

136

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 ⁴C₁ 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 H₂O 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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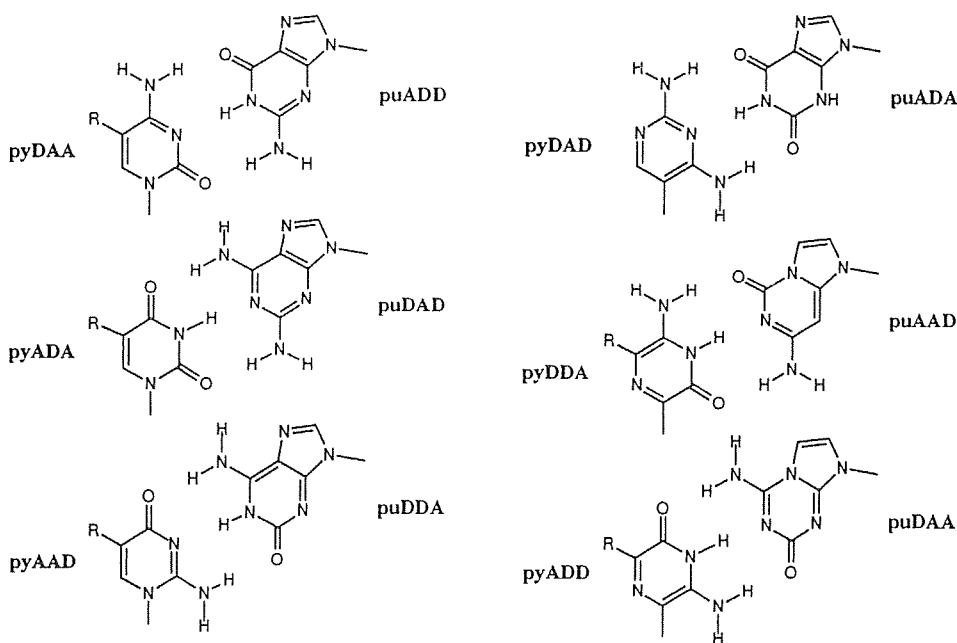


Fig. 1. Twelve bases possible in a DNA- or RNA-based 'alphabet' within the constraints of the Watson-Crick base-pair geometry. Pyrimidine-base analogs are designated by 'py', purine by 'pu'. The upper-case letters following the designation indicate the H-bonding pattern of acceptor (A) and donor (D) 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?

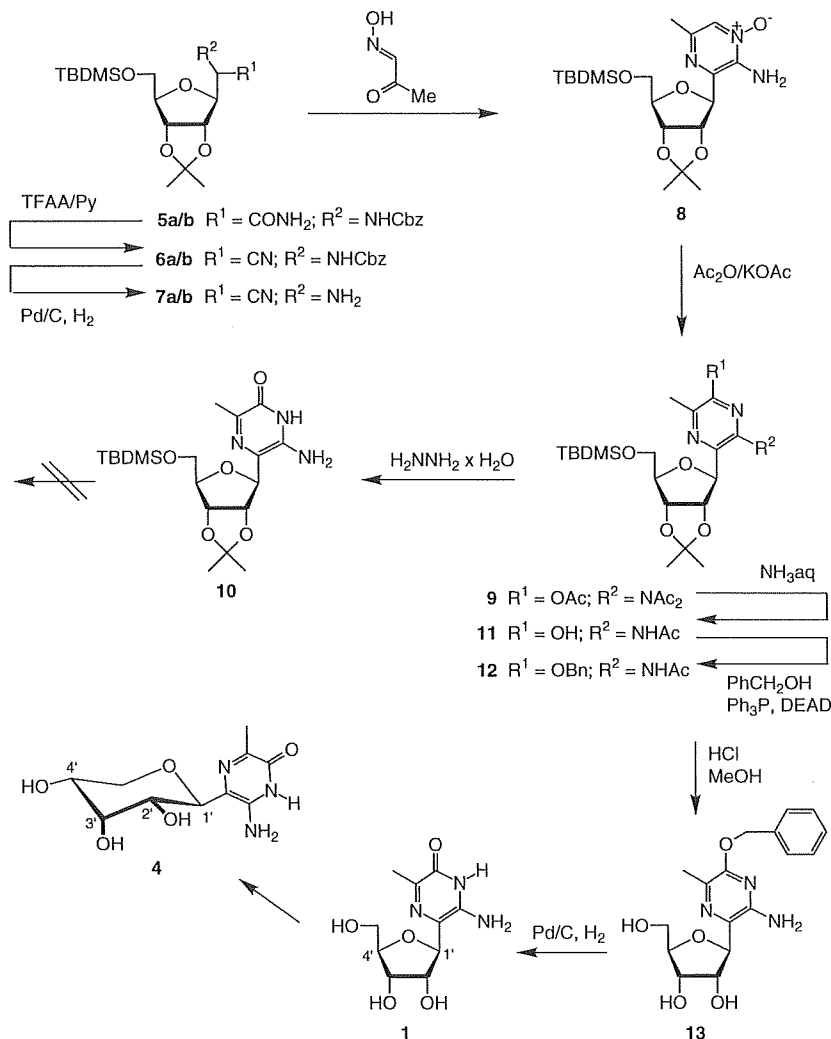
We have for some time been exploring this question by developing the chemistry of these non-standard base pairs, creating the enzymology and molecular biology of an expanded genetic alphabet, using non-standard base pairing to probe the molecular basis for the action of polymerases and the ribosome, 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 = $\text{PhCH}_2\text{OC(O)}$, TFAA = $(\text{CF}_3\text{CO})_2\text{O}$, DEAD = $\text{EtOOCN} = \text{NCOOEt}$

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 ¹H-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 ¹H-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 ⁴C₁-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 ⁴C₁ 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 ⁴C₁ form compared to the ¹C₄ 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 ⁴C₁ form compared to the ¹C₄ 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 ⁴C₁ 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*.

