Synthesis and Properties of *O*-β-D-ribofuranosyl-(1″ → 2′)-guanosine-5″-*O*-phosphate and Its Derivatives

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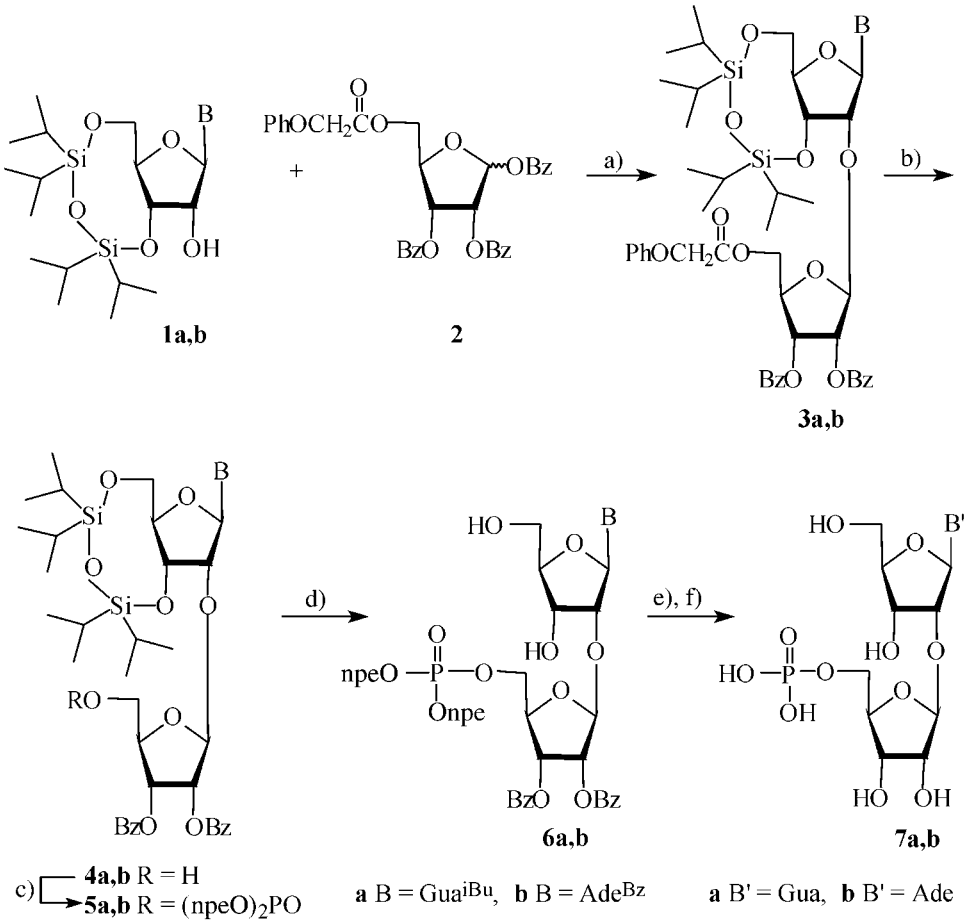
Dedicated to Professor *Wolfgang Pfleiderer* on the occasion of his 75th birthday

The efficient synthesis of *O*-β-D-ribofuranosyl-(1″ → 2′)-guanosine-5″-*O*-phosphate and *O*-β-D-ribofuranosyl-(1″ → 2′)-adenosine-5″-*O*-phosphate, minor tRNA components, have been developed, and their conformational properties were examined by NMR spectroscopy.

1. Introduction. – Nucleic acids consist of over 100 modified nucleosides, in addition to eight major ribo- and deoxyribonucleosides. Although a few modifications have been found in DNA, 81 modified nucleosides were found in tRNAs and 15 were found in other RNAs [1][2]. Most of the minor ribonucleosides have modified heterocyclic bases. Only two types of sugar-modified nucleosides were found in RNA, namely 2′-*O*-Me and 2′-*O*-β-D-ribofuranosyl derivatives. The last nucleotides *O*-β-D-ribofuranosyl-(1″ → 2′)-guanosine (and adenosine)-5″-*O*-phosphate (Grp and Arp) were isolated from initiator tRNAs of yeasts [3–5]. It was shown that this modification controlled the discrimination between their initiator *vs.* elongator function [4]. It should be mentioned that the charged components are usually located in tRNA loops, but Grp and Arp are located in position 64 of the stem region of T loop [6]. X-Ray analysis demonstrated that the additional phosphorylated ribofuranose moiety is located in the minor groove so that the 5″-phosphoryl group interacts with the 2-amino group of the neighboring guanosine residue [7]. The same position of the additional ribose moiety was found in solution of the self-complementary decaribonucleotide 5′r(GCGA*AUUCGC)-3′ containing 2′-*O*-β-D-ribofuranosyl-adenosine by NMR spectroscopy [8]. It was also shown that this modification has no effect on the thermal stability of the duplex [8].

2. Results and Discussion. – We have developed a general method for the preparation of disaccharide nucleosides [9–13]. Recently, 2′-*O*-β-D-ribofuranosyl-adenosine was prepared by condensation of N^6,3′,5′-*O*-protected adenosine **1b** with an excess of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in the presence of $SnCl_4$ and then converted into *O*-β-D-ribofuranosyl-(1″ → 2′)-adenosine-5″-*O*-phosphate (**7b**) in overall yield of 9% [14][15] (*Scheme*). The selective blocking of five OH groups in

Scheme



a) $SnCl_4$/$ClCH_2CH_2Cl$, 0°. b) 0.1M K_2CO_3 in MeOH. c) bis[2-(4-nitrophenyl)ethyl]phosphate/TPSCl/1-methyl-1*H*-imidazole/Py. d) Bu_4NF/THF. e) DBU/Py. f) NH_3/MeOH.

2′-*O*-*β*-D-ribofuranosyl-nucleosides is an essential strategy in the preparation of the title compound. Previously, the per-*O*-benzoylated disaccharide nucleoside was fully deprotected, tritylated at the 5″-position, acetylated, and detritylated, and the 5″-OH group (as in **4**) of the additional ribose residue was further phosphorylated [15]. This protocol is rather lengthy, but it can be shortened and simplified, when differentiatively blocked carbohydrates are utilized for *O*-glycosylation. Here, we present our results on the preparation of *O*-*β*-D-ribofuranosyl-(1″ → 2′)-guanosine-5″-*O*-phosphate (**7a**). To compare both methods and to develop efficient preparation of suitable synthons for oligonucleotide synthesis, the corresponding adenosine derivative was also prepared.

Our attempts to use 1,2,3-tri-*O*-benzoyl-D-ribofuranose and its 5-*O*-monomethoxytrityl derivative [16] resulted in a complex mixture with the formation of the desired disaccharide product in very low yield. The same results were obtained with 1,2,3-tri-*O*-

acetyl-5-*O*-bis[2-(4-nitrophenyl)ethyl]phosphorylyl-D-ribofuranose [17]. It should be mentioned that this synthon was used for the preparation of 5′-nucleotides under conditions developed by *Vorbruggen* [18] and for glycosylation of secondary OH groups in 2′-deoxynucleosides [19]. Therefore, we investigated the use of another synthon **2** for the glycosylation reaction of the hindered secondary OH group in nucleoside **1**, *i.e.*, the use of a 5-*O*-phenoxyacetyl group, which is 300 times less stable under alkaline conditions than the Bz group [20][21].

