Synthesis and antiviral evaluation of novel conformationally locked nucleosides and masked 5′-phosphate derivatives thereof

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As part of a programme towards evaluating the potential of conformationally locked 3′-deoxy- and 3′-azido-3′-deoxy-nucleoside derivatives as prodrugs of potential 5′-*O*-triphosphorylated anti-HIV drugs, novel nucleoside derivatives with locked *N*-type (*north*-type, C3′-*endo*) furanose conformation were prepared using convergent synthetic strategies. In addition, masked 5′-monophosphate derivatives of these, and of a conformationally restricted 3′-azido-3′-deoxynucleoside with *E*-type (*eastern*-type, O4′-*endo*) furanose conformation, were prepared in order to potentially circumvent the first phosphorylation step. However, neither the free 5′-hydroxy derivatives nor the masked 5′-monophosphates showed anti-HIV activity in MT-4 cells.

Introduction

In the treatment of HIV infection, 2′,3′-dideoxynucleosides such as 2′,3′-dideoxycytidine (ddC), 2′,3′-dideoxyinosine (ddI), 2′,3′-didehydro-3′-deoxythymidine (d4T) and 3′-azido-3′-deoxythymidine (AZT) (Fig. 1) are important drugs used in either monotherapy or in combination therapy.[1] These nucleosides are prodrugs of the active nucleoside triphosphates (NTPs) and therefore need to be triphosphorylated *in vivo* before interacting with the HIV-encoded enzyme HIV-1 reverse transciptase (HIV-1 RT). Unfortunately, their use is correlated with toxic side effects and the development of drug-resistant viruses. Therefore, there is a need for improved new anti-HIV drugs and a deeper understanding of the pharmacological effects of the different nucleoside analogues.

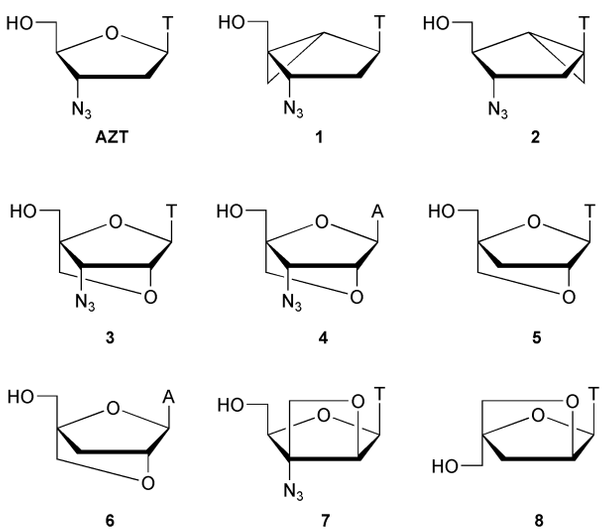

Fig. 1 Structures of AZT and conformationally locked bicyclic nucleoside analogues **1**–**8**. T = thymin-1-yl. A = adenin-9-yl.

Molecular modelling studies based on the crystal structure of HIV-1 RT complexed with AZT[2] and studies of conformationally restricted NTP analogues (Fig. 1; derivatives **1** and **2**) have shown that the polymerase domain of HIV-1 RT preferentially binds NTPs adopting an *N*-type (*north*-type; C3′-*endo*) furanose conformation (Fig. 2).[3,4] Analogues of AZT and other anti-HIV-active nucleosides with a locked *N*-type furanose conformation are therefore promising drug candidates to be studied. Marquez *et al.* showed that the bicyclic nucleoside **1** with a fixed *N*-type furanose conformation was devoid of activity whereas the corresponding 5′-triphosphorylated derivative showed activity similar to that of AZT (as an inhibitor of HIV-1 RT).[3] In contrast, the corresponding AZT analogue **2** with a locked *S*-type (*south*-type; C3′-*exo*) furanose conformation showed no activity either as the free nucleoside **2** or as the corresponding 5′-*O*-triphosphorylated derivative.[3] It was therefore suggested that the lack of activity of analogue **1** could be caused by the inability of kinases to convert

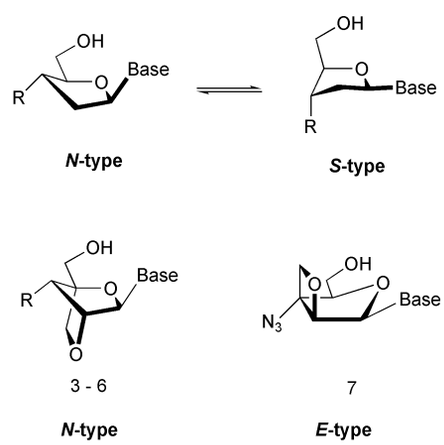

Fig. 2 Conformational equilibrium of a 2′,3′-dideoxynucleoside, the locked *N*-type furanose conformation of nucleoside analogues **3**–**6**, and the preferred *E*-type furanose conformation of AZT analogue **7**. For a detailed description of furanose conformations and the pseudorotational cycle the reader is referred to, *e.g.*, refs 3, 4, 7 and 8.

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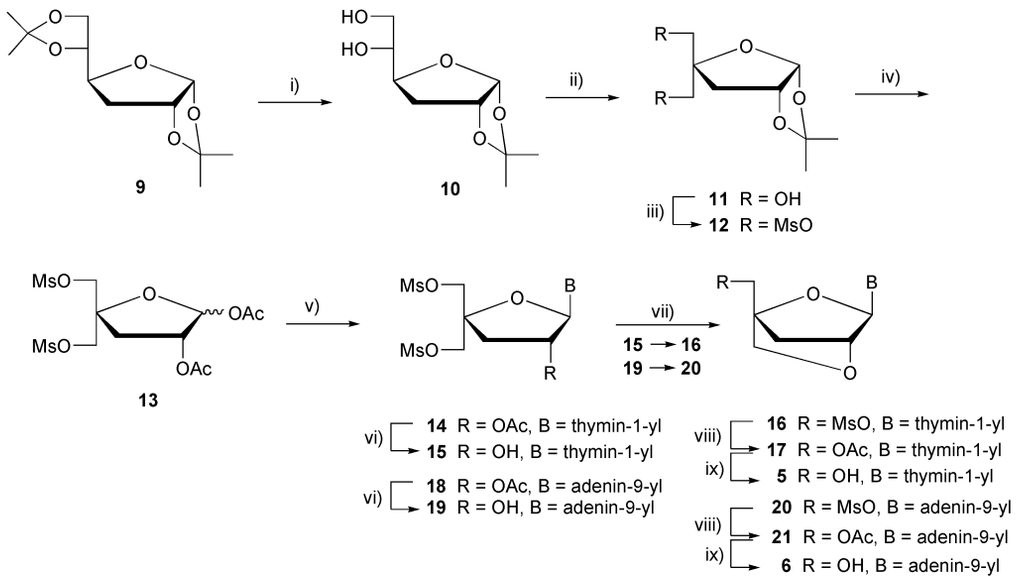


Scheme 1 *Reagents and conditions (and yields)*: i) 80% AcOH; ii) a) $NaIO_4$, H_2O, THF, b) CH_2O, NaOH, 1,4-dioxane; iii) MsCl, pyridine; iv) a) 80% TFA, b) Ac_2O, pyridine; v) a) thymine, *N,O*-bis(trimethylsilyl)acetamide, TMS triflate, acetonitrile (23% from **9**) or b) adenine, $SnCl_4$, acetonitrile; vi) saturated methanolic ammonia; vii) NaH, DMF [B = thymin-1-yl (64%, 2 steps); B = adenin-9-yl]; viii) KOAc, 18-crown-6, 1,4-dioxane [B = thymin-1-yl (66%), B = adenin-9-yl]; ix) saturated methanolic ammonia [B = thymin-1-yl (65%), B = adenin-9-yl (3% from **9**)].

