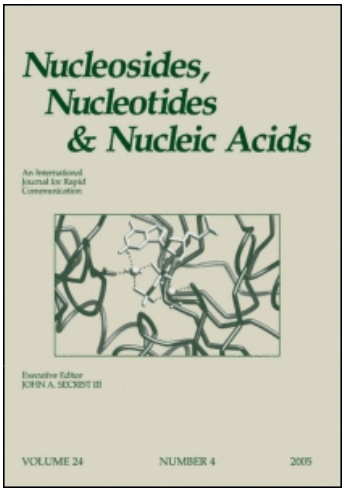

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Ribosylation of Pyrimidine 2'-Deoxynucleosides

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RIBOSYLATION OF PYRIMIDINE 2'-DEOXYNUCLEOSIDES

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ABSTRACT : The previously developed method for the preparation of 2'-*O*-*D*-ribofuranosyl-nucleosides is extended to ribosylation of 2'-deoxynucleosides. The scope and limitations of this reaction are discussed.

Several antibiotics (amicetin, hikizimycin, ezomycins, thuringiensin, tunicamycins) have a disaccharide structure. Their structure, synthesis and properties were recently reviewed [1,2]. Disaccharide nucleosides are also found as modified nucleosides in t-RNA [3-5]. Nevertheless, little information is available about the biological activity of these nucleosides and even the most simple representative of this series (3'-*O*-ribosylated-2'-deoxy nucleosides) were hitherto unknown. Therefore we started a systematic study of the synthesis of these disaccharide nucleosides and evaluated their antiviral activity.

Disaccharide nucleosides may be prepared either by coupling of suitably blocked disaccharides with nucleic acid bases or by condensation of protected nucleosides with monosaccharides. Most of complex nucleoside antibiotics have been prepared using the first route [1]. This route is rather lengthy. The synthesis of disaccharide nucleosides may be shortened when using natural nucleosides as starting compounds. Several attempts are described to use classical methods of glycosidic bond formation with blocked nucleosides as an alcohol component. The yields of these reactions were usually very low (20-30%), due to formation of several by-products [1].

Recently a general method has been developed [6-10] for the preparation of 2'-*O*-ribofuranosylnucleosides which were isolated from the yeast methionine initiator tRNAs [3-5]. It consists in condensation of preactivated 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranose with tin tetrachloride and N,3',5'-*O*-protected

ribonucleosides under the conditions proposed by S.Hanessian and J. Banoub [11] for the preparation of alkyl β-D-ribofuranosides. To get further insight into the scope and limitations of this reaction we decided to examine glycosylation of 2'-deoxynucleosides. The starting material, N,5'-O-protected **1a-e** were prepared by selective silylation [12,13] of the corresponding 2'-deoxynucleosides [14].

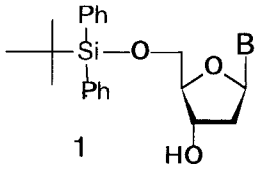
Condensation of **1a** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranose under the previously developed conditions [7,10] (dichloroethane, 0°C, under nitrogen) gave the desired **2a** in 79% yield. The reaction was completed in 40 min. Compound **2b** was prepared in the same yield (71%). The glycosylation reaction proceeded stereospecifically with β-glycosidic bond formation. NMR analysis of compounds **2a** and **2b** revealed that the coupling constant between H-1 and H-2 ($J_{1',2'}$) of the ribose residue is near zero. It should be mentioned that several by-products with higher R_f were formed in small quantities. This was also observed during the previously reported synthesis of 2'-*O*-ribofuranosylnucleosides [7,10]. The amounts of these by-products depended on the purity of the starting material and on the reaction conditions. Performing the reaction under strictly anhydrous conditions reduced the formation of by-products. The silyl group was selectively deblocked to yield partially protected **3a,b** which may be used for further modification. Deblocking with ammonia in methanol gave free disaccharide nucleosides **4a,b** in high overall yield.

Glycosylation of **1c-e** yielded an untractable mixture of products due to the known instability of glycosidic bond in purine 2'-deoxynucleosides [15]. This means that the present method [3-7] can not be applied for acid labile nucleosides without further modification.

The method has also been tested for the synthesis of β-*D*-ribopyranosyl derivatives. It is known that pyranoses are less reactive [16] than furanoses under similar reaction conditions. In this case the reaction was performed at 20°C for 2 hr and **5** was obtained in 38% yield. After deblocking respectively, first **6** and then **7** were obtained in a high overall yield. It should be mentioned that in this series only one disaccharide **7** was obtained in crystalline form. It is clear that in spite of much lower yields in condensation reaction this method may be used for the preparation of *O*-pyranosylnucleosides.

