Synthesis and evaluation of anti-HIV-1 activity of 3′-azido-3′-deoxy-2′-*O*,4′-*C*-methylene-linked bicyclic thymine nucleosides

Anne G. Olsen,[a] Vivek K. Rajwanshi,[a] Claus Nielsen[b] and Jesper Wengel[a,*†]

[a] *Center for Synthetic Bioorganic Chemistry, Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark*
[b] *Retrovirus Laboratory, Department of Virology, Statens Serum Institut, DK-2300 Copenhagen, Denmark*

Received (in Cambridge, UK) 7th July 2000, Accepted 8th September 2000
First published as an Advance Article on the web 16th October 2000

The two conformationally locked AZT analogues **4** and **5**, each containing a 2′-*O*,4′-*C*-methylene-linked bicyclic furanose moiety, are synthesized *via* the 3′-azido-3′-deoxy-4′-*C*-hydroxymethyl nucleoside **16**. The β-D-*ribo*-configured derivative **4** is shown by NOE experiments to exist in a north-type (^3E, C3′-*endo*) conformation and the α-L-*xylo*-configured derivative **5** in a south-type ($_3E$, C3′-*exo*) conformation. Both nucleosides were devoid of anti-HIV activity in MT-4 cells.

Introduction

The use of 2′,3′-dideoxynucleosides to combat HIV infection remains important in the treatment of AIDS patients. These nucleosides are prodrugs which need to be triphosphorylated at the 5′-hydroxy group *in vivo* in order be effective inhibitors of the HIV-encoded enzyme HIV-1 Reverse Transcriptase (HIV-1 RT).[1–3] 3′-Azido-3′-deoxythymidine (AZT, **1**, Fig. 1) was the first anti-HIV drug to be approved for treatment of AIDS but as with other anti-HIV drugs its use is hampered by toxic side-effects and the emergence of resistance. Therefore, the collection of additional knowledge on the pharmacological effect of various nucleosides and the development of improved anti-HIV drugs continues to be of immense importance.

A study published by Marquez *et al.* has furnished relevant insight into the relationship between conformation of the furanose ring and anti-HIV activity.[4] Thus, the two conformationally restricted bicyclic carbocyclic AZT analogues **2** and **3** (Fig. 1) were synthesized and their cyclopentane moieties shown to be locked into what corresponds to a north-type ($_2E$, C2′-*exo*) and a south-type ($_3E$, C3′-*exo*) conformation, respectively. Whereas the 5′-triphosphate of **3** was inactive as an inhibitor of HIV-1 RT, the inhibitory activity of the 5′-triphosphate of the north-type analogue **2** corresponded closely to that of AZT 5′-triphosphate.[4] In line with the earlier proposal that a south-type conformation is essential for efficient conversion of the nucleoside prodrugs to the bioactive 5′-triphosphates,[5] these results led to the conclusion that efficient 5′-triphosphorylation and subsequent inhibition of HIV-1-RT requires conformational flexibility of the pentofuranose ring (allowing the nucleoside prodrug to adopt a south-type conformation and the 5′-triphosphate drug to adopt a north-type conformation).[4]

To further evaluate the relation between furanose conformation and anti-HIV activity we have synthesized the two conformationally locked AZT analogues **4** and **5** each containing a 2′-*O*,4′-*C*-methylene-linked bicyclic furanose moiety (Fig. 1). Based on molecular modelling, NMR and X-ray crystallography of the corresponding nucleoside derivatives[6–9] having a 3′-hydroxy group instead of the 3′-azido group, the β-D-*ribo*-configured derivative **4** was predicted to exist in a north-type (^3E, C3′-*endo*) conformation and the α-L-*xylo*-configured-derivative **5** in a south-type ($_3E$, C3′-*exo*)‡ conformation (Fig. 1). These predictions were confirmed by NOE experiments on **4** and **5** (*vide supra*) and by results reported in a recent com-

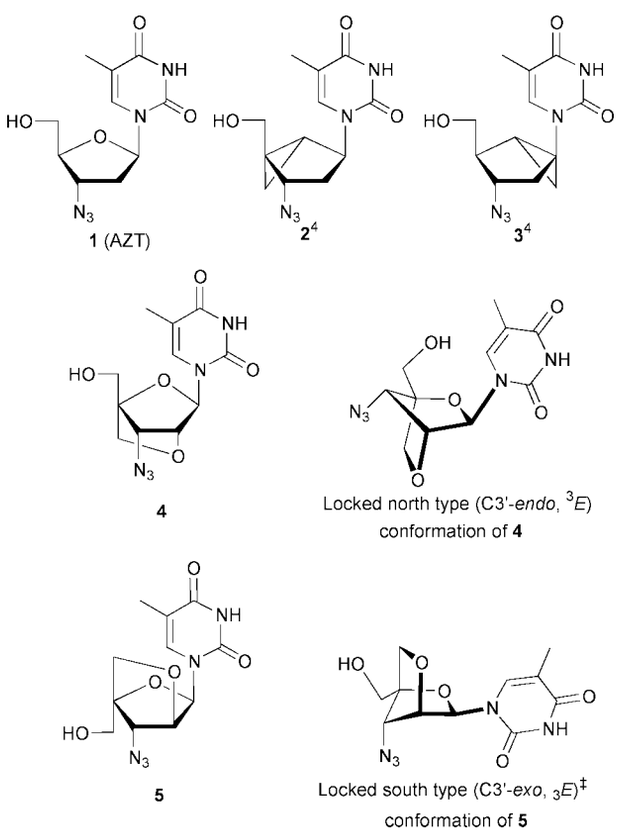


Fig. 1 Structures of AZT (**1**) and conformationally restricted bicylic AZT analogues **2**–**5**. The displayed conformations of nucleosides **4** and **5** are those found by molecular modelling (HyperChem™ program, Polak–Ribiere algorithm), and confirmed herein by NMR experiments (see Fig. 2).

