DOI: 10.1002/ejoc.200800716

Synthesis of the 3′-*C*-Hydroxymethyl-Branched Locked Nucleic Acid Thymidine Monomer

Surender Kumar,[a,b] Pawan K. Sharma,[a,b] Paul C. Stein,[a] and Poul Nielsen*[a]

Keywords: Locked nucleic acid / Nucleosides / Oligonucleotides / Ruthenium oxidation

A 3′-*C*-hydroxymethyl-branched Locked Nucleic Acid (LNA) monomer **3** was synthesized from diacetone-α-D-glucose taking advantage of a stereoselective Grignard reaction for the introduction of a vinyl group, an aldol/Cannizzaro sequence for introducing the 4′-substituent, oxidative cleavage of the vinyl group using a RuO_4-based protocol, Vorbrüggen-type nucleobase coupling and finally the ring closure by ether formation giving the bicyclic skeleton.

Introduction

Conformationally restricted nucleosides have been very successful for the construction of oligonucleotides that are preorganised for high-affinity nucleic acid recognition.[1] Especially nucleoside analogues with bicyclic carbohydrate moieties have been designed and applied as the monomers in conformationally restricted oligonucleotide sequences, which have displayed very promising results as compounds with improved recognition of complementary RNA and DNA sequences.[1] The prime example is Locked Nucleic Acid (LNA, Figure 1), in which the bicyclic nucleoside monomer **1** is a perfect mimic of the *N*-type nucleoside conformation.[2,3] This leads to the formation of thermally extremely stable A-type nucleic acid duplexes between LNA and complementary DNA and RNA sequences. The incorporation of just one or a few LNA monomers into an oligodeoxynucleotide (ODN) strongly increases the thermal stability of the duplexes due to conformational steering of neighbouring 2′-deoxynucleotides towards *N*-type conformations.[3,4] LNA has demonstrated very promising results as potential antisense therapeutics.[3,5]

Another approach in nucleic acid chemistry and nanotechnology is the introduction of branched nucleoside monomers as conjugation sites in order to introduce various decorations onto the nucleic acid duplex or to obtain branched nucleic acid nanostructures. An example of a building block is the 3′-*C*-hydroxymethyl-branched nucleoside monomer **2** (Figure 1), which have been incorporated into ODNs and found to improve the enzymatic stability of the ODN and to be more or less neutral concerning the binding affinity for complementary nucleic acid sequences.[6,7] Also other 3′-*C*-modifications have been prepared, and in general neutral or slightly decreased binding affinities were found.[7,8] A 3′-*C*-alkyl substituent is expected to be oriented in a pseudoequatorial position, driving the nucleoside monomer towards an *S*-type conformation.[6–8] The 3′-*C*-substituents point into the major groove of nucleic acid duplexes and are reasonably well tolerated in the duplex structure.[6–8]

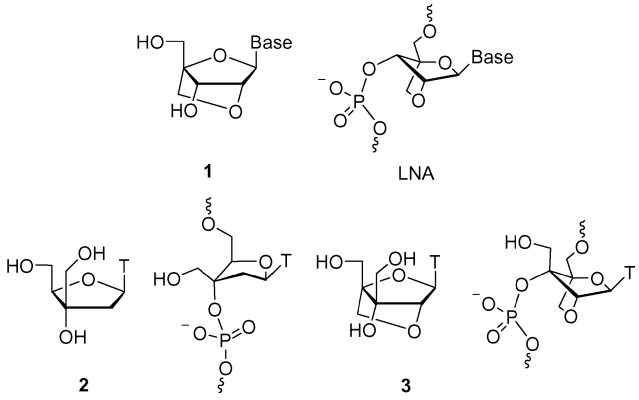


Figure 1. The general LNA nucleoside monomer **1** and the *N*-type conformation of LNA. 3′-*C*-(Hydroxymethyl)thymidine **2** and the 3′-*C*-hydroxymethyl LNA thymidine monomer **3** as well as their incorporation in nucleic acids as *S*-type and *N*-type mimics, respectively. T = thymin-1-yl.

Due to the conformationally locked nature of the LNA monomer in an *N*-type conformation, a 3′-*C*-substituent of LNA is forced into an axial position and into a very different orientation as compared to a 3′-*C*-substituted 2′-deoxynucleotide (Figure 1). Therefore, it was appealing to study this structural feature in the form of a 3′-*C*-hydroxymethyl LNA monomer. Longer alkyl groups in the same position have been recently studied in ODNs leading

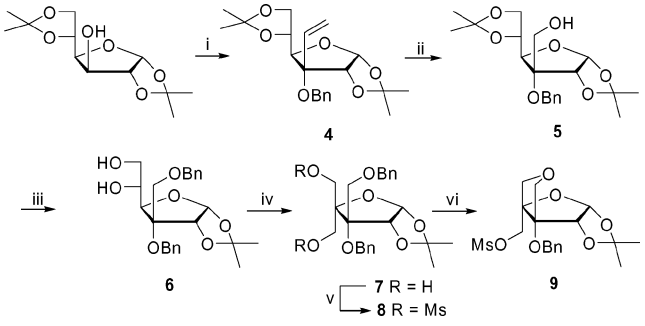
[a] Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark,
5230 Odense M, Denmark
Fax: +45-66158780
E-mail: pon@ifk.sdu.dk
[b] Department of Chemistry, Kurukshetra University, Kurukshetra 136119, India

to some decrease in affinity for complementary sequences as compared to LNA.[9] Foreseeing that the smaller and less hydrophobic 3′-hydroxymethyl group might be better accommodated into duplexes, we decided to attempt the synthesis of the 3′-*C*-hydroxymethyl LNA nucleoside **3**.[10] Besides the potential as a direct building block for ODN synthesis in order to obtain simple 3′-functionalized LNA, alternative incorporations into nucleic acid nanostructures, for instance branched LNA, as well as biological properties such as antiviral or anticancer therapeutic potential of the monomer can be envisioned. Recently, we have shown the synthesis of a complementary 6′-*C*-hydroxymethyl-branched LNA thymidine monomer using a mercury cyclisation.[11]

Results and Discussion

A convergent synthetic strategy was chosen for the synthesis of the target hydroxymethyl LNA nucleoside **3** in order to obtain the desired configuration at C-3′. A retrosynthetic analysis revealed the introductions of the 3′-*C*-substituent and the 4′-*C*-substituent, as well as the ring closure to form the bicyclic system, to be the key steps. It is well known, that insertion of the nucleobase can not be performed on a preformed bicyclo[2.2.1] skeleton,[12] wherefore a ring-closing reaction from the 2′-hydroxy group to an activated 4′-hydroxymethyl group was planned to be the last of the key steps. Diacetone-α-D-glucose was chosen as a cheap and commercially available starting material, on which the 3′-*C*-substituent can be easily introduced.[13] A vinyl group was envisioned as a precursor for the hydroxymethyl group based on our recently developed ruthenium-mediated protocol.[14] Either before or after the oxidative cleavage of the vinyl group, the 4′-*C*-hydroxymethyl group can be introduced by a known aldol condensation/Cannizzarro sequence.[15]