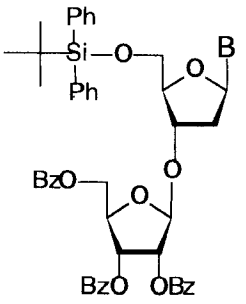
The glycosylation reaction was examined under different reaction conditions. When the condensation of **1a** with 1.2 eq. of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranose was carried out in acetonitrile at 0°C for 30 min only a minor quantity of **2a** was formed. The main product isolated in 28% yield (44% based on starting

sugar) proved to be trisaccharide 8. The ^{1}H NMR and ^{13}C NMR spectra clearly demonstrated the presence of two ribose residues (both with $J_{1',2'} = 0$ Hz). The same product 8 (38% based on the sugar) was obtained when the reaction was carried out in the presence of $ZnBr_2$. In this case the suspension was stirred at 20°C for 3 hr. It was shown previously that silyl blocking groups could be replaced by an acetyl in the reaction with acetic bromide in the presence of Lewis acid ($SnBr_2$) [17].



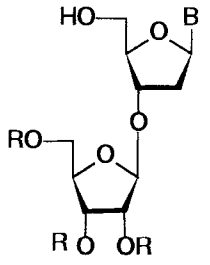
1

a. B = Thy
b. B = Cyt^{Bz}
c. B = Ade^{Bz}
d. B = Ade$^{Bz}_2$
e. B = Ade



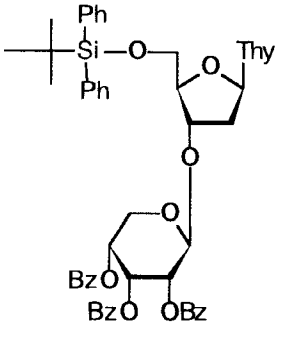
2

a. B = Thy
b. B = Cyt^{Bz}

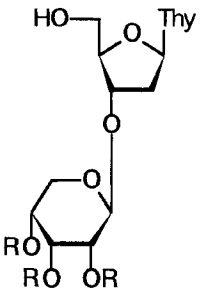


3 R = Bz
a. B = Thy
b. B = Cyt^{Bz}

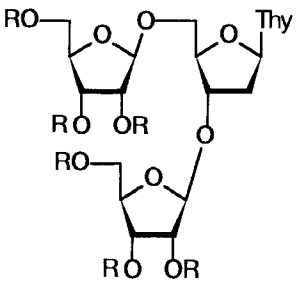
4 R = H
a. B = Thy
b. B = Cyt



5



6 R = Bz
7 R = H



8 R = Bz
9 R = H

The compounds 4a,b and 7 were found to be inactive against a number of viruses, including herpes simplex virus (HSV-1, HSV-2), vaccinia virus, vesicular stomatitis virus, parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie B-4 virus, Semliki forest virus and polio-1 virus. Nor did they exhibit any toxicity to the host cells [human embryonic skin-muscle (ESM) Hela, or Vero cells].

Conclusions

The general method for the preparation of O-ribofuranosylnucleosides was found to be applicable for the synthesis of pyrimidine 2'-deoxynucleosides. In the case of purine 2'-deoxynucleosides a complex mixture was formed evidently due to the glycosidic bond instability. We have demonstrated that the method may be extended to the preparation of *O*-pyranosylnucleosides and may be used for simple and efficient preparation of different disaccharide nucleosides for systematic SAR studies.

EXPERIMENTAL

Melting points (uncorrected) were determined with a Buchi-Tottoli instrument. UV spectra were recorded on a Philips PU8740 UV/VIS spectrophotometer. Optical rotations were measured with a JASCO DIP-360 automatic polarimeter at 20°C. Liquid secondary ion mass spectra (LSIMS) were obtained using a Kratos Concept 1H mass spectrometer. Column chromatography was performed on silica gel (0.06-0.20 mm), TLC was carried out on Kieselgel 260 F (Merck) with detection by UV light. NMR spectra were recorded using a Gemini 200 NMR spectrometer at 20°C. Chemical shifts were measured relative to solvent signals. The signals were assigned by the double resonance techniques.

General method for the preparation of 1a-e.

To a solution of 2'-deoxynucleoside (N-benzoyl-2'-deoxynucleoside) (10 mmol) and imidazole (10 mmol) in dry pyridine (20 ml), tert-butyldiphenylsilyl chloride (11 mmol) was added and the solution was kept at 20°C for 3-5 hrs until the reaction was complete according to TLC. The solvent was evaporated to dryness, the residue was dissolved in methylene chloride (in ethyl acetate for 1e), the organic layer was washed successfully with water, 10% aqueous solution of sodium bicarbonate and again with water. The organic layer was dried with Na_2SO_4, filtered, the filtrates were evaporated to dryness and evaporated with toluene. The products were purified by column chromatography on silica gel (compounds 1a and 1e were directly recrystallized from a mixture of methylene chloride - hexane).

5'-*O*-tert-Butyldiphenylsilyl-2'-deoxythymidine (1a).

