Formation of Trisaccharide Nucleosides During Disaccharide Nucleoside Synthesis

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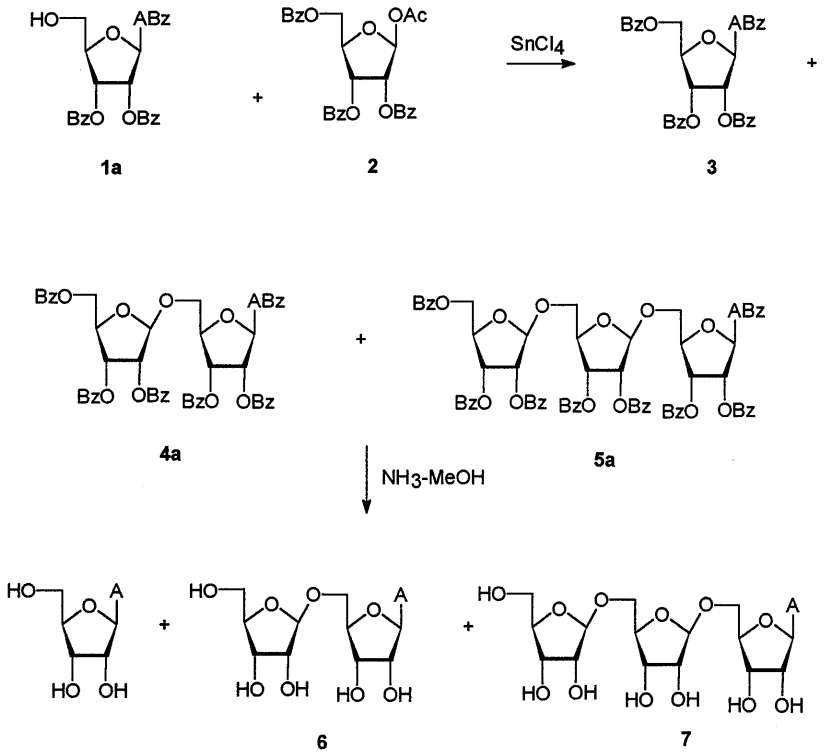
The unexpected formation of trisaccharide nucleosides during synthesis of disaccharide nucleosides with a purine base moiety has been observed. The occurrence of this side reaction can be explained in terms of the instability of the protected purine nucleosides in the presence of Lewis acids. The formation of these by-products can be avoided by using the 2,3-*O*-isopropylidene group instead of acyl groups for the blocking of the secondary hydroxyl groups of the nucleoside.

We have recently developed a general route for the preparation of 2′-*O*-β-D-ribofuranosyl nucleosides[1][2] by condensation of partially protected nucleosides with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in the presence of tin tetrachloride. Purine nucleosides having a *D*-ribofuranosyl substituent bonded to the 2′-hydroxy function of the nucleoside sugar moiety have recently been isolated from *t*-RNA[3][4]. Other types of disaccharide nucleosides have also shown biological activities, and several antibiotics have this type of structure[5]. This reaction was further extended to the synthesis of 3′-*O*-β-D-ribofuranosyl 2′-deoxypyrimidine nucleosides[6] and 5′-*O*-β-D-ribofuranosyl ribo (and 2′-deoxy) pyrimidine nucleosides[7]. It was shown that application of the reaction to the preparation of purine nucleosides in the latter two cases gave complex mixtures of products[6][7]. Therefore, we set about a more detailed investigation of this condensation reaction.

Scheme 1



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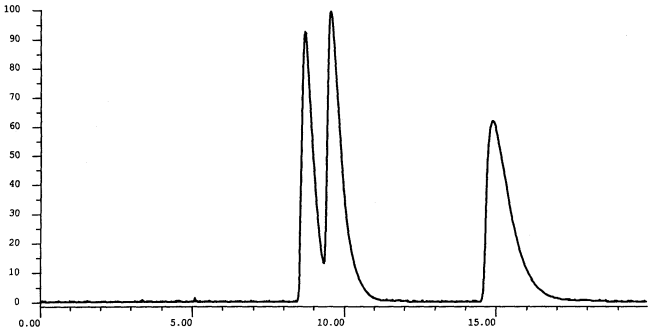
We report herein on the results of structure determinations of the compounds formed in this reaction and on the proposed mechanism of their formation. Condensation of **1a** with 1.2 equiv. of **2** in the presence of tin tetrachloride followed by standard work-up and silica gel column chromatography gave one main fraction with a higher R_F value by TLC than the starting nucleoside **1a**. According to its ^{1}H-NMR spectrum, this fraction represents a mixture of three products, which were tentatively assigned as **3**, **4a**, and **5a** (Scheme 1). This mixture was difficult to separate by silica gel column chromatography on a preparative scale. Therefore, after debenzoylation with ammonia in methanol, the resulting mixture of adenosine, **6**, and **7** was separated by means of HPLC (Figure 1). The spectrophotometrically determined ratio of these products was 3.7:3.6:2.7.

