Synthesis and evaluation of anti-HIV activity of 3-azido-4-(hydroxymethyl)tetrahydrofuran derivatives containing 2-(thymin-1-yl)methyl, 2-(cytosin-1-yl)methyl or 2-(adenin-9-yl)methyl substituents – a new series of AZT analogues

Anne G. Olsen,[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) 14th November 2000, Accepted 22nd January 2001
First published as an Advance Article on the web 16th February 2001

The three monocyclic AZT analogues **6**, **7** and **8** {3-azido-4-(hydroxymethyl)tetrahydrofuran derivatives containing 2-[(thymin-1-yl)methyl], 2-[(cytosin-1-yl)methyl] and 2-[(adenin-9-yl)methyl] substituents} are synthesized *via* methyl 3-azido-3-deoxy-2-*O*,4-*C*-methylene-D-ribofuranoside **13** as key intermediate. All nucleoside analogues proved to be devoid of anti-HIV activity in MT-4 cells.

Introduction

3′-Azido-3′-deoxythymidine (AZT, **1**, Fig. 1) was the first drug to be approved by the FDA (US Food and Drug Administration) for treatment of AIDS patients. AZT and the five approved 2′,3′-dideoxynucleosides[1] are prodrugs which need to be triphosphorylated *in vivo* at the 5′-hydroxy group to successfully interact with the virus-specific enzyme HIV-1 Reverse Transcriptase (HIV-1 RT).[2] The active triphosphate derivatives can either act as competitive inhibitors, preventing incorporation of the natural substrate, or as an alternative substrate thus causing viral DNA chain termination as no hydroxy group is present in the 3′-position.[3] AZT and the other nucleoside analogues continue to play an important role in the treatment of HIV infection but, due in part to the development of resistance and toxic side effects, they are often administered in combinations with other drugs.[4] Therefore, further investigations of the pharmacological effects of novel nucleosides and the development of new drug candidates are important.

The relation between the conformation of the furanose ring as described by the pseudorotational wheel[5] and the anti-HIV activity is a field of current interest. Investigations on solid-state conformations of nucleosides suggested that an *S*-type (*south*-type; *e.g.*, C2′-*endo*/C3′-*exo*) conformer of AZT is responsible for both its successful triphosphorylation and potent anti-HIV activity, thus assuming that a high level of AZT 5′-triphosphate would efficiently inhibit HIV-1 RT.[6,7] However, subsequent NMR studies revealed that AZT triphosphate and thymidine triphosphate, when bound to HIV-1 RT, both adopt an *N*-type (*north*-type; C4′-*exo*) conformation.[8] Marquez *et al.* have reported the synthesis of the two bicyclic AZT analogues **2** and **3** (Fig. 1) predominantly adopting an *N*-type and *S*-type conformation, respectively.[9] Neither **2** nor **3** showed anti-HIV activity, but when converted to their 5′-triphosphates *in vitro* the *N*-type analogue **2** showed an anti-HIV effect similar to that of AZT 5′-triphosphate while the *S*-type analogue **3** was still inactive. These results led to the conclusion that an *S*-type conformation is necessary for efficient triphosphorylation while subsequent inhibition of HIV-1 RT requires an *N*-type conformation.[9,10]

We and others have further investigated these results by synthesizing the two locked AZT analogues **4**[11,12] and **5**[12] (Fig. 1) adopting a C3′-*endo* conformation and a C3′-*exo* conformation, respectively. In line with the results of Marquez *et al.*[9,10] both **4** and **5** were inactive against HIV-1, thus supporting the assumption that conformational flexibility of the furanose ring is essential for anti-HIV-1 activity.[12]

Based on the above we decided to synthesize and study a series of novel monocyclic AZT analogues **6**–**8** (Fig. 1). These are considered as ring-opened derivatives of **4** and were chosen as synthetic targets in an attempt to discover new, conformationally more flexible, lead structures in the search for improved anti-HIV drugs.

Results and discussion

The anomeric methyl furanoside **13** (Scheme 1) was considered as a potential key intermediate in the synthesis of **6**–**8** as it has been reported that a Vorbrüggen-type coupling reaction[13,14] on a related methyl furanoside afforded ring-opened derivatives.[15] The favouring of the Lewis acid-mediated ring-opening reaction over the expected cleavage of the anomeric bond was explained by ring strain due to the bicyclic constitution.[15]

To prepare **13**, the known 3-azido-5-*O*-benzoyl-4-*C*-benzoyloxymethyl-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose **9** was prepared from D-glucose in several steps, as described.[16] The corresponding diol **10** was obtained as previously reported[11] by reaction with sodium methoxide in methanol (94% yield; reported[11] 86%). Mesylation of **10** using 2.1 equiv. of mesyl chloride in pyridine afforded an intermediate, tentatively assigned as the di-*O*-mesyl derivative **11**, which was subsequently treated with HCl in aq. methanol to give the anomeric mixture of methyl furanosides **12** in 79% yield (calculated from **10**). Treatment of **12** with a mixture of 1 M aq. NaOH and 1,4-dioxane (room temperature, 1 h) afforded an intermediate expected to be the bicyclic 5-*O*-mesylated derivative, which upon raising the temperature to 85 °C was converted into the anomeric key intermediate **13** in 87% yield (Scheme 1).

