Synthesis and restricted furanose conformations of three novel bicyclic thymine nucleosides: a *xylo*-LNA nucleoside, a 3′-*O*,5′-*C*-methylene-linked nucleoside, and a 2′-*O*,5′-*C*-methylene-linked nucleoside

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The *xylo*-LNA nucleoside 1-(2-*O*,4-*C*-methylene-β-D-xylofuranosyl)thymine (**9**) and the 2′-*O*,5′-*C*-methylene linked nucleoside 1-(2,6-anhydro-β-D-altrofuranosyl)thymine (**28**) were obtained in overall yields of 13% (8 steps) and 31% (7 steps) starting from furanose derivatives **1** and **21**, respectively. In the synthesis of 3′-*O*,5′-*C*-methylene-linked nucleoside derivatives, cyclization by intramolecular attack from the 6-hydroxy group on the 3-keto functionality to give C-3-hemiketal furanose **11** and its subsequent transformation into nucleoside **15** proved very efficient. It was, however, impossible to isolate the debenzylated 2′-hydroxy, 2′-*O*-methyl and 2′-*O*-*tert*-butyldimethylsilyl derivatives **16**, **18** and **20**, respectively, in analytically pure form. Solution-phase conformational analysis showed the bicyclic nucleosides **8**, **9**, **14**, **15**, **17** and **19** to exist predominantly in an N-type furanose conformation and bicyclic nucleotides **27** and **28** to adopt an S-type conformation.

Introduction

A successful approach towards oligonucleotide analogues capable of binding with high affinity to complementary single- stranded DNA and RNA has been the use of oligomers containing conformationally restricted monomers.[1,2] Thus, several oligonucleotide analogues containing bi- and tricyclic carbohydrate moieties have displayed enhanced duplex stabilities compared with the unmodified reference oligonucleotides.[3–18] LNA (Locked Nucleic Acid; Fig. 1) is a prime example displaying unprecedented increases in the thermal stability (melting temperature, T_m) of duplexes towards complementary single-stranded DNA and RNA (ΔT_m/modification = +3 °C to +11 °C).[10–17]† Conformationally restricted nucleosides are also useful as tools in evaluation of conformational preferences of nucleos(t)ide converting enzymes and thereby also of structure–activity relationships of, *e.g.*, antiviral nucleosides. As an example, Marquez *et al.* have recently found HIV-1 reverse transcriptase to discriminate between two conformationally locked carbocyclic AZT triphosphate analogues.[19] Most of the research conducted in these directions has been focused on restricting the conformation of the furanose ring, or structural entities mimicking the furanose ring, into either an S-type (*e.g.*, C-2′-*endo*) or an N-type (*e.g.*, C-3′-*endo*) conformation (Fig. 1).‡ Due to the low energy barrier (~2 kcal mol^{-1}[20]§) between the two dominating conformers a fast equilibrium between the N- and the S-type states exists[21–23] for unmodified monomeric nucleosides in solution. However, the furanose moieties in RNA normally exist in an N-type conformation leading to A-type duplexes and in DNA generally in an S-type conformation leading to B-type duplexes. In addition, one conformer is expected to be dominating for a nucleoside bound in the active sites of enzymes.[19]

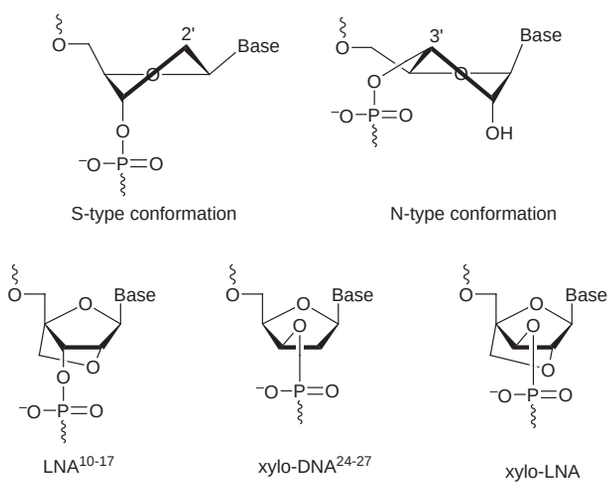


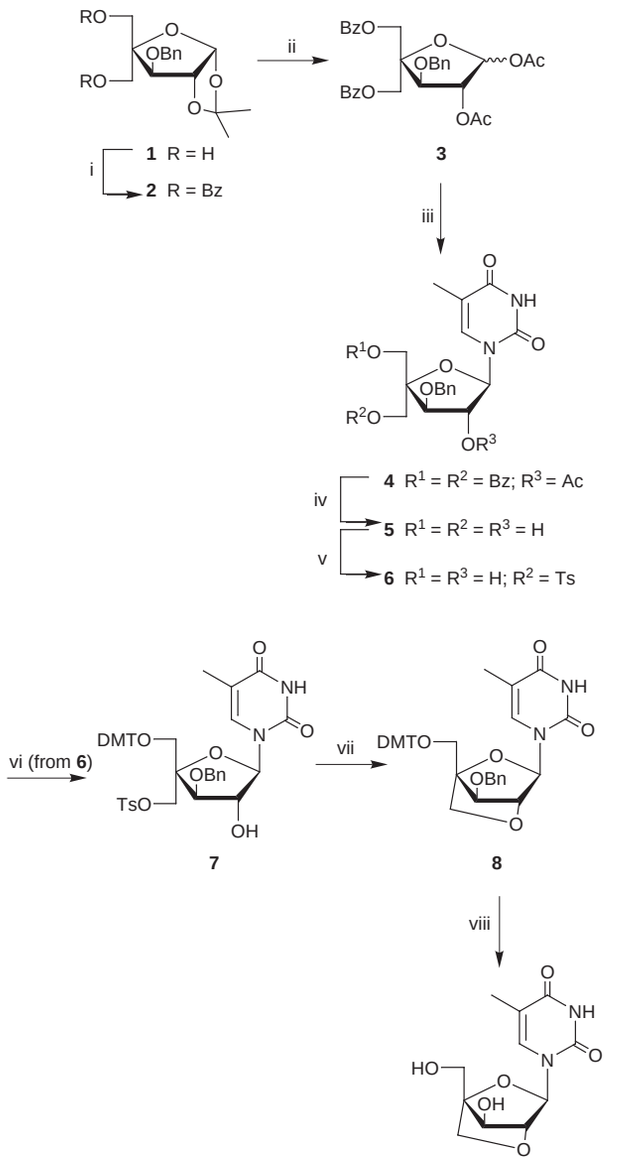
Fig. 1

The work in this area has been based on the assumption that complexation between an oligonucleotide analogue and its DNA or RNA target, and between a nucleoside analogue and a relevant enzyme, is favoured by the appropriate preorganization of the ligand. The results obtained[1–19] have shown this assumption to be valid, but in order to gain further knowledge of the natural systems and to be able to design improved therapeutic molecules, novel analogues are needed. In this paper we describe the synthesis of three novel classes of conformationally restricted nucleoside analogues all containing a bicyclic pentofuranose moiety and hydroxy groups positioned at the 3′- and 5′-positions allowing the formation of 3′-O- to 5′-O-linked oligonucleotide analogues and 5′-phosphorylated nucleoside derivatives thus mimicking the natural regiochemistry. Our interest in the *xylo*-LNA nucleoside **9** (Scheme 1) was stimulated by a series of papers in which Seela *et al.* have studied

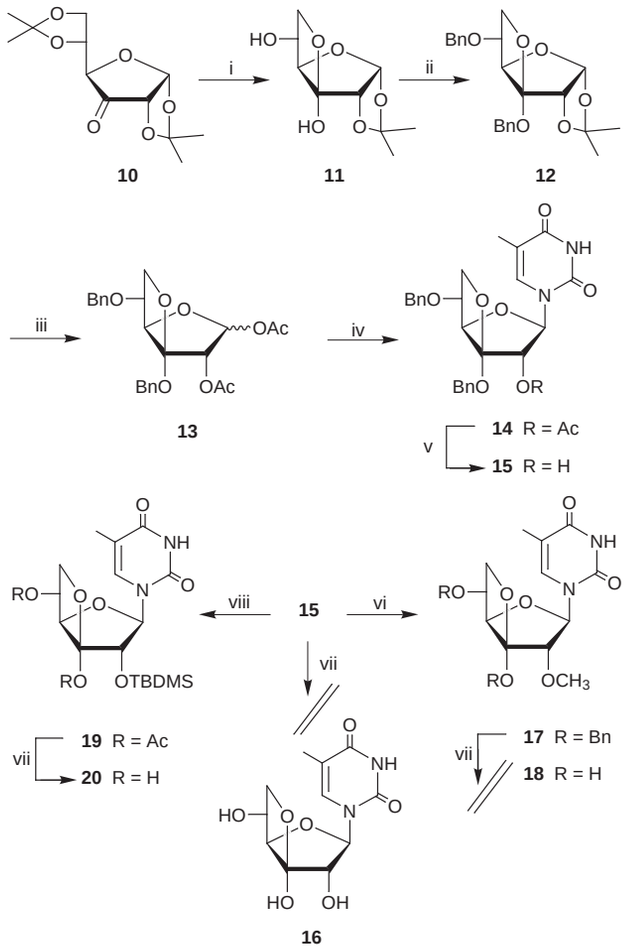
† We have defined LNA as an oligonucleotide containing one or more 2′-*O*,4′-*C*-methylene-linked bicyclic ribonucleoside monomers.