nucleosides with locked *N*-type conformation into the corresponding 5′-*O*-triphosphorylated derivatives[3] which requires three sequential enzymatic steps involving different cellular kinases.[5] Accordingly, further examples of non-phosphorylated free nucleoside analogues of AZT with a locked or restricted furanose conformation (Fig. 1; derivatives **3** and **7**) have been shown to be devoid of anti-HIV activity.[6,7]

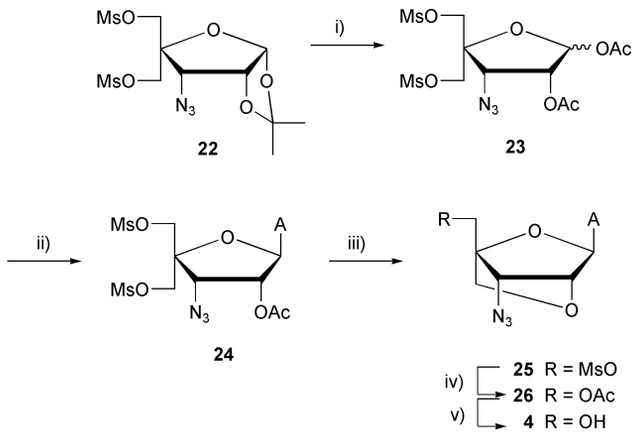
To further evaluate the relation between furanose conformation and anti-HIV activity, we describe herein synthesis of analogues of known anti-HIV active nucleosides with the furanose moieties locked in the *N*-type (Fig. 1; derivatives **3**,[6,9] **4**, **5**, **6** and **8**) or in the *E*-type (*east*-type; O4′-*endo*) (Fig. 1; derivative **7**[7]) furanose conformation. The 3′-deoxy-L-nucleoside analogue **8** (Fig. 1), also with locked *N*-type furanose conformation, was included in this study as a 3′-hydroxy derivative of **8** has been shown to structurally mimic the corresponding D-*ribo*-configured nucleoside.[10] In addition, we have synthesized masked 5′-monophosphate derivatives of nucleosides **3**, **4**, **5** and **7** in order to study if the first phosphorylation step is the major obstacle for *in vivo* activity of these derivatives. The masked monophosphates prepared are of the cyclosal-type introduced Meier *et al.*[11,12] and of the aryloxy phosphoramidate-type introduced by McGuigan *et al.*,[13–16] both of which have been successfully applied to other nucleosides being inactive unless masked.[11–16] We decided to prepare these novel compounds despite the results of an interesting molecular dynamics study indicating that a substituent in the 2′-α-position of a nucleoside NTP (*e.g.*, a hydroxy or a fluoro substituent) is likely to collide with a tyrosine moiety of the active site in HIV-1 RT, thereby preventing efficient binding and thus anti-HIV activity.[4] However, the 2′-deoxy-2′-fluororibonucleotide used in this molecular dynamics study[4] preferentially adopts a twisted C2′-*exo*-C3′-*endo* conformation which, though of the *N*-type, differs from the locked envelope-type C3′-*endo* furanose conformation of nucleosides **3**–**6**.

Results and discussion

In order to prepare the conformationally locked 3′-deoxynucleosides **5** and **6** (Scheme 1) we chose as the first step to remove the 3-hydroxy group of 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose to give the 3-deoxyfuranose **9** as described earlier.[17] Preparation of derivative **12** *via* **10** and **11** was accomplished by hydrolysis of the primary acetonide, oxidative cleavage of the generated diol, aldol reaction and Cannizzaro reaction,[18,19] and finally mesylation of the two primary hydroxy groups. The key intermediate **13** was obtained by cleavage of the isopropylidene group using 80% trifluoroacetic acid (TFA) followed by acetylation with acetic anhydride in pyridine. Coupling between furanose **13** and thymine by standard Vorbrüggen-type reaction[20] using trimethylsilyl triflate as Lewis acid and *N,O*-bis(trimethylsilyl)acetamide as silylating agent provided stereoselectively the thymine nucleoside **14** in 23% yield (from **9**). The acetyl group of **14** was removed by treatment with a mixture of saturated methanolic ammonia and methanol (1 : 2, v/v) to give nucleoside **15**, which was converted into the bicyclic derivative **16** using NaH (64% yield from **14**). The remaining methylsulfonyloxy group was replaced by an acetate group by treatment of nucleoside **16** with a mixture of potassium acetate and 18-crown-6 in 1,4-dioxane under reflux, affording nucleoside **17** in 66% yield. Deacetylation by treatment with a mixture of saturated methanolic ammonia and methanol (2 : 1, v/v) yielded the target bicyclic nucleoside **5** in 65% yield. The applicability of di-*O*-(methylsulfonyl)furanose intermediates for the synthesis of locked nucleic acid (LNA)-type nucleosides has been demonstrated earlier.[21,22]

Coupling of furanose **13** with adenine using $SnCl_4$ in acetonitrile afforded nucleoside **18**. As expected from the presence of the 2-acetoxy group in **13**, the Vorbrüggen-type coupling reaction also, in this case, proceeded stereoselectively with no indication of the formation of the anomeric nucleoside. It was possible to assign the obtained product as the N^9-regioisomer from the ^{13}C NMR data [δ_C 155.55 (C-6), 152.95 (C-2), 148.86 (C-4), 139.7 (C-8) and 120.04 (C-5)] and comparison of these with literature data.[23] The target adenine 3′-deoxynucleoside **6** was subsequently obtained in 3% yield (9 steps from **9**) *via* derivatives **19**–**21** using similar procedures as described above for the corresponding thymine 3′-deoxynucleoside **5**.

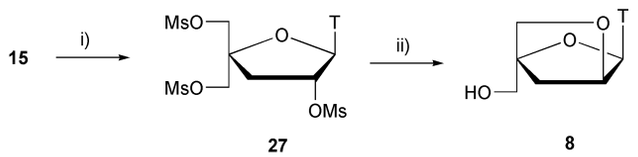
Synthesis of 1-(3-azido-3-deoxy-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)thymine **3**[6,9] was accomplished using a published procedure.[6] However, for the synthesis of 9-(3-azido-3-deoxy-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)adenine (**4**; Scheme 2) we found it more convenient to employ the furanoside **22** (with methylsulfonyloxy groups instead of benzoyl groups[6]) for the coupling reaction with adenine, and we used the known method for synthesis of the di-*O*-methylsulfonyl derivative **22**.[24] Treatment of **22** with 80% TFA to remove the isopropylidene group,



Scheme 2 *Reagents and conditions (and yields)*: i) a) 80% TFA, b) Ac_2O, pyridine (90%, 2 steps); ii) adenine, $SnCl_4$, acetonitrile, 60 °C (35%); iii) sat. methanolic ammonia; iv) 18-crown-6, KOAc, 1,4-dioxane (91%, 2 steps); v) saturated methanolic ammonia (79%).

