155. Synthesis and Characterization of Non-standard Nucleosides and Nucleotides Bearing the Acceptor-Donor-Donor Pyrimidine Analog 6-Amino-3-methylpyrazin-2(1*H*)-one

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6-Aminopyrazin-2(1*H*)-one, when incorporated as a pyrimidine-base analog into an oligonucleotide chain, presents a H-bond acceptor-donor-donor pattern to a complementary purine analog. When paired with the corresponding donor-acceptor-acceptor purine in oligonucleotides, the heterocycle selectively contributes to the stability of the duplex, presumably by forming a base pair of *Watson-Crick* geometry joined by a non-standard H-bonding pattern. Aspects of the nucleoside chemistry, including syntheses of the β-furanosyl ribonucleoside **1**, the ribonucleoside triphosphate **2** and the ribonucleoside bisphosphate **3** of 6-aminopyrazin-2(1*H*)-one are reported here. In aqueous solution, the ribonucleoside **1** was found to undergo acid- and base-catalyzed rearrangement with an apparent half-life of *ca.* 63 h at neutral pH and 30°. The rearrangement appears to be specific acid- and base-catalyzed. The thermodynamically most stable compound formed during this rearrangement reaction was isolated by HPLC and shown to be the β-pyranosyl form **4** of the 6-aminopyrazin-2(1*H*)-one nucleoside in its $^4C_1$ chair conformation. This reactivity of **1** under physiological conditions may explain why Nature does not use this particular heterocyclic system to implement an acceptor-donor-donor H-bonding pattern in the genetic alphabet.

Introduction. – According to the 'standard model' for DNA and RNA structure proposed four decades ago by *Watson* and *Crick*, recognition between the base pairs in complementary oligonucleotide strands is mediated by two rules of complementarity: size complementarity (a large purine pairs with a small pyrimidine) and H-bonding complementarity (H-bond donors are matched with H-bond acceptors). In addition, hydrophobicity and planarity in the bases are believed to be important for the stability of the double-helical structure. Hydrophobicity is believed to allow transfer of the bases from H_2O to the hydrophobic core of the double helix to make an energetic contribution to duplex formation. Planarity is believed to allow sequence-independent stacking of the bases to form an aperiodic crystalline-like structure necessary for duplex stability.

The standard model admits considerable structural flexibility within the *Watson-Crick* base pair. *E.g.*, in 1962 *Rich* pointed out that isocytosine (pyAAD, *Fig. 1*), which presents a H-bond acceptor-acceptor-donor pattern to the complementary strand, might fit the *Watson-Crick* geometry when paired with isoguanine (puDDA) [1]. *Zubay* also proposed a base pair joined by a non-standard H-bonding pattern [2], although in a ring system that was not planar, presumably cannot stack, and, therefore, does not precisely fit the *Watson-Crick* formalism.

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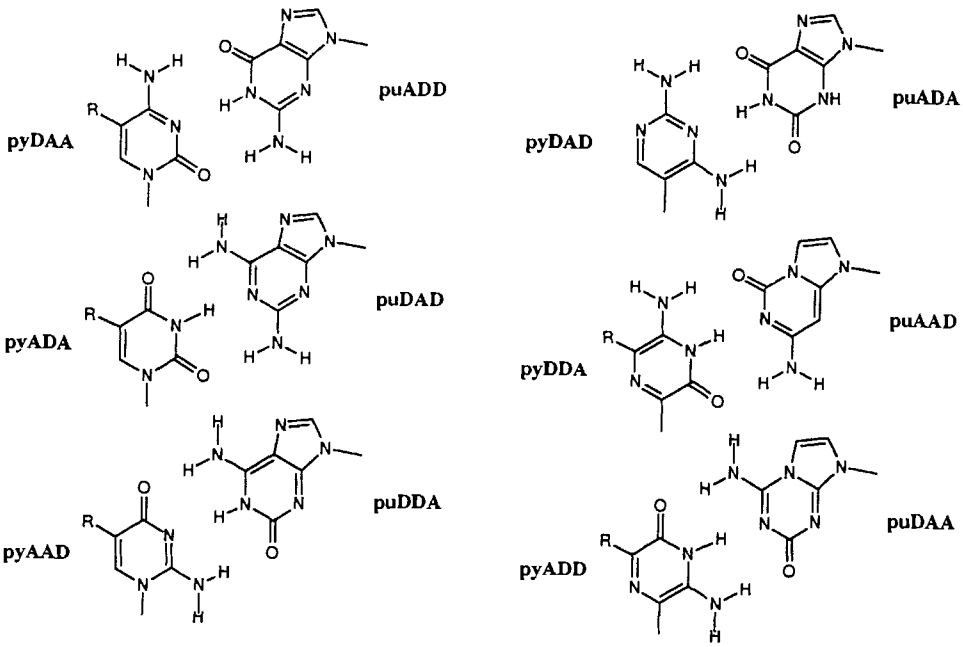


Fig. 1. *Twelve bases possible in a DNA- or RNA-based 'alphabet' within the constraints of the* Watson-Crick *base-pair geometry.* Pyrimidine-base analogs are designated by 'py', purine by 'pu'. The upper-case letters following the designation indicate the H-bonding pattern of acceptor (A) and donor (D) groups. The H-bonding patterns found in natural DNA and RNA are termed standard; the remainder are termed non-standard. The first two base pairs on the left represent the H-bonding patterns used in natural oligonucleotides.

Some time ago, we noted that the *Watson-Crick* formalism could be extended to include 12 independently replicatable bases joined in 6 base pairs by mutually independent H-bonding patterns, provided that some bases were joined to the sugar *via* a C–C bond (a '*C*-glycoside') (*Fig. 1*) [3]. In several cases, natural DNA and RNA polymerases catalyzed the template-directed incorporation of one or more of these non-standard base pairs into duplex DNA [4–7]. These results raised the question: Why does Nature not incorporate non-standard nucleobases into an expanded genetic alphabet?

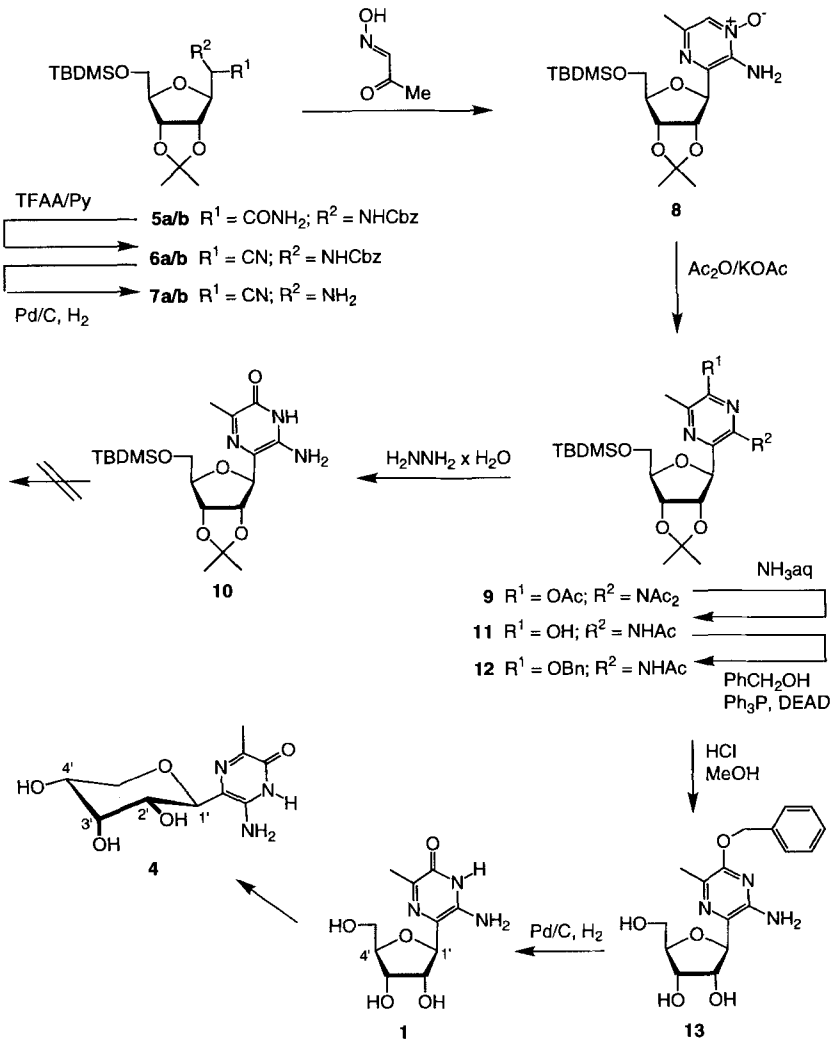
We have for some time been exploring this question by developing the chemistry of these non-standard base pairs, creating the enzymology and molecular biology of an expanded genetic alphabet, using non-standard base pairing to probe the molecular basis for the action of polymerases and the ribosome, and testing the technological potential of non-standard base pairs as components of diagnostic systems, nanostructures, and *in vitro* selection systems [8]. From these studies, we hope to learn why the encoding molecules found on earth contain the four bases that they do, and whether non-standard bases might be able to form alternative encoding systems.

Such questions can be addressed experimentally by preparing alternative implementations of various H-bonding donor and acceptor patterns using different heterocycle skeletons, and studying their reactivity. This paper reports the synthesis and properties of a pyrimidine analog, 6-aminopyrazin-2(1*H*)-one, implementing an acceptor-donor-

donor H-bonding pattern (pyADD, *Fig. 1*), in the form of ribonucleoside **1**, and the investigation of its physical properties. Syntheses are also described for the ribonucleoside 5′-triphosphate **2** and the ribonucleoside 3′,5′-bisphosphate **3**, which can be used respectively for *in vitro* transcription reactions with RNA polymerases [6] [7] [9] [10] and enzymatic oligoribonucleotide synthesis [11–14] (see also accompanying report).

Results. – *Synthesis and Structure Analysis of Ribonucleoside* **1** *and Rearrangement Product* **4**. The carboxamide group of **5a/b** [15] was converted to the nitrile group of **6a/b** by dehydration with trifluoroacetic anhydride in pyridine, and the product deprotected to yield **7a/b** (*Scheme 1*). The diastereoisomers of **5**–**7** were not separated, as they yield the

Scheme 1. *Synthesis of Ribonucleoside* **1**



TBDMS = (*t*-Bu)Me_2Si, Cbz = $PhCH_2OC(O)$, TFAA = $(CF_3CO)_2O$, DEAD = EtOOCN = NCOOEt

same aromatic pyrazine derivative **8** during the subsequent cyclization step. The α-amino nitrile **7a/b** was condensed with the 1-oxime of pyruvaldehyde (2-oxopropanal) to yield pyrazine *N*-oxide **8**. *N*-Oxide **8** was rearranged to yield **9**, and the acetyl groups of the pyrazine heterocycle were removed by treatment with hydrazine hydrate. All attempts to synthesize the free ribonucleoside **1** starting from **10** failed, however, as side products were generated under the acidic reaction conditions necessary to remove the acetonide protecting group. Therefore, a new protecting-group strategy, adapted from [15], was used.

Compound **9** was converted to the monoacetyl derivative **11** by brief treatment with aqueous ammonia. The resulting pyrazinone was then protected under *Mitsunobu* conditions [16] to yield **12**. Treatment with HCl in MeOH removed all protecting groups except the benzyl group. Intermediate **13** was purified by silica-gel chromatography followed by crystallization. Starting from analytically pure **13**, the benzyl group was removed in the last step at 0° under mild neutral conditions by catalytic hydrogenation to yield target molecule **1**.

Comparison of the ^{1}H-NMR chemical shift values and vicinal coupling constants of **1** (see *Exper. Part*) with the values reported for pseudouridine, an analogous *C*-glycoside having a ribofuranosyl form [17], suggested that the sugar of **1** adopted a furanose constitution. To determine the configuration at its anomeric center, **1** was analyzed by NOE spectroscopy. For β-D-ribonucleosides, a NOE between H–C(1′) and H–C(4′) is expected [18] [19], and for α-D-nucleosides, a characteristic NOE from H–C(1′) to H–C(3′) can be observed. The measured NOE from H–C(1′) to H–C(4′), observed in both directions, confirmed the assignment of the β-D-configuration of **1**.

When stored in solution at room temperature, **1** yielded a new compound slowly over time (*vide infra*). This compound was isolated by HPLC (see *Exper. Part*) and its structure again studied by ^{1}H-NMR and NOE spectroscopy. Its chemical-shift values and vicinal coupling constants were analyzed using empirically established rules for the NMR parameters of D-ribofuranosyl *C*-nucleosides [20] and the values reported for the α-D-furanosyl form of pseudouridine [17]. This analysis showed that the product could not be another furanosyl structure. Another set of empirical rules [21], established with *N*-glycosides, suggested that this product was the β-ribopyranosyl nucleoside **4**, predominantly in a $^4C_1$-chair conformation.

The signal for H–C(3′) of **4** appears as a small *t* at lower field than the *m* of H–C(2′) and H–C(4′); J(2′,3′), expected from the rules to be 2.8–3.4 Hz in the ribopyranosyl $^4C_1$ chair, is found to be only slightly lower (2.7 Hz), and J(3′,4′) (expected: 2.6–3.3 Hz; found: 2.8 Hz) is characteristically small for the $^4C_1$ form compared to the $^1C_4$ form (expected: 3.6–4.0 Hz). J(5′a,5′b) (expected: 10.8–10.9 Hz; found: 10.6 Hz) is smaller for the $^4C_1$ form compared to the $^1C_4$ form (expected: 13.4–14.0 Hz). J(4′,5′a) (found: 10.9 Hz) is near to the expected value (10.0 Hz). Only the value measured for J(1′,2′) (predicted: 7.0 Hz; found: 9.9 Hz) is somewhat large compared to the value expected for a β-D-ribopyranosyl nucleoside in a $^4C_1$ conformation. Considering that the empirical rules were based on spectra for *N*-nucleosides, this is not surprising, as larger J(1′,2′) coupling constants are generally observed for *C*-nucleosides [22]. The assignment for **4** was confirmed by the observed NOE's from H–C(2′) to H–C(4′) and from H–C(3′) to H–C(2′) and H–C(4′).