‡ A thorough introduction to the conformational aspects of nucleosides is given in ref. 19. The term '*endo*' designates the positioning of an atom 'above' the reference plane of the furanose ring, *e.g.*, the C-1′, C-2′, C-4′ and O atoms. The term '*exo*' designates the positioning of an atom below the reference plane.

§ A simple rule to evaluate the percentage S and percentage N conformer in a two-state model is given in ref. 23: %S = 10 × $J_{1',2'}$; %N = 10 × $J_{3',4'}$. The problem in the context of the bicyclic nucleosides prepared herein is that no value exists for $J_{3',4'}$ for compounds **8** and **9** (no H-4′ present) and **14**, **15**, **17**, **19** and **20** (no H-3′ present).



Scheme 1 *Reagents and conditions*: i) BzCl, pyridine (90%); ii) a) 80% AcOH, b) Ac_2O, pyridine (92%); iii) *N*,*O*-bis(trimethylsilyl)acetamide, thymine, trimethylsilyl triflate, acetonitrile (85%); iv) $NaOCH_3$, CH_3OH (89%); v) toluene-*p*-sulfonyl chloride, pyridine, CH_2Cl_2 (35%); vi) DMTCl, DMAP, pyridine (75%); vii) NaH, DMF (96%); viii) H_2, 10% Pd/C, CH_3OH (82%).



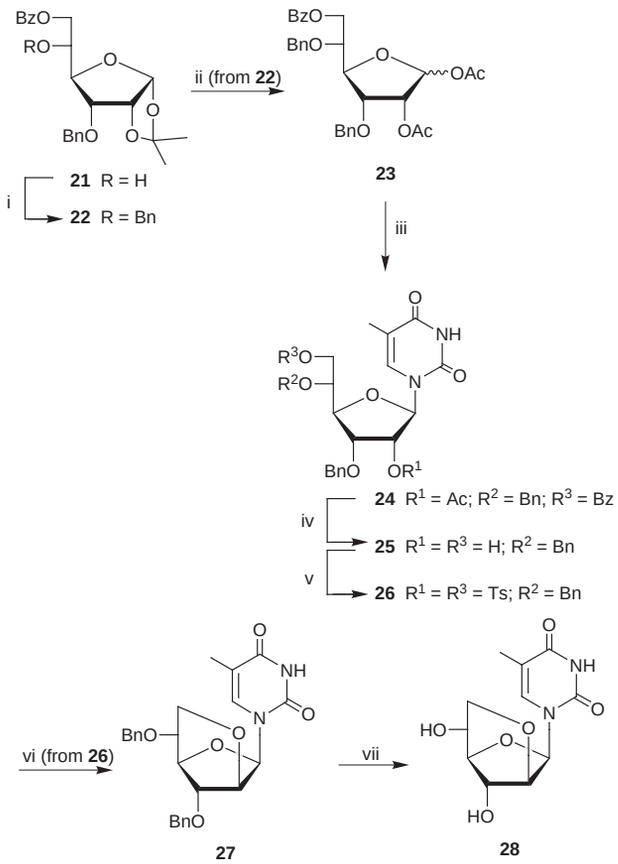
Scheme 2 *Reagents and conditions*: i) 80% AcOH (96%); ii) NaH, BnBr, DMF (93%); iii) a) 80% AcOH, b) Ac_2O, pyridine (57%); iv) *N*,*O*-bis(trimethylsilyl)acetamide, thymine, trimethylsilyl triflate, acetonitrile (85%); v) $NaOCH_3$, CH_3OH (90%); vi) NaH, CH_3I, CH_2Cl_2 (62%); vii) H_2, 20% $Pd(OH)_2$/C, ethanol (for **20**: 50%); viii) TBDMSCl, imidazole, DMF (85%).

xylo-DNA (Fig. 1) containing one or more 2′-deoxy-β-D-xylofuranosyl nucleotide monomers,[24–27] and by the remarkable properties of LNA. A convenient synthetic methodology towards *xylo*-LNA nucleosides should allow the synthesis of *xylo*-LNA (Fig. 1) containing one or more 2′-*O*,4′-*C*-methylene-β-D-xylofuranosyl nucleotide monomer(s) as a stereoisomer of LNA. As a continuation of our general interest in conformationally restricted nucleosides and oligonucleotide analogues, we decided also to synthesize bicyclic 3′-*O*,5′-*C*-methylene-linked and 2′-*O*,5′-*C*-methylene-linked nucleosides (Schemes 2 and 3).

Results and discussion

Chemical synthesis

Benzoylation of the 4-*C*-hydroxymethyl-β-L-*threo*-pentofuranose **1** [28] afforded in 90% yield the di-*O*-benzoyl derivative **2** which was subsequently converted into the 1,2-di-*O*-acetylated furanose **3** in 92% yield by acetolysis using 80% acetic acid followed by acetylation. Employing a modified Vorbrüggen methodology,[29,30] the thymine β-configured nucleoside **4** was stereoselectively obtained in 85% yield by *in situ* silylation of thymine and trimethylsilyl triflate-mediated coupling. Treatment of compound **4** with sodium methoxide resulted in deacylation to give nucleoside triol **5** in 89% yield. To prepare for cyclization, we investigated the selectivity of monotosylation by reaction using 1.1 equivalent of toluene-*p*-sulfonyl chloride and pyridine in anhydrous dichloromethane. As we have reported earlier for a structurally closely related nucleoside,[31] the 4′-*C*-hydroxymethyl group positioned at the α-face of the furanose ring is more reactive than the 5′-hydroxy group under such conditions. Accordingly, we could isolate 4′-*C*-tosyloxymethyl nucleoside **6** as the major product (35% yield) and one monotosylated and one ditosylated compound in only 7% and 6% yield, respectively (data not reported). To our surprise, cyclization of **6** (sodium hydride, anhydrous DMF) proved unsuccessful at room temperature even after stirring for several days using either DMF or THF as solvent. However, after DMT-protection of the 5′-hydroxy group [4,4′-dimethoxytrityl chloride (DMTCl) and 4-(dimethylamino)pyridine (DMAP) in anhydrous pyridine; 75% yield of nucleoside **7**] efficient cyclization was accomplished to give the protected bicyclic 2′-*O*,4′-*C*-methylene-β-D-xylofuranosyl nucleoside **8** in 96% yield. Apparently, the introduction of the bulky DMT group induces a conformational change favourable for the cyclization. Catalytic hydrogenation yielded the parent *xylo*-LNA nucleoside **9** [1-(2′-*O*,4′-*C*-methylene-β-D-xylofuranosyl)-thymine] in 82% yield by concomitant debenzylation and detritylation (Scheme 1).



Scheme 3 *Reagents and conditions*: i) NaH, BnBr, DMF (90%); ii) a) 80% AcOH, b) Ac_2O, pyridine (74%); iii) *N*,*O*-bis(trimethylsilyl)-acetamide, thymine, trimethylsilyl triflate, acetonitrile (81%); iv) $NaOCH_3$, CH_3OH (88%); v) toluene-*p*-sulfonyl chloride, DMAP, CH_2Cl_2 (71%); vi) NaOH, H_2O, ethanol (93%); vii) H_2, 20% $Pd(OH)_2$/C, ethanol (98%).