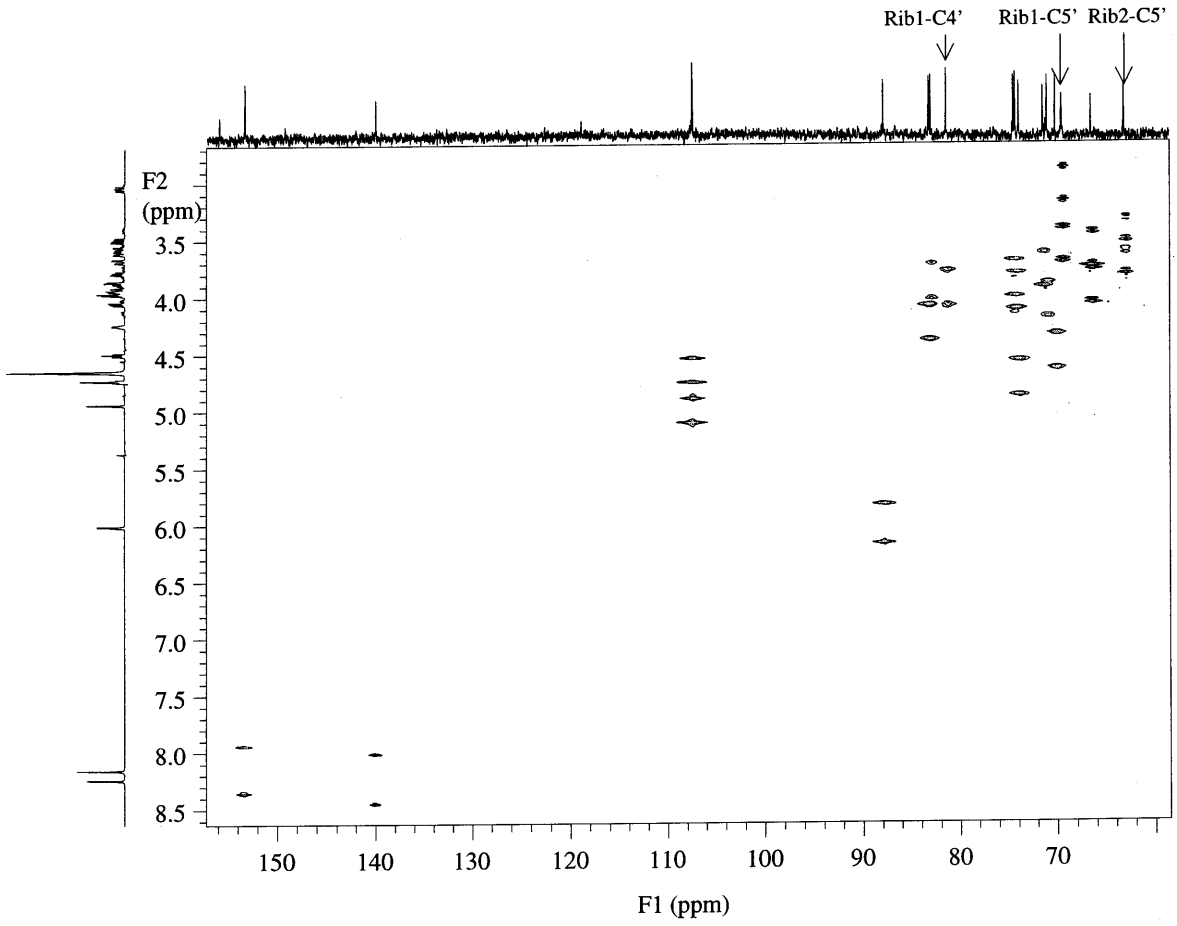
Figure 1. Semi-preparative reversed-phase HPLC separation of a mixture of **6**, **7**, and adenosine on a Silasorb C-18 column (10 × 250 mm, 13 μm) by isocratic elution with 5% acetonitrile in water at a flow rate of 4 ml/min.



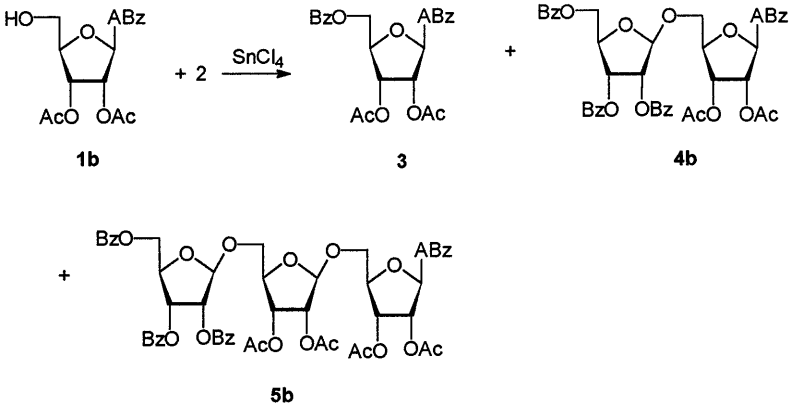
The structures of these compounds were proven by NMR spectroscopy and mass spectrometry. The ^{1}H- and ^{13}C-NMR spectra of **6** and **7** clearly indicated the presence of one and two additional ribose residues, respectively, linked by β-glycosidic bonds ($J_{1',2'}$ < 1.5 Hz). The ^{1}H signals of each individual ribose unit were assigned by a high-resolution GDQFCOPS experiment (0.5 Hz/point). The connectivity between the units Ado and Rib1 in structures **6**, **7**, and **10b** was determined by NOEs between 1′-H of Rib1 and 5′-H of Ado (3, 2 and 4%, respectively) in 1D-NOE experiments in which the isolated Rib1 1′-H proton was presaturated during 5 seconds. This proton also showed the expected NOE to 2′-H of Rib 1 (7, 6 and 5%, respectively). Presaturation of 5′b-H of Rib1 at δ = 3.03 resulted in an NOE to 1′-H of Rib2 at δ = 4.72 in compound **7**, proving the position of *O*-ribosylation of the second ribose moiety.

In an HSQC experiment, ^{13}C signals were correlated to ^{1}H signals, resulting in the ^{13}C assignments listed in Table 1. These data (Figure 2) show the highfield shift of C-4′ and the lowfield shift of C-5′ of Rib1 in compound **7** (cf.

Figure 2. Full HSQC spectrum of compound **7**; since no decoupling was used during acquisition, the signals are split in the F2 dimension by the ^{13}C-^{1}H coupling; typical shifts of Rib1-C4′, Rib1-C5′ and Rib2-C5′ are indicated above the ^{13}C spectrum along the F1 axis



Scheme 2

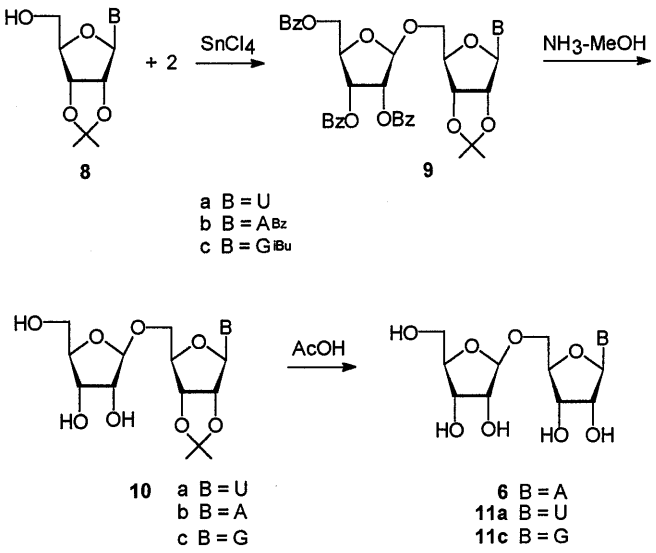


compound **6**). These changes are induced by glycosylation at the 5′-position of Rib1 of compound **7**. The shift of the C-5′ signal of Rib2 is typical of that of a free 5′-ribose unit.