Yield 88%. M.p. 162-163°C. Lit. [13]: M.p. 163-164°C. ^{1}H NMR ($CDCl_3$): 9.37 brs (1H, NH), 7.70-7.34 m (11H, H-6, Ph), 6.43 dd (1H, $J_{1',2'a}$ = 5.6 Hz, $J_{1',2'b}$ = 8.2 Hz, H-1'), 4.57 m (1H, H-3'), 4.04 m (1H, H-4'), 3.97 dd (1H, $J_{5'a,4'}$ = 2.8 Hz, $J_{5'a,5'b}$ = - 11.5 Hz, H-5'a), 3.85 dd (1H, $J_{5'b,4'}$ = 2.6 Hz, H-5'b), 3.00 brs (1H,

OH), 2.43 m (1H, H-2'a), 2.19 m (1H, H-2'b), 1.62 d (3H, $J_{5,6}$ = 1.2 Hz, Me-5), 1.08 s (9H, t-Bu). ^{13}C NMR ($CDCl_3$): 163.88 (C-4), 150.58 (C-2), 135.53 (C-6), 135.29, 132.87, 132.32, 130.15, 130.04 and 127.97 (Ph), 111.25 (C-5), 87.11 (C-4'), 84.71 (C-1'), 72.22 (C-3'), 64.14 (C-5'), 40.98 (C-2'), 26.96 (Me_3), 19.33 (Si*C*Me_3), 12.07 (Me-5).

5'-*O*-tert-Butyldiphenylsilyl-N^4-benzoyl-2'-deoxycytidine (1b).
Yield 80% (foam). ^1H NMR ($CDCl_3$): 8.86 brs (1H, NH), 8.34 d (1H, $J_{6,5}$ = 7.5 Hz, H-6), 7.92-7.35 m (16H, Ph, Bz, H-5), 6.38 t (1H, $J_{1',2'a} = J_{1',2'b}$ = 6.0 Hz, H-1'), 4.53 m (1H, H-3'), 4.13 m (1H, H-4'), 4.01 dd (1H, $J_{5'a,4'}$ = 2.8 Hz, $J_{5'a,5'b}$ = -11.6 Hz, H-5'a), 3.86 dd (1H, $J_{5'b,4'}$ = 2.9 Hz, H-5'b), 2.79 m (1H, H-2'a), 2.21 m (1H, H-2'b), 1.09 s (9H, t-Bu). ^{13}C NMR ($CDCl_3$): 166.66 (C-4), 162.17 (C=O), 155.03 (C-2), 144.51 (C-6), 135.57, 135.42, 133.07, 132.60, 132.35, 130.08, 129.94, 127.94 and 127.55 (Ph and Bz), 96.65 (C-5), 87.53 (C-4'), 87.14 (C-1'), 70.94 (C-3'), 63.44 (C-5'), 42.16 (C-2'), 26.91 (Me_3), 19.18 (Si*C*Me_3).

1-[5-*O*-tert-Butyldiphenylsilyl-3-*O*-(2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranosyl)-2-deoxy-β-*D*-ribofuranosyl]-thymine (2a).
To a cold solution (0°C) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranose (630 mg, 1.25 mmol) in 1,2-dichloroethane (12 ml) under nitrogen tin tetrachloride (0.16 ml, 1.38 mmol) was added and the solution was kept at 0°C for 10 min. After addition of nucleoside 1a (480 mg, 1 mmol) the resulting solution was kept at 0°C for 40 min. Then 10% aqueous solution of sodium bicarbonate (5 ml) was added and the suspension was stirred at 0°C for 20 min. The suspension was diluted with methylene chloride (20 ml), filtered through Hyflo Super Cel, the organic layer was separated, washed with water (10 ml), dried and evaporated to dryness. The residue was purified by column chromatography on silica gel (50 g). The column was washed with methylene chloride (500 ml) and then eluted with 1% methanol in methylene chloride to give 2a as a foam. Yield 730 mg (79%). LSIMS: (M + H) 925. ^1H NMR ($CDCl_3$): 8.68 brs (1H, NH), 8.10-7.30 m (26H, Ph, Bz, H-6), 6.29 dd (1H, $J_{1',2'a}$ = 5.6 Hz, $J_{1',2'b}$ = 8.1 Hz, H-1' Thd), 5.84 dd (1H, $J_{3',2'}$ = 5.0 Hz, $J_{3',4'}$ = 6.5 Hz, H-3' Rib), 5.66 d (1H, H-2' Rib), 5.32 s (1H, H-1' Rib), 4.82-4.56 m (4H, H-3',4',5'a,5'b Thd), 4.12 m (1H, H-4' Rib), 3.95 dd (1H, $J_{5'a,4'}$ = 3.1 Hz, $J_{5'a,5'b}$ = -11.5 Hz, H-5'a Rib), 3.83 dd (1H, $J_{5'b,4'}$ = 2.7 Hz, H-5'b Rib), 2.52 m (1H, H-2'a Thd), 2.18 m (1H, H-2'b Thd), 1.61 d (3H, $J_{5,6}$ = 1.0 Hz, Me-5), 1.06 s (9H, t-Bu). ^{13}C NMR ($CDCl_3$): 166.09, 165.29 and 165.16 (C=O), 163.61 (C-4), 150.04 (C-2), 135.51 (C-6), 135.29, 133.57, 133.45, 133.25, 132.82, 132.28, 130.04, 129.74, 129.05,

128.84, 128.40 and 127.96 (Ph and Bz), 111.05 (C-5), 104.69 (C-1' Rib), 84.55 (C-1' Thd), 84.24 (C-4' Thd), 79.40 (C-3' Thd), 77.66 (C-4' Rib), 75.74 (C-2' Rib), 72.21 (C-3' Rib), 64.73 (C-5' Thd), 63.88 (C-5' Rib), 38.57 (C-2' Thd), 26.93 (Me_3), 19.30 (Si*C*Me_3), 12.07 (Me-5).