The 3′-*O*,5′-*C*-methylene-linked nucleoside derivatives were synthesized as outlined in Scheme 2. Selective removal of the 5,6-*O*-isopropylidene protecting group of the known 3-ulose **10**[32] using 80% acetic acid at room temperature afforded smoothly the tricyclic hemiacetal furanose derivative **11** in 96% yield. To prepare for nucleoside coupling, 1,2-di-*O*-acetylated furanose **13** was prepared from **11** *via* **12** in 53% yield by benzylation, acetolysis and acetylation. Using the methodology described above for the synthesis of **4**, the thymine nucleoside **14** was stereoselectively obtained in 85% yield. Deacetylation using sodium methoxide gave in 90% yield the nucleoside derivative **15** with an unprotected 2′-hydroxylic functionality. Debenzylation of **15** to afford the bicyclic *ribo*-configured nucleoside triol **16** proved impossible in our hands. As judged from analytical TLC during the debenzylation (hydrogen in combination with various catalysts as well as transfer hydrogenation were attempted[11]) a major product having the expected R_f-value was formed during the reaction but all attempts to isolate this product in pure form were unsuccessful. Analogously, debenzylation of 2′-*O*-methyl and 2′-*O*-*tert*-butyldimethylsilyl derivatives **17** and **19**, respectively, proceeded efficiently according to analytical TLC but isolation proved troublesome in our hands and only the 2′-*O*-*tert*-butyldimethylsilyl derivative **20** could be isolated in acceptable purity (according to NMR; microanalysis could not be obtained).

The 2′-*O*,5′-*C*-linked thymine nucleoside **28** was synthesized as outlined in Scheme 3. Benzylation of the known furanose **21**[33] by reaction with benzyl bromide and sodium hydride in anhydrous DMF (**22**, 90% yield) followed by acetolysis and acetylation in 74% yield afforded the anomeric mixture **23** as a suitable substrate to give stereoselectively the fully protected novel thymine nucleoside **24** in 81% yield after coupling with thymine. Deacylation to give the 2′- and 6′-hydroxy derivative **25** was accomplished in 88% yield using sodium methoxide. To prepare for ring-closure, the 2′,6′-di-*O*-*p*-tolylsulfonyl nucleoside **26** was synthesized in 71% yield by reaction with excess toluene-*p*-sulfonyl chloride and DMAP in anhydrous dichloromethane. Ring-closure was subsequently attempted using sodium hydroxide in a mixture of H_2O and ethanol hoping for initial anhydro formation leading to *in situ* inversion at C-2′ and subsequent intramolecular nucleophilic attack from the liberated (α-face oriented) 2′-hydroxy group on the 6′-*O*-tosyl group. This strategy turned out to be very successful as the nucleoside **27** containing a novel 2′-*O*,5′-*C*-methylene-linked bicyclic furanose skeleton was obtained in 93% yield directly from the di-*O*-tosylated nucleoside **26**. Debenzylation was effected using hydrogen and 20% $Pd(OH)_2$/C as catalyst to give the unprotected bicyclic nucleoside **28** in 98% yield.

With the synthetic methods described herein, the *xylo*-LNA nucleoside **9** and the 2′-*O*,5′-*C*-methylene-linked nucleoside **28** was obtained in overall yields of 13% (8 steps) and 31% (7 steps) starting from the known furanose derivatives **1** and **21**, respectively. The methods are stereoselective, affording only one stereoisomer in the nucleobase-coupling steps and straightforward C-2′-inversion during the synthesis of **28**. The method depicted in Scheme 1 should be applicable for both pyrimidine and purine bases whereas the strategy towards nucleoside **28** is limited to pyrimidine derivatives because of the probable intermediacy of an 2,2′-*O*-anhydro compound during the C-2′-inversion. In the synthesis of the 3′-*O*,5′-*C*-methylene-linked derivatives, spontaneous cyclization by intramolecular attack on the 3-keto functionality and the subsequent trapping as the *ribo*-configured furanoside C-3-acetal proved very efficient in analogy with our results on a similar cyclization to give 2′-*O*,3′-*C*-ethylene-linked C-2′-acetal nucleosides which could be debenzylated in high yield.[34] However, after analogous debenzylation of the 2′-hydroxy, 2′-*O*-methyl and 2′-*O*-*tert*-butyldimethylsilyl derivatives **15**, **17** and **19**, respectively, the isolation of the pure products proved very difficult probably due to the instability of the hemiacetals on the column materials (silica gel, basic alumina; 2′-*O*-*tert*-butyldimethylsilyl derivative **20** appeared to be more stable than the 2′-hydroxy and 2′-*O*-methyl nucleosides **16** and **18**). Our interest in derivatives **18** and **20** originates from their potential as intermediates in the synthesis of phosphoramidite building blocks towards the corresponding 2′-*O*-methyl-RNA and RNA oligonucleotides, respectively. However, the problematic debenzylations render the chosen strategy inconvenient. It can therefore be summarized that quite efficient methods for synthesis of *xylo*-LNA nucleosides and 2′-*O*,5′-*C*-methylene-linked bicyclic nucleosides of the pyrimidine series have been developed and that further derivatizations of, *e.g.*, nucleosides **9** and **28** towards oligonucleotide building blocks[35] or functionalized nucleoside analogues should be possible.¶

Furanose conformations

Based on molecular modelling (Hyperchem™ Program, MM+ Molecular Mechanics Force Field; handmodel building), the nucleosides **8** and **9** were expected to adopt an N-type furanose conformation whereas **27** and **28** were expected to adopt an S-type furanose conformation. The situation was more dubious for compounds **14**, **15**, **17**, **19** and **20** as these bicyclic nucleosides consist of a bicyclo[3.3.0]octane system intrinsically more flexible compared with the bicyclo[2.2.1]heptane system of **8** and **9** and the bicyclo[3.2.1]octane system of **27** and **28**. From the coupling constants in the ^1H NMR spectrum, especially the $J_{1',2'}$- and $J_{3',4'}$-values, the furanose conformation of nucleosides

¶ Copies of the ^{13}C NMR spectra for compounds **9**, **20** and **28** were enclosed with this manuscript on submission to verify the purity and identity of these three bicyclic nucleosides.

in solutions can be estimated.[21–23] Following the prediction from modelling, the signals for H-1′ for the *xylo*-LNA nucleosides **8** and **9** were found to be singlets, revealing that these conformationally locked nucleosides exclusively adopt an N-type conformation. For the 3′-*O*,5′-*C*-methylene-linked nucleosides **14**, **15**, **17** and **19**, the $J_{1',2'}$-values were found to be 2.1 Hz (for **14**) and 0 Hz (for **15**, **17** and **19**). Thus, this 3′-*O*,5′-*C*-methylene linkage effectively introduces a novel class of N-type conformationally restricted nucleosides. The $J_{1',2'}$-values of 2.2 and 2.3 Hz found for the 2′-*O*,5′-*C*-methylene-linked nucleosides **27** and **28**, respectively, and the singlet for H-4′ found for **28** verify the expected S-type conformational preference for this class of nucleosides. It should, however, be mentioned that the analyses given herein are limited to the furanose puckering. Other structural features, *e.g.*, the orientation of the nucleobase and the 5′-hydroxy group, are likewise important aspects of nucleoside preorganization.

Conclusions

Efficient and stereoselective methods have been developed for synthesis of bicyclic nucleosides of the *xylo*-LNA type (*e.g.*, **9**) and of the 2′-*O*,5′-*C*-methylene-linked type (*e.g.*, **28**). Conformational analysis indicates the *xylo*-LNA-type nucleosides, and the 3′-*O*,5′-*C*-linked derivatives **14**, **15**, **17** and **19**, to adopt an N-type furanose conformation, whereas the 2′-*O*,5′-*C*-methylene-linked nucleosides adopt an S-type furanose conformation. These results suggest that the members of these novel classes of conformationally restricted nucleosides should be evaluated as building blocks in oligonucleotide analogues or as biologically active nucleosides, possibly after further functionalizations. This work shows the wide spectrum of restricted furanose conformations attainable for bicyclic nucleoside analogues.

Experimental

General

Reactions were conducted under an atmosphere of nitrogen when anhydrous solvents were used. Column chromatography was carried out using glass columns and Silica gel 60 (0.040–0.063 mm). After drying organic phases with Na_2SO_4, filtration was performed. Petroleum ether of distillation range 60–80 °C was used. Chemical-shift values δ are in ppm relative to tetramethylsilane as internal reference. *J*-Values are given in Hz. The names of the bicyclic nucleosides and tricyclic furanoses in this section are given according to the von Baeyer nomenclature. However, for easy comparison the assignments (when given based on 2D NMR experiments) of the signals of the NMR spectra are indicated using standard carbohydrate/nucleoside numbering with the thymine moiety having the highest priority. Microanalyses were performed at The Microanalytical Laboratory, Department of Chemistry, University of Copenhagen.

