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A ring-closing metathesis based synthesis of bicyclic nucleosides locked in *S*-type conformations by hydroxyl functionalised 3′,4′-*trans* linkages

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Abstract—A [4.3.0]bicyclic nucleoside that contains an unsaturated hydroxylated 3′,4′-*trans* linkage has been efficiently synthesised. Thus, from diacetone-D-glucose as the starting material, stereoselective Grignard reactions for the introduction of allyl groups, a nucleobase coupling and, subsequently, a ring-closing metathesis (RCM)-reaction were applied as the key reactions. The cyclohexene moiety introduced in this nucleoside reveals a large potential for further derivatisation, and as the first example, a stereoselective dihydroxylation followed by deprotection afforded a multihydroxylated bicyclic nucleoside. The configuration and conformational behaviour was determined by NMR spectroscopy and ab initio calculations, and both this bicyclic nucleoside and its unsaturated analogue were found to be strongly restricted in *S*-type conformations.

1. Introduction

The biological importance of the conformational equilibrium of nucleosides between *N*-type and *S*-type conformations following the pseudorotational cycle[1,2] has motivated the preparation of a significant number of synthetic nucleoside analogues mimicking these conformational ranges. Thus, nucleosides with conformationally restricted bi- or tricyclic carbohydrate parts have been intensively studied as building blocks in nucleic acid analogues,[3–5] for the study of enzymes and receptors with nucleoside or nucleotide substrates,[6–9] and/or for potential antiviral agents.[6,8–11] As a prime example, nucleosides with the [2.2.1]bicyclic core structure **1** are locked in an *N*-type conformation due to a 2′,4′-linkage (Fig. 1),[12,13] and oligonucleotides containing the monomer **1** (R=OH) have been defined as LNA (locked nucleic acid).[12] LNA has demonstrated high affinity recognition of DNA and RNA[12,14] and, subsequently, has shown promising results towards potential therapeutic applications.[14] Other *N*-type mimicking nucleosides based on the same structure (e.g. **1**, R=N_3)[15,16] or on bicarbocyclic structures[6–8,17] (e.g., **2**, R=H, OH, or N_3) have been used in the study of different receptors and enzymes, including the HIV reverse transcriptase.[6] Also bicyclic nucleosides that are restricted in the intermediate *E*-type conformations have been presented[5,9,11,18–20] and used in similar studies.[9]

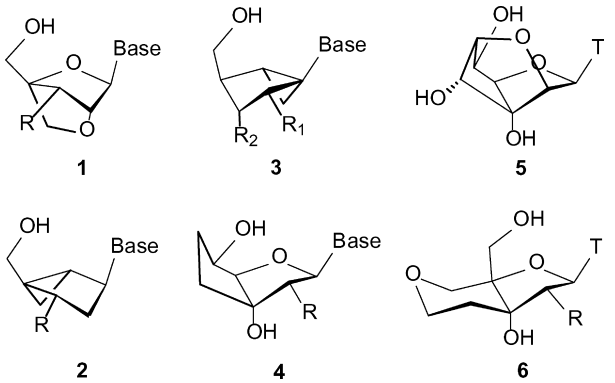

Figure 1. Selected bi- and tricyclic nucleoside analogues mimicking *N*-type (**1**, **2**) and *S*-type conformations (**3**–**6**). T=thymin-1-yl.

Bi- or tricyclic nucleoside analogues that are conformationally restricted towards *S*-type conformations have also been obtained.[4,5] Thus, nucleosides with the bicarbocyclic structure **3** have been used in the studies of several enzymes such as HIV reverse transcriptase as well as adenosine receptors.[6,7] Oligonucleotides containing the monomer **3** (R_1=H, R_2=OH), however, demonstrated slightly decreased recognition of DNA and RNA complements.[21] Similar results have been obtained with other bicyclic nucleoside monomers that are strongly restricted in *S*-type conformations due to a 1′,3′-*cis* linkage[22] or a 2′,3′-*trans* linkage.[18] However, the reason for the induced duplex

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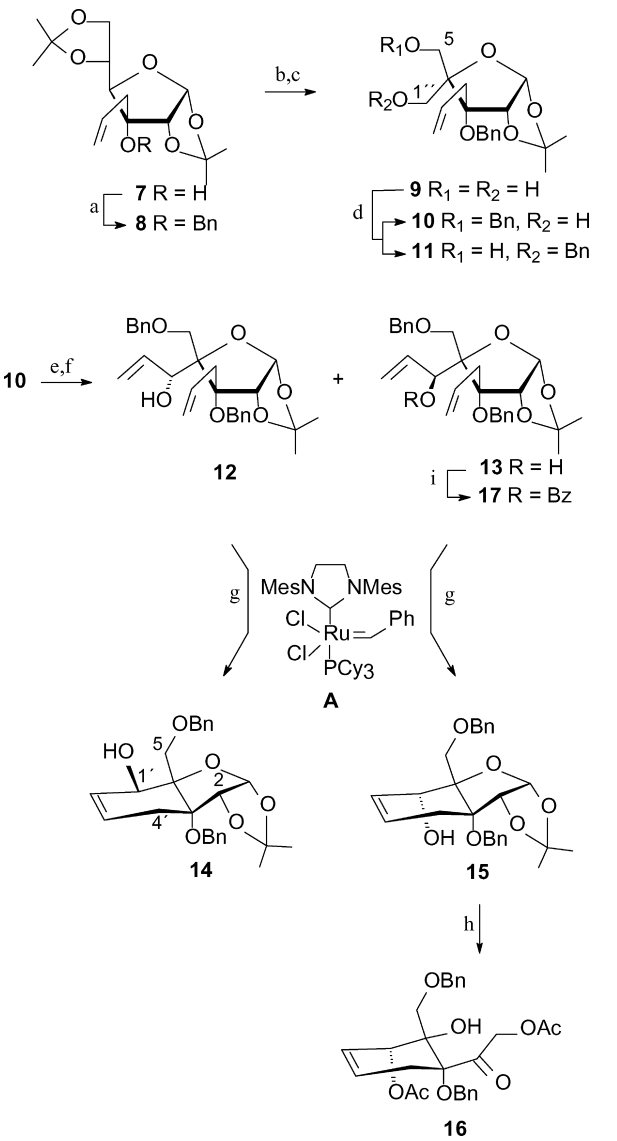
destabilisation might, in all three cases, be steric influence from the additional ring structures or distortion of duplex hydration patterns. On the other hand, the pioneering 3′,5′-linked bicyclic nucleoside **4** (R=H) has demonstrated slightly increased but varying affinities for DNA and RNA complements when incorporated into oligonucleotides.[23,24] The nucleoside monomers are restricted towards an *S*-type conformation but the C4′–C5′ bond, described by the standard torsion angle γ,[2] is restricted in the *ap* range which is not favourable for duplex formation by standard Watson–Crick base-pairing.[23,24] This has been significantly improved, however, by the introduction of an additional cyclopropane ring in a tricyclic nucleoside which, on the other hand, is no longer a true *S*-type mimic.[25]

We have recently introduced a new synthetic strategy based on ring-closing metathesis (RCM) towards **4** and its analogues[26–28] including the ribo-configured analogue (**4**, R=OH),[26] and a tricyclic nucleoside **5**.[27] Oligonucleotides containing **5**, however, displayed a significant destabilisation of duplexes with complementary DNA and RNA.[27] This result, in combination with the results of **4**,[23,24] demonstrates that the design of a nucleoside monomer for oligonucleotides that are preorganised for duplex formation should not combine a locked *S*-type conformation with γ in an inappropriate angle. Therefore, we have focused on the design of bicyclic nucleoside analogues with 3′,4′-*trans* linkages combining a locked *S*-type conformation with an unrestricted C4′–C5′-bond. The first 3′,4′-*trans* linked bicyclic nucleoside structure **6** (R=OH or OCH_3) was introduced recently as a perfect *S*-type mimic.[29,30] In the same communication,[30] we introduced a novel 3′,4′-*trans* linked nucleoside by a synthetic strategy that is based on RCM[31] and comparable with the one used successfully in the preparation of **4** and **5**. This synthesis leading to a nucleoside with a 3′,4′-*trans* fused cyclohexene ring with a large potential for further modification is hereby described in detail. Furthermore, the potential of this [4.3.0]-bicyclic core structure is exemplified by a stereoselective dihydroxylation and, subsequently, a highly hydroxylated and hydrophilic nucleoside analogue that is conformationally locked in an *S*-type conformation.



Scheme 1. Reagents and conditions: (a) NaH, BnBr, DMF, 92% (Ref. 32); (b) H_5IO_6, EtOAc; (c) H_2CO, NaOH, THF, H_2O then $NaBH_4$, 86% (2 steps); (d) NaH, BnBr, DMF, **10** 61% and **11** 22%; (e) PCC, CH_2Cl_2; (f) vinylMgBr, THF, **12** 17% and **13** 63% (2 steps); (g) **A** (2 mol%), CH_2Cl_2, **14** 76%, **15** 93%; (h) 80% aq. AcOH, then Ac_2O, pyridine, 47%; (i) BzCl, pyridine, 98%.

2. Results

2.1. Chemical synthesis

As a convenient and cheap starting material, diacetone-D-glucose was converted by oxidation and a stereoselective Grignard reaction to give the 3′-*C*-allyl derivative **7** (Scheme 1).[32] This was further protected as a benzyl ether to give **8**.[33] Treatment with periodic acid to give in situ regio-selective cleavage of the primary acetonide and subsequent cleavage of the diol was followed by an aldol condensation of the resulting aldehyde with formaldehyde and a Cannizzarro reaction to give the diol **9** in a high yield. Differentiation between the two primary alcohols of **9** was possible probably because of a weak steric shielding of the α-phase of the bicyclic system. Thus, the benzylation afforded a 3:1 ratio of bis-benzylic ethers of which **10** was obtained as the major isomer after chromatographic separation. The full assignments of **10** and its 4-epimer **11** were only indicated at this stage. Thus, a comparable ratio has been obtained on a similar substrate without the 3-allyl group, i.e., the *O*-benzylation of 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-di-*O*-isopropylidene-α-D-ribofuranose.[34] When exploring the ^{1}H NMR data given for that case, the H-1″ signals‡ of both isomers were seen to be shifted downfield compared to the H-5 signals.[34] We deduce this phenomenon to the deshielding by the electronegative 3-O atom. For **10**, the highest chemical shifts were observed for the signal coupling to an OH-signal hereby confirming the 5-*O*-benzylation, whereas in the 4-epimer **11** the situation is opposite. The ^{13}C NMR data supports this argument perfectly. Thus, the C-5 signal has shifted from 63.2 ppm in **9** to 68.7 ppm in **10** confirming the alkylation of the C-5 hydroxyl group, whereas the C-1″ signal has only shifted