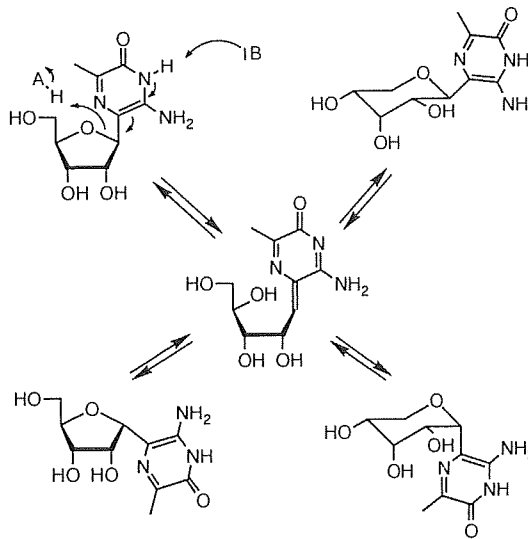


Fig. 2. Proposed mechanism for the rearrangement of **1**

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]: $\ln([\mathbf{1}] - [\mathbf{1}]_{\text{eq}}) = -(k_1 + k_{-1})t + \ln([\mathbf{1}]_0 - [\mathbf{1}]_{\text{eq}})$. A plot of $\ln([\mathbf{1}] - [\mathbf{1}]_{\text{eq}})$ 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 $t_{1/2} = 15\text{--}30$ 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.

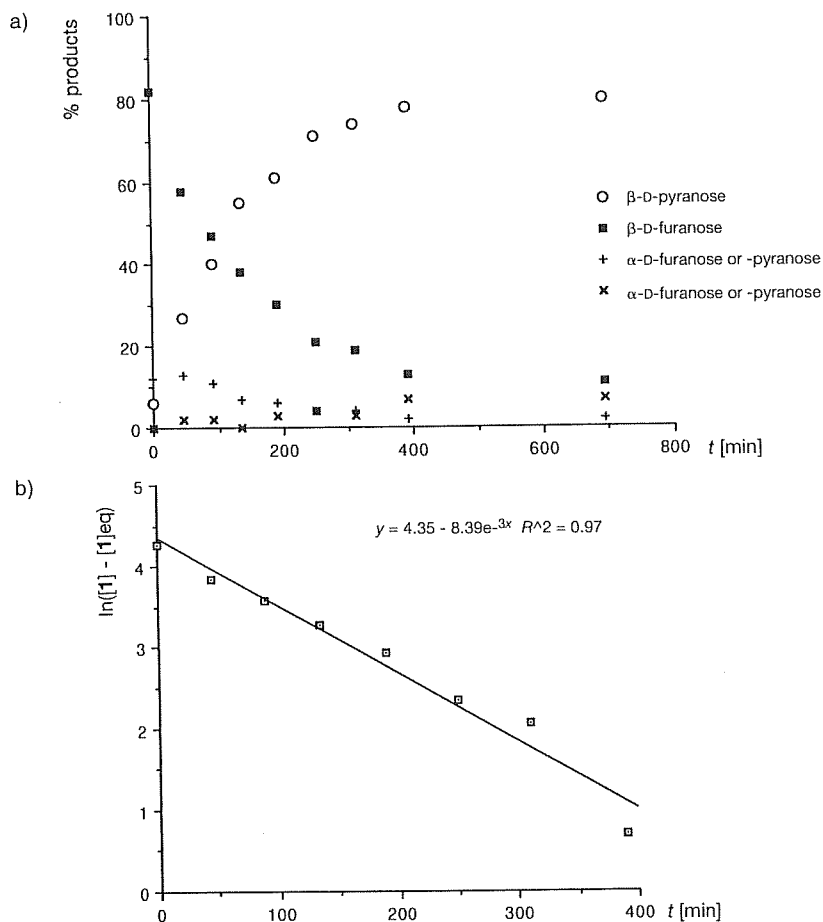


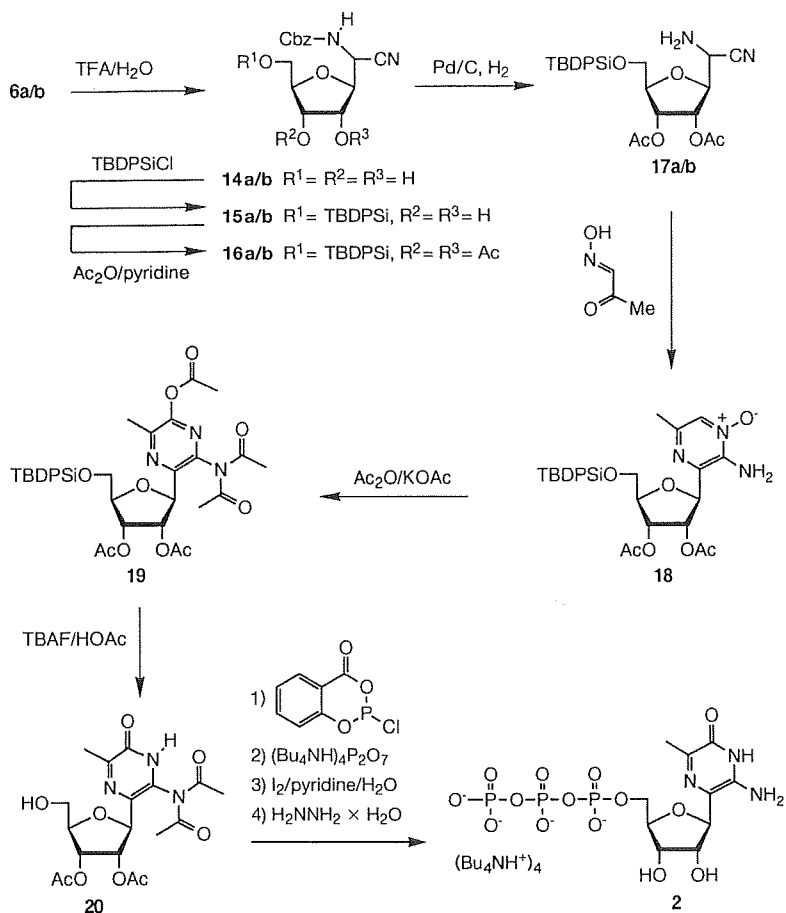
Fig. 3. a) Course of a typical rearrangement reaction of **1** at pH 4.3 in 1.0M (Et_3NH)OAc at 30.0° (data by HPLC, see Exper. Part) and b) quantitative analysis of the rearrangement reaction of **1** as a reversible first-order process using the experimentally determined values shown in Fig. 3,a