† *Present address*: Department of Chemistry, University of Southern Denmark, Odense, DK-5230 Odense M, Denmark. Fax: +45 66 15 87 80; E-mail: jwe@chem.sdu.dk

 DOI: 10.1039/b009109j

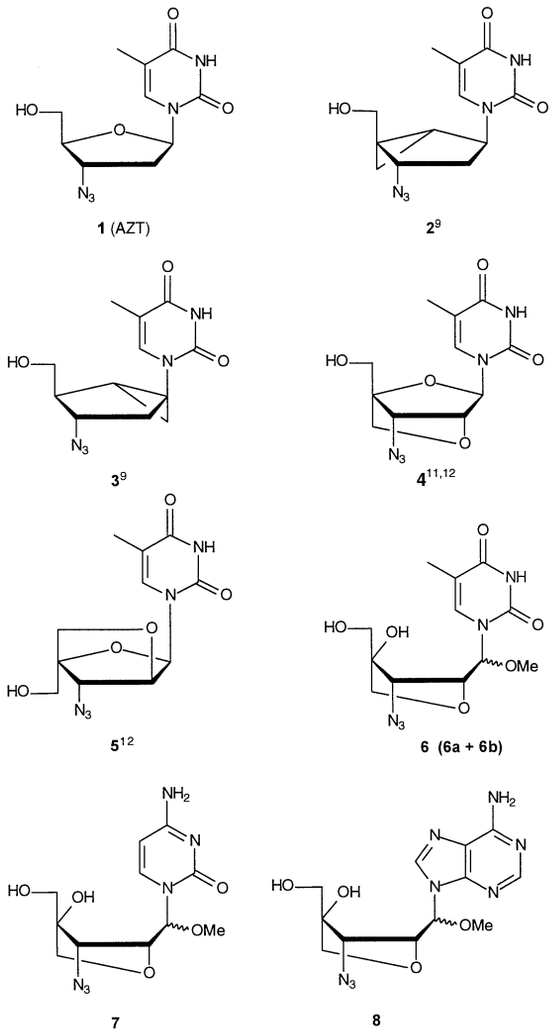
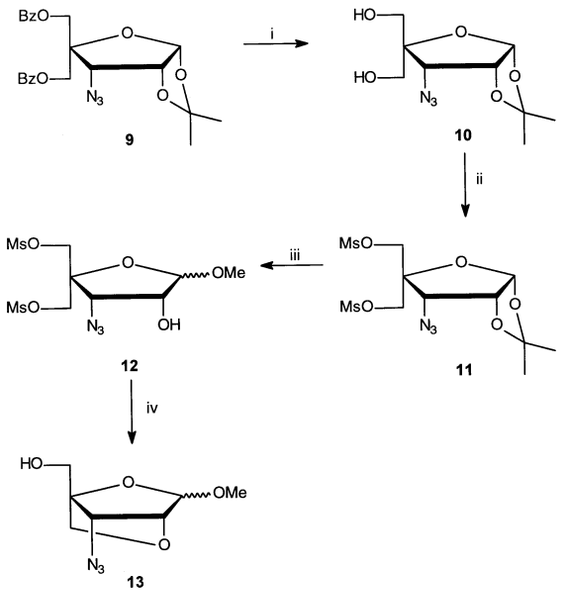


Fig. 1 Structures of AZT **1**, the conformationally restricted bicyclic AZT analogues **2**–**5**, and the monocyclic nucleoside analogues **6**–**8**.

The bicyclic conformationally locked structures of the anomers of **13** were verified by the appearance of the H1 and H2 signals as singlets in the ^{1}H NMR spectra. As expected from earlier results,[15] the Vorbrüggen-type coupling reaction[13,14] between thymine and the bicyclic furanoside **13** afforded the ring-opened target compound **6** as a crude mixture of diastereoisomers **6a** and **6b** in a yield of 61%. After column chromatographic purification followed by preparative reversed-phase HPLC, the individual diastereoisomers **6a** and **6b** were obtained in low yields but their configuration at the exocyclic asymmetric carbon atom could not be assigned. As expected, the strain in the bicyclic structure was relieved during the reaction with silylated thymine and the monocyclic constitution of **6a** and **6b** was verified by NMR studies (*e.g.*, by H1′ appearing as a doublet for both isomers and the presence of both 1′-*C*-methoxy and 4′-*C*-hydroxy groups). Owing to unsuccessful attempted Vorbrüggen-type coupling reaction between **13** and silylated cytosine, it was decided to protect the primary hydroxy group of **13** by reaction with acetic anhydride in pyridine to give the 5-*O*-acetylated product **14a** and the corresponding α-anomer **14b** as separated anomers in an overall yield of 88% (α/β = 1 : 3) after column chromatographic purification. Both anomers **14a** and **14b** should be applicable for subsequent introduction of nucleobases, but because of its predominance, the β-anomer **14a** was coupled with 4-*N*-acetylcytosine to give the ring-opened nucleoside analogue **15**, protected as a trimethylsilyl ether at the tertiary hydroxy group, in 60% yield as an intermediate diastereoisomeric mixture (Scheme 2). The complete deprotection of compound **15** by reaction with