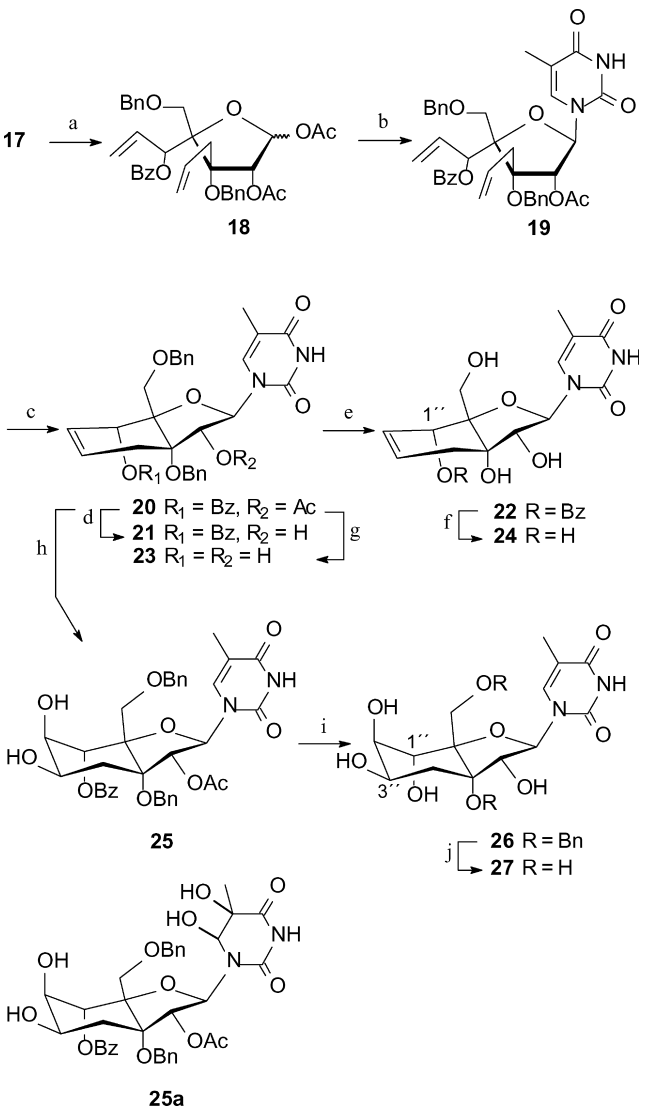
‡ For the numbering of the different carbon atoms, see the depictions in Scheme 1 and 2, as well as the introduction to Section 5.

from 63.5 ppm to 61.5 ppm. For **11**, the C-1″ signal has shifted to 71.6 ppm and the C-5 signal remains almost unchanged at 63.6 ppm.

Subsequently, **10** was oxidised using a simple and mild chromium(VI) mediated oxidation followed by another Grignard reaction to give the two epimers **12** and **13** in an approx. 1:4 ratio and in a high yield after chromatographic separation (Scheme 1). The configurations of the two epimers were not determined at this stage but we deduced this problem to be much easier solved after RCM-mediated ring-closure. Thus, both epimers were used as substrates for an RCM reaction using the second generation Grubbs' catalyst **A** (Scheme 1).[35] The major isomer **13** afforded smoothly the cyclohexene analogue **15** in 93% yield whereas **12** after longer reaction time and higher loading of **A** (3 mol% instead of 2 mol%) gave **14** in 76% yield. The configurations of **14** and **15** were determined by ^{1}H NMR spectroscopy hereby giving a determination also of **12** and **13** and a further confirmation of the configurations of **10** and **11**. Thus, from NOE-difference spectra strong mutual contacts between H-5 and H-2 as well as between H-5 and one H-4′ confirmed the C-4 configuration of **15**, and strong mutual contacts between H-5 and H-1′ on the cyclohexene ring confirmed the (*S*)-configuration of C-1′. Analogously, a significantly smaller contact between H-1′ and H-5 indicated the (*R*)-configuration of C-1′ in **14**. A very large difference in chemical shift for the H-1′ atoms (5.26 ppm in **14** and 3.83 ppm in **15**) further verified this determination, as H-1′ in **14** is expected to be strongly deshielded by the 3-O atom.

Problems with Lewis-acid promoted nucleobase coupling reactions have been observed before with constrained bicyclic carbohydrate substrates.[36,37] Nevertheless, we decided to use the major of the two tricyclic products **15** in our first investigation towards the preparation of the bicyclic nucleoside targets. Thus, we attempted the standard conversion using acetic acid followed by basic acetylation to give an anomeric mixture of bicyclic furanosyl acetates. However, instead of this mixture we obtained one major compound which from NMR spectroscopy was proved to be the cyclohexene **16**. This result can be deduced to the high ring strain of the bicyclic furanose due to the 3,4-*trans* fused cyclohexene ring. Thus, the intermediate free aldose derivative simply prefers its open form, and subsequently, the hydroxyaldehyde undergoes an α-ketol rearrangement to a more thermodynamically stable hydroxyketone derivative. Finally, acetylation of the primary and secondary alcohols gives **16**. This observation of ring strain gives a final confirmation of the determination of C-4 configuration (i.e., of the epimers **10** and **11**).

A better route towards the 3′,4′-*trans* linked bicyclic nucleosides was hereafter initiated and, obviously, the nucleobase coupling should be performed before the RCM reaction. Thus, the compatibility of RCM reactions and nucleosides has been demonstrated several times before.[31,38,39] Therefore, **13** was protected as its benzoic ester to give **17** in a high yield (Scheme 1) followed by hydrolysis and acetylation to give the anomeric mixture **18** (Scheme 2). Standard Vorbrüggen type coupling of thymine to this substrate gave exclusively the β-nucleoside **19** in a



Scheme 2. Reagents and conditions: (a) i, 80% aq. AcOH, ii, Ac_2O, pyridine, 92% (2 steps); (b) thymine, *N*,*O*-bis(trimethylsilyl)acetamide, TMS-OTf, CH_3CN, 93%; (c) **A** (2 mol%), $ClCH_2CH_2Cl$, 90%; (d) $NaOCH_3$, CH_3OH, 87%; (e) BCl_3, hexanes, CH_2Cl_2, **22** 55% and **24** 21% (from **21**), **24** 69% (from **23**); (f) $NaOCH_3$, CH_3OH, 48%; (g) $NaOCH_3$, CH_3OH, reflux, 69%; (h) OsO_4, NMO, THF, H_2O, 49%; (i) $NaOCH_3$, CH_3OH, 75%; (j) H_2, $Pd(OH)_2$–C, MeOH, 95%.

high yield due to anchimeric assistance from the 2′-*O*-acetyl group.[40,41] This was confirmed by the large coupling constant $^3J_{H1'H2'}$=8.5 Hz. A RCM reaction using the same catalyst as before afforded smoothly the bicyclic nucleoside **20** in 90% yield. The structure of this compound was confirmed by MS showing the expected loss of the mass of ethylene and NMR as the large coupling constant $^3J_{H1'H2'}$=7.6 Hz again confirms the nucleoside to be β-configured and restricted in an *S*-type conformation.[42] A basic treatment of **20** using methanolic ammonia or, alternatively, sodium methoxide afforded a selective cleavage of only the acetate ester to give **21**. A subsequent Lewis acid mediated debenzylation of **21** using boron trichloride afforded both the benzoate **22** and, surprisingly, the fully deprotected bicyclic nucleoside **24**. Finally, this target nucleoside **24** was further obtained after a new treatment of **22** with sodium methoxide. Hereby **24** was obtained in 41% combined yield over the three steps from

20. In order to obtain a better overall yield of **24**, a stronger treatment of **20** with sodium methoxide hydrolysed both esters simultaneously to give **23**, which subsequently was debenzylated using boron trichloride to give **24** in 46% yield over the two steps. The structure of **24** was confirmed by NMR spectroscopy. Thus, comparison of the spectra of **23** and **24** with the spectra of **15** revealed all expected similarities including the relatively low chemical shift of H-1″.

The double bond now incorporated in the 3′,4′-linkage of **24** opens the opportunity for further functionalisation. As the first example, the protected nucleoside **20** was used as the substrate in a dihydroxylation reaction. After treatment with osmium tetraoxide and *N*-methylmorpholine-*N*-oxide as a co-oxidant, the diol **25** was obtained in a medium 49% yield as an approximately 16:1 ratio of diastereomers. However, based on the recovery of 33% starting material this corresponds to a 73% yield. With a longer reaction time, the isolated yield of **25** could be slightly improved to 53% yield but with only 10% starting material recovered this displayed no improvement. Also a small amount of a bis-dihydroxylated product **25a** on which also the nucleobase has been dihydroxylated was isolated in 10% yield, or with a longer reaction time, 16% yield. This problem has been observed before for comparable nucleoside substrates.[20,28] The configurations of the two diastereomers of **25** were not determined at this stage and the stereoselective outcome of the dihydroxylation process was not obviously predicted from simple modelling. Nevertheless, the mixture was deprotected to give only the pure main diastereomer **26** and further debenzylated via hydrogenation to give **27** in 71% yield over the two steps as a fully deprotected multi-hydroxylated bicyclic nucleoside derivative. The absolute configuration of **27** was hereafter determined from NMR-studies (see below).

Table 1. Measured coupling constants for **27** together with possible torsions angles of the 'up'-isomer

		Possible torsion angles[a]	
	J, (Hz)	Allowed[b]	Not allowed[b]
H1′H2′	6.9	147°	0°, −147°
H1″H2″	2.0	76°, 127°	−106°, −50°
H2″H3″	4.7	36°, −38°	134°, −136°
$H3''H4''_{up}$	10.7	−166°	161°
$H3''H4''_{down}$	5.3	−50°	35°, 128°, −138°

[a] Possible torsion angles as determined by analysis of Karplus relationships.
[b] Torsion angles allowed or not allowed by the covalent geometry of the nucleoside.

2.2. Determination of the configuration and conformation of 27

To determine the configuration at C-2″ and C-3″ and the conformation of the [4.3.0]bicyclic skeleton in **27**, NMR investigations and molecular modelling were performed. The NMR-signals of **27** were determined from 2D-experiments and the assignment of the two different H-4″ signals was performed after an NOE-difference experiment. Thus, strong mutual contacts were observed between H-5′ and only one H-4″ hereafter determined to be $H\text{-}4''_{up}$. The $^3J_{HH}$ coupling constants of **27** were determined as given in Table 1. As no significant changes for any of the coupling constants were observed in the temperature range from −50 to 50 °C, we assume that **27** exists in only one conformer and not in a dynamic equilibrium between two or more conformers. All subsequent modelling was performed under this assumption. The Karplus relationships between $J_{H1'H2'}$, $J_{H1''H2''}$, $J_{H2''H3''}$, $J_{H3''H4''up}$, and $J_{H3''H4''down}$ and the corresponding torsion angles were derived for both of the possible configurations.[1,43] For each Karplus relationship there are, typically, four possible torsion angles corresponding to the given coupling constant. However, the restraints of the covalent geometry reduces this to two allowed torsion angles for $\theta_{H1''H2''}$ and $\theta_{H2''H3''}$, and considering the large $J_{H3''H4''}$ coupling constant and the fact that $H\text{-}4''_{up}$ and $H\text{-}4''_{down}$ are geminal, just one allowed geometry of the $H3''\text{–}C3''\text{–}C4''\text{–}H4''_{up/down}$ fragment for each possible configuration of **27**. The possible torsion angles for one of these, the 'up'-isomer (i.e., the 2″(*R*),3″(*R*)-isomer as depicted in Scheme 2), are included in Table 1.