The thermodynamic preference for the β-D-ribopyranosyl nucleoside **4** over the furanosyl form **1** can be rationalized. Six-membered rings (pyranoses) are generally more stable than five-membered rings (furanoses). In addition, the sterically demanding pyrazine base and three of four OH groups lie in an equatorial position, avoiding unfavorable 1,3-diaxial interactions as much as possible. No other chair or boat structure leads to an equally favorable array of the substituents.

Both the kinetic and thermodynamic behavior of **1** has precedent in the reactivity of other *C*-nucleosides [23–29]. *E.g.*, rearrangement of the naturally occurring *C*-nucleoside pseudouridine was first described by *Cohn* [27], and later shown to be acid- and base-catalyzed [25]. Pyranose forms of pseudouridine predominate at thermodynamic equilibrium (71% pyranoses; 29% furanoses). A postulated mechanism for the analogous rearrangement reaction of **1** is shown in *Fig. 2*.

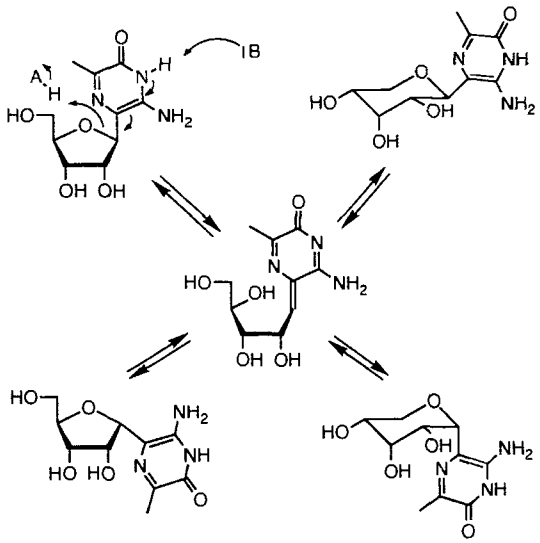


Fig. 2. *Proposed mechanism for the rearrangement of* **1**

Kinetic Studies on the Rearrangement Reaction of **1**. To obtain quantitative information concerning the reaction, the rearrangement of **1** was followed by HPLC (see *Fig. 3a* for the course of a typical reaction). Under both acidic and neutral conditions, the reaction can be described by a reversible first-order 'approach to equilibrium' process [30]: $\ln([\mathbf{1}] - [\mathbf{1}]_{eq}) = -(k_1 + k_{-1})t + \ln([\mathbf{1}]_o - [\mathbf{1}]_{eq})$. A plot of $\ln([\mathbf{1}] - [\mathbf{1}]_{eq})$ against t gives a line with the slope $-(k_1 + k_{-1})$ (see *Fig. 3,b*). The *Table* collects the calculated rate constants and half-lives ($t_{1/2}$) for the rearrangement of **1** under various conditions. Interestingly, the rearrangement of **1** is considerably more rapid than the analogous rearrangement seen with pseudouridine, which rearranges with a $t_{1/2} = 15$–30 min in 1M aqueous HCl at 100° (estimated from [27]).

Both acid and base influence the rate of the rearrangement reaction. The concentration of buffer, however, has virtually no effect on the rate constants. The reaction rate, therefore, seems subject to specific acid catalysis and specific base catalysis, but not general base catalysis, at least with this buffer. Under basic conditions, **1** undergoes decomposition in addition to the rearrangement. The decomposition reaction can be described by an irreversible first-order process; the products were not identified.

Synthesis of the Ribonucleoside Triphosphate **2**. Starting from α-amino nitrile **6a/b**, the protecting groups on the ribose moiety were exchanged to yield *via* **14a/b** and **15a/b** fully protected **16a/b** (*Scheme 2*). To facilitate interpretation of the spectroscopic data, the diastereoisomers **14a** and **14b** were separated by silica-gel column chromatography.

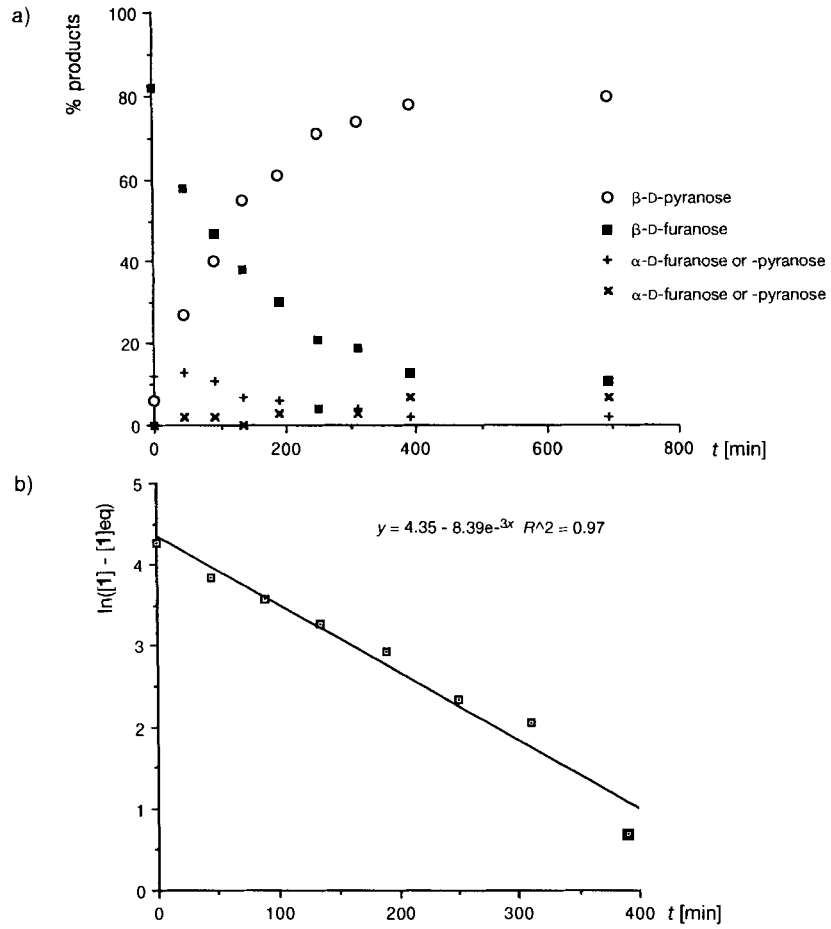


Fig. 3. a) *Course of a typical rearrangement reaction of* **1** *at pH 4.3 in 1.0* M *(Et_3NH)OAc at 30.0°* (data by HPLC, see *Exper. Part*) *and* b) *quantitative analysis of the rearrangement reaction of* **1** *as a reversible first-order process using the experimentally determined values shown in* Fig. 3, a

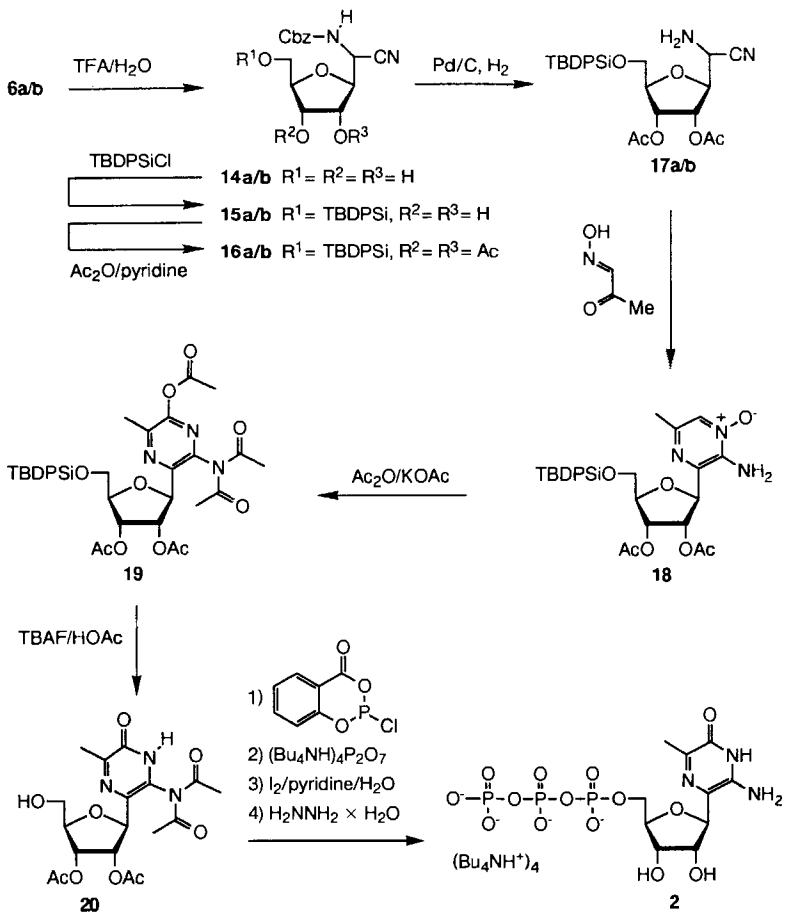
Table. *Velocity Constants and Half-Life Values for the Rearrangement Reaction of* **1** *at 30.0° at Different pH Values and (Et_3NH)OAc Concentrations.* Data by HPLC (see *Exper. Part*).

pH	Buffer conc. [M]	k(pseudo) [min^{-1}]	$t_{1/2}$(pseudo) [min]
4.2	0.5	$6.36 \cdot 10^{-3}$	109
5.6	0.5	$7.62 \cdot 10^{-4}$	909
6.5	0.5	$1.65 \cdot 10^{-4}$	4200
4.3	1.0	$8.39 \cdot 10^{-3}$	83
6.8	1.0	$1.84 \cdot 10^{-4}$	3770
pH	Buffer conc. [M]	k [min^{-1}]	$t_{1/2}$ [min]
9.8	0.5	$4.43 \cdot 10^{-4}$	1560
11.3	0.5	$4.95 \cdot 10^{-4}$	1400
10.1	1.0	$4.81 \cdot 10^{-4}$	1440

During the synthesis of pyrazine *N*-oxide **18**, the intermediate α-amino nitrile **17** was isolated. This allowed $CHCl_3$ to be used as solvent instead of dioxane, increasing the yields of the condensation reaction. Then **18** underwent rearrangement to **19**. Treatment with Bu_4NF removed the silyl protecting group [31] [32] to yield **20**. The free 5′-OH group of **20** was converted to the triphosphate by the method of *Ludwig* and *Eckstein* [33]; the acetyl groups were then removed by treatment with hydrazine hydrate. The free ribonucleoside triphosphate **2** was isolated by ion-exchange chromatography followed by HPLC. Spectroscopic analysis by ^{1}H- and ^{31}P-NMR showed no evidence that **2** underwent significant isomerization. Thus, **2** prepared by this route is useful as a substrate for RNA polymerases (see accompanying report).

Synthesis of the Ribonucleoside 3′,5′-Bisphosphate **3**. Compound **14a/b** was protected with the tetraisopropyldisiloxanediyl group (→**21a/b**) and the remaining 2′-OH group

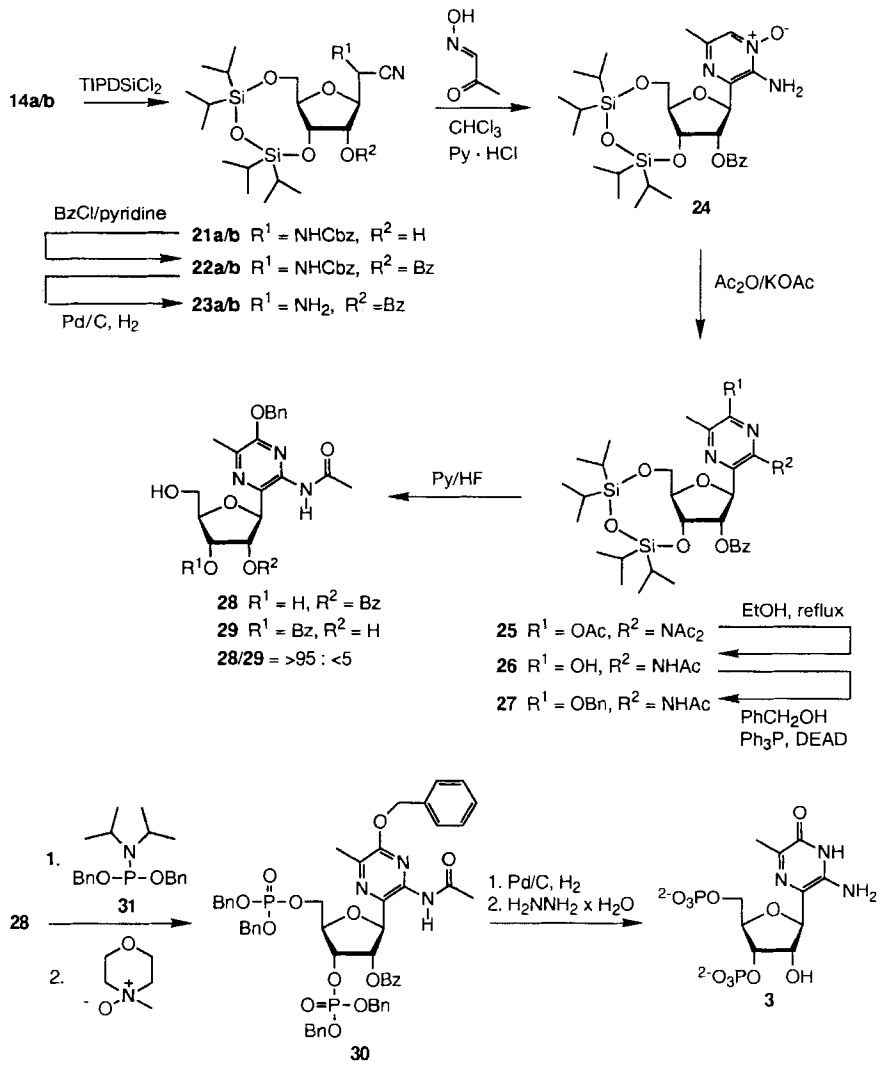
Scheme 2. *Synthesis of Ribonucleoside Triphosphate* **2**



TBDPSi = (*t*-Bu)Ph_2Si, TFA = CF_3COOH, Cbz = $PhCH_2OC(O)$, TBAF = Bu_4NF

benzoylated to yield **22a/b** (*Scheme 3*). Very low yields were noted during the cyclization of the free α-amino nitrile **23a/b** to pyrazine *N*-oxide **24**. Addition of catalytic amounts of pyridinium hydrochloride increased yields substantially. A weakly acidic catalyst for condensation reactions leading to highly substituted pyrazine *N*-oxides may be generally useful.