We have shown previously that some protecting groups are not completely stable under glycosylation conditions[6][7]. For example, condensation of 5′-*tert*-butyldiphenylsilyl-2′-deoxythymidine with **2** in the presence of a Lewis acid yielded a trisaccharide nucleoside[6]. To gain insight into the mechanism of formation of the trisaccharide and to determine the source of the additional ribofuranosyl moiety, we performed the reaction with another substrate **1b**, bearing 2′,3′-di-*O*-acetyl protecting groups. In this case, three main products were isolated following silica gel column chromatography: the known nucleoside **3**[8], **4b**, and **5b** (Scheme 2). The ratio of **4b** to **5b** in this case was 10:6. Their structures were also confirmed by mass spectrometry and NMR spectroscopy. Four acetyl group signals can clearly be seen in the ^{1}H-NMR spectrum of **5b**.

After deprotection of **4b** and **5b**, **6** and **7** were obtained. The formation of trisaccharides as the main by-products was evidently due to the instability of the glycosidic bonds of purine nucleosides **1a**,**b** (and/or those in disaccharide nucleosides **4a**,**b**) in the presence of the Lewis acid. This was confirmed by treatment of **1b** with tin tetrachloride for 16 h at 0°C. After standard work-up and deprotection, a mixture of adenine, adenosine, and **6** was obtained (overall yield 76%, ratio 9:81:10). It should be mentioned that the formation of disaccharide in this case is much slower due to the absence of a nucleobase acceptor (**2**). Therefore, we conclude that the purine nucleoside itself is the source of the additional riboses in **5b**. The formation of by-products could be prevented by using another substrate with a more stable glycosidic bond. We decided to investigate the reactions with 2′,3′-*O*-isopropylidene nucleosides, in view of the fact that this protecting group is stable under glycosylation conditions[9][10]. We first examined the coupling of a pyrimidine nucleoside (2′,3′-*O*-isopropylideneuridine **8a**) with **2**. The protected disaccharide nucleoside **9a** was indeed formed in good yields. After debenzoylation, the formed **10a** was further deprotected in refluxing 70% acetic acid or with Dowex-50 (H^+ form) (Scheme 3). Compound **11a**[7] was isolated in good yield. The acidic hydrolysis needs to be strictly controlled by TLC because of partial cleavage of the *O*-glycosidic bond[7].

Scheme 3



The reaction was then applied to the preparation of purine disaccharide nucleosides according to Scheme 3. The isolated yields of **9b**,**c** were more than 60%. After deprotection with ammonia in methanol, 2′,3′-*O*-isopropylidene derivatives **10b**,**c** were obtained and their structures were confirmed by their NMR spectra. The main differences between the NMR spectra of **10b** and **6** are smaller coupling constants $J_{1',2'}$ and $J_{3',4'}$ and lowfield shifts of 2′-H/3′-H and C-2′/C-3′ of the adenosine residue in the former, reflecting the attachment of the isopropylidene protecting group (Table 1). Further deprotection with 70% acetic acid followed by HPLC separation gave the desired compounds **6** and **11c** in good overall yields. In conclusion, the use of 2′,3′-*O*-isopropylidene protecting groups can prevent the formation of trisaccharide nucleosides during disaccharide nucleoside synthesis, and thus improve the purification procedure and yield of the desired synthesis.

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Experimental Section

NMR spectra were recorded using a Bruker AMX 400 spectrometer at 20°C. Chemical shifts were measured relative to solvent signals. UV spectra were recorded with a Cary-3 Bio UV/Vis spectrophotometer (Varian). Liquid secondary-ion mass spectra (LSIMS) were obtained using a Kratos Concept 1H mass spectrometer (Manchester, UK). Column chromatography was performed on silica gel (70−230 mesh, Aldrich); TLC was carried out on Kieselgel 260 F (Merck) with detection by UV light using the solvent systems: A: CH_2Cl_2; B: CH_2Cl_2/EtOH (99:1); C: CH_2Cl_2/EtOH (98:2); D: CH_2Cl_2/EtOH (95:5); E: CH_2Cl_2/EtOH (75:25); and F: CH_2Cl_2/EtOH (60:40).

NMR Experiments: A Varian 500 Unity spectrometer with a gradient indirect detection probe, operating at 499.6 MHz (^{1}H), was used for NMR experiments on compounds **6**, **7** and **10b** at 27°C. Data were processed with VNMR (Varian) and Felix 0.95 (Biosym Technologies Inc.) on Sun Sparc and Silicon Graphics Inc. workstations, respectively. Gradient double quantum filtered phase-sensitive ^{1}H-^{1}H correlation spectra (GDQFCOPS)[11] consisted of 2048 t_1 increments, each having 4096 data points. A 2000-Hz sweep width was used. The data were zero-filled to 4 K in the t_1 dimension and apodized with a shifted sine bell squared function in both dimensions. ^{1}H-detected ^{1}H-^{13}C heteronuclear correlation spectra (HSQC)[12] were recorded with 512 increments in t_1 and 2 K data points in t_2. The data were apodized with a shifted sine bell squared function in both dimensions. NOEs were measured with a standard (presaturation − −90° − acquire) pulse sequence. A presaturation time of 5 s was used.

9-(5-O-β-D-Ribofuranosyl-β-D-ribofuranosyl)adenine (**6**) *and 9-[5-O-(5-O-β-D-Ribofuranosyl)-β-D-ribofuranosyl-β-D-ribofuranosyl]adenine* (**7**): To a cold (0°C) solution of **2** (908 mg, 1.8 mmol) in 1,2-dichloroethane (15 ml) under nitrogen, tin tetrachloride (0.26 ml, 2.25 mmol) was added and the solution was kept at 0°C for 10 min. After the addition of nucleoside **1a** (869 mg, 1.5 mmol), the resulting mixture was kept at 0°C for a further 2 h. Then, a 10% aqueous solution of sodium bicarbonate (10 ml) was added and the suspension was stirred at 0°C for 20 min. The mixture was subsequently diluted with dichloromethane (30 ml) and filtered through Hyflo Super Cel. The organic layer was separated, washed with water (2 × 10 ml), dried, and concentrated to dryness. The residue was purified by column chromatography on silica gel (100 g). The column was washed with solvent system A (500 ml) and then eluted with system B. UV absorbing fractions were combined and concentrated to dryness to give a mixture of **3**, **4a**, and **5a** (984 mg), R_F = 0.22 (C). The mixture was dissolved in 5 M ammonia in methanol (20 ml), the resulting solution was stored for 3 d at 20°C, and then concentrated to dryness in vacuo. The residue was partitioned between dichloromethane (15 ml) and water (30 ml), and the aqueous layer was washed with dichloromethane (2 × 10 ml). The aqueous layer was then concentrated to dryness. The residue was separated on a Silasorb C-18 semi-preparative reversed-phase

