mmol), trifluoroacetic acid (0.14 mL, 1.8 mmol) and 1,3-dicyclohexylcarbodiimide (2.17 g, 10.5 mmol) and the reaction mixture was stirred overnight. Diphenyl (triphenylphosphoranylidenemethyl)phosphonate (2.67 g, 5.3 mmol) was added and the stirring was continued for 7 h. The reaction was quenched by the careful addition of oxalic acid (0.88 g, 7.0 mmol) in methanol (5 mL). The urea formed was removed by filtration and washed with toluene (150 mL). The combined filtrate and washings were washed with water (4 × 100 mL), dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography using toluene–ethyl acetate (2 : 1 v/v) as eluent (2.10 g, 75%).

Some diagnostic spectral data: ^{31}P NMR, δ 14.0; ^{1}H NMR, δ 5.34 (1H, dd, J 17.6 and 23.1 Hz, H-6′), 6.35 (1H, dd, J 5.5 and 9.9 Hz, H-1′), 7.99 (1H, m, H-5′); ^{13}C NMR, δ 26.9 ($CH_3^{t\text{-}Bu}$), 88.1 (C-1′).

Diphenyl [9-(3′-*O*-*tert*-butyldiphenylsilyl-2′,5′,6′-trideoxy-β-D-*erythro*-hexofuranosyl)-2-*N*-isobutyrylguanin-6′-yl]-phosphonate 3b

Vinylphosphonate **2b** (2.01 g, 2.5 mmol) was made anhydrous by evaporation of added pyridine and dissolved in the same solvent (25 mL). To this was added potassium azodicarboxylate (1.94 g, 10.0 mmol) followed by a slow addition (80 min) of a mixture of acetic acid (2.86 mL, 50.0 mmol) in pyridine (2.86 mL). The mixture was stirred overnight and then filtered. The filtrate was partitioned between 0.5 M aq. $NaHCO_3$ and chloroform (2 × 100 mL), and the combined organic phase was dried over Na_2SO_4 and evaporated. The residue was coevaporated with added toluene and then subjected to purification by silica gel column chromatography using a stepwise gradient of methanol (0–2%) in chloroform (1.44 g, 71%).

Some diagnostic spectral data: ^{31}P NMR, δ 29.2; ^{1}H NMR, δ 1.55–2.36 (5H, m, H-2′, H_2-5′ and H_2-6′), 6.27 (1H, dd, J 5.1 and 9.9 Hz, H-1′); ^{13}C NMR, δ 21.4 (C-6′, d, J 141.2 Hz), 87.6 (C-1′).

Phenyl [9-(3′-*O*-*tert*-butyldiphenylsilyl-2′,5′,6′-trideoxy-β-D-*erythro*-hexofuranosyl)-2-*N*-isobutyrylguanin-6′-yl]-phosphonate, triethylammonium salt 4b

Phosphonate diester **3b** (1.37 g, 1.7 mmol) was dissolved in the 1,4-dioxane–water (3 : 1 v/v; 15 mL), and pyridine-2-aldoxime (0.62 g, 5.1 mmol) and 1,1,3,3-tetramethylguanidine (0.64 mL, 5.1 mmol) were added. The reaction mixture was stirred overnight and then partitioned between 1 M aq. TEAB (75 mL) and chloroform (2 × 75 mL). The combined organic phase was evaporated and the residue was subjected to purification by silica gel column chromatography using a stepwise gradient of methanol (0–10%) in chloroform containing triethylamine (0.5%) (1.11 g, 78%).

Some diagnostic spectral data: ^{31}P NMR, δ 21.8; ^{1}H NMR, δ 2.88 (6H, q, J 7.3 Hz, 3 × CH_2^{TEAH}), 6.23 (1H, dd, J 5.1 and 9.9 Hz, H-1′); ^{13}C NMR, δ 45.4 (3 × CH_2^{TEAH}), 87.6 (C-1′).