Scheme 3. *Synthesis of Ribonucleoside Biphosphate* **3**



Bz = PhC(O), Bn = $PhCH_2$, $TIPSiCl_2 = O[Si(i\text{-}Pr)_2]Cl_2$, Cbz = $PhCH_2OC(O)$, DEAD = EtOOCN = NCOOEt

Rearrangement of **24** (→**25**) and exchange of the acetyl protecting group on the pyrazine O-atom for a benzyl group yielded **27** *via* **26**. Use of the mild pyridine/HF reagent for removal of the silyl protecting group of **27** almost completely prevented

migration of the acyl group from O–C(2′) to O–C(3′). The ^{1}H-NMR spectrum of crude **28** showed no traces of compound **29**, which would arise from an undesired benzoyl migration. The migration was observed, however, during both column chromatography and crystallization attempts. Crude **28** was, therefore, used directly for the following phosphitylation step. Oxidation with *N*-methylmorpholine *N*-oxide yielded the protected bisphosphotriester **30** in a one-pot reaction. Deprotection of **30** by catalytic hydrogenation and treatment with hydrazine hydrate followed by ion-exchange chromatography yielded the pure ribonucleoside 3′,5′-bisphosphate **3**.

Qualitative investigations on the stability of **3** in aqueous solution at pH 7.0 showed promising results. After being treated at 60° for 40 min, **3** yielded only traces of side products by HPLC and ^{31}P-NMR analysis. T4 RNA-Ligase accepted the ribonucleoside 3′,5′-bisphosphate **3** as a substrate. Thus, it proved possible to enzymatically synthesize oligoribonucleotides containing the labile unnatural pyrazine nucleoside (see accompanying report).

Discussion. – Several different types of heterocycles can be considered to implement an acceptor-donor-donor H-bonding scheme on the 'small' component of a *Watson-Crick* base pair [15]. This H-bonding pattern is not, however, used in oligonucleotides on planet Earth, and it is appropriate to ask whether this fact reflects simple historical omission, constraints imposed by prebiological reactions, or an intrinsic chemical problem with pairs involving this base. These questions can be answered, at least in part, by chemical synthesis in the laboratory, exploring possible alternative implementations of this H-bonding scheme.

On paper, the acceptor-donor-donor H-bonding scheme can be implemented on a pyridine ring system, in the form of a 6-aminopyridin-2(1*H*)-one 5-riboside. Preliminary work by *von Krosigk* in our laboratories [34a] confirmed observations made many years ago that this substituted pyridine ring system was extremely unstable to oxidative decomposition [34b]. In air, these derivatives rapidly color (still more rapidly under alkaline conditions), yielding decomposition product that remain undefined.

We had encountered analogous (although less severe) problems when attempting to implement a donor-acceptor-donor system base on a pyridine heterocycle [35]. These problems were resolved by implementing this H-bonding pattern on a pyrimidine skeleton instead of a pyridine skeleton. The additional N-atom in the ring makes the system less electron-rich, and increases the stability of the system overall. The pyDAD system implemented on a pyrimidine heterocycle has proven to be one of the most useful expansions of the genetic alphabet [4].

The acceptor-donor-donor H-bonding pattern cannot easily be implemented on a pyrimidine nucleus, however, as a component of a replication system. The 5-ribofuranosyl-4-aminopyrimidin-2(1*H*)-one system suffers from an unacceptable tautomeric ambiguity; movement of a proton from one ring N-atom to the other converts the acceptor-donor-donor H-bonding pattern to an acceptor-acceptor-donor H-bonding pattern.

The pyrazine ring system appears to have sufficient intrinsic stability to support this H-bonding pattern [15] [36]. Indeed, as we have shown previously, the pyrazine ring system described here, incorporated into an oligonucleotide, sustains sequence-specific recognition of a complementary strand following an extended set of *Watson-Crick* base-pairing rules ([37] and accompanying report).

Suppressing the oxidation reaction, however, allows another reactivity to emerge. With a free 5′-OH group, rearrangement of the furanose ring to give a pyranose is observed. When the 5′-OH group of **1** is protected, making pyranose formation impossible, simple epimerization is observed. This reaction occurs slowly when the pyrazine nucleoside **1** is incorporated in an oligoribonucleotide ([37] and accompanying report).

Such epimerization is not unknown in oligonucleotide chemistry. *E.g.*, pseudouridine, a component of natural tRNA and rRNA presenting an acceptor-donor-acceptor pattern to its neighbors, undergoes such an epimerization reaction, slowly interconverting the α-D- and β-D-forms of the riboside. 2′-Deoxyribo-*C*-nucleosides generally isomerize even more easily than the corresponding ribo-*C*-nucleosides [38]; *e.g.*, 2′-deoxypseudouridine epimerizes more rapidly than pseudouridine itself [39]. The corresponding deoxyribonucleoside derivative of **1** incorporated in a DNA molecule is, therefore, expected to undergo the epimerization even more easily. Such a reaction is clearly undesirable for an oligodeoxyribonucleotide intended for the storage of genetic information. Epimerization might be observed with any nucleoside where electrons from a ring N–H bond can flow *via* a π system to break the C(1′)–O(4′) bond in the sugar. It may explain why this H-bonding scheme is not found in oligonucleotides from organisms found naturally on earth.

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Experimental Part

1. *General. Fluka* solvents (*puriss. p.a.*, *puriss*, or *purum*) were used for all syntheses. Et_2O, THF, and dioxane were distilled over Na with benzophenone as indicator. $CHCl_3$, CH_2Cl_2, and DMF were dried over molecular sieves (4 Å, 2–3 mm). MeCN was distilled over CaH_2. All chemicals were purchased from *Fluka*, except *anti*-pyruvic aldehyde 1-oxime, which was from *Aldrich*, and 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one and triethylammonium pyrophosphate which were from *Sigma*. Flash chromatography (FC): standard flash silica gel *60* adsorbent. NMR Spectra: 93.94 kG (400 MHz for ^1H, 100 MHz for ^{13}C) or 70.46 kG (300 MHz for ^1H, 75 MHz for ^{13}C); $SiMe_4$ (δ 0.00 ppm) was used as internal standard for both ^1H and ^{13}C spectra; OH and NH assignments were confirmed by D_2O exchange MS: *m/z* (rel. intensity); 3-NOBA = 3-nitrobenzyl alcohol.

2. *Ribonucleoside* **1**. N-*(Benzyloxycarbonyl)-2-*{*5′*-O-*[(*tert-*butyl)dimethylsilyl]-2′,3′*-O-*isopropylidene-β*-D-*ribofuranosyl*}-D,L-*glycinenitrile* (**6a/b**). To a soln. of **5a/b** (21.7 g, 43.9 mmol; synthesized following [15]) in abs. dioxane (400 ml), pyridine (10.6 ml, 131.7 mmol) was added and the resulting soln. cooled to 10°. $(CF_3CO)_2O$ (10.5 ml, 74.6 mmol) was injected and the soln. afterwards warmed to r.t. and stirred for 14 h. The soln. was diluted with $CHCl_3$ and extracted with ice/H_2O, H_2O, and sat. aq. NaCl soln. the org. phase was dried ($MgSO_4$) and evaporated: **6a/b** 2:1 (22.53 g, quant.). Brown oil, which was used directly for the next reaction. IR ($CHCl_3$): 3430, 2960, 2930, 2860, 1730, 1500, 1375, 1285, 1260, 1130, 1080, 805, 785. ^1H-NMR ($CDCl_3$; assignment of the two diastereoisomers by COSY): 0.04, 0.05 (2*s*, 3 H, Me_2Si(**6a**)); 0.10 (*s*, 6 H, Me_2Si(**6b**)); 0.84 (*s*, 9 H, ^{t}Bu(**6a**)); 0.91 (*s*, 9 H, ^{t}Bu(**6b**)); 1.32, 1.52 (2*s*, 3 H, Me_2C(**6a**)); 1.34, 1.52 (2*s*, 3 H, Me_2C(**6b**)); 3.74 (*dd*, *J* = 23.9, 11.2, 1 H, H–C(5′)(**6b**)); 3.76 (*dd*, *J* = 2.2, 11.4, 1 H, H–C(5′)(**6a**)); 3.79 (*dd*, *J* = 3.5, 11.2, 1 H, H′–C(5′)(**6b**)); 3.90 (*dd*, *J* = 2.5, 11.4, 1 H, H′–C(5′)(**6a**)); 4.07 (*dd*, *J* = 4.3, 6.0, 1 H, H–C(1′)(**6b**)); 4.12 (*m*, 1 H, H–C(4′)(**6a**)); 4.18 (*m*, 1 H, H–C(4′)(**6b**)); 4.29 (br., 1 H, H–C(1′)(**6a**)); 4.48 (*dd*, *J* = 5.8, 5.9, 1 H, H–C(2′)(**6a**)); 4.57 (*dd*, *J* = 4.4, 6.4, 1 H, H–C(2′)(**6b**)); 4.67 (*dd*, *J* = 3.6, 6.2, 1 H, H–C(3′)(**6a**)); 4.70 (*dd*, *J* = 3.0, 6.5, 1 H, H–C(3′(**6b**)); 4.87 (br. *d*, *J* = 8.0, 2 H, CH(**6a/b**)); 5.10–5.17 (*m*, 4 H, PhCH_2(**6a/b**)); 5.42 (br. *d*, *J* = 8.0, 1 H, NH(**6b**)); 5.93 (br. *d*, *J* = 9.0,

1 H, NH(**6a**)); 7.31–7.38 (*m*, 10 H, arom. H(**6a/b**)). ^{13}C-NMR ($CDCl_3$): −5.45, −5.34 (2*q*, Me_2Si(**6b**)); −5.42, −5.38 (2*q*, Me_2Si(**6a**)); 18.50 (*s*, Me_3C(**6b**)); 18.59 (*s*, Me_3C(**6a**)); 25.37, 27.30 (2*q*, Me_2C(**6b**)); 25.47, 27.52 (2*q*, Me_2C(**6a**)); 26.00 (*q*, Me_3C(**6a, b**)); 45.06 (*d*, CH(**6a**)); 45.88 (*d*, CH(**6b**)); 63.38 (*t*, C(5′)(**6b**)); 63.80 (*t*, C(5′)(**6a**)); 67.89 (*t*, $PhCH_2$(**6b**)); 68.00 (*t*, $PhCH_2$(**6a**)); 80.88, 82.26, 83.92, 85.92 (4*d*, C(1′), C(2′), C(3′), C(4′)(**6b**)); 81.32, 82.03, 83.17, 85.47 (4*d*, C(1′), C(2′), C(3′), C(4′)(**6a**)); 114.32, (*s*, Me_2C(**6a**)); 114.43 (*s*, Me_2C(**6b**)); 116.53 (*s*, CN(**6b**)); 116.99 (*s*, CN(**6a**)); 128.34, 128.53 (2*d*, C_o, C_m(**6b**)); 128.50 (*d*, C_p(**6a**)); 128.57, 128.63 (2*d*, C_o(**6a**), C_m(**6b**)); 128.74 (*d*, C_p(**6b**)); 135.42 (*s*, C_{ipso}(**6a**)); 135.50 (*s*, C_{ipso}(**6b**)); 155.47 (*s*, $PhCH_2OC(O)$(**6b**)); 155.57 (*s*, $PhCH_2OC(O)$(**6a**)). MS: 477 (1, $[M+1]^+$), 476 (1, M^+), 311 (59), 302 (52), 258 (62), 117 (59), 108 (57), 92 (53), 91 (100), 89 (54), 79 (51), 75 (64), 73 (67), 59 (54), 43 (64).

*3-{5′-O-[(*tert*-Butyl)dimethylsilyl]-2′,3′-O-isopropylidene-β-*D*-ribofuranosyl}-5-methylpyrazin-2-amine 1-Oxide* (**8**). To a soln. of **6a,b** (100 mg, 0.21 mmol) in dioxane (2 ml), 10% Pd/C (10 mg) was added and the suspension stirred at r.t. under H_2 overnight. The Pd/C was removed by centrifugation. *anti*-Pyruvic aldehyde 1-oxime (27 mg, 0.31 mmol) was added, the soln. heated to reflux for 54 h, and the solvent evaporated. Chromatography (silica gel, AcOEt/hexane 8:2) yielded **8** (40 mg, 46% for two steps) as a brown oil. A larger-scale reaction (23 mmol) proceeded analogously. IR ($CHCl_3$): 3430, 3320, 2990, 2960, 2930, 2860, 1610, 1565, 1490, 1385, 1335, 1260, 1140, 1105, 1080, 985, 860, 840. ^{1}H-NMR ($CDCl_3$): 0.06 (*s*, 3 H, Me_2Si); 0.09 (*s*, 3 H, Me_2Si); 0.83 (*s*, ^{t}Bu); 1.39 (*s*, 3 H, Me_2C); 1.62 (*s*, 3 H, Me_2C); 2.37 (*s*, Me–C(5)); 3.82 (*dd*, *J* = 2.3, 11.3, 1 H–C(5′)); 3.91 (*dd*, *J* = 2.6, 11.3, 1 H–C(5′)); 4.24 (*m*, H–C(4′)); 4.83 (*dd*, *J* = 3.6, 6.5, H–C(3′)); 5.02–5.09 (*m*, H–C(1′), H–C(2′)); 6.28 (*s*, NH_2); 7.87 (*s*, H–C(6)). ^{13}C-NMR ($CDCl_3$): −5.53 (*q*, Me_2Si); 18.48 (*s*, Me_3C); 20.52 (*q*, *Me*–C(5)); 25.48 (*q*, Me_2C); 25.90 (*q*, Me_3C); 27.45 (*q*, Me_2C); 62.81 (*t*, C(5′)); 80.99, 82.93, 85.24, 87.40 (4*d*, C(1′), C(2′), C(3′), C(4′)); 114.88 (*s*, Me_2C); 129.13 (*d*, C(6)); 139.45, 141.26, 143.59 (3*s*, C(2), C(3), C(5)). MS: 411 (1, M^+), 208 (92), 150 (55), 134 (57), 133 (71), 117 (53), 75 (100), 73 (99).