Table 1. ^{13}C- and ^{1}H-chemical shifts and coupling constants in D_2O at 27°C (at 499.6 MHz); nd: the coupling constant could not be determined since it was smaller then the line width

Compound	5′-Rib-Ado (**6**)		5′-Rib-5′-Rib-Ado (**7**)			5′-Rib-IP-Ado (**10b**)	
moiety	Ado	Rib	Ado	Rib1	Rib2	Ado[d]	Rib
^{13}C chemical shifts[a]							
C-2	153.0		153.3			153.2	
C-4	148.9		149.3			148.9	
C-5	118.7		118.9			119.0	
C-6	155.6		155.9			156.1	
C-8	139.9		140.1			140.4	
C-1′	88.0	107.6	88.0	107.6	107.5	90.4	107.8
C-2′	74.1	74.6	74.0	74.4	74.6	83.9	74.5
C-3′	70.3	71.2	70.3	71.5	71.1	82.1	71.1
C-4′	83.2	83.2	83.3	81.5	83.1	85.8	83.3
C-5′	66.9	63.3	66.6	69.6	63.2	68.0	63.3
^{1}H chemical shifts[b][c]							
8-H	8.17		8.23			8.14	
2-H	8.05		8.14			8.10	
1′-H	5.95	4.93	6.00	4.93	4.72	6.10	4.77
2′-H	4.67	3.93	4.72	3.95	3.84	5.39	3.73
3′-H	4.43	3.78	4.48	3.77	4.04	5.03	3.63
4′-H	4.23	3.88	4.23	3.92	3.86	4.47	3.80
5′a-H	3.96	3.61	3.90	3.55	3.66	3.82	3.47
5′b-H	3.49	3.23	3.59	3.03	3.48	3.48	3.17
coupling constants[c]							
1′-2′	4.0	nd	3.9	1.5	1.5	2.5	0.8
2′-3′	4.1	4.9	4.4	4.7	4.7	6.1	4.8
3′-4′	5.3	7.0	6.3	6.8	6.8	2.8	7.0
4′-5′a	2.4	3.0	2.4	2.7	3.4	3.2	3.1
4′-5′b	4.0	7.0	3.3	7.6	6.4	4.9	6.9
5′a-5′b	−11.3	−11.9	−10.7	−11.0	−12.2	−11.2	−12.2

[a] Externally referenced to 10% acetone (v/v) in D_2O, the signals of the acetone methyl groups appeared at δ = 30.70. − [b] Referenced to the water signal at δ = 4.63. − [c] The present data were estimated from first-order data analysis. − [d] Other signals of **10b**: ^{13}C NMR: δ = 115.2 (CMe_2), 26.4 and 24.7 (Me); ^{1}H NMR: δ = 1.55 and 1.35 (Me).

HPLC column (10 × 250 mm, 13 μm) by isocratic elution with 5% acetonitrile in water at a flow rate of 4 ml/min. Compound **7** was eluted after 8.6 min. Yield: 180 mg, R_F = 0.24 (F). − LSIMS: calcd. for $C_{20}H_{29}N_5O_{12}$ + H^+ 532.1891; found 532.1920. − UV (pH = 7−13): λ_{max} = 260 nm; (pH = 1): λ_{max} = 258 nm. Further elution gave **6** after 9.5 min. Yield: 180 mg, R_F = 0.29 (F). − LSIMS: calcd. for $C_{15}H_{21}N_5O_8$ + H^+ 400.1468; found 400.1449. − UV (pH = 7−13): λ_{max} = 260 nm; (pH = 1): λ_{max} = 259 nm. Adenosine was eluted after 14.9 min. Yield: 120 mg, R_F = 0.47 (F).

N^6-Benzoyl-9-[2,3-di-O-acetyl-5-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]adenine (**4b**), *N^6-Benzoyl-9-{2,3-di-O-acetyl-5-O-[2,3-di-O-acetyl-5-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]-β-D-ribofuranosyl}adenine* (**5b**) *and N^6-Benzoyl-9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)adenine* (**3**): To a cold (0°C) solution of **2** (930 mg, 1.85 mmol) in 1,2-dichloroethane (15 ml) under nitrogen, tin tetrachloride (0.27 ml, 2.3 mmol) was added and the solution was kept at 0°C for 10 min. After the addition of nucleoside **1b** (700 mg, 1.54 mmol), the resulting mixture was kept at 0°C for a further 2 h. Then, a 10% aqueous solution of sodium bicarbonate (10 ml) was added and the suspension was stirred at 0°C for 20 min. The mixture was subsequently diluted with dichloromethane (30 ml) and filtered through Hyflo Super Cel. The organic layer was separated, washed with water (2 × 10 ml), dried, and concentrated to dryness. The residue was applied to a column of silica gel (70 g) and the column was washed with toluene (300 ml). Subsequent elution with a mixture of toluene/ethyl acetate (2:1) gave **3** as a foam. Yield: 187 mg (18%). − R_F = 0.23 (C). − ^{1}H NMR ($CDCl_3$): δ = 8.68 (s, 1 H, 8-H), 8.27 (s, 1 H, 2-H), 8.12−7.90 (m, 8 H, Bz), 7.64−7.34 (m, 12 H, Bz), 6.52 (d, 1 H, $J_{1',2'}$ = 5.4 Hz, 1′-H), 6.42 (dd, 1 H, $J_{2',3'}$ = 5.5 Hz, 2′-H), 6.26 (t, 1 H, $J_{3',4'}$ = 5.5 Hz, 3′-H), 4.95 (dd, 1 H, $J_{5'a,4'}$ = 3.2 Hz, $J_{5'a,5'b}$ = −12.2 Hz, 5′a-H), 4.87 (ddd, 1 H, 4′-H), 4.73 (dd, 1 H, $J_{5'b,4'}$ = 4.2 Hz, 5′b-H).