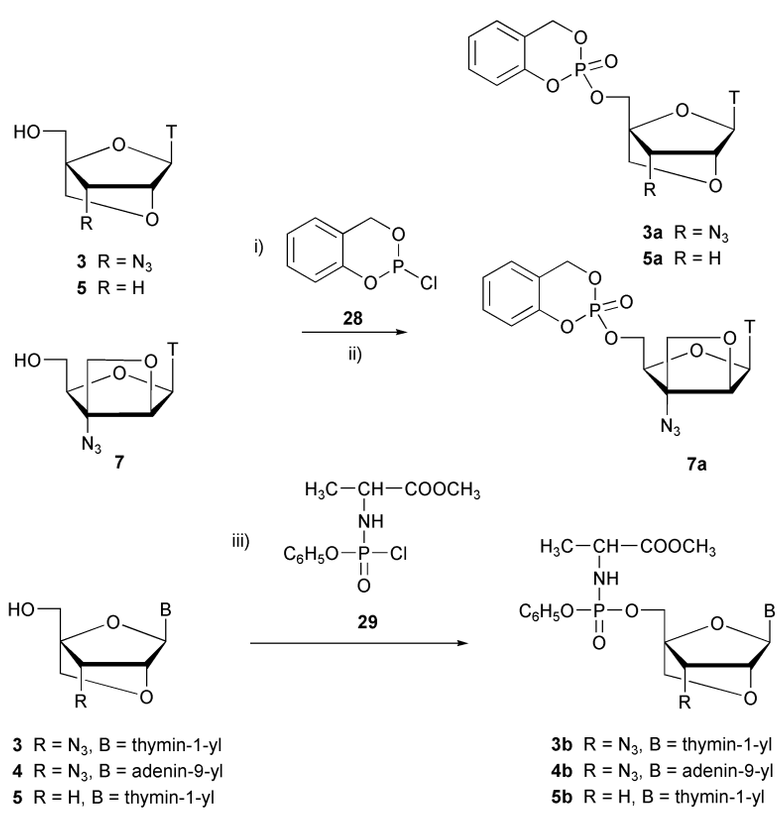
followed by acetylation using acetic anhydride in pyridine, afforded furanose **23** in 90% yield (2 steps). Coupling with adenine was performed under the same conditions as described above for nucleoside **18**, furnishing nucleoside **24** in limited (35%) yield. The product was subsequently dissolved in saturated methanolic ammonia. After 3 h, two reaction products were detected by analytical TLC, which were tentatively assigned as the desired ring-closed nucleoside **25** and the 2-*O*-deacetylated derivative of **24**. When the reaction mixture was instead stirred for three days only one product was observed according to analytical TLC. This product was not purified, but on the basis of NMR of the crude product it was identified as nucleoside **25**, and a subsequent substitution reaction using potassium acetate as described above (synthesis of nucleosides **17** and **21**) afforded nucleoside **26** in 91% yield (from **24**). Eventually, 5′-*O*-deacetylation by treatment with saturated methanolic ammonia furnished the target adenine 3′-azido-3′-deoxynucleoside **4** in 79% yield.

We also prepared the 3′-deoxynucleoside derivative **8**, a stereoisomer of derivative **5** (Scheme 3). Thus, the tri-*O*-(methylsulfonyl)nucleoside **27** was prepared by methylsulfonylation of derivative **15** using 2.6 equivalents of methylsulfonyl chloride in pyridine. Compound **27** was subsequently dissolved in a mixture of 1,4-dioxane and 1 M NaOH and heated at 90 °C for 18 h whereupon the thymine 3′-deoxyderivative **8** was isolated in 15% yield (from **14** *via* **15**), implying C2′-inversion, hydrolysis of the putative 2′-*C*,2-*O*-anhydro intermediate, cyclization, and conversion of the remaining methylsulfonyloxy group into a hydroxy group in a one pot reaction. We have previously reported similar cascade-type reactions during synthesis of stereoisomeric LNA nucleosides.[21]



Scheme 3 *Reagents and conditions (and yields)*: i) MsCl, pyridine; ii) 1 M NaOH, 1,4-dioxane (15% from **14** *via* **15**).

As none of the 5′-hydroxynucleosides **3**–**7** showed activity against HIV-1 (*vide infra*), we decided to synthesize masked 5′-monophosphate derivatives (Scheme 4) in order to investigate if the first phosphorylation step towards the corresponding 5′-triphosphates is the major obstacle for anti-HIV activity of these locked nucleosides. We chose two different strategies, namely the 'cyclosal approach' developed by Meier *et al.*[11,12] and the 'aryloxy phosphoramidate approach' developed McGuigan *et al.*,[13–15] both of which have previously been used successfully. The cyclosal approach allows release of the nucleoside 5′-monophosphate inside the cell by chemical cleavage of the cyclosal group functioning as a phosphate-protecting



Scheme 4 *Reagents and conditions (and yields)*: i) 2-chloro-4*H*-1,3,2-benzodioxaphosphorine **28**, DIPEA, acetonitrile; 0 °C; ii) *tert*-butyl hydroperoxide (yields **3a**: 34%, **5a**: 30%, **7a**: 58%, 2 steps); iii) phenyl methoxyalaninylphosphorochloridate **29**, *N*-methylimidazole, THF (**3b**: 38%, **4b**: 16%, **5b**: 22%).

group during penetration into the cell. The procedure used for synthesis of derivatives **3a**, **5a** and **7a** (Scheme 4) was adopted from the literature.[11] Thus, the relevant nucleoside was dissolved in acetonitrile containing *N,N*-diisopropylethylamine (DIPEA), and the phosphorylating reagent 2-chloro-4*H*-1,3,2-benzodioxaphosphorine (**28**)[11] was added followed after 1 h by addition of *tert*-butyl hydroperoxide to give, after troublesome column chromatographic purification, the masked 5′-monophosphate derivatives **3a** (34% yield), **5a** (30% yield) and **7a** (58% yield) as diastereoisomeric mixtures.

Many different derivatives of the aryloxy phosphoramidate-type of masked 5′-monophosphates have been prepared, *e.g.*, by introducing substituents into the phenyl moiety or by changing the amino acid involved.[14,15] They have been reported to be enzymatically cleaved inside the cell, liberating the free 5′-monophosphates.[13] We chose to synthesize the masked monophosphate derivatives **3b**, **4b** and **5b** (Scheme 4) containing the parent phosphoramidate group because of their relatively straightforward synthesis. The reagent phenyl methoxyalaninylphosphorochloridate (**29**)[13,25] was treated with nucleosides **3**, **4** and **5** in THF to give the target molecules **3b** (38%), **4b** (16%) and **5b** (22%) as diastereoisomeric mixtures.

The bicyclic nucleosides **3a**, **3b**, **4**, **4b**, **5**, **5a**, **5b**, **6**, **7a** and **8** were evaluated for antiviral activity against HIV-1 in MT-4 cells as described earlier.[26] All compounds were inactive against HIV-1 at 300 μM. Earlier, nucleosides **3** and **7** likewise tested inactive against HIV-1.[6,7] These results suggest that the parent nucleosides and/or the nucleoside 5′-monophosphates and/or the nucleoside diphosphates are not substrates for nucleoside kinases, and/or that the nucleoside triphosphates are not recognized by HIV-1 RT. However, as the 5′-triphosphate of the *N*-type mimicking AZT analogue **1** is active against HIV-1,[3] a possible explanation for the lack of activity for the *ribo*-configured derivatives **3**, **3a**, **3b**, **4**, **4b**, **5**, **5a**, **5b** and **6** is unfavourable steric interactions at the acitve site in HIV-1 RT preventing efficient binding and thus anti-HIV activity, as shown before for other *ribo*-configured nucleosides.[4] Likewise, the inactivity of the *E*-type nucleoside **7** and the derivatives **7a** and **8** suggests structural incompatibility between the core bicyclic structures of these nucleosides and the active site of one or more of the relevant enzymes.