*5-{5′-O-[(*tert*-Butyl)dimethylsilyl]-2′,3′-O-isopropylidene-β-*D*-ribofuranosyl}-6-(diacetylamino)-3-methylpyrazin-2-yl Acetate* (**9**). To a soln. of **8** (2.27 g, 5.51 mmol) in Ac_2O (2.0 ml), KOAc (0.54 g, 5.51 mmol) was added and the resulting suspension heated to reflux for 9 min. The mixture was cooled to r.t., and Ac_2O removed under reduced pressure. To remove last traces of Ac_2O, the resulting brown oil was dissolved in EtOH and evaporated. The crude product was dissolved in CH_2Cl_2 and excess KOAc removed by filtration. The product was adsorbed on silica gel (8 g) and submitted to chromatography (silica gel (200 g), Et_2O/hexane 4:6): **9** (1.96 g, 66%). Slightly yellow oil. IR ($CHCl_3$): 2960, 2930, 2860, 1790, 1170, 1725, 1420, 1370, 1260, 1170, 1140, 1075, 840. ^{1}H-NMR ($CDCl_3$): 0.02 (*s*, 3 H, Me_2Si); 0.01 (*s*, 3 H, Me_2Si); 0.83 (*s*, 9 H, ^{t}Bu); 1.38 (*s*, 3 H, Me_2C); 1.56 (*s*, 3 H, Me_2C); 2.09 (br. *s*, 3 H, Ac_2N); 2.38 (*s*, Me–C(3) or AcO); 2.49 (br. *s*, 3 H, Ac_2N); 2.54 (*s*, Me–C(3) or AcO); 3.66 (*AB*, 2 H–C(5′)); 4.19 (*m*, H–C(4′)); 4.75 (*dd*, *J* = 3.2, 6.3, H–C(3′)); 4.93 (*d*, *J* = 4.2, H–C(1′)); 5.22 (*dd*, *J* = 4.2, 6.3, H–C(2′)). ^{13}C-NMR ($CDCl_3$): −5.74 (*q*, Me_2Si); −5.41 (*q*, Me_2Si); 18.29 (*s*, Me_3C); 19.03 (*q*, *Me*–C(3)); 20.85 (*q*, *Me*COO); 25.61 (*q*, Me_2C); 25.84 (*q*, Me_3C); 26.45 (*q*, *Me*CON); 26.90 (*q*, *Me*CON); 27.58 (*q*, Me_2C); 63.42 (*t*, C(5′)); 82.22, 82.48, 83.42, 85.98 (4*d*, C(1′), C(2′), C(3′), C(4′)); 113.88 (*s*, Me_2C); 143.37, 147.68, 149.23, 151.40 (4*s*, C(2), C(3), C(5), C(6)); 168.01 (*s*, MeCOO); 171.88 (*s*, MeCON); 172.73 (*s*, MeCON). MS: 537 (1, M^+), 75 (63), 73 (66), 43 (100).

*6-Amino-5-{5′-O-[(*tert*-butyl)dimethylsilyl]-2′,3′-O-isopropylidene-β-*D*-ribofuranosyl}-3-methylpyrazin-2(1*H*)-one* (**10**). A soln. of **9** (100 mg, 0.19 mmol) in hydrazine hydrate (1 ml), was stirred for 2 h at r.t. The soln. was cooled to 0° and cautiously neutralized to pH 7 with 2N aq. HCl. An oil separated which, after trituration with H_2O, yielded **10** (62 mg, 80%). Yellow solid. ^{1}H-NMR ($CDCl_3$): 0.05 (*s*, 3 H, Me_2Si); 0.07 (*s*, 3 H, Me_2Si); 0.88 (*s*, ^{t}Bu); 1.37 (*s*, 3 H, Me_2C); 1.59 (*s*, 3 H, Me_2C); 2.25 (*s*, Me–C(3)); 3.76 (*dd*, *J* = 2.9, 11.2, 1 H–C(5′)); 3.85 (*dd*, *J* = 3.1, 11.2, 1 H–C(5′)); 4.10 (*m*, H–C(4′)); 4.76 (*dd*, *J* = 3.7, 6.8, H–C(3′)); 4.92 (*d*, *J* = 5.0, H–C(1′)); 5.03 (*dd*, *J* = 5.1, 6.7, H–C(2′)); 5.61 (br. *s*, NH_2). ^{13}C-NMR ($CDCl_3$): −5.32 (*q*, Me_2Si); −5.28 (*q*, Me_2Si); 18.7 (*s*, Me_3C); 18.8 (*q*, *Me*–C(3)); 25.9 (*q*, Me_2C); 26.1 (*q*, Me_3C); 27.9 (*q*, Me_2C); 63.3 (*t*, C(5′)); 81.2, 83.2, 84.9, 86.6 (4*d*, C(1′), C(2′), C(3′), C(4′)); 114.8 (*s*, Me_2C); 117.0, 136.2, 143.2, 157.0 (4*s*, C(2), C(3), C(5), C(6)). FAB-MS (3-NOBA): 412 ($[M+1]^+$).

*6-(Acetylamino)-5-{5′-O-[(*tert*-butyl)dimethylsilyl]-2′,3′-O-isopropylidene-β-*D*-ribofuranosyl}-3-methylpyrazin-2(1*H*)-one* (**11**). A soln. of **9** (294 mg, 0.55 mmol) in MeOH (6 ml) was diluted with conc. aq. NH_3 soln. (0.1 ml) and stirred for 12 min. The solvent was then evaporated and the crude product adsorbed on silica gel. Chromatography (silica gel (10 g), AcOEt/CH_2Cl_2 3:2) yielded **11** (236 mg, 95%). Amorphous solid. ^{1}H-NMR ($CDCl_3$): 0.00 (*s*, 3 H, Me_2Si); 0.02 (*s*, 3 H, Me_2Si); 0.83 (*s*, ^{t}Bu); 1.41 (*s*, 3 H, Me_2C); 1.59 (*s*, 3 H, Me_2C); 2.18, 2.38 (2*s*, Me–C(3), AcN); 3.68 (*dd*, *J* = 4.7, 11.5, 1 H–C(5′)); 3.76 (*dd*, *J* = 3.2, 11.5, 1 H–C(5′)); 4.29–4.31 (*m*, H–C(4′)); 4.63 (*dd*, *J* = 3.1, 6.5, H–C(3′)); 5.08 (*d*, *J* = 3.8, H–C(1′)); 5.19 (*dd*, *J* = 3.8, 6.5, H–C(2′)); 9.67 (br. *s*, NH); 12.20 (br. *s*, NH). ^{13}C-NMR ($CDCl_3$): −5.46 (*q*, Me_2Si); −5.44 (*q*, Me_2Si); 18.3 (*s*, Me_3C); 20.02 (*q*, *Me*–C(3)); 24.95 (*q*, *Me*CON); 25.44 (*q*, Me_2C); 25.77 (*q*, Me_3C); 27.36 (*q*, Me_2C); 63.54 (*t*, C(5′)); 81.04, 84.54,

85.75, 86.44 (4*d*, C(1′), C(2′), C(3′), C(4′)); 114.07 (*s*, Me_2C); 114.69, 133.59, 150.14, 153.48 (4*s*, C(2), C(3), C(5), C(6)); 171.05 (*s*, Me*C*ON). FAB-MS (3-NOBA): 454 ([*M* + 1]$^+$). Anal. calc. for $C_{21}H_{35}N_3O_6Si$ (453.61): C 55.61, H 7.78, N 9.26; found: C 55.50, H 7.86, N 9.06.

N-{*6-(Benzyloxy)-3-*{*5′*-O-*[(* tert-*butyl)dimethylsilyl]-2′,3′*-O-*isopropylidene-β*-D-*ribofuranosyl*}*-5-methylpyrazin-2-yl*}acetamide* (**12**). At 50°, **11** (468 mg, 1.03 mmol) and PPh_3 (406 mg, 1.55 mmol) were dried for 1.5 h under high vacuum and then dissolved in abs. THF (9 ml). Benzyl alcohol (128 µl, 1.24 mmol) was added and the resulting homogeneous soln. cooled to 0°. DEAD (243 µl, 1.55 mmol) was slowly added within 10 min. After being stirred for 30 min at 0°, the soln. was cooled in liq. N_2 and the solvent removed under high vacuum at low temp. Chromatography (silica gel (60 g), AcOEt/hexane 10:90, then 20:80, 25:75, and 30:70) yielded **12** (382 mg, 68%). Amorphous foam. ^{1}H-NMR ($CDCl_3$): −0.10 (*s*, 3 H, Me_2Si); −0.05 (*s*, 3 H, Me_2Si); 0.79 (*s*, ^{t}Bu); 1.41 (*s*, 3 H, Me_2C); 1.59 (*s*, 3 H, Me_2C); 2.30, 2.45 (2*s*, Me−C(5), AcN); 3.59 (*dd*, *J* = 4.5, 11.1, 1 H−C(5′)); 3.63 (*dd*, *J* = 4.3, 11.1, 1 H−C(5′)); 4.26–4.28 (*m*, H−C(4′)); 4.72 (*dd*, *J* = 2.7, 6.5, H−C(3′)); 5.07 (*d*, *J* = 4.1, H−C(1′)); 5.38 (*s*, PhCH_2); 5.45 (*dd*, *J* = 4.1, 6.5, H−C(2′)); 7.31–7.43 (*m*, 5 arom. H); 8.71 (br. *s*, NH). ^{13}C-NMR ($CDCl_3$): −5.56 (*q*, Me_2Si); −5.54 (*q*, Me_2Si); 18.28 (*s*, ^{t}Bu); 18.56 (*q*, *Me*−C(5)); 24.56 (*q*, *Me*CON); 25.56 (*q*, Me_2C); 25.78 (*q*, Me_2C); 27.43 (*q*, Me_2C); 63.47 (*t*, C(5′)); 68.18 (*t*, PhCH_2); 81.91, 82.76, 84.45, 85.82 (4*d*, C(1′), C(2′), C(3′), C(4′)); 113.80 (*s*, Me_2C); 127.58, 127.99, 128.52 (3*d*, arom. CH); 136.63 (*s*, C_{ipso}(Bn)); 132.54, 137.43, 141.7 (3*s*, C(2), C(3), C(5), C(6)); 155.75 (*s*, Me*C*ON). FAB-MS (3-NOBA): 544 ([*M* + 1]$^+$). Anal. calc. for $C_{28}H_{41}N_3O_6Si$ (543.74): C 61.85, H 7.60, N 7.73; found: C 62.12, H 7.82, N 7.88.

*6-(Benzyloxy)-5-methyl-3-β-*D-*ribofuranosylpyrazin-2-amine* (**13**). A soln. of **12** (147 mg, 0.27 mmol) in MeOH/HCl (2% HCl, 1.5 ml) at r.t. was stirred for 3.75 h. Solid $NaHCO_3$ was added until the production of CO_2(g) ceased. The inorg. salts were removed by filtration and the solvent evaporated. Chromatography (silica gel (115 g), CH_2Cl_2/MeOH 25:1, 20:1, and 15:1) yielded **13** (56 mg, 60%). White solid. An anal. sample for the final step was obtained by recrystallization from MeCN. M.p. 152–153°. ^{1}H-NMR (CD_3OD): 2.27 (*s*, Me−C(5)); 3.72 (*dd*, *J* = 2.8, 11.9, 1 H−C(5′)); 3.85 (*dd*, *J* = 2.8, 11.9, 1 H−C(5′)); 3.98–4.01 (*m*, H−C(4′)); 4.19 (*dd*, *J* = 4.5, 5.7, H−C(3′)); 4.31–4.35 (*m*(*t*′), H−C(2′)); 4.82 (*d*, *J* = 6.4, H−C(1′)); 5.34 (*s*, PhCH_2); 7.28–7.45 (*m*, 5 arom. H). ^{13}C-NMR (CD_3OD): 17.21 (*q*, *Me*−C(5)); 62.91 (*t*, C(5′)); 68.52 (*t*, PhCH_2); 72.53, 74.71, 84.34, 86.64 (4*d*, C(1′), C(2′), C(3′), C(4′)); 128.85, 128.92, 129.45 (*d*, arom. CH); 138.78 (*s*, C_{ipso}(Bn)); 128.11, 129.26, 151.73, 157.84 (4*s*, C(2), C(3), C(5), C(6)). FAB-MS (3-NOBA): 348 ([*M* + 1]$^+$). Anal. calc. for $C_{17}H_{21}N_3O_5$ (374.4): C 58.78, H 6.09, N 12.10; found: C 59.01, H 6.11, N 12.03.

*6-Amino-3-methyl-5-(β-*D-*ribofuranosyl)pyrazin-2-(1*H*)-one* (**1**). At r.t. **13** (21 mg, 0.060 mmol) was dissolved in MeOH (3 ml) and the soln. cooled to 0°. $Pd(OH)_2$/C was added and the suspension stirred under H_2 for 1 h at 0°. The catalyst was quickly removed by filtration and the product-containing soln. recovered in a precooled flask. The resulting colorless soln. was cooled in liq. N_2. Evaporation of the solvent at low temperature under high vacuum yielded **1** (18 mg, quant.) as amorphous white solid. Dissolution of the crude product at r.t. in H_2O followed by addition of MeCN and cooling yielded a few mg of crystalline material. The rest of the material was recovered as amorphous solid after evaporation of the solvent. M.p. 165° (dec.). IR (KBr): 3420 (br.), 3930, 1625 (br.), 1540, 1475, 1375, 1160 (sh), 1075 (br.), 960, 795. ^{1}H-NMR ((D_6)DMSO): 2.07 (*s*, Me−C(3)); 3.40–3.58 (*m*, 2 H−C(5′)); 3.75–3.78 (*m*(*q*′), H−C(4′)); 3.95 (very br. *s*, H−C(3′)); 4.16–4.17 (*m*(*q*′), H−C(2′)); 4.60 (*d*, *J* = 6.7, H−C(1′)); 4.75–4.79 (*m*, OH−C(2′), OH−C(3′)); 5.11–5.13 (br. *m*(*t*′), OH−C(5′)); 5.79 (br. *s*, NH_2); 11.1 (very br. *s*, NH). ^{1}H-NMR (D_2O): 2.19 (*s*, Me−C(3)); 3.79 (*dd*, *J* = 3.4, 12.5, 1 H−C(5′)); 3.84 (*dd*, *J* = 3.0, 12.5, 1 H−C(5′)); 4.07 (*ddd*, *J* = 3.0, 3.4, 3.7, H−C(4′)); 4.25 (*dd*, *J* = 3.7, 5.8, H−C(3′)); 4.37 (*dd*, *J* = 5.8, 7.7, H−C(2′)); 4.79 (*d*, *J* = 7.7, H−C(1′)). ^{13}C-NMR (D_2O): 19.91 (*q*, *Me*−C(3)); 64.06 (*t*, C(5′)); 73.59, 75.42, 82.94, 87.69 (4*d*, C(1′), C(2′) C(3′), C(4′)); 116.97, 141.05, 145.86, 160.09 (4*s*, C(2), C(3), C(5), C(6)). FAB-MS (glycerol): 258 ([*M* + 1]$^+$). Anal. calc. for $C_{10}H_{15}N_3O_5$ (257.25): C 46.69, H 5.88, N 16.33; found: C 46.31, H 5.82, N 15.82.

