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Metal-Ion-Mediated Base Pairing between Natural Nucleobases and Bidentate 3,5-Dimethylpyrazolyl-Substituted Purine Ligands

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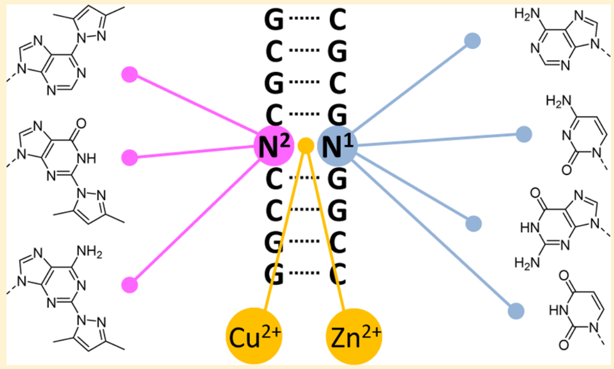
Supporting Information

ABSTRACT: The potential of three modified purine bases, namely, 6-(3,5-dimethylpyrazol-1-yl)purine, 2-(3,5-dimethylpyrazol-1-yl)hypoxanthine, and 2-(3,5-dimethylpyrazol-1-yl)adenine, for metal-ion-mediated base pairing within an oligonucleotide environment has been investigated. The respective modified nucleosides were incorporated in the middle of 9-mer 2′-*O*-methyl oligonucleotides and the hybridization of these modified oligonucleotides with their unmodified counterparts studied by UV and CD spectrometry in the absence and presence of Cu^{2+} or Zn^{2+}. All of the modified oligonucleotides formed more stable duplexes in the presence of divalent metal ions than in the absence thereof, but with different preferences for the complementary oligonucleotide. The oligonucleotide incorporating 2-(3,5-dimethylpyrazol-1-yl)hypoxanthine readily accepted any of the natural nucleobases opposite to this modified base regardless of whether Cu^{2+} or Zn^{2+} was used as the bridging metal ion. The other two oligonucleotides, on the other hand, were much more discriminating, exhibiting markedly elevated T_m values only in the presence of Cu^{2+} and only when certain natural nucleobases were paired with the modified one. The origin of the selectivity (or promiscuity) of the metal-ion-mediated base pairing is discussed in terms of the ability of the modified nucleobases, as well as their natural counterparts, to serve as anionic ligands.



■ INTRODUCTION

Replacement and augmentation of Watson−Crick base-pairing of nucleic acids by metal-ion-mediated base pairing has been the object of continuing attention during the past decade, mainly because of the potential of such base pairs in expanding the genetic alphabet and in DNA nanotechnology.[1−7] Our main interest, in turn, has been the application of metal ion chelates in discrimination between the natural nucleobases. Short metallo-oligonucleotides with enhanced affinity toward their natural counterparts could, for example, find use in recognition and inhibition of short noncoding RNAs.

We have previously demonstrated the ability of a tridentate chelating nucleoside, 2,6-bis(3,5-dimethylpyrazol-1-yl)purine riboside (**1**), to markedly increase the melting temperature of short double-stranded oligonucleotides in the presence of Cu^{2+} and, to a lesser extent, Zn^{2+}, without compromising the fidelity of base pairing in the Watson−Crick parts of the oligonucleotides.[8] The greatest effect was observed when the artificial nucleoside was placed in a terminal position or flanked by mismatched base pairs, that is, with the most flexible structures studied. In the middle of a fully matched double helix, introduction of Cu^{2+} barely overcame the destabilization caused by the bulky modified nucleoside. The sensitivity of the metal-ion-mediated base pairing of **1** to changes in the oligonucleotide environment is probably attributable to steric constraints imposed by the base stacking of double-helical nucleic acids. More specifically, at the monomeric level the 3 + 1 coordination of the **1**:M^{z+}:Urd ternary complexes allows alleviation of steric crowding by rotation about the metal−N3(Urd) bond (Figure 1), the most stable conformation likely being the one with the bases perpendicular to each other.[2] The base stack of a double-helical oligonucleotide, on the other hand, can only accommodate coplanar base pairs, offering an explanation for the preference of flexible over rigid structures.

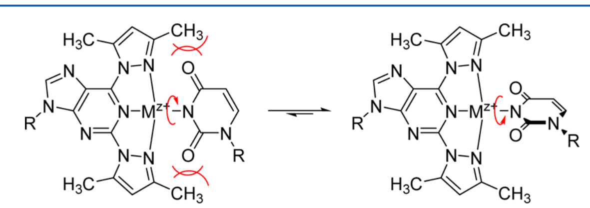


Figure 1. Rotation about the metal−N3(Urd) bond in the **1**:M^{z+}:Urd ternary complex.

To test the above hypothesis, three bidentate analogs of **1** have been synthesized and incorporated in the middle of 9-mer oligonucleotides, the melting temperatures of which have been measured in the presence (and absence) of Cu^{2+} and Zn^{2+}. The

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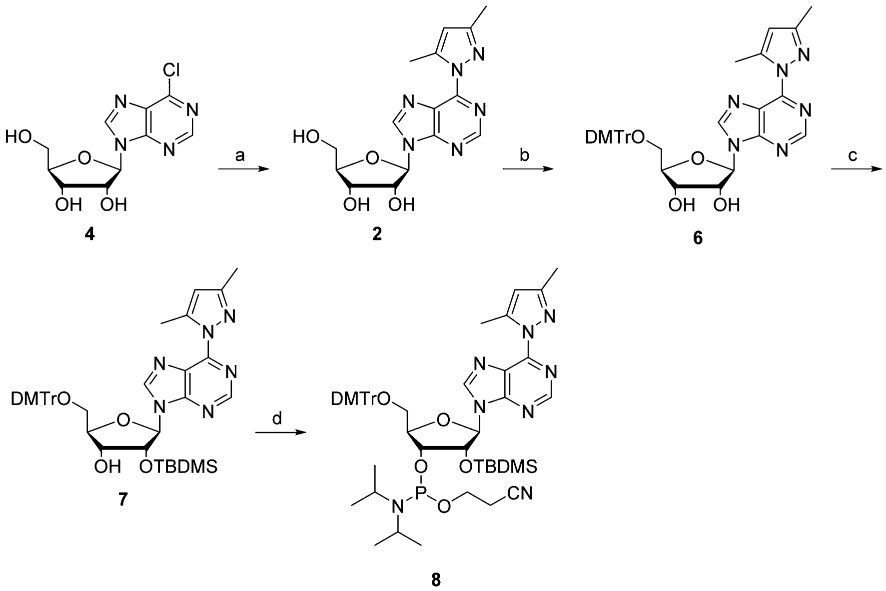
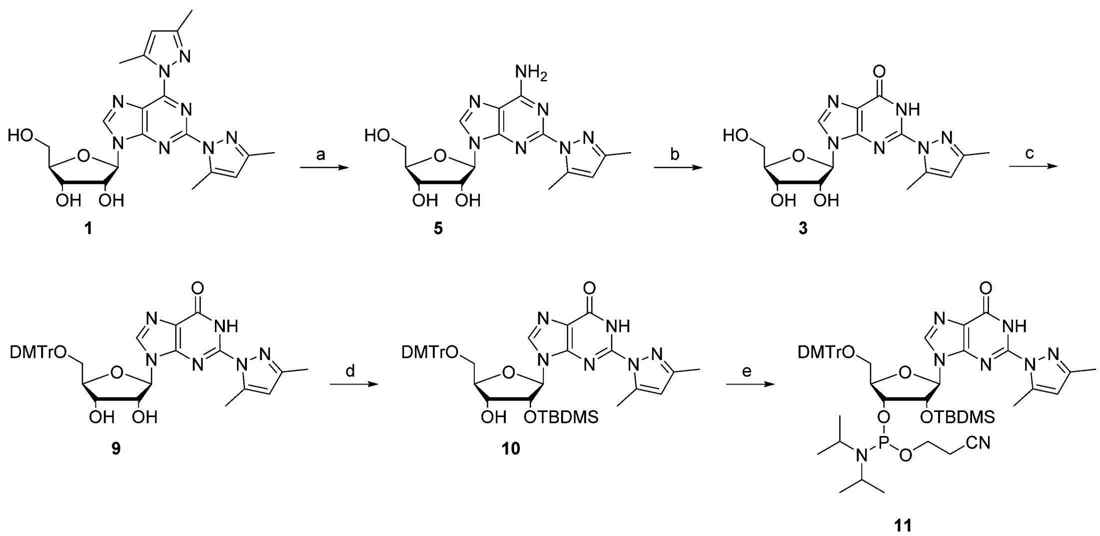
Scheme 1. Preparation of 9-(β-D-Ribofuranosyl)-6-(3,5-dimethylpyrazol-1-yl)purine and Its Conversion to a Protected Phosphoramidite Building Block[a]



[a]Reagents and conditions: (a) 1 $H_2NNH_2 \cdot H_2O$, 2 pentane-2,4-dione, TFA; (b) DMTrCl, pyridine; (c) TBDMSCl, imidazole, DMF; (d) 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, Et_3N, CH_2Cl_2.