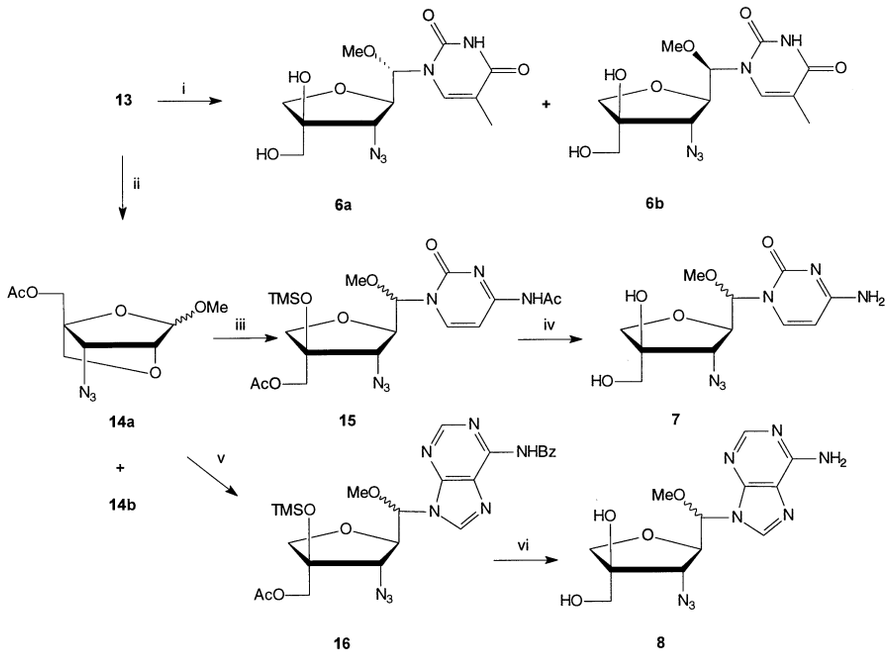
Scheme 1 *Reagents, conditions and yields*: i) $NaOCH_3$, CH_3OH (94%); ii) MsCl, pyridine; iii) 20% HCl in aq. methanol (79%, two steps); iv) aq. NaOH, 1,4-dioxane (87%).

sodium methoxide in methanol afforded the target nucleoside analogue **7** as a diastereoisomeric mixture in 57% yield. The mixture **7** was not separated into the two diastereoisomers but was submitted for biological testing as the isolated 1.0 : 1.3 mixture. The coupling of **14a** with 6-*N*-benzoyladenine was performed under thermodynamic control and furnished the desired N9 regioisomeric intermediate **16** as a diastereoisomeric mixture. The target diastereoisomeric compound **8** was subsequently obtained by reaction of nucleoside analogue **16** with sodium methoxide in methanol to give the monocyclic nucleoside derivative **8** (19% yield calculated from **14a**) which was assigned as the N9 regioisomers by comparing the obtained ^{13}C chemical shift values with published values for N7 and N9 regioisomeric β-D-ribofuranosyladenines **17**.[17] As with **7**, the diastereoisomeric mixture **8** (1.0 : 1.4) was tested as such.

The monocyclic nucleoside analogues **6a**, **6b**, **7** and **8** were evaluated for antiviral activity against HIV-1 in MT-4 cells as described earlier.[18] All compounds were inactive against HIV-1 at 300 μM and also proved to be non-toxic to the MT-4 cells at this concentration. Thus, although the structures of the three analogues allow considerable conformational flexibility of the tetrahydrofuran ring, they are devoid of activity. Possible explanations for the lack of activity, despite the configurations at all carbon atoms being identical with those of AZT, include a) the presence of the 1′-*C*-methoxy substituent, the 4′-*C*-hydroxy group and the 2′-*O*,4′-*C*-methylene bridge, and b) the less constrained relative positioning of the C5′ atom and the thymine moiety compared with AZT. Alone or in combination, these points may explain unfavorable binding interactions with relevant receptors.

Conclusions

The synthesis of a series of novel monocyclic AZT analogues **6**, **7** and **8** has been accomplished. All nucleoside analogues prepared were devoid of activity against HIV-1 in MT-4 cells. In theory, they should be conformationally flexible and be able to adopt what would correspond to the *N*-type and the *S*-type pentofuranose conformations of AZT, thereby possibly being 5′-*O*-triphosphorylated and eventually acting as inhibitors of HIV-1 RT. However, the structural requirements for anti-HIV-1 activity are apparently not fulfilled with these novel, rather drastically modified, AZT analogues.