N^4-Benzoyl-1-[5-*O*-tert-butyldiphenylsilyl-3-*O*-(2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranosyl)-2-deoxy-β-*D*-ribofuranosyl]-cytosine (2b).

Analogous condensation of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranose (1.32 g, 2.61 mmole) with **1b** (1.18 g, 2.07 mmol) in the presence of tin tetrachloride (0.33 ml, 2.83 mmol) in 1,2-dichloroethane (20 ml) at 0°C for 40 min gave **2b** as a foam. Yield 1.5 g (71%). LSIMS: (M + H) 10164. ^{1}H NMR ($CDCl_3$): 8.72 brs (1H, NH), 8.26 d (1H, $J_{6,5}$ = 7.5 Hz, H-6), 8.10-7.25 m (31H, Ph, Bz, H-5), 6.25 t (1H, $J_{1',2'a} = J_{1',2'b}$ = 5.6 Hz, H-1' dCyd), 5.81 dd (1H, $J_{3',2'}$ = 4.7 Hz, $J_{3',4'}$ = 6.7 Hz, H-3' Rib), 5.63 d (1H, H-2' Rib), 5.28 s (1H, H-1' Rib), 4.81-4.53 m (4H, H-3' dCyd, H-4',5'a,5'b Rib), 4.15 m (1H, H-4' dCyd), 4.02 dd (1H, $J_{5'a,4'}$ = 3.1 Hz, $J_{5'a,5'b}$ = -11.4 Hz, H-5'a dCyd), 3.85 dd (1H, $J_{5'b,4'}$ = 2.5 Hz, H-5'b dCyd), 2.79 ddd (1H, $J_{2'a,3'}$ = 5.1 Hz, $J_{2'a,2'b}$ = -14.0 Hz, H-2'a dCyd), 2.30 ddd (1H, $J_{2'b,3'}$ = 6.0 Hz, H-2'b dCyd), 1.08 s (9H, t-Bu). ^{13}C NMR ($CDCl_3$): 165.98 (C-4), 165.18 and 165.04 (C=O), 161.93 (C-2), 144.19 (C-6), 135.51, 135.36, 133.50, 133.35, 133.16, 133.03, 132.45, 132.14, 130.06, 129.67, 128.92, 128.76, 128.36, 128.29, 127.91 and 127.47 (Ph and Bz), 104.81 (C-1' Rib), 96.33 (C-5), 86.69 (C-1' dCyd), 84.69 (C-4' dCyd), 79.23 (C-3' dCyd), 76.01 (C-4' Rib), 75.59 (C-2' Rib), 72.12 (C-3' Rib), 64.57 (C-5' dCyd), 62.82 (C-5' Rib), 39.88 (C-2' dCyd), 26.85 (Me_3), 19.12 (Si*C*Me_3).

1-[3-*O*-(2,3,5-tri-*O*-Benzoyl-β-*D*-ribofuranosyl)-2-deoxy-β-*D*-ribofuranosyl]-thymine (3a).

Nucleoside 2a (670 mg, 0.72 mmol) was dissolved in 0.5 M tetrabutylammonium fluoride trihydrate in tetrahydrofuran (2 ml). The solution was kept for 45 min at 20°C, evaporated to dryness, coevaporated with chloroform (10 ml) and applied onto a silica gel column (20 g). The column was washed with methylene chloride (300 ml) and with 1% methanol in methylene chloride (200 ml) and then eluted with 2% methanol in methylene chloride to give **3a** as a foam. Yield 0.43 g (86%). LSIMS: (M + H) 687. ^{1}H NMR ($CDCl_3$): 9.01 brs (1H, NH), 8.10-7.25 m (16H, Bz, H-6), 5.95 t (1H, $J_{1',2'a} = J_{1',2'b}$ = 6.8 Hz, H-1' Thd), 5.84 dd (1H, $J_{3',2'}$ = 4.9 Hz, $J_{3',4'}$ = 6.4 Hz, H-3' Rib), 5.66 d (1H, H-2' Rib), 5.40 s (1H, H-1' Rib), 4.80-4.52 m (4H, H-3',5'a,5'b Thd, H-4' Rib), 4.10 m (1H, H-4' Thd), 3,91 dd (1H, $J_{5'a,4'}$ = 3.0 Hz, $J_{5'a,5'b}$ = - 12.3 Hz, H-5'a Rib), 3.77 dd (1H, $J_{5'b,4'}$ = 1.5 Hz, H-5'b

Rib), 3.21 brs (1H, HO-5'), 2.54-2.40 m (2H, H-2'a,2'b Thd), 1.88 d (3H, $J_{5,6}$ = 1.2 Hz, Me-5). ^{13}C NMR ($CDCl_3$): 166.15, 165.39 and 165.29 (C=O), 163.72 (C-4), 150.23 (C-2), 137.63 (C-6), 133.62, 133.51, 133.36, 129.71, 129.49, 128.93, 128.75, 128.50 and 128.40 (Ph), 110.89 (C-5), 105.31 (C-1' Rib), 88.01 (C-1' Thd), 84.82 (C-4' Thd), 79.35 (C-3' Thd), 78.24 (C-4' Rib), 75.72 (C-2' Rib), 72.03 (C-3' Rib), 64.63 (C-5' Thd), 62.35 (C-5' Rib), 37.60 (C-2' Thd), 12.41 (Me-5).