4-Methoxy-1-oxido-2-picolyl phenyl [9-(3′-*O*-*tert*-butyldiphenylsilyl-2′,5′,6′-trideoxy-β-D-*erythro*-hexofuranosyl)-2-*N*-isobutyrylguanin-6′-yl]phosphonate 5b

Phosphonate monoester **4b** (1.08 g, 1.3 mmol), 4-methoxy-1-oxidopyridine-2-methanol (0.22 g, 1.4 mmol) and 4-methoxypyridine 1-oxide (0.56 g, 3.9 mmol) were rendered anhydrous by evaporation of added pyridine and then dissolved in the same solvent (15 mL). 2-Chloro-5,5-dimethyl-2-oxo-2λ^5-1,3,2-dioxaphosphinane (0.72 g, 3.9 mmol) was added and the reaction mixture was stirred for 1 h. Concentration, partitioning between 0.5 M aq. $NaHCO_3$ (75 mL) and chloroform (2 × 75 mL), and evaporation to dryness afforded a residue, which was purified using silica gel column chromatography with a stepwise gradient of methanol (0–6%) in chloroform (1.07 g, 95%).

Some diagnostic spectral data: ^{31}P NMR, δ 32.8 and 33.2; ^{1}H NMR, δ 5.14–5.45 (2H, m, $CH_2^{\#}$), 6.19–6.26 (1H, m, H-1′); ^{13}C NMR, δ 62.0 and 62.3 ($CH_2^{\#}$, d, J_{PC} 5.5 Hz and d, J_{PC} 5.5 Hz), 87.1 and 87.2 (C-1′).

4-Methoxy-1-oxido-2-picolyl [9-(3′-*O*-*tert*-butyldiphenylsilyl-2′,5′,6′-trideoxy-β-D-*erythro*-hexofuranosyl)-2-*N*-isobutyrylguanin-6′-yl]phosphonate, triethylammonium salt 6b

Phosphonate diester **5b** (1.04 g, 1.2 mmol) was dissolved in 1,4-dioxane–water (3 : 1 v/v; 12 mL) and pyridine-2-aldoxime (0.44 g, 3.6 mmol) and 1,1,3,3-tetramethylguanidine (0.45 mL, 3.6 mmol) were added. The reaction mixture was stirred for 6 h and then partitioned between 1 M aq. TEAB (75 mL) and chloroform (2 × 75 mL). The combined organic phase was evaporated and the residue was purified by silica gel column chromatography using a stepwise gradient of methanol (0–25%) in chloroform containing triethylamine (0.5%) (0.82 g, 76%).

Some diagnostic spectral data: ^{31}P NMR, δ 25.1; ^{1}H NMR, δ 5.04 (2H, J 7.0 Hz, $CH_2^{\#}$), 6.21 (1H, dd, J 3.7 and 7.1 Hz, H-1′); ^{13}C NMR, δ 60.5 ($CH_2^{\#}$), 87.5 (C-1′).

4-Methoxy-1-oxido-2-picolyl {9-[2′,5′,6′-trideoxy-3′-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-hexafuranosyl]-2-*N*-isobutyrylguanin-6′-yl}phosphonate, triethylammonium salt 7b

Phosphonate **6b** (0.80 g, 0.90 mmol) was dissolved in freshly distilled tetrahydrofuran (10 mL) containing tetrabutylammonium fluoride trihydrate (1.14 g, 3.6 mmol) and the reaction

mixture was stirred for 5.5 h. The reaction was quenched by the addition of water (1 mL) and the mixture was concentrated. The residue was taken up into water (30 mL) and washed with diethyl ether (30 mL). The removal of tetrabutylammonium ions from the reaction mixture was carried out as described above for **7a**. The obtained crude product was coevaporated with pyridine and then dissolved in pyridine (10 mL). 4,4′-Dimethoxytrityl chloride (0.34 g, 0.99 mmol) and 4-(dimethylamino)pyridine (11 mg, 0.090 mmol) were added and the reaction mixture was stirred at 70 °C overnight, allowed to attain room temperature, and then methanol (1 mL) was added. The reaction mixture was concentrated and partitioned between 1 M aq. TEAB (50 mL) and chloroform (2 × 50 mL). Product **7b** was obtained by silica gel column chromatography using a stepwise gradient of methanol (0–25%) in chloroform (0.35 g, 41%).