Scheme 2 *Reagents, conditions and yields*: i) *N*,*O*-bis(trimethylsilyl)acetamide, thymine, TMS triflate, acetonitrile (61%, **6a** + **6b**); ii) Ac_2O, pyridine (88%, **14a** + **14b**); iii) *N*,*O*-bis(trimethylsilyl)acetamide, 4-*N*-acetylcytosine, TMS triflate, acetonitrile (60%); iv) $NaOCH_3$, CH_3OH (57%); v) *N*,*O*-bis(trimethylsilyl)acetamide, 6-*N*-benzoyladenine, TMS triflate, acetonitrile; vi) $NaOCH_3$, CH_3OH (19%, two steps).

Experimental

General

All reagents and solvents were obtained from commercial suppliers and were used without further purification. Petroleum ether of distillation range 60–80 °C was used. Reactions were conducted under an atmosphere of nitrogen when anhydrous solvents were used. All reactions were monitored by TLC. During work-up, organic phases were combined and either dried (Na_2SO_4) and filtered or co-evaporated with anhydrous toluene 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. NMR spectra were recorded on a 250 MHz spectrometer (^{1}H NMR at 250 MHz and ^{13}C NMR at 62.9 MHz) for compounds **10**–**13** and on a 400 MHz spectrometer (^{1}H NMR at 400 MHz and ^{13}C NMR at 100.6 MHz) for all other compounds. Chemical shifts are reported in ppm relative to tetramethylsilane as internal standard, and coupling constants *J* are given in Hz. Complete assignments of NMR spectra when given are based on 2D spectra recorded on a 400 MHz spectrometer. The atom numbering used throughout the NMR assignments follows standard carbohydrate/nucleoside style. When mixtures of isomers were obtained, signals obtained for the major isomer are marked with an asterisk (*). NOE spectra were recorded on a 250 MHz spectrometer. NOE and 2D spectra were obtained in the same solvent as the corresponding ^{1}H and ^{13}C spectra. Fast-atom bombardment (high-resolution) mass spectra [FAB-(HR)MS] were recorded in positive ion mode. Microanalyses were performed at The Microanalytical Laboratory, Department of Chemistry, University of Copenhagen.

3-Azido-3-deoxy-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-*α*-D-*erythro*-pentofuranose 10

To a stirred solution of compound **9**[16] (8.52 g, 0.0188 mol) in anhydrous methanol (200 cm^3) was added sodium methoxide (2.13 g, 0.0394 mol). The reaction mixture was stirred for 1 h at room temperature whereupon the mixture was 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 (97 : 3 v/v) as eluent, to give furanose **10** (4.34 g, 94%) as a white solid material, δ_H (CD_3OD) 5.82 (1H, d, *J* 3.9, 1-H), 4.88 (1H, dd, *J* 3.9, 5.63, 2-H), 4.27 (1H, d, *J* 5.7, 3-H), 3.90 (1H, d, *J* 11.9), 3.68 (1H, d, *J* 11.9), 3.66 (1H, d, *J* 11.8), 3.56 (1H, d, *J* 11.9) (together 5-, 1′-H_2), 1.57 (3H, s, CH_3), 1.34 (3H, s, CH_3); δ_C (CD_3OD) 114.6 [*C*H(CH_3)$_2$], 106.0 (C-1), 89.3 (C-4), 81.9 (C-2), 64.0, 63.4, 63.0 (C-3, -5, -1′), 26.8, 26.3 (2 × CH_3); FAB-MS *m*/*z* 246 [M + H]$^+$. Selected IR signal: ν_{max} 2117 cm^{-1} (azido group). Furanose **10** had been synthesized earlier, but no data were reported.[11]

3-Azido-3-deoxy-1,2-*O*-isopropylidene-5-*O*-mesyl-4-*C*-mesyloxymethyl-*α*-D-*erythro*-pentofuranose 11

To a stirred solution of furanose **10** (4.25 g, 0.0173 mol) in anhydrous pyridine (125 cm^3) was added a solution of methanesulfonyl chloride (3.25 cm^3, 0.0418 mol) in anhydrous pyridine (5.0 cm^3). After stirring of the mixture for 1.5 h at room temperature, ice-cold water (50 cm^3) was added at 0 °C and the mixture was evaporated to dryness under reduced pressure. The residue was dissolved in dichloromethane (125 cm^3), washed with saturated aq. sodium hydrogen carbonate (3 × 50 cm^3) and evaporated to dryness under reduced pressure to give an intermediate tentatively assigned as **11** which was used without further purification in the next step; δ_C ($CDCl_3$) 113.4 [*C*H(CH_3)$_2$], 103.8 (C-1), 82.6, 78.5, 67.5, 67.3, 62.8 (C-2, -3, -4, -5, -1′), 37.1, 36.8 (2 × Ms), 25.0, 24.6 (2 × CH_3). FAB-MS *m*/*z* 402 [M + H]$^+$; selected IR signal: ν_{max} 2120 cm^{-1} (azido group).