For each of the two possible configurations of **27**, the four possible combinations of torsion angles in the cyclohexane ring were included as restraints in a simulated annealing (SA) protocol, thus yielding eight calculations (Table 2). An SA protocol was employed to assure that the global minimum was found for each possible combination of torsion angles. Only the calculation for one of the eight possible combinations (**1**) yielded geometries in accordance with all experimental observations hereby establishing the configuration of **27** to be the hydroxylation *anti* to the

Table 2. Summary of modelling performed on either configuration of nucleoside **27**[a]

	Torsion angle combination[b]	Sum of restraint violations (Å)	E_{AMBER} (kcal/mol)
'up'-isomer	**1**	1.4	39
	2	44	56
	3	14	46
	4	24	51
'down'-isomer	**1**	33	63
	2	42	56
	3	36	62
	4	24	92

[a] Shown is the torsion angle combination employed as restraints in calculations, the sum of restraint violations and the force field energy (E_{AMBER}) returned in calculations.
[b] The torsion angle combinations are as follows (given as $\theta_{H1''H2''}$, $\theta_{H2''H3''}$, $\theta_{H3''H4''up}$, $\theta_{H3''H4''down}$); up-isomer **1**: 76°, 36°, −166°, −50°; **2**: 76°, −38°, −166°, −50°; **3**: 127°, 36°, −166°, −50°; **4**: 127°, −38°, −166°, −50°; down-isomer **1**: 66°, 38°, −159°, −38°; **2**: 66°, −36°, −159°, −38°; **3**: −65°, 38°, −159°, −38°; **4**: −65°, −36°, −159°, −38°.

1″-*O*-substituent. In the calculations, no constraints were employed for the furanose ring, so the value of $\theta_{H1'H2'}$ obtained should serve as to validate the conformation determined. Indeed, we observe $\theta_{H1'H2'}=151°$ which is in good agreement with the value of 147° obtained by analysis of the Karplus relationship between $J_{H1'H2'}$ and $\theta_{H1'H2'}$ (Table 1).

Thus, we have also determined the conformation of the bicyclic ring system. The furanose ring is locked in a C-3′-*exo* (*S*-type) conformation ($_3$E, P=207°, $\Phi_{max}=46.7°$)[1,2] while the cyclohexane ring is found in a very slightly twisted chair conformation. In our modelling procedure, we have included no constraints for the γ and ε backbone torsion angles or for the glycosidic angle, χ, as no relevant experimental information is available. However, to assess if the nucleoside **27** might be compatible with the standard genus of backbone angles of right-handed nucleic acid duplexes, we performed an ab initio geometry optimisation (HF 3-21G level) with a nucleoside structure where the γ, ε and χ torsion angles were initially located in the +*sc*, *ap*, and *anti* ranges, respectively. As this calculation returned a geometry optimised structure with the γ, ε and χ angles in identical ranges, it appears that there are no apparent arguments against incorporating **27** or preferably a 2′-deoxy derivative thereof into a nucleic acid duplex. In Figure 2 is given a view of **27** obtained from the ab initio geometry optimisation.

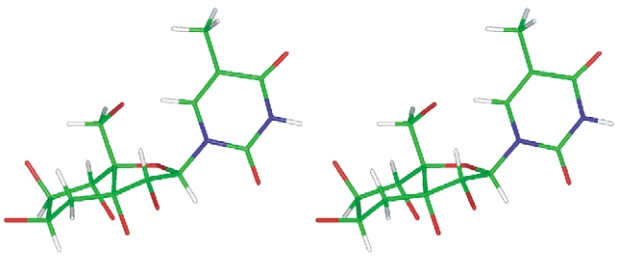


Figure 2. Stereoview of the structure of **27** obtained from an ab initio calculation. Hydrogens of the hydroxyl functionalities have been removed.

3. Discussion

In summary, the fully protected bicyclic nucleoside **20** has been synthesised in 11 steps and a satisfying 18% overall yield from diacetone-D-glucose. The general strategy was only slightly hampered, however, by the demand for separation of isomers after the benzylation of **9** as well as after the Grignard reaction giving **12** and **13**. Nevertheless, all RCM reactions afforded the constrained bicyclic carbohydrate or nucleoside derivatives. The ring strain of the RCM-generated cyclohexene ring was illuminated by the acidic ring-opening of the furanose in **15**. Simple deprotection of **20** afforded **24**, but the nucleoside **20** is also a key intermediate towards the construction of other bicyclic nucleosides e.g., saturated or 2′-deoxygenated derivatives. Alternatively, the olefinic moiety gives the potential for further derivatisation e.g., mono- or dihydroxylation, amination and/or the attachment of other groups. Hereby, a plethora of conformationally restricted nucleoside analogues might be constructed from **20**, and herein, we have shown the first example, the multihydroxylated bicyclic nucleoside **27**. Furthermore, the precursor **18** can be potentially used in the construction of similar bicyclic nucleosides with other nucleobases.

All the bicyclic nucleosides obtained are restricted in *S*-type conformations as indicated by their large $^3J_{H1'H2'}$ coupling constants.[29,30,42] Thus, both **24** and **27** are locked *S*-type mimics, and we determined the pseudorotation angle P of **27** to be 207° by molecular modelling. Furthermore, the flexibility of the C4′–C5′ bond is indicated to be unchanged compared to natural nucleosides. The strong restriction in an *S*-type conformation combined with flexibility of the C4′–C5′ bond is expected to be very favourable in the preparation of oligonucleotides with strong recognition of complementary nucleic acids in the formation of B-type duplexes and triplexes. Thus, the first bicyclic nucleosides to be incorporated into oligodeoxynucleotides[23,24] had the C4′–C5′ bond restricted in unnatural torsion angles and, hence, the affinity towards complementary nucleic acids were very dependent on the nucleotide sequences. Furthermore, the flexibility of the C4′–C5′ bond in combination with a locked *N*-type conformation might be a key factor for the impressing hybridisation behaviour of LNA. In order to obtain a bicyclic deoxynucleoside analogue better suited for B-type duplex formation, the preparation of 2′-deoxygenated analogues of **24** and **27** is in progress. The additional hydroxy functionalities on the cyclohexane ring might, on the other hand, favour duplex formation, and especially duplex hydration, by their hydrophilic nature. Thus, the cyclohexane is expected to be positioned at the brim of the minor groove in a B-type duplex, and hydrophilicity might be a significant advantage over the rather hydrophobic bicyclic structures investigated before, e.g., **2**, **3**, **4** (and its tricyclic analogue), **6** and others.[5,18–21,23–25,29,30] Finally, **24**, **27** and related compounds of the same [4.3.0]bicyclic skeleton might be useful molecules for the studies of protein/nucleoside(tide) interactions.

4. Conclusion

In conclusion, we have synthesised a bicyclic nucleoside structure with a functionalised 3′,4′-*trans* linkage and a locked *S*-type conformation. We expect this general bicyclic nucleoside structure to be an important tool in the study of biological versus conformational behaviour of nucleoside and nucleotides as well as in the development of conformationally restricted oligonucleotides with high affinity for complementary nucleic acids and thereby potential therapeutic, diagnostic and biotechnological applications. The construction of other nucleosides based on the key intermediate **20** is in progress.

5. Experimental

5.1. General

All commercial reagents were used as supplied. When necessary, reactions were performed under an atmosphere of nitrogen. Column chromatography was carried out on glass columns using silica gel 60 (0.040–0.063 mm). NMR spectra were recorded on a Varian Gemini 2000 spectrometer or at a Varian Unity 500 spectrometer. ^{1}H NMR

spectra were recorded at 300 or 500 MHz, ^{13}C NMR spectra were recorded at 75.5 MHz. Values for δ are in ppm relative to tetramethylsilane as internal standard. Fast-atom bombardment mass spectra (FAB-MS) were recorded in positive ion mode on a Kratos MS50TC spectrometer and MALDI mass spectra were recorded on an Ionspec Ultima Fourier Transform mass spectrometer. Microanalyses were performed at The Microanalytical Laboratory, Department of Chemistry, University of Copenhagen. Assignments of NMR spectra when given are based on $^{1}H,^{1}H$-COSY, $^{1}H,^{13}C$-COSY and/or DEPT spectra and follow standard carbohydrate and nucleoside style; i.e., the carbon atom next to a nucleobase is assigned C-1′; in the compounds **9**–**13**, **17**–**18**, the numbering continues from C-3 to C-1′ (2′ and 3′) and from C-4 to C-1″ (2″ and 3″); in the nucleoside **19** the numbering continues from C-3′ to C-1″ (2″ and 3″) and from C-4′ to C-1‴ (2‴ and 3‴); in compounds **14**–**15** the carbons in the cyclohexene ring are numbered from C-4 to C-1′, 2′, 3′ and 4′ next to C-3; in the bicyclic nucleosides **20**–**27** the carbons in the cyclohexene ring are numbered from C-4′ to C-1″, 2″, 3″ and 4″ next to C-3′. However, compound names for bi- and tricyclic compounds are given according to the von Baeyer nomenclature. ^{1}H NOE difference spectra were recorded for compounds **14**, **15**, **24** and **27**.

5.1.1. Preparation of 3-*C*-allyl-3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-di-*O*-isopropylidene-α-D-ribofuranose (9). The furanose **8** (6.08 g, 15.6 mmol) was dissolved in anhydrous ethyl acetate (150 mL), and H_5IO_6 (4.26 g, 18.7 mmol) was added. The mixture was stirred at room temperature for 1.5 h and then filtered through a layer of celite. The combined filtrates were evaporated to dryness under reduced pressure, and the residue was dissolved in anhydrous THF (50 mL). An aqueous solution of formaldehyde (4.40 mL, 49.5 mmol, 36% (w/v) containing 10% MeOH) and an aqueous solution of NaOH (2 M, 17.7 mL, 35.4 mmol) were added dropwise, and the reaction mixture was stirred at room temperature for 24 h. The mixture was cooled to 0 °C, $NaBH_4$ (0.88 g, 23.4 mmol) was added, and the mixture was stirred at room temperature for 1 h. The mixture was neutralised with 4 M acetic acid and extracted with ethyl acetate. The combined organic phases were washed with a saturated aqueous solution of $NaHCO_3$, dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using petrol ether–ethyl acetate (7:3) as eluent to give the product as a clear oil (4.67 g, 86%) (Found: C, 64.99; H, 7.49% $C_{19}H_{26}O_6$ requires C, 65.13; H, 7.48%); ^{1}H NMR ($CDCl_3$) δ 7.39–7.26 (5H, m, Ph), 5.96 (1H, m, H-2′), 5.72 (1H, d, J=4.2 Hz, H-1), 5.30–5.20 (2H, m, H-3′), 4.72, 4.65 (2H, AB system, J=10.4 Hz, Bn), 4.54 (1H, d, J=4.3 Hz, H-2), 4.14 (2H, br s, H-1″), 3.86 (2H, br s, H-5), 2.77 (1H, dd, J=8.1, 15.0 Hz, H-1′), 2.62 (1H, dd, J=5.6, 15.0 Hz, H-1′), 1.65 (3H, s, CH_3), 1.32 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 138.0 (Ph), 131.9 (C-2′), 128.4, 127.7, 127.6, 127.6, 127.4 (Ph), 119.6 (C-3′), 112.6 ($C(CH_3)_2$), 104.1 (C-1), 87.5, 85.9 (C-3, C-4), 83.1 (C-2), 67.1 (Bn), 63.5, 63.2 (C-5, C-1″), 36.7 (C-1′), 26.1, 25.6 ($C(CH_3)_2$); m/z (FAB) 373 (M+Na).