Scheme 2. Preparation of 2-(3,5-Dimethylpyrazol-1-yl)inosine and Its Conversion to a Protected Phosphoramidite Building Block[a]



[a]Reagents and conditions: (a) NH_3, H_2O; (b) $NaNO_2$, H_2O, AcOH; (c) DMTrCl, pyridine; (d) TBDMSCl, imidazole, DMF; (e) 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, Et_3N, CH_2Cl_2.

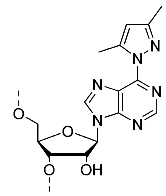
2 + 2 coordination of the putative metal-ion-mediated base pairs between these structures and native nucleobases would force them to adopt a coplanar geometry, presumably making them more compatible with oligonucleotide hybridization.

■ RESULTS

Synthesis of the Modified Nucleosides and Their Phosphoramidite Building Blocks. Syntheses of the protected phosphoramidite building blocks of 9-(β-D-ribofuranosyl)-6-(3,5-dimethylpyrazol-1-yl)purine (**2**) and 2-(3,5-dimethylpyrazol-1-yl)inosine (**3**) are outlined in Schemes 1 and 2, respectively. Compound **2** was prepared by first displacing the chloro substituent of 6-chloropurine riboside (**4**) with hydrazine and then converting the hydrazino substituent to a 3,5-dimethylpyrazol-1-yl substituent by treatment with pentane-2,4-dione. Compound **3**, in turn, was obtained by displacement of the 6-(3,5-dimethylpyrazol-1-yl) substituent of compound **1** with ammonia, followed by conversion of the amino group of the 2-(3,5-dimethylpyrazol-1-yl)adenosine (**5**) thus formed to an oxo group by treatment with sodium nitrite in aq. acetic acid. Both of the artificial nucleosides were protected as 4,4′-dimethoxytrityl ethers at the 5′-position and *tert*-butyldimethylsilyl ethers at the 2′-position. Finally, the 3′-OH was phosphitylated by conventional methods to afford the phosphoramidite building blocks **8** and **11**.

Table 1. Structures of the 2′-*O*-Methyl-RNA Oligonucleotides Used in This Study

	Sequence
ON1a	5′-GCGC**A**CCGG-3′
ON1p	5′-GCGC**P**CCGG-3′
ON1q	5′-GCGC**Q**CCGG-3′
ON1x	5′-GCGC**X**CCGG-3′
ON1z	5′-GCGC**Z**CCGG-3′
ON2a	5′-CCGG**A**GCGC-3′
ON2c	5′-CCGG**C**GCGC-3′
ON2g	5′-CCGG**G**GCGC-3′
ON2u	5′-CCGG**U**GCGC-3′
ON2s	5′-CCGG**S**GCGC-3′
ON3x	5′-UCAG**X**-3′
ON3q	5′-UCAG**Q**-3′

P =


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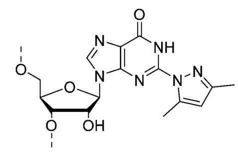

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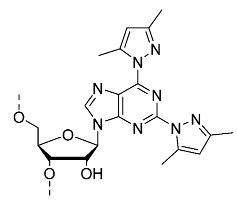

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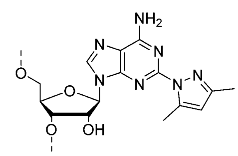

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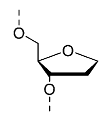


Oligonucleotide Synthesis. The 9-mer 2′-*O*-methyl-RNA oligonucleotides **ON1p** and **ON1q** (Table 1), bearing a modified nucleoside (**2** or **3**, respectively) in the middle of the strand, were synthesized from commercial 2′-O-methylated protected phosphoramidite building blocks by an automated synthesizer, except for the modified building block, which was coupled manually with an exceptionally long coupling time (60 min). The coupling yield for the modified building blocks **8** and **11** were 40% and 44%, respectively, the other couplings proceeding with normal efficiency. For removal of the phosphate and base protections and release of the oligonucleotides from the support, conventional treatment with 33% aq. NH_3 (4 h at 55 °C) was used. The third 9-mer oligonucleotide **ON1z** (Table 1), incorporating 2-(3,5-dimethylpyrazol-1-yl)-adenosine (**5**) as the modified nucleoside, was prepared from the previously synthesized 2,6-bis(3,5-dimethylpyrazol-1-yl)-purine-containing oligonucleotide **ON1x** in approximately 40% yield by treatment with 33% aq. NH_3 (6 h at 55 °C). As previously described, under these conditions the dimethylpyrazol-1-yl substituent at position 6 of the modified nucleoside **1** is displaced by an amino group.[8]

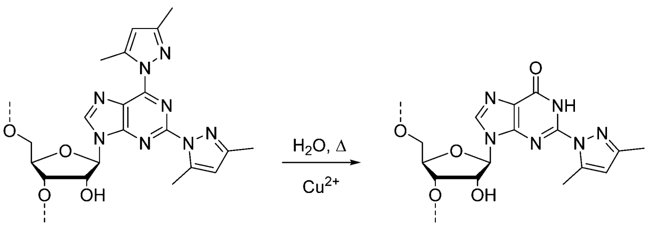
The 5-mer 2′-O-methyl-RNA oligonucleotide **ON3x** bearing the artificial nucleoside **1** at the 3′-terminus was synthesized as previously described.[8] Accordingly, the 5′-*O*-DMTr- and 3′-*O*-TBDMS-protected derivative of **1** was immobilized on aminoalkyl-CPG via a succinyl linker and the oligonucleotide chain assembled on the solid support thus obtained from commercial 2′-*O*-methylated protected phosphoramidite building blocks by an automated synthesizer. The couplings proceeded on this support with normal efficiency. Release of the oligonucleotide from support and removal of the base and phosphate protections was achieved by treatment with 33% aq. NH_3 (2 h at 55 °C).

In all cases, the TBDMS protection was removed by treatment with 1.5 M triethylamine trihydrofluoride in DMSO (2 h at 55 °C), after which the crude oligonucleotides were purified by RP-HPLC. The purified products were characterized by electrospray ionization mass spectrometry (ESI-MS) and their concentrations were determined UV-spectrophotometrically at 260 nm using molar absorptivities calculated by an implementation of the nearest-neighbors method.[9,10]

Hydrolytic Stability of the Modified Oligonucleotides. The relative lability of **1** in concentrated aq. NH_3, discovered during deprotection of the respective modified oligonucleotides,[8] calls into question the hydrolytic stability of the 3,5-dimethylpyrazolyl-substituted purine nucleosides at the high temperatures of the T_m measurements. While H_2O is an inferior nucleophile compared to NH_3, coordination of a metal ion presumably makes the purine ring more susceptible to nucleophilic attack, possibly to the point where hydrolysis becomes significant. To verify the hydrolytic stability of the modified oligonucleotides under conditions of the T_m studies, the samples were analyzed by HPLC and ESI-MS before and after the measurement (3 cycles from 0 to 90 °C and back). Oligonucleotides **ON1p**, **ON1q**, and **ON1z** proved to be stable in all the cases studied but the previously studied[8,11] **ON1x** had been converted almost quantitatively to the hydrolysis product

ON1q in the presence of Cu^{2+} (Scheme 3). In the presence of Zn^{2+} or in the absence of divalent metal ions, on the other hand, no changes in the **ON1x**-containing samples were observed.