† *Present address*: Department of Chemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark.

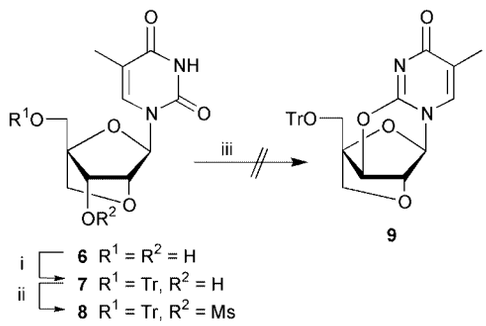
‡ The conformation of the furanose ring of nucleoside **5** could also be given as C3′-*endo*, reflecting the positioning of the 4′-*C*-hydroxymethyl group and the C3′ atom at the same face of the plane created by the atoms C1′, C2′, C4′ and O4′. However, to allow direct comparison with nucleosides **1**–**4** the conformation of **5** is given as C3′-*exo*.

 DOI: 10.1039/b005469k

munication[10] by Obika *et al.* who independently synthesized derivative **4**. The 2,5-dioxabicyclo[2.2.1]heptane derivatives **4** and **5** therefore mimic the north- and south-type pentofuranose conformations of AZT **1**, respectively, although the configuration at C4′ of the α-L-*xylo*-configured derivative **5** is opposite to that of AZT.

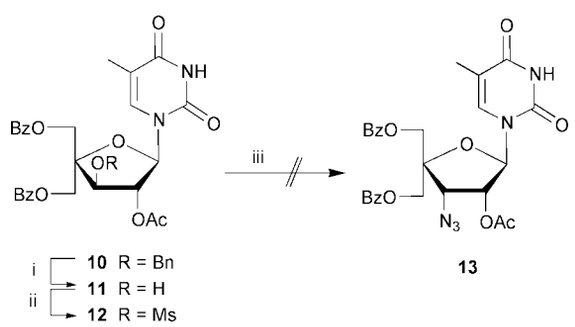
Results and discussion

Our first approach (Scheme 1) towards the synthesis of 1-(3-azido-3-deoxy-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)thymine **4** was planned to involve nucleophilic opening of the 2,3′-anhydro nucleoside **9** using azide ion, followed by detritylation. As the first step, the known bicyclic nucleoside diol **6**[8] was monotritylated at the 5′-position by reaction with less than two equivalents of trityl chloride in pyridine to give the novel nucleoside **7** in 91% yield. However, activation of the 3′-hydroxy group by formation of the 3′-*O*-mesyl derivative **8** (mesyl chloride, pyridine; 87% yield) and subsequent treatment with one equivalent of base following standard conditions for anhydro-nucleoside formation (aq. NaOH in ethanol)[11,12] failed to yield the desired anhydro nucleoside **9**. Analogously, attempted conversion of the 5′-*O*-(*tert*-butyldimethylsilyl) derivative of **8** into the corresponding 2,3′-anhydro nucleoside, as well as direct conversion of the 5′-*O*-(*tert*-butyldimethylsilyl) derivative of **7** using Mitsunobu conditions, failed (data not shown). These results are in contrast to those of Marquez *et al.* as they successfully used a 2,3′-anhydro nucleoside as an intermediate during synthesis of the north-type conformer **2**.[4] However, probably because of complete lack of conformational mobility in the furanose ring of compounds **7** and **8**, the necessary geometry for intramolecular nucleophilic attack at the 3′-position is unattainable.



Scheme 1 *Reagents and conditions (and yields)*: i) TrCl, pyridine (91%); ii) MsCl, pyridine (87%); iii) NaOH, aq. ethanol.

In a second approach (Scheme 2), the known 1-(2-*O*-acetyl-5-*O*-benzoyl-4-*C*-benzoyloxymethyl-3-*O*-benzyl-α-L-*threo*-pentofuranosyl)thymine[13] **10** was used as starting material. Debenzylation by catalytic hydrogenation afforded nucleoside **11** in 70% yield. To prepare for introduction of a 3′-azido group with inversion of configuration, the 3′-*O*-mesyl derivative **12** was prepared in 78% yield by reaction of **11** with mesyl chloride and 4-(*N*,*N*-dimethyl)aminopyridine (DMAP) in pyridine. However, attempted conversion of **12** into the 3′-azido nucleoside **13** (NaN_3, DMF), a possible intermediate towards synthesis of the target molecule **4**, was unsuccessful in our hands. Likewise, reaction of the 3′-*O*-trifluoromethanesulfonate derivative of **11** (data not shown) with sodium azide in anhydrous DMF failed to give nucleoside **13**.