For the preparation of **2**, readily available in three steps from D-ribose, 1,2,3-tri-*O*-benzoyl-D-ribofuranose [16] was chosen as a starting compound. Anomers of **2**, which may be easily separated on silica-gel column, were used for the next glycosylation step under standard conditions [9–13]. The *O*-glycosylation reaction proceed stereospecifically with the formation of β-anomers **3a**,**b**, in which the coupling constants ($J(1',2')$) in the additional ribofuranose moiety are less than 0.5 Hz. Selective deblocking of the phenoxyacetyl group was achieved with 0.1M K_2CO_3 in MeOH. The overall yields for the preparation of **4a**,**b** were 46–38%, higher then the earlier multistep conversion mentioned above (26%) [15]. For the protection of the additional phosphate residue, the 2-(4-nitrophenyl)ethyl (npe) group [22][23] was chosen. Phosphorylation with bis[2-(4-nitrophenyl)ethyl]phosphate in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) and 1-methyl-1*H*-imidazole gave phosphotriesters **5a**,**b** in good yields. After desilylation [15], **6a**,**b** were converted *via* its dimethoxytrityl derivatives to the corresponding phosphoramidites. Their incorporation into oligonucleotides will be published shortly. Subsequent cleavage of the npe groups in triester **6a** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) [22][23] in dry pyridine followed by deacylation with NH_3 in MeOH gave free monophosphate **7a** in high overall yield (18% starting from **1a**). The structures of the compounds were supported by NMR spectroscopy and mass spectrometry. The chemical shifts were assigned by double-resonance techniques and COSY experiments. It should be mentioned that the ^{1}H-NMR spectra of the compouds obtained are rather complicated due to the presence of two ribofuranose residues and diastereotopic protons of the npe $(CH_2)_2$ group in phosphates **5a**,**b** and **6a**,**b**. Several conclusions were drawn from the ^{1}H-NMR analysis. In disaccharides nucleosides **3a**,**b** and **4a**,**b** with the 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl group, the coupling constants $J(1',2')$ of both ribose and nucleoside moieties are less than 0.5 Hz.

The removal of the silyl group and introduction of a bulky substituent at the 5′-*O*-position of the additional ribofuranosyl residue resulted in significant conformational changes. The presence of bis[2-(4-nitrophenyl)ethyl]phosphoryl groups in disaccharides **5a**,**b** and **6a**,**b** increases the coupling constant $J(1',2')$ in the ribofuranosyl residue up to 1.9 Hz. In ^{13}C-NMR spectra of these derivatives, the vicinal coupling constants $J(C(4),P)$ are larger than the geminal $J(C(5),P)$ ones [24].

The conformation of a five-membered ring can be completely described by two independent parameters: phase angle and puckering amplitude. Since the puckering amplitude always remains within a narrow range and the pseudorotational angle can adopt values ranging from 0 to 360 degrees, the different conformations are mostly visualized by using the pseudorotational wheel.

It is widely accepted that furanose rings, which are flexible entities, will show a high-speed equilibrium between two low-energy conformations in solution. One of those

conformations is situated in the northern hemisphere of the pseudorotational wheel (the so called *N* conformer), and the other in the southern hemisphere (the *S* conformer) (*Fig. 1*). A complete description of this system now requires five independent parameters: four parameters describing the conformers and one parameter describing the fraction of one of the conformers.

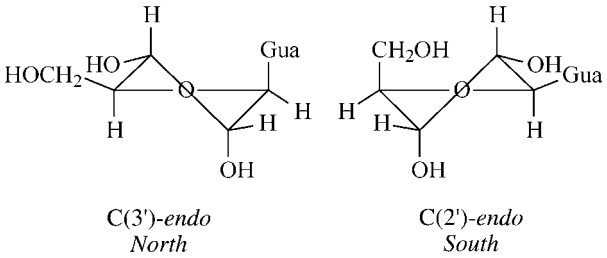


Fig. 1. *N and S conformations of a ribose*

The two conformers and their relative abundance can be resolved by NMR spectroscopy, through measurement of the ^3J(H,H) coupling constants. Because the equilibrium, as mentioned above, can be considered as fast on the NMR time-scale, average coupling constants and average chemical shifts will be observed. Assuming the two-state model described above, it is possible to find the conformations and their populations that best fit the experimentally observed ^3J(H,H) coupling constants [25][26].

A 2D-TOCSY experiment [27][28] was performed for the assignment of all signals (*Table 1*). The presence of a phosphate group was confirmed by ^{31}P-NMR spectroscopy, and its correct attachment to the molecule was evident from a characteristic downfield shift of $H_a-C(5')$ and $H_b-C(5')$ of the ribose moiety and their couplings with the P-atom (J(H,P)). The correct glycosidic linkage of C(1′) of the ribose moiety with C(2′) of the nucleoside moiety could be confirmed by a NOE contact between H−C(1′) of the ribose and H−C(2′) of the nucleoside.

A high-resolution 1D ^{1}H-NMR spectrum was used to measure the coupling constants. The ^3J(H,H) coupling constants of both ribose moieties, determined from spectra recorded at 292 K, are presented in *Table 2*. Analysis of these data with Pseurot 6.2 [29] generated the results shown in *Table 3*.

It was shown that the additional ribose moiety of **7a** will appear predominantly in its *N* conformation, which we could expect because of stereoelectronic effects due to the 2′-OH group [30]. The ribose ring of the nucleoside shows a remarkably high percentage of *S* conformer (58%), where the large group that is attached to C(2′) occurs in an energetically favorable equatorial-like position. This high percentage of the *S* conformer can also be explained by the *syn*-orientation about the N-glycosidic bond, which is preferred by guanosine nucleosides, and which drives the equilibrium towards the *S* conformer due to steric and stereoelectronic effects and intramolecular H-bond formation [31].