Scheme 3. Hydrolytic Conversion of a 2,6-Bis(3,5-dimethylpyrazol-1-yl)purine Riboside Residue to a 2-(3,5-Dimethylpyrazol-1-yl)inosine Residue



To obtain more quantitative data on the reactivity of the modified building block **1** within an oligonucleotide structure, hydrolysis of oligonucleotide **ON3x** was studied at 37 °C in a 20 mmol L^{-1} cacodylate buffer (pH = 7.4) in the absence and presence of 1 equiv of either $CuSO_4$ or $Zn(NO_3)_2$, the ionic strength being adjusted to 0.1 mol L^{-1} with $NaClO_4$. The starting concentration of **ON3x** was 2.0 μmol L^{-1} in each experiment. Analysis of the composition of aliquots withdrawn from the reaction solutions at appropriate time intervals was carried out by RP HPLC. The pseudo-first-order rate constants for the hydrolysis of **ON3x** were obtained by applying the integrated first-order rate law to the time-dependent decrease of the relative signal area of **ON3x**, using potassium 4-nitrobenzenesulfonate as an internal standard. The logarithmic time profiles thus obtained are presented in Figure 2. In all cases, disappearance of **ON3x** was accompanied by the appearance of a new, faster-eluting, signal, identified by ESI-MS analysis as the expected product **ON3q**.

The half-lives obtained for the hydrolysis of **ON3x** at 37 °C in the presence of Cu^{2+} and Zn^{2+} and in the absence of divalent metal ions were 69, 875, and 1481 h, respectively. These kinetic results are in good agreement with those of the HPLC and MS analysis of the T_m samples: significant decomposition of **ON1x** was only detected in the presence of Cu^{2+}. The most likely explanation to the promotion by hydrolysis by Zn^{2+} and, especially, Cu^{2+} is that coordination of a metal ion makes the purine ring less electron-rich and, hence, more susceptible to nucleophilic attack. Whether the nucleophile is a free water molecule or an aquo (or hydroxo) ligand of the divalent metal ion is beyond the scope of this study.

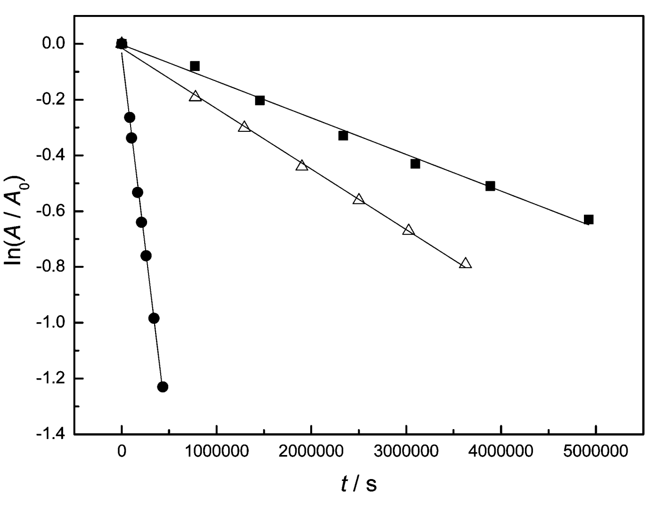


Figure 2. Logarithmic time profiles for the decomposition of **ON3x** in the absence (■) and presence of Cu^{2+} (●) or Zn^{2+} (△); [**ON3x**] = [$CuSO_4$] = [$Zn(NO_3)_2$] = 2.0 μM; $I(NaClO_4)$ = 0.1 mol L^{-1}; pH = 7.4; T = 37 °C.

Hybridization Efficiency of the Modified Oligonucleotides. Melting temperatures of the duplexes formed by the modified oligonucleotides **ON1p**, **ON1q**, and **ON1z** with their unmodified complements **ON2a**, **ON2c**, **ON2g**, and **ON2u** were measured in a 20 mmol L^{-1} cacodylate buffer (pH = 7.4) in the presence and absence of 1 eq. of either Cu^{2+} or Zn^{2+} ions. To break the observed changes in T_m down to contributions from base stacking and (metal-ion-mediated) base pairing, respective measurements were also carried out using the modified oligonucleotide **ON2s** as the other strand. In **ON2s**, the central nucleoside was replaced by a 2-(hydroxymethyl)-tetrahydrofuran-3-ol spacer, creating an abasic site opposite to the metal-ion-chelating modified nucleoside. The concentration of the oligonucleotides was 3.0 μmol L^{-1} and the ionic strength was adjusted to 0.1 mol L^{-1} with $NaClO_4$. Table 2 summarizes the results of these measurements. For reference, the previously determined T_m values for the corresponding unmodified duplexes have also been included.[11]

In the absence of divalent metal ions, the duplexes formed by the modified oligonucleotide **ON1p** with **ON2a**, **ON2c**, **ON2g**, and **ON2u** exhibited T_m values of 50−53 °C, that is, 3−9 °C lower than their unmodified counterparts with a single mismatch in the middle of the chain. The **ON1p**:**ON2s** duplex, pairing the modified 6-(3,5-dimethylpyrazol-1-yl)purine base with an abasic site, was considerably more stable, with a T_m of more than 58 °C. In the presence of 1 eq. of Cu^{2+}, the **ON1p**:**ON2a**, **ON1p**:**ON2c**, and **ON1p**:**ON2g** duplexes were somewhat stabilized, but no more than the unmodified ones. The melting temperature of **ON1p**:**ON2u**, in turn, was increased by nearly 7 °C, suggesting a specific interaction between the modified nucleoside **2**, Cu^{2+} ion, and uridine. In contrast to Cu^{2+}, no changes in T_m were observed with 1 equiv of Zn^{2+} in any of the cases studied.

Oligonucleotide **ON1q** formed fairly stable duplexes with all of the complementary unmodified strands even when no divalent metal ions were added, with melting temperatures of approximately 66 °C. Hybridization with **ON2s** was somewhat stronger but the difference was less marked than with **ON1p**. Introduction of 1 equiv of Cu^{2+} resulted in further stabilization of 5−9 °C, bringing the T_m values of all the duplexes formed by **ON1q** to the level of the fully matched unmodified one (**ON1a**:**ON2u**). With these duplexes, very similar increases in T_m were also observed in the presence of 1 equiv of Zn^{2+}. The sole exception was **ON1q**:**ON2s**, which was not stabilized by either Cu^{2+} or Zn^{2+}.

The melting temperatures of the duplexes formed by oligonucleotide **ON1z** with **ON2a**, **ON2c**, **ON2g**, and **ON2u** in the absence of divalent metal ions were approximately 63 °C, that is 3 °C lower than the respective values obtained with **ON1q** under the same conditions. **ON1z**:**ON2s**, on the other hand, was as stable as **ON1q**:**ON2s**. In the presence of Cu^{2+}, sigmoidal UV melting curves could only be obtained for **ON1z**:**ON2c** and **ON1z**:**ON2u**. Of these duplexes, the former was stabilized by approximately 7 °C whereas hardly any effect was detected with the latter. In contrast to **ON1q**, the T_m values of the duplexes formed by **ON1z** were largely insensitive

Table 2. Melting Temperatures for the Duplexes Formed between the Modified 2′-*O*-Methyl-RNA Oligonucleotides ON1p, ON1q, and ON1z with their Unmodified Counterparts ON2a, ON2c, ON2g, ON2u, and ON2s[a]