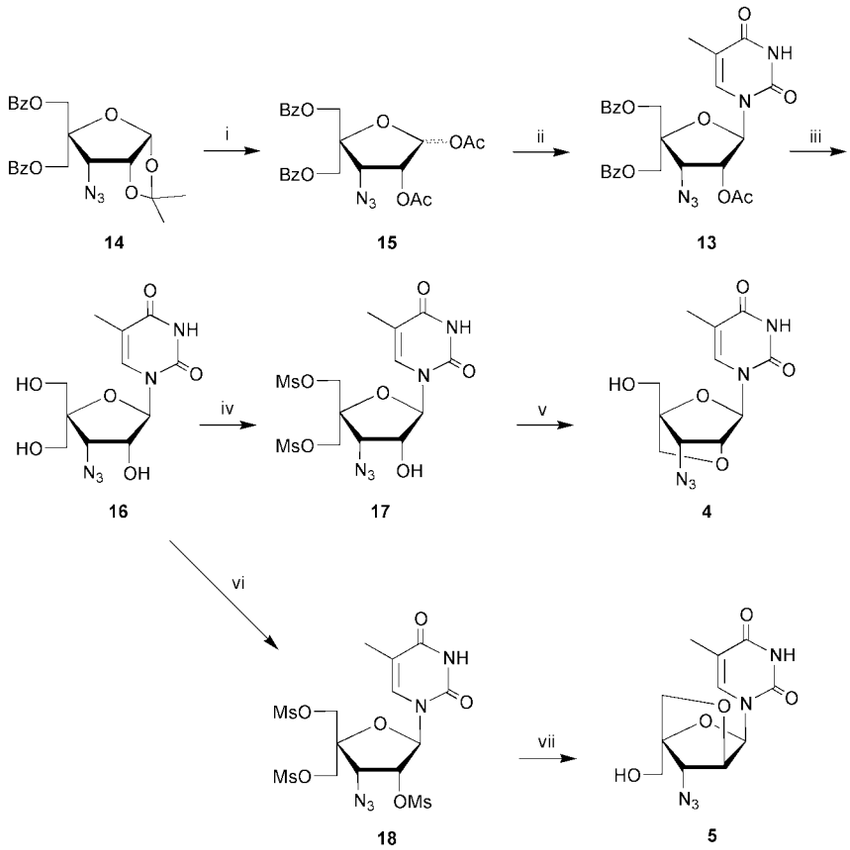


Scheme 2 *Reagents and conditions (and yields)*: i) H_2, 10% Pd/C, CH_3OH (70%); ii) MsCl, DMAP, pyridine (78%); iii) NaN_3, DMF.

With two unsuccessful attempts at introducing the azido substituent at the nucleoside level we decided to use the known 3-azido-3-deoxyfuranose **15**,[14] easily obtainable from D-glucose *via* 1,2-*O*-isopropylidene derivative **14**,[14] as starting material (Scheme 3). Reaction of **15** with thymine, *N*,*O*-bis(trimethylsilyl)acetamide and trimethylsilyl triflate in acetonitrile[15,16] afforded nucleoside **13** in 68% yield. As expected from the presence of a 2-*O*-acetyl group in **15**, the Vorbrüggen-type coupling reaction[15,16] proceeded stereoselectively with no indication of the formation of the anomeric nucleoside. Complete deacylation of nucleoside **13** by reaction with sodium methoxide in methanol afforded triol **16** in 85% yield. Mesylation of **16** using 2.1 equivalents of mesyl chloride in pyridine afforded an intermediate, tentatively assigned as derivative **17** with the two primary hydroxy groups mesylated, which subsequently in a 1:1 mixture of 1,4-dioxane and 1 M aqueous NaOH (room temperature, 15 min; 90 °C, 12 h) was directly converted into the target compound 3′-azido-3′-deoxy-2′-*O*,4′-*C*-methylene-β-D-ribofuranosyl)thymine **4** in 36% yield (calculated from **16**). We have recently reported a similar, successful, one-pot ring-closure and 5′-*O*-demesylation reaction during the synthesis of 2′-*O*,4′-*C*-methylene-α-L-ribofuranosyl)thymine.[9] The overall yield for the five-step synthesis of nucleoside **4** from furanose **14** was 19% as compared with 38% for the reported[10] alternative seven-step synthesis likewise starting from furanose **14**.

Towards the synthesis of 3′-azido-3′-deoxy-2′-*O*,4′-*C*-methylene-α-L-xylofuranosyl)thymine **5**, the tri-*O*-mesyl intermediate **18** was prepared in 79% yield by trimesylation of derivative **16** using 3.5 equivalents of mesyl chloride in pyridine. Subsequent treatment of **18** with first a mixture of 1,4-dioxane and 1 M aq. NaOH (5:2; 90 °C, 24 h), to give a putative 5′-*O*-mesyl intermediate, and then with a mixture of 1 M aq. NaOH, EtOH and water (1:5:5; 80 °C, 24 h; then 90 °C, 17 h) effected formation of the target nucleoside **5** in 40% yield. We have recently reported a similar conversion for the 3′-hydroxy derivative[9] of **18** likewise involving 2′-epimerization, cyclization and demesylation. Mechanistically *en route* from **18** to **5**, a reaction cascade including 2,2′-anhydro-nucleoside formation, hydrolysis of this intermediate to give the 2′-epimer of **17**, and ring-closure by intramolecular nucleophilic attack followed by demesylation seems likely. This efficient synthesis of nucleoside **5** underlines the importance of anhydro nucleoside intermediates in nucleoside chemistry. However, the necessity of 2′-epimerization and the fact that anhydro-intermediates are generally unattainable for purine derivatives suggests the strategy used for synthesis of **5** to be viable for pyrimidine derivatives only.