Methyl 3-azido-3-deoxy-5-*O*-mesyl-4-*C*-mesyloxymethyl-D-*erythro*-pentofuranoside 12

Intermediate **11** (6.96 g) was stirred in a mixture of HCl in methanol (225 cm^3; 20% w/w) and water (30 cm^3) for 72 h at room temperature, then neutralized with sodium hydrogen carbonate (s). Extraction was performed with dichloromethane (3 × 100 cm^3) and the combined organic phase was washed with saturated aq. sodium hydrogen carbonate (3 × 100 cm^3) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography, using dichloromethane–methanol (100 : 0 to 97 : 3 v/v) as eluent, to give the

anomeric furanoside **12** (≈1.0 : 2.5; 5.16 g, 79%, two steps) as a light yellow oil; δ_H (CD_3OD) 4.91 (d, *J* 4.2, 1-H), 4.84* (*J* 12.8, 1-H), 4.50–4.12 (m, 2-, 3-, 5-, 1′-H), 3.44 (s, CH_3O), 3.38* (s, CH_3), 3.18, 3.15, 3.14 (3 × s, 4 × Ms); δ_C (CD_3OD) 107.9, 103.0 (C-1), 81.7, 81.0, 74.4, 72.2, 69.1, 68.7, 68.6, 68.5, 65.3, 62.7 (C-2, -3, -4, -5, -1′), 54.4, 53.9 (CH_3O), 35.6, 35.4 (Ms); FAB-MS *m*/*z* 376 $[M + H]^+$; selected IR signal: ν_{max} 2121 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.

Methyl 3-azido-3-deoxy-2-*O*,4-*C*-methylene-D-ribofuranoside 13

Furanoside **12** (5.16 g, 0.0137 mol) was stirred in a mixture of 1,4-dioxane (150 cm^3) and 1 M aq. NaOH (137 cm^3). After 1 h at room temperature, analytical TLC (methanol–dichloromethane; 1.0 : 12.5 v/v) showed quantitative conversion of starting material into an intermediate with a slightly lower mobility. The reaction mixture was subsequently stirred at 85 °C for 96 h and then evaporated to dryness under reduced pressure. The residue was dissolved in methanol–water (1 : 1 v/v), mixed with silica gel and evaporated to dryness under reduced pressure. The silica gel containing absorbed product was applied to a silica gel column and purification was performed using dichloromethane–methanol (99 : 1 to 98 : 2 v/v) as eluent to give anomeric furanoside **13** (≈1.0 : 1.8; 2.40 g, 87%) as a clear oil; δ_H (CD_3OD) 5.10* (s, 1-H), 4.78 (s, 1-H), 4.32* (s, 2-H), 4.23 (s, 2-H), 4.06–3.57 (m, 3-, 5-, 1′-H), 3.46* (s, CH_3O), 3.39 (s, CH_3O); δ_C (CD_3OD) 106.0, 105.7 (C-1), 91.6, 88.5, 80.2, 80.0, 73.0, 72.4, 65.3, 63.8 (C-2, -3, -4, -5), 59.1, 58.8, 56.5, 55.7 (CH_3O, C-1′); FAB-MS *m*/*z* 202 $[M + H]^+$; selected IR signal: ν_{max} 2114 cm^{-1} (azido group) [Found: (%) C, 41.4; H, 5.8; N, 20.0. $C_7H_{11}N_3O_4 \cdot 0.25H_2O$ requires C, 40.9; H, 5.6; N, 20.4].

Methyl 5-*O*-acetyl-3-azido-3-deoxy-2-*O*,4-*C*-methylene-β-D-ribofuranoside 14a and methyl 5-*O*-acetyl-3-azido-3-deoxy-2-*O*,4-*C*-methylene-α-D-ribofuranoside 14b

To a stirred solution of furanoside **13** (2.33 g, 0.0116 mol) in anhydrous pyridine (75 cm^3) was added acetic anhydride (2.74 cm^3, 0.0290 mol). After stirring of the mixture for 5 h at room temperature, ice-cold water (60 cm^3) was added at 0 °C and the mixture was evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate (150 cm^3), washed with saturated aq. sodium hydrogen carbonate (3 × 50 cm^3) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography, using ethyl acetate–petroleum ether (0 : 100 to 30 : 70 v/v) as eluent, to give the β-anomer **14a** and the corresponding α-anomer **14b** as individual compounds (**14a** : **14b** = 3 : 1; 2.48 g combined yield, 88%), both as clear oils.