ON1 5′−G C G C N² C C G G−3′
ON2 3′−C G C G N¹ G G C C−5′

		N¹				
M^{2+}	N²	A	C	G	U	S
no metal	A	56.3 ± 0.2[b]	59.0 ± 0.2[b]	61.2 ± 0.3[b]	75.1 ± 0.3[b]	55.6 ± 0.3
	P	53.1 ± 0.4	50.1 ± 0.5	53.0 ± 0.4	51.9 ± 0.4	58.3 ± 0.2
	Q	66.5 ± 0.8	66.3 ± 0.6	66.1 ± 0.8	67.0 ± 0.6	69.3 ± 0.4
	Z	62.7 ± 0.6	63.4 ± 0.7	63.8 ± 2.1	63.4 ± 0.9	69.6 ± 0.7
Cu^{2+}	A	58.8 ± 0.3 (+2.5)[b]	61.7 ± 0.2 (+2.7)[b]	62.5 ± 0.1 (+1.3)[b]	75.8 ± 0.1 (+0.7)[b]	55.6 ± 0.2 (+0)
	P	54.5 ± 0.5 (+1.4)	53.3 ± 0.6 (+3.2)	56.3 ± 0.6 (+3.3)	58.7 ± 0.8 (+6.8)	58.0 ± 0.3 (−0.3)
	Q	75 ± 2 (+8.5)	73 ± 2 (+6.7)	72 ± 1 (+5.9)	72.2 ± 0.3 (+5.2)	69.6 ± 0.4 (+0.3)
	Z	N/A[c]	70.6 ± 1.0 (+7.2)	N/A[c]	64.8 ± 1.0 (+1.4)	N/A[c]
Zn^{2+}	A	56.4 ± 0.1 (+0.1)[b]	61.4 ± 0.1 (+2.4)[b]	62.3 ± 0.1 (+1.1)[b]	76.0 ± 0.3 (+0.9)[b]	55.4 ± 0.2 (−0.2)
	P	52.8 ± 0.8 (−0.3)	51.3 ± 0.6 (+1.2)	54.0 ± 0.8 (+1.0)	51.8 ± 0.4 (−0.1)	58.4 ± 0.1 (+0.1)
	Q	73 ± 2 (+6.5)	72 ± 2 (+5.7)	73 ± 2 (+6.9)	74 ± 2 (+7.0)	69.4 ± 0.1 (+0.1)
	Z	63.6 ± 0.8 (+0.9)	62.5 ± 0.5 (−0.9)	65.5 ± 1.1 (+1.7)	65.8 ± 1.0 (+2.4)	69.5 ± 1.5 (−0.1)

[a]Conditions: pH = 7.4 (20 mM cacodylate buffer); [oligonucleotides] = 3.0 μM; [metal ions] = 0 or 3.0 μM; $I(NaClO_4)$ = 0.10 M. For the structures of the P, Q, Z, and S residues, see Table 1. [b]From ref 8. [c]No sigmoidal melting curve was obtained.

to addition of 1 equiv of Zn^{2+}, with the possible exception of a very modest stabilization in the case of **ON1z**:**ON2u**.

With all the duplexes studied, folding to the expected secondary structure was verified by recording their CD spectra over a wide temperature range (6−94 °C) under the same conditions as the T_m measurements. In all cases, CD spectra characteristic of an A-type double helix were obtained at low temperatures (Figure 3A).[12−15] On increasing temperature, the intensity of the CD signals gradually decreased following sigmoidal curves with similar inflection points to those of the UV melting curves (Figure 3B).

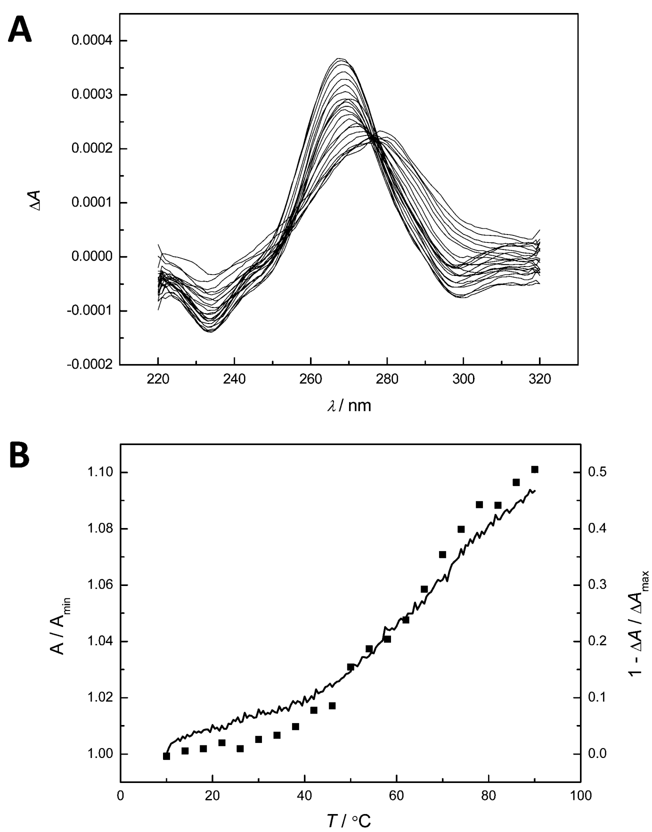


Figure 3. (A) CD spectra of the **ON1z**:**ON2c** duplex in the presence of Cu^{2+}, recorded at 4 °C intervals between 6 and 94 °C and (B) thermal hyperchromicity at 260 nm (solid line) and loss of ellipticity (■) of the same duplex; [**ON1z**] = [**ON2c**] = [Cu^{2+}] = 3.0 μM; $I(NaClO_4)$ = 0.1 M; pH = 7.4.

■ DISCUSSION

A distinct pattern arises from the melting temperatures of the oligonucleotide duplexes studied in the absence of divalent metal ions: the modified nucleoside **2**, bearing a 3,5-dimethylpyrazol-1-yl substituent at position 6, is destabilizing and the 2-substituted analogs **3** and **5** are stabilizing. It is interesting to note that the previously studied 2,6-bis-substituted derivative **1** also promoted hybridization relative to mispaired canonical nucleosides but considerably less than **3** or **5**. As the stabilization of these nucleosides is in all likelihood attributable to enhanced base stacking, the results suggest that the pyrazole ring at position 2, but not at position 6, may engage in stacking interactions with the neighboring nucleobases. The fact that replacing the opposing canonical residue with an abasic site is stabilizing with **2**, **3**, and **5**, but not with adenosine, indicates that all of the modified nucleobases are sterically more demanding than the natural ones. In particular, the 6-substituted derivative **2** seems to stand out also in this respect.

In line with previous results,[8] all of the modified oligonucleotide duplexes exhibit their highest melting temperatures in the presence of Cu^{2+}. Considerable increases in T_m are observed with just 1 equiv of Cu^{2+}, consistent with formation of a stable Cu^{2+}-mediated base pair between the modified nucleoside residue and the one opposite to it. This interpretation is further borne out by the fact that no stabilization by Cu^{2+} (or Zn^{2+}) was observed with duplexes placing an abasic site opposite to the metal-ion-chelating nucleobase. Several base pairs of this type have been described in the literature, typically with square-planar (or octahedral) geometry around the Cu^{2+} center.[16−22] In the present study, the modified nucleosides **2**, **3**, and **5** have been designed to serve as bidentate ligands, leaving the other two coordination sites vacant for the natural nucleobase. Numerous NMR, EPR

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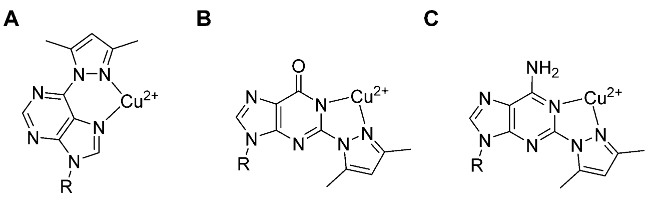
and IR spectrometric and X-ray crystallographic studies have established N1 and N7 of purines[23−34] and N3 of pyrimidines[35−38] as the preferred binding sites for Cu^{2+}.[39−43] At physiological pH, adenosine coordinates predominantly through N7 and guanosine, with concomitant deprotonation, through N1. Of the potential exocyclic ligands, the oxo substituents tend to be favored over the amino substituents, consistent with the lone pair of the latter being engaged in the aromatic π-electron system.[44,45] In the case of the modified nucleosides **2**, **3**, and **5**, the dimethylpyrazolyl substituent presents an additional binding site (note that the free electron pair on N2 of the pyrazole ring is not part of the π-electron system and is, hence, readily available for coordination). The most likely binding modes would, hence, be through N7 and pyrazole-N2 for **2** and N1 and pyrazole-N2 for **3** and **5** (Figure 4). Recent studies with the Cu^{2+} complex of related 6-furylpurine 2′-deoxyriboside appear to confirm these predictions at least in the case of **2**.[46] The interactions of Zn^{2+} with nucleosides are generally similar to those of Cu^{2+}, but weaker.[34,43,47]



2:Cu^{2+} **3**:Cu^{2+} **5**:Cu^{2+}

Figure 4. Coordination of Cu^{2+} by the modified nucleosides **2** (A), **5** (B), and **3** (C).