To compare the structural characteristics of Guo2′Rib5′p (**7a**) with Ade2′Rib5′p (**7b**), described earlier by *Rodionov et al.* [15], we performed a Pseurot 6.2 analysis

Table 1. *Measured Chemical Shifts* [ppm] *in the Ribose and Nucleoside Moieties of* **7a** *and* **7b**

	Grp (**7a**)		Arp (**7b**)	
Nucleus	Ribose moiety	Nucleoside moiety	Ribose moiety	Nucleoside moiety
H–C(1′)	5.02 (*d*)	6.01 (*d*)	5.02 (*d*)	6.25 (*d*)
H–C(2′)	4.11 (*dd*)	4.72 (*dd*)	4.20 (*dd*)	4.93 (*dd*)
H–C(3′)	4.18 (*dd*)	4.48 (*dd*)	4.24 (*dd*)	4.63 (*dd*)
H–C(4′)	3.94 (*ddd*)	4.10 (*ddd*)	3.99 (*ddd*)	4.32 (*ddd*)
H_a–C(5′)	3.76 (*ddd*)	3.81 (*dd*)	3.73 (*ddd*)	3.90 (*dd*)
H_b–C(5′)	3.56 (*ddd*)	3.73 (*dd*)	3.54 (*ddd*)	3.87 (*dd*)
H–C(8)	–	7.93 (*s*)	–	8.42 (*s*)
H–C(2)	–	–	–	8.28 (*s*)
C(1′)	108.50 (*s*)	88.01 (*s*)	108.20 (*s*)	88.40 (*s*)
C(2′)	75.28 (*s*)	80.40 (*s*)	75.20 (*s*)	80.20 (*s*)
C(3′)	71.80 (*s*)	70.13 (*s*)	71.50 (*s*)	70.50 (*s*)
C(4′)	82.89 (*d*)[a])	85.63 (*s*)	82.67 (*d*)[c])	86.70 (*s*)
C(5′)	65.88 (*d*)[b])	61.90 (*s*)	65.70 (*d*)[d])	62.68 (*s*)
C(2)	–	154.58 (*s*)	–	156.54 (*s*)
C(4)	–	152.03 (*s*)	–	149.15 (*s*)
C(5)	–	117.39 (*s*)	–	119.99 (*s*)
C(6)	–	159.80 (*s*)	–	153.31 (*s*)
C(8)	–	139.61 (*s*)	–	142.6 (*s*)
P	1.98	–	1.93	–

[a]) J(C(4′),P) = 6.4 Hz. [b]) J(C(5′),P) = 5.1 Hz. [c]) J(C(4′),P) = 6.7 Hz. [d]) J(C(5′),P) = 5.0 Hz.

Table 2. *Measured Coupling Constants* [Hz] *in the Ribose and Nucleoside Moieties of* **7a** *and* **7b**

	Grp (**7a**)		Arp (**7b**)	
	Ribose moiety	Nucleoside moiety	Ribose moiety	Nucleoside moiety
J(H–C(1′),H–C(2′))	1.5	5.4	1.4	6.1
J(H–C(2′),H–C(3′))	4.6	5.0	4.6	5.2
J(H–C(3′),H–C(4′))	6.7	4.8	6.6	3.4
J(H–C(4′),H_a–C(5′))	4.3	2.7	4.3	2.6
J(H–C(4′),H_b–C(5′))	5.7	4.3	5.8	3.5
J(H_a–C(5′),H_b–C(5′))	−12.9	−12.1	−12.8	−11.6
J(P,H_a–C(5′))	6.4	–	6.3	–
J(P,H_b–C(5′))	7.0	–	7.0	–

of the ^3J(H,H) couplings of the latter compound. Results in *Table 2* show that the substitution of the adenine by a guanine drives the conformational equilibrium of the nucleoside moiety towards the *N* conformer. A comparison of the ribose moieties in both compounds showed only minor differences. It seems that the substitution of the adenine by a guanine has no effects on the conformation of the ribose moiety.

To probe the O- and N-glycosidic-bond populations, NOESY spectra were recorded with 150, 300, and 500 ms mixing times respectively. No relevant data could be obtained, most likely due to fast rotation around these bonds in a fast equilibrium

Table 3. *Ring Conformers of the Ribose and Nucleoside Moieties of* **7a** *and* **7b**

	Guo2′Rib5′p (**7a**)		Ade2′Rib5′p (**7b**)	
	Ribose moiety	Nucleoside moiety	Ribose moiety	Nucleoside moiety
P_N[a])	2.8	25	4.6	27
N[b])	$^3T_2$	^3E	$^3T_2$	^3E
X_N[c])	0.82	0.42	0.80	0.36
P_S[a])	178	154	177	156
S[b])	$^2T_3$	^2E	$^2T_3$	^2E
X_S[c])	0.18	0.58	0.20	0.64
RMSD[d])	0.051	0.095	0.054	0.094

[a]) Phase angle of pseudorotation [degrees]. [b]) Type of conformer. [c]) Molar fraction of the conformer [%]. [d]) Root Mean Square Deviation. The puckering amplitude was kept 38 degrees in all cases.

between several low-energy conformations in solution. This was confirmed by a Monte Carlo conformational search, performed with Macromodel 5.0, which showed the existence of two equally populated, low-energy conformations about the N-glycosidic bond, consistent with the *syn*- and *anti*-conformation of the guanine moiety (*Fig. 2*). About the O-glycosidic bond, three low-energy conformations were seen. (+*g:* 30%, −*g:* 26%, *t:* 44%). The most-populated conformation (*trans*) was also observed in the solution structure of an RNA dodecamer duplex containing 9-[2-*O*-(β-D-ribofuranosyl]adenine [8]. To obtain more experimental information on the relative populations of these bond conformations, experiments on a ^{13}C-labeled sample will be necessary.

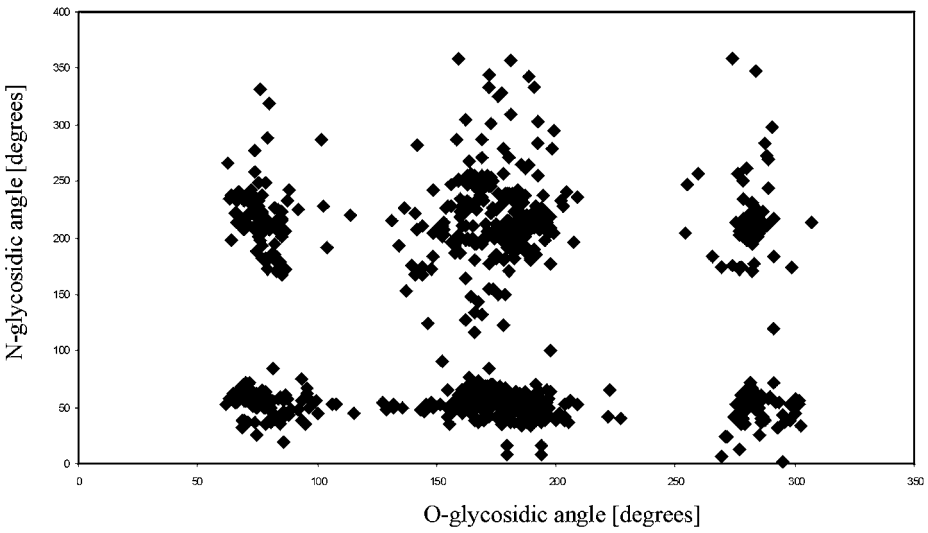


Fig. 2. *Low-energy conformations around the O- and N-glycosidic bonds.* Points represent conformations within 20 kJ of a local minimum. N-Glycosidic angle: C(4)–N(9)–C(1′)–O(4′); O-glycosidic angle: C(2′)–C(1′)–O(1′)–C(2′).

3. Conclusions. – The efficient synthesis of *O*-β-D-ribofuranosyl-(1″ → 2′)guanosine-5″-*O*-phosphate and *O*-β-D-ribofuranosyl-(1″ → 2′)-adenosine-5″-*O*-phosphate, minor tRNA components, has been developed, and their conformational properties were examined by NMR spectroscopy. These molecules are rather flexible and show free rotation around the O- and N-glycosidic bonds. The ribose moiety of the nucleoside shows a high percentage of *S*-type conformation.