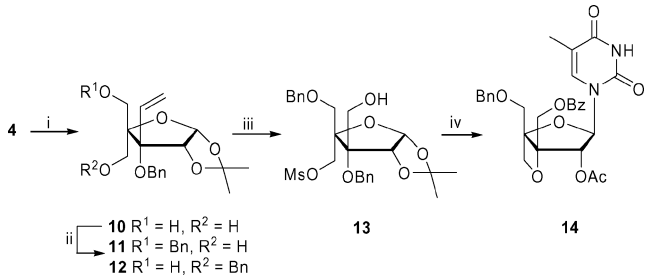
Following this analysis, diacetone-α-D-glucose was converted into the known 3-*C*-vinyl derivative **4** in three standard steps (oxidation, stereoselective Grignard and benzylation) in 68% yield (Scheme 1).[13] As the first strategy, we decided to perform the cleavage reaction before inserting the 4′-*C*-hydroxymethyl group. Oxidative cleavage of the double bond of **4** was achieved by the ruthenium-mediated protocol based on RuO_4 formed in situ, oxidative cleavage and a final reduction[14] to give the hydroxymethyl derivative **5** in 74% yield. The dibenzylfuranose **6**, was obtained in another 74% yield after benzylation using a standard procedure and selective cleavage of the 5,6-*O*-isopropylidene ring with 80% aqueous acetic acid. Oxidative cleavage of the diol **6** using $NaIO_4$ and subsequent aldol condensation between the resulting aldehyde and formaldehyde followed by an in situ Cannizzarro reaction afforded the diol **7** in 93% yield. The mesylation of the two primary hydroxy groups was achieved using excess of methanesulfonyl chloride to give derivative **8** in quantitative yield. We decided to investigate a nucleophilic displacement of the mesyl group by NaOBz – a methodology that has been applied in an optimised synthesis of LNA monomers.[16] We attempted this as a selective reaction using one equivalent of NaOBz, expecting the formation of a monobenzoyl furanoside. However, the bicyclic furanoside **9** was obtained under the reaction conditions used. The constitution of **9** was proved by NMR-studies. The application of HMBC-spectra proved the presence of the 3-*O*-benzyl group, the removal of the 3-benzyloxymethylene group and the formation of a five-membered ring. The formation of this bicyclic product might be based on the preorganisation for forming a five-membered ring from the benzyl ether oxygen to the mesyl-activated carbon and the destabilisation of the benzyl ether in the presence of the benzoate ion. Therefore, we decided to follow another strategy, whereby the vinyl group would be converted into a hydroxymethyl group at a later stage of the reaction sequence after the 4′-alkylation, and where the 5-*O*-mesyl group is avoided.



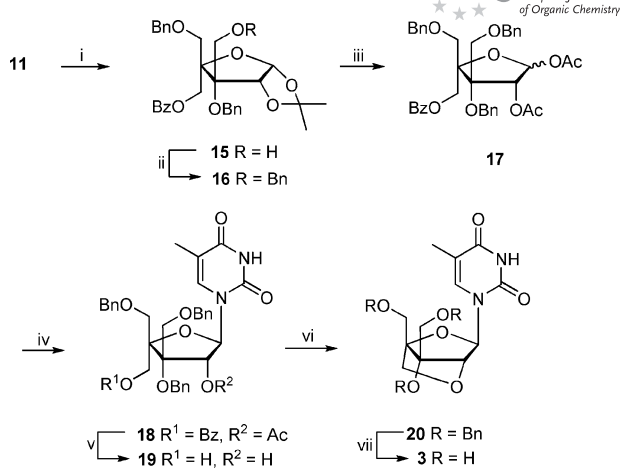
Scheme 1. Reagents and conditions: i. ref.[13], 68%; ii. a) $RuCl_3 \cdot xH_2O$, $NaIO_4$, H_2O, EtOAc, CH_3CN; b) $NaBH_4$, H_2O, THF; c) $NaIO_4$; d) $NaBH_4$, 74%; iii. a) BnBr, NaH, DMF; b) 80% aq. CH_3COOH, 74%; iv. a) $NaIO_4$, H_2O, THF; b) HCHO, NaOH, 93%; v. MsCl, pyridine, quantitative; vi. NaOBz, DMF, 82%.

Starting from compound **4** conversion to the diol **10** was performed in two standard steps in 94% yield (Scheme 2). Selective benzylation of diol **10** was achieved using sodium hydride and 1.2 equiv. of benzyl bromide to give 5-*O*-benzyl derivative **11** in 60% yield along with the 4-epimer **12** in 28% yield. Mesylation of the free hydroxy group of 3,5-di-*O*-benzylfuranose **11** gave the corresponding mesyl derivative, which was converted into a 3-hydroxymethyl derivative **13** by the same ruthenium-mediated protocol for the oxidative cleavage as above in 54% yield from **11**. Benzoylation of the primary hydroxy group of **13**, followed by acetolysis and acetylation, afforded the anomeric mixture of di-*O*-acetates, which was subsequently used as a glycosyl donor in a modified Vorbrüggen[17] reaction. However, the major product obtained in 20% yield after the chromatographic separation of a crude mixture was characterized as the bicyclic nucleoside **14**. It seems that the preorganisation of a 3-*C*-benzyloxy group in proximity to the 4-*C*-mesyloxymethyl group by Lewis acid activation lead to the formation of an oxetane ring. Approx. 10% of a crude compound indicated by MS to be a nucleoside with two thymines was also ob-

tained. This could be due to the opening of the oxetane in **14** by a second thymine – a reaction observed before with a similar bicyclic system.[18] It was hereby clear that the activation of alcohols with mesyl groups should be avoided until needed for the final ring-closing step.



Scheme 2. Reagents and conditions: i. a) H_5IO_6, EtOAc; b) HCHO, NaOH, $NaBH_4$, 94%; ii. NaH, BnBr, DMF, 60% **11** and 28% **12**; iii. a) MsCl, Pyridine; b) $RuCl_3{\cdot}xH_2O$, $NaIO_4$, H_2O, EtOAc, CH_3CN; c) $NaBH_4$, H_2O, THF; d) $NaIO_4$; e) $NaBH_4$; 54%; iv. a) BzCl, pyridine; b) 80% aq. CH_3COOH; c) Ac_2O, pyridine; d) thymine, BSA, TMS-triflate, CH_3CN, 20%.



Scheme 3. Reagents and conditions: i. a) BzCl, pyridine; b) $RuCl_3{\cdot}xH_2O$, $NaIO_4$, H_2O, EtOAc, CH_3CN; c) $NaBH_4$, H_2O, THF; d) $NaIO_4$; e) $NaBH_4$; 71%; ii. BnBr, NaH, DMF, 75%; iii. a) 80% aq. CH_3COOH; b) Ac_2O, pyridine, 91%; iv. Thymine, BSA, TMS-triflate, CH_3CN, 93%; v. $NaOCH_3$, MeOH, 86%; vi. a) MsCl, CH_2Cl_2, pyridine; b) NaH, 1,4-dioxane, 72%; vii. H_2, $Pd(OH)_2$/C, EtOH, quantitative.

In the third strategy, the free hydroxy group of **11** was protected as a benzoyl ester followed by ruthenium-mediated oxidative cleavage[14] of the vinyl group. This resulted in the formation of 3-*C*-(hydroxymethyl)furanose **15** in 71% yield from **11** (Scheme 3). In order to be able to deprotect the 4′-*C*-hydroxymethyl group selectively, we decided to introduce another convenient benzyl group for the 3′-*C*-hydroxymethyl group, even though differentiation between the two primary positions of the final product was lost. Benzylation of the primary hydroxy group afforded **16** in 75% yield, which was treated with 80% aqueous acetic acid to remove the 1,2-*O*-isopropylidene protective group followed by acetylation of the resulting two hydroxy groups to give the anomeric mixture of diacetates **17** in 91% yield. Coupling between **17** and thymine using the modified Vorbrüggen conditions[17] provided nucleoside **18** in 93% yield. Cleavage of the acetyl and benzoyl esters in a single transesterification step using sodium methoxide in methanol resulted in the formation of nucleoside **19** in 86% yield. The selective mesylation of **19** followed by the ring formation using sodium hydride in anhydrous 1,4-dioxane resulted in the formation of bicyclic nucleoside **20** in 72% yield. Debenzylation using 20% palladium hydroxide over carbon under hydrogen atmosphere proceeded efficiently to yield the target branched LNA nucleoside monomer **3** in quantitative yield. The constitution of **3** was proved by ^{1}H NMR spectroscopy showing e.g. $^3J_{H1'-H2'} = 0$ Hz and thereby the locked *N*-type conformation of an LNA-monomer.

We are currently investigating different ways for the differentiation and protection of the two primary alcohols and for the phosphitylation of the tertiary alcohol in order to make a phosphoramidite building block of the 3′-branched LNA-monomer **3** for oligonucleotide synthesis.

Conclusions

We have successfully synthesized a 3′-*C*-hydroxymethyl-branched LNA nucleoside analogue **3** in an overall yield of 11% after 19 steps, using a convergent synthesis starting from diacetone-α-D-glucose as a cheap starting material. This nucleoside has a broad potential as a monomer for the functionalisation of LNA.