5.1.2. Preparation of 3-*C*-allyl-3,5-di-*O*-benzyl-4-*C*-hydroxymethyl-1,2-di-*O*-isopropylidene-α-D-ribofuranose (10) and 3-*C*-allyl-3-*O*-benzyl-4-*C*-benzyloxymethyl-1,2-di-*O*-isopropylidene-α-D-ribofuranose (11). The diol **9** (4.66 g, 13.3 mmol) was dissolved in anhydrous DMF (20 mL) and stirred at −5 °C. A 60% oily dispersion of NaH (638 mg, 16.0 mmol) was added in small portions. Benzylbromide (1.90 mL, 16.0 mmol) was added dropwise and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured on ice and water (12 mL) and extracted with ethyl acetate. The combined organic fractions were dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using petrol ether–ethyl acetate (3:1) as eluent to give the two products as clear oils.

10: (3.59 g, 61%) (Found: C, 70.85; H, 7.39% $C_{26}H_{32}O_6$ requires C, 70.89; H, 7.39%); ^{1}H NMR ($CDCl_3$) δ 7.34–7.23 (10H, m, 2×Ph), 5.90 (1H, m, H-2′), 5,72 (1H, d, J=4.2 Hz, H-1), 5.19–5.14 (2H, m, H-3′), 4.71, 4.66 (2H, AB system, J=11.6 Hz, Bn), 4.62, 4.54 (2H, AB system, J=11.8 Hz, Bn), 4.53 (1H, d, J=4.2 Hz, H-2), 4.30 (1H, dd, J=4.5, 11.6 Hz, H-1″) 3.91 (1H, dd, J=9.1, 11.6 Hz, H-1″), 3.72, 3.57 (2H, AB system, J=9.9 Hz, H-5), 2.79 (1H, dd, J=7.2, 15.1 Hz, H-1′), 2.49 (1H, dd, J=6.3, 15.1 Hz, H-1′), 2.25 (1H, dd, J=4.5, 9.1 Hz, 1″-OH), 1.63 (3H, s, CH_3), 1.31 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 138.4, 137.9 (Ph), 132.2 (C-2′), 128.3, 128.2, 127.7, 127.6, 127.3, 127.1 (Ph), 119.2 (C-3′), 112.7 ($C(CH_3)_2$), 103.7 (C-1), 88.4, 85.5 (C-3, C-4), 83.4 (C-2), 73.7 (Bn), 68.7 (C-5), 66.7 (Bn), 61.5 (C-1″), 36.3 (C-1′), 26.2, 25.8 ($C(CH_3)_2$); m/z (FAB) 441 (M+H).

11: (1.26 g, 22%) (Found: C, 70.39; H, 7.36% $C_{26}H_{32}O_6$ 1/4H_2O requires C, 70.17; H, 7.37%); ^{1}H NMR ($CDCl_3$) δ 7.33–7.23 (10H, m, 2×Ph), 5.95 (1H, m, H-2′), 5.71 (1H, m, H-1), 5.29 5.18 (2H, m, H-3′), 4.69 (2H, m, Bn), 4.59–4.45 (3H, m, Ph, H-2), 4.16–4.02 (2H, m, H-1″), 3.92–3.80 (2H, m, H-5), 2.84 (1H, dd, J=7.3, 15.3 Hz, H-1′), 2.58–2.50 (2H, m, H-1′, 5-OH), 1.51 (3H, s, CH_3), 1.30 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 138.5, 137.9 (Ph), 132.2 (C-2′), 128.3, 128.1, 127.9, 127.6, 127.2, 127.1 (Ph), 119.4 (C-3′), 112.5 ($C(CH_3)_2$), 103.6 (C-1), 88.0, 85.1 (C-3, C-4), 83.5 (C-2), 73.7 (Bn), 71.6 (C-1″), 66.6 (Bn), 63.6 (C-5), 36.1 (C-1′), 26.2, 25.8 ($C(CH_3)_2$); m/z (FAB) 463 (M+Na).

5.1.3. Preparation of 3-*C*-allyl-3,5-di-*O*-benzyl-4-*C*-(1(*R*)-hydroxyallyl)-1,2-di-*O*-isopropylidene-α-D-ribofuranose (12) and 3-*C*-allyl-3,5-di-*O*-benzyl-4-*C*-(1(*S*)-hydroxyallyl)-1,2-di-*O*-isopropylidene-α-D-ribofuranose (13). The bis-benzylic ether **10** (4.47 g, 10.1 mmol) was dissolved in anhydrous CH_2Cl_2 (250 mL) and PCC (6.56 g, 30.4 mmol) was added. The reaction mixture was stirred at room temperature for 42 h and diluted with ethyl acetate (25 mL). After stirring for another 30 min the mixture was filtered though a layer of silica and the filter was rinsed with ethyl acetate. The combined filtrates were evaporated to dryness under reduced pressure and the residue was dissolved in anhydrous THF (100 mL). The solution was cooled to 0 °C and a 1 M solution of vinylMgBr in THF (18.3 mL) was added dropwise. The reaction mixture was stirred at room temperature for 17 h, poured on ice and water (50 mL) and neutralised with 4 M acetic acid. The mixture was concentrated under reduced pressure and extracted with CH_2Cl_2. The combined organic fractions were dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel

column chromatography using petrol ether–ethyl acetate (9:1) as eluent to give the two products as clear oils.

12: (0.79 g, 17%) (Found: C, 72.23; H, 7.51% $C_{28}H_{34}O_6$ requires C, 72.08; H, 7.34%); ^{1}H NMR ($CDCl_3$) δ 7.37–7.24 (10H, m, 2×Ph), 6.09–5.91 (2H, m, H-2′, H-2″), 5.81 (1H, d, J=4.4 Hz, H-1), 5.32 (1H, m, H-3″), 5.19–5.09 (3H, m, H-3′, H-1″), 5.00 (1H, m, H-3″), 4.75, 4.70 (2H, AB system, J=10.9 Hz, Bn), 4.56 (1H, d, J=4.4 Hz, H-2), 4.43 (2H, br s, Bn), 3.56, 3.53 (2H, AB system, J=10.7 Hz, H-5), 3.26 (1H, d, J=1.4 Hz, 1″-OH), 2.85 (1H, dd, J=7.7, 15.1 Hz, H-1′), 2.68 (1H, dd, J=6.1, 15.1 Hz, H-1′), 1.63 (3H, s, CH_3), 1,33 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 138.3, 137.9 (Ph), 136.7, 133.0 (C-2′, C-2″), 128.3, 128.2, 127.7, 127.5, 127.4 (Ph), 118.9, 114.2 (C-3′, C-3″), 113.2 (C(CH3)2), 104.1 (C-1), 89.7 86.0 (C-3, C-4), 83.6 (C-2), 73.5 (Bn), 71.3 (C-1″), 69.8 (C-5), 67.1 (Bn), 37.3 (C-1′), 26.1, 26.0 ($C(CH_3)_2$); m/z (FAB) 489 (M+Na).

13: (2.95 g, 63%) (Found: C, 72.01; H, 7.46% $C_{28}H_{34}O_6$ requires C, 72.08; H, 7.34%); ^{1}H NMR ($CDCl_3$) δ 7.40–7.22 (10H, m, 2×Ph), 6.24 (1H, m, H-2″), 6.04 (1H, m, H-2′), 5.81 (1H, d, J=4.0 Hz, H-1), 5.41–5.13 (5H, m, H-1″, H-3″, H-3′), 4.82, 4.72 (2H, AB system, J=10.3 Hz, Bn), 4.52 (1H, d, J=4.4 Hz, H-2), 4.52, 4.45 (2H, AB system, J=11.9 Hz, Bn), 3.88, 3.68 (2H, AB system, J=11.4 Hz, H-5), 3.30 (1H, d, J=1.6 Hz, 1″-OH), 2.90 (1H, dd, J=7.8, 15.0 Hz, H-1′), 2.78 (1H, dd, J=6.0, 15.0 Hz, H-1′), 1.60 (3H, s, CH_3), 1.34 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 138.2, 137.8 (Ph), 133.1 (C-2′), 128.5, 128.2, 127.8, 127.7, 127.6, 127.4 (Ph), 118.8, 114.6 (C-3′, C-3″), 112.9 ($C(CH_3)_2$), 104.7 (C-1), 88.8, 87.0 (C-3, C-4), 83.7 (C-2), 73.7 (Bn), 72.3 (C-1″), 68.8 (C-5), 67.5 (Bn), 37.9 (C-1′), 26.3, 26.1 ($C(CH_3)_2$); m/z (FAB) 489 (M+Na).

5.1.4. Preparation of (1*S*,3*R*,7*R*,8*S*,12*R*)-8-benzyloxy-1-benzyloxymethyl-5,5-dimethyl-12-hydroxy-2,4,6-trioxa-tricyclo[6.4.0.0^{3,7}]dodec-10-ene (14). The bis-allylic compound **12** (53.5 mg, 0.12 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL) and Grubb's catalyst **A** (2.0 mg, 2.4×10^{-6} mol, 2 mol%) added. The reaction mixture was stirred at reflux for 48 h, another 1 mol% of the catalyst was added and the mixture was stirred for another 48 h. The mixture was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using petrol ether–ethyl acetate (2:1) as eluent to give the product as off-white crystals (39.9 mg, 76%) (Found: C, 70.87; H, 7.18% $C_{26}H_{30}O_6$ 1/4H_2O requires C, 70.49; H, 6.83%); ^{1}H NMR ($CDCl_3$) δ 7.37–7.15 (10H, m, 2×Ph), 6.03 (1H, d, J=5.0 Hz, H-1), 5.69–5.58 (2H, m, H-2′, H-3′), 5.26 (1H, br s, H-1′), 4.99 (1H, d, J=5.0 Hz, H-2), 4.73, 4.37 (2H, AB system, J=10.7 Hz, Bn), 4.53, 4.44 (2H, AB system, J=11.9 Hz, Bn), 3.82, 3.73 (1H, AB system, J=11.0 Hz, H-5), 2.86 (1H, dd, J=4.0, 18.3 Hz, H-4′), 2.38 (1H, br s, OH), 2.24 (1H, m, H-4′), 1.34 (3H, s, CH_3), 1.31 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 139.1, 137.7 (Ph), 132.5, 124.9 (C-2′, C-3′), 128.4, 127.9, 127.7, 127.5, 127.0, 126.8 (Ph), 115.6 (C(CH3)2), 106.0 (C-1), 92.6, 82.2 (C-3, C-4), 85.5 (C-2), 74.0 (Bn), 73.4 (C-5), 73.0 (C-1′), 67.1 (Bn), 29.2 (C-4′), 27.6, 26.5 ($C(CH_3)_2$).