Conclusions

The synthesis of a series of novel conformationally locked 3′-deoxy- and 3′-azido-3′-deoxy-nucleoside analogues has been accomplished. The analogues were all devoid of any activity against HIV-1 in MT-4 cells. Although nucleosides are known preferentially to adopt *N*-type furanose conformation when bound to the active site of HIV-1 RT, even masked 5′-monophosphate derivatives of the locked *N*-type nucleosides containing a 2′-oxygen atom are inactive. The results obtained herein indicate that the subsequent phosphorylation steps and/or the interaction with HIV-1 RT are incompatible with the *ribo*-configured locked *N*-type nucleoside structure. Unfortunately, also the masked 5′-monophosphate derivative **7a** of the conformationally restricted but somewhat flexible 3′-azido-3′-deoxy *E*-type nucleoside **7** is devoid of anti-HIV activity.

Experimental ‡

General

Reactions were conducted under an atmosphere of nitrogen when anhydrous solvents were used. All reagents were obtained from commercial suppliers and were used without further purification. Petroleum ether of the distillation range 60–80 °C was used. The silica gel (0.040–0.063 mm) used for column chromatography was purchased from Merck. After drying of organic phases using Na_2SO_4, filtration was performed. After column chromatography, fractions containing product were pooled, evaporated under reduced pressure, and dried overnight under high vacuum (oil pump) to give the product unless otherwise specified. ^{1}H NMR spectra were recorded at 300 MHz, ^{13}C NMR spectra at 75.5 MHz, and ^{31}P NMR spectra at 121.5 MHz. Chemical shifts are reported in ppm relative to either tetramethylsilane or the deuterated solvent as internal standard for ^{1}H NMR and ^{13}C NMR, and relative to 85% H_3PO_4 as external standard for ^{31}P NMR. *J*-values are given in Hz. Assignments of NMR spectra, when given, are based on 2D spectra and follow the standard carbohydrate/nucleoside nomenclature. Fast-atom bombardment mass spectra (FAB-MS) were recorded in positive-ion mode.

3-Deoxy-5-*O*-methylsulfonyl-4-[(methylsulfonyloxy)methyl]-1,2-*O*-isopropylidene-*α*-D-*glycero*-pentofuranose (12)

3-Deoxy-1,2;5,6-di-*O*-isopropylidene-*α*-D-*ribo*-hexofuranose[17] (**9**) (11.0 g, 45.0 mmol) was dissolved in 80% acetic acid (250 cm^3) and stirring was continued for 20 h. The mixture was evaporated to dryness under reduced pressure and the residue was coevaporated with EtOH (3 × 100 cm^3). The residue was dissolved in a mixture of H_2O (100 cm^3) and THF (100 cm^3), the resulting mixture was cooled to 0 °C, and sodium periodate (9.10 g, 42.5 mmol) was added. The mixture was stirred for 1 h at 0 °C and then for 30 min at rt whereupon ethylene glycol (6 cm^3) was added. The mixture was filtered and the aqueous phase was extracted with EtOAc (3 × 250 cm^3). The combined organic phase was evaporated to dryness under reduced pressure. The residue was suspended in 1,4-dioxane (100 cm^3), and formaldehyde (37%; 9.00 cm^3, 111 mmol) was added together with 1 M NaOH (80 cm^3). The mixture was stirred for 20 h and was then extracted with EtOAc (6 × 150 cm^3). The combined organic phase was dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was dissolved in pyridine (150 cm^3) and MsCl (10.5 cm^3, 135 mmol) was added. The mixture was stirred for 2 h at rt whereupon H_2O (10 cm^3) was added. The mixture was evaporated to dryness under reduced pressure, the residue was dissolved in DCM (300 cm^3), and washing was performed using saturated aq. $NaHCO_3$ (3 × 150 cm^3). The organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure to give a crude product, which was purified by column chromatography and elution with 0 ⟶ 2% MeOH in DCM (v/v) to give pentofuranose **12** as a white solid material, which was used without further purification in the next step; $\delta_C(CDCl_3)$ 112.98, 107.06, 84.22, 80.61, 70.24, 69.72, 37.68, 35.48, 26.60, 25.59; $\delta_H(CDCl_3)$ 5.90 (1H, d, *J* 4.0), 4.84 (1H, d, *J* 2.1), 4.59–4.16 (4H, m), 3.13 (3H, s), 3.07 (3H, s), 1.61 (3H, s), 1.32 (3H, s); FAB-MS *m*/*z* 361 $[M + H]^+$.

3-Deoxy-1,2-di-*O*-acetyl-5-*O*-methylsulfonyl-4-[(methylsulfonyloxy)methyl]-*α*,*β*-D-*glycero*-pentofuranose (13)

Pentofuranose **12** (10.0 g) was dissolved in 80% TFA (100 cm^3) and stirring at rt was continued for 2 h at which point analytical TLC showed complete conversion of the starting material. The mixture was evaporated to dryness under reduced pressure and the residue was coevaporated first with EtOH (2 × 100 cm^3) and then with pyridine (200 cm^3). The residue was dissolved in anhydrous pyridine (250 cm^3) at rt and Ac_2O (20.0 cm^3, 211 mmol) was added. The reaction was quenched after 22 h at rt by addition of H_2O (20 cm^3). The mixture was evaporated to dryness under reduced pressure and the residue was dissolved in EtOAc (500 cm^3) and washed with saturated aq. $NaHCO_3$ (3 × 150 cm^3). The organic phase was dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The product was obtained as a white solid material after dry column vacuum

‡ Copies of the ^{13}C NMR spectra of compounds **4**, **5**, **6**, **8**, **18**, **24**, **25**, **26** and ^{31}P NMR spectra of compounds **3a**, **3b**, **4b**, **5a**, **5b** and **7a** were enclosed with this manuscript on submission in order to verify satisfactory purities.

chromatography,[27] and elution with 0 ⟶ 100% EtOAc in petroleum ether (v/v) to give pentofuranose **13** (7.70 g) as a white solid material. The not completely clean mixture was used without any further purification in the next step; $\delta_C(CDCl_3)$ 170.29, 169.95, 169.69, 169.20, 99.59, 94.14, 84.52, 82.57, 79.55, 76.95, 70.76, 70.52, 68.97, 68.37, 64.15, 63.52, 37.87, 37.60, 37.52, 20.95, 20.88, 20.74, 20.54; FAB-MS *m*/*z* 421 $[M + H]^+$.

1-{2-*O*-Acetyl-3-deoxy-5-*O*-methylsulfonyl-4-[(methylsulfonyloxy)methyl]-*β*-D-*glycero*-pentofuranoyl}thymine (14)

Pentofuranose **13** (1.9 g) and thymine (1.2 g, 9.5 mmol) were suspended in anhydrous acetonitrile (40 cm^3) and *N,O*-bis-(trimethylsilyl)acetamide (9.0 cm^3, 36.4 mmol) was added. The mixture was heated under reflux for 1 h and was then cooled to 0 °C. Trimethylsilyl trifluoromethanesulfonate (2.70 cm^3, 17.5 mmol) was added dropwise to the stirred mixture at 0 °C whereupon the temperature was raised to 60 °C (for 24 h) and then to reflux (for 48 h). After cooling of the mixture to rt, additional trimethylsilyl trifluoromethanesulfonate (2.00 cm^3, 11.1 mmol) was added and the mixture was heated under reflux for an additional 24 h. After cooling to rt, the mixture was evaporated to dryness under reduced pressure, and the residue was dissolved in EtOAc (200 cm^3) and washed with saturated aq. $NaHCO_3$ (3 × 75 cm^3). The organic phase was dried (Na_2SO_4), and evaporated to dryness under reduced pressure to give a crude product, which was purified by column chromatography and elution with 0 ⟶ 3% MeOH in DCM (v/v) to give nucleoside **14** (1.12 g, 23% from **9**) as a white solid material, $\delta_C(CDCl_3)$ 170.55, 163.81, 150.44, 138.08, 111.62, 95.23, 84.24, 69.82, 68.89, 37.74, 37.49, 35.46, 20.76, 12.22 76; $\delta_H(CDCl_3)$ 9.64 (1H, s), 7.28 (1H, s), 7.09 (1H, s), 5.65 (1H, d, *J* 3.0), 5.51–5.31 (1H, m), 4.47–4.27 (3H, m), 3.11 (3H, s), 3.09 (3H, s), 2.88–2.80 (1H, m), 2.22–2.16 (1H, m), 2.13 (3H, s), 1.92 (3H, s); FAB-MS *m*/*z* 471 $[M + H]^+$ [Found (%): C, 38.41; H, 4.71; N, 6.14. $C_{15}H_{22}N_2O_{11}S_2$ requires C, 38.29; H, 4.71; N, 5.95%].