Experimental Section

General: All reagents were obtained from commercial suppliers and used without further purification except dichloromethane which was distilled. Reactions were performed under an atmosphere of nitrogen when anhydrous solvents were used. All reactions were followed on TLC plates made of aluminium sheets with silica gel 60 F254 from Merck. The plates were visualized under UV-light and then developed in a 5% solution of H_2SO_4 in MeOH. Column chromatography was carried out on glass columns using silica gel 60 (0.040–0.063 mm) which was purchased from Merck. NMR spectra were recorded at 200, 300 or 500 MHz for ^{1}H NMR and 75 or 125 MHz for ^{13}C NMR spectroscopy. Chemical shifts are in ppm relative to tetramethylsilane as internal standard (for ^{1}H and ^{13}C NMR). Assignments of the NMR spectroscopic data were based on 2D spectra (COSY, HETCOR, HSQC and/or HMBC) and follow standard carbohydrate and nucleoside nomenclature and numbering, i.e. the carbon atom next to a nucleobase is assigned C-1′, etc. In nucleosides, C-1′′ designates the carbon atom in the 3′-branch and C-5′′ the carbon atom in the 4′-branch; otherwise, C-1′ designates the carbon atom in the 3-branch and C-5′ the carbon atom in the 4-branch. Compound names for the bi- and tricyclic compounds are given according to the von Baeyer nomenclature. High-resolution MALDI mass determinations were performed on an Ionspec Ultima Fourier transform mass spectrometer.

3-*O*-Benzyl-1,2:5,6-di-*O*-isopropylidene-3-*C*-(hydroxymethyl)-α-D-allofuranose (5): A solution of sodium periodate (321 mg, 1.50 mmol) in water (2 mL) was stirred at 0 °C and $RuCl_3 \cdot xH_2O$ (18 mg, 0.07 mmol) was added. In another flask, benzylated compound **4** (376 mg, 1.0 mmol) was dissolved in a mixture of ethyl acetate (5 mL) and acetonitrile (5 mL) and the mixture was stirred for 5 min at 0 °C. The aqueous solution of $RuCl_3 \cdot xH_2O$ and sodium periodate was added and the slurry was stirred for 4 min. The reaction was quenched by the addition of a saturated aqueous solution of $Na_2S_2O_3$ (5 mL). Phases were separated and the aqueous layer was extracted with ethyl acetate (3 × 4 mL). The combined organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was dissolved in a mixture of THF (3 mL) and water (3 mL). Sodium borohydride (76 mg, 2.0 mmol) was added, and a flocculent black powder began to separate after 5 min. The mixture was stirred for 20 min at room temperature, water (3 mL) was added, and the mixture was extracted with dichloromethane (3 × 4 mL). The combined organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 3 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was dissolved in THF (3 mL) and water (3 mL) at 0–5 °C. Sodium periodate (430 mg, 2.0 mmol) was added in small portions and the solution was stirred for 2 h at room temperature. The reaction mixture was diluted with water (3 mL). The mixture was extracted with ethyl acetate (3 × 4 mL), and the combined organic phase was dried (Na_2SO_4), and concentrated under reduced pressure. The residue was redissolved in a mixture of THF (3 mL) and water (3 mL), and sodium borohydride (76 mg, 1.0 mmol) was added. The reaction mixture was stirred at room temperature for 1 h, water (4 mL) was added, and the mixture was extracted with dichloromethane (3 × 4 mL). The combined organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 2 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–1.5% methanol in dichloromethane) to give **5** (281 mg, 74%) as a colourless oil: R_f = 0.50 (19:1, CH_2Cl_2/CH_3OH). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.41–7.25 (m, 5 H, Ph), 5.76 (d, *J* = 3.9 Hz, 1 H, 1-H), 4.74 (s, 2 H, CH_2Ph), 4.49 (d, *J* = 3.9 Hz, 1 H, 2-H), 4.37 (m, 1 H, 5-H), 4.18 (d, *J* = 6.9 Hz, 1 H, 4-H), 4.17–4.02 (m, 2 H, 6-H), 3.90 (dd, *J* = 12.0, 6.0 Hz, 1 H, 1′-H), 3.80 (dd, *J* = 12.0, 6.3 Hz, 1 H, 1′-H), 3.07 (dd, *J* = 6.0, 6.3 Hz, 1 H, OH), 1.59 (s, 3 H, CH_3), 1.43 (s, 3 H, CH_3), 1.36 (s, 3 H, CH_3), 1.34 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.7, 128.3, 127.6, 127.6 (Ph), 112.9 ($C(CH_3)_2$), 110.0 [$C(CH_3)_2$], 104.1 (C-1), 84.7 (C-3), 82.0 (C-2), 80.2 (C-6), 73.8 (C-4), 67.2 (3′-OCH_2Ph), 66.8 (C-5), 61.4 (C-1′), 27.0 (CH_3), 26.8 (CH_3), 26.4 (CH_3), 24.9 (CH_3) ppm. HR-MALDI MS: *m*/*z* 403.1720 ([M + Na]$^+$, $C_{20}H_{28}O_7Na^+$ calcd. 403.1727).

3-*O*-Benzyl-3-*C*-(benzyloxymethyl)-1,2-di-*O*-isopropylidene-α-D-allofuranose (6): To a solution of **5** (10.49 g, 27.60 mmol) in anhydrous DMF (75 mL) at 0 °C was added a 60% oil dispersion of NaH (1.66 g, 41.40 mmol) and the mixture was stirred for 2 h. Benzyl bromide (6.60 mL, 55.21 mmol) was added dropwise, and the reaction mixture was stirred overnight at room temperature. The reaction was quenched by slow addition of water (150 mL) at 0 °C and the resulting mixture was extracted with ethyl acetate (3 × 75 mL). The combined organic phase was washed successively with a saturated aqueous solution of $NaHCO_3$ (100 mL), brine (100 mL) and water (100 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure, and the residue was coevaporated with toluene (3 × 25 mL). The residue was dissolved in 80% aqueous acetic acid (70 mL) and the reaction mixture was stirred for 45 h at room temperature. The mixture was concentrated under reduced pressure and dissolved in ethyl acetate (100 mL). The organic phase was washed with water (2 × 50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was coevaporated with toluene and purified by silica gel column chromatography (0–45% ethyl acetate in petroleum ether) to give **6** (8.73 g, 74%) as a white solid: R_f = 0.25 (1:1, ethyl acetate/petroleum ether). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.40–7.25 (m, 10 H, Ph), 5.68 (d, *J* = 3.9 Hz, 1 H, 1-H), 4.73–4.67 (m, 2 H, 3-OCH_2Ph), 4.61, 4.50 (AB, *J* = 12.0 Hz, 2 H, 1′-OCH_2Ph), 4.45 (d, *J* = 3.9 Hz, 1 H, 2-H), 4.05 (d, *J* = 8.7 Hz, 1 H, 4-H), 3.88 (m, 1 H, 5-H), 3.77–3.62 (m, 4 H, 1′-H, 6-H), 3.31 (d, *J* = 2.7 Hz, 1 H, 5′-OH), 2.33 (m, 1 H, 6′-OH), 1.59 (s, 3 H, CH_3), 1.34 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.3, 136.8, 128.7, 128.4, 128.3, 128.1, 127.7, 127.6 (Ph), 113.1 [$C(CH_3)_2$], 104.0 (C-1), 84.9 (C-3), 79.9 (C-2), 79.4 (C-4), 74.1 (1′-OCH_2Ph), 70.3, 68.1, 67.9, 64.7 (3-OCH_2Ph, C-5, C-1′, C-6), 26.8 (CH_3) ppm. HR-MALDI MS: *m*/*z* 453.1870 ([M + Na]$^+$, $C_{24}H_{30}O_7Na^+$ calcd. 453.1884).