5-*O*-Benzoyl-4-*C*-benzoyloxymethyl-3-*O*-benzyl-1,2-*O*-isopropylidene-β-L-*threo*-pentofuranose 2

To a stirred, ice-cold solution of 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-β-L-*threo*-pentofuranose **1**[28] (5.00 g, 0.016 mol) in anhydrous pyridine (60 cm^3) was added benzoyl chloride (4.1 cm^3, 0.035 mol). After stirring at room temperature for 4 h, the reaction mixture was cooled to 0 °C, H_2O (50 cm^3) was added, and the mixture was extracted with dichloromethane (100 cm^3 × 3). The combined organic phase was washed successively with saturated aqueous sodium hydrogen carbonate (30 cm^3 × 3) and brine (20 cm^3 × 3), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using, first, petroleum ether–dichloromethane (1 : 1, v/v), and then dichloromethane–methanol (99 : 1, v/v), as eluent to give furanose **2** (7.50 g, 90%) as a yellowish oil after evaporation of the solvents under reduced pressure; $\delta_H(CDCl_3)$ 8.02–7.23 (15H, m), 6.08 (1H, d, *J* 4.2), 4.81–4.50 (7H, m), 4.22 (1H, d, *J* 1.0), 1.59 (3H, s), 1.37 (3H, s); $\delta_C(CDCl_3)$ 166.1, 165.8, 136.7, 133.1, 133.0, 129.9, 129.7, 129.6, 129.5, 128.5, 128.4, 128.3, 128.0, 127.9, 113.3, 105.4, 86.4, 85.1, 83.8, 72.3, 64.3, 63.8, 27.0, 26.4; FAB-MS *m*/*z* 521 $[M + H]^+$ [Found (%): C, 69.1; H, 5.9. $C_{30}H_{32}O_8$ requires C, 69.2; H, 6.2].

1,2-Di-*O*-acetyl-5-*O*-benzoyl-4-*C*-benzoyloxymethyl-3-*O*-benzyl-L-*threo*-pentofuranose 3

A solution of furanose **2** (7.40 g, 0.014 mol) in 80% acetic acid (60 cm^3) was stirred for 9 h at 90 °C. The mixture was evaporated to dryness under reduced pressure, and the residue was coevaporated with toluene (10 cm^3 × 3) and dissolved in anhydrous pyridine (80 cm^3). Acetic anhydride (5.5 cm^3) was added and the solution was stirred for 46 h at room temperature. The mixture was evaporated to dryness under reduced pressure, and the residue was coevaporated with toluene (10 cm^3 × 3) and dissolved in dichloromethane (150 cm^3). The solution was washed successively with saturated aqueous sodium hydrogen carbonate (30 cm^3 × 3) and brine (30 cm^3 × 3), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using, first, petroleum ether–dichloromethane (1 : 1, v/v), and then dichloromethane–methanol (99 : 1, v/v), as eluent to give the anomeric mixture **3** (α : β = 3 : 1; 7.33 g, 92%) as a clear oil after evaporation of the solvents under reduced pressure. This oil was used in the next step without further purification; $\delta_C(CDCl_3)$ 169.4, 169.0, 165.8, 165.6, 137.0, 133.2, 133.1, 133.0, 129.6, 129.5, 129.2, 128.3, 127.8, 127.7, 127.4, 99.4, 92.3, 87.0, 83.2, 82.2, 80.7, 77.4, 76.9, 76.3, 73.2, 72.4, 20.9, 20.8, 20.6, 20.3; FAB-MS *m*/*z* 562 $[M]^+$.

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

To a stirred suspension of the anomeric mixture **3** (7.26 g, 0.013 mol) and thymine (3.25 g, 0.028 mol) in anhydrous acetonitrile (80 cm^3) was added *N*,*O*-bis(trimethylsilyl)acetamide (19.1 cm^3, 0.077 mol). The reaction mixture was stirred at 60 °C for 1 h and then cooled to 0 °C. Trimethylsilyl triflate (4.1 cm^3, 0.023 mol) was added dropwise during 10 min and the mixture was subsequently heated for 22 h under reflux. After cooling of the mixture to room temperature, saturated aqueous sodium hydrogen carbonate (30 cm^3) was added and extraction was performed using dichloromethane (100 cm^3 × 3). The combined organic phase was washed successively with saturated aqueous sodium hydrogen carbonate (30 cm^3 × 3) and brine (50 cm^3 × 3), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane–methanol (0.5–2.0% methanol, v/v) as eluent to give nucleoside **4** (6.88 g, 85%) as a white solid after evaporation of the solvents under reduced pressure; $\delta_H(CDCl_3)$ 8.97 (1H, br s), 8.04–7.23 (16H, m), 6.37 (1H, d, *J* 3.6), 5.42 (1H, t, *J* 3.1), 4.89–4.56 (6H, m), 4.22 (1H, d, *J* 2.6), 2.13 (3H, s), 1.74 (3H, d, *J* 0.8); $\delta_C(CDCl_3)$ 169.9, 166.0, 165.7, 163.4, 150.4, 136.2, 135.2, 133.5, 133.4, 129.8, 129.7, 129.6, 129.5, 129.0, 128.6, 128.4, 128.2, 112.0, 87.4, 86.0, 81.3, 80.3, 72.6, 63.1, 62.9, 20.8, 12.3; FAB-MS *m*/*z* 629 $[M + H]^+$ [Found (%): C, 64.4; H, 4.9; N, 4.4. $C_{34}H_{32}N_2O_{10}\cdot 0.25H_2O$ requires C, 64.5; H, 5.1; N, 4.4].

1-(3-*O*-Benzyl-4-*C*-hydroxymethyl-α-L-*threo*-pentofuranosyl)-thymine 5

To a stirred solution of nucleoside **4** (9.00 g, 0.014 mol) in methanol (130 cm^3) was added sodium methoxide (3.87 g, 0.0716 mol). The reaction mixture was stirred at room temper-

ature for 4 h and then neutralized with dilute hydrochloric acid. The mixture was evaporated to dryness under reduced pressure followed by coevaporation using toluene (15 cm^3 × 3). The residue was purified by silica gel column chromatography using dichloromethane–methanol (4–15% methanol, v/v) as eluent to give nucleoside triol **5** (4.82 g, 89%) as a white solid after evaporation of the solvents under reduced pressure; $\delta_H(CD_3OD)$ 7.89 (1H, d, *J* 1.2), 7.40–7.24 (5H, m), 5.97 (1H, d, *J* 6.2), 4.83–4.65 (2H, m), 4.53 (1H, t, *J* 6.2), 4.21 (1H, d, *J* 6.2), 3.84 (1H, d, *J* 12.0), 3.63 (1 H, d, *J* 12.0), 3.59 (2H, d, *J* 2.6), 1.82 (3H, d, *J* 1.1); $\delta_C(CD_3OD)$ 164.4, 150.9, 137.5, 136.6, 127.5, 127.0, 126.9, 109.8, 86.7, 86.4, 82.8, 78.0, 72.1, 62.3, 61.1, 10.5; FAB-MS *m*/*z* 379 $[M + H]^+$ [Found (%): C, 56.2; H, 6.0; N, 7.0. $C_{18}H_{22}N_2O_7 \cdot 0.25H_2O$ requires C, 56.5; H, 5.9; N, 7.3].

1-[3-*O*-Benzyl-4-*C*-(*p*-tolylsulfonyloxymethyl)-β-D-xylofuranosyl]thymine 6

To a solution of nucleoside **5** (7.25 g, 0.0192 mol) in anhydrous pyridine (20 cm^3) and dichloromethane (70 cm^3) at −30 °C was added dropwise during 1.5 h a solution of toluene-*p*-sulfonyl chloride (4.38 g, 0.023 mol) in dichloromethane (8 cm^3). The temperature was raised to 0 °C for 2 h, whereupon additional toluene-*p*-sulfonyl chloride (1.80 g, 0.0094 mol) was added at −20 °C and the mixture was stirred for 12 h at −20 °C. At that time further toluene-*p*-sulfonyl chloride (0.736 g, 3.86 mmol) was added and stirring was continued at −20 °C for an additional 24 h. The reaction mixture was diluted with dichloromethane (75 cm^3) and H_2O (75 cm^3), and extraction was performed with dichloromethane (75 cm^3 × 3). The combined organic phase was washed successively with saturated aqueous sodium hydrogen carbonate (30 cm^3 × 3) and brine (40 cm^3 × 3). The aqueous phase was extracted with ethyl acetate (30 cm^3 × 3), and these extracts were combined with the dichloromethane extracts, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane–methanol (1.5–3.5% methanol, v/v) as eluent to give nucleoside **6** (3.56 g, 35%) as a white solid after evaporation of the solvents under reduced pressure; $\delta_H(CDCl_3)$ 10.23 (1H, s), 7.78–7.26 (10H, m), 5.84 (1H, d, *J* 5.5), 4.84 (1H, d, *J* 11.5), 4.59 (1H, d, *J* 11.5), 4.53 (1H, t, *J* 5.5), 4.19 (1H, d, *J* 5.6), 4.09 (1H, d, *J* 10.6), 4.03 (1H, d, *J* 10.6), 3.85 (1H, d, *J* 12.4), 3.67 (1H, d, *J* 12.4), 2.39 (3H, s), 1.78 (3H, d, *J* 0.6); $\delta_C(CDCl_3)$ 164.1, 151.5, 145.3, 137.0, 136.2, 132.3, 130.0, 128.6, 128.2, 128.0, 111.0, 88.5, 85.4, 83.8, 79.8, 73.2, 69.4, 63.0, 21.6, 12.5; FAB-MS *m*/*z* 533 $[M + H]^+$ [Found (%): C, 56.7; H, 5.4; N, 4.9. $C_{25}H_{28}N_2O_9S$ requires C, 56.4; H, 5.3; N, 5.2].