Experimental Part

General. Column chromatography (CC): silica gel (0.06–0.20 mm). TLC: *Kieselgel 260 F* (*Merck*); eluents: CH_2Cl_2 (*A*); CH_2Cl_2/MeOH 98:2 (*B*); CH_2Cl_2/MeOH 95:5 (*C*); detection by UV light. NMR Spectra: *Bruker AMX-400* and *Varian Unity 500* NMR spectrometers; at 300 K; chemical shifts δ in ppm relative to the solvent signals (^{1}H and ^{13}C) and relative external reference (H_4PO_3 (capil.); ^{31}P); coupling constants *J* in Hz; the signals were assigned by double-resonance techniques and COSY experiments. MS and exact mass measurements: quadrupole/orthogonal-acceleration time-of-flight tandem mass spectrometer (*Q-Tof-2, Micromass*, Manchester, UK) equipped with a standard electrospray ionization (ESI) interface.

1,2,3-Tri-O-*benzoyl-5*-O-*(phenoxyacetyl)*-D-*ribofuranose* (**2**). Phenoxyacetic anhydride (2.08 g, 7.25 mmol) was added to a soln. of 1,2,3-tri-*O*-benzoyl-D-ribofuranose [12] (2.58 g, 5.58 mmol) in pyridine/1,2-dichloroethane 1:4 (25 ml). The mixture was kept for 1 h at 20°, then MeOH (1 ml) was added, and, after storage for 30 min at 20°, diluted with CH_2Cl_2, washed with 10% aq. soln. of $NaHCO_3$ (30 ml) and H_2O (30 ml), dried (Na_2SO_4), evaporated *in vacuo*, and co-evaporated with toluene (2 × 10 ml). The residue was purified by flash chromatography on silica gel to give 2.83 g (85%) of **2** as a mixture of anomers 1:1. Oil. This mixture was separated by CC (100 g) with 0.2% MeOH/CH_2Cl_2.

β-Isomer of **2**. Oil. R_f (CH_2Cl_2/MeOH 99.5:0.5) 0.7. ^{1}H-NMR ($CDCl_3$): 8.09–6.75 (*m*, 20 arom. H); 6.65 (*s*, H–C(1)); 5.93 (*m*, H–C(2), H–C(3)); 4.76 (*ddd*, *J*(4,3) = 6.3, *J*(4,5a) = 3.7, *J*(4,5b) = 4.8, H–C(4)); 4.65 (*dd*, *J*(5a,5b) = −12.1, H_a–C(5)); 4.53 (*d*, *J* = −16.0, 1 H, PhOCH_2); 4.42 (*dd*, H_b–C(5)); 4.40 (*d*, 1 H, PhOCH_2). ^{13}C-NMR ($CDCl_3$): 168.54, 165.50, 165.14, 164.68 (C=O); 157.61 (Ph); 133.94, 133.76, 129.96, 129.84, 129.57, 129.02, 128.72, 128.63, 128.57 (Bz); 121.80, 114.67 (Ph); 98.97 (C(1)); 79.73 (C(4)); 75.0 (C(2)); 71.26 (C(3)); 64.83 (C(5)); 64.19 (CH_2).

α-Isomer of **2**. Oil. R_f (CH_2Cl_2/MeOH 99.5:0.5) 0.56. ^{1}H-NMR ($CDCl_3$): 8.08–6.95 (*m*, 20 arom. H); 6.75 (*d*, *J*(1,2) = 4.3, H–C(1)); 5.68 (*dd*, *J*(3,2) = 6.6, *J*(3,4) = 2.2, H–C(3)); 5.42 (*dd*, H–C(2)); 4.76 (*m*, H–C(4), CH_2); 4.64 (*dd*, *J*(5a,4) = 2.9, *J*(5a,5b) = −12.1, H_a–C(5)); 4.53 (*dd*, *J*(5b,4) = 3.3, H_b–C(5)). ^{13}C-NMR ($CDCl_3$): 168.84, 165.81, 165.17, 164.96 (C=O); 157.70 (Ph); 133.66, 133.54, 129.96, 129.87, 129.75, 129.20, 128.44 (Bz, Ph); 121.92, 114.54 (Ph); 94.88 (C(1)); 82.40 (C(4)); 71.36 (C(2)); 70.69 (C(3)); 65.04 (C(5)); 64.13 (CH_2).

9-[2-O-*[2,3-di*-O-*benzoyl-3,5*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-5*-O-*(phenoxyacetyl)-β*-D-*ribofuranosyl]-β*-D-*ribofuranosyl]*-N^2-*isobutyryl guanine* (**3a**). To a cooled soln. (0°) of **2** (1.35 g, 2.26 mmol) under N_2 in 1,2-dichloroethane (25 ml), $SnCl_4$ (0.32 ml, 2.71 mmol) was added, and the soln. was kept at 0° for 10 min. After addition of N^2-*isobutyryl-9-[3,5*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β*-D-*ribofuranosyl]guanine* (**1a**; 1.16 g, 1.95 mmol), the resulting soln. was kept at 0° for 16 h. The mixture was diluted with CH_2Cl_2 (25 ml), 10% aq. soln. of $NaHCO_3$ (30 ml) was added, and the suspension was stirred at 20° for 20 min. The suspension was filtered through *Hyflo Super Cel*, the org. layer was separated, washed with H_2O (20 ml), dried (Na_2SO_4), and evaporated to dryness. The residue was purified by CC (silica gel (50 g)). The column was washed with system *A*, and then elution with 1% MeOH/CH_2Cl_2 gave 1.41 g (68%) of **3a**. Foam. R_f (*C*) 0.62. ^{1}H-NMR ($CDCl_3$): 12.19 (br. *s*, NH); 9.64 (br. *s*, NH); 8.12 (*s*, H–C(8)); 7.99–7.86 (*m*, 4 H, Bz); 7.62–7.21 (*m*, 8 H, Bz, Ph); 6.95 (*t*, *J* = 7.3, 1 H, Ph); 6.82 (*d*, *J* = 8.1, 2 H, Ph); 5.98 (*s*, H–C(1′) (Guo)); 5.93 (*dd*, *J*(3′,2′) = 5.3, *J*(3′,4′) = 5.9, H–C(3′)(Rib)); 5.85 (*s*, H–C(1′)(Rib)); 5.78 (*d*, H–C(2′)(Rib)); 4.93 (*dd*, *J*(5′a,4′) = 4.7, *J*(5′a,5′b) = −11.5, H_a–C(5′)(Rib)); 4.88 (*d*, *J* = 16.5, 1 H, PhOCH_2); 4.79 (*d*, 1 H, PhOCH_2); 4.61 (*m*, H–C(2′), H–C(3′)(Guo)); 4.42 (*dd*, *J*(5′b,4′) = 3.4, H_b–C(5′)(Rib)); 4.29 (*m*, H_a–C(5′)(Guo), H–C(4′)(Rib)); 4.19 (*dd*, *J*(4′,3′) = 9.3, *J*(4′,5′b) = 2.5, H–C(4′)(Guo)); 4.03 (*dd*, *J*(5′b,5′a) = −13.7, H_b–C(5′)(Guo)); 2.61 (*sept.*, *J* = 6.8, CH (i-Bu)); 1.17–0.93 (*m*, 34 H, i-Bu, i-Pr). ^{13}C-NMR ($CDCl_3$): 179.43, 171.01, 165.40, 164.92 (C=O); 157.36 (Ph); 155.20 (C(6)); 148.47 (C(2)); 147.26 (C(4)); 135.84 (C(8)); 133.59, 129.72, 129.68, 129.04, 128.85, 128.50, 128.46 (Bz, Ph); 122.14 (Ph); 121.18 (C(5)); 114.35 (Ph); 105.39 (C(1′)(Rib)); 87.55 (C(1′)(Guo)); 81.27 (C(4′)(Guo)); 79.46 (C(4′)(Rib)); 79.40 (C(2′)(Guo)); 75.91