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 ^1H - 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).

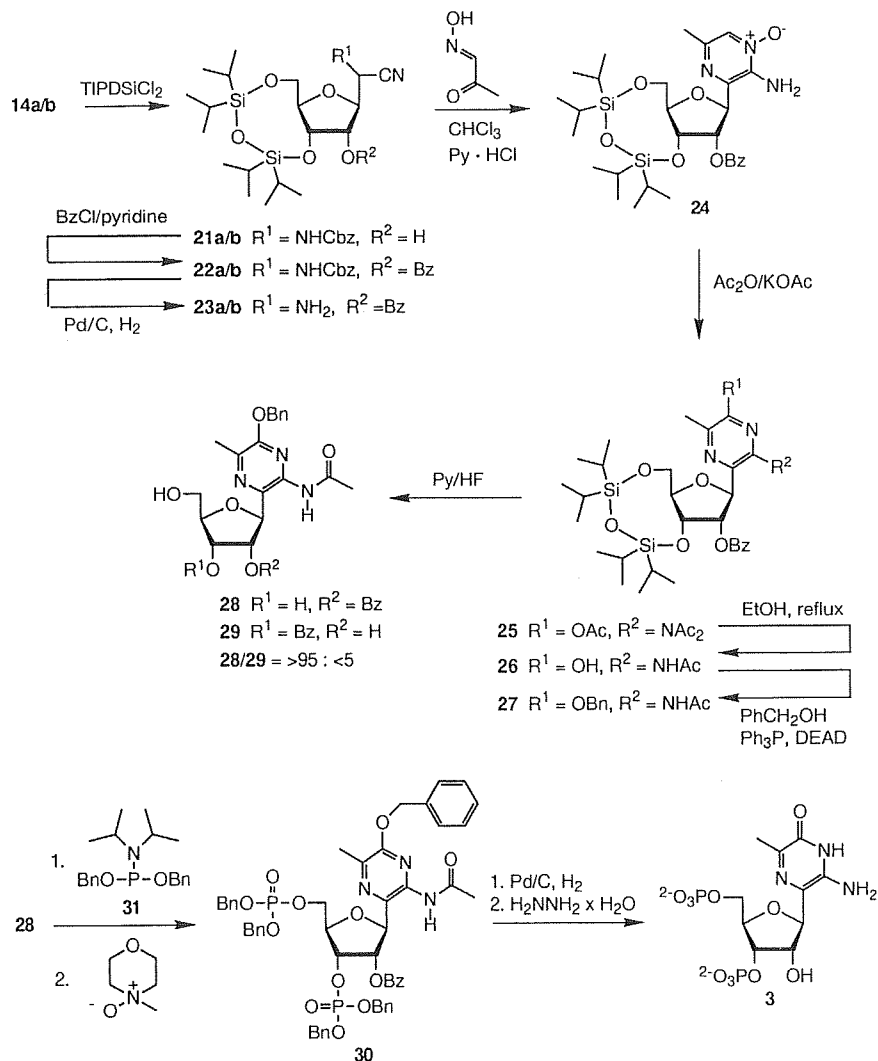
Synthesis of the Ribonucleoside 3',5'-Bisphosphate 3. Compound **14a/b** was protected with the tetraisopropylidisiloxanediyl group (\rightarrow **21a/b**) and the remaining 2'-OH group

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

TBDPSi = (*t*-Bu) $_2\text{PhSi}$, TFA = CF_3COOH , Cbz = $\text{PhCH}_2\text{OC(O)}$, 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 = PhCH₂, TIPDSiCl₂ = O[Si(i-Pr)₂]₂Cl₂, Cbz = PhCH₂OC(O), DEAD = EtOOCN = NCOOEt