Elution with toluene/ethyl acetate (1:1) produced **4b** as a foam. Yield: 405 mg (29%). − R_F = 0.16 (C). − LSIMS: calcd. for $C_{47}H_{41}N_5O_{14}$ + H^+ 900.2728; found 900.2726. − ^{1}H NMR ($CDCl_3$): δ = 8.84 (s, 1 H, 8-H), 8.41 (s, 1 H, 2-H), 8.05−7.87 (m, 8 H, Bz), 7.62−7.30 (m, 12 H, Bz), 6.25 (d, 1 H, $J_{1',2'}$ = 5.2 Hz, 1′-H Ado), 5.98 (dd, 1 H, $J_{2',3'}$ = 5.4 Hz, 2′-H Ado), 5.79 (dd, 1 H, $J_{3',2'}$ = 4.9 Hz, $J_{3',4'}$ = 6.9 Hz, 3′-H Rib), 5.70 (m, 2 H, 3′-H Ado, 2′-H Rib), 5.36 (s, 1 H, 1′-H Rib), 4.75 (ddd, 1 H, $J_{4',5'a}$ = 4.0 Hz, $J_{4',5'b}$ = 5.9 Hz, 4′-H Rib), 4.65 (dd, 1 H, $J_{5'a,5'b}$ = −12.0 Hz, 5′a-H Rib), 4.59 (dd, 1 H, 5′b-H Rib), 4.42 (ddd, 1 H, $J_{4',3'}$ = 4.7 Hz, $J_{4',5'a}$ = 2.9 Hz, $J_{4',5'b}$ = 5.0 Hz, 4′-H Ado), 4.16 (dd, 1 H, $J_{5'a,5'b}$ = −11.4 Hz, 5′a-H Ado), 3.93 (dd, 1 H, 5′b-H Ado), 2.13 (s, 3 H, Ac), 2.06 (s, 3 H, Ac). − ^{13}C NMR ($CDCl_3$): δ = 169.65, 169.34, 166.13, 165.33, 165.17 and 164.68 (C=O), 152.58 (C-6), 151.55 (C-2), 149.60 (C-4), 141.77 (C-8), 133.56, 133.46, 133.07, 129.79, 129.74, 129.66, 128.81, 128.48, 128.37, 128.32 and 128.01 (Bz), 122.34 (C-5), 106.20 (C-1′ Rib), 86.34 (C-1′ Ado), 81.74 (C-4′ Ado), 79.33 (C-4′ Rib), 75.17 (C-2′ Ado), 73.22 (C-2′ Rib), 72.05 (C-3′ Ado), 70.65 (C-3′ Rib), 67.70 (C-5′ Ado), 64.83 (C-5′ Rib), 20.55 (Me), 20.37 (Me).

Further elution with the same solvent system gave **5b** as a foam. Yield 299 mg (17%), R_F = 0.15 (C). − LSIMS: calcd. for $C_{56}H_{53}N_5O_{20}$ + H^+ 1116.3362; found 1116.3346. − ^{1}H NMR ($CDCl_3$): δ = 8.82 (s, 1 H, 8-H), 8.67 (s, 1 H, 2-H), 8.09−7.69 (m, 8 H, Bz), 7.54−7.22 (m, 12 H, Bz), 6.37 (d, 1 H, $J_{1',2'}$ = 6.2 Hz, 1′-H Ado), 5.85 (m, 2 H, 2′-H Ado, 3′-H Rib), 5.79 (d, 1 H, $J_{2',3'}$ = 4.9 Hz, 2′-H Rib), 5.61 (dd, 1 H, $J_{3',2'}$ = 5.3 Hz, $J_{3',4'}$ = 3.9 Hz, 3′-H Ado), 5.35 (s, 1 H, 1′-H Rib), 5.27 (m, 2 H, 2′-H, 3′-H Rib), 5.12 (s, 1 H, 1′-H Rib), 4.68 (m, 2 H, 4′-H, 5′a-H Rib), 4.60 (dd, 1 H, $J_{5'b,4'}$ = 6.5 Hz, $J_{5'a,5'b}$ = −11.8 Hz, 5′b-H Rib), 4.40 (m, 1 H, 4′-H Ado), 4.34 (m, 1 H, 4′-H Rib), 4.12 (dd, 1 H, $J_{5'a,4'}$ = 2.4 Hz, $J_{5'a,5'b}$ = −11.8 Hz, 5′a-H Ado), 4.06 (dd, 1 H, $J_{5'a,4'}$ = 2.6 Hz, $J_{5'a,5'b}$ = −10.8 Hz, 5′a-H Rib), 3.88 (dd, 1 H, $J_{5'b,4'}$ = 2.9 Hz, 5′b-H Ado), 3.66 (dd, 1 H, $J_{5'b,4'}$ = 7.1 Hz, $J_{5'b,5'a}$ = −10.8 Hz, 5′b-H Rib), 2.13 (s, 3 H, Ac), 2.10 (s, 3 H, Ac), 2.02 (s, 3 H, Ac), 1.98 (s, 3 H, Ac). − ^{13}C NMR ($CDCl_3$): δ = 169.76, 169.37, 166.15, 165.27, and 165.08 (C=O), 152.50 (C-6), 151.35 (C-2), 149.61 (C-4), 141.80 (C-8), 133.69, 133.46, 133.36, 133.07, 129.66, 128.95, 128.61, 128.42, 128.35, and 128.18 (Bz), 122.68 (C-5), 106.53 and 105.68 (C-1′ Rib), 85.00 (C-1′ Ado), 82.10 (C-4′ Ado), 79.71 and 79.34 (C-4′ Rib), 75.46, 74.42 and 73.32 (C-2′ Ado, C-2′ Rib), 72.48 and 70.85, 70.83 (C-3′ Ado, C-3′ Rib), 68.70 (C-5′ Ado), 67.91 and 65.29 (C-5′ Rib), 20.59 (Me), 20.32 (Me).

Treatment of **1b** *with Tin Tetrachloride:* To a solution of nucleoside **1b** (100 mg, 0.22 mmol) in 1,2-dichloroethane (3 ml) under nitrogen, tin tetrachloride (0.04 ml, 0.33 mmol) was added and the mixture was kept at 0°C for 16 h. Then, a 10% aqueous solution of sodium bicarbonate (5 ml) was added and the suspension was stirred at 0°C for 20 min. The mixture was diluted with dichloromethane (15 ml) and filtered through Hyflo Super Cel. The organic layer was separated, washed with water (2 × 5 ml), dried, and concentrated to dryness. The residue was dissolved in 5 M ammonia in methanol (5 ml) and the resulting solution was stored for 3 d at 20°C, then concentrated to dryness in vacuo. The residue was partitioned between dichloromethane (5 ml) and water (15 ml), and the aqueous layer was washed with dichloromethane (2 × 5 ml). The aqueous layer was then concentrated to dryness. The total yield, 76%, was estimated by UV spectrophotometry (A_{260} = 2340 o.u.). The mixture was analyzed by reversed-phase HPLC on a Nucleosil C-18 column (4 × 250 mm, 5 μm) using a concentration gradient of 0−10% acetonitrile in 0.05 M sodium acetate, pH = 4.2, with a flow rate of 1 ml/min. According to HPLC analysis, the ratio of adenine (t_R = 15.8 min) to nucleoside **6** (t_R = 17.3 min) to adenosine (t_R = 19.5 min) was 0.09:0.10:0.81.