Stability of the metal-ion-mediated base pairs appears to correlate with the potential of the bases to serve as anionic ligands: the greatest increases in T_m on addition of Cu^{2+} or Zn^{2+} are observed in cases where at least one member may deprotonate to give an anion. At physiological pH, the natural nucleosides guanosine and uridine, and in all likelihood the modified nucleoside **3**, deprotonate upon coordination of Cu^{2+} or (to a lesser extent) Zn^{2+}. Accordingly, **ON1q**, incorporating the modified nucleoside **3**, hybridizes strongly with all the complementary oligonucleotides. The modified nucleosides of **ON1p** and **ON1z** (**2** and **5**, respectively) are not readily deprotonated under the experimental conditions and, hence, favor hybridization with complementary oligonucleotides that can supply an anionic ligand to the metal-ion-mediated base pair, that is, **ON2g** and **ON2u**. The sole exception from this behavior is **ON1z** in the presence of Cu^{2+}, in which case **ON2c**, rather than **ON2u**, is the favored complement. It should be noted, however, that no T_m values could be obtained for the **ON1z:ON2a** and **ON1z:ON2g** duplexes under the same conditions, casting doubt also on the reliability of the respective data for **ON1z:ON2c** and **ON1z:ON2u**. Finally, while some of the mismatched unmodified duplexes (notably **ON1a:ON2a** and **ON1a:ON2c**) enjoy some stabilization by Cu^{2+} or Zn^{2+}, the effects are very modest compared to the duplexes between modified and unmodified oligonucleotides (ΔT_m = +2.5 and +7 °C, respectively).

The most probable structures for the putative metal-ion-mediated base pairs formed by the modified nucleosides **2**, **3** and **5** with their natural counterparts are presented in Figures 5−7, respectively. Besides the electronic effects discussed

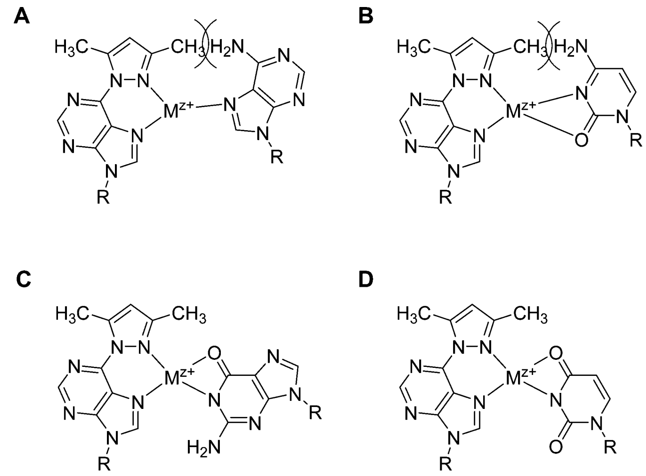


Figure 5. Proposed metal-ion-mediated base pairs between **2** and adenosine (A), cytidine (B), guanosine (C), and uridine (D).

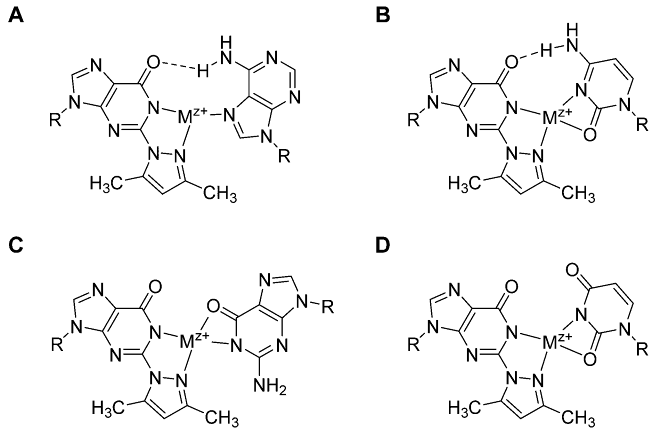


Figure 6. Proposed metal-ion-mediated base pairs between **3** and adenosine (A), cytidine (B), guanosine (C), and uridine (D).

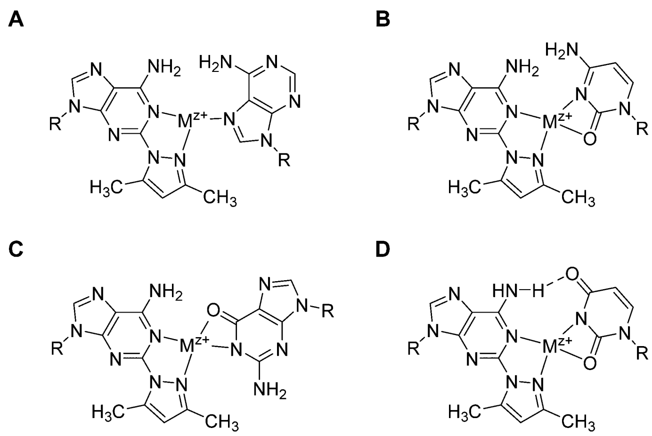


Figure 7. Proposed metal-ion-mediated base pairs between **5** and adenosine (A), cytidine (B), guanosine (C), and uridine (D).

above, steric clash between the amino and methyl substituents is evident in the case of the **2**:M^{z+}:A and **2**:M^{z+}:C pairs (Figures 5A and 5B, respectively). In fact, whether the former base pair forms at all is questionable in light of the insensitivity of the T_m of **ON1p:ON2a** to the presence of Cu^{2+} or Zn^{2+}.

The ability of Zn^{2+} to replace Cu^{2+} in the duplexes formed by **ON1q** without any decrease in T_m is in striking contrast to the other modified oligonucleotides of the present study and also to previous reports on related systems. For example, while the stability of a double-stranded DNA oligonucleotide incorporating a pyridine-2,6-dicarboxylate:pyridine base pair was dramatically increased on addition of 1 equiv of Cu^{2+}, none of the other metal ion studied (including Zn^{2+}) had any impact.[18] With hydroxypyridone homobase pairs, as well as hydroxypyridone:pyridopurine base pairs, considerable stabilization by Zn^{2+} was observed but even in those cases the effect was much less pronounced than with Cu^{2+}.[48] The fact that marked stabilization by Zn^{2+} is unique to the duplexes of **ON1q** suggests exceptionally high affinity of the modified nucleoside **3** for Zn^{2+} but the present data does not allow further elaboration of this point.

■ CONCLUSIONS

3,5-Dimethylpyrazol-1-yl substituted purine derivatives exhibit different preferences in metal-ion-mediated base pairing with natural nucleobases depending on whether the chelating pyrazolyl substituent is located at position 2 or 6 and, more importantly, whether or not the purine derivative is readily deprotonated to an anionic ligand. Accordingly, the 6-oxo derivative **3**, with a relatively acidic proton at N1, is much more stabilizing than the other modifies nucleosides studied and shows little discrimination between its unmodified counterparts. In line with a number of previous studies, Cu^{2+} is generally the superior bridging metal ion. A notable exception are the metal-ion-mediated base pairs between **3** and natural nucleosides, where Zn^{2+} can replace Cu^{2+} with no decrease in the melting temperature of the respective oligonucleotide duplex.