N^4-Benzoyl-1-[3-*O*-(2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranosyl)-2-deoxy-β-*D*-ribofuranosyl]-cytosine (3b).

Analogous desilylation of **2b** yielded **3b** as a foam (89%). LSIMS: (M + H) 776. ^1H NMR ($CDCl_3$): 8.82 brs (1H, NH), 8.14-7.27 m (22H, Bz, H-5,6), 6.02 t (1H, $J_{1',2'a} = J_{1',2'b}$ = 6.2 Hz, H-1' dCyd), 5.83 dd (1H, $J_{3',2'}$ = 4.8 Hz, $J_{3',4'}$ = 6.5 Hz, H-3' Rib), 5.67 d (1H, H-2' Rib), 5.41 s (1H, H-1' Rib), 4.78-4.52 m (4H, H-3' dCyd, H-4',5'a,5'b Rib), 4.19 m (1H, H-4' dCyd), 4.00 dd (1H, $J_{5'a,4'}$ = 2.0 Hz, $J_{5'a,5'b}$ = -11.5 Hz, H-5'a dCyd), 3.85 dd (1H, $J_{5'b,4'}$ = 2.5 Hz, H-5'b dCyd), 2.72-2.51 m (2H, H-2'a,2'b dCyd). ^{13}C NMR ($CDCl_3$): 166.08 (C-4), 165.28 and 162.23 (C=O), 154.83 (C-2), 146.16 (C-6), 133.52, 133.40, 133.25, 133.06, 129.68, 128.90, 128.44, 128.33 and 127.55 (Bz), 105.27 (C-1' Rib), 96.61 (C-5), 89.54 (C-1' dCyd), 85.49 (C-4' dCyd), 79.20 (C-3' dCyd), 77.64 (C-4' Rib), 75.69 (C-2' Rib), 72.11 (C-3' Rib), 64.61 (C-5' dCyd), 61.83 (C-5' Rib), 38.75 (C-2' dCyd).

1-(3-*O*-β-*D*-Ribofuranosyl-2-deoxy-β-*D*-ribofuranosyl)-thymine (4a).

A solution of nucleoside **3a** (350 mg, 0.51 mmol) in 5 M ammonia in methanol (10 ml) was kept for 5 days at 20°C and then concentrated in vacuo to dryness. The residue was partitioned between methylene chloride (10 ml) and water (20 ml), and the aqueous layer was washed with methylene chloride (2 x 10 ml). The aqueous layer was evaporated to dryness, the residue was coevaporated with methanol (2 x 5 ml) to give a hygroscopic foam. Yield 190 mg (99%). $[\alpha]_D$ -28° (c 1.0, water). UV (pH 1-7): λ max 267 nm (ε 9600); (pH 13): λ max 267 nm (ε 7300). LSIMS: ($C_{15}H_{22}N_2O_9$ + H). Calc. 375.1403. Found 375.1419. ^1H NMR (D_2O): 7.50 d (1H, $J_{6,5}$ = 1.2 Hz, H-6), 6.11 t (1H, $J_{1',2'a} = J_{1',2'b}$ = 6.8 Hz, H-1' Thd), 4.99 s (1H, H-1' Rib), 4.33 m (1H, H-3' Thd), 4.10 dd (1H, $J_{3',2'}$ = 4.8 Hz, $J_{3',4'}$ = 6.3 Hz, H-3' Rib), 4.01-3.87 m (3H, H-4',5'a,5'b Thd), 3.76-3.65 m (3H, H-2',4',5'a Rib), 3.57 dd (1H, $J_{5'b,4'}$ = 6.6 Hz, $J_{5'b,5'a}$ = -12.4 Hz, H-5'b Rib), 2.50-2.12 m (2H, H-2'a,2'b Thd), 1.76 d (3H, Me-5). ^{13}C NMR (D_2O): 167.48 (C-4), 152.69 (C-2), 138.41 (C-6), 112.44 (C-5), 107.64 (C-1' Rib), 86.22 (C-1' Thd), 85.73 (C-4' Thd), 83.96 (C-3' Thd), 78.55 (C-4' Rib), 75.63 (C-2' Rib), 71.82 (C-3' Rib), 63.88 (C-5' Thd), 62.33 (C-5' Rib), 38.33 (C-2' Thd), 12.60 (Me-5).