3-*O*-Benzyl-3-*C*-(benzyloxymethyl)-4-*C*-(hydroxymethyl)-1,2-di-*O*-isopropylidene-α-D-ribofuranose (7): To a stirred solution of **6** (8.69 g, 20.20 mmol) in a mixture of THF and water (66 mL, 1:1 v/v) at 0 °C was added sodium periodate (4.76 g, 22.22 mmol) in small portions over a period of 30 min, and the mixture was stirred for 3 h. The precipitate was filtered off and washed with diethyl ether (3 × 20 mL). The phases were separated and the aqueous phase was extracted with diethyl ether (3 × 20 mL). The combined organic phase was washed with brine (2 × 10 mL), concentrated under reduced pressure and redissolved in 1,4-dioxane (20 mL). Formaldehyde (37% solution, 5 mL) and NaOH (2 M, 21 mL) were added and the reaction mixture was stirred overnight. Dichloromethane (50 mL) was added and the phases were separated. The aqueous phase was extracted with dichloromethane (3 × 50 mL). The combined organic phase was washed with brine (2 × 50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Crystallization from CH_2Cl_2/petroleum ether afforded **7** (8.04 g, 93%) as white solid: R_f = 0.25 (1:1, ethyl acetate/petroleum ether). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.39–7.24 (m, 10 H, Ph), 5.75 (d, *J* = 4.5 Hz, 1 H, 1-H), 4.74, 4.63 (AB, *J* = 10.8 Hz, 2 H, 3-OCH_2Ph), 4.57–4.52 (m, 3 H, 2-H, 1′-OCH_2Ph), 4.14–4.02 (m, 2 H, 5′-H), 3.82–3.72 (m, 4 H, 5-H, 1′-H), 2.86 (m, 1 H, 5-OH), 2.33 (m, 1 H, 5′-OH), 1.63 (s, 3 H, CH_3), 1.31 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.4, 136.8, 128.7, 128.4, 128.3, 128.1, 127.7, 127.4 (Ph), 113.0 [$C(CH_3)_2$], 104.4 (C-1), 87.4, 86.1 (C-3, C-4), 81.2 (C-2), 74.0 (1′-OCH_2Ph), 69.3, 68.0, 62.9, 62.6 (3-OCH_2Ph, C-1′, C-5, C-5′), 26.3 (CH_3), 25.9 (CH_3) ppm. HR-MALDI MS: *m*/*z* 453.1869 ([M + Na]$^+$, $C_{24}H_{30}O_7Na^+$ calcd. 453.1884).

3-*O*-Benzyl-3-*C*-(benzyloxymethyl)-1,2-di-*O*-isopropylidene-5-*O*-(methylsulfonyl)-4-*C*-(methylsulfonyloxymethyl)-α-D-ribopentofuranose (8): To a solution of **7** (8.0 g, 18.60 mmol) in anhydrous dichloromethane (10 mL) and anhydrous pyridine (7.5 mL, 93.01 mmol) at 0 °C, methanesulfonyl chloride (3.20 mL, 40.93 mmol) was added dropwise, and the reaction mixture was stirred at room temperature for 1.5 h. A saturated aqueous solution of $NaHCO_3$ (20 mL) was added and the mixture was stirred for 15 min. The reaction mixture was diluted with dichloromethane (20 mL) and the phases were separated. The organic phase was washed with 1 M HCl (3 × 10 mL) and a saturated aqueous solution of $NaHCO_3$ (5 mL), dried (Na_2SO_4) and evaporated under reduced pressure to give **8** (10.90 g, quantitative) as a white foam: R_f = 0.28 (1:1, ethyl acetate/petroleum ether). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.40–7.25 (m, 10 H, Ph), 5.79 (d, *J* = 3.9 Hz, 1 H, 1-H), 4.83, 4.62 (AB, *J* = 11.7 Hz, 2 H), 4.74, 4.60 (AB, *J* = 11.1 Hz, 2 H, 3-OCH_2Ph, 5′-H), 4.70, (d, *J* = 3.9 Hz, 1 H, 2-H), 4.58, 4.51 (AB, *J*

= 11.4 Hz, 2 H, 1′-OCH_2Ph), 4.39, 4.34 (AB, *J* = 9.9 Hz, 2 H, 5-H), 3.81, 3.79 (AB, *J* = 9.9 Hz, 2 H, 1′-H), 2.99 (s, 3 H, SO_2CH_3), 2.98 (s, 3 H, SO_2CH_3), 1.70 (s, 3 H, CH_3), 1.30 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.0, 136.8, 128.7, 128.5, 128.3, 128.0, 127.8, 127.4 (Ph), 113.5 [$C(CH_3)_2$], 105.2 (C-1), 85.6, 85.1 (C-3, C-4), 82.1 (C-2), 74.0 (1′-OCH_2Ph), 69.2, 68.8, 68.5, 65.9 (3-OCH_2Ph, C-1′, C-2, C-5′, C-5), 38.0 (SO_2CH_3), 37.4 (SO_2CH_3), 26.3 (CH_3), 25.9 (CH_3) ppm. HR-MALDI MS: *m*/*z* 609.1441 ([M + Na]$^+$, $C_{26}H_{34}O_{11}S_2Na^+$ calcd. 609.1435).

(*1S*,*2S*,*6R*,*8R*)-1-(Benzyloxy)-8-(methylsulfonyloxymethyl)-4,4-dimethyl-3,5,7,10-tetraoxatricyclo[6.3.0.0^{2,6}]undecane (9): Sodium benzoate (12.3 mg, 0.09 mmol) was added to a solution of compound **8** (50 mg, 0.09 mmol) in DMF (2 mL). The mixture was stirred for 5 h at 100 °C, cooled to room temperature, and filtered. The solvent was evaporated under reduced pressure, and the residue was suspended in ethyl acetate (5 mL), washed with water (3 × 2 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–25% ethyl acetate in petroleum ether) to give **9** (28 mg, 82%) as a white solid: R_f = 0.15 (1:1, ethyl acetate/petroleum ether). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.45–7.30 (m, 5 H, Ph), 5.96 (d, *J* = 3.6 Hz, 1 H, 1-H), 4.82 (d, *J* = 11.8 Hz, 1 H, 5′-H), 4.73–4.69 (m, 2 H, CH_2Ph, 2-H), 4.49 (d, *J* = 11.8 Hz, 1 H, 5′-H), 4.44 (d, *J* = 10.5 Hz, 1 H, CH_2Ph), 4.15 (d, *J* = 10.5 Hz, 1 H, 1′-H), 4.06 (d, *J* = 10.5 Hz, 1 H, 5-H), 3.97 (d, *J* = 10.5 Hz, 1 H, 1′-H), 3.88 (d, *J* = 10.5 Hz, 1 H, 5-H), 3.06 (s, 3 H, SO_2CH_3), 1.68 (s, 3 H, CH_3), 1.38 (s, 3 H, CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 137.4, 128.7, 128.3, 127.8 (Ph), 114.2 [$C(CH_3)_2$], 105.8 (C-1), 93.1 (C-4), 92.1 (C-3), 81.4 (C-2), 76.4 (C-5′), 75.6 (C-1′), 71.7 (C-5), 68.7 (CH_2Ph), 38.0 (SO_2CH_3), 26.8 (CH_3), 26.2 (CH_3) ppm. HR-MALDI MS: *m*/*z* 423.1078 ([M + Na]$^+$, $C_{18}H_{24}O_8SNa^+$ calcd. 423.1084).

3-*O*-Benzyl-4-*C*-(hydroxymethyl)-1,2-di-*O*-isopropylidene-3-*C*-vinyl-α-D-ribofuranose (10): The furanose **4** (32.00 g, 85.1 mmol) was dissolved in anhydrous ethyl acetate (400 mL), and H_5IO_6 (23.29 g, 102.1 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 concentrated under reduced pressure, and the residue was dissolved in THF (250 mL). An aqueous solution of formaldehyde [24 mL, 37% (w/v) containing 10% CH_3OH] and an aqueous solution of NaOH (2 M, 96.6 mL, 193.2 mmol) were added dropwise, and the reaction mixture was stirred at room temperature for 24 h. The mixture was cooled to 0 °C, $NaBH_4$ (4.85 g, 127.7 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 (3 × 100 mL). The combined organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–45% ethyl acetate in petroleum ether) to give **10** (26.73 g, 94%) as a colourless viscous oil: R_f = 0.20 (1:1, ethyl acetate/petroleum ether). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.36–7.26 (m, 5 H, Ph), 5.93 (dd, *J* = 11.4, 18.0 Hz, 1 H, 1′-H), 5.88 (d, *J* = 3.9 Hz, 1 H, 1-H), 5.50 (d, *J* = 11.4 Hz, 1 H, 2′-H), 5.32 (d, *J* = 18.0 Hz, 1 H, 2′-H), 4.73 (d, *J* = 3.9 Hz, 1 H, 2-H), 4.71, 4.57 (AB, *J* = 10.5 Hz, 2 H, CH_2Ph), 4.33 (dd, *J* = 6.3, 11.7 Hz, 1 H, 5′-H), 4.07 (dd, *J* = 7.5, 11.7 Hz, 1 H, 5′-H), 3.76–3.68 (m, 2 H, 5-H), 2.58 (m, 1 H, OH), 2.45 (m, 1 H, OH), 1.67 (s, 3 H, CH_3), 1.37 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 135.7 (C-1′), 138.0, 128.6, 127.9, 127.5 (Ph), 117.7 (C-2′), 113.2 [$C(CH_3)_2$], 104.7 (C-1), 87.8, 87.1 (C-3, C-4), 82.3 (C-2), 67.7 (CH_2Ph), 65.5, 63.8 (C-5, C-5′), 26.4 (CH_3), 25.9 (CH_3) ppm. HR-MALDI MS: *m*/*z* 359.1478 ([M + Na]$^+$, $C_{18}H_{24}O_6Na^+$ calcd. 359.1465).