■ EXPERIMENTAL SECTION

General Methods. 6-Chloro-2-iodo-9-(2,3,5-tri-*O*-acetyl-*β*-D-ribofuranosyl)purine and 6-chloro-9-(-*β*-D-ribofuranosyl)purine were commercial products that were used as received. The NMR spectra were recorded on a 400 or 500 MHz spectrometer and the chemical shifts are given in ppm. The NMR signals were assigned based on COSY, HSQC, and HMBC spectra. The mass spectra were acquired on a TOF-Q ESI-MS system. Oligonucleotides were synthesized by an automated DNA/RNA synthesizer. Solvents were dried over 3 Å molecular sieves and triethylamine over calcium hydride. For the preparation of HPLC elution buffers, freshly distilled triethylamine was used.

9-(*β*-D-Ribofuranosyl)-6-(3,5-dimethylpyrazol-1-yl)purine (2). 6-Chloropurine riboside (500 mg, 1.74 mmol) was dissolved in hydrazine hydrate (4.0 mL). The mixture was stirred at room temperature for 48 h, after which volatiles were evaporated under reduced pressure. Dry pentane-2,4-dione (10 mL, 102 mmol) and trifluoroacetic acid (5.0 μL, 0.029 mmol) were added and the mixture was stirred at room temperature for 24 h. The volatiles were removed under reduced pressure and the residue was purified by silica gel chromatography eluting with a mixture of MeOH and CH_2Cl_2 (15:85, *v*/*v*). Yield: 461 mg (76%). ^{1}H NMR (δ_H) (500 MHz, DMSO-d_6): 8.86 (s, 1H, H2), 8.85 (s, 1H, H8), 6.24 (s, 1H, pyrazole), 6.09 (d, 1H, J = 5.7 Hz, H1′), 5.58 (d, 1H, J = 6.0 Hz, 2′-OH), 5.27 (d, 1H, J = 5.0, 3′−OH), 5.13 (dd, 1H, J_1 = 5.8 Hz, J_2 = 5.1 Hz, 5′−OH), 4.63 (ddd, 1H, J_1 = 6.0 Hz, J_2 = 5.7 Hz, J_3 = 4.8 Hz, H2′), 4.21 (ddd, 1H, J_1 = 5.0 Hz, J_2 = 4.8 Hz, J_3 = 3.7 Hz, H3′), 4.00 (ddd, 1H, J_1 = 4.3 Hz, J_2 = 3.9 Hz, J_3 = 3.7 Hz, H4′), 3.72 (ddd, 1H, J_1 = 12.0, J_2 = 5.1 Hz, J_3 = 4.3 Hz, H5′), 3.60 (ddd, 1H, J_1 = 12.0 Hz, J_2 = 5.8 Hz, J_3 = 3.9 Hz, H5″), 2.57 (s, 3H, pyrazole-CH_3), 2.26 (s, 3H, pyrazole-CH_3). ^{13}C NMR (δ_C)(125 MHz, DMSO-d_6): 154.0 (C4), 151.6 (C2), 151.3 (pyrazole-C3), 149.2 (C6), 145.3 (C8), 142.8 (pyrazole-C5), 125.4 (C5), 109.9 (pyrazole-C4), 88.2 (C1′), 86.1 (C4′), 74.3 (C2′), 70.7 (C3′), 61.6 (C5′), 14.0 (pyrazole-CH_3), 13.9 (pyrazole-CH_3). HRMS (ESI-TOF-Q) m/z: $[M + Na]^+$ Calcd for $C_{15}H_{18}N_6NaO_4$ 369.1282; Found 369.1305.

2-(3,5-Dimethylpyrazol-1-yl)inosine (3). A solution of $NaNO_2$ (1.6 g) in water (30 mL) was added to a suspension of **5** (1.03 g, 2.85 mmol) in acetic acid (150 mL). The reaction mixture was stirred at room temperature for 16 h, after which it was evaporated to dryness. The residue was washed with water (4 × 50 mL) and dried in vacuum. Yield: 958 mg (93%). ^{1}H NMR (δ_H) (400 MHz, DMSO-d_6): 11.83 (s, 1H, NH), 8.33 (s, 1H, H8), 6.26 (s, 1H, pyrazole), 5.82 (d, 1H, J = 5.4 Hz, H1′), 5.51 (d, 1H, J = 6.0 Hz, 2′−OH), 5.23 (d, 1H, J = 5.1 Hz, 3′−OH), 5.05 (dd, 1H, J_1 = 5.4 Hz, J_2 = 5.2 Hz, 5′−OH), 4.46 (ddd, 1H, J_1 = 6.0 Hz, J_2 = 5.4 Hz, J_3 = 4.9 Hz, H2′), 4.10 (ddd, 1H, J_1 = 5.1 Hz, J_2 = 4.9 Hz, J_3 = 4.7 Hz, H3′), 3.93 (ddd, 1H, J_1 = 4.7 Hz, J_2 = 4.0 Hz, J_3 = 3.8 Hz, H4′), 3.65 (ddd, 1H, J_1 = 11.9 Hz, J_2 = 5.4 Hz, J_3 = 4.0 Hz, H5′), 3.55 (ddd, 1H, J_1 = 11.9 Hz, J_2 = 5.2 Hz, J_3 = 3.8 Hz, H5″), 2.61 (s, 3H, pyrazole-CH_3), 2.23 (s, 3H, pyrazole-CH_3). ^{13}C NMR (δ_C) (100 MHz, D_2O): 158.1 (C2), 150.6 (pyrazole-C3), 148.5 (C4), 147.2 (C2), 142.6 (pyrazole-C5), 138.7 (C8), 122.3 (C5), 110.6 (pyrazole-C4), 88.0 (C1′), 85.9 (C4′), 74.6 (C2′), 70.6 (C3′), 61.6 (C5′), 14.4 (pyrazole-CH_3), 13.8 (pyrazole-CH_3). HRMS (ESI-TOF-Q) m/z: $[M + H]^+$ Calcd for $C_{15}H_{19}N_6O_5$ 363.1411; Found 363.1409.

2-(3,5-Dimethylpyrazol-1-yl)adenosine (5). Compound **1**[11] (4.0 g, 9.09 mmol) was dissolved in 30% aq. ammonia (400 mL). The resulting mixture was stirred at 60 °C for 24 h, after which it was cooled to 20 °C and filtered. The filtrate was evaporated and the oily residue was triturated with a mixture of CH_2Cl_2, MeOH, and H_2O (90:9:1, *v*/*v*, 50 mL). The resulting precipitate was collected by filtration, washed with a mixture of CH_2Cl_2, MeOH, and H_2O (90:9:1, *v*/*v*, 3 × 50 mL) and dried in vacuum. Yield: 1.03 g (31%). ^{1}H NMR (δ_H) (500 MHz, DMSO-d_6): 8.38 (s, 1H, H8), 7.62 (s, 2H, NH_2), 6.05 (s, 1H, pyrazole), 5.88 (d, 1H, J = 6.1 Hz, H1′), 5.48 (d, 1H, J = 5.7 Hz, 2′−OH), 5.22 (d, 1H, J = 5.0 Hz, 3′−OH), 5.07 (m, 1H, 5′−OH), 4.60 (ddd, 1H, J_1 = 6.1 Hz, J_2 = 5.7 Hz, J_3 = 4.1 Hz, H2′), 4.14 (m, 1H, H3′), 3.95 (ddd, 1H, J_1 = 4.5 Hz, J_2 = 3.7 Hz, J_3 = 3.5 Hz, H4′), 3.66 (ddd, 1H, J_1 = 12.2 Hz, J_2 = 4.9 Hz, J_3 = 3.5 Hz, H5′), 3.56 (ddd, 1H, J_1 = 12.2 Hz, J_2 = 5.3 Hz, J_3 = 3.7 Hz, H5″), 2.54 (s, 3H, pyrazole-CH_3), 2.18 (s, 3H, pyrazole-CH_3). ^{13}C NMR (δ_C) (125 MHz, DMSO-d_6): 156.7 (C2), 152.6 (C6), 150.6 (C4), 148.3 (pyrazole-C3), 141.4 (pyrazole-C5), 140.4 (C8), 117.7 (C5), 108.6 (pyrazole-C4), 87.7 (C1′), 86.1 (C4′), 74.1 (C2′), 70.9 (C3′), 61.9 (C5′), 14.6 (pyrazole-CH_3), 13.8 (pyrazole-CH_3). HRMS (ESI-TOF-Q) m/z: $[M + H]^+$ Calcd for $C_{15}H_{20}N_7O_4$ 362.1571; Found 362.1570.