1-{3-Deoxy-5-*O*-methylsulfonyl-4-[(methylsulfonyloxy)methyl]-*β*-D-*glycero*-pentofuranosyl}thymine (15)

Nucleoside **14** (1.00 g, 2.12 mmol) was dissolved in a mixture of MeOH (70 cm^3) and saturated methanolic ammonia (40 cm^3). The mixture was stirred for 5 h and then evaporated to dryness under reduced pressure. The crude product tentatively assigned as nucleoside **15** was coevaporated with EtOH (3 × 100 cm^3) and used without further purification in the next step; $\delta_C(CDCl_3)$ 164.39, 151.14, 136.23, 110.91, 93.02, 82.86, 79.45, 73.70, 69.76, 69.49, 48.24, 36.79, 11.49.

1-(3-Deoxy-5-*O*-methylsulfonyl-2-*O*,4-*C*-methylene-*β*-D-*erythro*-pentofuranosyl)thymine (16)

Nucleoside **15** (200 mg) was dissolved in anhydrous DMF (8 cm^3), and NaH (60% suspension in oil; 110 mg, 2.75 mmol) was added. After stirring for 3 h at rt the mixture was diluted with DCM (100 cm^3) and the resulting organic phase was washed with saturated aq. $NaHCO_3$ (3 × 50 cm^3). The organic phase was dried (Na_2SO_4), and evaporated to dryness under reduced pressure to give a crude product, which was purified by column chromatography and elution with 3% MeOH in DCM (v/v) to give nucleoside **16** (101 mg, 64% from **14**) as a white solid material, $\delta_C(CD_3OD)$ 163.80, 149.91, 134.47, 108.58, 87.60, 86.44, 77.85, 72.81, 72.72, 67.07, 36.83, 33.33, 12.20; $\delta_H(CD_3OD)$ 11.37 (1H, s), 7.44 (1H, s), 5.53 (1H, s), 4.94 (1H, d, *J* 12.0), 4.82 (1H, d, *J* 11.6), 4.60 (1H, s), 3.75 (2H, dd, *J* 7.6 and 19.6), 3.29 (3H, s), 1.91–1.75 (5H, m); FAB-MS *m*/*z* 333 $[M + H]^+$ [Found (%): C, 43.30; H, 4.83; N, 8.47. $C_{12}H_{16}N_2O_7S$ requires C, 43.37; H, 4.85; N, 8.43].

1-(5-*O*-Acetyl-3-deoxy-2-*O*,4-*C*-methylene-*β*-D-*erythro*-pentofuranosyl)thymine (17)

Nucleoside **16** (370 mg, 1.1 mmol) was dissolved in anhydrous 1,4-dioxane (20 cm^3). KOAc (870 mg, 8.90 mmol) and 18-crown-6 (450 mg, 1.70 mmol) were added to the solution and the mixture was heated under reflux for 6 h. The mixture was then cooled to rt and the solvent was removed under reduced pressure. The residue was dissolved in DCM (200 cm^3) and washed with saturated aq. $NaHCO_3$ (3 × 100 cm^3). The organic phase was dried (Na_2SO_4), and evaporated to dryness under reduced pressure to give a crude product, which was purified by dry column vacuum chromatography and elution with 50 ⟶ 100% EtOAc in petroleum ether (v/v) followed by 0 ⟶ 2.0% MeOH in EtOAc (v/v) to give nucleoside **17** (220 mg, 66%) as a white solid material, $\delta_C(CDCl_3)$ 170.25, 163.97, 149.94, 134.40, 110.19, 88.40, 87.32, 78.37, 73.68, 61.04, 33.78, 20.77, 12.88; $\delta_H(CDCl_3)$ 8.52 (1H, s), 7.58 (1H, d, *J* 1.1), 5.67 (1H, s), 4.70 (1H, d, *J* 1.4), 3.95 (1H, d, *J* 10.9), 3.83–3.61 (3H, m), 1.93 (3H, s), 1.89–1.76 (1H, m), 1.32–1.24 (4H, m); FAB-MS *m*/*z* 297 $[M + H]^+$ [Found (%): C, 52.52; H, 5.44; N, 9.16. $C_{13}H_{16}N_2O_6$ requires C, 52.70; H, 5.44; N, 9.16].

1-(3-Deoxy-2-*O*,4-*C*-methylene-*β*-D-*erythro*-pentofuranosyl)-thymine (5)

Nucleoside **17** (50 mg, 0.17 mmol) was dissolved in saturated methanolic ammonia (5 cm^3). The mixture was stirred for 4 h at rt and was then evaporated to dryness under reduced pressure. The residue was coevaporated with EtOH (3 × 5 cm^3) and the crude product was purified by column chromatography and elution with 3% MeOH in DCM (v/v), affording nucleoside **5** (28 mg, 65%) as a white solid material, $\delta_C(CD_3OD)$ 166.50, 151.87, 136.90, 110.49, 91.51, 89.53, 79.73, 74.69, 59.77, 33.78, 12.59: $\delta_H(CD_3OD)$ 7.52 (1H, d, *J* 1.2), 5.43 (1H, s), 4.67 (1H, s), 3.94 (1H, d, *J* 12.8), 3.85 (1H, d, *J* 12.8), 3.61–3.54 (1H, m), 1.78–1.66 (5H, m); MALDI-HRMS *m*/*z* 277.0794 ($[M + Na]^+$, $C_{11}H_{14}N_2O_5{\cdot}Na^+$ Calc. 277.0795).

1-[3-Deoxy-2-*O*,4-*C*-methylene-5-*O*-(2-oxo-4*H*-1,3,2-benzodioxaphosphorin-2-yl)-*β*-D-*erythro*-pentofuranosyl]thymine (5a)

Nucleoside **5** (65 mg, 0.25 mmol) was dissolved in anhydrous acetonitrile (4 cm^3) under stirring and DIPEA (0.1 cm^3) was added. After cooling of the mixture to 0 °C, 2-chloro-4*H*-1,3,2-benzodioxaphosphorine[11] (**28**, 107 mg, 0.56 mmol) was added. After 45 min at 0 °C the mixture was treated with *tert*-butyl hydroperoxide (0.1 cm^3, 5.0–6.0 M solution in THF) added slowly. The mixture was allowed to reach rt, was stirred for an additional 1 h, and then was evaporated to dryness under reduced pressure. The crude product was purified by column chromatography and elution with 0 ⟶ 1.75% MeOH in DCM (v/v) to give nucleoside **5a** (32 mg, 30%) as a clear oil, $\delta_P(CDCl_3)$ −7.65, −7.87; $\delta_C(CDCl_3)$ 163.88, 149.83, 134.32, 130.17, 125.47, 124.72, 118.62, 118.50, 110.11, 88.27, 88.21, 87.69, 87.63, 87.60, 87.53, 78.35, 73.15, 73.10, 68.72, 68.63, 65.08, 65.01, 64.87, 64.80, 33.48, 33.36, 12.51, 12.37; MALDI-HRMS *m*/*z* 445.0772 ($[M + Na]^+$, $C_{18}H_{19}N_2O_8P{\cdot}Na^+$ Calc. 445.0771).