3,5-Di-*O*-benzyl-4-*C*-(hydroxymethyl)-1,2-di-*O*-isopropylidene-3-*C*-vinyl-α-D-ribofuranose (11) and 3-*O*-Benzyl-4-*C*-(benzyloxymethyl)-1,2-di-*O*-isopropylidene-3-*C*-vinyl-α-D-ribofuranose (12): The diol **10** (29.61 g, 88.13 mmol) was dissolved in anhydrous DMF (400 mL) and stirred at –20 °C. A 60% oily dispersion of NaH (4.23 g, 105.8 mmol) was added and the mixture was stirred at –20 °C for 30 min. Benzyl bromide (12.56 mL, 105.8 mmol) was added dropwise, and the reaction mixture was stirred for 30 min at –20 °C. The reaction was quenched by the dropwise addition of water (5 mL) followed by stirring for 15 min. The reaction mixture was concentrated under reduced pressure and the residue was partitioned between ethyl acetate (200 mL) and water (100 mL). The aqueous phase was extracted with ethyl acetate (2 × 50 mL). The combined organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–45% ethyl acetate in petroleum ether) to give the two products **11** and **12** as clear viscous oils. **11** (22.37 g, 60%): R_f = 0.45 (1:1, petroleum ether/ethyl acetate). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.36–7.24 (m, 10 H, Ph), 5.89 (d, *J* = 3.9 Hz, 1 H, 1-H), 5.84 (dd, *J* = 11.1, 18.0 Hz, 1 H, 1′-H), 5.40 (d, *J* = 11.1 Hz, 1 H, 2′-H), 5.27 (d, *J* = 18.0 Hz, 1 H, 2′-H), 4.72, 4.56 (AB, *J* = 11.4 Hz, 2 H, CH_2Ph), 4.70 (d, *J* = 3.9 Hz, 1 H, 2-H), 4.57, 4.48 (AB, *J* = 12.0 Hz, 2 H, CH_2Ph), 4.45–4.11 (m, 2 H, 5′-H), 3.54 (s, 2 H, 5-H), 2.46 (t, *J* = 6.9 Hz, 1 H, 5′-OH), 1.67 (s, 3 H, CH_3), 1.36 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 135.9 (C-1′), 138.5, 138.4, 128.4, 128.3, 127.6, 127.5, 127.4, 127.2 (Ph), 117.5 (C-2′), 113.3 [$C(CH_3)_2$], 104.8 (C-1), 88.6, 87.2 (C-3, C-4), 82.5 (C-2), 73.6 (5-OCH_2Ph), 71.3 (C-5), 67.4 (3-OCH_2Ph), 62.1 (C-5′), 26.5 (CH_3), 26.1 (CH_3) ppm. HR-MALDI MS: *m*/*z* 449.1940 ([M + Na]$^+$, $C_{25}H_{30}O_6Na^+$ calcd. 449.1935). **12** (10.51 g, 28%): R_f = 0.42 (1:1, ethyl acetate/petroleum ether). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.36–7.24 (m, 10 H, Ph), 5.96 (dd, *J* = 11.4, 18.0 Hz, 1 H, 1′-H), 5.88 (d, *J* = 4.2 Hz, 1 H, 1-H), 5.47 (d, *J* = 11.4 Hz, 1 H, 2′-H), 5.32 (d, *J* = 18.0 Hz, 1 H, 2′-H), 4.72 (d, *J* = 4.2 Hz, 1 H, 2-H), 4.70, 4.58 (AB, *J* = 11.1 Hz, 2 H, CH_2Ph), 4.60, 4.53 (AB, *J* = 12.0 Hz, 2 H, CH_2Ph), 4.20, 4.11 (AB, *J* = 10.5 Hz, 2 H, 5′-H), 3.78–3.65 (m, 2 H, 5-H), 2.56 (dd, *J* = 6.0, 7.2 Hz, 1 H, 5-OH), 1.52 (s, 3 H, CH_3), 1.35 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 136.2 (C-1′), 138.8, 138.2, 128.4, 128.3, 128.0, 127.7, 127.4, 127.1 (Ph), 117.6 (C-2′), 113.2 [$C(CH_3)_2$], 104.7 (C-1), 88.4, 86.4 (C-3, C-4), 82.5 (C-2), 73.8 (5′-OCH_2Ph), 71.4 (C-5′), 67.3 (3-OCH_2Ph), 65.0 (C-5′), 26.4 (CH_3), 26.2 (CH_3) ppm. HR-MALDI MS: *m*/*z* 449.1915 ([M + Na]$^+$, $C_{25}H_{30}O_6Na^+$ calcd. 449.1935).

3,5-Di-*O*-benzyl-3-*C*-(hydroxymethyl)-1,2-di-*O*-isopropylidene-4-*C*-(methylsulfonyloxymethyl)-α-D-ribofuranose (13): To a solution of **11** (409 mg, 0.96 mmol) in anhydrous dichloromethane (1 mL) and anhydrous pyridine (0.40 mL, 4.80 mmol) at 0 °C, methanesulfonyl chloride (82.0 μL, 1.06 mmol) was added dropwise and the reaction mixture was stirred at room temperature for 1.5 h. A saturated aqueous solution of $NaHCO_3$ (5 mL) was added and the mixture was stirred for 15 min. The reaction mixture was diluted with dichloromethane (10 mL) and the phases were separated. The organic phase was washed with 1 M HCl (3 × 4 mL) and a saturated aqueous solution of $NaHCO_3$ (5 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give the mesylated derivative in quantitative yield as a white foam. In a separate flask, a solution of sodium periodate (64 mg, 0.30 mmol) in water (1 mL) at 0 °C was added $RuCl_3{\cdot}xH_2O$ (3.50 mg, 0.01 mmol). The mesylated compound (100 mg, 0.20 mmol) was dissolved in a mixture of ethyl acetate (3 mL) and acetonitrile (3 mL) and the mixture was stirred

for 5 min at 0 °C. To this solution, the aqueous solution of $RuCl_3 \cdot xH_2O$ and sodium periodate was added in one portion and the slurry was stirred for 5 min. The reaction was quenched by the addition of a saturated aqueous solution of $Na_2S_2O_3$ (5 mL). The phases were separated and the aqueous layer was extracted with ethyl acetate (3 × 5 mL). The combined organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was dissolved in a mixture of THF (2.5 mL) and water (2.5 mL). Sodium borohydride (15 mg, 0.40 mmol) was added, and a flocculent black powder began to separate after 5 min. The mixture was stirred for 20 min at room temperature, water (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 5 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was dissolved in THF (2.5 mL) and water (2.5 mL) at 0–5 °C. Sodium periodate (85 mg, 0.40 mmol) was added in small portions, and the solution was stirred for 2 h at room temperature. The reaction mixture was diluted with water (10 mL). The mixture was extracted with ethyl acetate (3 × 5 mL). The combined organic phase was dried (Na_2SO_4), and concentrated under reduced pressure. The residue was redissolved in a mixture of THF (2.5 mL) and water (2.5 mL), and sodium borohydride (15 mg, 0.40 mmol) was added. The reaction mixture was stirred at room temperature for 1 h, water (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 5 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–30% ethyl acetate in petroleum ether) to give **13** (54 mg, 54%) as a white solid: R_f = 0.35 (1:1, ethyl acetate/petroleum ether). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.36–7.25 (m, 10 H, Ph), 5.87 (d, J = 4.2 Hz, 1 H, 1-H), 4.97 (d, J = 11.7 Hz, 1 H), 4.75–4.53 (m, 5 H), 4.34 (d, J = 11.7 Hz, 1 H), 3.86–3.62 (m, 5 H), 3.07 (s, 3 H, SO_2CH_3), 1.54 (s, 3 H, CH_3), 1.36 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.0, 136.5, 128.9, 128.8, 128.5, 128.3, 127.9, 127.5 (Ph), 113.1 [$C(CH_3)_2$], 105.2 (C-1), 87.0, 86.5, 81.8, 74.6, 69.9, 67.8, 67.8, 62.6 (C-2, C-3, C-4, C-5, C-1′, C-5′, 2× CH_2Ph), 38.3 (SO_2CH_3), 26.0 (CH_3), 25.6 (CH_3) ppm. HR-MALDI MS: m/z 531.1641 ([M + Na]$^+$, $C_{25}H_{32}O_9SNa^+$ calcd. 531.1659).