The configuration and conformation of the two bicyclic nucleosides **4** and **5** were evaluated by ^{1}H NMR and NOE experiments (Fig. 2). Thus, for the β-D-*ribo*-configured derivative **4**, mutual strong NOEs between H6 and H3′ (8%/8%) indicate *anti* conformation of the thymine moiety and C3′-*endo* conformation of the furanose ring. In addition, comparable NOEs between the pairs H1′ and H2′ (5%/5%) and H2′ and H3′ (5%/4%), together with the fact that these three protons appear as singlets in the ^{1}H NMR spectrum, indicate a C3′-*endo* conformation of nucleoside **4** in analogy to what was found earlier for **4** by a similar analysis and by molecular modelling.[10] For the α-L-*xylo*-configured derivative **5**, mutual strong NOEs between H6 and one of the two 2′-*O*,4′-*C*-methylene protons (11%/7%) suggest an *anti* conformation of



Scheme 3 *Reagents and conditions (and yields)*: i) a, 80% AcOH; b, Ac_2O, pyridine (89%); ii) *N*,*O*-bis(trimethylsilyl)acetamide, thymine, TMS triflate, acetonitrile (68%); iii) $NaOCH_3$, CH_3OH, (85%); iv) MsCl, pyridine; v) NaOH, aq. 1,4-dioxane (36%, two steps); vi) MsCl, pyridine (79%); vii) a, NaOH, aq. 1,4-dioxane; b, NaOH, aq. EtOH (40%).

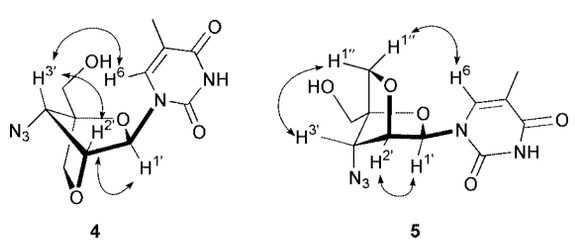


Fig. 2 Key NOE effects (indicated by the curved arrows) observed for nucleosides **4** and **5**. For **4**: 8% and 8% (between H6 and H3′), 5% and 5% (between H1′ and H2′), 5% and 4% (between H2′ and H3′). For **5**: 11% and 7% (between H6 and one of the two H1″ protons), 7% and 2% (between H3′ and the other H1″ proton), 9% and 8% (between H1′ and H2′).

the thymine moiety in addition to supporting the assigned structure and C3′-*exo* conformation of this nucleoside. In addition, mutual NOEs between the other 2′-*O*,4′-*C*-methylene proton and H3′ (7%/2%) and between H1′ and H2′ (9%/8%) add support for the assigned structure and conformation of **5**. The locked conformations for nucleosides **4** and **5** were further verified by molecular modelling (see Fig. 1).

The bicyclic nucleosides **4** and **5** were evaluated for antiviral activity against HIV-1 in MT-4 cells as described earlier.[17] Both compounds were inactive against HIV-1 at 300 μM. Thus, although derivative **4** is structurally closely related to AZT and efficiently locked in a north-type conformation corresponding to the conformation of AZT triphosphate when interacting with HIV-1-RT, no anti-HIV activity of **4** was found. This, in line with the earlier reported results, suggests that a south-type furanose conformation is essential for efficient 5′-*O*-phosphorylation *in vivo*.[5] As the configuration at C4′ of the south-type conformer **5** is of the unnatural L-type it is more difficult to draw conclusions on the basis of the lack of anti-HIV activity of **5**. For example, efficient 5′-*O*-phosphorylation *in vivo* may *a priori* not be anticipated for nucleoside derivative **5** despite its furanose ring being in a south-type conformation.

Conclusions

Synthesis of the two conformationally locked AZT analogues **4** and **5** has been accomplished. The β-D-*ribo* configured derivative **4** adopts a north-type (^3E, C3′-*endo*) conformation, and the α-L-*xylo*-configured derivative **5** a south-type ($_3E$, C3′-*exo*) conformation, while the thymine moieties adopt *anti* conformations. Both nucleosides were inactive against HIV-1 in MT-4 cells. These results are in line with the results of Marquez *et al.*[4] indicating that conformational flexibility of the furanose ring of 5′-hydroxy nucleoside prodrugs is essential for anti-HIV-1 activity.

Experimental

General

All reagents and solvents were obtained from commercial suppliers and were used without further purification. Reactions were conducted under an atmosphere of nitrogen when anhydrous solvents were used. During work-up, organic phases were combined, dried (Na_2SO_4) and filtered before evaporation. After column chromatographic purification (glass columns; silica gel 60, Merck, 0.040–0.063 mm), fractions containing product were pooled, evaporated to dryness under reduced pressure, and dried under vacuum to give the product. Petroleum ether of distillation range 60–80 °C was used. NMR spectra were recorded on a Varian 400 MHz NMR instrument and chemical shifts are reported in ppm relative to tetramethylsilane as internal standard for ^1H (400 MHz) and ^{13}C (100.6 MHz) except for the ^{13}C spectrum (62.9 MHz) of nucleoside **4** which was recorded on a Bruker 250 MHz NMR instrument.