^{31}P NMR, δ 22.7; ^{1}H NMR, δ 1.14 (1H, d, J 7.0 Hz, CH_3^{ibu}), 1.09 (3H, d, J 6.3 Hz, CH_3^{ibu}), 1.27 (9H, t, J 7.4 Hz, 3 × CH_3^{TEAH}), 1.34–2.27 (5H, m, H-2′, H_2-5′ and H_2-6′), 2.48 (1H, m, H-2″), 2.97 (6H, q, J 7.4 Hz, 3 × CH_2^{TEAH}), 3.07 (1H, m, CH^{ibu}), 3.77 (6H, s, 2 × CH_3O), 3.78 (3H, s, CH_3O), 3.96 (1H, m, H-4′), 4.13 (1H, m, H-3′), 5.07 (2H, d, J 4.7 Hz, $CH_2^{\#}$), 6.11 (1H, dd, J 5.2 and 8.6 Hz, H-1′), 6.74 (1H, m, H-5$^{\#}$), 6.81 (4H, m, 4 × ArH), 7.11 (1H, m, H-3$^{\#}$), 7.19–7.53 (9H, m, 9 × ArH), 7.58 (1H, s, H-8), 8.08 (1H, d, J 7.4 Hz, H-6$^{\#}$), 12.42 (1H, br, NH), 12.61 (1H, br, NH), 12.89 (1H, br, NH^{TEAH}); ^{13}C NMR, δ 8.8 (3 × CH_3^{TEAH}), 18.9 and 20.0 (2 × CH_3^{ibu}), 23.4 (C-6′, d, J 136.3 Hz), 29.2 (C-5′), 35.1 (CH^{ibu}), 37.2 (C-2′), 45.8 (3 × CH_2^{TEAH}), 55.5 (2 × CH_3O), 56.3 (CH_3O), 60.8 ($CH_2^{\#}$), 78.4 (C-3′), 87.9 (C-4′, d, J 17.4 Hz), 87.5 (C^{DMT}), 87.7 (C-1′), 108.3 (C-3$^{\#}$), 110.4 (C-5$^{\#}$), 123.1 (C-5), 139.0 (C-8), 139.8 (C-6$^{\#}$), 148.4 (C-2 and C-4), 151.3 (C-2$^{\#}$), 156.2 (C-6), 158.5 (C-4$^{\#}$), 181.6 (C=O^{ibu}), 113.4, 113.5, 127.3, 128.2, 128.5, 130.2, 130.4 (methine Cs of DMT), 136.4, 136.6, 145.3, 158.9, 159.0 (tertiary Cs of DMT); HRMS [M + Na]$^{+}$, Found: 877.3007. $C_{43}H_{47}N_4NaO_{11}P$ requires m/z, 877.2938.

Diphenyl [9-(3′-*O*-*tert*-butyldiphenylsilyl-2′,5′,6′-trideoxy-β-D-*erythro*-hex-5-enofuranosyl)-6-*N*-benzoyladenin-6′-yl]phosphonate 2c

3′-Silylated nucleoside **1c** (5.64 g, 9.5 mmol) was rendered anhydrous by evaporated of added pyridine and dissolved in dimethyl sulfoxide (40 mL). To this were added 1,3-dicyclohexylcarbodiimide (5.88 g, 28.5 mmol), pyridine (0.77 mL, 9.5 mmol) and trifluoroacetic acid (0.37 mL, 4.8 mmol) and the reaction mixture was stirred for 7 h. Diphenyl (triphenylphosphoranylidenemethyl)phosphonate was added and the stirring was continued overnight. Oxalic acid (2.40 g, 19.0 mmol) as a solution in methanol (10 mL) was added carefully. The urea formed was removed *via* filtration, washed with toluene (200 mL) and the combined organic phases were washed with water (4 × 100 mL), dried over Na_2SO_4 and evaporated. The residue was purified using silica gel column chromatography with toluene–ethyl acetate (2:1, v/v) as eluent (6.07 g, 78%).

Some diagnostic spectral data: ^{31}P NMR, δ 11.6; ^{1}H NMR, δ 5.95 (1H, ddd, J 1.8, 16.9 and 21.6, H-6′), 6.55–6.77 (2H, m, H-1′ and -5′); ^{13}C NMR, δ 26.9 (3 × $CH_3^{t\text{-}Bu}$), 85.3 (C-1′).

Diphenyl [9-(3′-*O*-*tert*-butyldiphenylsilyl-2′,5′,6′-trideoxy-β-D-*erythro*-hexafuranosyl)-6-*N*-benzoyladenin-6′-yl]phosphonate 3c

To vinylphosphonate **2c** (3.12 g, 3.8 mmol), coevaporated with added pyridine and dissolved in the same solvent (40 mL), was added potassium azodicarboxylate (2.95 g, 15.2 mmol), followed by a slow addition (135 min) of a mixture of acetic acid (4.35 mL, 76.0 mmol) in pyridine (4.35 mL). The reaction mixture was left for 3 h and then an additional portion of acetic acid (4.35 mL, 15.2 mmol) in pyridine (4.35 mL) was added dropwise during 30 min. After an additional 30 min, the reaction mixture was filtered, concentrated and partitioned between saturated aq. $NaHCO_3$ (150 mL) and chloroform (2 × 150 mL). The combined organic phase was dried over Na_2SO_4 and concentrated. The residue was coevaporated with toluene and then purified by silica gel column chromatography using a stepwise gradient of methanol (0–2%) in chloroform (2.74 g, 88%).