1-(3-Deoxy-2-*O*,4-*C*-methylene-5-*O*-{phenoxy[1-(methoxycarbonyl)ethylamino]phophoryl}-*β*-D-*erythro*-pentofuranosyl)-thymine (5b)

Nucleoside **5** (20.0 mg, 0.107 mmol) was dissolved in a solution of phenyl methoxyalaninylphosphorochloridate[13,25] (**29**, 0.184 M solution in anhydrous THF; 2 cm^3, 0.736 mmol). *N*-Methylimidazole (0.1 cm^3) and anhydrous pyridine (1 cm^3) were added to the solution and stirring was continued for 20 h at rt. The temperature was raised to 45 °C and the mixture was stirred for an additional 3 h. The mixture was evaporated to dryness under reduced pressure and the residue was re-dissolved in DCM (10 cm^3) and washed successively with 1 M aq. HCl (2 × 5 cm^3)

and saturated aq. $NaHCO_3$ (2 × 5 cm^3). The organic phase was dried (Na_2SO_4), and evaporated to dryness under reduced pressure to give a crude product, which was purified by column chromatography: 0 ⟶ 3% MeOH in DCM (v/v) to give compound **5b** as a white solid material (12 mg, 22%), $\delta_P(CDCl_3)$ 3.70, 3.48; MALDI-HRMS *m/z* 518.1284 ($[M + Na]^+$, $C_{21}H_{26}N_3O_9P{\cdot}Na^+$ Calc. 518.1299).

1-[3-Azido-3-deoxy-2-*O*,4-*C*-methylene-5-*O*-(2-oxo-4*H*-1,3,2-benzodioxaphosphorin-2-yl)-*β*-D-ribofuranosyl]thymine (3a)

1-(3-Azido-2-*O*,4-*C*-methylene-*β*-D-ribofuranosyl)thymine[6] **3** (50 mg, 0.17 mmol) was dissolved in anhydrous acetonitrile (4 cm^3) and DIPEA (0.1 cm^3) was added. The mixture was cooled to 0 °C, and 2-chloro-4*H*-1,3,2-benzodioxaphosphorine[11] (**28**, 107 mg, 0.56 mmol) was added. Stirring was continued for 45 min at 0 °C, whereupon *tert*-butyl hydroperoxide (0.15 cm^3; 5.0–6.0 M solution in THF) was slowly added. The mixture was allowed to reach rt and was then stirred for an additional 1 h. After evaporation to dryness under reduced pressure and coevaporation with anhydrous acetonitrile (5 cm^3), the crude product was purified by column chromatography and elution with 1 ⟶ 1.5% MeOH in DCM (v/v) to give nucleoside **3a** (16 mg, 34%) as a white solid material, $\delta_P(CDCl_3)$ −8.01, −8.16; $\delta_C(CDCl_3)$ 163.50, 149.61, 133.83, 130.14, 125.39, 124.72, 118.60, 118.46, 110.75, 87.30, 78,47, 70.95, 68.67, 62.42, 60.27, 12.26; MALDI-HRMS *m/z* 486.0765 ($[M + Na]^+$, $C_{18}H_{18}N_5O_8P{\cdot}Na^+$ Calc. 486.0785).

1-(3-Azido-3-deoxy-2-*O*,4-*C*-methylene-5-*O*-{phenoxy-[1-(methoxycarbonyl)ethylamino]phosphoryl}-*β*-D-ribofuranosyl)thymine (3b)

1-(3-Azido-2-*O*,4-*C*-methylene-*β*-D-ribofuranosyl)thymine[6] **3** (50 mg, 0.17 mmol) was dissolved in a solution of phenyl methoxyalaninylphosphorochloridate[13,25] (**29**, 0.184 M solution in anhydrous THF; 4 cm^3, 0.736 mmol). To the mixture were added *N*-methylimidazole (0.1 cm^3) and anhydrous pyridine (1 cm^3). Stirring was continued for 20 h at rt whereupon the temperature was raised to 45 °C and stirring was continued for an additional 3 h. The mixture was evaporated to dryness under reduced pressure, the residue was dissolved in DCM (20 cm^3), and the solution was washed successively with 1 M aq. HCl (2 × 7 cm^3) and saturated aq. $NaHCO_3$ (2 × 7 cm^3). The organic phase was dried (Na_2SO_4), and evaporated to dryness under reduced pressure to give a crude product, which was purified by column chromatography and elution with 3% MeOH in DCM (v/v). The crude product obtained was again purified by column chromatography; this time, elution with 0 ⟶ 3% MeOH in DCM (v/v) afforded compound **3b** (35mg, 38%), $\delta_P(CDCl_3)$ 3.58, 3.50; MALDI-HRMS *m/z* 559.1291 ($[M + Na]^+$, $C_{21}H_{25}N_6O_9P{\cdot}Na^+$ Calc. 559.1313).

9-{2-*O*-Acetyl-3-deoxy-5-*O*-methylsulfonyl-4-[(methylsulfonyloxy)methyl]-*β*-D-*glycero*-pentofuranosyl}adenine (18)

Pentofuranose **13** (1.0 g) was coevaporated with anhydrous acetonitrile (15 cm^3) and dissolved in anhydrous acetonitrile (15 cm^3). Adenine (675 mg, 5.0 mmol) and then $SnCl_4$ (0.75 cm^3, 6.4 mmol) were added under stirring at rt. After 6 h, additional $SnCl_4$ (0.75 cm^3, 6.4 mmol) was added and stirring was continued for an additional 20 h. Saturated aq. $NaHCO_3$ (approx. 100 cm^3) was slowly added until evolution of gas ceased. The mixture was filtered through compressed Celite 545 which, after filtration, was flushed with DCM (800 cm^3). The combined organic phase was separated, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The crude product was purified by dry column vacuum chromatography and elution with, first, 20 ⟶ 100% EtOAc in petroleum ether (v/v) and then 0 ⟶ 5.0% MeOH in EtOAc (v/v) to give nucleoside **18** (604 mg) as a white solid material, and this was used without any further purification in the next step; $\delta_C(CDCl_3)$ 169.92 (C=O), 155.55 (C-6), 152.95 (C-2), 148.86 (C-4), 139.70 (C-8), 120.04 (C-5), 90.67 (C-1′), 84.69 (C-4′), 76.38 (C-2′), 69.03, 68.27 (C-5′,C-5″), 37.56, 37.17 (2 × Ms), 35.22 (C-3′), 20.57 (CH_3); $\delta_H(CDCl_3)$ 8.33 (1H, s, H-8), 7.90 (1H, s, H-2), 7.28 (2H, s, NH_2), 6.15 (1H, d *J* 2.1, H-1), 5.90 (1H, m, H-2′), 4.56–4.13 (4H, m, H_2-5′, H_2-5″), 3.13 (3H, s, Ms), 2.95 (3H, s, Ms), 3.26–2.98 (2H, m, H_2-3′), 2.15 (3H, s, CH_3CO); FAB-MS *m/z* 480 [M + H]$^+$.