Coupling constants J are given in Hz. Assignments of NMR spectra when given are based on 2D spectra. Nuclear Overhauser enhancement (NOE) experiments were recorded on a Bruker 250 MHz NMR instrument (nucleoside **4** in non-degassed CD_3OD) and nucleoside **5** [in non-degassed $(CD_3)_2SO$]. Fast-atom bombardment mass spectra (FAB-MS) were recorded in positive ion mode. Microanalyses were performed at The Microanalytical Laboratory, Department of Chemistry, University of Copenhagen.

1-(2-*O*,4-*C*-Methylene-5-*O*-trityl-β-D-ribofuranosyl)thymine 7

To a solution of 1-(2-*O*,4-*C*-methylene-β-D-ribofuranosyl)-thymine[8] **6** (0.39 g, 1.45 mmol) in anhydrous pyridine (8 cm^3) at room temperature was added trityl chloride (0.68 g, 2.32 mmol). The reaction mixture was heated at 110 °C for 20 h, and then evaporated to dryness under reduced pressure. The residue was dissolved in dichloromethane (35 cm^3; containing 0.5% pyridine, v/v), washing was performed successively with saturated aq. of sodium hydrogen carbonate (3 × 8 cm^3) and brine (3 × 10 cm^3), and the organic phase was evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane–methanol–pyridine (98.0 : 1.5 : 0.5 to 97.0 : 2.5 : 0.5, v/v/v) as eluent to give an intermediate tentatively assigned as nucleoside **7** (white solid material, 0.67 g, 91%). FAB-MS *m/z* 513 $[M + H]^+$. This intermediate was used in the next step without further purification.

1-(2-*O*,4-*C*-Methylene-3-*O*-methylsulfonyl-5-*O*-trityl-β-D-ribofuranosyl)thymine 8

To an ice-cold solution of nucleoside **7** (0.25 g, 0.49 mmol) in anhydrous pyridine (3 cm^3) was added mesyl chloride (0.06 cm^3, 0.73 mmol). The solution was stirred at 10 °C for 24 h whereupon additional mesyl chloride (0.007 cm^3, 0.09 mmol) was added. After being stirred at 10 °C for an additional 20 h, the reaction mixture was concentrated to half-volume, and the resulting mixture was poured into ice-cold water (125 cm^3) under vigorous stirring to give nucleoside **8** (0.25 g, 87%) as a white solid material after filtration, washing with water (250 cm^3) and coevaporation with anhydrous toluene (3 × 5 cm^3); δ_H ($CDCl_3$) 8.83 (1H, br s, NH), 7.62–7.25 (16H, m, 6-H, Tr), 5.70 (1H, s, 1′-H), 5.08 (1H, s, 3′-H), 4.80 (1H, s, 2′-H), 3.89–3.83 (2H, m), 3.62 (1H, d, *J* 11.1), 3.43 (1H, d, *J* 11.1), 3.00 (3H, s, Ms), 1.66 (3H, s, CH_3). δ_C ($CDCl_3$) 163.5 (C-4), 149.7 (C-2), 142.8, 133.6, 128.5, 128.3, 128.1, 127.9, 127.6, 127.2 (C-6, Tr), 111.3 (C-5), 87.6, 87.2, 77.7, 75.2, 72.0, 57.6 (C-1′, -2′, -3′, -4′, -5′, -1″), 39.0 (Ms), 12.6 (CH_3); FAB-MS *m/z* 591 $[M + H]^+$ [Found: (%) C, 63.4; H, 5.1; N, 4.5. $C_{31}H_{30}N_2O_8S$ requires C, 63.0; H, 5.1; N, 4.7].

1-(2-*O*-Acetyl-5-*O*-benzoyl-4-*C*-benzoyloxymethyl-α-L-*threo*-pentofuranosyl)thymine 11

1-(2-*O*-Acetyl-5-*O*-benzoyl-4-*C*-benzoyloxymethyl-3-*O*-benzyl-α-L-*threo*-pentofuranosyl)thymine[13] **10** (1.00 g, 1.60 mmol) was dissolved in methanol (4.0 cm^3) and 10% Pd/C (0.25 g) suspended in methanol (3.0 cm^3) was added. The mixture was degassed and then stirred for 21 h under an atmosphere of hydrogen. Additional Pd/C (0.75 g) suspended in methanol (4 cm^3) was added followed by degassing and subsequent stirring for 46 h under an atmosphere of hydrogen. The mixture was filtered [silica gel; washing was performed using dichloromethane–methanol (250 cm^3; 1 : 1, v/v], and the combined filtrate was evaporated to dryness under reduced pressure. The residue was subjected to column chromatography on silica gel using dichloromethane–methanol (99 : 1, v/v) as eluent to give nucleoside **11** (0.60 g, 70%) as a white solid material, δ_H ($CDCl_3$) 9.03 (1H, br s, NH), 8.06–7.27 (11H, m, 6-H, Bz), 5.92 (1H, d, *J* 3.8, 1′-H), 5.35–5.37 (1H, m, 2′-H), 4.80–4.43 (5H, m, 3′-H, 5′-, 1″-H_2), 2.13 (3H, s, $COCH_3$), 2.13 (3H, s, CH_3); δ_C ($CDCl_3$) 170.5, 166.0, 165.8 (C=O), 163.3 (C-4), 150.0 (C-2), 137.3, 133.3, 129.6, 129.5, 129.0, 128.9, 128.4, 128.1 (C-6, Bz), 111.8 (C-5), 100.0, 90.5, 86.0, 83.0 (C-1′, -2′, -3′, -4′), 63.0, 62.7 (C-5′, -1″), 20.7 ($COCH_3$), 12.3 (CH_3); FAB-MS *m/z* 539 $[M + H]^+$ [Found: (%) C, 60.8; H, 4.9; N, 5.0. $C_{27}H_{26}N_2O_{10}$ requires C, 60.2; H, 4.9; N, 5.2].