Data for **14a**: δ_H (CD_3OD) 4.79 (1H, s, 1-H), 4.45 (1H, d, *J* 12.5, 5- or 1′-H), 4.32 (1H, d, *J* 12.5, 1′- or 5-H), 4.25 (1H, s, 2-H), 4.09 (1H, s, 3-H), 3.83 (1H, d, *J* 7.9, 5- or 1′-H), 3.62 (1H, d, *J* 7.9, 1′- or 5-H), 3.37 (3H, s, CH_3O), 2.08 (3H, s, CH_3CO); δ_C (CD_3OD) 172.1 (*C*OCH_3), 106.2 (C-1), 85.9 (C-4), 80.1 (C-2), 72.4 (C-5 or -1′), 64.4 (C-3), 61.0 (C-1′ or -5), 55.7 (CH_3O), 20.6 (*C*H_3CO); FAB-MS *m*/*z* 244 $[M + H]^+$ [Found: (%) C, 43.9; H, 5.5; N, 17.1. $C_9H_{13}N_3O_6$ requires C, 43.6; H, 5.5; N, 17.0].

Data for **14b**: δ_H (CD_3OD) 5.11 (1H, s, 1-H), 4.35 (1H, s, 2-H), 4.39, 4.29 (2H, 2 × d, *J* 12.7, 5- or 1′-H_2), 4.08 (1H, s, 3-H), 3.88, 3.83 (2H, 2 × d, *J* 8.2, 1′- or 5-H_2), 3.45 (3H, s, CH_3O), 2.08 (3H, s, CH_3CO); δ_C (CD_3OD) 172.0 (*C*OCH_3), 105.9 (C-1), 88.8 (C-4), 79.9 (C-2), 73.0 (C-5 or -1′), 65.9 (C-3), 61.4 (C-1′ or -5), 56.6 (CH_3O), 20.6 (*C*H_3CO); FAB-MS *m*/*z* 244 $[M + H]^+$ [Found: (%) C, 43.8; H, 5.7; N, 17.2. $C_9H_{13}N_3O_6 \cdot 0.25H_2O$ requires C, 43.6; H, 5.5; N, 17.0].

Data for the anomeric mixture: Selected IR signal: ν_{max} 2115 cm^{-1} (azido group).

(2*R*,3*S*,4*S*)-3-Azido-4-hydroxy-4-hydroxymethyl-2-[(*S*)-(thymin-1-yl)(methoxy)methyl]tetrahydrofuran 6a and (2*R*,3*S*,4*S*)-3-azido-4-hydroxy-4-hydroxymethyl-2-[(*R*)-(thymin-1-yl)-(methoxy)methyl]tetrahydrofuran 6b

To a stirred solution of the anomeric mixture **13** (0.108 g, 0.537 mmol) and thymine (0.135 g, 1.07 mmol) in anhydrous acetonitrile (10 cm^3) was added *N*,*O*-bis(trimethylsilyl)-acetamide (0.79 cm^3, 3.22 mmol). The reaction mixture was stirred and heated with reflux for 1 h. After cooling to 0 °C, TMS triflate (0.26 cm^3, 1.34 mmol) was added dropwise. The mixture was heated to 75 °C and stirring was continued for 48 h whereupon additional TMS triflate (0.10 cm^3, 0.537 mmol) was added and the reaction mixture was stirred for 72 h at 75 °C. Half-saturated aq. sodium hydrogen carbonate (10 cm^3) was added and the mixture was evaporated to dryness under reduced pressure. The residue was dissolved in methanol–water (2 : 1 v/v), mixed with silica gel and evaporated to dryness under reduced pressure. The silica gel containing absorbed product was applied to a silica gel column and purification was performed using dichloromethane–methanol (98 : 2 to 97 : 3 v/v) as eluent to give nucleoside analogues **6a** + **6b** as a diastereoisomeric mixture (0.180 g, 61%). This mixture was purified by repeated silica gel column chromatography as described above, and by preparative reversed-phase HPLC (C_{18} column, eluting with ethanol–water (1 : 40 to 1 : 10 v/v)) to yield the two individual diastereoisomers **6a** and **6b** (4.0 mg, 2.2%, t_R 43 min; 5.0 mg, 2.8%, t_R 66 min) as individual but not assigned compounds, both as white solid materials.

Data for the more polar diastereoisomer: δ_H ($(CD_3)_2SO$) 11.4 (1H, br s, NH), 7.45 (1H, s, 6-H), 5.53 (1H, d, *J* 6.2, 1′-H), 5.41 (1H, br s, OH), 4.90 (1H, br s, OH), 3.88–3.46 (6H, m, 2′-, 3′-, 5′-, 1″-H), 3.27 (3H, s, CH_3O), 1.78 (3H, s, CH_3); FAB-HRMS *m*/*z* 328.1263 $[C_{12}H_{18}N_5O_6, M + H]^+$. Calc. 328.1257.

Data for the less polar diastereoisomer: δ_H ($(CD_3)_2SO$) 11.3 (1H, br s, NH), 7.53 (1H, s, 6-H), 5.73 (1H, d, *J* 7.9, 1′-H), 5.40 (1H, br s, OH), 4.91 (1H, br s, OH), 4.04–3.42 (6H, m, 2′-, 3′-, 5′-, 1″-H), 3.28 (3H, s, CH_3O), 1.79 (3H, s, CH_3); FAB-HRMS *m*/*z* 328.1255 $[C_{12}H_{18}N_5O_6, M + H]^+$. Calc. 328.1257.