9-(3-Deoxy-2-*O*,4-*C*-methylene-*β*-D-*erythro*-pentofuranosyl)-adenine (6)

Nucleoside **18** (600 mg) was dissolved in saturated methanolic ammonia (50 cm^3) and the solution was stirred under N_2 for 1 h. The mixture was then evaporated to dryness under reduced pressure and the residue was coevaporated with EtOH (2 × 50 cm^3). This gave an intermediate white solid material tentatively assigned as nucleoside **19**, which was dissolved in anhydrous DMF (20 cm^3) followed by addition of NaH (60% suspension in oil; 250 mg, 6.2 mmol). After stirring for 3 h at rt the mixture was diluted with DCM (100 cm^3) and the mixture was washed with saturated aq. $NaHCO_3$ (3 × 50 cm^3). The combined water phase was extracted with DCM (50 cm^3), and the combined organic phases were dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by dry column vacuum chromatography and elution, first, with 80 ⟶ 100% EtOAc (v/v) in petroleum ether and then 0 ⟶ 1.0% MeOH in EtOAc (v/v) to give a slightly yellow oil tentatively assigned as nucleoside **20**. The product **20** was dissolved in anhydrous 1,4-dioxane (15 cm^3), KOAc and 18-crown-6 (450 mg, 1.7 mmol) were added under stirring, and the mixture was heated with reflux for 3 h. The mixture was then cooled to rt and evaporated to dryness under reduced pressure. The residue was dissolved in EtOAc (60 cm^3) and washed with saturated aq. $NaHCO_3$ (3 × 20 cm^3). The organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by column chromatography and elution with 2 ⟶ 5% MeOH in DCM (v/v) to give an intermediate tentatively assigned as nucleoside **21** (110 mg) as a white solid material. This material (100 mg) was dissolved in saturated methanolic ammonia (10 cm^3) and the mixture was stirred at rt for 2 h and then evaporated to dryness under reduced pressure. The residue was coevaporated with EtOH (3 × 5 cm^3) and the crude product was purified by column chromatography and elution with 3% MeOH in DCM (v/v) to give nucleoside **6** (42 mg, 3% from **9**) as a white solid material, $\delta_C((CD_3)_2SO)$ 155.86, 152.55, 148.30, 137.55, 89.30, 86.61, 78.07, 73.38, 58.29, 33.86; $\delta_H((CD_3)_2SO)$ 8.18 (1H, s), 8.14 (1H, s), 7.29 (2H, br s), 5.93 (1H, s), 4.76 (1H, s), 3.92 (2H, m), 3.76 (1H, d, *J* 7.8), 3.73 (1H, d, *J* 7.4), 3.31 (1H, s, overlapping with H_2O signal), 2.04 (1H, d, *J* 11.6), 1.94 (1H, d, *J* 11.3); MALDI-HRMS *m/z* 286.0910 ($[M + Na]^+$, $C_{11}H_{13}N_5O_3{\cdot}Na^+$ Calc. 286.0911).

3-Azido-3-deoxy-1,2-di-*O*-acetyl-6-*O*-methylsulfonyl-4-[(methylsulfonyloxy)methyl]-*α*,*β*-D-*erythro*-pentofuranose (23)

Ribofuranose **22**[24] (700 mg, 1.7 mmol) was dissolved in TFA (50 cm^3; 80%) and after stirring for 1 h the mixture was evaporated to dryness under reduced pressure. The residue was coevaporated successively with CH_3CN (2 × 50 cm^3) and pyridine (50 cm^3). The residue was dissolved in anhydrous pyridine (50 cm^3), and Ac_2O (5 cm^3, 52.8 mmol) was added. The mixture was stirred at rt for 42 h whereupon H_2O (15 cm^3) was added. The mixture was evaporated to dryness under reduced pressure and the residue was dissolved EtOAc (50 cm^3) and washing was performed using saturated aq. $NaHCO_3$ (3 × 20 cm^3). The organic phase was dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The crude product was purified by dry column vacuum chromatography and elution with 0 ⟶ 70%

EtOAc in petroleumether (v/v) to afford ribofuranose **23** (687 mg, 90%) as a white solid material, which was not completely clean but was used without any further purification in the next step; $\delta_C(CDCl_3)$ (data for major anomer) 169.26, 168.74, 97.54, 83.45, 75.17, 68.20, 67.84, 63.43, 37.95, 37.85, 20.99, 20.61; MALDI-MS *m*/*z* 486 $[M + Na]^+$.

9-{(2-*O*-Acetyl-3-azido-3-deoxy-5-*O*-methylsulfonyl-4-[(methylsulfonyloxy)methyl]-*β*-D-*erythro*-pentofuranosyl}adenine (24)

Ribofuranose **23** (600mg, 1.34 mmol) was dissolved in anhydrous CH_3CN (15 cm^3) and adenine (435 mg, 3.2 mmol) was added. To the mixture was added $SnCl_4$ (0.75 cm^3, 6.4 mmol) and the temperature was raised to 60 °C. After continued stirring for 16 h at 60 °C, the temperature was changed to rt and saturated aq. $NaHCO_3$ (approx. 80 cm^3) was added until evolution of gas ceased. The mixture was filtered through compressed Celite 545 which, after filtration, was flushed with DCM (500 cm^3). The combined organic phase was separated, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The product was purified by dry column vacuum chromatography and elution, first, with 20 ⟶ 100% EtOAc in petroleum ether (v/v) and then 0 ⟶ 5.0% MeOH in EtOAc (v/v) to give nucleoside **24** (247 mg, 35%) as a white solid material, $\delta_C((CD_3)_2SO)$ 169.411, 156.19, 152.93, 149.05, 139.67, 118.98, 85.28, 73.60, 69.06, 67.76, 62.08, 36.84, 36.72, 20.18; $\delta_H((CD_3)_2SO)$ 8.31 (1H, s), 8.13 (1H, s), 7.36 (2H, s), 6.27 (1H, d, *J* 5.3), 6.14 (1H, d, *J* 5.7), 5.21 (1H, d, *J* 6.5), 4.48 (2H, s), 4.38 (2H, s), 3.27 (3H, s), 3.23 (3H, s), 2.06 (3H, s); MALDI-MS *m*/*z* 543 $[M + Na]^+$.

9-(5-*O*-Acetyl-3-azido-3-deoxy-2-*O*,4-*C*-methylene-*β*-D-ribofuranosyl)adenine (26)

Nucleoside **24** (200 mg, 0.38 mmol) was dissolved in saturated methanolic ammonia (20 cm^3). The mixture was stirred for 72 h at rt whereupon the mixture was evaporated to dryness under reduced pressure. The product was dried *in vacuum* (oil pump) to give a white solid material tentatively assigned as nucleoside **25**, which was used in the next step without further purification. Data for **25**: $\delta_C((CD_3)_2SO)$ 156.08, 152.84, 148.12, 138.17, 118.84, 85.62, 85.20, 78.97, 71.20, 65.58, 62.03, 36.91; $\delta_H((CD_3)_2SO)$ 8.30 (1H, s), 8.15 (1H, s), 7.34 (2H, s), 6.04 (1H, s), 4.92 (1H, s), 4.77 (1H, d, *J* 11.6), 4.76 (1H, s), 4.63 (1H, d, *J* 12.1), 3.94 (2H, dd, *J* 8.5 and 3.2), 3.24 (3H, s).