1-[3-*O*-Benzyl-5-*O*-(4,4′-dimethoxytrityl)-4-*C*-(*p*-tolylsulfonyloxymethyl)-β-D-xylofuranosyl]thymine 7

To a solution of nucleoside **6** (3.66 g, 6.88 mmol) in anhydrous pyridine (25 cm^3) were added DMAP (0.84 g, 6.81 mmol) and DMTCl (3.5 g, 13.2 mmol) and the mixture was stirred for 23 h at room temperature. Additional DMAP (0.250 g, 2.06 mmol) and DMTCl (0.700 g, 2.06 mmol) were added, and stirring was continued for 36 h at room temperature. Ice-cold H_2O (50 cm^3) was added and the reaction mixture was diluted with dichloromethane (150 cm^3). The organic phase was separated, and washed successively with saturated aqueous sodium hydrogen carbonate (25 cm^3 × 3) and brine (40 cm^3 × 3), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane–methanol–pyridine (0.75–1.5% methanol; 0.5% pyridine, v/v/v) as eluent to afford nucleoside **7** (4.28 g, 75%) as a white solid after evaporation of the solvents under reduced pressure; $\delta_H(CDCl_3)$ 9.40 (1H, s), 7.72–6.68 (23H, m), 5.77 (1H, d, *J* 4.2), 4.86 (1H, d, *J* 11.3), 4.49–4.43 (2H, m), 4.23–4.12 (3H, m), 3.76 (3H, s), 3.75 (3H, s), 3.45 (1H, d, *J* 10.2), 3.17 (1H, d, *J* 10.2), 2.37 (3H, s), 1.44 (3H, s); $\delta_C(CDCl_3)$ 163.7, 158.5, 151.0, 144.9, 144.4, 137.1, 135.8, 135.2, 135.0, 132.5, 130.1, 129.8, 128.3, 128.0, 127.8, 127.7, 126.9, 113.1, 110.0, 90.2, 87.1, 86.4, 83.3, 79.9, 72.9, 68.7, 62.2, 55.2, 21.6, 12.0; FAB-MS *m*/*z* 835 $[M + H]^+$ [Found (%): C, 66.0; H, 5.7; N, 3.3. $C_{46}H_{46}N_2O_{11}S$ requires C, 66.1; H, 5.5; N, 3.4].

(1*R*,3*R*,4*R*,7*R*)-7-Benzyloxy-1-(4,4′-dimethoxytrityloxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane 8

To a solution of nucleoside **7** (4.22 g, 5.06 mmol) in anhydrous DMF (25 cm^3) at 0 °C was added a 60% suspension of sodium hydride in mineral oil (w/w; 0.607 g, 15.7 mmol, added in four portions during 20 min) and the reaction mixture was stirred at room temperature for 25 h, cooled to 0 °C and diluted with dichloromethane–pyridine (100 cm^3; 99.5 : 0.5, v/v). Saturated aqueous sodium hydrogen carbonate (120 cm^3) was added whereupon extraction was performed using dichloromethane (75 cm^3 × 2). The combined organic phase was washed successively with saturated aqueous sodium hydrogen carbonate (60 cm^3 × 3) and brine (40 cm^3 × 3), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane–methanol–pyridine (0.5–1.5% methanol; 0.5% pyridine, v/v/v) as eluent to yield nucleoside **8** (3.20 g, 96%) as a white solid material after evaporation of the solvents under reduced pressure; $\delta_H(CDCl_3)$ 13.24 (1H, s, NH), 7.70–7.19 (19H, m, Bn, DMT, 6-H), 6.15 (1H, s, 1′-H), 4.98 (1H, s, 2′-H), 4.55 (1H, d, *J* 11.2, Bn), 4.42 (1H, d, *J* 11.2, Bn), 4.40 (1H, s, 3′-H), 4.34 (1H, d, *J* 8.0, 1″-H^a), 4.17 (1H, d, *J* 8.0, 1″-H^b), 3.94 (2H, s, 5′-H_2), 3.67 (3H, s, OCH_3), 3.64 (3H, s, OCH_3), 1.75 (3H, d, *J* 0.7, CH_3); $\delta_C(CDCl_3)$ 165.0, 159.2, 151.5, 145.5, 137.4, 136.6, 136.0, 130.6, 128.7, 128.6, 128.4, 128.3, 127.3, 113.8, 108.1, 89.3, 88.6, 86.7, 80.6, 77.0, 73.8, 73.0, 59.8, 55.2, 12.7; FAB-MS *m*/*z* 663 $[M + H]^+$ [Found (%): C, 70.4; H, 5.8; N, 4.0. $C_{39}H_{38}N_2O_8$ requires C, 70.7; H, 5.7; N, 4.2].

(1*S*,3*R*,4*R*,7*R*)-7-Hydroxy-1-hydroxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane 9

Nucleoside **8** (3.09 g, 4.66 mmol) was dissolved in methanol (40 cm^3) and 10% Pd/C [3 g, suspended in methanol (20 cm^3)] was added. The mixture was degassed, and stirred under an atmosphere of hydrogen. After 26 h, the mixture was filtered [silica gel, washed with dichloromethane–methanol (700 cm^3; 1 : 3, v/v)] and the volume of the filtrate was reduced to 25% of its initial volume. After repeated filtration, the filtrate was evaporated to dryness under reduced pressure and the residue was subjected to column chromatography on silica gel using dichloromethane–methanol (5–12% methanol, v/v) as eluent which furnished nucleoside **9** (1.03 g, 82%) as a white solid after evaporation of the solvents under reduced pressure; $\delta_H(CD_3OD)$ 7.73 (1H, d, *J* 1.1, 6-H), 5.56 (1H, s, 1′-H), 4.32 (1H, d, *J* 2.2, 2′-H), 4.21 (1H, d, *J* 2.2, 3′-H), 4.06 (1H, d, *J* 8.2, 1″-H^a), 4.01 (2H, s, 5′-H_2), 3.86 (1H, d, *J* 8.2, 1″-H^b), 1.85 (3H, d, *J* 1.1, CH_3); $\delta_C(CD_3OD)$ 166.8, 139.4, 108.4, 91.0, 90.3, 79.6, 74,5, 70.0, 59.0, 12.6; FAB-MS *m*/*z* 271 $[M + H]^+$ [Found (%): C, 47.8; H, 5.5; N, 9.5. $C_{11}H_{14}N_2O_6 \cdot 0.5H_2O$ requires C, 47.3; H, 5.4; N, 10.0].

(1*S*,2*R*,6*R*,8*R*,9*R*)-1,9-Dihydroxy-4,4-dimethyl-3,5,7,11-tetraoxatricyclo[6.3.0.$0^{2,6}$]undecane 11

1,2;5,6-Di-*O*-isopropylidene-α-D-ribofuranos-3-ulose [32] **10** (10.0 g, 38.7 mmol) was stirred for 19 h at room temperature in 80% acetic acid. The mixture was evaporated to dryness under reduced pressure and the residue was coevaporated with toluene (2 × 100 cm^3) and acetonitrile (2 × 50 cm^3) to give compound **11** (8.26 g, 96%) as a clear oil; $\delta_H(CDCl_3)$ 5.98 (1H, d, *J* 3.9), 4.53–4.43 (3H, m), 4.28 (1H, dd, *J* 9.2 and 6.3), 3.81–3.75 (1H, dd, *J* 9.2 and 5.5), 3.70–3.50 (2H, br s), 1.58 (3H, s), 1.40 (3H, s); $\delta_C(CDCl_3)$ 113.9, 110.8, 106.9, 84.0, 82.6, 73.6, 70.9,

27.0; FAB-MS *m*/*z* 219 $[M + H]^+$ [Found (%): C, 48.8; H, 6.4. $C_9H_{14}O_6 \cdot 0.25H_2O$ requires C, 48.5; H, 6.6].