Rearrangement of **24** (\rightarrow **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 ¹H-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 ³¹P-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. Et₂O, THF, and dioxane were distilled over Na with benzophenone as indicator. CHCl₃, CH₂Cl₂, and DMF were dried over molecular sieves (4 Å, 2-3 mm). MeCN was distilled over CaH₂. 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 ¹³C) or 70.46 kG (300 MHz for ¹H, 75 MHz for ¹³C); SiMe₄ (δ 0.00 ppm) was used as internal standard for both ¹H and ¹³C spectra; OH and NH assignments were confirmed by D₂O 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°. (CF₃CO)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 CHCl₃ and extracted with ice/H₂O, H₂O, and sat. aq. NaCl soln. the org. phase was dried (MgSO₄) and evaporated: **6a/b** 2:1 (22.53 g, quant.). Brown oil, which was used directly for the next reaction. IR (CHCl₃): 3430, 2960, 2930, 2860, 1730, 1500, 1375, 1285, 1260, 1130, 1080, 805, 785. ¹H-NMR (CDCl₃): assignment of the two diastereoisomers by COSY: 0.04, 0.05 (2s, 3 H, Me₂Si(**6a**)); 0.10 (s, 6 H, Me₂Si(**6b**)); 0.84 (s, 9 H, 'Bu(**6a**)); 0.91 (s, 9 H, 'Bu(**6b**)); 1.32, 1.52 (2s, 3 H, Me₂C(**6a**)); 1.34, 1.52 (2s, 3 H, Me₂C(**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, PhCH₂(**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)). $^{13}\text{C-NMR}$ (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_2C (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_o (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*, $\text{PhCH}_2\text{OC}(\text{O})(6b)$); 155.57 (*s*, $\text{PhCH}_2\text{OC}(\text{O})(6a)$). 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. $^1\text{H-NMR}$ (CDCl_3): 0.06 (*s*, 3 H, Me_2Si); 0.09 (*s*, 3 H, Me_2Si); 0.83 (*s*, ^tBu); 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)). $^{13}\text{C-NMR}$ (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), Et₂O/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. $^1\text{H-NMR}$ (CDCl_3): 0.02 (*s*, 3 H, Me_2Si); 0.01 (*s*, 3 H, Me_2Si); 0.83 (*s*, 9 H, ^tBu); 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')). $^{13}\text{C-NMR}$ (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*, MeCOO); 25.61 (*q*, Me_2C); 25.84 (*q*, Me_3C); 26.45 (*q*, MeCON); 26.90 (*q*, MeCON); 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(1H)-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 2*N* aq. HCl. An oil separated which, after trituration with H_2O , yielded 10 (62 mg, 80%). Yellow solid. $^1\text{H-NMR}$ (CDCl_3): 0.05 (*s*, 3 H, Me_2Si); 0.07 (*s*, 3 H, Me_2Si); 0.88 (*s*, ^tBu); 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). $^{13}\text{C-NMR}$ (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(1H)-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. $^1\text{H-NMR}$ (CDCl_3): 0.00 (*s*, 3 H, Me_2Si); 0.02 (*s*, 3 H, Me_2Si); 0.83 (*s*, ^tBu); 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). $^{13}\text{C-NMR}$ (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*, MeCON); 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 (4d, C(1'), C(2'), C(3'), C(4')); 114.07 (s, Me₂C); 114.69, 133.59, 150.14, 153.48 (4s, C(2), C(3), C(5), C(6)); 171.05 (s, MeCON). FAB-MS (3-NOBA): 454 ([M + 1]⁺). Anal. calc. for C₂₁H₃₅N₃O₆Si (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 PPh₃ (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. N₂ 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. ¹H-NMR (CDCl₃): -0.10 (s, 3 H, Me₂Si); -0.05 (s, 3 H, Me₂Si); 0.79 (s, 'Bu); 1.41 (s, 3 H, Me₂C); 1.59 (s, 3 H, Me₂C); 2.30, 2.45 (2s, 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, PhCH₂); 5.45 (dd, J = 4.1, 6.5, H-C(2')); 7.31-7.43 (m, 5 arom. H); 8.71 (br. s, NH). ¹³C-NMR (CDCl₃): -5.56 (q, Me₂Si); -5.54 (q, Me₂Si); 18.28 (s, 'Bu); 18.56 (q, Me-C(5)); 24.56 (q, MeCON); 25.56 (q, Me₂C); 25.78 (q, Me₂C); 27.43 (q, Me₂C); 63.47 (t, C(5')); 68.18 (t, PhCH₂); 81.91, 82.76, 84.45, 85.82 (4d, C(1'), C(2'), C(3'), C(4')); 113.80 (s, Me₂C); 127.58, 127.99, 128.52 (3d, arom. CH); 136.63 (s, C_{ipso}(Bn)); 132.54, 137.43, 141.7 (3s, C(2), C(3), C(5), C(6)); 155.75 (s, MeCON). FAB-MS (3-NOBA): 544 ([M + 1]⁺). Anal. calc. for C₂₈H₄₁N₃O₆Si (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 NaHCO₃ was added until the production of CO₂(g) ceased. The inorg. salts were removed by filtration and the solvent evaporated. Chromatography (silica gel (115 g), CH₂Cl₂/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°. ¹H-NMR (CD₃OD): 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, PhCH₂); 7.28-7.45 (m, 5 arom. H). ¹³C-NMR (CD₃OD): 17.21 (q, Me-C(5)); 62.91 (t, C(5')); 68.52 (t, PhCH₂); 72.53, 74.71, 84.34, 86.64 (4d, 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 (4s, C(2), C(3), C(5), C(6)). FAB-MS (3-NOBA): 348 ([M + 1]⁺). Anal. calc. for C₁₇H₂₁N₃O₅ (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-(1H)-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 H₂ for 1 h at 0°. The catalyst was quickly removed by filtration and the product-containing soln. recovered in a pre-cooled flask. The resulting colorless soln. was cooled in liq. N₂. 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 H₂O 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. ¹H-NMR ((D₆)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, NH₂); 11.1 (very br. s, NH). ¹H-NMR (D₂O): 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')). ¹³C-NMR (D₂O): 19.91 (q, Me-C(3)); 64.06 (t, C(5')); 73.59, 75.42, 82.94, 87.69 (4d, C(1'), C(2'), C(3'), C(4')); 116.97, 141.05, 145.86, 160.09 (4s, C(2), C(3), C(5), C(6)). FAB-MS (glycerol): 258 ([M + 1]⁺). Anal. calc. for C₁₀H₁₅N₃O₅ (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 (Et₃NH)OAc) was mixed with (Et₃NH)OAc buffer (900 μl; 0.5 and 1.0M (Et₃NH)OAc, resp., pH adjusted with Et₃N), 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 (Et₃NH)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-furanosyl-nucleoside (20), 1 (22), α-D-pyranosyl- or α-D-furanosyl-nucleoside (29), 4-amino-2-nitrobenzene-1-sulfonic acid (37). As internal standard 4-amino-2-nitrobenzene-1-sulfonic acid (recrystallized from H₂O) 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(1H)-one (4). Isolation by semiprep. HPLC (*Supelco, LC18-DB*, particle size 5 μm, 25 × 10 mm; eluent *A*, 0.1M (Et₃NH)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 H₂O as eluent. ¹H-NMR (D₂O): 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*-(Benzoyloxycarbonyl)-2-(β-D-ribofuranosyl)-D,L-glycinenitrile (**14a/b**). A soln. of **6a/b** (5.18 g, 10.9 mmol) CF₃COOH/H₂O 4:1 was stirred for 1 h at r.t. The solvent was removed by distillation, and last traces of CF₃COOH were removed by co-evaporation with EtOH. Chromatography (silica gel, CH₂Cl₂/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: ¹H-NMR (D₆)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*, CH₂); 7.34–7.39 (*m*, 5 arom. H); 8.06 (d, *J* = 8.4, NH). ¹³C-NMR ((D₆)DMSO): 44.56 (d, CH); 61.31 (t, C(5')); 66.33 (t, CH₂); 70.96, 71.24, 81.65, 85.01 (4d, C(1'), C(2'), C(3'), C(4')); 118.22 (s, CN); 127.89, 128.04, 128.44 (3d, C_o, C_m, C_p); 136.39 (s, C_{ipso}); 155.74 (s, PhCH₂OC(O)). MS: 322 (0.5, M⁺), 108 (33), 107 (28), 91 (100), 79 (31), 77 (20).