Compound **25** was dissolved in anhydrous 1,4-dioxane (10 cm^3) together with 18-crown-6 (200 mg, 0.75 mmol) and KOAc (300 mg, 3.00 mmol). The mixture was heated under reflux for 3 h and was then cooled to rt. The mixture was evaporated to dryness under reduced pressure and the residue was dissolved in EtOAc (20 cm^3). Washing was performed using saturated aq. $NaHCO_3$ (3 × 8 cm^3), and the organic phase was dried (Na_2SO_4), and evaporated to dryness under reduced pressure to afford nucleoside **26** (122 mg, 91% from **24**) as a white solid material, $\delta_C(CDOD_3)$ 170.94, 153.36, 138.20, 87.04, 86.98, 79.22, 72.27, 62.36, 59.62, 20.70; $\delta_H(CDOD_3)$ 8.28 (1H, s), 8.06 (1H, s), 6.08 (1H, s), 5.00 (1H, s), 4.58 (1H, d, *J* 13.0), 4.50 (1H, d, *J* 12.7), 4.39 (1H, s), 4.28 (1H, s), 4.09 (1H, d, *J* 8.5), 4.04 (1H, d, *J* 8.5), 2.18 (3H, s); MALDI-MS *m*/*z* 369.4 $[M + Na]^+$.

9-(3-Azido-3-deoxy-2-*O*,4-*C*-methylene-*β*-D-ribofuranosyl)-adenine (4)

Nucleoside **26** (122 mg, 0.35 mmol) was dissolved in saturated methanolic ammonia (5 cm^3). The mixture was stirred for 24 h at rt and then evaporated to dryness under reduced pressure. The residue was crystallized from 40% MeOH in DCM to give nucleoside **4** (85 mg, 79%) as a white solid material. Selected IR signal: ν_{max} 2118 cm^{-1} (azido group); $\delta_C((CD_3)_2SO)$ 156.03, 152.72, 148.71, 138.06, 118.95, 88.96, 85.09, 78.74, 71.46, 61.39, 56.57; $\delta_H((CD_3)_2SO)$ 8.30 (1H, s), 8.16 (1H, s), 7.35 (2H, s), 5.99 (1H, s), 5.29 (1H, br s), 4.80 (1H, s), 4.58 (1H, s), 3.90–3.81 (4H, m); MALDI-MS *m*/*z* 305.1 $[M + H]^+$, 327.1 $[M + Na]^+$.

3′-Azido-3′-deoxy-2′-*O*,4′-*C*-methylene-5′-*O*-{phenoxy-[1-(methoxycarbonyl)ethylamino]phosphoryl}adenosine (4b)

Nucleoside **4** (20.0 mg, 0.065 mmol) was dissolved in a solution of phenyl methoxyalaninylphosphorochloridate[13,25] **29** in anhydrous THF (0.184 M solution; 1.1 cm^3, 0.20 mmol). To the solution was added *N*-methylimidazole (0.05 cm^3) and the mixture was stirred at rt for 16 h before being evaporated to dryness under reduced pressure. The residue was re-dissolved in DCM (15 cm^3) and washing was performed using saturated aq. $NaHCO_3$ (3 × 8 cm^3). The organic phase was dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography and elution with 3% MeOH in DCM (v/v) to give compound **4b** (6 mg, 16%) as a white solid material and starting nucleoside **4** (recovery 10 mg), $\delta_P((CD_3)_2SO)$ 4.84, 4.63; MALDI-HRMS *m*/*z* 568.1432 ($[M + Na]^+$, $C_{21}H_{24}N_9O_7P{\cdot}Na^+$ Calc. 568.1429).

1-{3-Deoxy-2,5-di-*O*-methylsulfonyl-4-[(methylsulfonyloxy)-methyl]-*β*-D-*glycero*-pentofuranosyl}thymine (27)

Nucleoside **15** (700 mg) was dissolved in anhydrous pyridine (20 cm^3) and MsCl (0.2 cm^3, 2.6 mmol) was added. The mixture was stirred at rt for 48 h and was then evaporated to dryness. The residue was dissolved in EtOAc (150 cm^3) and washing was performed using saturated aq. $NaHCO_3$ (3 × 50 cm^3). The organic phase was dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The crude product was purified by dry column vacuum chromatography and elution with, first, 50 ⟶ 100% EtOAc in petroleum ether (v/v) and then 0 ⟶ 1.5% MeOH in EtOAc (v/v) to give nucleoside **27** (320 mg) as a white solid material. This was used without any further purification in the next step; $\delta_C(CD_3OD)$ 164.14, 151.32, 136.56, 110.62, 90.07, 81.25, 72.16, 71.09, 71.01, 37.23, 36.46, 32,85, 12.51; $\delta_H(CD_3OD)$ 11.38 (1H, s), 7.49 (1H, d, *J* 0.71), 5.88 (1H, d, *J* 5.9), 5.73 (1H, d, *J* 4.8), 4.46–4.27 (4H, m), 3.59 (3H, s), 3.27 (3H, s), 3.25 (3H, s), 2.56–2.32 (2H, m), 1.79 (3H, s); FAB-MS *m*/*z* 507 $[M + H]^+$.

1-(3-Deoxy-2-*O*,4-*C*-methylene-*α*-L-*erythro*-pentofuranosyl)-thymine (8)

Nucleoside **27** (300 mg, 0.59 mmol) was dissolved in 1,4-dioxane (10 cm^3) and to the solution was added 1 M aq. NaOH (6 cm^3). The mixture was stirred for 4 h at rt and then for 18 h at 90 °C. After neutralization using 10% aq. HCl, the mixture was evaporated to dryness and the crude product was purified by column chromatography and elution with 3 ⟶ 4% MeOH in DCM (v/v) to give nucleoside **8** (61 mg, 15% from **14**) as a white solid material, $\delta_C(CD_3OD)$ 166.54, 153.07, 138.52, 110.37, 93.55, 92.40, 79.39, 76.67, 61.83, 61.72, 14.91; $\delta_H(CD_3OD)$ 7.60 (1H, m), 5.82 (1H, d, *J* 4.8), 4.55 (1H, m), 4.04–3.76 (4H, m), 2.20–1.73 (5H, m); MALDI-HRMS *m*/*z* 277.09797 ($[M + Na]^+$, $C_{11}H_{14}N_2O_5{\cdot}Na^+$ Calc. 277.0795).

1-[3-Azido-3-deoxy-2-*O*,3-*C*-methylene-5-*O*-(2-oxo-4*H*-1,3,2-benzodioxaphosphorin-2-yl)-*β*-D-arabinofuranosyl]thymine (7a)

Nucleoside **7**[7] (50 mg, 0.19 mmol) was dissolved in anhydrous acetonitrile (4 cm^3) and DIPEA (0.1 cm^3) was added. The stirred mixture was cooled to 0 °C and 2-chloro-4*H*-1,3,2-benzodioxaphosphorine[11] **28** (90 mg, 0.47 mmol) was added. The mixture was stirred for 1.5 h at 0 °C whereupon a solution of *tert*-butyl hydroperoxide THF (5.0–6.0 M in anhydrous THF; 0.1 cm^3, 0.5–0.6 mmol) was added. The resulting mixture was stirred for an additional 2 h during which time the mixture was allowed to reach rt. The mixture was evaporated to dryness under reduced pressure and the crude product was purified by column chromatography and elution with 0 ⟶ 1% MeOH in

DCM (v/v) to give compound **7a** (46 mg, 58%), $\delta_P(CDCl_3)$ −8.43; $\delta_C(CDCl_3)$ 163.74, 150.31, 137.04, 136.98, 130.15, 130.13, 125.49, 124.77, 118.84, 118.79, 118.72, 118.66, 110.49, 110.45, 87.15, 83.57, 83.52, 78.29, 78.19, 72.77, 72.70, 69.13, 69.04, 68.95, 66.80, 66.76, 64.81, 64.73, 64.66, 12.67; MALDI-HRMS *m/z* 486.0787 ($[M + Na]^+$, $C_{18}H_{18}N_5O_8P{\cdot}Na^+$ Calc. 486.0785).

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