(1*S*,2*R*,6*R*,8*R*,9*R*)-1,9-Dibenzyloxy-4,4-dimethyl-3,5,7,11-tetraoxatricyclo[6.3.0.0^{2,6}]undecane 12

A solution of derivative **11** (4.45 g, 20.4 mmol) in anhydrous DMF (100 cm^3) at 0 °C was added dropwise during 20 min to a mixture of NaH (3.3 g; 60% suspension in mineral oil, w/w; 81.6 mmol) and anhydrous DMF (50 cm^3). The mixture was stirred at room temperature for 45 min whereupon a solution of benzyl bromide (10.0 cm^3, 84.1 mmol) in anhydrous DMF (10 cm^3) was added dropwise. After 2 h, ethyl acetate (10 cm^3) was added and the mixture was evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using ethyl acetate–petroleum ether (0–5% ethyl acetate, v/v) as eluent. After evaporation of the solvents under reduced pressure, compound **12** (7.56 g, 93%) was obtained as a yellowish solid, which was used without further purification in the next step; $\delta_H(CDCl_3)$ 7.37–7.22 (10H, m), 6.02 (1H, d, *J* 3.8), 4.72–3.93 (9H, m), 1.60 (3H, s), 1.39 (3H, s); $\delta_C(CDCl_3)$ 137.8, 137.5, 128.4, 128.3, 128.0, 127.9, 127.7, 115.0, 113.5, 107.5, 81.7, 80.3, 78.0, 72.6, 71.5, 66.5, 27.4, 27.2; FAB-MS *m*/*z* 399 $[M + H]^+$.

(1*S*,4*R*,5*R*,7*RS*,8*S*)-7,8-Diacetoxy-1,4-dibenzyloxy-2,6-dioxabicyclo[3.3.0]octane 13

Derivative **12** (10.32 g, 25.9 mmol) was stirred in 80% acetic acid (125 cm^3) for 26 h at 80 °C. After evaporation of the solvents under reduced pressure and coevaporation first with absolute ethanol (100 cm^3) and then toluene (2 × 100 cm^3), the residue was dissolved in a mixture of anhydrous pyridine (90 cm^3) and acetic anhydride (40 cm^3). The mixture was stirred at room temperature for 19 h and then evaporated to dryness under reduced pressure after addition of a mixture of ice and H_2O (40 cm^3). The residue was purified by silica gel column chromatography using ethyl acetate–petroleum ether (5–10% ethyl acetate, v/v) as eluent to afford the anomeric mixture **13** (6.60 g, 57.0%; ~2:1 ratio between diastereoisomers) as a yellowish oil after evaporation of the solvents under reduced pressure; $\delta_C(CDCl_3)$ 169.3, 169.2, 168.4, 137.3, 137.3, 128.3, 128.2, 127.9, 127.8, 127.6, 127.3, 114.2, 112.9, 101.0, 96.9, 84.7, 82.3, 77.5, 76.4, 75.2, 72.5, 72.4, 72.4, 71.7, 71.6, 66.5, 66.3, 20.8, 20.6, 20.5, 20.3; FAB-MS *m*/*z* 441.4 $[M - H]^-$ [Found (%): C, 64.7; H, 5.9. $C_{24}H_{26}O_8 \cdot 0.25H_2O$ requires C, 64.5; H, 6.0].

(1*S*,4*R*,5*R*,7*R*,8*S*)-8-Acetoxy-1,4-dibenzyloxy-7-(thymin-1-yl)-2,6-dioxabicyclo[3.3.0]octane 14

The bicyclic furanose **13** (218 mg, 0.49 mmol) and thymine (125 mg, 0.99 mmol) were dissolved in anhydrous acetonitrile (5 cm^3). *N*,*O*-Bis(trimethylsilyl)acetamide (0.85 cm^3, 3.45 mmol) was added and the mixture was stirred for 1 h under reflux. After cooling of the mixture to −30 °C, trimethylsilyl triflate (0.25 cm^3, 1.38 mmol) was added dropwise during 20 min. The temperature was allowed to rise to room temperature and the mixture was stirred for 40 h at 50 °C and subsequently for 1 h under reflux. After cooling of the mixture to room temperature, ice-cold saturated aqueous sodium hydrogen carbonate (5 cm^3) was added. Extraction was performed using dichloromethane (3 × 5 cm^3) and the combined organic phase was washed successively with saturated aqueous sodium hydrogen carbonate (5 cm^3) and brine (5 cm^3), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using methanol–dichloromethane as eluent (0–1% methanol, v/v). The fractions containing the product were collected, and evaporated to 10% volume under reduced pressure. Petroleum ether (10 cm^3) was added and precipitation yielded the product nucleoside **14** (219 mg, 85%) as a white solid after filtration; $\delta_H(CDCl_3)$ 8.65 (1H, br s), 7.60 (1H, d, *J* 1.2), 7.37–7.24 (10H, m), 6.18 (1H, d, *J* 2.1), 5.42 (1H, d, *J* 2.1), 4.70–4.39 (6H, m), 4.16 (1H, dd, *J* 9.5 and 7.3), 3.99 (1H, dd, *J* 9.5 and 5.2), 2.09 (3H, s), 1.86 (3H, d, *J* 1.1); $\delta_C(CDCl_3)$ 169.1, 163.5, 150.0, 137.1, 137.0, 135.7, 128.6, 128.5, 128.2, 128.0, 128.0, 127.5, 113.4, 111.3, 91.6, 83.0, 78.4, 75.1, 72.8, 72.6, 66.8, 20.7, 12.6; FAB-MS *m*/*z* 509.1 $[M + H]^+$ [Found (%): C, 64.0; H, 5.5; N, 5.3. $C_{27}H_{28}N_2O_8$ requires C, 63.8; H, 5.6; N, 5.5].

(1*R*,4*R*,5*R*,7*R*,8*S*)-1,4-Dibenzyloxy-8-hydroxy-7-(thymin-1-yl)-2,6-dioxabicyclo[3.3.0]octane 15

Sodium methoxide (1.32 g, 24.4 mmol) was added to a solution of nucleoside **14** (4.10 g, 8.10 mmol) in anhydrous methanol (50 cm^3) and the resulting mixture was stirred for 24 h at room temperature. After neutralization (4 M hydrochloric acid), the mixture was evaporated to dryness under reduced pressure and dichloromethane (250 cm^3) was added. The resulting mixture was washed using saturated aqueous sodium hydrogen carbonate (2 × 100 cm^3) and the combined aqueous phase was extracted using dichloromethane (4 × 50 cm^3). The combined organic phase was dried (Na_2SO_4), and evaporated to dryness under reduced pressure to give nucleoside **15** (3.46 g, 90%) as a yellowish solid; $\delta_H(CDCl_3)$ 10.18 (1H, br s, NH), 8.02 (1H, d, *J* 1.2, H-6), 7.39–7.25 (10 H, m, 2 × Bn), 5.97 (1H, s, H-1′), 5.41 (1H, d, *J* 3.9, OH), 4.82 (1H, d, *J* 4.9, H-4′), 4.75–4.48 (6H, m, H-5′, -2′, Bn), 4.26–4.19 (1H, dd, *J* 10.0 and 7.2, H^a-6′), 3.92–3.87 (1H, dd, *J*, 10.0 and 3.7, H^b-6′), 1.78 (3H, d, *J* 1.1, CH_3); $\delta_C(CDCl_3)$ 164.5 (C-4), 150.7 (C-2), 137.3, 137.0 (Bn), 136.4 (C-6), 128.4, 128.3, 128.0, 128.0, 127.8, 127.7 (Bn), 115.4 (C-3′), 109.8 (C-5), 96.0 (C-1′), 84.0 (C-4′), 79.0, 75.1 (C-2′, -5′), 73.2 (C-6′), 72.5 (Bn), 66.3 (Bn), 12.2 (CH_3); FAB-MS *m*/*z* 467.2 $[M + H]^+$ [Found (%): C, 62.9; H, 5.7; N, 5.7. $C_{25}H_{26}N_2O_7 \cdot 0.5H_2O$ requires C, 63.2; H, 5.5; N, 5.9].