3. *Studies on the Rearrangement Reaction of* **1**. *General Description.* Aq. *ca.* 5 mM **1** (100 µl; containing some (Et_3NH)OAc) was mixed with (Et_3NH)OAc buffer (900 µl; 0.5 and 1.0M (Et_3NH)OAc, resp., pH adjusted with Et_3N), and the resulting pH was measured exactly. The reaction was immediately incubated at 30.0° and periodically analyzed by HPLC (*Supelco, LC18-DB*, particle size 5 µm, 25 cm × 10 mm; *A*, 0.1M (Et_3NH)OAc pH 7.5, eluent *B*, MeCN, gradient 0–5% *B* within 30 min then to 25% within 10 min; flow rate 2 ml/min for 30 min, then to 3 ml/min within 1 min and remaining at this rate; detection: UV at 254 and 350 nm, integration of the signals at 350 nm (assumption of identical extinction coefficients for the different isomers); t_R (min): **4** (16), α-D-pyranosyl- or α-D-furanosylnucleoside (20), **1** (22), α-D-pyranosyl- or α-D-furanosylnucleoside (29), 4-amino-2-nitrobenzene-1-sulfonic acid (37). As internal standard 4-amino-2-nitrobenzene-1-sulfonic acid (recrystallized from H_2O) was added in a concentration which led to a UV absorption at 350 nm in between the initial and the equilibrium concentration of **1**. The internal standard, which was stable under the reaction conditions, allowed the exact determination of the relative concentration of **1** independent of small variations during the periodical injections on

the HPLC column. The equilibrium concentration of **1** was determined to be 11% from two independent experiments at pH < 7 (at higher pH, an irreversible decomposition took place).

*6-Amino-3-methyl-5-(β-*D*-ribofuranosyl)pyrazin-2(1*H*)-one* (**4**). Isolation by semiprep. HPLC (*Supelco, LC18-DB*, particle size 5 μm, 25 × 10 mm; eluent *A*, 0.1M (Et_3NH)OAc pH 7.5, no gradient; flow rate 4 ml/min; detection: UV at 254 and 350 nm; t_R 7 min). Product-containing fractions were pooled and the solvent evaporated under pH control (periodically readjusted to 7). To remove buffer salts, the product was filtered through *RP-C18*-silica gel with H_2O as eluent. ^{1}H-NMR (D_2O): 2.20 (*s*, Me–C(3)); 3.68 (*dd*, *J* = 10.9, 10.6, H_a–C(5′)); 3.77 (*dd*, *J* = 5.4, 10.6, H_b–C(5′)); 3.92 (*ddd*, *J* = 2.8, 5.4, 10.9, H–C(4′)); 4.05 (*dd*, *J* = 9.9, 2.7, H–C(2′)); 4.27 (‘*t*’, *dd*, *J* = 2.7, 2.8, H–C(3′)); 4.51 (*d*, *J* = 9.9, H–C(1′)).

4. *Synthesis of the Ribonucleoside 5′-Triphosphate.* N-*(Benzyloxycarbonyl)-2-(β-*D*-ribofuranosyl)-*D,L*-glycinenitrile* (**14a/b**). A soln. of **6a/b** (5.18 g, 10.9 mmol) CF_3COOH/H_2O 4:1 was stirred for 1 h at r.t. The solvent was removed by distillation, and last traces of CF_3COOH were removed by co-evaporation with EtOH. Chromatography (silica gel, CH_2Cl_2/EtOH 13:1) of the resulting oil yielded **14a/b** (1.80 g, 83%) as two separable oils (first eluting product = diastereoisomer **14a**).

Diastereoisomer **14a**: ^{1}H-NMR ((D_6)DMSO): 3.38–3.47 (*m*, 1 H–C(5′)); 3.52–3.58 (*m*, 1 H–C(5′)); 3.73–3.77 (*m*, 1 H), 3.80–3.85 (*m*, 2 H), 3.91–3.94 (*m*, 1 H; H–C(1′), H–C(2′), H–C(3′), H–C(4′)); 4.78 (*dd*, *J* = 4.1, 8.4, CH); 4.87 (*dd*, *J* = 5.1, 5.2, OH); 4.96 (*d*, *J* = 4.7, OH); 5.06 (*d*, *J* = 5.0, OH); 5.05–5.15 (*m*, CH_2); 7.34–7.39 (*m*, 5 arom. H); 8.06 (*d*, *J* = 8.4, NH). ^{13}C-NMR ((D_6)DMSO): 44.56 (*d*, CH); 61.31 (*t*, C(5′)); 66.33 (*t*, CH_2); 70.96, 71.24, 81.65, 85.01 (4*d*, C(1′), C(2′), C(3′), C(4′)); 118.22 (*s*, CN); 127.89, 128.04, 128.44 (3*d*, C_o, C_m, C_p); 136.39 (*s*, C_{ipso}); 155.74 (*s*, $PhCH_2OC(O)$). MS: 322 (0.5, M^+), 108 (33), 107 (28), 91 (100), 79 (31), 77 (20).

Diastereoisomer **14b**: ^{1}H-NMR ((D_6)DMSO): 3.37–3.51 (*m*, 2 H–C(5′)); 3.74–3.92 (*m*, H–C(1′), H–C(2′), H–C(3′), H–C(4′)); 4.67 (*dd*, *J* = 6.1, 8.4, CH); 4.74 (*dd*, *J* = 5.3. 5.4, OH); 4.96 (*d*, *J* = 5.4, OH); 5.08–5.10 (*m*, CH_2, OH); 7.33–7.38 (*m*, 5 arom. H); 8.34 (*d*, *J* = 8.4, NH). ^{13}C-NMR ((D_6)DMSO): 45.73 (*d*, CH); 61.39 (*t*, C(5′)); 66.21 (*t*, CH_2); 70.76, 71.32, 81.23, 84.82 (*d*, C(1′), C(2′), C(3′), C(4′)); 117.74 (*s*, CN); 127.80, 127.98, 128.42 (3*d*, C_o, C_m, C_p); 136.48 (*s*, C_{ipso}); 155.57 (*s*, $PhCH_2OC(O)$). MS: 323 (1.2, $[M + 1]^+$), 322 (3.0, M^+), 108 (28), 107 (21), 97 (17), 92 (17), 91 (100), 79 (17).

N-*(Benzyloxycarbonyl)-2-{5′-[(*tert*-butyl)diphenylsilyl]-β-*D*-ribofuranosyl}-*D,L*-glycinenitrile* (**15a**). A soln. of **14a** (1.63 g, 5.0 mmol) and 1*H*-imidazole (0.68 g, 10.0 mmol) in DMF (16.0 ml) was cooled to 0°. (*t*-Bu)Ph_2SiCl (1.55 ml, 6.24 mmol) was slowly added within 0.5 h and the soln. warmed to r.t. and stirred for 3 h. H_2O was added and the soln. extracted by addition of CH_2Cl_2. The org. phase was extracted with H_2O (2×) and sat. aq. NaCl soln. After drying ($MgSO_4$), the solvent was evaporated to yield crude **15a**. The product was used directly for the next reaction. A reaction with diastereoisomer **14b** proceeded analogously.

N-*(Benzyloxycarbonyl)-2-{2′,3′-di-*O*-acetyl-5′-*O*-[(*tert*-butyl)diphenylsilyl]-β-*D*-ribofuranosyl}-*D,L*-glycinenitrile* (**16a/b**). A soln. of **15a** in pyridine (8.1 ml, 100 mmol) and Ac_2O (14.2 ml, 150 mmol) was stirred at r.t. for 20 h. The solvent was removed by repeated co-evaporation with toluene and the resulting oil dried overnight under high vacuum. Chromatography (silica gel, AcOEt/hexane 3:7) yielded **16a** (2.77 g, 82% over 2 steps) as a clear oil. An analogous reaction with **14b** gave **6b** (78% over 2 steps).

Diastereoisomer **16a**: IR ($CHCl_3$): 3420 (br.), 2960, 2930, 2890, 2860, 1745 (br.), 1500, 1430, 1370, 1115, 1105, 1055, 930, 905. ^{1}H-NMR ($CDCl_3$): 1.00 (*s*, ^{t}Bu); 2.07 (*s*, Ac); 2.09 (*s*, Ac); 3.77 (*dd*, *J* = 3.0, 11.7, 1 H–C(5′)); 3.91 (*dd*, *J* = 2.5, 11.7, 1 H–C(5′)); 4.08–4.10 (*m*, H–C(1′) or H–C(4′)); 4.27–4.30 (*m*, H–C(1′) or H–C(4′)); 4.92–4.98 (*m*, H–C(2′) or H–C(3′), CH); 5.11–5.19 (*m*, CH_2); 5.33 (*dd*, *J* = 4.5, 5.6, H–C(2′) or H–C(3′)); 5.49 (*d*, *J* = 8.7, NH); 7.24–7.44, 7.63–7.68 (2*m*, 15 arom. H). ^{13}C-NMR ($CDCl_3$): 19.11 (*s*, Me_3C); 20.43, 20.52 (2*q*, *Me*COO); 26.84 (*q*, Me_3C); 44.57 (*d*, CH); 63.03 (*t*, C(5′)); 67.99 (*t*, CH_2); 70.71, 71.19, 79.99, 83.54 (4*d*, C(1′), C(2′), C(3′), C(4′)); 116.71 (*s*, CN); 127.85, 127.91, 127.95, 128.39, 128.53, 129.94, 130.02, 135.45, 135.68 (9*d*, C_o, C_m, C_p); 132.52, 132.76, 135.36 (3*s*, C_{ipso}); 155.38 (*s*, $PhCH_2OC(O)$); 169.56, 169.67 (2*s*, MeCOO). MS: 587 (15, $[M - 57(t\text{-Bu})]^+$), 241 (52), 199 (47), 91 (100).

Diastereoisomer **16b**: IR ($CHCl_3$): 3430 (br.), 2960, 2930, 2890, 2860, 1745 (br.), 1500, 1430, 1375, 1115, 1105, 1060, 1020, 980, 940. ^{1}H-NMR ($CDCl_3$): 1.06 (*s*, ^{t}Bu); 2.07 (*s*, Ac); 2.08 (*s*, Ac); 3.76 (*dd*, *J* = 3.9, 11.6, 1 H–C(5′)); 3.82 (*dd*, *J* = 3.6, 11.6, 1 H–C(5′)); 4.06–4.13 (*m*, H–C(1′), H–C(4′)); 4.83–5.00 (*m*, CH); 5.06 (*d*, *J* = 12.0, 1 H, CH_2); 5.13 (*d*, *J* = 12.0, 1 H, CH_2); 5.20 (*d*, *J* = 5.8, 7.2, H–C(2′) or H–C(3′)); 5.40 (*d*, *J* = 4.0, 5.8, H–C(2′) or H–C(3′)); 5.52 (*d*, *J* = 7.8, NH); 7.31–7.44, 7.64–7.69 (2*m*, 15 arom. H). ^{13}C-NMR ($CDCl_3$): 19.14 (*s*, Me_3C); 20.45, 20.56 (2*q*, *Me*COO); 26.83 (*q*, Me_3C); 44.97 (*d*, CH); 63.23 (*t*, C(5′)); 67.92 (*t*, CH_2); 77.38, 71.68, 79.48, 83.53 (4*d*, C(1′), C(2′), C(3′), C(4′)); 115.61 (*s*, CN); 127.85 (double intensity), 128.36, 128.49, 128.60, 129.86, 129.91, 135.65, 135.67 (8*d*, C_o, C_m, C_p); 132.72, 132.80, 137.47 (*s*, C_{ipso}); 155.07 (*s*, $PhCH_2OC(O)$); 169.51, 169.89 (2*s*, MeCOO). MS: 645 (3.3, $[M + 1]^+$), 587 (17, $[M - 57(^t\text{Bu})]^+$), 135 (31), 91 (100).