1-(3-*O*-β-*D*-Ribofuranosyl-2-deoxy-β-*D*-ribofuranosyl)-cytosine (4b).
Analogous debenzoylation of **3b** after evaporation with methanol yielded **4b** as a hygroscopic foam (95%). $[\alpha]_D$ -2.2° (c 0.55, water). UV (pH 1): λ max 279 nm (ε 12100); (pH 7-13): λ max 270 nm (ε 8600). LSIMS: ($C_{14}H_{21}N_3O_8$ + H). Calc. 360.1407. Found 360.1431. ^{1}H NMR (D_2O): 7.68 d (1H, $J_{6,5}$ = 7.7 Hz, H-6), 6.10 t (1H, $J_{1',2'a} = J_{1',2'b}$ = 6.6 Hz, H-1' dCyd), 5.91 d (1H, H-5), 5.00 s (1H, H-1' Rib), 4.30 m (1H, H-3' dCyd), 4.11 dd (1H, $J_{3',2'}$ = 4.7 Hz, $J_{3',4'}$ = 6.7 Hz, H-3' Rib), 4.05-3.88 m (3H, H-4',5'a,5'b dCyd), 3.77-3.65 m (3H, H-2',4',5'a Rib), 3.55 dd (1H, $J_{5'b,4'}$ = 6.6 Hz, $J_{5'b,5'a}$ = -12.3 Hz, H-5'b Rib), 2.60-2.08 m (2H, H-2'a,2'b dCyd). ^{13}C NMR (D_2O): 167.12 (C-4), 158.40 (C-2), 142.45 (C-6), 107.59 (C-1' Rib), 97.16 (C-5), 87.29 (C-1' dCyd), 85.85 (C-4' dCyd), 83.97 (C-3' dCyd), 78.62 (C-4' Rib), 75.64 (C-2' Rib), 71.85 (C-3' Rib), 63.93 (C-5' dCyd), 62.40 (C-5' Rib), 39.11 (C-2' dCyd).

1-[5-*O*-tert-Butyldiphenylsilyl-3-*O*-(2,3,4-tri-*O*-benzoyl-β-*D*-ribopyranosyl)-2-deoxy-β-*D*-ribofuranosyl]-thymine (5).
To a solution of 1,2,3,4-tetra-O-O-acetyl-β-D-ribopyranose (716 mg, 2.25 mmol) in 1,2-dichloroethane (20 ml) under nitrogen, tin tetrachloride (0.29 ml, 2.5 mmol) was added and the solution was kept at 20°C for 10 min. Nucleoside **1a** (680 mg, 1,415 mmol) was added in three portions over 40 min under stirring and the resulting yellow solution was kept further for 1.3 hr at 20°C. Then 10% aqueous solution of sodium bicarbonate (5 ml) was added and the suspension was stirred at 20°C for 20 min. The suspension was diluted with methylene chloride (20 ml), filtered through Hyflo Super Cel, the organic layer was separated, washed with water (10 ml), dried and evaporated to dryness. The residue was purified by column chromatography on silica gel (50 g). The column was washed with methylene chloride (500 ml) and eluted with 1% methanol in methylene chloride to give 5 as a foam. Yield 400 mg (38%). LSIMS: (M + H) 739. ^{1}H NMR ($CDCl_3$): 8.95 brs (1H, NH), 7.70-7.38 m (11H, Ph, H-6), 6.30 dd (1H, $J_{1',2'a}$ = 5.6 Hz, $J_{1',2'b}$ = 8.2 Hz, H-1' Thd), 5.41 dd (1H, $J_{3',2'}$ = 3.2 Hz, $J_{3',4'}$ = 3.6 Hz, H-3' Rib), 5.16 m (1H, H-4' Rib), 4.98 dd (1H, $J_{2',1'}$ = 4.0 Hz, H-2' Rib), 4.85 d (1H, H-1' Rib), 4.48 m (1H, H-3' Thd), 4.09 m (1H, H-4' Thd), 4.05-3.76 m (4H, H-5'a,5'b Thd, H-5'a,5'b Rib), 2.54 ddd (1H, $J_{2'a,2'b}$ = - 13.8 Hz, $J_{2'a,3'}$ = 2.1 Hz, H-2'a Thd), 2.18 m (1H, H-2'b Thd), 2.11 s (3H, Ac), 2.10 s (3H, Ac), 2.06 s (3H, Ac), 1.63 d (3H, $J_{5,6}$ = 1.0 Hz, Me-5), 1.10 s (9H, t-Bu). ^{13}C NMR ($CDCl_3$): 169.91 and 169.62 (C=O), 163.68 (C-4), 150.16 (C-2), 135.04 (C-6), 135.46, 135.28, 132.70, 132.29, 130.11 and 127.98 (Ph), 111.15 (C-5), 97.88 (C-1' Rib), 84.73 (C-1' Thd), 84.52 (C-4' Thd), 78.18 (C-3'

Thd), 68.27 (C-2' Rib), 66.48 (C-3' Rib), 65.98 (C-4' Rib), 63.93 (C-5' Thd), 61.63 (C-5' Rib), 38.65 (C-2' Thd), 26.96 (Me_3), 20.76 and 20.63 (Me, Ac), 19.31 (Si*C*Me_3), 12.06 (Me-5).