1-[2,3-Isopropylidene-5-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]uracil (**9a**): To a cold (0°C) solution of **2** (1.21 g, 2.4 mmol) in 1,2-dichloroethane (30 ml) under nitrogen, tin tetrachloride (0.35 ml, 3.0 mmol) was added and the solution was kept at 0°C for 10 min. After the addition of nucleoside **8a** (569 mg, 2.0 mmol), the resulting mixture was kept at 0°C for 2 h. After standard work-up, the residue was purified by column chromatography on silica gel (100 g). The column was washed with solvent system A (500 ml) and then eluted with system B to give **9a** as a foam. Yield: 918 mg (63%). − R_F = 0.49 (D). − LSIMS: calcd. for $C_{38}H_{36}N_2O_{13}$: 729.2296; found 729.2291. − ^{1}H NMR ($CDCl_3$): δ = 9.10 (br. s, 1 H, NH), 8.04−7.89 (m, 6 H, Bz), 7.60−7.32 (m, 10 H, 6-H, Bz), 5.85 (d, 1 H, $J_{5,6}$ = 8.1 Hz, 5-H), 5.82 (dd, 1 H, $J_{3',2'}$ = 4.8 Hz, $J_{3',4'}$ = 7.4 Hz, 3′-H Rib), 5.79 (d, 1 H, $J_{1',2'}$ = 2.3 Hz, 1′-H Urd), 5.71 (d, 1 H, 2′-H Rib), 5.31 (s, 1 H, 1′-H Rib), 4.88 (dd, 1 H, $J_{2',3'}$ = 6.5 Hz, 2′-H Urd), 4.81 (dd, 1 H, $J_{3',4'}$ = 4.1 Hz, 3′-H Urd), 4.77 (ddd, 1 H, $J_{4',5'a}$ = 4.0 Hz, $J_{4',5'b}$ = 5.6 Hz, 4′-H Rib), 4.67 (dd, 1 H, $J_{5'a,5'b}$ = −11.9 Hz, 5′a-H Rib), 4.58 (dd, 1 H, 5′b-H Rib), 4.30 (ddd, 1 H, $J_{4',5'a}$ = 3.2 Hz, $J_{4',5'b}$ = 5.4 Hz, 4′-H Urd), 4.11 (dd, 1 H, $J_{5'a,5'b}$ = −11.1 Hz, 5′a-H Urd), 3.73 (dd, 1 H, 5′b-H Urd), 1.54 (s, 3 H, Me), 1.34 (s, 3 H, Me). − ^{13}C NMR ($CDCl_3$): δ = 166.15, 165.33 and 165.21 (C=O), 163.10 (C-4), 149.93 (C-2), 141.52 (C-6), 133.59, 133.46, 133.20, 129.76, 129.73, 129.43, 128.94, 128.68, 128.50 and 128.35 (Bz), 114.61 (*C*-Me_2), 105.81 (C-1′ Rib), 102.88 (C-5), 93.09 (C-1′ Urd), 85.34 (C-4′ Urd), 84.38 (C-4′ Rib), 80.36 (C-2′ Urd), 78.89

(C-3′ Urd), 75.18 (C-2′ Rib), 71.95 (C-3′ Rib), 67.93 (C-5′ Urd), 64.59 (C-5′ Rib), 27.11 (Me), 25.27 (Me).

N^6-Benzoyl-9-[2,3-isopropylidene-5-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]adenine (**9b**): Analogous condensation of **2** (1.21 g, 2.4 mmol) with **8b** (823 mg, 2.0 mmol) in the presence of tin tetrachloride (0.35 ml, 3.0 mmol) in 1,2-dichloroethane (15 ml) at 0°C for 1.5 h gave **9b** as a foam. Yield: 1.10 g (64%). − R_F = 0.48 (D). − LSIMS: calcd. for $C_{46}H_{41}N_5O_{12}$ + H^+ 856.2830; found 856.2885. − ^{1}H NMR ($CDCl_3$): δ = 9.48 (br. s, 1 H, NH), 8.80 (s, 1 H, 8-H), 8.25 (s, 1 H, 2-H), 8.01−7.76 (m, 8 H, Bz), 7.60−7.22 (m, 12 H, Bz), 6.18 (d, 1 H, $J_{1',2'}$ = 1.9 Hz, 1′-H Ado), 5.71 (dd, 1 H, $J_{3',2'}$ = 5.0 Hz, $J_{3',4'}$ = 6.4 Hz, 3′-H Rib), 5.55 (d, 1 H, 2′-H Rib), 5.41 (dd, 1 H, $J_{2',3'}$ = 6.2 Hz, 2′-H Ado), 5.22 (s, 1 H, 1′-H Rib), 5.00 (dd, 1 H, $J_{3',4'}$ = 2.9 Hz, 3′-H Ado), 4.71−4.49 (m, 4 H, 4′-H Ado, 4′-, 5′a-, 5′b-H, Rib), 4.09 (dd, 1 H, $J_{5'a,4'}$ = 3.6 Hz, $J_{5'a,5'b}$ = −11.1 Hz, 5′a-H Ado), 3.72 (dd, 1 H, $J_{5'b,4'}$ = 5.6 Hz, 5′b-H Ado), 1.59 (s, 3 H, Me), 1.37 (s, 3 H, Me). − ^{13}C NMR (D_2O): δ = 166.11, 165.32 and 165.13 (C=O), 155.64 (C-6), 153.05 (C-2), 150.36 (C-4), 140.21 (C-8), 133.51, 133.40, 133.15, 129.80, 129.75, 129.59, 129.05, 129.93, 128.47 and 128.35 (Bz), 118.55 (C-5), 114.78 (*C*-Me_2), 107.54 (C-1′ Rib), 90.12 (C-1′ Ado), 86.45 (C-4′ Ado), 83.96 (C-4′ Rib), 82.92 (C-2′ Ado), 81.31 (C-3′ Ado), 75.71 (C-2′ Rib), 70.52 (C-3′ Rib), 68.32 (C-5′ Ado), 62.69 (C-5′ Rib), 27.14 (Me), 25.28 (Me).