Diastereoisomer 14b: ¹H-NMR ((D₆)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*, CH₂, OH); 7.33–7.38 (*m*, 5 arom. H); 8.34 (d, *J* = 8.4, NH). ¹³C-NMR ((D₆)DMSO): 45.73 (d, CH); 61.39 (t, C(5')); 66.21 (t, CH₂); 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 (3d, C_o, C_m, C_p); 136.48 (s, C_{ipso}); 155.57 (s, PhCH₂OC(O)). MS: 323 (1.2, [M + 1]⁺), 322 (3.0, M⁺), 108 (28), 107 (21), 97 (17), 92 (17), 91 (100), 79 (17).

N-(Benzoyloxycarbonyl)-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)Ph₂SiCl (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. H₂O was added and the soln. extracted by addition of CH₂Cl₂. The org. phase was extracted with H₂O (2×) and sat. aq. NaCl soln. After drying (MgSO₄), 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-(Benzoyloxycarbonyl)-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 Ac₂O (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 (CHCl₃): 3420 (br.), 2960, 2930, 2890, 2860, 1745 (br.), 1500, 1430, 1370, 1115, 1105, 1055, 930, 905. ¹H-NMR (CDCl₃): 1.00 (s, ^tBu); 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*, CH₂); 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). ¹³C-NMR (CDCl₃): 19.11 (s, Me₃C); 20.43, 20.52 (2*q*, MeCOO); 26.84 (*q*, Me₃C); 44.57 (d, CH); 63.03 (t, C(5')); 67.99 (t, CH₂); 70.71, 71.19, 79.99, 83.54 (4d, 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 (9d, C_o, C_m, C_p); 132.52, 132.76, 135.36 (3s, C_{ipso}); 155.38 (s, PhCH₂OC(O)); 169.56, 169.67 (2s, MeCOO). MS: 587 (15, [M - 57(*t*-Bu)]⁺), 241 (52), 199 (47), 91 (100).

Diastereoisomer 16b: IR (CHCl₃): 3430 (br.), 2960, 2930, 2890, 2860, 1745 (br.), 1500, 1430, 1375, 1115, 1105, 1060, 1020, 980, 940. ¹H-NMR (CDCl₃): 1.06 (s, ^tBu); 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, CH₂); 5.13 (d, *J* = 12.0, 1 H, CH₂); 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). ¹³C-NMR (CDCl₃): 19.14 (s, Me₃C); 20.45, 20.56 (2*q*, MeCOO); 26.83 (*q*, Me₃C); 44.97 (d, CH); 63.23 (t, C(5')); 67.92 (t, CH₂); 77.38, 71.68, 79.48, 83.53 (4d, 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 (8d, C_o, C_m, C_p); 132.72, 132.80, 137.47 (s, C_{ipso}); 155.07 (s, PhCH₂OC(O)); 169.51, 169.89 (2s, MeCOO). MS: 645 (3.3, [M + 1]⁺), 587 (17, [M - 57(^tBu)]⁺), 135 (31), 91 (100).