1-(2-*O*-Acetyl-5-*O*-benzoyl-4-*C*-benzoyloxymethyl-3-*O*-methylsulfonyl-α-L-*threo*-pentofuranosyl)thymine 12

To an ice-cold solution of nucleoside **11** (0.10 g, 0.18 mmol) in anhydrous pyridine (0.5 cm^3) was added mesyl chloride (0.03 cm^3, 0.37 mmol). The solution was stirred at room temperature for 26 h whereupon DMAP (0.01 g, 0.09 mmol) and additional mesyl chloride (0.01 cm^3, 0.13 mmol) were added. After being stirred at room temperature for 22 h, the reaction mixture was diluted with dichloromethane (25 cm^3), and ice-cold water (5.0 cm^3) was added. The phases were separated and the aqueous phase was extracted with dichloromethane (3 × 15 cm^3). The combined organic phase was washed successively with saturated aq. sodium hydrogen carbonate (3 × 10 cm^3) and brine (3 × 10 cm^3) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane–methanol (99.5 : 0.5 to 99 : 1, v/v) as eluent to give nucleoside **12** (0.09 g, 78%) as a white solid material, δ_H ($CDCl_3$) 8.73 (1H, br s, NH), 8.10–7.30 (11H, m, 6-H, Bz), 6.33 (1H, d, *J* 6.0, 1′-H), 5.70 (1H, dd, *J* 6.0 and 5.2, 2′-H), 5.47 (1H, d, *J* 5.2, 3′-H), 4.90–4.56 (4H, m, 5′-, 1″-H_2), 3.14 (3H, s, Ms), 2.15 (3H, s, $COCH_3$), 1.7 (3H, s, CH_3); FAB-MS *m/z* 617 $[M + H]^+$ [Found: (%) C, 55.0; H, 4.7; N, 4.3. $C_{28}H_{28}N_2O_{12}S$ requires C, 54.6; H, 4.6; N, 4.5].

1-(2-*O*-Acetyl-3-azido-5-*O*-benzoyl-4-*C*-benzoyloxymethyl-3-deoxy-β-D-*erythro*-pentofuranosyl)thymine 13

To a stirred solution of the anomeric mixture 1,2-*O*-acetyl-3-azido-5-*O*-benzoyl-4-*C*-benzoyloxymethyl-3-deoxy-D-*erythro*-pentofuranose[14] **15** (0.64 g, 1.28 mmol) and thymine (0.32 g, 2.57 mmol) in anhydrous acetonitrile (15 cm^3) was added *N*,*O*-bis(trimethylsilyl)acetamide (2.19 cm^3, 8.98 mmol). The reaction mixture was stirred and heated under reflux for 1 h. After cooling of the mixture to 0 °C, TMS triflate (0.67 cm^3, 3.47 mmol) was added dropwise. After being stirred for 10 min at 0 °C, the mixture was heated to 60 °C and stirring was continued for 16 h. The reaction mixture was evaporated to dryness under reduced pressure, and the residue was dissolved in ethyl acetate (50 cm^3), washed with saturated aq. sodium hydrogen carbonate (3 × 20 cm^3) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using ethyl acetate–petroleum ether (2 : 3 to 1 : 1, v/v) as eluent to give nucleoside **13** (0.49 g, 68%) as a white foam, δ_H ($CDCl_3$) 8.83 (1H, s, NH), 8.08–7.44 (10H, m, Bz), 7.02 (1H, s, 6-H), 5.90 (1H, d, *J* 4.8, 1′-H), 5.75 (1H, dd, *J* 4.8 and 6.6, 2′-H), 4.92 (1H, d *J* 6.6, 3′-H), 4.88–4.39 (4H, m, 5′-, 1″-H_2), 2.20 (3H, s, $COCH_3$), 1.72 (3H, s, CH_3); δ_C ($CDCl_3$) 170.0, 165.9, 165.8 (C=O), 163.3 (C-4), 149.8 (C-2), 133.6, 133.3, 129.7, 129.6, 129.2, 128.9, 128.6, 128.4 (Bz), 136.8 (C-6), 111.7 (C-5), 90.0 (C-1′), 84.1 (C-4′), 74.5 (C-2′), 65.0, 62.8, 62.7 (C-3′, -5′, -1″), 20.4 ($COCH_3$), 12.0 (CH_3); FAB-MS *m/z* 564 $[M + H]^+$. Selected IR signal: ν_{max} 2118 cm^{-1} (azido group) [Found: (%) C, 57.3; H, 4.8; N, 11.6. $C_{27}H_{25}N_5O_9 \cdot 0.5EtOAc$ requires C, 57.3; H, 4.8; N, 11.5].