Some diagnostic spectral data: ^{31}P NMR, δ 26.5; ^{1}H NMR, δ 1.82–2.05 (4H, m, H_2-5′ and H_2-6′), 6.44 (1H, t, J 6.8 Hz, H-1′); ^{13}C NMR, δ 22.4 (C-6′, d, J_{PC} 144.8 Hz), 84.9 (C-1′).

Phenyl [9-(3′-*O*-*tert*-butyldiphenylsilyl-2′,5′,6′-trideoxy-β-D-*erythro*-hexafuranosyl)-6-*N*-benzoyladenin-6′-yl]phosphonate, triethylammonium salt 4c

To phosphonate diester **3c** (2.72 g, 3.3 mmol) dissolved in 1,4-dioxane–water (3:1 v/v; 30 mL) were added pyridine-2-aldoxime (1.21 g, 9.9 mmol) and 1,1,3,3-tetramethylguanidine (1.24 mL, 9.9 mmol) were added. The mixture was stirred for 7 h, partitioned between 1 M aq. TEAB (100 mL) and chloroform (2 × 100 mL), and the combined organic phase was evaporated. The residue was subjected to silica gel column chromatography using a stepwise gradient of methanol (0–14%) in chloroform (2.28 g, 81%).

Some diagnostic spectral data: ^{31}P NMR, δ 19.9; ^{1}H NMR, δ 2.95 (6H, q, J 7.3 Hz, 3 × CH_2^{TEAH}), 6.52 (1H, t, J 7.0 Hz, H-1′); ^{13}C NMR, δ 45.8 (3 × CH_2^{TEAH}), 85.2 (C-1′).

4-Methoxy-1-oxido-2-picolyl phenyl [9-(3′-*O*-*tert*-butyldiphenylsilyl-2′,5′,6′-trideoxy-β-D-*erythro*-hexafuranosyl)-6-*N*-benzoyladenin-6′-yl]phosphonate 5c

Phosphonate monoester **4c** (2.21 g, 2.6 mmol), 4-methoxy-1-oxidopyridine-2-methanol (0.44 g, 2.9 mmol) and 4-methoxypyridine 1-oxide (1.12 g, 7.8 mmol) were rendered anhydrous by evaporation of added pyridine and then dissolved in the same solvent (25 mL). 2-Chloro-5,5-dimethyl-2-oxo-2λ^5-1,3,2-dioxaphosphinane (1.44 g, 7.8 mmol) was added, and the reaction mixture was stirred for 3 h, and then partitioned between 0.5 M aq. $NaHCO_3$ (150 mL) and chloroform (2 × 100 mL). The organic layer was dried over Na_2SO_4, evaporated, and the residue was subjected to silica gel column chromatography using a stepwise gradient of methanol (0–5%) in chloroform (2.26 g, 98%).

Some diagnostic spectral data: ^{31}P NMR, δ 30.8 and 30.9; ^{1}H NMR, δ 5.22–5.28 (2H, m, $CH_2^{\#}$), 6.42–6.49 (1H, m, H-1′); ^{13}C NMR, δ 62.2 ($CH_2^{\#}$), 84.8 and 84.9 (C-1′).

4- Methoxy-1-oxido-2-picolyl [9-(3′-*O*-*tert*-butyldiphenylsilyl-2′,5′,6′-trideoxy-β-D-*erythro*-hexafuranosyl)-6-*N*-benzoyladenin-6′-yl]phosphonate, triethylammonium salt 6c

To phosphonate diester **5c** (2.21 g, 2.5 mmol), dissolved in 1,4-dioxane–water (3:1 v/v; 20 mL), were added pyridine-2-aldoxime (0.92 g, 7.5 mmol) and 1,1,3,3-tetramethylguanidine (0.94 mL, 7.5 mmol). The reaction mixture was stirred for 6.5 h, concentrated, and the residue was partitioned between 1 M aq. TEAB (100 mL) and chloroform (2 × 100 mL). The combined organic phase was evaporated and the residue was purified by silica gel column chromatography using a stepwise gradient of methanol (0–25%) in chloroform (1.87 g, 82%).