5.1.5. Preparation of (1*S*,3*R*,7*R*,8*S*,12*S*)-8-Benzyloxy-1-benzyloxymethyl-5,5-dimethyl-12-hydroxy-2,4,6-trioxa-tricyclo[6.4.0.0^{3,7}]dodec-10-ene (15). The bis-allylic compound **13** (259 mg, 0.56 mmol) was dissolved in anhydrous CH_2Cl_2 (25 mL) and Grubb's catalyst **A** (9.4 mg, 1.1×10^{-5} mol, 2 mol%) added. The reaction mixture was stirred at reflux for 24 h. The mixture was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using petrol ether–ethyl acetate (2:1) as eluent to give the product as off-white crystals (228 mg, 93%); ^{1}H NMR ($CDCl_3$) δ 7.39–7.21 (10H, m, 2×Ph), 6.12 (1H, d, J=5.0 Hz, H-1), 6.02 (1H, m, H-2′), 5.70 (1H, m, H-3′), 4.94 (1H, d, J=5.1 Hz, H-2), 4.66, 4.39 (2H, AB system, J=10.0 Hz, Bn), 4.51, 4.44 (2H, AB system, J=11.9 Hz, Bn), 4.18 (1H, d, J=10.9 Hz, 1′-OH), 3.83 (1H, m, H-1′), 3.44, 3.39 (2H, AB system, J=10.3 Hz, H-5), 2.96 (1H, dd, J=5.5, 18.6 Hz, H-4′), 2.21 (1H, m, 4′-H), 1.43 (3H, s, CH_3), 1.35 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 137.7, 137.2 (Ph), 132.6 (C-2′), 128.5, 128.4, 127.9, 127.8, 127.6 (Ph), 123.7 (C-3′), 115.8 ($C(CH_3)_2$), 105.8 (C-1), 90.6, 82.3 (C-3, C-4), 84.9 (C-2), 75.8 (C-5), 73.9 (Bn), 68.7 (C-1′), 67.1 (Bn), 29.1 (C-4′), 27.4, 26.7 ($C(CH_3)_2$); m/z (FAB) 461 (M+Na).

5.1.6. Preparation of 3-acetyloxy-5-acetyloxymethylcarbonyl-5-benzyloxy-4-benzyloxymethyl-4-hydroxycyclohexene (16). The alcohol **15** (94.8 mg, 0.22 mmol) was dissolved in an 80% aqueous solution of acetic acid (1.0 mL). The reaction mixture was stirred at 90 °C for 6 h and then concentrated under reduced pressure. The residue was co-evaporated with anhydrous ethanol, toluene and pyridine and then redissolved in anhydrous pyridine (0.4 mL). Acetic anhydride (0.33 mL, 3.49 mmol) was added dropwise and the reaction mixture was stirred at room temperature for 48 h and then quenched by the addition of ice and water (2 mL). The mixture was extracted with CH_2Cl_2 and the combined organic phases were washed with a saturated aqueous solution of $NaHCO_3$, dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The residue was purified by silica gel chromatography using petrol ether–ethyl acetate (4:1) as eluent to give the product as a clear oil (48.9 mg, 47%) (Found: C, 66.18; H, 6.26% $C_{27}H_{30}O_8$ 1/2H_2O requires C, 65.98; H, 6.36%); ^{1}H NMR ($CDCl_3$) δ 7.41–7.30 (10H, m, 2×Ph), 5.83–5.78 (2H, m, H-1, H-2), 5.34 (1H, br s, H-3), 5.26, 5.09 (2H, AB system, J=17.5 Hz, 4-CH_2), 4.52–4.42 (4H, m, Bn), 3.45, 3.35 (2H, AB system, J=10.1 Hz, 5-$COCH_2O$), 3.37 (1H, s, 4-OH), 2.79–2.53 (2H, m, H-6), 2.15 (3H, s, $COCH_3$), 2.06 (3H, s, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 203.3 (5-CO), 170.6 (CH_3CO), 170.2 (CH_3CO), 137.6, 137.1, 128.3, 128.2, 127.7, 127.7, 127.7, 127.5, 127.4 (Ph), 126.1 (C-2), 124.9 (C-1), 83.8, 75.6 (C-4, C-5), 73.7 (Bn), 70.6 ($COCH_2O$), 69.4 (C-3), 68.1 (4-CH_2O), 66.1 (Bn), 26.9 (C-6), 21.0 (CH_3CO), 20.5 (CH_3CO); m/z (FAB) 505 (M+Na).

5.1.7. Preparation of 3-*C*-allyl-3,5-di-*O*-benzyl-4-*C*-(1(*S*)-benzoyloxy)allyl-1,2-di-*O*-isopropyliden-α-D-ribofuranose (17). The alcohol **13** (3.73 g, 7.99 mmol) was dissolved in anhydrous pyridine (30 mL) and cooled to 0 °C. Benzoyl chloride (2.78 mL, 24.0 mmol) was added, and the reaction mixture was stirred at room temperature for 21 h. Another portion of benzoyl chloride (0.93 mL, 8.0 mmol) was added, and the reaction mixture was stirred for another 4 h and then concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 and extracted with a

saturated aqueous solution of $NaHCO_3$ and brine. The combined organic phases were dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The residue was purified by silica gel chromatography using petrol ether–ethyl acetate (9:1) as eluent to give the product as a clear oil (4.48 g, 98%) (Found: C, 73.35; H, 6.74% $C_{35}H_{38}O_7$ requires C, 73.66; H, 6.71%); ^{1}H NMR ($CDCl_3$) δ 7.77–7.74 (2H, m, Ph), 7.49–7.22 (9H, m, 2×Ph), 7.09–6.97 (4H, m, 2×Ph), 6.63 (1H, m, H-1″), 6.35 (1H, ddd, J=17.4, 11.0, 3.6 Hz, H-2″), 5.91 (1H, m, H-2′), 5.90 (1H, d, J=4.6 Hz, H-1), 5.20–5.01 (4H, m, H-3′, H-3″), 4.71, 4.54 (2H, AB system, J=10.9 Hz, Bn), 4.63–4.47 (2H, m, Bn), 4.56 (1H, d, J=4.6 Hz, H-2), 4.04, 3.85 (2H, AB system, J=10.8 Hz, H-5), 2.85 (1H, dd, J=15.6, 7.7 Hz, H-1′), 2.73 (1H, dd, J=15.6, 5.8 Hz, H-1′), 1.65 (3H, s, CH_3), 1.36 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 164.6 (CO), 138.0, 137.9 (Ph), 134.4 (C-2″), 133.6 (C-2′), 132.3, 130.3, 129.6, 128.3, 127.9, 127.7, 127.7, 127.6, 127.3, 126.8 (Ph), 118.3, 115.0 (C-3′, C-3″), 113.5 ($C(CH_3)_2$), 104.8 (C-1), 88.7, 85.6 (C-3, C-4), 85.0 (C-2), 73.9 (Bn), 73.4 (C-1″), 70.1 (C-5), 67.0 (Bn), 37.2 (C-1′), 26.6, 26.4 ($C(CH_3)_2$); m/z (FAB) 593 (M+H).

5.1.8. Preparation of 1,2-di-*O*-acetyl-3-*C*-allyl-3,5-di-*O*-benzyl-4-*C*-(1(*S*)-benzoyloxy)allyl-(α/β)-D-ribofuranose (18). The isopropylidene protected furanose **17** (4.48 g, 7.85 mmol) was dissolved in 80% aqueous acetic acid (38 mL) and stirred for 90 min. at 90 °C. The reaction mixture was concentrated under reduced pressure and co-evaporated with anhydrous ethanol, toluene and pyridine. The residue was redissolved in anhydrous pyridine (20 mL) and acetic anhydride (11 mL) was added dropwise. The reaction mixture was stirred at room temperature for 48 h and the reaction was then quenched by the addition of ice and water. The mixture was extracted with CH_2Cl_2 and the combined organic phases were washed with a saturated aqueous solution of $NaHCO_3$, dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The residue was purified by silica gel chromatography using CH_2Cl_2–methanol (99.5:0.5) as eluent to give the product as a mixture of anomers (4.46 g, 92%; β:α ~5:1) (Found: C, 70.68; H, 6.08% $C_{36}H_{38}O_9$ requires C, 70.34; H, 6.23%); ^{1}H NMR ($CDCl_3$) δ 7.95–7.92 (m), 7.57–7.52 (m), 7.45–7.22 (m), 6.42 (d, J=5.2 Hz), 6.30 (d, J=3.9 Hz), 6.28–5.86 (m), 5.58 (d, J=5.1 Hz), 5.21–4.56 (m), 4.01–3.96 (m), 3.77–3.69 (m), 2.91–2.81 (m), 2.07–1.93 (m); ^{13}C NMR ($CDCl_3$) δ (for the major β anomer) 169.9, 169.4 (CH_3CO), 164.1 (CO), 138.0, 137.6 (Ph), 133.0, 129.6, 128.4, 128.3, 128.1, 127.7, 127.5, 127.3, 127.3 (Ph, C-2′, C-2″), 118.3, 116.7 (C-3′, C-3″), 98.7 (C-1), 90.9, 84.9 (C-3, C-4), 79.8 (C-2), 74.0, 73.7, 70.5, 67.5 (Ph, C-5, C-1″), 35.8 (C-1′), 21.1, 20.9 ($C(CH_3)_2$); m/z (FAB) 637 (M+H).

5.1.9. Preparation of 1-(2-*O*-acetyl-3-*C*-allyl-3,5-di-*O*-benzyl-4-*C*-(1(*S*)-benzoyloxy)allyl-β-D-ribofuranosyl)-thymine (19). A solution of the bis-acetate **18** (1.02 g, 1.65 mmol) and thymine (417 mg, 3.30 mmol) in anhydrous CH_3CN (17 mL) was stirred at room temperature and *N*,*O*-bis(trimethylsilyl)acetamide (2.05 mL, 8.26 mmol) was added dropwise. The reaction mixture was stirred at reflux for 30 min. and cooled to 0 °C. TMS-OTf (0.51 mL, 2.81 mmol) was added dropwise and the reaction mixture was stirred at 40 °C for 17 h. The reaction was quenched by the addition of ice-cold aqueous solution of $NaHCO_3$ (20 mL) and the mixture was extracted with CH_2Cl_2. The combined organic phases were washed with a saturated aqueous solution of $NaHCO_3$ and brine, dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The residue was purified by silica gel chromatography using petrol ether–ethyl acetate (4:1) as eluent to give the product as a white solid (1.05 g, 93%) (Found: C, 68.45; H, 6.13; N, 3.91% $C_{39}H_{40}O_9N_2$ requires C, 68.81; H, 5.92; N, 4.12%); ^{1}H NMR ($CDCl_3$) δ 8.60 (1H, br s, NH), 8.00–7.98 (m, 2H, Ph), 7.71 (1H, d, J=0.9 Hz, H-6), 7.64–7.59 (1H, m, Ph), 7.50–7.26 (m, 12H, Ph), 6.43 (1H, d, J=8.5 Hz, H-1′), 6.22 (1H, m, H-1‴), 6.02 (1H, ddd, J=17.6, 11.1, 4.1 Hz, H-2‴), 5.85 (1H, m, H-2″), 5.88 (1H, d, J=8.5 Hz, H-2′), 5.21–4.59 (6H, m, Ph, H-3″, H-3‴), 4.75, 4.67 (2H, AB system, J=11.5 Hz, Bn), 4.01, 3.95 (2H, AB system, J=11.0 Hz, H-5′), 2.98 (1H, dd, J=16.0, 6.5 Hz, H-1″), 2.83 (1H, dd, J=16.0, 7.6 Hz, H-1″), 2.09 (3H, s, CH_3CO), 1.41 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 169.8 (CH_3CO), 163.8, 163.6 (C-2, CO), 150.9 (C-4), 137.7, 136.5, 136.0 (Ph), 133.2, 132.9, 132.7 (C-6, C-2″, C-2‴), 129.7, 129.6, 128.8, 128.5, 128.3, 128.3, 127.9, 127.7, 127.4 (Ph), 118.4, 116.4 (C-3″, C-3‴), 111.2 (C-5), 89.3, 83.2 (C-3′, C-4′), 83.6 (C-1′), 78.0 (C-2′), 73.8 (Bn), 73.2 (C-1‴), 70.7 (C-5′), 68.0 (Bn), 34.9 (C 1″), 20.8 (CH_3CO), 11.8 (CH_3); m/z (FAB) 703 (M+Na).