*2-{2′,3′-Di-O-acetyl-5′-O-[(*tert*-butyl)diphenylsilyl]-β-*D*-ribofuranosyl}-*D,L*-glycinenitrile* (**17a**). To a soln. of **16a** (1.52 g, 2.36 mmol) in dioxane (15 ml), 10% Pd/C (0.3 g) was added. The soln. was stirred at r.t. for 8 h under H_2. Pd/C was removed by filtration and the solvent evaporated followed by co-evaporation with AcOEt. Chromatography (silica gel (200 g), AcOEt/hexane 1:2) yielded **17a** (877 mg, 73%). Light yellow oil. IR ($CHCl_3$): 3410, 3330, 2960, 2930, 2890, 2860, 1750 (br.), 1470, 1430, 1370, 1250, 1115, 1090, 1020, 980. ^{1}H-NMR ($CDCl_3$): 1.06 (*s*, ^{t}Bu); 1.78 (*d*, *J* = 7.32, NH_2); 2.07 (*s*, Ac); 2.09 (*s*, Ac); 3.74 (*dd*, *J* = 3.0, 11.7, 1 H–C(5′)); 3.97 (*dd*, *J* = 2.7, 11.7, 2 H, 1 H–C(5′), overlayed with CH); 4.09–4.13 (*m*, H–C(1′) or H–C(4′)); 4.20–4.23 (*m*, H–C(1′) or H–C(4′)); 5.34–5.43 (*m*, H–C(2′), and H–C(3′)); 7.37–7.47, 7.64–7.70 (2*m*, 10 arom. H). ^{1}H-NMR ($CDCl_3$, D_2O exchange): 1.07 (*s*, ^{t}Bu); 2.07 (*s*, Ac); 2.09 (*s*, Ac); 3.75 (*dd*, *J* = 3.0, 11.6, 1 H–C(5′)); 3.93 (*d*, *J* = 2.6, CH); 3.97 (*dd*, *J* = 2.8, 11.6, 1 H–C(5′)); 4.10–4.14 (*m*, H–C(1′) or H–C(4′)); 4.20–4.23 (*m*, H–C(1′) or H–C(4′)); 5.34–5.42 (*m*, H–C(2′), H–C(3′)); 7.37–7.46, 7.65–7.71 (2*m*, 10 arom. H). ^{13}C-NMR ($CDCl_3$): 19.23 (*s*, Me_3C); 20.52 (*q* with sh, 2 MeCOO); 26.84 (*q*, Me_3C); 44.54 (*d*, CH); 63.57 (*t*, C(5′)); 70.89, 71.89, 82.25, 82.61 (4*d*, C(1′), C(2′), C(3′), C(4′)); 119.96 (*s*, CN); 127.89 (double intensity), 129.89, 129.97, 135.47, 135.70 (5*d*, C_o, C_m, C_p); 132.64, 133.95 (2*s*, C_{ipso}); 169.62, 170.03 (*s*, 2 Me*C*OO). MS: 512 (18, $[M + 2]^+$), 511 (49, $[M + 1]^+$), 199 (61), 137 (50), 135 (100).

*3-{2′,3′-Di-O-acetyl-5′-O-[(*tert*-butyl)diphenylsilyl]-β-*D*-ribofuranosyl}-5-methylpyrazin-2-amine 1-Oxide* (**18**). A soln. of **17a** (788 mg, 1.54 mmol) and *anti*-pyruvic aldehyde 1-oxime (175 mg, 2.0 mmol) in abs. $CHCl_3$ (4.0 ml) was heated under reflux for 7 days. The mixture was evaporated and then co-evaporated with AcOEt. Chromatography (silica gel (80 g), AcOEt) yielded **18** (514 mg, 58%). Slightly yellow semicrystalline foam. UV (EtOH): 255 (sh, 6880), 345 (7660). IR (KBr): 3450, 3330, 2960, 2930, 2860, 1745 (br.), 1610, 1560, 1490, 1470, 1430, 1370, 1335, 1250, 1140, 1110, 1090, 1040, 980, 900, 845. ^{1}H-NMR ($CDCl_3$): 1.06 (*s*, ^{t}Bu); 2.08 (*s*, Ac); 2.09 (*s*, Ac); 2.31 (*d*, *J* = 0.5, Me–C(5)); 3.86 (*dd*, *J* = 2.7, 11.6, 1 H–C(5′)); 3.93 (*dd*, *J* = 2.5, 11.6, 1 H–C(5′)); 4.20 (*ddd*, *J* = 2.5, 2.7, 3.7, H–C(4′)); 5.15 (*d*, *J* = 7.6, H–C(1′)); 5.55 (*dd*, *J* = 3.7, 5.7, H–C(3′)); 5.78 (*dd*, *J* = 5.7, 7.6, H–C(2′)); 6.13 (br. *s*, NH_2); 7.34–7.46, 7.52–7.60, 7.66–7.68 (3*m*, 10 arom. H); 7.87 (*s*, H–C(6)). ^{13}C-NMR ($CDCl_3$): 19.18, (*s*, Me_3C); 20.50, 20.56, 20.66 (3*q*, *Me*–C(3), 2 *Me*COO); 26.89 (*q*, Me_3C); 63.47 (*t*, (5′)); 71.67, 72.11, 81.76, 84.07 (4*d*, C(1′), C(2′), C(3′), C(4′)); 127.87, 127.95, 135.41, 135.72 (4*d*, C_o, C_m); 129.49 (*d*, C(6)); 129.98, 130.08 (2*d*, C_p); 132.24, 132.58 (2*s*, C_{ipso}); 137.61, 141.48, 143.71 (3*s*, C(2), C(3), C(5)); 169.55, 169.74 (2*s*, 2 Me*C*OO). FAB-MS (3-NOBA): 580 ($[M + 1]^+$).

*6-(Diacetylamino)-5-{2′,3′-di-O-acetyl-5-O-[(*tert*-butyl)diphenylsilyl]-β-*D*-ribofuranosyl}-3-methylpyrazin-2-yl Acetate* (**19**). KOAc (104 mg, 1.06 mmol) was suspended in Ac_2O (5.0 ml) and **18** (560 mg, 0.96 mmol) added. The resulting suspension was heated 15 min to reflux at 140°. After cooling to r.t., the resulting oil was treated with CH_2Cl_2. KOAc was removed by filtration and the product adsorbed on silica gel (2 g). Chromatography (silica gel (30 g), AcOEt/hexane 3:7) yielded **19** (585 mg, 82%). Foam. UV (EtOH): 273 (7800), 285 (5560). IR ($CHCl_3$): 2960, 2930, 2860, 1785 (br.), 1745 (br.), 1730 (br.), 1470, 1430, 1370, 1325, 1170, 1150, 1110, 1090, 1050, 1010, 900 (br.). ^{1}H-NMR ($CDCl_3$): 1.01 (*s*, ^{t}Bu); 2.03 (br. *s*, 3 H, Ac_2N); 2.05 (*s*, 1 AcO); 2.09 (*s*, 1 AcO); 2.39 (*s*, 1 AcO–C(2) or Me–C(3)); 2.40 (*s*, AcO–C(2) or Me–C(3)); 2.56 (br. *s*, 3 H, Ac_2N); 3.72 (*dd*, *J* = 3.2, 11.3, 1 H–C(5′)); 3.75 (*dd*, *J* = 3.3, 11.3, 1 H–C(5′)); 4.18 (*ddd*, *J* = 3.2, 3.3, 4.0, 1 H–C(4′)); 4.99 (*d*, *J* = 6.4, H–C(1′)); 5.69 (*dd*, *J* = 4.0, 5.4, H–C(3′)); 6.04 (*dd*, *J* = 5.4, 6.4, H–C(2′)); 7.26–7.42, 7.55–7.57, 7.60–7.63 (3*m*, 10 arom. H). ^{13}C-NMR ($CDCl_3$): 18.92 (*q*, *Me*–C(3)); 19.09 (*s*, Me_3C); 20.57, 20.76, 20.84 (*s*, 3 *Me*COO); 26.73 (*q*, Me_3C); 26.31, 27.00 (2*q*, $(MeCO)_2N$); 63.41 (*t*, C(5′)); 72.21, 73.25, 77.78, 83.80 (4*d*, C(1′), C(2′), C(3′), C(4′)); 127.63, 127.73, 135.58, 135.69 (4*d*, C_o, C_m); 129.71, 129.81 (2*d*, C_p); 132.78, 132.93 (2*s*, C_{ipso}); 143.87, 147.68, 148.34, 151.85 (4*s*, C(2), C(3), C(5), C(6)); 167.91, 169.45, 169.81 (3*s*, 3 Me*C*OO); 171.91, 172.93 (2*s*, 2 $(MeCO)_2N$). FAB-MS (3-NOBA): 706 ($[M + 1]^+$).

*6-(Diacetylamino)-5-(2′,3′-di-O-acetyl-β-*D*-ribofuranosyl)-3-methylpyrazin-2(1*H*)-one* (**20**). Silyl ether **19** (100 mg, 0.14 mmol), Bu_4NF (67 mg, 0.21 mmol) and AcOH (16 µl, 0.28 mmol) were dissolved in abs. THF (1 ml) and stirred at r.t. for 23 h. The solvent was evaporated, the resulting oil dissolved in CH_2Cl_2 and extracted with H_2O, and the org. layer dried ($MgSO_4$) and evaporated. Chromatography (silica gel (8.5 g), CH_2Cl_2/EtOH 10:1) yielded **20** (63 mg, 60%). Slightly yellow oil. IR ($CHCl_3$): 3290 (br.), 2930, 2870, 1745 (br.), 1725 (sh), 1660, 1625, 1545, 1460, 1430, 1370, 1110, 1080, 1060, 1035, 1010, 950, 910. ^{1}H-NMR ($CDCl_3$): 2.00 (*s*, AcO); 2.13 (*s*, AcO); 2.29 (br. *s*, 3 H, Ac_2N); 2.42 (br. *s*, 3 H, Ac_2N); 2.52 (*s*, Me–C(3)); 3.73 (*dd*, *J* = 1.7, 12.6, 1 H–C(5′)); 3.90 (*dd*, *J* = 2.5, 12.6, 1 H–C(5′)); 4.25–4.27 (*m*, H–C(4′)); 4.87 (*d*, *J* = 6.7, H–C(1′)); 5.54 (*dd*, *J* = 2.7, 5.3, H–C(3′)); 5.62 (*dd*, *J* = 5.3, 6.7, H–C(2′)). ^{13}C-NMR ($CDCl_3$): 19.58, 20.46, 20.81 (3*q*, *Me*–C(3), 2 *Me*COO); 26.03, 26.36 (2*q*, 2 *Me*CON); 62.93 (*t*, C(5′)); 73.58, 75.29, 76.82, 85.17 (4*d*, C(1′), C(2′), C(3′), C(4′)); 136.80 (double intensity), 152.02, 158.15 (3*s*, C(2), C(3), C(5), C(6)); 169.40, 170.04 (2*s*, 2 Me*C*OO); 171.66, 172.31 (2*s*, 2 Me*C*ON). FAB-MS (3-NOBA): 426 ($[M + 1]^+$).

*6-Amino-3-methyl-5-(β-*D*-ribofuranosyl)pyrazin-2(1*H*)-one 5′-Triphosphate* (**2**). Pyrazinone **20** (24 mg, 56 µmol) was dried by repeated evaporation from abs. pyridine, followed by placing it 1 h over P_2O_5 under high

vacuum. Abs. pyridine (56 µl) and abs. dioxane (166 µl) were added under Ar. To the yellow soln. was added a soln. of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (67.8 µl, 62 mmol), prepared from reagent (188 mg) in abs. dioxane. After 10 min stirring at r.t., a soln. of triethylammonium pyrophosphate (32 mg, 56 µmol) and Bu_3N (56.4 µl, 237 µmol) in abs. DMF (171 µl) was injected. The mixture was stirred for 10 min at r.t. and then diluted with 1% I_2 soln. in pyridine/H_2O 98:2 (1.12 ml). After 15 min, the excess I_2 was quenched by addition of 5% aq. sodium pyrosulfite soln. The solvent was evaporated and the product dried 2 h under high vacuum. The product was dissolved in H_2O (1 ml) and the soln. allowed to stand for 30 min at r.t. The solvent was removed by evaporation and the residue dissolved in hydrazine hydrate (*Fluka*; 0.5 ml) and stirred for 2 h at r.t. Chromatography (DEAE *Sephadex-A25*; column 19 × 3 cm; eluent *A*, 400 ml of 0.1M $(Et_3NH)HCO_3$ pH 7.0 eluent *B*, 400 ml of 1.0M $(Et_3NH)HCO_3$ pH 7.0, linear gradient 0–100% *B*; flow 3–4 ml/min; detection by UV absorption at 254 and 360 nm) followed by lyophilization yielded **2** (15 mg) as a slightly yellow solid. For further purification, reversed-phase HPLC was performed (*Supelco LC-18-DB*, particle size 5 µm, 25 cm × 10 mm; eluent *A*, 0.1M $(Et_3NH)HCO_3$ pH 7.0, eluent *B*, MeCN; linear gradient 0–4% *B* in 30 min, flow 4.0 ml/min; detection by UV absorption at 254 nm; t_R 23 min): **2** (3 mg). White solid. ^{1}H-NMR (D_2O): 2.16 (*s*, Me–C(3)); 4.13–4.20 (*m*, H–C(4′), 1 H–C(5′)); 4.27 (*ddd*, *J* = 3.5, 12.0, *J*(H,P) = 6.4, 1 H–C(5′)); 4.40 (*dd*, *J* = 3.0, 6.2, H–C(3′)); 4.43 (*dd*, *J* = 6.2, 8.3, H–C(2′)); 4.70 (*d*, *J* = 8.3, H–C(1′)). ^{1}H-NMR (^{31}P-decoupled, D_2O): 2.16 (*s*, Me–C(3)); 4.13–4.20 (*m*, H–C(4′), 1 H–C(5′)); 4.27 (*dd*, *J* = 3.5, 12.0, 1 H–C(5′)); 4.40 (*dd*, *J* = 3.0, 6.2, H–C(3′)); 4.44 (*dd*, *J* = 6.2, 8.3, H–C(2′)); 4.69 (*d*, *J* = 8.3, H–C(1′)). ^{31}P-NMR (D_2O): −21.62 (*t*, *J* = 19.3, 20.5, P(β)); −10.69 (*d*, *J* = 19.3, P(α)); −5.56 (*d*, *J* = 20.5, P(γ)). FAB-MS (glycerol, neg. mode): 496 (monoanion).