2-{2',3'-Di-O-acetyl-5'-O-[(tert-butyl)diphenylsilyl]- β -D-ribofuranosyl}-D,L-glycine nitrile (**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 H₂. 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 (CHCl₃): 3410, 3330, 2960, 2930, 2890, 2860, 1750 (br.), 1470, 1430, 1370, 1250, 1115, 1090, 1020, 980. ¹H-NMR (CDCl₃): 1.06 (s, 'Bu); 1.78 (d, *J* = 7.32, NH₂); 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'), overlaid 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 (2m, 10 arom. H). ¹H-NMR (CDCl₃, D₂O exchange): 1.07 (s, '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 (2m, 10 arom. H). ¹³C-NMR (CDCl₃): 19.23 (s, Me₃C); 20.52 (q with sh, 2 MeCOO); 26.84 (q, Me₃C); 44.54 (d, CH); 63.57 (t, C(5')); 70.89, 71.89, 82.25, 82.61 (4d, C(1'), C(2'), C(3'), C(4')); 119.96 (s, CN); 127.89 (double intensity), 129.89, 129.97, 135.47, 135.70 (5d, C_o, C_m, C_p); 132.64, 133.95 (2s, C_{ipso}); 169.62, 170.03 (s, 2 MeCOO). 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. CHCl₃ (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. ¹H-NMR (CDCl₃): 1.06 (s, '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, NH₂); 7.34-7.46, 7.52-7.60, 7.66-7.68 (3m, 10 arom. H); 7.87 (s, H-C(6)). ¹³C-NMR (CDCl₃): 19.18 (s, Me₃C); 20.50, 20.56, 20.66 (3q, Me-C(3), 2 MeCOO); 26.89 (q, Me₃C); 63.47 (t, (5')); 71.67, 72.11, 81.76, 84.07 (4d, C(1'), C(2'), C(3'), C(4')); 127.87, 127.95, 135.41, 135.72 (4d, C_o, C_m); 129.49 (d, C(6)); 129.98, 130.08 (2d, C_p); 132.24, 132.58 (2s, C_{ipso}); 137.61, 141.48, 143.71 (3s, C(2), C(3), C(5)); 169.55, 169.74 (2s, 2 MeCOO). 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 Ac₂O (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 CH₂Cl₂. 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 (CHCl₃): 2960, 2930, 2860, 1785 (br.), 1745 (br.), 1730 (br.), 1470, 1430, 1370, 1325, 1170, 1150, 1110, 1090, 1050, 1010, 900 (br.). ¹H-NMR (CDCl₃): 1.01 (s, 'Bu); 2.03 (br. s, 3 H, Ac₂N); 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, Ac₂N); 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 (3m, 10 arom. H). ¹³C-NMR (CDCl₃): 18.92 (q, Me-C(3)); 19.09 (s, Me₃C); 20.57, 20.76, 20.84 (s, 3 MeCOO); 26.73 (q, Me₃C); 26.31, 27.00 (2q, (MeCO)₂N); 63.41 (t, C(5')); 72.21, 73.25, 77.78, 83.80 (4d, C(1'), C(2'), C(3'), C(4')); 127.63, 127.73, 135.58, 135.69 (4d, C_o, C_m); 129.71, 129.81 (2d, C_p); 132.78, 132.93 (2s, C_{ipso}); 143.87, 147.68, 148.34, 151.85 (4s, C(2), C(3), C(5), C(6)); 167.91, 169.45, 169.81 (3s, 3 MeCOO); 171.91, 172.93 (2s, 2 (MeCO)₂N). FAB-MS (3-NOBA): 706 ([M + 1]⁺).

6-(Diacetylamino)-5-(2',3'-di-O-acetyl- β -D-ribofuranosyl)-3-methylpyrazin-2(1H)-one (**20**). Silyl ether **19** (100 mg, 0.14 mmol), Bu₄NF (67 mg, 0.21 mmol) and AcOH (16 μ l, 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 CH₂Cl₂ and extracted with H₂O, and the org. layer dried (MgSO₄) and evaporated. Chromatography (silica gel (8.5 g), CH₂Cl₂/EtOH 10:1) yielded **20** (63 mg, 60%). Slightly yellow oil. IR (CHCl₃): 3290 (br.), 2930, 2870, 1745 (br.), 1725 (sh), 1660, 1625. ¹H-NMR (CDCl₃): 2.00 (s, AcO); 2.13 (s, AcO); 2.29 (br. s, 3 H, Ac₂N); 2.42 (br. s, 3 H, Ac₂N); 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')). ¹³C-NMR (CDCl₃): 19.58, 20.46, 20.81 (3q, Me-C(3), 2 MeCOO); 26.03, 26.36 (2q, 2 MeCON); 62.93 (t, C(5')); 73.58, 75.29, 76.82, 85.17 (4d, C(1'), C(2'), C(3'), C(4')); 136.80 (double intensity), 152.02, 158.15 (3s, C(2), C(3), C(5), C(6)); 169.40, 170.04 (2s, 2 MeCOO); 171.66, 172.31 (2s, 2 MeCON). FAB-MS (3-NOBA): 426 ([M + 1]⁺).

6-Amino-3-methyl-5-(β -D-ribofuranosyl)pyrazin-2(1H)-one 5'-Triphosphate (**2**). Pyrazinone **20** (24 mg, 56 μ mol) was dried by repeated evaporation from abs. pyridine, followed by placing it 1 h over P₂O₅ under high