N^2-Isobutyryl-9-[2,3-isopropylidene-5-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]guanine (**9c**): Analogous condensation of **2** (656 mg, 1.3 mmol) with **8c** (392 mg, 1.0 mmol) in the presence of tin tetrachloride (0.164 ml, 1.4 mmol) in 1,2-dichloroethane (10 ml) at 0°C for 4 h gave **9c** as a foam. Yield: 550 mg (64%). − R_F = 0.15 (D). − LSIMS: calcd. for $C_{43}H_{43}N_5O_{13}$ + H^+ 838.2936; found 838.2929 (M + H^+). − ^{1}H NMR ($CDCl_3$): δ = 12.15 (br. s, 1 H, NH), 9.49 (s, 1 H, 8-H), 8.06−7.89 (m, 6 H, Bz), 7.60−7.27 (m, 9 H, Bz), 5.89 (d, 1 H, $J_{1',2'}$ = 2.6 Hz, 1′-H Guo), 5.80 (dd, 1 H, $J_{3',2'}$ = 5.1 Hz, $J_{3',4'}$ = 6.6 Hz, 3′-H Rib), 5.71 (d, 1 H, 2′-H Rib), 5.31 (s, 1 H, 1′-H Rib), 5.20 (dd, 1 H, $J_{2',3'}$ = 6.2 Hz, 2′-H Guo), 4.94−4.72 (m, 3 H, 3′-H Guo, 4′-, 5′a-H Rib), 4.59 (dd, 1 H, $J_{5'b,4'}$ = 6.0 Hz, $J_{5'b,5'a}$ = −11.7 Hz, 5′b-H Rib), 4.37 (m, 1 H, 4′-H Guo), 4.08 (dd, 1 H, $J_{5'a,4'}$ = 7.2 Hz, $J_{5'a,5'b}$ = −10.3 Hz, 5′a-H Guo), 3.72 (dd, 1 H, $J_{5'b,4'}$ = 5.2 Hz, 5′b-H Guo), 2.67 (sept, 1 H, *J* = 6.8 Hz, CH, *i*Bu), 1.56 (s, 3 H, Me), 1.27 (s, 3 H, Me), 1.21 (d, 3 H, Me, *i*Bu), 1.18 (d, 3 H, Me, *i*Bu). − ^{13}C NMR (D_2O): δ = 169.73, 167.77, 165.27 (C=O), 157.95 (C-6), 148.03 (C-2), 147.48 (C-4), 138.98 (C-8), 133.93, 133.48, 129.73, 129.69, 128.99, 128.83, 128.64, 128.45 and 128.39 (Bz), 120.50 (C-5), 114.65(*C*-Me_2), 106.84 (C-1′ Rib), 89.97 (C-1′ Guo), 85.23 (C-4′ Guo), 83.41 (C-4′ Rib), 82.14 (C-2′ Guo), 81.59 (C-3′ Guo), 74.62 (C-2′ Rib), 71.02 (C-3′ Rib), 66.98 (C-5′ Guo), 62.84 (C-5′ Rib), 36.05 (CH, *i*Bu), 27.62 (Me), 25.19 (Me), 19.13 (Me, *i*Bu), 18.85 (Me, *i*Bu).

1-(2,3-Isopropylidene-5-O-β-D-ribofuranosyl-β-D-ribofuranosyl)-uracil (**10a**): A solution of nucleoside **9a** (918 mg, 1.26 mmol) in 5 M ammonia in methanol (10 ml) was kept for 3 d at 20°C and then concentrated to dryness in vacuo. The residue was partitioned between dichloromethane (15 ml) and water (30 ml), and the aqueous layer was washed with dichloromethane (2 × 10 ml). The aqueous layer was then concentrated to dryness and the residue was treated with methanol, which was evaporated to leave **10a** as a foam. Yield: 420 mg (80%). − R_F = 0.80 (E). − LSIMS: calcd. for $C_{17}H_{24}N_2O_{10}$ + H^+ 417.1509; found 417.1526. − ^{1}H NMR (D_2O): δ = 7.72 (d, 1 H, $J_{6,5}$ = 8.1 Hz, 6-H), 5.83 (d, 1 H, 5-H), 5.81 (d, 1 H, $J_{1',2'}$ = 2.3 Hz, 1′-H Urd), 5.03 (dd, 1 H, $J_{2',3'}$ = 6.2 Hz, 2′-H Urd), 4.98 (s, 1 H, 1′-H Rib), 4.89 (dd, 1 H, $J_{3',4'}$ = 3.2 Hz, 3′-H Urd), 4.48 (ddd, 1 H, $J_{4',5'a}$ = 3.0 Hz, $J_{4',5'b}$ = 6.3 Hz, 4′-H Urd), 4.10 (dd, 1 H, $J_{3',2'}$ = 4.7 Hz, $J_{3',4'}$ = 7.0 Hz, 3′-H Rib), 4.00 (m, 3 H, 2′-, 4′-, 5′a-H Rib), 3.77 (dd, 1 H, $J_{5'a,5'b}$ = −12.3 Hz, 5′a-H Urd), 3.63 (dd, 1 H, $J_{5'b,4'}$ = 5.5 Hz, $J_{5'b,5'a}$ = −11.3 Hz, 5′b-H Rib), 3.57 (dd, 1 H, 5′b-H Urd), 1.56 (s, 3 H, Me), 1.37 (s, 3 H, Me). − ^{13}C NMR (D_2O): δ = 167.23 (C-4), 152.04 (C-2), 143.97 (C-6), 115.46 (*C*-Me_2), 108.22 (C-1′ Rib), 102.32 (C-5), 94.38 (C-1′ Urd), 86.27 (C-4′ Urd), 85.30 (C-4′ Rib), 83.68 (C-2′ Urd), 81.67 (C-3′ Urd), 75.13 (C-2′ Rib), 71.45 (C-3′ Rib), 68.62 (C-5′ Urd), 63.37 (C-5′ Rib), 26.86 (Me), 25.10 (Me).

9-(2,3-Isopropylidene-5-O-β-D-ribofuranosyl-β-D-ribofuranosyl)adenine (**10b**): Analogous debenzoylation of **9b** (630 mg, 0.74 mmol) and evaporation of methanol from the product yielded **10b** as a foam. Yield: 270 mg (83%). − R_F = 0.53 (E). − LSIMS: calcd. for $C_{18}H_{25}N_5O_8$ + H 440.1781; found 440.1752.