5. *Synthesis of the Ribonucleoside 3′,5′-Bisphosphate. 2-[2′-*O*-Benzoyl-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-*D*-ribofuranosyl]-*N*-(benzyloxycarbonyl)-*D*,*L*-glycinenitrile* (**22a**/**b**). A soln. of crude **14a**/**b** (impure; 9.58 g, ≤ 25.8 mmol) and 1*H*-imidazole (7.0 g, 103.2 mmol) in. abs. DMF (96 ml) was cooled to 0°. Within 30 min, $TIPSiCl_2$ (8.1 ml, 25.8 mmol) was sowly added at 0°. After being stirred for 1 h, the soln. was poured on H_2O (400 ml) and extracted with CH_2Cl_2. The org. layer was extracted sequentially with sat. aq. NH_4Cl and sat. aq. $NaHCO_3$ soln., H_2O, and sat. aq. NaCl soln., and dried ($MgSO_4$), and the solvent removed by distillation to yield crude **21a**/**b** (16.80 g). Thereof, 7.0 g (12.4 mmol) were dried by repeated evaporation from toluene and placing overnight under high vacuum. Abs. pyridine (30 ml) was added and the resulting soln. cooled to 0°. Benzoyl chloride (1.73 ml, 14.9 mmol) was added slowly, the soln. warmed to r.t., stirred for 3 h at r.t., and then cooled in ice/H_2O. MeOH (7 ml) was added and the soln. poured on H_2O. After extraction with CH_2Cl_2 and washing with sat. aq. $NaHCO_3$ and sat. aq. NH_4Cl soln., H_2O, and sat. aq. NaCl soln., the org. layer was dried ($MgSO_4$) and evaporated: **22a**/**b** (8.0 g, 95%). An anal. sample of the diastereoisomer mixture was obtained by chromatography (silica gel, AcOEt/petroleum ether 3:1) major diastereoisomer **22a**, minor diastereoisomer **22b**. ^{1}H-NMR ($CDCl_3$): 0.84–1.11 (*m*, 2 × 28 H, i-Pr); 3.93–3.96 (*m*, 2 H, H–C(4′)(**22a**,**b**)); 4.01 (*dd*, *J* = 2.6, 13.1, 1 H, H–C(5′)(**22a**)); 4.04 (*dd*, *J* = 3.0, 12.9, 1 H–C(5′)(**22b**)); 4.08 (*dd*, *J* = 3.8, 12.9, 1 H–C(5′)(**22b**)); 4.13 (*dd*, *J* = 2.4, 13.1, 1 H–C(5′)(**22a**)); 4.23 (*d*, *J* = 2.7, 3.7, H–C(1′)(**22b**)); 4.30 (very br. *s*, H–C(1′)(**22a**)); 4.40–4.44 (br. *m*(‘*t*’), H–C(3′)(**22a**)); 4.58 (*dd*, *J* = 6.2, 8.6, H–C(3′)(**22b**)); 5.05–5.11 (*m*, 2 H, CH); 5.13–5.19 (*m*, 4 H, CH_2); 5.23 (*dd*, *J* = 1.9, 6.1, H–C(2′)(**22a**)); 5.35–5.38 (*m*, H–C(2′)(**22b**)); 5.64 (br. *d*, *J* = 9.1, NH(**22b**)); 5.90 (br. *d*, *J* = 9.4, NH(**22a**)); 7.31–7.38 (*m*, 10 H, *Ph*CH_2); 7.43–7.47 (*m*, 4 H, H_m(Bz)); 7.58–7.61 (*m*, 2 H, H_p(Bz)); 8.04–8.07 (*m*, 4 H, H_o(Bz)). ^{13}C-NMR ($CDCl_3$): 12.65, 12.68, 12.76, 12.86, 12.96, 13.06, 13.21, 13.23 (8*d*, Me_2CH); 16.83, 16.85, 18.86, 16.95, 16.96, 17.02, 17.21, 17.29, 17.33, 17.48 (10*q*, Me_2CH); 44.86 (*d*, CH(**22a**)); 45.77 (*d*, CH(**22b**)); 60.00 (*t*, C(5′)(**22a**)); 60.93 (*t*, C(5′)(**22b**)); 67.89 (*t*, CH_2(**22b**)); 68.04 (*t*, CH_2(**22a**)); 69.98, 74.14, 81.87, 82.56 (4*d*, C(1′), C(2′), C(3′), C(4′)(**22a**)); 70.74, 73.76, 81.73, 82.00 (4*d*, C(1′), C(2′), C(3′), C(4′)(**22b**)); 116.02 (*s*, CN(**22b**)); 116.80 (*s*, CN(**22a**)); 128.28, 128.48, 128.52, 128.61, 126.63, 129.34, 129.80, 129.84, 133.43, 133.58 (10*d*, arom. CH); 134.54, 135.48 (2*s*, C_{ipso}(**22a**/**b**)); 154.99 (*s*, $PhCH_2OC(O)$(**22b**)); 155.40 (*s*, $PhCH_2OC(O)$(**22a**)); 165.81 (*s*, Ph*C*O(**22b**)); 166.29 (*s*, Ph*C*O(**22a**)). FAB-MS (3-NOBA): 669 ($[M + 1]^+$).

*2-[2′-*O*-Benzoyl-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-*D*-ribofuranosyl]-*D*,*L*-glycinenitrile* (**23a**/**b**). Pd/C (200 mg) was suspended under Ar in MeOH (5 ml), and a soln. of **22a**/**b** (4.0 g, 5.9 mmol) in THF (5 ml) and MeOH (35 ml) was added. The suspension was stirred under H_2 overnight. The catalyst was removed by filtration on *Celite* and the solvent evaporated. Chromatography (silica gel, AcOEt/petroleum ether 8:2, 7:3, and 1:1) of the crude material (3.2 g) resulted in separation into the major diastereoisomer **23a** (eluting first; 1.23 g, 39%) and the minor diastereoisomer **23b** (0.61 g, 19%). An anal. sample of **23a** was obtained by recrystallization from pentane. Experiments with higher H_2 pressure (10 bar) and reduced reaction time (4 h) did not result in improved yields.

23a: M.p. 111–112°. ^{1}H-NMR ($CDCl_3$): 0.86–1.11 (*m*, 28 H, i-Pr); 1.87 (br. *d*, *J* = 8.6, NH_2); 3.97 (*ddd*, *J* = 2.4, 2.4, 9.1, H–C(4′)); 4.01 (*dd*, *J* = 2.7, 13.0, 1 H–C(5′)); 4.04–4.07 (*m*, CH); 4.16 (*dd*, *J* = 2.2, 13.0, 1 H–C(5′)); 4.29–4.30 (*m*, H–C(1′)); 4.54 (*dd*, *J* = 5.9, 9.1, H–C(3′)); 5.41 (*dd*, *J* = 1.7, 5.9, H–C(2′)); 7.44–7.47 (*m*,(‘*t*’), 2 H_m); 7.57–7.60 (*m*(‘*t*’), H_p); 8.05 (*dd*, *J* = 1.3, 8.4, 2 H_o). ^{13}C-NMR ($CDCl_3$): 12.69, 12.70, 13.02, 13.10,

13.33 (5*d*, Me_2CH); 16.87, 16.91, 16.93, 17.02, 17.28, 17.33, 17.37, 17.47 (8*q*, *Me*$_2$CH); 44.96 (*d*, CH); 60.22 (*t*, C(5′)); 69.86, 74.68, 81.96, 83.57 (4*d*, C(1′), C(2′), C(3′), C(4′)); 120.23 (*s*, CN); 128.46, 129.74 (2*d*, C_o, C_m); 129.81 (*s*, C_{ipso}); 133.30 (*d*, C_p); 166.17 (*s*, Ph*C*O). FAB-MS (3-NOBA): 535 ($[M + 1]^+$). Anal. calc. for $C_{26}H_{42}N_2O_6Si_2$ (534.80): C 58.39, H 7.92, N 5.24; found: C 58.24, H 7.87, N 5.25.

23b: ^{1}H-NMR ($CDCl_3$): 0.81–1.12 (*m*, 28 H, i-Pr); 1.92 (br. *s*, NH_2); 3.96 (*ddd*, *J* = 3.1, 3.4, 8.7, H–C(4′)); 4.05 (*dd*, *J* = 2.9, 12.8, 1 H–C(5′)); 4.09–4.13 (*m*, CH, 1 H–C(5′)); 4.22 (*dd*, *J* = 2.5, 6.3, H–C(1′)); 4.55 (*dd*, *J* = 6.1, 8.7, H–C(3′)); 5.41 (*dd*, *J* = 2.4, 6.1, H–C(2′)); 7.43–7.47 (*m*(*t*′), 2 H_m); 7.56–7.60 (*m*(*t*′), H_p); 8.05–8.07 (*m*(*d*′), 2 H_o). ^{13}C-NMR ($CDCl_3$): 12.68, 12.72, 13.05, 13.25 (4*d*, Me_2CH); 16.84, 16.86, 16.96, 17.03, 17.29, 17.34, 17.47 (7*q*, *Me*$_2$CH); 46.29 (*d*, CH); 60.94 (*t*, C(5′)); 70.68, 74.15, 81.80, 83.40 (4*d*, C(1′), C(2′), C(3′), C(4′)); 119.14 (*s*, CN); 128.43, 129.74 (2*d*, C_o, C_m); 129.71 (*s*, C_{ipso}); 133.28 (*d*, C_p); 165.83 (*s*, Ph*C*O). FAB-MS (3-NOBA): 535 ($[M + 1]^+$).

*3-[2-*O*-Benzoyl-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-*D*-ribofuranosyl]-5-methylpyrazin-2-amine 1-Oxide* (**24**). Dry **23a** (1.12 g, 2.09 mmol), *anti*-pyruvic aldehyde 1-oxime (235 mg, 2.7 mmol) and pyridine hydrochloride (23 mg, 0.2 mmol) in abs. $CHCl_3$ were heated under reflux overnight. The solvent was evaporated. Chromatography (silica gel (150 g), AcOEt/petroleum ether 3:7, 1:1, and 7:3) of the dark brown oil (1.58 g) yielded **24** (676 mg, 54%) as a foam. Reaction of diastereoisomer **23b** yielded the same product. ^{1}H-NMR ($CDCl_3$): 0.83–1.13 (*m*, 28 H, i-Pr); 2.35 (*d*, *J* = 0.5, Me–C(5)); 4.02–4.18 (*m*, H–C(4′), 2 H–C(5′)); 4.60 (*dd*, *J* = 5.2, 8.9, H–C(3′)); 5.31 (*d*, *J* = 1.3, H–C(1′)); 5.94 (*dd*, *J* = 1.3, 5.2, H–C(2′)); 6.13 (br. *s*, NH_2); 7.44–7.48 (*m*(*t*′), 2 H_o); 7.57–7.61 (*m*(*t*′), H_p); 7.88 (*s*, H–C(6)); 8.09–8.11 (*m*(*d*′), 2 H_m). ^{13}C-NMR ($CDCl_3$): 12.64, 12.92, 13.04, 13.29 (4*d*, Me_2CH); 16.77, 16.80, 16.86, 16.92, 17.18, 17.30, 17.34, 17.51 (8*q*, *Me*$_2$CH); 20.52 (*q*, *Me*–C(5)); 60.36 (*t*, C(5′)); 70.58, 76.48, 81.93, 83.51 (4*d*, C(1′), C(2′), C(3′), C(4′)); 128.38, 129.77 (2*d*, C_o, C_m); 129.21 (*d*, C(6)); 130.02 (*s*, C_{ipso}); 133.14 (*d*, C_p); 138.84, 141.78, 143.65 (3*s*, C(2), C(3), C(5)); 165.73 (*s*, Ph*C*O). FAB-MS (3-NOBA): 604 ($[M + 1]^+$). Anal. calc. for $C_{29}H_{45}N_3O_7Si_2$ (603.86): C 57.68, H 7.51, N 6.96; found: C 57.88, H 7.51, N 6.93.

*5-[2-*O*-Benzoyl-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-*D*-ribofuranosyl]-6-(diacetylamino)-3-methylpyrazin-2-yl Acetate* (**25**). A suspension of **24** (676 mg, 1.12 mmol) and KOAc (275 mg, 2.8 mmol) in Ac_2O (8 ml) was heated under reflux for 20 min. The mixture was cooled to r.t. and evaporated under high vacuum, the dark brown residue suspended in CH_2Cl_2, and KOAc removed by filtration. Chromatography (silica gel (80 g), petroleum ether/AcOEt 8:2) yielded **25** (490 mg, 60%). Foam. ^{1}H-NMR ($CDCl_3$): 0.82–1.14 (*m*, 28 H, i-Pr); 2.07 (br. *s*, 3 H, Ac_2N); 2.38, 2.52 (2*s*, Me–C(3), AcO); 2.54 (br. *s*, 3 H, Ac_2N); 3.94 (*dd*, *J* = 2.7, 12.9, 1 H–C(5′)); 4.01 (*dd*, *J* = 2.8, 12.9, 1 H–C(5′)); 4.07 (*ddd*, *J* = 2.7, 2.7, 9.1, H–C(4′)); 4.74 (*dd*, *J* = 4.9, 9.1, H–C(3′)); 5.06 (*d*, *J* = 1.1, H–C(1′)); 4.92 (br. *d*, *J* = 4.9, H–C(2′)); 7.43–7.47 (*m*(*t*′), 2 H_o); 7.55–7.59 (*m*(*t*′), H_p); 8.06–8.09 (*m*(*d*′), 2 H_m). ^{13}C-NMR ($CDCl_3$): 12.52, 12.72, 13.04, 13.34 (4*d*, Me_2CH); 16.79, 16.84, 17.03, 17.10, 17.23, 17.27, 17.30, 17.38 (8*q*, *Me*$_2$CH); 18.92, 20.85 (2*q*, *Me*–C(3), *Me*COO); 26.30, 26.99 (2*q*, (*Me*′CO)$_2$N); 60.55 (*t*, C(5′)); 70.81, 75.87, 79.58, 81.99 (4*d*, C(1′), C(2′), C(3′), C(4′)); 128.33, 129.76 (2*d*, C_o, C_m); 130.20 (*s*, C_{ipso}); 132.97 (*d*, C_p); 143.27, 147.82, 148.96, 151.57 (4*s*, C(2), C(3), C(5), C(6)); 165.46, 167.92 (2*s*, Ph*C*O, Me*C*OO); 172.16, 172.93 (*s*, Me*C*O)$_2$N). FAB-MS (3-NOBA): 730 ($[M + 1]^+$). Anal. calc. for $C_{35}H_{51}N_3O_{10}Si_2$ (729.98): C 57.59, H 7.04, N 5.76; found: C 57.87, H 7.24, N 5.82.