1-(3-Azido-3-deoxy-4-*C*-hydroxymethyl-β-D-*erythro*-pentofuranosyl)thymine 16

To a stirred solution of nucleoside **13** (1.00 g, 1.77 mmol) in anhydrous methanol (20 cm^3) was added sodium methoxide (0.58 g, 10.6 mmol). The reaction mixture was stirred for 30 min at room temperature, and was then neutralized with a 7% (w/w) solution of HCl in 1,4-dioxane. After evaporation to dryness

under reduced pressure, the residue was purified by silica gel column chromatography using dichloromethane–methanol (96:4 to 94:6, v/v) as eluent to give nucleoside **16** (0.47 g, 85%) as a white foam, δ_H (CD_3OD) 7.78 (1H, s, 6-H), 5.93 (1H, d, *J* 6.6, 1′-H), 4.69 (1H, m, 2′-H), 4.31 (1H, d, *J* 6.1, 3′-H), 3.78–3.60 (4H, m, 5′-, 1″-H_2), 1.88 (3H, s, CH_3); δ_C (CD_3OD) 164.3 (C-4), 150.8 (C-2), 136.5 (C-6), 110.0 (C-5), 87.6 (C-1′), 86.4 (C-4′), 74.0 (C-2′), 63.6 (C-3′), 63.2, 61.7 (C-5′, -1″), 10.5 (CH_3); FAB-MS *m*/*z* 314 $[M + H]^+$. Selected IR signal: ν_{max} 2117 cm^{-1} (azido group) [Found: (%) C, 40.2; H, 4.8; N, 20.4. $C_{11}H_{15}N_5O_6 \cdot H_2O$ requires C, 39.9; H, 5.2; N, 21.1].

1-(3-Azido-3-deoxy-5-*O*-mesyl-4-*C*-mesyloxymethyl-β-D-*erythro*-pentofuranosyl)thymine 17

To a stirred solution of nucleoside **16** (0.082 g, 0.26 mmol) in anhydrous pyridine (10 cm^3) at −20 °C was added a solution of mesyl chloride (0.04 cm^3, 0.55 mmol) in anhydrous pyridine (1.0 cm^3) dropwise during 45 min. After 2 h, ice-cold water (6.0 cm^3) was added and the mixture was evaporated to dryness under reduced pressure to give an intermediate tentatively assigned as crude **17**, which after co-evaporation with anhydrous toluene was used without further purification in the next step.

1-(3-Azido-3-deoxy-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)-thymine 4

Intermediate **17** (0.122 g, 0.26 mmol) was stirred in a mixture of 1,4-dioxane (4.0 cm^3) and 1 M aq. NaOH (2.25 cm^3). After 15 min at room temperature, analytical TLC (methanol–dichloromethane; 1:19, v/v) showed quantitative conversion of starting material into an intermediate with a slightly lower mobility. The reaction mixture was subsequently stirred at 90 °C for 12 h, then neutralized with a 7% (w/w) solution of HCl in 1,4-dioxane and finally evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane–methanol (97:3, v/v) as eluent to give nucleoside **4** (28 mg, 36%, two steps) as a white solid material, δ_H (CD_3OD) 7.70 (1H, s, 6-H), 5.59 (1H, s, 1′-H), 4.57 (1H, s, 2′-H), 4.02 (1H, s, 3′-H), 3.95–3.76 (4H, m, 5′-, 1″-H_2), 1.89 (3H, s, CH_3). These δ_H (CD_3OD) data are in accord with previously published δ_H (CD_3OD) data;[10] δ_C (CD_3OD) 164.2 (C-4), 150.0 (C-2), 134.6 (C-6), 109.1 (C-5), 89.2 (C-4′), 86.5 (C-1′), 78.1 (C-2′), 59.5 (C-3′), 70.6, 55.5 (C-5′, -1″), 10.8 (CH_3); FAB-HRMS *m*/*z* 296.0995. Calc. 296.0995 $[M + H]^+$. Selected IR signal: ν_{max} 2120 cm^{-1} (azido group).

1-(3-Azido-3-deoxy-2,5-di-*O*-mesyl-4-*C*-mesyloxymethyl-β-D-*erythro*-pentofuranosyl)thymine 18

To a stirred solution of nucleoside **16** (206 mg, 0.66 mmol) in anhydrous pyridine (10 cm^3) was added a solution of mesyl chloride (0.18 ml, 2.30 mmol) in anhydrous pyridine (1 cm^3). After stirring of the mixture for 1.5 h, ice-cold water (8 cm^3) was added and the mixture was evaporated to dryness under reduced pressure. The residue was dissolved in dichloromethane (50 cm^3), washed with saturated aq. sodium hydrogen carbonate (3 × 20 cm^3) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane–methanol (49:1, v/v) as eluent to give nucleoside **18** (283 mg, 79%) as a white solid material, δ_H [$(CD_3)_2SO$] 11.54 (1H, s, NH), 7.55 (1H, s, 6-H), 6.06 (1H, d, *J* 5.0, 1′-H), 5.68 (1H, dd, *J* 5.0 and 6.6, 2′-H), 5.03 (1H, d, *J* 6.6, 3′-H), 4.43 (4H, m, 5′-, 1″-H_2), 3.37, 3.31, 3.28 (9H, 3 × s, Ms), 1.79 (3H, s, CH_3); δ_C [$(CD_3)_2SO$] 163.7 (C-4), 150.5 (C-2), 136.6 (C-6), 110.5 (C-5), 87.8 (C-1′), 82.7 (C-4′), 78.1 (C-2′), 68.1, 67.4 (C-5′, -1″), 61.5 (C-3′), 37.8, 37.0, 37.0 (3 × Ms), 12.1 (CH_3); FAB-MS *m*/*z* 548 $[M + H]^+$.