9-[5-*O*-(4,4′-Dimethoxytrityl)-*β*-D-ribofuranosyl]-6-(3,5-dimethylpyrazol-1-yl)purine (6). To a solution of crude compound **2** (821 mg, 2.37 mmol) in dry pyridine (15 mL), DMTrCl (884 mg, 2.61 mmol) was added. The reaction mixture was stirred for 16 h at room temperature, after which it was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (60 mL) and washed with saturated aq. $NaHCO_3$ (100 mL). The organic phase was dried with Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel chromatography eluting with a mixture of MeOH, CH_2Cl_2, and Et_3N (5:94:1, *v*/*v*). Yield: 930 mg (60%). ^{1}H NMR (δ_H)(400 MHz, DMSO-d_6): 8.77 (s, 1H, H2), 8.70 (s, 1H, H8), 7.35 (d, 2H, J = 7.3 Hz, Ar), 7.28−7.14 (m, 7H, Ar), 6.82 (d, 2H, J = 8.6 Hz, Ar), 6.80 (d, 2H, J = 8.6 Hz, Ar), 6.23 (s, 1H, pyrazole), 6.11 (d, 1H, J = 4.9 Hz, H1′), 5.64 (d, 1H, J = 5.6 Hz, 2′-OH), 5.28 (d, 1H, J = 5.8 Hz, 3′−OH), 4.78 (ddd, 1H, J_1 = 5.6 Hz, J_2 = 5.2 Hz, J_3 = 4.9 Hz, H2′), 4.34 (ddd, 1H, J_1 = 5.8 Hz, J_2 = 5.2 Hz, J_3 = 4.0 Hz, H3′), 4.13 (ddd, 1H, J_1 = 5.0 Hz, J_2 = 4.5 Hz, J_3 = 4.0 Hz, H4′), 3.71 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 3.24 (m, 2H, H5′ and H5″), 2.55 (s, 3H, pyrazole-CH_3), 2.25 (s, 3H, pyrazole-CH_3). ^{13}C NMR (δ_C) (100 MHz, DMSO-d_6): 158.5 (OAr), 158.4 (OAr), 153.9 (C4), 151.6 (C2), 151.3 (pyrazole-C3), 149.2 (C6), 145.5 (C8), 145.3 (Ar), 142.8 (pyrazole-C5), 136.0 (Ar), 135.9 (Ar), 130.2 (Ar), 130.1 (Ar), 128.2 (Ar), 128.1 (Ar), 127.1 (Ar), 125.5 (C5), 113.6 (Ar), 113.6 (Ar), 109.9 (pyrazole-C4), 88.9 (C1′), 85.9 (Ar_3C), 83.8 (C4′), 73.4 (C2′), 70.8 (C3′), 64.2(C5′), 55.5 (OCH_3), 55.3 (OCH_3), 13.9 (pyrazole-CH_3), 13.8 (pyrazole-CH_3). HRMS (ESI-TOF-Q) m/z: $[M + Na]^+$ Calcd for $C_{36}H_{36}N_6NaO_6$ 671.2589; Found 671.2611.

9-[2-*O*-*tert*-Butyldimethylsilyl-5-*O*-(4,4′-dimethoxytrityl)-*β*-D-ribofuranosyl]-6-(3,5-dimethylpyrazol-1-yl)purine (7). To a solution of compound **6** (800 mg, 1.23 mmol) in dry DMF (10 mL), a solution of imidazole (830 mg, 12.2 mmol) in dry DMF (15 mL) was added, followed by TBDMSCl (204 mg, 1.35 mmol). After being stirred for 4 days, the reaction was quenched by adding MeOH (5.0 mL). Stirring was continued for 10 min, after which EtOAc (60 mL) and water (100 mL) were added and the phases separated. The organic phase was dried with Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel chromatography eluting with a mixture of EtOAc, CH_2Cl_2, and Et_3N (19:80:1, *v*/*v*). Yield: 240 mg (26%). ^{1}H NMR (δ_H)(500 MHz, DMSO-d_6): 8.78 (s, 1H, H2), 8.73 (s, 1H, H8), 7.41 (m, 2H, Ar), 7.31−7.19 (m, 7H, Ar), 6.84 (m, 4H, Ar), 6.25 (s, 1H, pyrazole), 6.14 (d, 1H, *J* = 4.9 Hz, H1′), 5.24 (d, 1H, *J* = 6.0 Hz, 3′-OH), 4.90 (dd, 1H, J_1 = J_2 = 4.9 Hz, H2′), 4.30 (ddd, 1H, J_1 = 6.0 Hz, J_2 = 4.9 Hz, J_3 = 4.7 Hz, H3′), 4.17 (ddd, 1H, J_1 = 4.7 Hz, J_2 = 4.2 Hz, J_3 = 3.8 Hz, H4′), 3.73 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 3.31 (m, 2H, H5′ and H5″), 2.56 (*s*, 3H, pyrazole-CH_3), 2.26 (*s*, 3H, pyrazole-CH_3), 0.77 (s, 9H, $SiCCH_3$), −0.00 (s, 3H, $SiCH_3$), −0.10 (s, 3H, $SiCH_3$). ^{13}C NMR (δ_C)(125 MHz, DMSO-d_6): 158.5 (OAr), 158.5 (OAr), 153.8 (C4), 151.6 (C2), 151.4 (pyrazole-C3), 149.3 (C6), 145.3 (C8), 145.3 (Ar), 142.8 (pyrazole-C5), 136.0 (Ar), 135.9 (Ar), 130.2 (Ar), 130.2 (Ar), 128.3 (Ar), 128.1 (CAr), 127.2 (Ar), 125.4 (C5), 113.6 (Ar), 110.0 (pyrazole-C4), 88.9 (C1′), 86.0 (Ar_3C), 84.1 (C4′), 75.4 (C2′), 70.6 (C3′), 63.9 (C5′), 55.5 (OCH_3), 55.5 (OCH_3), 26.0 ($SiC\underline{C}H_3$), 18.3 ($Si\underline{C}CH_3$), 13.9 (pyrazole-CH_3), 13.9 (pyrazole-CH_3), −4.3 ($SiCH_3$), −4.8 ($SiCH_3$). HRMS (ESI-TOF-Q) *m*/*z*: $[M + H]^+$ Calcd for $C_{42}H_{51}N_6O_6Si$ 763.3634; Found 763.3648.