vacuum. Abs. pyridine (56 μ l) and abs. dioxane (166 μ l) 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 μ l, 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 μ mol) and Bu₃N (56.4 μ l, 237 μ mol) in abs. DMF (171 μ l) was injected. The mixture was stirred for 10 min at r.t. and then diluted with 1% I₂ soln. in pyridine/H₂O 98:2 (1.12 ml). After 15 min, the excess I₂ 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 H₂O (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 \times 3 cm; eluent *A*, 400 ml of 0.1M (Et₃NH)HCO₃ pH 7.0 eluent *B*, 400 ml of 1.0M (Et₃NH)HCO₃ 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 μ m, 25 cm \times 10 mm; eluent *A*, 0.1M (Et₃NH)HCO₃ 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. ¹H-NMR (D₂O): 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')). ¹H-NMR (³¹P-decoupled, D₂O): 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')). ³¹P-NMR (D₂O): –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-(tetraisopropylidisiloxane-1,3-diyl)- β -D-ribofuranosyl]-N-(benzyloxycarbonyl)-D,L-glycinenitrile (22a/b)*. A soln. of crude **14a/b** (impure; 9.58 g, \leq 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, TIPSCl₂ (8.1 ml, 25.8 mmol) was slowly added at 0°. After being stirred for 1 h, the soln. was poured on H₂O (400 ml) and extracted with CH₂Cl₂. The org. layer was extracted sequentially with sat. aq. NH₄Cl and sat. aq. NaHCO₃ soln., H₂O, and sat. aq. NaCl soln., and dried (MgSO₄), 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/H₂O. MeOH (7 ml) was added and the soln. poured on H₂O. After extraction with CH₂Cl₂ and washing with sat. aq. NaHCO₃ and sat. aq. NH₄Cl soln., H₂O, and sat. aq. NaCl soln., the org. layer was dried (MgSO₄) 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**. ¹H-NMR (CDCl₃): 0.84–1.11 (*m*, 2 \times 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, CH₂); 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*CH₂); 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_{*p*}(Bz)). ¹³C-NMR (CDCl₃): 12.65, 12.68, 12.76, 12.86, 12.96, 13.06, 13.21, 13.23 (8*d*, Me₂CH); 16.83, 16.85, 18.86, 16.95, 16.96, 17.02, 17.21, 17.29, 17.33, 17.48 (10*q*, Me₂CH); 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*, CH₂(**22b**)); 68.04 (*t*, CH₂(**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*, PhCH₂OC(O)(**22b**)); 155.40 (*s*, PhCH₂OC(O)(**22a**)); 165.81 (*s*, PhCO(**22b**)); 166.29 (*s*, PhCO(**22a**)). FAB-MS (3-NOBA): 669 ([*M* + 1]⁺).

2-[2'-O-Benzoyl-3',5'-O-(tetraisopropylidisiloxane-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 H₂ 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 H₂ pressure (10 bar) and reduced reaction time (4 h) did not result in improved yields.

23a: M.p. 111–112°. ¹H-NMR (CDCl₃): 0.86–1.11 (*m*, 28 H, *i*-Pr); 1.87 (br. *d*, *J* = 8.6, NH₂); 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_{*p*}). ¹³C-NMR (CDCl₃): 12.69, 12.70, 13.02, 13.10,

13.33 (*5d*, Me₂CH); 16.87, 16.91, 16.93, 17.02, 17.28, 17.33, 17.37, 17.47 (*8g*, Me₂CH); 44.96 (*d*, CH); 60.22 (*t*, C(5')); 69.86, 74.68, 81.96, 83.57 (*4d*, C(1'), C(2'), C(3'), C(4')); 120.23 (*s*, CN); 128.46, 129.74 (*2d*, C_o, C_m); 129.81 (*s*, C_{ipso}); 133.30 (*d*, C_p); 166.17 (*s*, PhCO). FAB-MS (3-NOBA): 535 (*[M + 1]*⁺). Anal. calc. for C₂₆H₄₂N₂O₆Si₂ (534.80): C 58.39, H 7.92, N 5.24; found: C 58.24, H 7.87, N 5.25.

23b: ¹H-NMR (CDCl₃): 0.81–1.12 (*m*, 28 H, i-Pr); 1.92 (*br. s*, NH₂); 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). ¹³C-NMR (CDCl₃): 12.68, 12.72, 13.05, 13.25 (*4d*, Me₂CH); 16.84, 16.86, 16.96, 17.03, 17.29, 17.34, 17.47 (*7q*, Me₂CH); 46.29 (*d*, CH); 60.94 (*t*, C(5')); 70.68, 74.15, 81.80, 83.40 (*4d*, C(1'), C(2'), C(3'), C(4')); 119.14 (*s*, CN); 128.43, 129.74 (*2d*, C_o, C_m); 129.71 (*s*, C_{ipso}); 133.28 (*d*, C_p); 165.83 (*s*, PhCO). FAB-MS (3-NOBA): 535 (*[M + 1]*⁺).