(2*R*,3*S*,4*R*)-4-Acetoxymethyl-2-[(4-*N*-acetylcytosin-1-yl)-(methoxy)methyl]-3-azido-4-trimethylsilyloxytetrahydrofuran 15

To a stirred solution of furanoside **14a** (0.161 g, 0.662 mmol) and 4-*N*-acetylcytosine (0.203 g, 1.32 mmol) in anhydrous acetonitrile (15 cm^3) was added *N*,*O*-bis(trimethylsilyl)-acetamide (0.97 cm^3, 3.97 mmol). The reaction mixture was stirred and heated with reflux for 1 h. After cooling to 0 °C, the mixture was treated dropwise with TMS triflate (0.38 cm^3, 1.99 mmol). After being stirred for 10 min at 0 °C, the mixture was heated to 60 °C and stirring was continued for 48 h and subsequently at 70 °C for 24 h. Additional TMS triflate (0.12 cm^3, 0.662 mmol) was added and the reaction mixture was stirred for 20 h at 70 °C. Half-saturated aq. sodium hydrogen carbonate (10 cm^3) was added and the mixture was evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate, mixed with silica gel and evaporated to dryness under reduced pressure. The silica gel containing absorbed product was applied to a silica gel column and purification was performed using dichloromethane–methanol (100 : 0 to 97.5 : 2.5 v/v) as eluent to give the diastereoisomeric intermediate **15** as a light yellow oil (0.187 g, 60%); δ_C (CD_3OD) 173.0, 172.1, 172.1, 164.5, 164.4 (3 × C=O), 159.3, 158.6, 146.4, 146.1, 98.9, 98.6 (C-4, -5, -6), 88.6, 88.0, 84.6, 84.4, 84.0, 82.8, 75.3, 74.8, 72.6, 71.3, 66.4, 66.3 (C-1′, -2′, -3′, -4′, -5′, -1″), 58.1, 57.8 (CH_3O), 24.7, 22.2, 21.0, 20.9 (2 × *C*H_3C=O), 1.93 (TMS); selected IR signal: ν_{max} 2113 cm^{-1} (azido group).

(2*R*,3*S*,4*S*)-3-Azido-2-[(cytosin-1-yl)(methoxy)methyl]-4-hydroxy-4-hydroxymethyltetrahydrofuran 7

To a stirred solution of derivative **15** (50.0 mg, 0.117 mmol) in anhydrous methanol (5 cm^3) was added sodium methoxide (22.0 mg, 0.410 mmol). The reaction mixture was stirred for 3 h at room temperature whereupon the mixture was 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 (95 : 5 to 92 : 8 v/v) as eluent, to give the diastereoisomeric nucleoside analogue **7** (ratio between diastereoisomers = 1.0 : 1.3; 21.0 mg, 57%) as a white solid material; δ_H (CD_3OD) 7.64 (d, *J* 7.5, 6-H), 7.62* (d, *J* 7.5, 6-H), 5.97 (d, *J* 7.5, 5-H), 5.96* (d, *J* 7.5, 5-H), 5.93 (d, *J* 8.4, 1′-H), 5.80* (d, *J* 5.7, 1′-H), 4.08–3.59 (m, 2′-, 3′-, 5′-, 1″-H), 3.36* (s, CH_3O), 3.34 (s, CH_3O); δ_C (CD_3OD) 167.6, 167.5 (C-4), 159.7, 159.0 (C-2), 142.7, 142.4 (C-6), 97.0, 96.7 (C-5), 88.0, 87.5 (C-1′), 85.4, 84.8, 82.9, 82.7, 75.7, 75.6, 71.9, 71.0, 64.2, 64.0 (C-2′, -3′, -4′, -5′, -1″), 57.4, 57.2 (CH_3O); FAB-HRMS *m*/*z* 313.1243. Calc. 313.1260 $[M + H]^+$; selected IR signal: ν_{max} 2113 cm^{-1} (azido group).

(2*R*,3*S*,4*S*)-2-[(Adenin-9-yl)(methoxy)methyl]-3-azido-4-hydroxy-4-hydroxymethyltetrahydrofuran 8

To a stirred solution of furanoside **14** (75.0 mg, 0.308 mmol) and 6-*N*-benzoyladenine (0.148 g, 0.617 mmol) in anhydrous acetonitrile (10 cm^3) was added *N*,*O*-bis(trimethylsilyl)-acetamide (0.45 cm^3, 1.85 mmol). The reaction mixture was stirred and heated under reflux for 1 h. After the mixture had cooled to 0 °C, TMS triflate (0.18 cm^3, 0.925 mmol) was added dropwise. After being stirred for 10 min at 0 °C, the mixture was heated to 70 °C and stirring was continued for 90 h. Half-saturated aq. sodium hydrogen carbonate (5 cm^3) was added and the mixture was evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate, mixed with silica gel, and evaporated to dryness under reduced pressure. The silica gel containing absorbed product was applied to a silica gel column and purification was performed using dichloromethane–methanol (98.5 : 1.5 v/v) as eluent to give an intermediate tentatively assigned as **16** (74.0 mg).