1-(3-Azido-3-deoxy-2-*O*,4-*C*-methylene-α-L-xylofuranosyl)-thymine 5

Nucleoside **18** (20.0 mg, 0.0365 mmol) was stirred in a mixture of 1,4-dioxane (1.0 cm^3) and 1 M NaOH (0.4 cm^3). After 15 min at room temperature, analytical TLC (methanol–dichloromethane; 2:23, v/v) showed no conversion. The reaction mixture was subsequently stirred at 90 °C for 24 h and then evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane–methanol (97.5:2.5 to 97:3, v/v) as eluent to give an intermediate. This intermediate was stirred in a mixture of water–ethanol (1 cm^3; 1:1 v/v) and 2 M NaOH (0.2 cm^3) at 80 °C for 24 h and subsequently at 90 °C for 17 h. After neutralization using a 7% (w/w) solution of HCl in 1,4-dioxane, the mixture was evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane–methanol (97:3, v/v) as eluent to give nucleoside **5** (4.3 mg, 40%) as a white solid material, δ_H [$(CD_3)_2SO$] 11.3 (1H, br s, NH), 7.61 (1H, s, 6-H), 5.91 (1H, s, 1′-H), 5.26 (1H, dd, *J* 5.5 and 5.7, OH), 4.60 (1H, d, *J* 2.7, 3′-H), 4.54 (1H, d, *J* 2.6, 2′-H), 4.09 (1H, d, *J* 8.8, 5′-H^a), 4.03 (1H, d, *J* 8.8, 5′-H^b), 3.78 (2H, m, 1″-H_2), 1.82 (3H, s, CH_3); δ_C [$(CD_3)_2SO$] 163.8 (C-4), 150.3 (C-2), 136.0 (C-6), 108.3 (C-5), 89.3, 88.5 (C-1′, C-4′), 76.8 (C-2′), 73.6 (C-5′), 65.7 (C-3′), 56.9 (C-1″), 12.3 (CH_3); FAB-MS *m*/*z* 296 $[M + H]^+$. Selected IR signal: ν_{max} 2123 cm^{-1} (azido group). To verify the purity of this compound, a copy of the ^{13}C NMR spectrum was enclosed when submitting this manuscript.

Acknowledgements

The Danish Natural Science Research Council and The Danish Technical Research Council are thanked for financial support.

References

1 E. De Clercq, *J. Med. Chem.*, 1995, **38**, 2491.
2 K. Luzuriaga, Y. Bryson, P. Krogstad, J. Robinson, B. Stechenberg, M. Lamson, S. Cort and J. L. Sullivan, *New Engl. J. Med.*, 1997, **336**, 1343.
3 E. De Clercq, *Collect. Czech. Chem. Commun.*, 1998, **63**, 449.
4 V. E. Marquez, A. Ezzitouni, P. Russ, M. A. Siddiqui, H. Ford, Jr., R. J. Feldman, H. Mitsuya, C. George and J. J. Barchi, Jr., *J. Am. Chem. Soc.*, 1998, **120**, 2780.
5 P. Van Roey, E. W. Taylor, C. K. Chu and R. F. Schinazi, *Ann. N. Y. Acad. Sci.*, 1990, **616**, 29.
6 S. Obika, D. Nanbu, Y. Hari, K. Morio, Y. In, T. Ishida and T. Imanishi, *Tetrahedron Lett.*, 1997, **38**, 8735.
7 S. K. Singh, P. Nielsen, A. A. Koshkin and J. Wengel, *Chem. Commun.*, 1998, 455.
8 A. Koshkin, S. K. Singh, P. Nielsen, V. K. Rajwanshi, R. Kumar, M. Meldgaard, C. E. Olsen and J. Wengel, *Tetrahedron*, 1998, **54**, 3607.
9 A. E. Håkansson, A. A. Koshkin, M. D. Sørensen and J. Wengel, *J. Org. Chem.*, 2000, **65**, 5161.
10 S. Obika, J. Andoh, T. Sugimoto, K. Miyashita and T. Imanishi, *Tetrahedron Lett.*, 1999, **40**, 6465.
11 N. C. Yung and J. J. Fox, *J. Am. Chem. Soc.*, 1961, **83**, 3060.
12 P. Nielsen, H. M. Pfundheller, C. E. Olsen and J. Wengel, *J. Chem. Soc., Perkin Trans. 1*, 1997, 3423.
13 V. K. Rajwanshi, R. Kumar, M. Kofod-Hansen and J. Wengel, *J. Chem. Soc., Perkin Trans. 1*, 1999, 1407.
14 S. A. Surzhykov and A. A. Krayevsky, *Nucleosides Nucleotides*, 1994, **13**, 2283.
15 H. Vorbrüggen, K. Krolikiewicz and B. Bennua, *Chem. Ber.*, 1981, **114**, 1234.
16 H. Vorbrüggen and G. Höfle, *Chem. Ber.*, 1981, **114**, 1256.
17 K. Danel, E. B. Pedersen and C. Nielsen, *J. Med. Chem.*, 1998, **41**, 191.