Some diagnostic spectral data: ^{31}P NMR, δ 25.8; ^{1}H NMR, δ 5.05 (2H, d, J 7.0 Hz, $CH_2^{\#}$), 6.54 (1H, t, J 6.8 Hz, H-1′); ^{13}C NMR, δ 60.7 ($CH_2^{\#}$, d, J_{PC} 3.7 Hz), 84.5 (C-1′).

4-Methoxy-1-oxido-2-picolyl {9-[2′,5′,6′-trideoxy-3′-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-hexafuranosyl]-6-*N*-benzoyladenin-6′-yl}phosphonate, triethylammonium salt 7c

To phosphonate **6c** (0.51g, 0.56 mmol), coevaporated twice with added acetonitrile (20 mL) and then dissolved in freshly distilled tetrahydrofuran (3 mL), was added triethylamine trishydrofluoride (0.36 mL, 2.2 mmol), and the reaction mixture

was stirred for 48 h. After addition to diethyl ether–acetonitrile (3:1 v/v; 200 mL) at −70 °C, the formed precipitate was collected, evaporated twice with added acetonitrile, and dissolved in a mixture of tetrahydrofuran–dimethylformamide (1:1 v/v; 10 mL). To this were added $AgNO_3$ (0.23 g, 1.3 mmol) and 4,4′-dimethoxytrityl chloride (0.45 g, 1.3 mmol) and the reaction mixture was kept in the dark overnight. Methanol (0.5 mL) was added, the reaction mixture was stripped of low boiling contents, and the residue was filtered through a pad of Celite and washed with methylene dichloride (100 mL). The organic phase was washed with 1 M aq. TEAB (100 mL) and then the aqueous layer was extracted once with methylene dichloride (100 mL). The organic layers were combined and evaporated, and the residue was purified using silica gel column chromatography with a stepwise gradient of methanol (0–20%) in methylene dichloride containing triethylamine (0.5%) (0.36 g, 67%).

^{31}P NMR, δ 26.3; ^{1}H NMR, δ 1.25 (9H, t, J 7.6 Hz, $3 \times CH_3^{TEAH}$), 1.43–1.91 (5H, m, H-2′, H_2-5′ and H_2-6′), 2.20–2.29 (1H, m, H-2″), 3.00 (6H, q, J 7.6 Hz, $3 \times CH_2^{TEAH}$), 3.76–3.79 (9H, m, $3 \times CH_3O$), 5.08 (2H, d, J 7.0 Hz, $CH_2^{\#}$), 6.45 (1H, dd, J 8.2 and 5.9 Hz, H-1′), 6.69 (1H, dd, J 2.9 and 7.0 Hz, H-5$^{\#}$), 6.82–6.85 (1H, m, 4 × ArH), 7.18–7.59 (13H, m, H-3$^{\#}$ and 12 × ArH), 8.02–8.08 (3H, m, 2 × ArH and H-6$^{\#}$), 8.12 and 8.72 (2H, 2s, H-8 and H-2), 9.47 (1H, br, NH), 12.8 (1H, br, TEAH); ^{13}C NMR, δ 8.7 ($3 \times CH_3^{TEAH}$), 23.8 (C-6′, d, J_{PC} 137.0 Hz), 28.8 (C-5′), 38.6 (C-2′), 45.5 ($3 \times CH_2^{TEAH}$), 55.4 ($2 \times CH_3O$), 56.4 (CH_3O), 60.8 ($CH_2^{\#}$), 77.4 (C-3′), 84.7 (C-1′), 87.0 (C-4′, d, J_{PC} 17.3 Hz), 87.4 (C^{DMT}), 108.7 (C-3$^{\#}$), 110.5 (C-5$^{\#}$), 124.1 (C-5), 139.9 (C-6$^{\#}$), 141.7 (C-8), 150.0 (C-4), 151.3 (C-2$^{\#}$, d, J 7.5 Hz), 151.9 (C-6), 152.4 (C-2), 159.1 (C-4$^{\#}$), 165.7 ($C{=}O^{bz}$), 113.4, 113.5, 127.2, 128.1, 128.3, 128.4, 128.7, 130.3, 130.4, 132.6 (methine Cs of Ar), 133.9, 136.2, 136.4, 145.2, 158.8, 158.9 (tertiary Cs of Ar); HRMS $[M + Na]^+$, Found: 895.2899. $C_{46}H_{45}N_6NaO_{10}P$ requires m/z, 895.2833.

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