(1*R*,4*R*,5*R*,7*R*,8*S*)-1,4-Dibenzyloxy-8-methoxy-7-(thymin-1-yl)-2,6-dioxabicyclo[3.3.0]octane 17

A solution of nucleoside **15** (900 mg, 1.93 mmol) and sodium hydride (200 mg; 60% suspension in mineral oil, w/w; 5.00 mmol) in dichloromethane (10 cm^3) was stirred for 20 min at room temperature. Methyl iodide (0.90 cm^3, 14.5 mmol) was added and stirring at room temperature was continued for 48 h whereupon additional sodium hydride (600 mg; 60% suspension in mineral oil, w/w; 15.0 mmol) and methyl iodide (4 cm^3, 64.3 mmol) were added. After 168 h, ethyl acetate (10 cm^3) was added together with methanol (5 cm^3), dichloromethane (10 cm^3) and silica gel (10 g), and the mixture was evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography and elution, first with ethyl acetate–petroleum ether (10–50% ethyl acetate, v/v) and then with methanol–dichloromethane (1:99, v/v), yielded nucleoside **17** (573 mg, 62%) as a white solid after evaporation of the solvents; $\delta_H(CDCl_3)$ 8.97 (1H, br s, NH), 7.90 (1H, d, *J* 1.2, H-6), 7.41–7.27 (10H, m, Bn), 6.1 (1H, s, H-1′), 4.71 (1H, d, *J* 11.2, Bn), 4.65 (1H, d, *J* 4.8, H-4′), 4.62–4.46 (4H, m, Bn, H-5′), 4.22 (1H, dd, *J* 9.8 and 7.3, H^a-6′), 3.99 (1H, s, H-2′), 3.91 (1H, dd, *J* 9.8 and 4.1, H^b-6′), 3.65 (3H, s, OCH_3), 1.82 (3H, d, *J* 1.1, CH_3); $\delta_C(CDCl_3)$ 163.9, 150.1, 137.2, 137.1, 136.5, 128.6, 128.5, 128.2, 128.1, 128.0, 114.7, 110.0, 92.2, 83.6, 79.0, 77.2, 73.0, 72.7, 66.7, 58.5, 12.4; FAB-MS *m*/*z* 481.2 $[M + H]^+$ [Found (%): C, 65.1; H, 6.0; N, 5.8. $C_{26}H_{28}N_2O_7$ requires C, 65.0; H, 5.9; N, 5.8].

(1*S*,4*R*,5*R*,7*R*,8*S*)-8-(*tert*-Butyldimethylsilyloxy)-1,4-dibenzyloxy-7-(thymin-1-yl)-2,6-dioxabicyclo[3.3.0]octane 19

Nucleoside **15** (500 mg, 2.04 mmol) was dissolved in anhydrous DMF (5 cm^3). *tert*-Butyldimethylsilyl chloride (TBDMSCl) (485 mg, 3.2 mmol) and imidazole (292 mg, 4.3 mmol) were

added and the mixture was stirred for 24 h at room temperature. After addition of MeOH (1 cm^3), the mixture was evaporated to dryness under reduced pressure and the residue was purified by silica gel column chromatography using ethyl acetate–petroleum ether (0–12% ethyl acetate, v/v) as eluent to give product nucleoside **19** (527 mg, 85%) as a white solid after evaporation of the solvents; $\delta_H(CDCl_3)$ 8.76 (1H, br s), 7.89 (1H, d, *J* 1.1), 7.35–7.26 (10H, m), 5.90 (1H, s), 4.73–4.43 (7H, m), 4.14 (1H, dd, *J* 9.9 and 7.1), 3.88 (1H, dd, *J* 9.9 and 4.2), 1.80 (3H, d, *J* 1.1), 0.93 (9H, s), 0.24 (3H, s), 0.18 (3H, s); $\delta_C(CDCl_3)$ 163.8, 151.0, 137.4, 137.0, 136.5, 128.4, 128.3, 128.0, 127.7, 127.6, 127.4, 114.8, 109.7, 94.5, 83.2, 78.7, 76.0, 72.8, 72.5, 65.6, 25.6, 17.8, 12.2 (signals below 0 ppm not shown); FAB-MS *m*/*z* 581.3 $[M + H]^+$ [Found (%): C, 64.1; H, 6.7; N, 4.6. $C_{31}H_{40}N_2O_7Si$ requires C, 64.1; H, 6.9; N, 4.8].

(1*S*,4*R*,5*R*,7*R*,8*S*)-8-(*tert*-Butyldimethylsilyloxy)-1,4-dihydroxy-7-(thymin-1-yl)-2,6-dioxabicyclo[3.3.0]octane 20

Nucleoside **19** (181 mg, 0.43 mmol) was dissolved in absolute ethanol (3 cm^3) and 20% $Pd(OH)_2/C$ (90 mg) was added. After degassing, the solution was stirred in an atmosphere of hydrogen for 26 h at room temperature. The mixture was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in methanol–dichloromethane (2 cm^3; 1:99, v/v) and quickly filtered through a bed of silica gel. After washing with methanol–dichloromethane (1:99, v/v), nucleoside diol **20** (63 mg, 50%) was obtained as a white solid. This product decomposed during prolonged column chromatographic purification (silica gel or basic alumina); $\delta_H(CDCl_3)$ 8.84 (1H, br s), 7.21 (1H, d, *J* 1.3), 5.76 (1H, d, *J* 7.0), 4.45–4.40 (3H, m), 4.31 (1H, dd, *J* 10.2 and 3.9), 4.06 (1H, dd, *J* 10.2 and 2.5), 1.94 (3H, d, *J* 1.3), 0.89 (9H, s), 0.08 (3H, s), 0.01 (3H, s); $\delta_C(CDCl_3)$ 163.1, 150.1, 136.8, 112.0, 107.9, 93.9, 85.3, 76.0, 75.7, 71.1, 25.4, 17.8, 12.3, −5.0, −5.1; FAB-MS *m*/*z* 401.2 $[M + H]^+$.

6-*O*-Benzoyl-3,5-di-*O*-benzyl-1,2-di-*O*-isopropylidene-α-D-allofuranose 22

To a stirred solution of furanose **21**[33] (4.60 g, 11.1 mmol) in anhydrous DMF (20 cm^3) at 0 °C was added a 60% suspension of sodium hydride in mineral oil (w/w, 0.67 g, 16.7 mmol, added in four portions during 20 min). After stirring of the mixture for 30 min, benzyl bromide (1.99 cm^3, 16.7 mmol) was added and stirring was continued for 2 h at room temperature. The mixture was cooled to 0 °C, H_2O (30 cm^3) was added and extraction was performed using dichloromethane (50 cm^3 × 3). The combined organic phase was washed successively with saturated aqueous sodium hydrogen carbonate (30 cm^3) and brine (20 cm^3), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using ethyl acetate–petroleum ether (1:9, v/v) as eluent to give furanose **22** as a yellowish oil (5.0 g, 90%) after evaporation of the solvents under reduced pressure. This oil was used in the next step without further purification; $\delta_H(CDCl_3)$ 7.99 (2H, m), 7.58–7.21 (13H, m), 5.77 (1H, d, *J* 3.6), 4.77–4.00 (10H, m), 1.59 (3H, s), 1.35 (3H, s); $\delta_C(CDCl_3)$ 166.2, 138.4, 137.4, 133.0, 130.1, 129.7, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.5, 113.1, 102.2, 79.2, 77.6, 76.5, 76.3, 73.7, 72.2, 64.3, 26.9; FAB-MS *m*/*z* 505 $[M + H]^+$.

1,2-Di-*O*-acetyl-6-*O*-benzoyl-3,5-di-*O*-benzyl-D-allofuranose 23

A solution of furanose **22** (5.00 g, 9.92 mmol) in 80% acetic acid (75 cm^3) was stirred for 10 h at 80 °C. The solvent was removed under reduced pressure and the residue was coevaporated with toluene (10 cm^3 × 3) and dissolved in a mixture of anhydrous pyridine (30 cm^3) and dichloromethane (30 cm^3). Acetic anhydride (5.0 cm^3) was added and the solution was stirred for 20 h at room temperature. The mixture was evaporated to dryness under reduced pressure and the residue was dissolved in dichloromethane (150 cm^3), washed successively with saturated aqueous sodium hydrogen carbonate (60 cm^3) and brine (30 cm^3), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using petroleum ether–dichloromethane (1:1, v/v) as eluent to afford the anomeric mixture **23** as a clear oil (4.50 g, 74%) after evaporation of the solvents under reduced pressure. This oil was used in the next step without further purification; $\delta_C(CDCl_3)$ 169.9, 169.2, 166.2, 138.1, 137.0, 133.2, 133.1, 133.0, 129.9, 129.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 98.6, 94.3, 84.7, 82.3, 82.0, 77.7, 76.5, 76.4, 76.3, 74.7, 74.1, 73.8, 73.3, 73.1, 72.8, 71.8, 70.0, 63.8, 63.2, 21.2, 20.8, 20.8, 20.6; FAB-MS *m*/*z* 547 $[M - H]^-$.