(1*S*,3*R*,4*R*,5*S*)-4-(Acetyloxy)-5-(benzoyloxymethyl)-1-(benzyloxymethyl)-3-(thymin-1-yl)-2,6-dioxabicyclo[3.2.0]heptane (14): To a stirred solution of **13** (50 mg, 0.10 mmol) in anhydrous pyridine (2 mL) at 0 °C was added benzoyl chloride (24 μL, 0.20 mmol). The reaction mixture was stirred at room temperature for 2 h, concentrated under reduced pressure and coevaporated with toluene (3 × 5 mL). The residue was partitioned between ethyl acetate (5 mL) and water (3 mL). The organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 5 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was dissolved in 80% aqueous acetic acid (2 mL) and the solution was stirred at 90 °C for 24 h. The mixture was concentrated under reduced pressure, and the residue was coevaporated with 99% ethanol (3 × 5 mL), toluene (3 × 5 mL) and anhydrous pyridine (2 × 5 mL), and redissolved in anhydrous pyridine (2 mL). Acetic anhydride (0.5 mL) was added, and the solution was stirred at room temperature for 20 h. The reaction was quenched with ice-cold water (10 mL) and extracted with dichloromethane (2 × 10 mL). The combined extracts were washed with a saturated aqueous solution of $NaHCO_3$ (3 × 5 mL), dried (Na_2SO_4) and concentrated under reduced pressure. To a stirred solution of the residue and thymine (15 mg, 0.12 mmol) in anhydrous acetonitrile (1 mL) was added *N*,*O*-bis(trimethylsilyl)acetamide (71.0 μL, 0.29 mmol). The reaction mixture was stirred under reflux for 30 min. After cooling to 0 °C, trimethylsilyl triflate (21 μL, 0.12 mmol) was added dropwise, and the solution was stirred for 24 h at 60 °C. The reaction was quenched by the addition of a cold saturated aqueous solution of $NaHCO_3$ (10 mL), and the resulting mixture was extracted with dichloromethane (3 × 5 mL). The combined extracts were washed with a saturated aqueous solution of $NaHCO_3$ (2 × 5 mL) and brine (2 × 5 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–45% ethyl acetate in petroleum ether) to give nucleoside **14** (10.6 mg, 20%) as a white foam: R_f = 0.40 (3:1, ethyl acetate/petroleum ether). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.40 (br. s, 1 H, NH), 7.99–7.96 (m, 2 H, Ph), 7.65–7.24 (m, 9 H, Ph, 6-H), 6.68 (d, J = 6.9 Hz, 1 H, 1′-H), 5.17 (d, J = 6.9 Hz, 1 H, 2′-H), 4.84 (d, J = 12.3 Hz, 1 H), 4.76 (d, J = 7.5 Hz, 1 H), 4.65–4.40 (m, 4 H), 3.97 (d, J = 10.5 Hz, 1 H), 3.73 (d, J = 10.5 Hz, 1 H), 2.09 (s, 3 H, $COCH_3$), 1.75 (d, J = 0.9 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.4 ($COCH_3$), 165.8 (COPh), 163.2 (C-4), 150.5 (C-2), 136.8 (C-6), 134.8, 133.6, 129.7, 128.8, 128.7, 128.6, 128.4, 127.9 (Ph), 112.4 (C-5), 91.3, 86.3, 85.4, 76.9, 75.2, 74.0, 68.2, 63.2 (C-1′, C-2′, C-3′, C-4′, C-5′, C-1′′, C-5′′, CH_2Ph), 20.5 ($COCH_3$), 12.6 (CH_3) ppm. HR-MALDI MS: m/z 559.1676 ([M + Na]$^+$, $C_{28}H_{28}N_2O_9Na^+$ calcd. 559.1687).

4-*C*-(Benzoyloxymethyl)-3,5-di-*O*-benzyl-3-*C*-(hydroxymethyl)-1,2-di-*O*-isopropylidene-α-D-ribofuranose (15): To a stirred solution of **11** (1.12 g, 2.63 mmol) in anhydrous pyridine (10 mL) at 0 °C was added benzoyl chloride (0.46 mL, 3.94 mmol). The reaction mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure and coevaporated with toluene (3 × 5 mL). The residue was partitioned between ethyl acetate (15 mL) and water (9 mL). The organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 5 mL), dried (Na_2SO_4), and concentrated under reduced pressure to give the benzoylated compound as an oily residue. In a separate flask, a solution of sodium periodate (844 mg, 3.94 mmol) in water (5 mL) was stirred at 0 °C, and $RuCl_3 \cdot xH_2O$ (46.50 mg, 0.18 mmol) was added. The benzoylated compound was dissolved in a mixture of ethyl acetate (15 mL) and acetonitrile (15 mL), and the mixture was stirred for 5 min at 0 °C. The aqueous solution of $RuCl_3 \cdot xH_2O$ and sodium periodate was added and the slurry was stirred for 4 min. The reaction was quenched by the addition of a saturated aqueous solution of $Na_2S_2O_3$ (25 mL). The phases were separated and the aqueous phase was extracted with ethyl acetate (3 × 15 mL). The combined organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was dissolved in a mixture of THF (12.5 mL) and water (12.5 mL). Sodium borohydride (200 mg, 5.26 mmol) was added, and a flocculent black powder began to separate after 5 min. The mixture was stirred for 20 min at room temperature, water (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 15 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was dissolved in THF (12.5 mL) and water (12.5 mL) at 0–5 °C. Sodium periodate (1.13 g, 5.26 mmol) was added in small portions, and the solution was stirred for 2 h at room temperature. The reaction mixture was diluted with water (15 mL), and extracted with ethyl acetate (3 × 20 mL). The combined organic phase was dried (Na_2SO_4), and concentrated under reduced pressure. The residue was redissolved in a mixture of THF (12.5 mL) and water (12.5 mL), and sodium borohydride (200 mg, 2.63 mmol) was added. The reaction mixture was stirred at room temperature for 1 h, water (15 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phase

was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–22% ethyl acetate in petroleum ether) to give **15** (540 mg, 71%) as a clear viscous oil: R_f = 0.45 (1:1, ethyl acetate/petroleum ether). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.96–7.94 (m, 2 H, Ph), 7.54–7.50 (m, 1 H, Ph), 7.41–7.24 (m, 12 H, Ph), 5.89 (d, J = 4.2 Hz, 1 H, 1-H), 5.06 (d, J = 12.6 Hz, 1 H, 5′-H), 4.82 (d, J = 10.8 Hz, 1 H, CH_2Ph), 4.71–4.65 (m, 2 H, CH_2Ph), 4.63 (d, J = 4.2 Hz, 1 H, 2-H), 4.55 (d, J = 12.0 Hz, 1 H, CH_2Ph), 4.45 (d, J = 12.6 Hz, 1 H, 5′-H), 3.89 (d, J = 6.9 Hz, 2 H, 1′-H), 3.88–3.83 (m, 2 H, 5-H), 3.67 (t, J = 6.9 Hz, 1 H, 1′-OH), 1.66 (s, 3 H, CH_3), 1.34 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 4.2 Hz, H-1), 5.02 (d, 1 H, J = 12.0 Hz), 4.82–4.65 (m, 4 H), 4.45–4.25 (m, 4 H), 3.85–3.65 (m, 3 H), 3.58 (d, 1 H, J = 9.9 Hz), 1.62 (s, 3 H, CH_3), 1.32 (s, 3 H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.4 (CO), 139.1, 137.8, 137.4, 132.7, 130.6, 129.8, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.7, 127.2, 127.0, 126.9 (Ph), 113.5 [$C(CH_3)_2$], 104.9 (C-1), 87.3, 85.5, 82.8, 73.7, 73.5, 69.8, 68.7, 68.0, 64.8 (C-2, C-3, C-4, C-5, C-5′, C-1′, 3 × CH_2Ph), 26.7 (CH_3), 26.5 (CH_3) ppm. HR-MALDI MS: m/z 647.2596 ([M + Na]$^+$, $C_{38}H_{40}O_8Na^+$ calcd. 647.2615).