To a stirred solution of this intermediate (64.0 mg) in anhydrous methanol (5 cm^3) was added sodium methoxide (22.0 mg, 0.410 mmol). The reaction mixture was stirred for 3 h at room temperature, additional sodium methoxide (11.0 mg, 0.202 mmol) was added, and stirring was continued for 19 h whereupon the mixture was 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 (97.5 : 2.5 to 95 : 5 v/v) as eluent, to give the diastereoisomeric nucleoside analogue **8** (ratio between diastereoisomers = 1.0 : 1.4; 17 mg, 19%, 2 steps) as a white solid material, δ_H(CD_3OD) 8.33 (s), 8.28, 8.26* (2 × s) (together 8-, 2-H), 5.88 (d, *J* 6.2, 1′-H), 5.87* (d, *J* 7.9, 1′-H), 4.56–3.66 (m, 2′-, 3′-, 5′-H, 1″-H_2), 3.41 (s, CH_3O), 3.40* (s, CH_3O); δ_C (CD_3OD) 157.5, 157.5, 154.3, 154.1, 151.6, 151.1, 141.3, 141.2 (C-6, -2, -4, -8), 120.1, 120.1 (C-5), 87.9, 87.7, 85.4, 85.2, 83.3, 82.7, 75.9, 75,9, 71.9, 71.1, 64.0, 63.9 (C-1′, -2′, -3′, -4′, -5′, -1″), 57.6, 57.4 (CH_3O); FAB-HRMS *m*/*z* 337.1360. Calc. 337.1373 $[M + H]^+$; selected IR signal: ν_{max} 2113 cm^{-1} (azido group).

Acknowledgements

Dedicated to the memory of Professor Göran Magnusson.

The Danish Natural Science Research Council, The Danish Technical Research Council, and Exiqon A/S are thanked for financial support.

References

1 Information about FDA-approved anti-HIV drugs can be found at www.niaid.nih.gov/daids/dtpdb
2 E. De Clercq, *Collect. Czech. Chem. Commun.*, 1998, **63**, 449.
3 E. De Clercq, *Antiviral Res.*, 1989, **12**, 1.
4 E. De Clercq, *J. Med. Chem.*, 1995, **38**, 2491.
5 C. S. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, 1972, **94**, 8205.
6 P. Van Roey, E. W. Taylor, C. K. Chu and R. F. Schinazi, *Ann. N.Y. Acad. Sci.*, 1990, **616**, 29.
7 P. Van Roey, J. M. Salerno, C. K. Chu and R. F. Schinazi, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 3929.
8 G. R. Painter, A. E. Aulabaugh and C. W. Andrews, *Biochem. Biophys. Res. Commun.*, 1993, **191**, 1166.
9 V. E. Marquez, A. Ezzitouni, P. Russ, M. A. Siddiqui, H. Ford, R. J. Feldman, H. Mitsuya, C. George and J. J. Barchi, *J. Am. Chem. Soc.*, 1998, **120**, 2780.
10 V. E. Marquez, A. Ezzitouni, P. Russ, M. A. Siddiqui, H. Ford, R. J. Feldman, H. Mitsuya, C. George and J. J. Barchi, *Nucleosides, Nucleotides*, 1998, **17**, 1881.
11 S. Obika, J. Andoh, T. Sugimoto, K. Miyashita and T. Imanishi, *Tetrahedron Lett.*, 1999, **40**, 6465.
12 A. G. Olsen, V. K. Rajwanshi, C. Nielsen and J. Wengel, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3610. The furanose conformation of compound **5** could alternatively be assigned as *N*-type (C3′-*endo*, ^3E) because of its L-configuration. However, for a direct comparison with the conformations of the natural DNA/RNA monomers, the furanose conformation of **5** is considered herein as equivalent to an *S*-type (C3′-*exo*, $_3E$) conformation.
13 H. Vorbrüggen, K. Krolikiewicz and B. Bennua, *Chem. Ber.*, 1981, **114**, 1234.
14 H. Vorbrüggen and G. Höfle, *Chem. Ber.*, 1981, **114**, 1256.
15 P. Nielsen and J. Wengel, *Chem. Commun.*, 1998, 2645.
16 S. A. Surzhykov and A. A. Krayevsky, *Nucleosides, Nucleotides*, 1994, **13**, 2283.
17 M. Chenon, R. J. Pugmire, D. M. Grant, R. P. Panzica and L. B. Townsend, *J. Am. Chem. Soc.*, 1975, **97**, 4627.
18 K. Danel, E. B. Pedersen and C. Nielsen, *J. Med. Chem.*, 1998, **41**, 191.