(C(2′)(Rib)); 72.12 (C(3′)(Rib)); 69.64 (C(3′)(Guo)); 65.18 (C(5′)(Rib)); 64.75 (CH_2); 59.55 (C(5′)(Guo)); 36.03 (CH (i-Bu)); 18.85 (Me (i-Bu)); 17.47, 17.34, 17.29, 17.25, 17.13, 16.98, 16.70, 13.39, 13.09, 12.87, 12.61 (i-Pr). ESI-MS (pos.): 1070.4235 ($[C_{53}H_{67}N_5O_{15}Si_2+H]^+$; calc. 1070.4250).

N^6-*Benzoyl-9-[2*-O-*(2,3-di*-O-*benzoyl-3,5*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-5*-O-*(phenoxyacetyl)-β*-D-*ribofuranosyl]-β*-D-*ribofuranosyl]adenine* (**3b**). As described for **3a**, with **2** (840 mg, 1.41 mmol), $SnCl_4$ (0.2 ml, 1.71 mmol) and N^6-*benzoyl-9-[3,5*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl]-β*-D-*ribofuranosyl)adenine* (**1b**; 720 mg, 1.17 mmol): 765 mg (60%) of **3b**. Foam. R_f (*C*) 0.59. ^{1}H-NMR ($CDCl_3$): 9.10 (br. *s*, NH), 8.76 (*s*, H–C(8)); 8.30 (*s*, H–C(2)); 8.04–7.85 (*m*, 6 H, Bz); 7.61–7.18 (*m*, 11 H, Bz, Ph); 6.88 (*m*, 3 H, Ph); 6.02 (*s*, H–C(1′)(Ado)); 5.80 (*m*, H–C(1′), H–C(2′), H–C(3′)(Rib)); 4.98 (*dd*, *J*(3′,2′) = 4.8, *J*(3′,4′) = 8.8, H–C(3′)(Ado)); 4.79–4.56 (*m*, H–C(2′)(Ado), H–C(4′), CH_2(5′)(Rib), CH_2); 4.26–3.98 (*m*, H–C(4′), CH_2(5′)(Ado)); 1.08–0.97 (*m*, 28 H (i-Pr)). ^{13}C-NMR ($CDCl_3$): 168.78, 165.44, 165.05, 164.62 (C=O); 157.67 (Ph); 152.82 (C(2)); 150.93 (C(6)); 149.48 (C(4)); 142.01 (C(8)); 133.54, 129.75, 129.51, 128.87, 128.50, 128.44, 127.87 (Bz, Ph); 123.56 (C(5)); 121.74, 114.60 (Ph); 105.80 (C(1′)(Rib)); 88.75 (C(1′)(Ado)); 81.40 (C(4′)(Ado)); 79.67 (C(4′)(Rib)); 78.88 (C(2′)(Ado)); 75.51 (C(2′)(Rib)); 72.27 (C(3′)(Rib)); 69.81 (C(3′)(Ado)); 65.47 (C(5′)(Rib)); 65.13 (CH_2); 59.73 (C(5′)(Ado)); 17.30, 17.15, 16.97, 16.76, 16.66, 13.20, 12.78, 12.69, 12.45 (i-Pr). ESI-MS (pos.): 1088.4103 ($[C_{56}H_{65}N_5O_{14}Si_2+H]^+$; calc. 1088.4144).

9-[2-O-*(2,3-Di*-O-*benzoyl-β*-D-*ribofuranosyl)-3,5*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β*-D-*ribofuranosyl]*-N^2-*isobutyryl guanine* (**4a**). A soln. of **3a** (1.41 g, 1.32 mmol) in 0.1M K_2CO_3 in MeOH (13.2 ml) was kept for 10 min at 20°, and then 10% AcOH in MeOH was added to adjust pH 7.0. The resulting soln. was concentrated *in vacuo* to dryness, and the residue was partitioned between AcOEt (100 ml) and H_2O (30 ml), the org. layer was washed with H_2O (30 ml), dried (Na_2SO_4), and evaporated to dryness. The residue was applied to CC (silica gel (30 g)). The column was washed with system *A* and then eluted with system *B* to give 840 mg (68%) of **4a** Foam. R_f (*C*) 0.5. ^{1}H-NMR ($CDCl_3$): 12.10 (br. *s*, NH); 9.96 (br. *s*, NH); 8.05 (*s*, H–C(8)); 7.99–7.89 (*m*, 4 H, Bz); 7.61–7.33 (*m*, 6 H, Bz); 5.91 (*s*, H–C(1′)(Guo)); 5.90 (*dd*, *J*(3′,2′) = 4.9, *J*(3′,4′) = 7.6, H–C(3′)(Rib)); 5.87 (*s*, H–C(1′)(Rib)); 5.84 (*d*, H–C(2′)(Rib)); 4.59 (*ddd*, *J*(4′,5′a) = 2.1, *J*(4′,5′b) = 4.4, H–C(4′)(Rib)); 4.53 (*dd*, *J*(3′,2′) = 4.1, *J*(3′,4′) = 9.5, H–C(3′)(Guo)); 4.47 (*d*, (Guo)); 4.33 (*dd*, *J*(5′a,4′) = 1.0, *J*(5′a,5′b) = –13.7, H_a–C(5′)(Guo)); 4.24 (*ddd*, *J*(4′,5′b) = 2.4, H–C(4′)(Guo)); 4.16 (*dd*, *J*(5′a,5′b) = –12.5, H_a–C(5′)(Rib)); 4.04 (*dd*, H_b–C(5′)(Guo)); 3.97 (*dd*, H_b–C(5′)(Rib)); 2.62 (*sept.*, *J* = 6.9, CH (i-Bu)); 1.28 (*d*, Me (i-Bu)); 1.27 (*d*, Me (i-Bu)); 1.11–0.93 (*m*, 28 H, i-Pr). ^{13}C-NMR ($CDCl_3$): 178.64, 165.46, 164.90 (C=O); 155.23 (C(6)); 148.58 (C(2)); 146.41 (C(4)); 135.72 (C(8)); 133.47, 133.50, 129.69, 129.15, 128.96, 128.48, 128.40 (Bz); 121.15 (C(5)); 104.55 (C(1′)(Rib)); 88.73 (C(1′)(Guo)); 82.23 (C(4′)(Rib)); 81.43 (C(4′)(Guo)); 77.59 (C(2′)(Guo)); 75.86 (C(2′)(Rib)); 70.17 (C(3′)(Rib)); 69.58 (C(3′)(Guo)); 61.47 (C(5′)(Rib)); 59.34 (C(5′)(Guo)); 36.74 (CH (i-Bu)); 18.83, 18.78 (Me (i-Bu)); 17.44, 17.27, 17.18, 16.98, 16.72, 13.38, 12.89, 12.69 (i-Pr). ESI-MS (pos.): 936.3907 ($[C_{45}H_{61}N_5O_{13}Si_2+H]^+$; calc. 936.3882).