1-[3-*O*-(2,3,4-tri-*O*-Acetyl-β-*D*-ribopyranosyl)-2-deoxy-β-*D*-ribofuranosyl]-thymine(6).

Nucleoside 5 (750 mg, 1.015 mmol) was dissolved in 0.5 M tetrabutylammonium fluoride trihydrate in tetrahydrofuran (3 ml), the solution was kept for 20 min at 20°C, evaporated to dryness, coevaporated with chloroform (10 ml) and applied onto a silica gel column (20 g). The column was washed with methylene chloride (300 ml) and with 1% methanol in methylene chloride (200 ml) and then eluted with 2% methanol in methylene chloride to give **6** as a foam. Yield 0.45 g (89%). LSIMS: (M + H) 501, (M + Na) 523. ^{1}H NMR ($CDCl_3$): 9.03 brs (1H, NH), 7.35 q (1H, $J_{6,5}$ = 1.0 Hz, H-6), 6.05 t (1H, $J_{1',2'a}$ = $J_{1',2'b}$ = 6.9 Hz, H-1' Thd), 5.41 dd (1H, $J_{3',2'}$ = 3.5 Hz, $J_{3',4'}$ = 3.1 Hz, H-3' Rib), 5.13 m (1H, H-4' Rib), 4.98 dd (1H, $J_{2',1'}$ = 4.1 Hz, H-2' Rib), 4.91 d (1H, H-1' Rib), 4.53 m (1H, H-3' Thd), 4.08 m (1H, H-4' Thd), 4.02-3.71 m (4H, H-5'a,5'b Thd, H-5'a,5'b Rib), 3.02 brs (1H, OH), 2.47 m (2H, H-2'a,2'b Thd), 2.12 s (3H, Ac), 2.10 s (3H, Ac), 2.08 s (3H, Ac), 1.90 d (3H, Me-5). ^{13}C NMR ($CDCl_3$): 169.96 and 169.77 (C=O), 163.72 (C-4), 150.28 (C-2), 137.28 (C-6), 111.05 (C-5), 98.14 (C-1' Rib), 87.69 (C-1' Thd), 84.78 (C-4' Thd), 77.98 (C-3' Thd), 68.34 (C-2' Rib), 66.44 (C-3' Rib), 66.09 (C-4' Rib), 62.31 (C-5' Thd), 61.61 (C-5' Rib), 37.96 (C-2' Thd), 20.79 and 20.67 (Me, Ac), 12.44 (Me-5).

1-(3-*O*-β-*D*-Ribopyranosyl-2-deoxy-β-*D*-ribofuranosyl)-thymine (7).

A solution of nucleoside **6** (300 mg, 0.60 mmol) in 5 M ammonia in methanol (10 ml) was kept for 2 days at 20°C and then concentrated in vacuo to dryness. The residue was partitioned between methylene chloride (10 ml) and water (20 ml), and the aqueous layer was washed with methylene chloride (2 x 10 ml). The aqueous layer was concentrated to dryness and acetone was added. After 2 days at 0°C hygroscopic crystals were filtered off, washed with acetone and dried. Yield 180 mg (80%). M.p. 126-128°C. $[\alpha]_D$ -40.7° (c 0.73, water). UV (pH 1-7): λ max 267 nm (ε 9400); (pH 13): λ max 267 nm (ε 7100). LSIMS: ($C_{15}H_{22}N_2O_9$ + H). Calc. 375.1403. Found 375.1380.

Anal. Calc. for $C_{15}H_{22}N_2O_9.0.25H_2O$: C 47.56; H 5.99; N 7.39. Found: C 47.60; H 6.01; N 7.31.

^{1}H NMR (D_2O): 7.55 q (1H, $J_{6,5}$ = 1.0 Hz, H-6), 6.18 dd (1H, $J_{1',2'a}$ = 6.2 Hz, $J_{1',2'b}$ = 7.3 Hz, H-1' Thd), 4.77 d (1H, $J_{1',2'}$ = 5.5 Hz, H-1' Rib), 4.41 ddd (1H,

$J_{3',2a'}$ = 3.6 Hz, $J_{3',2'b'}$ = 6.8 Hz, $J_{3',4'}$ = 2.5 Hz, H-3' Thd), 4.09 m (1H, H-4' Thd), 4.00 t (1H, $J_{3',2'}$ = $J_{3',4'}$ = 2.9 Hz, H-3', Rib), 3.85-3.53 m (6H, H-5'a,5'b Thd, H-2',4',5'a,5'b Rib), 2.44 ddd (1H, $J_{2'a,2'b}$ = -14.1 Hz, H-2'a Thd), 2.32 ddd (1H, H-2'b Thd), 1.80 d (3H, Me-5). ^{13}C NMR (D_2O): 166.90 (C-4), 152.10 (C-2), 137.84 (C-6), 111.91 (C-5), 100.55 (C-1' Rib), 85.70 (C-1' Thd), 85.22 (C-4' Thd), 78.69 (C-3' Thd), 70.61 (C-2' Rib), 68.40 (C-3' Rib), 68.03 (C-4' Rib), 63.87 (C-5' Thd), 61.76 (C-5' Rib), 37.59 (C-2' Thd), 12.00 (Me-5).