3-[2-O-Benzoyl-3',5'-O-(tetraisopropylidisiloxane-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. CHCl₃ 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. ¹H-NMR (CDCl₃): 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*, NH₂); 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). ¹³C-NMR (CDCl₃): 12.64, 12.92, 13.04, 13.29 (*4d*, Me₂CH); 16.77, 16.80, 16.86, 16.92, 17.18, 17.30, 17.34, 17.51 (*8q*, Me₂CH); 20.52 (*q*, Me–C(5)); 60.36 (*t*, C(5')); 70.58, 76.48, 81.93, 83.51 (*4d*, C(1'), C(2'), C(3'), C(4')); 128.38, 129.77 (*2d*, 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 (*3s*, C(2), C(3), C(5)); 165.73 (*s*, PhCO). FAB-MS (3-NOBA): 604 (*[M + 1]*⁺). Anal. calc. for C₂₉H₄₅N₃O₇Si₂ (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-(tetraisopropylidisiloxane-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 Ac₂O (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 CH₂Cl₂, and KOAc removed by filtration. Chromatography (silica gel (80 g), petroleum ether/AcOEt 8:2) yielded **25** (490 mg, 60%). Foam. ¹H-NMR (CDCl₃): 0.82–1.14 (*m*, 28 H, i-Pr); 2.07 (*br. s*, 3 H, Ac₂N); 2.38, 2.52 (*2s*, Me–C(3), AcO); 2.54 (*br. s*, 3 H, Ac₂N); 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). ¹³C-NMR (CDCl₃): 12.52, 12.72, 13.04, 13.34 (*4d*, Me₂CH); 16.79, 16.84, 17.03, 17.10, 17.23, 17.27, 17.30, 17.38 (*8q*, Me₂CH); 18.92, 20.85 (*2q*, Me–C(3), MeCOO); 26.30, 26.99 (*2q*, MeCO₂N); 60.55 (*t*, C(5')); 70.81, 75.87, 79.58, 81.99 (*4d*, C(1'), C(2'), C(3'), C(4')); 128.33, 129.76 (*2d*, C_o, C_m); 130.20 (*s*, C_{ipso}); 132.97 (*d*, C_p); 143.27, 147.82, 148.96, 151.57 (*4s*, C(2), C(3), C(5), C(6)); 165.46, 167.92 (*2s*, PhCO, MeCOO); 172.16, 172.93 (*s*, MeCO₂N). FAB-MS (3-NOBA): 730 (*[M + 1]*⁺). Anal. calc. for C₃₅H₅₁N₃O₁₀Si₂ (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-(tetraisopropylidisiloxane-1,3-diyl)-β-D-ribofuranosyl]-3-methylpyrazin-2-(1H)-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. ¹H-NMR (CDCl₃): 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). ¹³C-NMR (CDCl₃): 12.48, 12.66, 12.97, 13.50 (*4d*, Me₂CH); 16.82, 16.86, 16.90, 16.91, 16.96, 17.03, 17.19, 17.30 (*8q*, Me₂CH); 19.94, 24.69 (*2q*, Me–C(3), MeCON); 59.71 (*t*, C(5')); 68.93, 77.82, 81.85, 83.94 (*4d*, C(1'), C(2'), C(3'), C(4')); 128.40, 129.72 (*2d*, 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*, PhCO); 171.54 (*s*, MeCON). FAB-MS (3-NOBA): 645 (*M*⁺).

N-{3-[2'-O-Benzoyl-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-β-D-ribofuranosyl]-6-(benzyloxy)-5-methylpyrazin-2-yl}acetamide (27). Dry **26** (323 mg, 0.50 mmol), PPh₃ (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. ¹H-NMR (CDCl₃): 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, CH₂); 5.40 (*d*, *J* = 12.7, 1 H, CH₂); 6.08 (*br. d*, *J* = 4.8, H–C(2')); 7.30–7.48 (*m*, 7 H, PhCH₂),

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). ¹³C-NMR (CDCl₃): 12.53, 12.72, 13.06, 13.28 (4*d*, Me₂CH); 16.83, 16.87, 17.01, 17.11, 17.18, 17.22, 17.27, 17.37 (8*q*, Me₂CH); 18.58, 23.94 (2*q*, Me–C(3), MeCON); 61.19 (*t*, C(5')); 68.23 (*t*, PhCH₂); 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*, PhCO, MeCON). FAB-MS (3-NOBA): 736 ([*M* + 1]⁺). Anal. calc. for C₃₈H₅₃N₃O₃Si₂ (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. MeOSiMe₃ (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'). ¹H-NMR ((D₆)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 (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*, CH₂); 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, PhCH₂, 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). ¹³C-NMR ((D₆)DMSO): 18.46, 23.02 (2*q*, Me–C(5), MeCON); 61.51 (*t*, C(5')); 67.56 (*t*, PhCH₂); 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*, PhCO, MeCON). 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 P₂O₅ 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. ¹H-NMR (CDCl₃): 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, POCH₂); 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, PhCH₂O–C(5)); 5.35 (*d*, *J* = 12.6, 1 H, PhCH₂O–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). ¹³C-NMR (CDCl₃): 18.71, 24.01 (2*q*, Me–C(5), MeCON); 66.74 (*td*, *J*(C,P) = 5.1, C(5')); 67.56 (*t*, PhCH₂O–C(5)); 69.27, 69.32, 69.39, 69.44, 69.63, 69.69, 69.75, 69.81 (4*d*, 4 CH₂OP); 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*, PhCO, MeCON). ³¹P-NMR (¹H-decoupled; CDCl₃): –0.98 (*s*, PO₄), –1.64 (*s*, PO₄). FAB-MS (3-NOBA): 1014 ([*M* + 1]⁺). Anal. calc. for C₅₄H₅₃N₃O₁₃P₂ (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), 0.2*M* aq. (Et₃NH)HCO₃ (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 H₂. 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. N₂, and evaporated at low temp. Ion-exchange chromatography (DEAE *Sephadex-A25*, column 55 × 1.8 cm; eluent *A*, H₂O (1 l), eluent *B*, 1.0*M* (Et₃NH)HCO₃ 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 H₂O followed by lyophilization (to remove the volatile buffer), 3 (25 mg) was obtained as a light brown amorphous solid. ¹H-NMR (D₂O): 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')). ¹H-NMR (³¹P-decoupled, D₂O): 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')). ¹³C-NMR (D₂O): 19.78 (*q*, Me–C(3)); 67.19 (*d*, *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)). ³¹P-NMR (D₂O): 2.14 (*d*, *J* = 8.0, PO₄–C(3')); 1.53 (very br. *s*, PO₄–C(5')). ³¹P-NMR (¹H-decoupled; D₂O): 2.14 (*s*, PO₄–C(3')); 1.54 (*s*, PO₄–C(5')). FAB-MS (*neg. mode*; glycerol): 416 (monoanion).

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