*6-(Acetylamino)-5-[2′-*O*-benzoyl-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-*D*-ribofuranosyl]-3-methylpyrazin-2-(1*H*)-one* (**26**). A soln. of **25** (458 mg, 0.63 mmol) in abs. EtOH (46 ml) was heated at reflux for 28 h. The solvent was evaporated and crude **26** (433 mg) isolated as an oil, which was used for the next reaction without further purification. Chromatography (silica gel, petroleum ether/AcOEt 2:3) yielded an anal. sample. ^{1}H-NMR ($CDCl_3$): 0.83–1.13 (*m*, 28 H, i-Pr); 2.21, 2.36 (*s*, AcN, Me–C(3)); 4.06 (*dd*, *J* = 2.4, 13.3, 1 H–C(5′)); 4.14–4.17 (*m*, H–C(4′)); 4.21 (br. *d*, *J* = 13.4, 1 H–C(5′)); 4.49 (*dd*, *J* = 4.7, 9.3, H–C(3′)); 5.30 (*d*, *J* = 0.8, H–C(1′)); 5.83 (br. *d*, *J* = 4.7, H–C(2′)); 7.45–7.49 (*m*(*t*′), H_o); 7.52–7.61 (*m*(*t*′), H_p); 8.08–8.11 (*m*(*d*′), 2 H_m); 9.95, 12.28 (br. *s*, 2 NH). ^{13}C-NMR ($CDCl_3$): 12.48, 12.66, 12.97, 13.50 (4*d*, Me_2CH); 16.82, 16.86, 16.90, 16.91, 16.96, 17.03, 17.19, 17.30 (8*q*, *Me*$_2$CH); 19.94, 24.69 (2*q*, *Me*–C(3), *Me*CON); 59.71 (*t*, C(5′)); 68.93, 77.82, 81.85, 83.94 (4*d*, C(1′), C(2′), C(3′), C(4′)); 128.40, 129.72 (2*d*, C_o, C_m); 130.07 (*s*, C_{ipso}); 133.14 (*d*, C_p); 112.83, 133.46, 150.97, 153.74 (*s*, C(2), C(3), C(5), C(6)); 165.53 (*s*, Ph*C*O); 171.54 (*s*, Me*C*ON). FAB-MS (3-NOBA): 645 (M^+).

*N-{3-[2′-*O*-Benzoyl-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-*D*-ribofuranosyl]-6-(benzyloxy)-5-methylpyrazin-2-yl}acetamide* (**27**). Dry **26** (323 mg, 0.50 mmol), PPh_3 (197 mg, 0.75 mmol), and benzyl alcohol (124 µl, 0.60 mmol) were dissolved in abs. THF (6 ml) and cooled to 0°. DEAD (118 µl, 0.75 mmol) was added within 5 min. The soln. was warmed to r.t., stirred for 30 min, and evaporated. The crude product was adsorbed to silica gel. Chromatography (silica gel, petroleum ether/AcOEt 8:2) yielded **27** (206 mg, 56%). Amorphous solid. ^{1}H-NMR ($CDCl_3$): 0.90–1.12 (*m*, 28 H, i-Pr); 2.24, 2.43 (2 br. *s*, AcN, Me–C(5)); 4.00 (*dd*, *J* = 2.9, 12.7, 1 H–C(5′)); 4.06 (*dd*, *J* = 3.4, 12.7, 1 H–C(5′)); 4.11–4.15 (*m*, H–C(4′)); 4.74 (*dd*, *J* = 5.0, 8.8, H–C(3′)); 5.24 (*d*, *J* = 1.2, H–C(1′)); 5.37 (*d*, *J* = 12.7, 1 H, CH_2); 5.40 (*d*, *J* = 12.7, 1 H, CH_2); 6.08 (br. *d*, *J* = 4.8, H–C(2′)); 7.30–7.48 (*m*, 7 H, *Ph*CH_2,

2 H_m(Bz)); 7.56–7.60 (*m*('*t*'), H_p(Bz)); 8.09–8.12 (*m*('*d*'), 2 H_o(Bz)); 8.15 (br. *s*, NH). ^{13}C-NMR ($CDCl_3$): 12.53, 12.72, 13.06, 13.28 (4*d*, Me_2CH); 16.83, 16.87, 17.01, 17.11, 17.18, 17.22, 17.27, 17.37 (8*q*, Me_2CH); 18.58, 23.94 (2*q*, *Me*–C(3), *Me*CON); 61.19 (*t*, C(5')); 68.23 (*t*, Ph*C*H_2); 70.94, 77.20, 80.32, 81.81 (4*d*, C(1'), C(2'), C(3'), C(4')); 127.59, 128.00, 128.34, 128.51, 129.75 (5*d*, arom. CH); 130.17 (*s*, C_{ipso}(Bz)); 133.04 (*d*, C_p(Bz)); 133.48, 139.40, 140.65 (3*s*, C(2), C(3), C(5), C(6)); 136.48 (*s*, C_{ipso}(Bn)); 156.05, 166.07 (2*s*, Ph*C*O, Me*C*ON). FAB-MS (3-NOBA): 736 ([*M* + 1]$^+$). Anal. calc. for $C_{38}H_{53}N_3O_8Si_2$ (735.9): C 62.01, H 7.26, N 5.71; found: C 62.17, H 7.38, N 5.80.

N-*[3-(2'-*O*-Benzoyl-β-*D*-ribofuranosyl)-6-(benzyloxy)-5-methylpyrazin-2-yl]acetamide* (**28**). In a precooled (0°) soln. of pyridine/HF (200 µl, 6%) **27** (100 mg, 136 µmol) was dissolved and stirred overnight at 0°. The deprotected product partially precipitated as a white solid. $MeOSiMe_3$ (230 µl) was added at 0°. The solvent was evaporated at < 0° to yield **28** (69 mg, quant.) as a slightly impure white solid. Attempts to further purify the product (chromatography on silica gel; recrystallization) failed due to partial acyl migration from O–C(2') to O–C(3'). ^{1}H-NMR ((D$_6$)DMSO): 2.05, 2.41 (2 br. *s*, AcN, Me–C(5)); 3.48–3.54 (*m*, 1 H–C(5')); 3.65–3.70 (*m*, 1 H–C(5')); 3.92–3.96 (*m*, H–C(4')); 4.41–4.37 (*m*, H–C(3')); 4.89 (*dd*, *J* = 4.6, 6.5, OH); 5.24 (*d*, *J* = 4.1, H–C(1')); 5.36 (*s*, CH_2); 5.41 (*d*, *J* = 6.0, OH); 5.54 (*dd*, *J* = 4.2, 5.3, H–C(2')); 7.32–7.56 (*m*, 7 H, *Ph*CH_2, 2 H_m(Bz)); 7.64–7.69 (*m*('*t*'), H_p(Bz)); 8.00–8.03 (*m*('*d*'), 2 H_o(Bz)); 10.01 (br. *s*, NH). ^{13}C-NMR ((D$_6$)DMSO): 18.46, 23.02 (2*q*, *Me*–C(5), *Me*CON); 61.51 (*t*, C(5')); 67.56 (*t*, Ph*C*H_2); 69.73, 77.00, 78.15, 84.22 (4*d*, C(1'), C(2'), C(3'), C(4')); 127.77, 127.87, 128.34, 128.50, 129.32 (5*d*, arom. CH); 129.76 (*s*, C_{ipso}(Bz)); 133.17 (*d*, C_p(Bz)); 136.39 (*s*, C_{ipso}(Bn)); 137.36, 139.84, 140.31, 149.51 (4*s*, C(2), C(3), C(5), C(6)); 155.70, 164.88 (2*s*, Ph*C*O, Me*C*ON). FAB-MS (3-NOBA): 494 ([*M* + 1]$^+$).

N-*{3-[2'-*O*-Benzoyl-3',5'-*O*-bis(dibenzyloxyphosphoryl)-β-*D*-ribofuranosyl-5-(benzyloxy)-5-methylpyrazin-2-yl}acetamide* (**30**). A mixture of crude **28** (68 mg, 136 µmol; from the above reaction), 1*H*-tetrazole (39 mg, 552 µmol), and 3-Å molecular sieves was dried overnight under high vacuum above P_2O_5 and then dissolved in a mixture of abs. DMF (2.1 ml) and abs. MeCN (1.5 ml). At r.t., 250 µl of a soln. prepared from **31** (275 mg, 797 µmol) in abs. MeCN (1.0 ml) was added. The mixture was stirred for 1 h at r.t. Then, 4-methylmorpholine 4-oxide monohydrate (187 mg, 1.38 mmol) was added and the resulting mixture again stirred at r.t. for 5.5 h and then filtered through a bed of *Celite*. The solvent was evaporated under high vacuum and the crude product adsorbed on silica gel (500 mg). Chromatography (silica gel (25 g), AcOEt/petroleum ether 1:1 then 3:1) yielded **30** (95 mg, 70%). Foam. ^{1}H-NMR ($CDCl_3$): 2.24, 2.30 (2*s*, AcN, Me–C(5)); 4.16–4.22 (*m*, 1 H–C(5')); 4.26–4.32 (*m*, 1 H–C(5')); 4.40–4.43 (*m*, H–C(4')); 4.82–5.00 (*m*, 8 H, $POCH_2$); 5.11–5.16 (*m*, H–C(3')); 5.29 (*d*, *J* = 4.2, H–C(1')); 5.30 (*d*, *J* = 12.6, 1 H, PhCH_2O–C(5)); 5.35 (*d*, *J* = 12.6, 1 H, PhCH_2O–C(5)); 5.88–5.90 (*m*,('*t*'), H–C(2')); 7.15–7.43 (*m*, 22 arom. H); 7.55–7.60 (*m*('*t*'), H_p(Bz)); 8.03–8.05 (*m*('*d*'), 2 H_o); 8.28 (br. *s*, NH). ^{13}C-NMR ($CDCl_3$): 18.71, 24.01 (2*q*, *Me*–C(5), *Me*CON); 66.74 (*td*, *J*(C,P) = 5.1, C(5')); 67.56 (*t*, Ph*C*H_2O–C(5)); 69.27, 69.32, 69.39, 69.44, 69.63, 69.69, 69.75, 69.81 (4*d*, 4 CH_2OP); 74.49, 79.94 (2*d*, C(1'), C(2')); 74.52 (*dd*, *J*(C,P) = 5.1, C(3')); 81.03 (*m*('*t*'), C(4')); 127.76, 127.80, 127.87, 127.92, 127.96, 128.07, 128.45, 128.48, 128.53, 128.56, 128.63 (11*d*, arom. CH); 129.26 (*s*, C_{ipso}(Bz)); 129.93 (*d*, arom. CH); 133.53 (*d*, C_p(Bz)); 135.30, 138.38, 135.60, 135.67, 135.73, 139.75, 140.98 (7*s*, arom. C); 136.39 (*s*, C_{ipso}(BnO–C(5)); 156.42, 165.79 (*s*, Ph*C*O, Me*C*ON). ^{31}P-NMR (^{1}H-decoupled; $CDCl_3$): –0.98 (*s*, PO_4), –1.64 (*s*, PO_4). FAB-MS (3-NOBA): 1014 ([*M* + 1]$^+$). Anal. calc. for $C_{54}H_{53}N_3O_{13}P_2$ (1013.9): C 63.97, H 5.27, N 4.14; found: C 63.07, H 5.24, N 4.05.

*6-Amino-3-methyl-5-(β-*D*-ribofuranosyl)pyrazin-2(1*H*)-one 3',5'-Bisphosphate* (**3**). To a soln. of **30** (133 mg, 0.131 mmol) in THF (2.6 ml), 0.2M aq. $(Et_3NH)HCO_3$ (pH 7.4; 1.3 ml) was added. Under Ar, Pd(OH_2)/C (*ca.* 15 mg; 20%) was added and the mixture stirred overnight at r.t. under H_2. The catalyst was removed by filtration and the solvent evaporated. After drying under high vacuum, the resulting yellow oil was dissolved in hydrazine hydrate (1.2 ml) and the soln. stirred for 1 h at r.t., cooled in liq. N_2, and evaporated at low temp. Ion-exchange chromatography (DEAE *Sephadex-A25*, column 55 × 1.8 cm; eluent *A*, H_2O (1 l), eluent *B*, 1.0M $(Et_3NH)HCO_3$ pH 8.0 (1 l), linear gradient 0–100% *B*; flow 3–5 ml min; fraction size 20–25 ml; detection by UV absorption at 254 and 360 nm). The product fractions (*Fr. 42–46*) were evaporated, and after repeated cycles of dissolution in H_2O followed by lyophilization (to remove the volatile buffer), **3** (25 mg) was obtained as a light brown amorphous solid. ^{1}H-NMR (D_2O): 2.18 (*s*, Me–C(3)); 4.03 (*ddd*, *J* = 11.5, 3.3, 2.3, 1 H–C(5')); 4.11 (*ddd*, *J* = 11.5, 5.6, 2.4, 1 H–C(5')); 4.36 (very br. *s*, with sh, H–C(4')); 4.45 (*dd*, *J* = 8.9, 5.8, H–C(2')); 4.63 (*ddd*, *J* = 7.9, 5.8, 2.3, H–C(3')); 4.78 (*d*, *J* = 8.9, H–C(1')). ^{1}H-NMR (^{31}P-decoupled, D_2O): 2.16 (*s*, Me–C(3)); 4.01 (*dd*, *J* = 11.5, 2.3, H–C(5')); 4.1 (*dd*, *J* = 11.5, 2.4, 1 H–C(5')); 4.35–4.36 (*m*('*q*'), H–C(4')); 4.44 (*dd*, *J* = 9.0, 5.7, H–C(2')); 4.62 (*dd*, *J* = 5.7, 2.4, H–C(3')); 4.77 (*d*, *J* = 9.0, H–C(1')). ^{13}C-NMR (D_2O): 19.78 (*q*, *Me*–C(3)); 67.19 (*td*, *J*(C,P) = 4.6, C(5')); 73.87 (*dd*, *J*(C,P) = 4.9, C(2') or C(3')); 77.12 (*dd*, *J*(C,P) = 4.9, C(2') or C(3')); 85.10 (*d*, C(1')); 86.49 (*ddd*, *J*(C,P) = 3.2, 8.4, C(4')); 116.30, 10.14, 145.81, 159.90 (4*s*, C(2), C(3), C(5), C(6)). ^{31}P-NMR (D_2O): 2.14 (*d*, *J* = 8.0, PO_4–C(3')); 1.53 (very br. *s*, PO_4–C(5')). ^{31}P-NMR (^{1}H-decoupled; D_2O): 2.14 (*s*, PO_4–C(3')); 1.54 (*s*, PO–C(5')). FAB-MS (neg. mode; glycerol): 416 (monoanion).

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