N^6-*Benzoyl-9-[2*-O-*(2,3-di*-O-*benzoyl-β*-D-*ribofuranosyl)-3,5*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β*-D-*ribofuranosyl]adenine* (**4b**). As described for **4a**, with **3b** (544 mg, 0.5 mmol): 300 mg (63%) of **4b**. Foam. R_f (*C*) 0.42. ^{1}H-NMR ($CDCl_3$): 9.18 (br. *s*, NH); 8.76 (*s*, H–C(8)); 8.47 (*s*, H–C(2)); 8.01–7.85 (*m*, 6 H, Bz); 7.57–7.26 (*m*, 9 H, Bz); 6.20 (*s*, H–C(1′)(Ado)); 6.03 (*dd*, *J*(3′,2′) = 4.7, *J*(3′,4′) = 7.8, H–C(3′)(Rib)); 5.79 (*d*, H–C(2′)(Rib)); 5.57 (*s*, H–C(1′)(Rib)); 4.49 (*m*, H–C(2′), H–C(3′)(Ado), H–C(4′)(Rib)); 4.29 (*d*, *J*(5′a,5′b) = –13.5, H_a–C(5′)(Ado)); 4.25 (*dd*, *J*(3′,4′) = 9.0, *J*(4′,5′b) = 2.5, H–C(4′)(Ado)); 4.08 (*dd*, *J*(5′a,4′) = 1.5, *J*(5′a,5′b) = –12.5, H_a–C(5′)(Rib)); 4.01 (*dd*, H_b–C(5′)(Ado)); 3.79 (*dd*, *J*(5′b,4′) = 3.1, H_b–C(5′)(Rib)); 1.05–0.87 (*m*, 28 H (i-Pr)). ^{13}C-NMR ($CDCl_3$): 165.53, 165.05, 164.68 (C=O); 152.39 (C(2)); 150.05 (C(6)); 149.84 (C(4)); 140.68 (C(8)); 133.66, 133.27, 132.88, 129.72, 129.42, 128.90, 128.44, 128.35, 127.93 (Bz); 123.98 (C(5)); 104.65 (C(1′)(Rib)); 89.08 (C(1′)(Ado)); 82.65 (C(4′)(Rib)); 81.74 (C(4′)(Ado)); 77.24 (C(2′)(Ado)); 75.70 (C(2′)(Rib)); 69.99 (C(3′)(Rib)); 69.08 (C(3′)(Ado)); 60.31 (C(5′)(Rib)); 59.37 (C(5′)(Ado)); 17.27, 17.18, 16.88, 16.72, 16.66, 13.26, 12.72, 12.54 (i-Pr). ESI-MS (pos.): 954.3787 ($[C_{48}H_{59}N_5O_{12}Si_2+H]^+$; calc. 954.3777).

9-[2-O-*[2,3-Di*-O-*benzoyl-5*-O-*bis[2-(4-nitrophenyl)ethyl]phosphorylyl-β*-D-*ribofuranosyl]-3,5*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β*-D-*ribofuranosyl]*-N-*isobutyryl guanine* (**5a**). A mixture of **4a** (1.17 g, 1.25 mmol) and a 0.5M soln. of the triethylammonium salt of bis[2-(4-nitrophenyl)ethyl] phosphate (5 ml, 2.5 mmol) was co-evaporated with dry pyridine (3 × 5 ml) and finally dissolved in pyridine (5 ml). Then, 1-methyl-1*H*-imidazole (1 ml, 12.5 mmol) and TPSCl (1.51 g, 5 mmol) were added. The mixture was stirred for 4 h at 20°, and then concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ (100 ml) and washed with 0.06M phosphate buffer (pH 7.0, 2 × 30 ml). The org. layer was dried (Na_2SO_4), evaporated *in vacuo*, co-evaporated with toluene (2 × 10 ml), and purified by CC (silica gel (30 g)). The column was washed with Et_2O (300 ml) and eluted with system *B* to give 1.12 g (68%) of **5a**. Foam. R_f (*C*) 0.65. ^{1}H-NMR ($CDCl_3$): 12.36 (br. *s*, NH); 10.86

(br. *s*, NH); 8.14–7.83 (*m*, 9 H, H–C(8), Bz, Ph); 7.60–7.27 (*m*, 10 H, Bz, Ph); 5.86 (*m*, H–C(1′)(Guo), H–C(3′)(Rib)); 5.81 (*d*, *J*(1′,2′) = 1.5, H–C(1′)(Rib)); 5.68 (*d*, *J*(2′,3′) = 5.4, H–C(2′)(Rib)); 4.63 (*dd*, *J*(3′,2′) = 4.2, *J*(3′,4′) = 9.3, H–C(3′)(Guo)); 4.47 (*d*, H–C(2′)(Guo)); 4.44–4.36 (*m*, H_a–C(5′)(Rib), CH_2O); 4.28–4.16 (*m*, H–C(4′), H_b–C(5′)(Rib), H_a–C(5′)(Guo), CH_2O); 4.09 (*dd*, *J*(4′,5′b) = 2.3, H–C(4′)(Guo)); 4.01 (*dd*, *J*(5′b,5′a) = –13.6, H_b–C(5′)(Guo)); 3.12 (*m*, CH_2); 3.01 (*m*, CH_2); 2.83 (*sept.*, *J* = 6.8, CH (i-Bu)); 1.24–0.93 (*m*, 34 H, i-Bu, i-Pr). ^{13}C-NMR ($CDCl_3$): 180.08, 165.32, 164.87 (C=O); 155.58 (C(6)); 148.84 (C(2)); 147.54 (C(4)); 144.38 (Ph); 135.90 (C(8)); 133.74, 133.62, 129.78, 129.67, 129.58, 128.79, 128.50, 128.46, 124.00, 123.79, 123.74 (Bz, Ph); 121.28 (C(5)); 105.67 (C(1′)(Rib)); 87.09 (C(1′)(Guo)); 81.23 (C(4′)(Guo)); 80.73 (*d*, *J*(C,P) = 9.8, C(4′)(Rib)); 79.50 (C(2′)(Guo)); 75.86 (C(2′)(Rib)); 72.35 (C(3′)(Rib)); 69.45 (C(3′)(Guo)); 68.04 (*d*, *J*(C,P) = 4.6, CH_2O); 67.77 (*d*, *J*(C,P) = 5.5, CH_2O); 67.20 (*d*, *J*(C,P) = 3.9, C(5′)(Rib)); 59.55 (C(5′)(Guo)); 36.18 (CH_2); 35.46 (CH (i-Bu)); 19.08, 19.02 (Me (i-Bu)); 17.44, 17.31, 17.26, 17.12, 16.95, 16.78, 13.35, 13.10, 12.82, 12.61 (i-Pr). ESI-MS (pos.): 1314.4503 ($[C_{61}H_{76}N_7O_{20}PSi_2 + H]^+$; calc. 1314.4499).