1-[Bis-3,5-*O*-(2,3,5-tri-*O*-Benzoyl-β-*D*-ribofuranosyl)-2-deoxy-β-*D*-ribofuranosyl]-thymine (8).

A. To a cold solution (0°C) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranose (1.21 g, 2.4 mmol, 1.27 eq.) in dry acetonitile (20 ml) under nitrogen tin tetrachloride (0.304 ml, 2.6 mmol, 1.38 eq.) was added and the solution was kept at 0°C for 10 min. After addition of nucleoside 1a (905 mg, 1.883 mmol, 1 eq.) the resulting solution was kept at 0°C for 30 min. After usual work-up the product 8 was purified by column chromatography on silica gel (50 g). The column was washed with methylene chloride (500 ml) and then eluted with 0.8% methanol in methylene chloride. Yield 600 mg (foam) (28% based on starting 1a; 44% based on sugar).

B. A mixture of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranose (353 mg, 0.7 mmol), nucleoside 1a (226 mg, 0.47 mmol) and zinc bromide (450 mg, 2 mmol) in dry 1,2-dichloroethane (10 ml) was stirred under nitrogen for 3 hr at 20°C. After usual work-up the product 8 was obtained (yield 28% on nucleoside, 38% on sugar).

LSIMS: (M + H) 1131. ^1H NMR ($CDCl_3$): 8.38 brs (1H, NH), 8.07-7.26 m (31H, Bz, H-6), 6.16 t (1H, $J_{1',2'a}$ = $J_{1',2'b}$ = 6.6 Hz, H-1' Thd), 5.83 dd (1H, $J_{3',2'}$ = 5.1 Hz, $J_{3',4'}$ = 6.3 Hz, H-3' Rib), 5.82 dd (1H, $J_{3',2'}$ = 5.4 Hz, $J_{3',4'}$ = 6.6 Hz, H-3' Rib), 5.70 d (1H, $J_{2',3'}$ = 5.1 Hz, H-2' Rib), 5.65 d (1H, $J_{2',3'}$ = 5.4 Hz, H-2' Rib), 5.40 s (1H, H-1' Rib), 5.34 s (1H, H-1' Rib), 4.80-3.68 m (10H6, H-3',4',5'a,5'b Thd, 2xH-4',5'a,5'b Rib), 2.42 m (1H, H-2'a Thd), 2.23 m (1H, H-2'b Thd), 1.90 d (3H, $J_{5,6}$ = 1.0 Hz, Me-5). ^{13}C NMR ($CDCl_3$): 166.02 and 165.51 (C=O), 163.12 (C-4), 150.11 (C-2), 135.02 (C-6), 133.39, 133.20, 129.68, 128.94, 128.75, 128.33 and 127.84 (Bz), 111.14 (C-5), 106.08 (C-1' Rib), 104.96 (C-1' Rib), 84.95 (C-1' Thd), 82.39 (C-4 Thd), 79.32 (2xC-4' Rib), 77.44 (C-3' Thd), 75.72 (C-2' Rib), 75.23 (C-2' Rib), 72.00 (2xC-3' Rib), 67.29 (C-5' Thd), 64.58 (C-5' Rib), 64.41 (C-5' Rib), 37.92 (C-2' Thd), 12.48 (Me-5).

1-(Bis-3,5-*O*-β-D-ribofuranosyl-2-deoxy-β-*D*-ribofuranosyl)-thymine (9). Analogous debenzoylation of 8 with 5 M ammonia in methanol for 4 days yielded after evaporation with methanol 9 as a hygroscopic foam (93%). $[\alpha]_D$ -46.4° (c 0.91, water). UV (pH 1-7): λ max 267 nm (ε 9300); (pH 13): λ max 267 nm (ε 6900). LSIMS: ($C_{20}H_{30}N_2O_{13}$ + H). Calc. 507.1826. Found 507.1812. ^1H NMR (D_2O): 7.41 d (1H, $J_{6,5}$ = 1.0 Hz, H-6), 6.12 t (1H, $J_{1',2'a} = J_{1',2'b}$ = 6.6 Hz, H-1' Thd), 5.00 s (1H, H-1' Rib), 4.95 s (1H, H-1' Rib), 4.38 m (1H, H-3' Thd), 4.11-3.43 m (13H, H-4',5'a,5'b Thd, 2xH-2',3',4',5'a,5'b Rib), 2.52-2.21 m (2H, H-2'a,2'b Thd), 1.80 d (3H, Me-5). ^{13}C NMR (D_2O): 166.85 (C-4), 152.08 (C-2), 137.74 (C-6), 111.80 (C-5), 107.72 (C-1' Rib), 107.07 (C-1' Rib), 85.73 (C-1' and C-4' Thd), 83.36 (2xC-4' Rib), 78.04 (C-3' Thd), 74.99 (C-2' Rib), 74.74 (C-2' Rib), 71.31 (C-3' Rib), 71.20 (C-3' Rib), 67.82 (C-5' Thd), 63.22 (2xC-5' Rib), 37.48 (C-2' Thd), 12.07 (Me-5).

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