9-(2,3-Isopropylidene-5-O-β-D-ribofuranosyl-β-D-ribofuranosyl)guanine (**10c**): Analogous debenzoylation of **9c** (500 mg, 0.6 mmol) and evaporation of methanol from the product yielded **10c** as a hygroscopic foam. Yield: 220 mg (81%). − R_F = 0.30 (E). − LSIMS: calcd. for $C_{18}H_{25}N_5O_9$ + H^+ 456.1731; found 456.1725. − ^{1}H NMR (D_2O): δ = 7.87 (s, 1 H, 8-H), 6.02 (d, 1 H, $J_{1',2'}$ = 1.4 Hz, 1′-H Guo), 5.40 (dd, 1 H, $J_{2',3'}$ = 6.0 Hz, 2′-H Guo), 5.10 (dd, 1 H, $J_{3',4'}$ = 2.8 Hz, 3′-H Guo), 4.89 (s, 1 H, 1′-H Rib), 4.50 (m, 1 H, 4′-H Guo), 3.89 (m, 2 H, 5′a-H Guo, 4′-H Rib), 3.84 (d, 1 H, $J_{2',3'}$ = 4.6 Hz, 2′-H Rib), 3.77 (dd, 1 H, $J_{3',4'}$ = 7.0 Hz, 3′-H Rib), 3.60 (dd, 1 H, $J_{5'a,4'}$ = 2.9 Hz, $J_{5'a,5'b}$ = −12.1 Hz, 5′a-H Rib), 3.54 (dd, 1 H, $J_{5'b,4'}$ = 4.9 Hz, $J_{5'b,5'a}$ = −11.4 Hz, 5′b-H Guo), 3.32 (dd, 1 H, $J_{5'b,4'}$ = 7.0 Hz, 5′b-H Rib), 1.59 (s, 3 H, Me), 1.40 (s, 3 H, Me). − ^{13}C NMR (D_2O): δ = 159.76 (C-6), 148.30 (C-2), 146.07 (C-4), 138.61 (C-8), 121.70 (C-5), 115.59 (*C*-Me_2), 108.38 (C-1′ Rib), 90.87 (C-1′ Guo), 86.65 (C-4′ Guo), 84.75 (C-4′ Rib), 83.94 (C-2′ Guo), 82.19 (C-3′ Guo), 75.22 (C-2′ Rib), 71.72 (C-3′ Rib), 68.67 (C-5′ Guo), 64.04 (C-5′ Rib), 27.02 (Me), 25.39 (Me).

1-(5-O-β-D-Ribofuranosyl-β-D-ribofuranosyl)uracil (**11a**): Method A: A solution of **10a** (63 mg, 0.15 mmol) in 70% acetic acid (6 ml) was kept at 90°C for 1 h. The reaction mixture was concentrated in vacuo and the residue was dried by repeated evaporation of 2-propanol (4 × 5 ml). The product was purified on a Silasorb C-18 semi-preparative reversed-phase HPLC column (10 × 250 mm, 13 μm) by isocratic elution with 5% acetonitrile in water at a flow rate of 4 ml/min. The peak eluted at t_R = 8.2 min was collected and concentrated to dryness. The residue was treated with methanol, which was evaporated to leave **11a** as a foam. Yield: 35 mg (62%). − R_F = 0.64 (F).

Method B: To a solution of **10a** (118 mg, 0.28 mmol) in water (5 ml) was added Dowex-50 (H^+ form) (0.2 ml) and the mixture was kept at 70°C for 40 min. The resin was then filtered off and washed with water. The combined filtrate and washings were concentrated to dryness and the residue was purified according to Method A. Yield: 90 mg (85%). UV, mass and NMR spectra were identical to spectral data obtained for previously prepared nucleoside **11a**[7].

9-(5-O-β-D-Ribofuranosyl-β-D-ribofuranosyl)adenine (**6**): A solution of **10b** (100 mg, 0.228 mmol) in 70% acetic acid (6 ml) was kept at 90°C for 3 h. The reaction mixture was then concentrated in vacuo and the residue was dried by repeated evaporation of 2-propanol (4 × 5 ml). The product was purified by HPLC according to the procedure for the preparation of **11a** (Method A). The peak eluted at t_R = 9.5 min was collected and concentrated to dryness. The residue was treated with methanol, which was evaporated to leave **6** as a foam. Yield: 68 mg (65%).

9-(5-O-β-D-Ribofuranosyl-β-D-ribofuranosyl)guanine (**11c**): A solution of **10c** (200 mg, 0.44 mmol) in 70% acetic acid (15 ml) was kept at 90°C for 2 h. The solution was then concentrated in vacuo and the residue was dried by repeated evaporation of 2-propanol (4 × 10 ml). The product was purified by HPLC according to the procedure for the preparation of **11a** (Method A). The peak eluted at t_R = 8.0 min. was collected and concentrated to dryness. The residue was treated with methanol, which was evaporated to leave **11c** as a foam. Yield: 100 mg (55%). – R_F = 0.23 (F). – LSIMS: calcd. for $C_{15}H_{21}N_5O_9$ + H^+ 416.1418; found 416.1420 (M + H^+). – UV (pH = 1): λ_{max} = 256 nm; (pH = 7): λ_{max} = 253 nm; (pH = 13): λ_{max} = 263 nm. – ^{1}H NMR (D_2O): δ = 7.88 (s, 1 H, 8-H), 5.81 (d, 1 H, $J_{1',2'}$ = 3.5 Hz, 1′-H Guo), 4.98 (s, 1 H, 1′-H Rib), 4.68 (dd, 1 H, $J_{2',3'}$ = 5.1 Hz, 2′-H Guo), 4.49 (dd, 1 H, $J_{3',4'}$ = 5.4 Hz, 3′-H Guo), 4.23 (m, 1 H, 4′-H Guo), 4.00 (m, 2 H, 5′a-H Guo, 2′-H Rib), 3.93 (ddd, 1 H, $J_{4',3'}$ = 6.2 Hz, $J_{4',5'a}$ = 2.4 Hz, $J_{4',5'b}$ = 7.1 Hz, 4′-H Rib), 3.85 (dd, 1 H, $J_{3',2'}$ = 5.1 Hz, 3′-H Rib), 3.64 (dd, 1 H, $J_{5'b,4'}$ = 3.9 Hz, $J_{5'b,5'a}$ = −11.4 Hz, 5′b-H Guo), 3.59 (dd, 1 H, $J_{5'a,5'b}$ = −12.1 Hz, 5′a-H Rib), 3.33 (dd, 1 H, 5′b-H Rib). – ^{13}C NMR (D_2O): δ = 158.26 (C-6), 153.43 (C-2), 151.09 (C-4), 136.80 (C-8), 115.68 (C-5), 106.90 (C-1′ Rib), 87.28 (C-1′ Guo), 82.68 (C-4′ Guo), 82.48 (C-4′ Rib), 74.00 (C-2′ Guo), 73.39 (C-2′ Rib), 70.65 (C-3′ Guo), 69.67 (C-3′ Rib), 66.16 (C-5′ Guo), 62.82 (C-5′ Rib).

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