1,2-Di-*O*-acetyl-4-*C*-(benzoyloxymethyl)-3,5-di-*O*-benzyl-3-*C*-(benzyloxymethyl)-(α/β)-D-ribofuranose (17): Compound **16** (1.78 g, 2.85 mmol) was dissolved in 80% aqueous acetic acid (20 mL) and the mixture was stirred for 3 h at 90 °C. The reaction mixture was concentrated under reduced pressure and coevaporated with anhydrous ethanol (4 mL), toluene (4 mL) and pyridine (4 mL). The residue was redissolved in anhydrous pyridine (20 mL) and acetic anhydride (4.6 mL) was added dropwise. The reaction mixture was stirred overnight at room temperature and quenched by the addition of ice-water (10 mL). The mixture was extracted with dichloromethane (3 × 10 mL) and the combined organic phase was washed with a saturated aqueous solution of $NaHCO_3$, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–16% ethyl acetate in petroleum ether) to give **17** as a mixture of anomers (1.73 g, 91%; β:α ≈2.5:1) as a clear viscous oil: R_f = 0.60 (1:1, petroleum ether/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.97–7.93 (m, 2 H, Ph), 7.58–7.45 (m, 1 H, Ph), 7.40–7.23 (m, 17 H, Ph), 6.45 (d, J = 5.4 Hz, 1α-H), 6.28 (d, J = 3.0 Hz, 1β-H), 5.81 (d, J = 3.0 Hz, 2β-H), 5.60 (d, J = 5.4 Hz, 2α-H), 4.83–4.60 (m), 4.55–4.25 (m), 4.05–3.55 (m), 2.03, 2.00, 1.98, 1.97 (each s, CH_3) ppm. HR-MALDI MS: m/z 691.2481 ([M + Na]$^+$, $C_{39}H_{40}O_{10}Na^+$ calcd. 691.2514).

1-[2-*O*-Acetyl-4-*C*-(benzoyloxymethyl)-3,5-di-*O*-benzyl-3-*C*-(benzyloxymethyl)-β-D-ribofuranosyl]thymine (18): To a stirred solution of **17** (1.69 g, 2.52 mmol) and thymine (636 mg, 5.05 mmol) in anhydrous CH_3CN (20 mL) was added *N*,*O*-bis(trimethylsilyl)acetamide (3.12 mL, 12.62 mmol). The reaction mixture was stirred for 30 min. After cooling to 0 °C, trimethylsilyl triflate (1.37 mL, 7.57 mmol) was added dropwise, and the solution was stirred overnight at 70 °C. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate (20 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (3 × 10 mL). The combined organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–2% methanol in dichloromethane) to give nucleoside **18** (1.72 g, 93%) as a white foam: R_f = 0.40 (19:1, CH_2Cl_2/CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.37 (br. s, 1 H, NH), 8.00–7.98 (m, 2 H, Ph), 7.66 (s, 1 H, 6-H), 7.55–7.25 (m, 18 H, Ph), 6.52 (d, J = 8.1 Hz, 1 H, 1′-H), 5.69 (d, J = 8.1 Hz, 1 H, 2′-H), 4.90, 4.81 (AB, J = 10.5 Hz, 2 H), 4.74 (s, 2 H), 4.52, 4.32 (AB, J = 12.0 Hz, 2 H), 4.39, 4.25 (AB, J = 11.4 Hz, 2 H), 3.98–3.67 (m, 4 H), 2.07 (s, 3 H, $COCH_3$), 1.42 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.3 ($COCH_3$), 166.2 ($COPh$), 163.6 (C-4), 150.8 (C-2), 138.2 (C-6), 136.8, 136.7, 136.1, 133.1, 130.0, 129.8, 128.9, 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 127.8, 127.7, 127.6, 127.5 (Ph), 111.3 (C-5), 88.6, 84.1, 76.5, 73.8, 73.8, 73.6, 71.0, 67.8, 67.0, 66.6 (C-1′, C-2′, C-3′, C-4′, C-5′, C-1′′, C-5′′, 3 × CH_2Ph), 20.8 ($COCH_3$), 12.0 (CH_3) ppm. HR-MALDI MS: m/z 757.2760 ([M + Na]$^+$, $C_{42}H_{42}N_2O_{10}Na^+$ calcd. 757.2732).

1-[3,5-Di-*O*-benzyl-3-*C*-(benzyloxymethyl)-4-*C*-(hydroxymethyl)-β-D-ribofuranosyl]thymine (19): Sodium methoxide (134 mg, 2.48 mmol) was added to a solution of **18** (455 mg, 0.62 mmol) in anhydrous methanol (5 mL), and the reaction mixture was stirred for 2 h at room temperature. Excess of sodium methoxide was neutralized with dilute aqueous hydrochloric acid. The mixture was extracted with dichloromethane (2 × 20 mL) and the combined extract was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 15 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–2% methanol in dichloromethane) to give nucleoside **19** as a white foam (312 mg, 86%): R_f = 0.50 (9:1, CH_2Cl_2/CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.50 (br. s, 1 H, NH), 7.62 (s, 1 H, 6-H), 7.38–7.18 (m, 15 H, Ph), 6.04 (d, J = 7.8 Hz, 1 H, 1′-H), 5.06, 4.87 (AB, J = 11.1 Hz, 2 H), 4.53, 4.36 (AB, J = 10.5 Hz, 2 H), 4.30–4.25 (m, 1 H), 4.27 (d, J = 7.8 Hz, 1 H, 2′-H), 4.20–4.10 (m, 2 H), 4.00 (dd, J = 4.2, 10.8 Hz, 1 H, 5′′-H), 3.86–3.82 (m, 1 H), 3.81, 3.72 (AB, J = 10.5 Hz, 2 H), 3.58 (dd, J = 8.7, 10.8 Hz, 1 H, 5′′-H), 3.50 (br. s, 1 H, 2′-OH), 2.87 (dd, J = 4.2, 8.7 Hz, 1 H, 5′′-OH), 1.48 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.9 (C-4), 151.6 (C-2), 136.8 (C-6), 138.7, 136.3, 136.2, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 127.9, 127.6, 127.5 (Ph), 111.1 (C-5), 90.0, 87.2, 84.1, 78.7, 73.8, 73.6, 72.7, 68.1, 67.0, 63.7 (C-1′, C-2′, C-3′, C-4′, C-5′, C-1′′, C-5′′, 3 × CH_2Ph), 12.1 (CH_3) ppm. HR-MALDI MS: m/z 611.2379 ([M + Na]$^+$, $C_{33}H_{36}N_2O_8Na^+$ calcd. 611.2364).