5.1.10. Preparation of (1*S*,5*S*,6*S*,8*R*,9*R*)-9-acetyloxy-5-benzoyloxy-1-benzyloxy-6-benzyloxymethyl-8-(thymin-1-yl)-7-oxabicyclo[4.3.0]non-3-ene (20). A solution of the protected nucleoside **19** (1.01 g, 1.48 mmol) in anhydrous $ClCH_2CH_2Cl$ (15 mL) was added the precatalyst **A** (19 mg, 22 μmol, 2 mol%) and the reaction mixture was stirred at reflux for 25 h and then concentrated under reduced pressure. The residue was purified by silica gel chromatography using CH_2Cl_2–methanol (99:1) as eluent to give the product as an off-white solid (873 mg, 90%) (Found: C, 67.97; H, 5.72; N, 4.30% $C_{37}H_{36}O_9N_2$ requires C, 68.09; H, 5.56; N, 4.29%); ^{1}H NMR ($CDCl_3$) δ 8.59 (1H, m, NH), 7.80 (1H, s, H-6), 7.55–7.31 (13H, m, Ph) 7.10–7.04 (2H, m, Ph), 6.71 (1H, d, J=7.4 Hz, H-1′), 6.17 (1H, d, J=7.4 Hz, H-2′), 6.00–5.96 (2H, m, H-2″, H-3″), 5.32 (1H, s, H-1″), 4.78 (1H, d, J=9.1 Hz, Bn), 4.65 (2H, s, Bn), 4.32 (1H, d, J=9.1 Hz, Bn), 3.81, 3.67 (2H, AB system, J=10.8 Hz, H-5′), 2.96 (1H, dd, J=18.9, 4.6 Hz, H-4″), 2.47 (1H, d, J=18.9 Hz, H-4″), 2.14 (3H, s, CH_3CO), 1.54 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 170.6 (CH_3CO), 166.6, 163.6 (C-2, CO), 150.8 (C-4), 137.9, 136.4, 136.0 (Ph), 132.6, 129.8, 129.5, 128.8, 128.6, 128.5, 128.3, 128.1, 127.9, 127.8, 127.4, 126.5 (Ph, C-2″, C-3″, C-6), 110.7 (C-5), 87.0, 80.8 (C 3′, C-4′), 86.9 (C-1′), 78.3 (C-2′), 74.7, 73.9 (Ph, C 5′), 67.8 (Bn), 67.1 (C-1″), 27.7 (C-4″), 20.8 (CH_3CO), 12.1 (CH_3); m/z (MALDI) 675 (M+Na).

5.1.11. Preparation of (1*S*,5*S*,6*S*,8*R*,9*R*)-5-benzoyloxy-1-benzyloxy-6-benzyloxymethyl-9-hydroxy-8-(thymin-1-yl)-7-oxabicyclo[4.3.0]non-3-ene (21). A solution of **20** (301 mg, 0.46 mmol) in methanol (13 mL) was added NaOMe (37.0 mg, 0.69 mmol) and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel chromatography using petrol ether–ethyl acetate (2:3) as eluent to give the product as a white solid (243 mg, 87%) (Found: C, 68.21; H, 5.67; N, 4.67% $C_{35}H_{34}O_8N_2 \cdot 1/4H_2O$ requires C, 68.34; H, 5.65;

N, 4.55%); ^{1}H NMR ($CDCl_3$) δ 9.39 (1H, br s, NH), 7.73 (1H, s, H-6), 7.65 (2H, d, J=7.6 Hz, Ph), 7.51–7.23 (m, 11H, Ph), 7.11 (2H, t, J=7.7 Hz, Ph), 6.02–5.94 (2H, m, H-2″, H-3″), 5.95 (1H, d, J=5.4 Hz, H-1′), 5.63 (1H, d, J=3.1 Hz, H-1″), 5.15 (1H, d, J=9.5 Hz, Bn), 4.85 (1H, d, J=5.7 Hz, H-2′), 4.71 (1H, br s, 2′-OH), 4.51, 4.44 (2H, AB system, J=11.5 Hz, Bn), 4.33 (1H, d, J=9.5 Hz, Bn), 3.61, 3.55 (2H, AB system, J=10.8 Hz, H-5′), 3.14 (1H, dd, J=18.8, 4.5 Hz, H-4″), 2.23 (1H, d, J=18.8 Hz, H-4″), 1.78 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 166.7, 164.0 (C-2, CO), 152.1 (C-4), 138.9, 136.5, 136.4 (Ph), 132.6, 129.8, 128.6, 128.2, 128.2, 128.1, 127.4, 127.3, 126.7 (Ph, C-2″, C-3″), 129.9 (C-6), 109.7 (C-5), 94.6 (C-1′), 88.4, 81.9 (C-3′, C 4′), 81.2 (C-2′), 73.9 (Bn), 73.3 (C-5′), 67.7 (Bn), 67.2 (C 1″), 27.6 (C-4″), 12.4 (CH_3); *m/z* (FAB) 611 (M+H).

5.1.12. Preparation of (1*S*,5*S*,6*S*,8*R*,9*R*)-5-benzoyloxy-1,9-dihydroxy-6-hydroxymethyl-8-(thymin-1-yl)-7-oxabicyclo[4.3.0]non-3-ene (22). A solution of **21** (91.0 mg, 0.15 mmol) in anhydrous CH_2Cl_2 (2.50 mL) was stirred at −78 °C. A solution of BCl_3 in hexane (1 M, 0.40 mL, 0.40 mmol) was added dropwise and the reaction mixture was stirred at −78 °C for 4 h. An additional amount of BCl_3 (0.40 mL, 0.40 mmol) was added and the reaction mixture was allowed to reach room temperature and stirred for another 16 h. The reaction was quenched by the addition of methanol (2 mL) and water (0.1 mL) and the mixture was stirred for 1 h and concentrated under reduced pressure. The residue was purified by silica gel chromatography using CH_2Cl_2–methanol (19:1) as eluent to give the product as a white solid as well as the debenzoylated product **24** (10.5 mg, 21%).

22: (35.6 mg, 55%); ^{1}H NMR ($CDCl_3$) δ 9.85 (1H, br s, NH), 8.02 (2H, d, J=7.3 Hz, Ph), 7.56–7.38 (4H, m, H-6), 6.07–5.96 (2H, m, H-2″, H-3″), 5.76 (1H, d, J=4.1 Hz, H-1″), 5.67 (1H, d, J=6.7 Hz, H-1′), 4.94 (1H, m, H-2′), 4.26 (1H, br s, 2′ OH), 3.93–3.59 (4H, m, H-5′, 5′-OH, 3′-OH), 2.58 (1H, dd, J=17.6, 4.2 Hz, H-4″), 2.34 (1H, d, J=17.6 Hz, H-4″), 1.75 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 165.9, 164.2 (C-2, CO), 151.4 (C-4), 139.5 (C-6), 133.3, 129.8, 129.7, 129.6, 128.5 (Ph), 130.6, 125.2 (C-2″, C-3″), 110.6 (C-5), 95.4 (C-1′), 86.4, 77.2 (C-3′, C-4′), 75.0 (C-2′), 67.4 (C-1″), 64.7 (C-5′), 32.9 (C-4″), 12.2 (CH_3); *m/z* (FAB) 431 (M+H).

5.1.13. Preparation of (1*S*,5*S*,6*S*,8*R*,9*R*)-1-benzyloxy-6-benzyloxymethyl-5,9-dihydroxy-8-(thymin-1-yl)-7-oxabicyclo[4.3.0]non-3-ene (23). A solution of **20** (250 mg, 0.38 mmol) in methanol (11 mL) was added NaOMe (41 mg, 0.76 mmol) and stirred at reflux for 24 h. An additional amount of NaOMe (41 mg, 0.76 mmol) was added and the reaction mixture was stirred at reflux for another 24 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel chromatography using petrol ether–ethyl acetate (1:3) as eluent to give the product as a white solid (133 mg, 69%); ^{1}H NMR ($CDCl_3$) δ 9.78 (1H, br s, NH), 7.82 (1H, d, J=1.3 Hz, H-6), 7.38–7.16 (10H, m, Ph), 6.06 (1H, m, H-2″), 5.89 (1H, d, J=5.3 Hz, H-1′), 5.81 (1H, m, H-3″), 5.10 (1H, d, J=9.4 Hz, Bn), 4.92 (1H, br s, OH), 4.78 (1H, d, J=5.3 Hz, H-2′), 4.43, 4.33 (2H, AB system, J=16.7 Hz, Bn), 4.31 (1H, d, J=9.4 Hz, Bn), 4.06–3.99 (2H, m, H-1″, OH), 3.43, 3.41 (2H, AB system, J=11.1 Hz, H-5′), 3.07 (1H, dd, J=18.6, 5,4 Hz, H-4″), 2.15 (1H, d, J=18.6 Hz, H-4″), 1.80 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 164.1, 152.2 (C-1, C-4), 137.5 (Ph), 136.5, 136.4 (Ph, C-6), 131.9 (C 2″), 128.5, 128.5, 128.4, 128.2, 128.0, 127.3 (Ph), 123.1 (C-3″), 109.6 (C-5), 94.9 (C-1′), 88.5, 84.5 (C-3′, C 4′), 82.0 (C 2′), 73.8 (Bn), 72.9 (C-5′), 68.2, 68.1 (Ph, C-1″), 27.7 (C-4″), 12.4 (CH_3); *m/z* (FAB) 507 (M+H).