1-(2-*O*-Acetyl-6-*O*-benzoyl-3,5-di-*O*-benzyl-β-D-allofuranosyl)-thymine 24

To a stirred suspension of the anomeric mixture **23** (4.50 g, 8.21 mmol) and thymine (1.55 g, 12.31 mmol) in anhydrous acetonitrile (50 cm^3) was added *N*,*O*-bis(trimethylsilyl)acetamide (12.2 cm^3, 49.3 mmol). The reaction mixture was stirred at 60 °C for 1 h and then cooled to 0 °C. Trimethylsilyl triflate (2.97 cm^3, 16.4 mmol) was added dropwise during 10 min and the mixture was heated for 2 h under reflux. The reaction mixture was allowed to cool to room temperature and the volume was reduced by 50% under reduced pressure. After cooling of the mixture to 0 °C, saturated aqueous sodium hydrogen carbonate (100 cm^3) was added and extraction was performed with dichloromethane (3 × 50 cm^3). The combined organic phase was washed with brine (50 cm^3), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane–methanol (99.5:0.5, v/v) as eluent to give nucleoside **24** as a white solid (4.06 g, 81%) after evaporation of the solvents under reduced pressure; $\delta_H(CDCl_3)$ 8.74 (1H, br s), 8.01 (2H, m), 7.61–7.11 (14H, m), 6.09 (1H, d, *J* 5.3), 5.32 (1H, m), 4.86 (1H, d, *J* 11.7), 4.65 (1H, d, *J* 11.7), 4.55–4.10 (7H, m), 2.10 (3H, s), 1.59 (3H, s); $\delta_C(CDCl_3)$ 170.1, 166.1, 163.4, 150.2, 137.4, 137.0, 135.7, 133.3, 129.7, 128.6, 128.5, 128.1, 128.0, 127.9, 127.7, 127.3, 126.9, 111.7, 87.6, 82.6, 76.7, 75.3, 73.7, 73.1, 73.0, 63.3, 20.7, 12.0; FAB-MS *m*/*z* 615 $[M + H]^+$ [Found (%): C, 66.4; H, 5.6; N, 4.4. $C_{34}H_{34}N_2O_9$ requires C, 66.4; H, 5.6; N, 4.6].

1-(3,5-Di-*O*-benzyl-β-D-allofuranosyl)thymine 25

To a stirred solution of nucleoside **24** (3.00 g, 4.88 mmol) in methanol (50 cm^3) was added sodium methoxide (0.79 g, 14.7 mmol). The reaction mixture was stirred for 14 h at room temperature and subsequently neutralized with dilute hydrochloric acid whereupon ice-cold H_2O (50 cm^3) was added. The resulting mixture was extracted using ethyl acetate (3 × 100 cm^3) and the combined organic phase was evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane–methanol (98.5:1.5, v/v) as eluent to give nucleoside **25** as a white solid (2.00 g, 88%) after evaporation of the solvents under reduced pressure; $\delta_H(CDCl_3)$ 9.39 (1H, br s), 7.38–7.15 (11H, m), 5.80 (1H, d, *J* 4.6), 4.80–3.55 (10H, m), 1.59 (3H, s); $\delta_C(CDCl_3)$ 163.7, 150.8, 137.7, 136.8, 136.3, 128.7, 128.4, 128.2, 128.0, 127.3, 111.4, 90.4, 82.7, 78.8, 76.5, 72.9, 72.5, 72.4, 60.7, 12.0; FAB-MS *m*/*z* 469 $[M + H]^+$ [Found (%): C, 64.4; H, 6.1; N, 5.5. $C_{25}H_{28}N_2O_7$ requires C, 64.1; H, 6.0; N, 6.0].

1-[3,5-Di-*O*-benzyl-2,6-di-*O*-(*p*-tolylsulfonyl)-β-D-allofuranosyl]thymine 26

To a stirred solution of nucleoside **25** (0.60 g, 1.28 mmol) in dichloromethane (70 cm^3) at room temperature was added

DMAP (0.63 g, 5.12 mmol) and toluene-*p*-sulfonyl chloride (0.73 g, 3.84 mmol). After stirring of the mixture for 3 h, ice-cold H_2O (50 cm^3) was added and extraction was performed using dichloromethane (3 × 75 cm^3). The combined organic phase was dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane–methanol (99.5 : 0.5, v/v) as eluent to give nucleoside **26** as a white solid (0.71 g, 71%) after evaporation of the solvents under reduced pressure; $\delta_H(CDCl_3)$ 8.83 (1H, br s), 7.73–7.12 (18H, m), 6.58 (1H, d, *J* 1.2), 5.88 (1H, d, *J* 6.9), 5.0 (1H, m), 4.73–3.82 (9H, m), 2.40 (3H, s), 2.35 (3H, s), 1.48 (3H, d, *J* 0.9); $\delta_C(CDCl_3)$ 163.1, 149.8, 145.8, 145.2, 137.1, 137.0, 135.6, 132.4, 132.3, 130.0, 128.7, 128.5, 128.3, 128.1, 128.0, 127.8, 127.2, 111.4, 86.9, 83.1, 77.7, 75.3, 73.1, 72.5, 67.4, 21.7, 11.9; FAB-MS *m*/*z* 777 $[M + H]^+$ [Found (%): C, 60.6; H, 5.2; N, 3.5. $C_{39}H_{40}N_2O_{11}S_2$ requires C, 60.3; H, 5.2; N, 3.6].

(1*S*,4*R*,5*R*,7*R*,8*R*)-4,8-Dibenzyloxy-7-(thymin-1-yl)-2,6-dioxabicyclo[3.2.1]octane 27

To a stirred solution of nucleoside **26** (0.63 g, 0.81 mmol) in a mixture of ethanol and H_2O (40 cm^3; 1 : 1, v/v) at room temperatue was added an aqueous solution of sodium hydroxide (1 M; 7 cm^3). The resulting mixture was heated under reflux for 16 h and then neutralized by addition of dilute hydrochloric acid. The volume of the mixture was reduced to 50% and extraction was performed using dichloromethane (50 cm^3 × 3). The combined organic phase was dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane–methanol (99 : 1, v/v) as eluent to give nucleoside **27** as a white solid (0.40 g, 93%) after evaporation of the solvents under reduced pressure; $\delta_H(CDCl_3)$ 8.69 (1H, br s), 7.90 (1H, d, *J* 1.1), 7.39–7.25 (10H, m), 5.85 (1H, d, *J* 2.2), 4.78–4.47 (6H, m), 3.87–3.38 (4H, m), 1.87 (3H, s); $\delta_C(CDCl_3)$ 163.9, 149.9, 137.3, 137.1, 136.8, 128.6, 128.5, 128.2, 128.1, 127.8, 127.7, 109.4, 88.6, 79.9, 79.7, 74.5, 73.5, 71.4, 70.8, 65.0, 12.5; FAB-MS *m*/*z* 451 $[M + H]^+$ [Found (%): C, 66.3; H, 5.7; N, 6.1. $C_{25}H_{26}N_2O_6$ requires C, 66.7; H, 5.8; N, 6.2].

(1*S*,4*R*,5*R*,7*R*,8*R*)-4,8-Dihydroxy-7-(thymin-1-yl)-2,6-dioxabicyclo[3.2.1]octane 28

Nucleoside **27** (0.27 g, 0.60 mmol) was dissolved in absolute ethanol (20 cm^3) and 20% $Pd(OH)_2/C$ (0.25 g) was added. The mixture was degassed and placed under an atmosphere of hydrogen. After stirring the mixture for 26 h the catalyst was filtered off (silica gel; washed with methanol, 400 cm^3) and the filtrate was concentrated to dryness under reduced pressure. The residue was subjected to column chromatography on silica gel using dichloromethane–methanol (94 : 6, v/v) as eluent to give nucleoside **28** as white solid (0.16 g, 98%) after evaporation of the solvents under reduced pressure; $\delta_H(CD_3OD)$ 8.06 (1H, d, *J* 1.2, 6-H), 5.57 (1H, d, *J* 2.3, 1′-H), 4.50 (1H, m, 2′-H), 4.42 (1H, s, 4′-H), 4.03 (1H, m, 3′-H), 3.93–3.80 (2H, m, 5′-H, 6′-H[a]), 3.21 (1H, m, 6′-H[b]), 1.91 (3H, d, *J* 1.2, CH_3); $\delta_C(CD_3OD)$ 166.8 (C-4), 152.0 (C-2), 139.2 (C-6), 110.2 (C-5), 90.2 (C-1′), 87.3 (C-4′), 77.0 (C-2′), 74.7 (C-3′), 68.5 (C-5′), 67.4 (C-6′), 12.5 (CH_3); FAB-MS *m*/*z* 271 $[M + H]^+$.

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