(1*S*,3*R*,4*R*,7*S*)-1,7-Bis(benzyloxymethyl)-7-(benzyloxy)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (20): A solution of **19** (526 mg, 0.89 mmol) in anhydrous dichloromethane (3 mL) and anhydrous pyridine (0.29 mL) was cooled to –40 °C. Methanesulfonyl chloride (104 μL, 1.34 mmol) was added dropwise, and the mixture was stirred at –40 °C to 0 °C for 7 h. Water (3 mL) was added followed by a saturated aqueous solution of $NaHCO_3$ (10 mL). The resulting mixture was extracted with dichloromethane (3 × 5 mL) and the organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was dissolved in anhydrous 1,4-dioxane (4 mL) and the solution was stirred at 10 °C. A 60% oil dispersion of NaH (90 mg, 2.23 mmol) was added in one portion. The reaction mixture was stirred at 50 °C for 12 h and then quenched by the addition of a saturated aqueous solution of NH_4Cl (2 mL). The reaction mixture was extracted with CH_2Cl_2 (4 × 5 mL). The combined organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–3% methanol in dichloromethane) affording nucleoside **20** (369 mg, 72%) as a white foam: R_f = 0.30 (19:1, CH_2Cl_2/CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.22 (s, 1 H, NH), 7.50 (d, J = 0.9 Hz, 1 H, 6-H), 7.36–7.11 (m, 15 H, Ph), 5.54 (s, 1 H, 1′-H), 5.39 (s, 1 H, 2′-H), 4.77, 4.70 (AB, J = 11.1 Hz, 2 H), 4.57 (s, 2 H), 4.24, 3.99 (AB, J = 7.5 Hz, 2 H), 4.20 (s, 2 H), 3.82–3.68 (m, 4 H), 1.87 (d, J = 0.9 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.5 (C-4), 149.8 (C-2), 138.3 (C-6), 137.7, 137.0, 134.5, 128.6, 128.6, 128.4, 128.0, 128.0, 127.8, 127.7, 127.6, 127.4 (Ph), 109.4 (C-5), 90.1 (C-4′), 89.0 (C-1′), 84.8 (C-3′), 78.1 (C-2′), 74.6, 73.8, 73.8, 70.6, 69.3, 65.1 (C-5′′, C-1′′, C-5′, 3 ×

CH_2Ph), 12.6 (CH_3) ppm. HR-MALDI MS: *m*/*z* 593.2246 ([M + Na]$^+$, $C_{33}H_{34}N_2O_7Na^+$ calcd. 593.2258).

(1*S*,3*R*,4*R*,7*S*)-7-Hydroxy-1,7-bis(hydroxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (3): To a stirred solution of nucleoside **20** (502 mg, 0.88 mmol) in ethanol (70 mL) was added 20% palladium hydroxide over carbon (500 mg). The mixture was degassed several times with argon and placed under a hydrogen atmosphere. The reaction mixture was stirred at room temperature for 20 h and then filtered through celite. The filterate was evaporated under reduced pressure to give nucleoside **3** (283 mg, quantitative yield) as a white foam: R_f = 0.20 (9:1, CH_2Cl_2/MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.77 (d, J = 0.9 Hz, 1 H, 6-H), 5.41 (s, 1 H, 1′-H), 4.69 (s, 1 H, 2′-H), 4.13 (d, J = 8.1 Hz, 1 H), 3.90–3.88 (m, 3 H), 3.75, 3.59 (AB, J = 12.3 Hz, 2 H), 1.91 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 166.6 (C-4), 152.1 (C-2), 136.7 (C-6), 110.7 (C-5), 91.4 (C-4′), 89.6 (C-1′), 82.1, 81.9 (C-2′, C-3′), 74.5, 61.9, 57.9 (C-5′′, C-1′′, C-5′), 12.5 (CH_3) ppm. HR-MALDI MS: *m*/*z* 323.0864 ([M + Na]$^+$, $C_{12}H_{16}N_2O_7Na^+$ calcd. 323.0850).

Acknowledgments

The work was supported by the Sixth Framework Program Marie Curie Host Fellowships for Early Stage Research Training under contract MEST-CT-2004-504018, by the Danish National Research Council, by the Danish National Research Foundation, and by Møllerens Fond. Mrs. Birthe Haack is thanked for technical assistance. Furthermore, the Nucleic Acid Center is funded by the Danish National Research Foundation for studies on nucleic acid chemical biology.

[1] a) M. Meldgaard, J. Wengel, *J. Chem. Soc. Perkin Trans. 1* **2000**, 3539–3554; b) C. J. Leumann, *Bioorg. Med. Chem.* **2002**, *10*, 841–854.

[2] a) S. K. Singh, P. Nielsen, A. A. Koshkin, J. Wengel, *Chem. Commun.* **1998**, 455–456; b) A. A. Koshkin, S. K. Singh, P. Nielsen, V. K. Rajwanshi, R. Kumar, M. Meldgaard, C. E. Olsen, J. Wengel, *Tetrahedron* **1998**, *54*, 3607–3630; c) S. Obika, D. Nanbu, Y. Hari, J. Andoh, K. Morio, T. Doi, T. Imanishi, *Tetrahedron Lett.* **1998**, *39*, 5401–5404.

[3] M. Petersen, J. Wengel, *Trends Biotechnol.* **2003**, *21*, 74–81.

[4] M. Petersen, K. Bondensgaard, J. Wengel, J. P. Jacobsen, *J. Am. Chem. Soc.* **2002**, *124*, 5974–5982.

[5] a) J. S. Jepsen, J. Wengel, *Curr. Opin. Drug Dis. Dev.* **2004**, *7*, 188–194; b) H. Kaur, B. R. Babu, S. Maiti, *Chem. Rev.* **2007**, *107*, 4672–4697; c) A. Grünweller, R. K. Hartmann, *Biodrugs* **2007**, *21*, 235–243.

[6] P. N. Jørgensen, P. C. Stein, J. Wengel, *J. Am. Chem. Soc.* **1994**, *116*, 2231–2232.

[7] G. Wang, P. J. Middleton, L. He, V. Stoisavljevic, W. E. Seifert, *Nucleosides Nucleotides* **1997**, *16*, 445–454.

[8] a) P. N. Jørgensen, M. L. Svendsen, C. Scheuer-Larsen, J. Wengel, *Tetrahedron* **1995**, *51*, 2155–2164; b) P. N. Jørgensen, U. S. Sørensen, H. M. Pfundheller, C. E. Olsen, J. Wengel, *J. Chem. Soc. Perkin Trans. 1* **1997**, 3275–3284; c) H. M. Pfundheller, P. N. Jørgensen, U. S. Sørensen, S. K. Sharma, M. Grimstrup, C. Stroch, P. Nielsen, G. Viswanadham, C. E. Olsen, J. Wengel, *J. Chem. Soc. Perkin Trans. 1* **1998**, 1409–1421.

[9] M. Meldgaard, F. G. Hansen, J. Wengel, *J. Org. Chem.* **2004**, *69*, 6310–6322.

[10] Parts of the present work have been shown in a conference proceeding: P. K. Sharma, S. Kumar, P. Nielsen, *Nucleosides Nucleotides Nucleic Acids* **2007**, *26*, 1505–1508.

[11] G. Enderlin, P. Nielsen, *J. Org. Chem.* **2008**, *73*, 6891–6894.

[12] P. Nielsen, J. Wengel, *Chem. Commun.* **1998**, 2645–2646.

[13] a) K. P. Gable, G. A. Benz, *Tetrahedron Lett.* **1991**, *32*, 3473–3476; b) P. Nielsen, M. Petersen, J. P. Jacobsen, *J. Chem. Soc. Perkin Trans. 1* **2000**, 3706–3713; c) J. Ravn, P. Nielsen, *J. Chem. Soc. Perkin Trans. 1* **2001**, 985–993.

[14] a) P. K. Sharma, P. Nielsen, *J. Org. Chem.* **2004**, *69*, 5742–5745; b) P. K. Sharma, S. Kumar, P. Kumar, P. Nielsen, *Tetrahedron Lett.* **2007**, *48*, 8704–8708.

[15] R. D. Youssefyeh, J. P. H. Verheyden, J. G. Moffatt, *J. Org. Chem.* **1979**, *44*, 1301–1309.

[16] A. A. Koshkin, J. Fensholdt, H. M. Pfundheller, C. Lomholt, *J. Org. Chem.* **2001**, *66*, 8504–8512.

[17] a) H. Vorbrüggen, K. Krolikiewicz, B. Bennua, *Chem. Ber.* **1981**, *114*, 1234–1255; b) H. Vorbrüggen, G. Höfle, *Chem. Ber.* **1981**, *114*, 1256–1268.

[18] S. Obika, K. Morio, D. Nanbu, Y. Hari, H. Itoh, T. Imanishi, *Tetrahedron* **2002**, *58*, 3039–3049.

Received: July 18, 2008
Published Online: October 17, 2008