N^6-*Benzoyl-9-[2-*O*-(2,3-di-*O*-benzoyl-5-*O*-{bis[2-(4-nitrophenyl)ethyl]phosphorylyl}-β-*D*-ribofuranosyl)-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-*D*-ribofuranosyl]adenine* (**5b**). As described for **5a**, with **4b** (220 mg, 0.23 mmol): 200 mg (65%) of **5b**. Foam. R_f (*C*) 0.66. ^{1}H-NMR ($CDCl_3$): 9.03 (br. *s*, NH); 8.72 (*s*, H–C(8)); 8.34 (*s*, H–C(2)); 8.01–7.83 (*m*, 10 H, Bz, Ph); 7.61–7.30 (*m*, 13 H, Bz, Ph); 6.08 (*s*, H–C(1′)(Ado)); 5.85 (*dd*, *J*(3′,2′) = 4.9, *J*(3′,4′) = 6.3, H–C(3′)(Rib)); 5.79 (*d*, H–C(2′)(Rib)); 5.77 (*s*, H–C(1′)(Rib)); 5.07 (*dd*, *J*(3′,2′) = 4.6, *J*(3′,4′) = 9.0, H–C(3′)(Ado)); 4.84 (*d*, H–C(2′)(Ado)); 4.50 (*m*, H–C(4′)(Rib)); 4.30–4.11 (*m*, H–C(4′), H_a–C(5′)(Ado), CH_2(5′)(Rib), CH_2O); 4.02 (*dd*, *J*(5′b,4′) = 2.7, *J*(5′b,5′a) = –13.2, H_b–C(5′)(Ado)); 3.00 (*m*, CH_2); 2.97 (*m*, CH_2); 1.08–0.95 (*m*, 28 H, i-Pr). ^{13}C-NMR ($CDCl_3$): 165.47, 165.02, 164.59 (C=O); 152.60 (C(2)); 150.96 (C(6)); 149.51 (C(4)); 146.96, 144.68 (Ph); 142.80 (C(8)); 133.63, 132.88, 129.78, 128.93, 128.54, 127.84, 123.71 (Bz, Ph); 123.69 (C(5)); 105.56 (C(1′)(Rib)); 88.84 (C(1′)(Ado)); 81.40 (C(4′)(Ado)); 80.16 (*d*, *J*(C,P) = 7.2, C(4′)(Rib)); 78.70 (C(2′)(Ado)); 75.39 (C(2′)(Rib)); 71.66 (C(3′)(Rib)); 70.05 (C(3′)(Ado)); 67.17 (*m*, C(5′)(Rib), CH_2O); 59.85 (C(5′)(Ado)); 36.07, 36.06 (CH_2), 17.15, 16.97, 16.76, 13.17, 12.78, 12.66, 12.48 (i-Pr). ESI-MS (pos.): 1332.4365 ($[C_{64}H_{74}N_7O_{19}PSi_2 + H]^+$; calc. 1332.4394).

*9-[2-*O*-(2,3-di-*O*-benzoyl-5-*O*-{bis[2-(4-nitrophenyl)ethyl]phosphorylyl}-β-*D*-ribofuranosyl)-β-*D*-ribofuranosyl]-*N^2*-isobutyryl guanine* (**6a**). The protected triester **5a** (830 mg, 0.63 mmol) was dissolved in THF (3 ml), and a soln. of $Bu_4NF \cdot 3\,H_2O$ (554 mg, 1.76 mmol) in THF (2 ml) was added. After 15 min at 20°, H_2O (1 ml) and *Dowex-50* (Na^+ form; 1.5 ml) were added, and the mixture was stirred for 30 min at 20°. The resin was filtered off and washed with AcOEt. The filtrate was washed with 0.06M phosphate buffer (pH 7.0; 2 × 30 ml). The org. layer was dried (Na_2SO_4), evaporated to dryness, and subjected to CC (silica gel (30 g)). The column was washed with system *A*, then *B*, and eluted with system *C* to give 520 mg (77%) of **6a**. Foam. R_f (*C*) 0.25. ^{1}H-NMR ($CDCl_3$): 12.30 (br. *s*, NH); 10.83 (br. *s*, NH); 8.56 (*s*, H–C(8)); 8.10–7.79 (*m*, 8 H, Bz, Ph); 7.56–7.26 (*m*, 10 H, Bz, Ph); 6.14 (*d*, *J*(1′,2′) = 2.9, H–C(1′)(Guo)); 5.82 (*dd*, *J*(3′,2′) = 5.1, *J*(3′,4′) = 6.1, H–C(3′)(Rib)); 5.68 (*dd*, *J*(2′,1′) = 1.2, H–C(2′)(Rib)); 5.62 (*d*, H–C(1′)(Rib)); 4.81 (*dd*, *J*(3′,2′) = 4.6, *J*(3′,4′) = 8.0, H–C(3′)(Guo)); 4.56 (*dd*, H–C(2′)(Guo)); 4.51 (*m*, H–C(4′)(Guo)); 4.42 (*m*, CH_2O); 4.36 (*dddd*, *J*(4′,5′a) = 4.4, *J*(4′,5′b) = 3.9, *J*(4′,P) = 1.9, H–C(4′)(Rib)); 4.30 (*ddd*, *J*(5′a,5′b) = –11.0, *J*(5′a,P) = 4.6, H_a–C(5′)(Rib)); 4.16 (*m*, CH_2O); 4.11 (*ddd*, *J*(5′b,P) = 4.6, H_b–C(5′)(Rib)); 4.06 (*m*, H_a–C(5′)(Guo)); 3.98 (*m*, H_b–C(5′)(Guo)); 3.11 (*m*, CH_2); 2.97 (*m*, CH_2); 2.75 (*sept.*, *J* = 6.8, CH (i-Bu)); 2.10 (*m*, HO–C(3′), HO–C(5′)(Guo)); 1.19 (*d*, Me (i-Bu)); 1.17 (*d*, Me (i-Bu)). ^{13}C-NMR ($CDCl_3$): 180.14, 165.29, 164.83 (C=O); 155.93 (C(6)); 148.51 (C(2)); 146.97 (C(4)); 144.97, 144.46 (Ph); 135.50 (C(8)); 133.63, 129.84, 129.77, 129.70, 129.63, 128.74, 128.50, 123.99, 123.69, 123.64 (Bz, Ph); 120.47 (C(5)); 106.02 (C(1′)(Rib)); 87.14 (C(1′)(Guo)); 84.36 (C(4′)(Guo)); 81.47 (C(2′)(Guo)); 79.99 (*d*, *J*(C,P) = 9.8, C(4′)(Rib)); 75.69 (C(2′)(Rib)); 71.24 (C(3′)(Rib)); 70.00 (C(3′)(Guo)); 68.08 (*d*, *J*(C,P) = 3.9, CH_2O); 67.56 (*d*, *J*(C,P) = 4.9, CH_2O); 66.47 (*d*, *J*(C,P) = 3.7, C(5′)(Rib)); 60.69 (C(5′)(Guo)); 36.14, 36.09 (CH_2); 35.67 (CH (i-Bu)); 19.01, 18.92 (Me (i-Bu)). ESI-MS (pos.): 1094.2791 ($[C_{49}H_{50}N_7O_{19}P + Na]^+$; calc. 1094.2797).