5.1.14. Preparation of (1*S*,5*S*,6*S*,8*R*,9*R*)-1,5,9-trihydroxy-6-hydroxymethyl-8-(thymin-1-yl)-7-oxabicyclo-[4.3.0]non-3-ene (24). *Method A*: A solution of **22** (35.0 mg, 0.8 mmol) in methanol (2.3 mL) was added NaOMe (6.50 mg, 0.12 mmol) and stirred at room temperature for 1 h. An additional amount of NaOMe (9.00 mg, 0.17 mmol) was added and the reaction mixture was stirred at 50 °C for 2 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel chromatography using CH_2Cl_2–methanol (9:1) as eluent to give the product as a white solid (12.6 mg, 48%).

Method B: A solution of **23** (22.5 mg, 0.045 mmol) in anhydrous CH_2Cl_2 (0.90 mL) was stirred at −78 °C. A solution of BCl_3 in hexane (1 M, 0.20 mL, 0.20 mmol) was added dropwise and the reaction mixture was stirred at −78 °C for 4 h. An additional amount of BCl_3 (0.15 mL, 0.15 mmol) was added and the reaction mixture was allowed to reach room temperature and stirred for another 16 h. The reaction was quenched by the addition of methanol (0.7 mL) and water (0.1 mL) and the mixture was stirred for 1 h and concentrated under reduced pressure. The residue was purified by silica gel chromatography using CH_2Cl_2–methanol (9:1) as eluent to give the product as a white solid (10.2 mg, 69%); ^{1}H NMR (DMSO-d_6) δ 11.31 (1H, br s, NH), 8.17 (1H, br s, H-6), 6.10 (1H, d, J=7.4 Hz, H-1′), 5.81 5.71 (3H, m, H-2″, H-3″, 3′-OH), 5.48 (1H, br s, 5′-OH), 5.29 (1H, d, J=6.3 Hz, 2′-OH), 5.10 (1H, d, J=8.9 Hz, 1″-OH), 4.64 (1H, t, J=6.7 Hz, H-2′), 3.76 (1H, m, H-1″), 3.56–3.35 (2H, m, H-5′), 2.32–2.26 (2H, m, H-4″), 1.79 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.6, 151.4 (C-2, C-4), 137.4 (C-6), 129.9, 126.2 (C-2″, C-3″), 109.2 (C-5), 88.1 (C-1′), 85.1, 78.3 (C-3′, C-4′), 74.3 (C-2′), 67.1 (C-1″), 64.9 (C-5′), 32.4 (C-4″), 12.2 (CH_3); HiRes MALDI FT-MS *m/z* (M+Na) found/calcd 349.1000/349.1006.

5.1.15. Preparation of (1*S*,3*R*,4*R*,5*S*,6*S*,8*R*,9*R*)-9-acetyloxy-5-benzoyloxy-1-benzyloxy-6-benzyloxymethyl-3,4-dihydroxy-8-(thymin-1-yl)-7-oxabicyclo[4.3.0]nonane (25). A solution of **20** (640 mg, 0.980 mmol) in a mixture of THF and water (1:1, 10 mL) was added *N*-methylmorpholine-*N*-oxide (172 mg, 1.47 mmol) and a 2.5% solution of OsO_4 in *tert*-butanol (0.49 mL, 0.039 mmol). The reaction mixture was stirred at 50 °C for 7 h and then quenched by the addition of a saturated aqueous solution of $NaHSO_3$ (3 mL). The mixture was stirred for 30 min at room temperature and then partly concentrated under reduced pressure. The aqueous residue was extracted with ethyl acetate, and the combined organic extracts were dried (Na_2SO_4). The residue was purified by silica gel chromatography using CH_2Cl_2–methanol (49:1) as eluent to give the product as a white solid and a (16:1)-mixture of stereoisomers (330 mg, 49%), in addition to a side product

25a as a white solid (71 mg, 10%) and unreacted starting material **20** (208 mg, 33%).

25: ^{1}H NMR ($CDCl_3$) δ (Major isomer) 8.30 (1H, s, NH), 7.81 (1H, s, H-6), 7.81–7.79 (2H, m, Ph), 7.54–7.24 (13H, m, Ph), 6.68 (1H, d, J=7.7 Hz, H-1′), 6.17 (1H, d, J=7.7 Hz, H-2′), 4.99 (1H, d, J=10.1 Hz, Bn), 4.78 (1H, d, J=11.6 Hz, Bn), 4.65 (1H, d, J=4.5 Hz, H-1″), 4.61 (1H, d, J=11.6 Hz, Bn), 4.54 (1H, d, J=11.1 Hz, H-5′), 4.40 (1H, d, J=10.1 Hz, Bn), 4.38 (1H, m, H-3″), 4.33 (1H, m, H-2″), 3.88 (1H, br s, OH), 3.84 (1H, d, J=11.1 Hz, H-5′), 3.31 (1H, br s, OH), 3.01 (1H, dd, J=9.1, 15.7 Hz, H-4″$_{down}$), 2.16 (1H, d, J=15.7 Hz, H-4″$_{up}$), 2.11 (3H, s, CH_3CO), 1.50 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 170.2 (CH_3CO), 167.8, 163.5 (C-4, CO), 150.8 (C-2) 137.3, 136.4, 133.3, 129.9, 129.3, 128.7, 128.5, 128.4, 128.2, 127.9, 127.6, 127.5 (C-6, Ph), 110.9 (C-5), 87.1, 86.9, 82.7 (C-1′, C-3′, C-4′), 78.2, 77.8 (C-2′, C-1″) 73.8, 73.7, 73.5, 67.4, 65.5 (C-5′, C-2″, C-3″, Bn), 29.8 (C-4″), 20.8 (CH_3CO), 12.0 (CH_3); HiRes MALDI FT-MS m/z (M+Na) found/calcd 709.2350/709.2368.

(1*S*,3*R*,4*R*,5*S*,6*S*,8*R*,9*R*)-9-Acetyloxy-5-benzoyloxy-1-benzyloxy-6-benzyloxymethyl-3,4-dihydroxy-8-(5,6-dihydroxypyrimidine-2,4-dion-1-yl)-7-oxabicyclo-[4.3.0]nonane 25: ^{1}H NMR ($CDCl_3$) δ (Major isomer) 7.82–7.78 (3H, m, Ph, NH), 7.52–7.21 (13H, m, Ph), 6.50 (1H, d, J=8.0 Hz, H-1′), 6.10 (1H, d, J=8.0 Hz, H-2′), 5.51 (1H, d, J=2.2 Hz, H-6), 4.99 (1H, d, J=10.5 Hz, Bn), 4.71 (1H, d, J=11.0 Hz, Bn), 4.59 (1H, d, J=4.3 Hz, H-1″), 4.51 (1H, d, J=11.0 Hz, Bn), 4.43 (2H, d, J=11.3 Hz, H-5′), 4.40 (1H, m, H-3″), 4.39 (1H, d, J=10.5 Hz, Bn), 4.28 (1H, m, H-2″), 4.02 (1H, d, J=2.8 Hz, 2″-OH), 3.71 (1H, d, J=2.2 Hz, 6-OH), 3.68 (1H, d, J=11.3 Hz, H-5′), 3.37 (1H, s, OH), 3.39 (1H, s, OH), 2.99 (1H, dd, J=9.7 Hz, 15.8 Hz, H-4″$_{down}$), 2.17 (1H, m, H-4″$_{up}$), 2.11 (3H, s, CH_3CO), 1.30 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 173.5 (CH_3CO), 171.5, 167.9 (C-4, CO), 150.9 (C-2), 137.4, 136.2, 133.2, 130.0, 129.9, 129.5, 129.5, 128.8, 128.7, 128.5, 128.3, 127.8, 127.5 (Ph), 86.2, 85.5, 82.4 (C-1′, C-3′, C-4′), 78.3, 78.2, 78.1, 74.0, 73.9, 73.7, 72.2, 67.3, 65.4 (C-5, C-6, C-1′, C-2′, C-5′, C-1″, C-2″, C-3″, Bn), 29.7 (C-4″), 21.9 (CH_3), 20.9 (CH_3CO); HiRes MALDI FT-MS m/z (M+Na) found/calcd 743.2439/743.2423.

5.1.16. Preparation of (1*S*,3*R*,4*R*,5*S*,6*S*,8*R*,9*R*)-1-benzyloxy-6-benzyloxymethyl-3,4,5,9-tetrahydroxy-8-(thymin-1-yl)-7-oxabicyclo[4.3.0]nonane (26). A solution of **25** (72 mg, 0.10 mmol) in methanol (2.0 mL) was added NaOMe (28 mg, 0.50 mmol) and stirred at 65 °C for 9 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel chroma-tography using CH_2Cl_2–methanol (49:1) as eluent to give the product as a white solid (43 mg, 75%); ^{1}H NMR (DMSO-d_6) δ 11.34 (1H, s, NH), 7.76 (1H, s, H-6), 7.45–7.29 (10H, m, Ph), 6.24 (1H, d, J=7.6 Hz, H-1′), 5.85 (1H, d, J=4.5 Hz, 2′-OH), 5.36 (1H, d, J=10.8 Hz, Bn), 5.11 (1H, d, J=4.9 Hz, 2″-OH), 4.92 (1H, dd, J=4.5, 7.6 Hz, H-2′), 4.83 (1H, d, J=6.3 Hz, 3″-OH), 4.59 (1H, d, J=10.8 Hz, Bn), 4.53 (2H, s, Bn), 4.16 (1H, d, J=11.4 Hz, H-5′), 4.10 (1H, m, H-3″), 4.01 (1H, d, J=9.9 Hz, 1″-OH), 3.86 (1H, dd, J=4.9, 7.3 Hz, H-2″), 3.79 (1H, d, J=11.4 Hz, H-5′), 3.57 (1H, dd, J=2.7, 9.9 Hz, H-1″), 2.71 (1H, dd, J=6.5, 14.5 Hz, H-4″$_{down}$), 1.62 (1H, dd, J=8.6, 14.5 Hz, H-4″$_{up}$), 1.58 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.6 (C-4), 151.4 (C-2), 138.3, 137.9, 136.7 (C-6, Ph), 128.5, 128.4, 128.3, 127.7, 127.6, 127.2 (Ph), 109.3 (C-5), 88.7 (C-1′), 85.8, 85.3 (C-3′, C-4′), 76.3, 74.5, 74.4, 73.1, 72.5, 66.6, 64.4 (C-2′, C-5′, C-1″, C-2″, C-3″, Bn), 29.4 (C-4″), 11.9 (CH_3); m/z (MALDI) 563 (M+Na).

5.1.17. Preparation of (1*S*,3*R*,4*R*,5*S*,6*S*,8*R*,9*R*)-6-hydroxymethyl-1,3,4,5,9-pentahydroxy-8-(thymin-1-yl)-7-oxabicyclo[4.3.0]nonane (27). A degassed solution of **26** (19 mg, 36 μmol) in anhydrous methanol (3 mL) was added 20% $Pd(OH)_2$/C (9 mg, 12 μmol) and stirred at room temperature. The mixture was bubbled with a stream of hydrogen for 10 min and then stirred under an atmosphere of hydrogen for 24 h. The reaction mixture was filtered through a layer of celite which was rinsed with methanol. The residue was concentrated under reduced pressure to give the product as a white solid (12.1 mg, 95%); mp 168–72 °C; ^{1}H NMR (DMSO-d_6) δ 11.28 (1H, br s, NH), 8.09 (1H, s, H-6), 6.20 (1H, br s, 3′-OH), 6.02 (1H, d, J=7.5 Hz, H-1′), 5.45 (1H, d, J=7.8 Hz, 1″-OH), 5.27 (1H, br s, 2′-OH), 4.97 (1H, br s, 5′-OH), 4.90 (1H, br s, 2″-OH), 4.63–4.59 (2H, m, H-2′, 3″-OH), 4.25 (1H, m, H-3″), 3.98 (1H, d, J=12.0 Hz, H-5′), 3.86 (1H, d, J=3.5 Hz, H-2″), 3.72 (1H, d, J=6.1 Hz, H-1″), 3.63 (1H, dd, J=3.9, 12.0 Hz, H-5′), 1.96 (1H, dd, J=5.5, 12.7 Hz, H-4″$_{down}$), 1.78 (3H, s, CH_3), 1.44 (1H, t, J=12.7 Hz, H-4″$_{up}$); ^{13}C NMR (DMSO-d_6) δ 163.8 (C-4), 151.6 (C-2), 137.7 (C-6), 109.0 (C-5), 89.2 (C-1′), 84.5, 81.2 (C-3′, C-4′), 74.1, 73.9, 73.4, 64.4, 63.3 (C-2′, C-5′, C-1″, C-2″, C-3″), 33.3 (C-4″), 12.3 (CH_3); ^{1}H NMR (CD_3OD) δ 8.07 (1H, d, J=1.3 Hz, H-6), 6.05 (1H, d, J=6.9 Hz, H-1′), 4.71 (1H, d, J=6.9 Hz, H-2′), 4.44 (1H, m, H-3″), 4.09 (1H, dd, J=2.0, 4.7 Hz, H-2″), 4.05 (1H, d, J=2.0 Hz, H-1″), 3.99 (1H, d, J=12.5 Hz, H-5′), 3.98 (1H, d, J=12.5 Hz, H-5′), 2.17 (1H, dd, J=5.3, 12.8 Hz, H-4″$_{down}$), 1.89 (3H, d, J=1.3 Hz, CH_3), 1.60 (1H, dd, J=10.7, 12.8 Hz, H-4″$_{up}$); ^{13}C NMR (CD_3OD) δ 153.5 (C-4), 145.8 (C-2), 139.7 (C-6), 111.3 (C-5), 93.2 (C-1′), 86.6, 82.8 (C-3′, C-4′), 76.9 (C-2′), 74.9 (C-2″), 74.7 (C-1″), 66.4 (C-3″), 64.4 (C-5′), 34.3 (C-4″), 12.5 (CH_3); HiRes MALDI FT-MS m/z (M+Na) found/calcd 383.1044/383.1061.

5.2. Measurement of three-bond coupling constants of 27

1D ^{1}H NMR spectra of the nucleoside studied were acquired on a Varian Unity 500 MHz spectrometer. The nucleoside **27** was dissolved in CD_3OD and spectra were obtained in the temperature range from −50 to +50 °C. Coupling constants were measured as the splitting of multiplet components, thereby limiting the accuracy to within 10% of the linewidth (~0.1 Hz).

5.3. Karplus relationships

Karplus relationships correlating $^3J_{HH}$ and torsion angles were constructed employing a state-of-art generalised Karplus equation for nucleosides and nucleotides developed by Altona and co-workers:[43–45]

$$J_{HH}(\theta) = \sum_{m=0}^{3} C_m \cos(m\theta) + \sum_{n=1}^{3} S_n \sin(n\theta)$$

where the electronegativity of the HCCH-fragment substituents is accounted for in the coefficients C_m and S_n.

5.4. Force field calculations

Briefly described, the torsion angle restraints were employed as flat-well potentials in the calculations, with the flat-well part being the value obtained from Karplus relationships $\pm 5°$, and force constants of 50 kcal/(mol rad^2). In the simulated annealing protocol, each structure was initially restrained energy minimised before being subjected to 40 ps molecular dynamics (40,000 steps of 1 fs) with the temperature being lowered from 2000 to 250 K. Finally, each structure was restrained energy minimised again. In this manner, 10 structures were generated for each of the eight calculations (as detailed in Table 2) by randomly varying initial atomic velocities.

5.5. Ab initio calculation

The ab initio quantum mechanical calculation was performed using the Gaussian94 program.[46] The geometry optimisation was carried out at the 3-21G* level using the restricted Hartree–Fock procedure.

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References and notes

1. (a) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205. (b) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1973**, *95*, 2333.
2. For definitions, nomenclature and conformational behaviour of nucleosides and nucleotides, see also Saenger, W. *Principles of nucleic acid structure*; Springer: New York, 1984.
3. Herdewijn, P. *Biochim. Biophys. Acta* **1999**, *1489*, 167.
4. Leumann, C. *J. Bioorg. Med. Chem.* **2002**, *10*, 841.
5. Meldgaard, M.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3539.
6. Marquez, V. E.; Ezzitouni, A.; Russ, P.; Siddiqui, M. A.; Ford, Jr. H.; Feldman, R. J.; Mitsuya, H.; George, C.; Barchi, Jr. J. J. *J. Am. Chem. Soc.* **1998**, *120*, 2780.
7. Kim, H. S.; Ravi, R. G.; Marquez, V. E.; Maddileti, S.; Wihlborg, A.-K.; Erlinge, D.; Malmsjö, M.; Boyer, J. L.; Harden, T. K.; Jacobson, K. A. *J. Med. Chem.* **2002**, *45*, 208.
8. Russ, P.; Schelling, P.; Scapozza, L.; Folkers, G.; De Clercq, E.; Marquez, V. E. *J. Med. Chem.* **2003**, *46*, 5045.
9. Sørensen, M. H.; Nielsen, C.; Nielsen, P. *J. Org. Chem.* **2001**, *66*, 4878.
10. Lescop, C.; Huet, F. *Tetrahedron* **2000**, *56*, 2995.
11. Chamorro, C.; Luengo, S. M.; Bonache, M.-C.; Velázquez, S.; Pérez-Pérez, M.-J.; Camarasa, M.-J.; Gago, F.; Jimeno, M.-L.; San-Félix, A. *J. Org. Chem.* **2003**, *68*, 6695.
12. Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607.
13. Obika, S.; Nanbu, D.; Hari, Y.; Andoh, J.; Morio, K.; Doi, T.; Imanishi, T. *Tetrahedron Lett.* **1998**, *39*, 5401.
14. Petersen, M.; Wengel, J. *Trends Biotech.* **2003**, *21*, 74.
15. Obika, S.; Andoh, J.; Sugimoto, T.; Miyashita, K.; Imanishi, T. *Tetrahedron Lett.* **1999**, *40*, 6465.
16. Olsen, A. G.; Rajwanshi, V. K.; Nielsen, C.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3610.
17. Kim, H. S.; Jacobson, K. A. *Org. Lett.* **2003**, *5*, 1665.
18. Nielsen, P.; Pfundheller, H. M.; Olsen, C. E.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3423.
19. Christensen, N. K.; Petersen, M.; Nielsen, P.; Jacobsen, J. P.; Olsen, C. E.; Wengel, J. *J. Am. Chem. Soc.* **1998**, *120*, 5458.
20. Christensen, N. K.; Andersen, A. K. L.; Schultz, T. R.; Nielsen, P. *Org. Biomol. Chem.* **2003**, *1*, 3738.
21. Altmann, K.-H.; Imwinkelried, R.; Kesselring, R.; Rihs, G. *Tetrahedron Lett.* **1994**, *35*, 7625.
22. Kværnø, L.; Wightman, R. H.; Wengel, J. *J. Org. Chem.* **2001**, *66*, 5106.
23. Tarköy, M.; Bolli, M.; Schweizer, B.; Leumann, C. *Helv. Chim. Acta* **1993**, *76*, 481.
24. Bolli, M.; Litten, J. C.; Schütz, R.; Leumann, C. *J. Chem. Biol.* **1996**, *3*, 197.
25. Renneberg, D.; Leumann, C. J. *J. Am. Chem. Soc.* **2002**, *124*, 5993.
26. Ravn, J.; Nielsen, P. *J. Chem. Soc., Perkin Trans. 1* **2001**, 985.
27. Ravn, J.; Thorup, N.; Nielsen, P. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1855.
28. Ravn, J.; Freitag, M.; Nielsen, P. *Org. Biomol. Chem.* **2003**, *1*, 811.
29. Obika, S.; Sekiguchi, M.; Osaki, T.; Shibata, N.; Masaki, M.; Hari, Y.; Imanishi, T. *Tetrahedron Lett.* **2002**, *43*, 4365.
30. Thomasen, H.; Meldgaard, M.; Freitag, M.; Petersen, M.; Wengel, J.; Nielsen, P. *Chem. Commun.* **2002**, 1888.
31. A less restricted bicyclic nucleoside with a 3′,4′-*cis* linkage has been recently obtained by an RCM-strategy Montembault, M.; Bourgourgnon, N.; Lebreton, J. *Tetrahedron Lett.* **2002**, *43*, 8091.
32. Patra, R.; Bar, N. C.; Roy, A.; Achari, B.; Ghosal, N.; Mandal, S. B. *Tetrahedron* **1996**, *52*, 11265.
33. Bar, N. C.; Roy, A.; Achari, B.; Mandal, S. B. *J. Org. Chem.* **1997**, *62*, 8948.
34. Waga, T.; Nishizaki, T.; Miyakawa, I.; Ohrui, H.; Meguro, H. *Biosci. Biotech. Biochem.* **1993**, *57*, 1433.
35. Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953.
36. Nielsen, P.; Wengel, J. *Chem. Commun.* **1998**, 2645.
37. Obika, S.; Morio, K.; Hari, Y.; Imanishi, T. *Chem. Commun.* **1999**, 2423.
38. Sørensen, A. M.; Nielsen, K. E.; Vogg, B.; Jacobsen, J. P.; Nielsen, P. *Tetrahedron* **2001**, *57*, 10191.
39. Chen, X.; Wiemer, D. F. *J. Org. Chem.* **2003**, *68*, 6597.
40. Vorbrüggen, H.; Krolikewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234.
41. Wilson, L. J.; Hager, M. W.; El-Kattan, Y. A.; Liotta, D. C. *Synthesis* **1995**, 1465.
42. Koole, L. H.; Buck, H. M.; Vial, J.-M.; Chattopadhyaya, J. *Acta Chem. Scand.* **1989**, *43*, 665.
43. Donders, L. A.; de Leeuw, F. A. A. M.; Altona, C. *Magn. Res. Chem.* **1989**, *27*, 556.
44. Altona, C.; Ippel, J. H.; Westra Hoekzema, A. J. A.; Erkelens,

C.; Groesbeek, M.; Donders, L. A. *Magn. Res. Chem.* **1989**, *27*, 564.
45. Altona, C.; Francke, R.; de Haan, R.; Ippel, J. H.; Daalmans, G. J.; Westra Hoekzema, A. J. A.; van Wijk, J. *Magn. Res. Chem.* **1994**, *32*, 670.
46. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T. A.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Comperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. Gaussian94 (Rev D.3), Gaussian Inc: Pittsburg, PA, 1995.