N^6-*Benzoyl-9-[2-*O*-(2,3-di-*O*-benzoyl-5-*O*-{bis[2-(4-nitrophenyl)ethyl]phosphorylyl}-β-*D*-ribofuranosyl)-β-*D*-ribofuranosyl]adenine* (**6b**). As described for **6a**, with **5b** (90 mg, 0.068 mmol): 53 mg (72%) of **6b**. Foam. R_f (*C*) 0.27. ^{1}H-NMR ($CDCl_3$): 9.07 (br. *s*, NH); 8.77 (*s*, H–C(8)); 8.39 (*s*, H–C(2′)); 8.11–7.83 (*m*, 10 H, Bz, Ph); 7.61–7.32 (*m*, 13 H, Bz, Ph); 6.17 (*d*, *J*(1′,2′) = 6.9, H–C(1′)(Ado)); 5.61 (*dd*, *J*(3′,2′) = 5.0, *J*(3′,4′) = 5.6, H–C(3′)(Rib)); 5.55 (*dd*, *J*(2′,1′) = 1.9, H–C(2′)(Rib)); 5.17 (*d*, H–C(1′)(Rib)); 5.15 (*dd*, *J*(2′,3′) = 4.7, H–C(2′)(Ado)); 4.65 (*d*, H–C(3′)(Ado)); 4.33–4.18 (*m*, H–C(4′)(Ado), H–C(4′)(Rib), CH_2O); 4.06 (*ddd*, *J*(5′a,4′) = 3.7, *J*(5′a,5′b) = –10.9, *J*(5′a,P) = 5.9, H_a–C(5′)(Rib)); 3.98 (br. *d*, *J*(5′a,5′b) = –12.8, H_a–C(5′)(Ado)); 3.89 (*ddd*, *J*(5′b,4′) = 4.9, *J*(5′a,P) = 5.6, H_b–C(5′)(Rib)); 3.78 (br. *d*, H_b–C(5′)(Ado)); 3.07

(*m*, CH_2); 3.03 (*m*, CH_2). ^{13}C-NMR ($CDCl_3$): 165.39, 165.33, 164.72 (C=O); 151.99 (C(2)); 150.65 (C(6)); 150.20 (C(4)); 144.56, 144.47 (Ph); 141.67 (C(8)); 133.82, 133.78, 133.41, 132.91, 129.77, 129.66, 128.84, 128.53, 128.44, 127.98 (Bz, Ph); 124.14 (C(5)); 123.72 (Ph); 106.53 (C(1′)(Rib)); 89.37 (C(1′)(Ado)); 87.15 (C(4′)(Ado)); 81.25 (C(2′)(Ado)); 80.68 (*d*, *J*(C,P) = 7.0, C(4′)(Rib)); 75.63 (C(2′)(Rib)); 71.76 (C(3′)(Rib)); 71.31 (C(3′)(Ado)); 67.63 (*d*, *J*(C,P) = 6.4, CH_2OP); 67.51 (*d*, *J*(C,P) = 5.7, CH_2OP); 66.55 (*d*, *J*(C,P) = 5.7, C(5′)(Rib)); 62.99 (C(5′)(Ado)); 36.27, 36.20 (CH_2). ESI-MS (pos.): 1090.2864 ($[C_{52}H_{48}N_7O_{18}P + H]^+$; calc. 1090.2871).

O-*β*-D-*Ribofuranosyl-(1″ → 2′)-guanosine-5″*-O-*phosphate* (**7a**). A soln. of **6a** (86 mg, 0.08 mmol) in 0.5M DBU in dry pyridine (6.4 ml, 3.2 mmol) was stored for 24 h at 20°, neutralized with AcOH (0.182 ml, 3.2 mmol), and evaporated. The residue was dissolved in 5M NH_3 in MeOH (5 ml) and kept for 3 d at 20° and then concentrated *in vacuo*, dissolved in H_2O (30 ml), and washed with CH_2Cl_2 (2 × 10 ml). The aq. layer was concentrated *in vacuo*, diluted with H_2O (50 ml), and then applied to a column of DEAE-cellulose (200 ml, $HCOy_3^-$ form). The column was washed with H_2O (500 ml) and a 0.05M soln. of NH_4HCO_3, and eluted with a 0.1M soln. of NH_4HCO_3. The UV-absorbing fractions were combined, evaporated *in vacuo*, and co-evaporated with H_2O (5 × 10 ml). The residue was dissolved in H_2O (2 ml), applied to a column of *Dowex 50* (Na^+ form) (2 ml), eluted with H_2O, and freeze-dried: 0.061 mmol (76%) of **7a** was obtained as its Na salt. R_f (i-PrOH/NH_4OH/H_2O 7:1:2) 0.16. UV (pH 1): λ_{max} 257 (11900). UV (pH 7): λ_{max} 252 (13300). UV (pH 13): λ_{max} 260 (11000). ESI-MS (pos.): 496.1068 ($[C_{15}H_{22}N_5O_{12}P + H]^+$; calc. 496.1080).

NMR Spectra. Samples were prepared with 3 mg of **7a** and 3 mg of **7b**, resp., dissolved in 250 µl of D_2O. The pD was adjusted to 7.2. Spectra were recorded with a *Varian 500 Unity* spectrometer at 292 K. Chemical shifts were measured relative to the H_2O signal. The coupling constants were measured in Hz. The signals were assigned by double-resonance techniques and TOCSY experiments [28][29]. The TOCSY consisted of 2048 datapoints in t_2 and 256 increments in t_1. The data were apodized with a shifted sine-bell square function in both dimensions and processed to a 2K × 1K matrix.

Analysis of the couplings was performed with Pseurot. 6.2 [29]. This program uses the *Altona–Sundaralingam* formalism [25][26] to describe the ring puckering and a generalized *Karplus* equation developed by *Diez et al*. in collaboration with *Donders et al*. [32] to describe the relation between coupling and dihedral angle. RMS was minimized by a *Newton Raphson* minimization.

NOESY Spectra [33] were recorded with 150, 300, and 500 ms mixing times, resp. The NOESY consisted of 2048 datapoints in t_2 and 256 increments in t_1. The data were apodized with a shifted sine-bell square function in both dimensions and processed to a 2K × 1K matrix.

Conformational Search. A 3D structure was created with Insight II and exported as a pdb file. A Monte Carlo conformational search was performed with Macromodel 5.0, generating 5000 random structures by changing dihedral angles and by ring opening and closure. These 5000 structures were minimized with the Amber Forcefield [34] and a *Newton Raphson* minimization.

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