9-{2-*O*-*tert*-Butyldimethylsilyl-3-O-[(2-cyanoethoxy)(*N*,*N*-diisoproylamino)phosphinyl]-5-*O*-(4,4′-dimethoxytrityl)-*β*-D-ribofuranosyl}-6-(3,5-dimethylpyrazol-1-yl)purine (8). To a solution of compound **7** (232 mg, 0.30 mmol) in dry CH_2Cl_2 (912 *μ*L), dry Et_3N (210 *μ*L, 1.5 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (75 *μ*L, 0.31 mmol) were added. The resulting mixture was stirred under nitrogen atmosphere for 1 h, after which the reaction was quenched by addition of MeOH (200 *μ*L). Stirring was continued for 10 min, after which CH_2Cl_2 (60 mL) was added and the resulting solution washed with saturated aq. $NaHCO_3$ (100 mL). The organic phase was dried with Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel chromatography eluting with a mixture of EtOAc, CH_2Cl_2, and Et_3N (19:80:1, *v*/*v*). Yield: 86.3 mg (29%). ^{31}P NMR (δ_P) (162 MHz, $CDCl_3$, faster eluting diastereomer): 151.3; (δ_P)(162 MHz, $CDCl_3$, slower eluting diastereomer): 149.3. ^{1}H NMR (δ_H)(500 MHz, $CDCl_3$, faster eluting diastereomer): 8.69 (s, 1H, H2), 8.40 (s, 1H, H8), 7.51 (d, 2H, *J* = 7.4 Hz, Ar), 7.40 (d, 4H, *J* = 8.8 Hz, Ar), 7.30 (m, 2H, Ar), 7.24 (m, 1H, Ar), 6.84 (d, 4H, *J* = 7.6 Hz, Ar), 6.11 (s, 1H, pyrazole), 6.11 (d, 1H, *J* = 6.1 Hz, H1′), 5.12 (dd, 1H, J_1 = 5.8 Hz, J_2 = 5.3 Hz, H2′), 4.47 (dd, 1H, J_1 = 3.1 Hz, J_2 = 6.3 Hz, H4′), 4.38 (ddd, J_1 = 9.6 Hz, J_2 = 4.7 Hz, J_3 = 3.2 Hz, H3′), 3.80 (br, 6H, OCH_3), 3.70−3.54 (m, 5H, H5′ and $NC\underline{H}CH_3$ and $POCH_2$), 3.30 (dd, 1H, J_1 = 10.6 Hz, J_2 = 4.1 Hz, H5″), 2.72 (s, 3H, pyrazole-CH_3), 2.43 (s, 3H, pyrazole-CH_3), 2.31 (m, 2H, CH_2CN), 1.22 (d, 6H, *J* = 6.8 Hz, $NCHC\underline{H_3}$), 1.19 (d, 6H, *J* = 6.8 Hz, $NCHC\underline{H_3}$), 0.79 (s, 9H, $SiCCH_3$), 0.02 (s, 3H, $SiCH_3$), −0.15 (s, 3H, $SiCH_3$); (δ_H)(500 MHz, $CDCl_3$, slower eluting diastereomer): 8.69 (s, 1H, H2), 8.38 (s, 1H, H8), 7.50 (d, 2H, *J* = 7.3 Hz, Ar), 7.38 (m, 4H, Ar), 7.30 (m, 2H, Ar), 7.23 (m, 1H, Ar), 6.83 (d, 4H, *J* = 8.6 Hz, Ar), 6.18 (d, 1H, *J* = 6.3 Hz, H1′), 6.12 (s, 1H, pyrazole), 5.10 (dd, 1H, J_1 = 6.1 Hz, J_2 = 4.4 Hz, H2′), 4.46−4.32 (m, 2H, H3′ and H4′), 3.99 (m, 1H, POCH), 3.90 (m, 1H, POCH), 3.80 (br, 6H, OCH_3), 3.62 (m, 2H, $NC\underline{H}CH_3$), 3.56 (dd, 1H, J_1 = 10.3 Hz, J_2 = 3.3 Hz, H5′), 3.35 (dd, 1H, J_1 = 10.6 Hz, J_2 = 3.8 Hz, H5″), 2.71 (s, 3H, pyrazole-CH_3), 2.68 (m, 2H, CH_2CN), 2.42 (s, 3H, pyrazole-CH_3), 1.20 (d, 6H, *J* = 6.7 Hz, $NCHC\underline{H_3}$), 1.06 (d, 6H, *J* = 6.7 Hz, $NCHC\underline{H_3}$), 0.80 (s, 9H, $SiCCH_3$), −0.00 (s, 3H, $SiCH_3$), −0.16 (s, 3H, $SiCH_3$). ^{13}C NMR (δ_H) (125 MHz, $CDCl_3$, faster eluting diastereomer): 158.6 (OAr), 158.6 (OAr), 153.7 (C4), 152.9 (pyrazole-C3), 151.4 (C2), 149.6 (C6), 144.6 (Ar), 143.7 (C8), 143.6 (pyrazole-C5), 135.8 (Ar), 135.7 (Ar), 130.2 (Ar), 130.1 (Ar), 128.2 (Ar), 127.9 (Ar), 126.9 (Ar), 124.8 (C5), 117.2 (CN), 113.2 (Ar), 113.2 (Ar), 110.3 (pyrazole-C4), 88.5 (C1′), 86.6 (Ar_3C), 84.2 (C4′), 74.4 (d, *J* = 5.5 Hz, C2′), 73.3 (d, *J* = 10.0 Hz, C3′), 63.3 (C5′), 57.7 (d, *J* = 20.8 Hz, $POCH_2$), 55.2 (OCH_3), 43.4 ($N\underline{C}HCH_3$), 43.4 ($N\underline{C}HCH_3$), 25.6 ($SiC\underline{C}H_3$), 24.8 ($NCH\underline{C}H_3$), 24.7 ($NCH\underline{C}H_3$), 24.7 ($NCH\underline{C}H_3$), 24.7 ($NCH\underline{C}H_3$), 20.1 (d, *J* = 7.1 Hz, $\underline{C}H_2CN$), 17.9 ($Si\underline{C}CH_3$), 14.7 (pyrazole-CH_3), 14.2 (pyrazole-CH_3), −4.6 ($SiCH_3$), −5.0 ($SiCH_3$); (δ_H) (125 MHz, $CDCl_3$, faster eluting diastereomer) 158.6 (OAr), 153.7 (C4), 153.0 (pyrazole-C3), 151.4 (C2), 149.6 (C6), 144.5 (Ar), 143.6 (C8), 143.6 (pyrazole-C5), 135.6 (Ar), 135.6 (Ar), 130.1 (Ar), 130.1 (Ar), 128.1 (Ar), 127.9 (Ar), 127.0 (Ar), 124.7 (C5), 117.6 (CN), 113.2 (Ar), 113.2 (Ar), 110.3 (pyrazole-C4), 88.2 (C1′), 86.7 (Ar_3C), 83.8 (d, *J* = 3.7 Hz, C4′), 75.1 (d, *J* = 2.5 Hz, C2′), 72.7 (d, *J* = 14.7 Hz, C3′), 63.4 (C5′), 58.8 (d, *J* = 16.6 Hz, $POCH_2$), 55.2 (OCH_3), 43.0 ($N\underline{C}HCH_3$), 42.9 ($N\underline{C}HCH_3$), 25.7 ($SiC\underline{C}H_3$), 24.7 ($NCH\underline{C}H_3$), 24.6 ($NCH\underline{C}H_3$), 24.6 ($NCH\underline{C}H_3$), 24.6 ($NCH\underline{C}H_3$), 20.4 (d, *J* = 6.3 Hz, $\underline{C}H_2CN$), 17.9 ($Si\underline{C}CH_3$), 14.7 (pyrazole-CH_3), 14.2 (pyrazole-CH_3), −4.6 ($SiCH_3$), −5.0 ($SiCH_3$). HRMS (ESI-TOF-Q) *m*/*z*: $[M + H]^+$ Calcd for $C_{51}H_{68}N_8O_7PSi$ 963.4712; Found 963.4753.

5′-*O*-(4,4′-Dimethoxytrityl)-2-(3,5-dimethylpyrazol-1-yl)-inosine (9). To a solution of crude compound **3** (907 mg, 2.51 mmol) in dry pyridine (30 mL), DMTrCl (930 mg, 2.75 mmol) was added. The reaction mixture was stirred at room temperature for 16 h, after which it was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (60 mL) and washed with saturated aq. $NaHCO_3$ (100 mL). The organic phase was dried with Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel chromatography eluting with a mixture of CH_2Cl_2, MeOH, and Et_3N (stepwise gradient from 98:1:1 to 80:19:1, *v*/*v*). Yield: 1.55 g (93%). ^{1}H NMR (δ_H) (500 MHz, $CDCl_3$): 7.80 (s, 1H, H8), 7.41 (d, 2H, *J* = 7.3 Hz, Ar), 7.30 (d, 2H, *J* = 8.6 Hz, Ar), 7.30 (d, 2H, *J* = 8.6 Hz, Ar), 7.23 (dd, 2H, J_1 = 8.0 Hz, J_2 = 7.2 Hz, Ar), 7.16 (t, 1H, *J* = 7.3 Hz, Ar), 6.79 (d, 4H, *J* = 8.8 Hz, Ar), 6.01 (d, 1H, *J* = 5.1 Hz, H1′), 5.96 (s, 1H, pyrazole), 4.71 (dd, 1H, J_1 = 5.1 Hz, J_2 = 4.5 Hz, H2′), 4.38 (dd, 1H, J_1 = 4.9 Hz, *J* = 4.5 Hz, H3′), 4.32 (m, 1H, H4′), 3.75 (s, 6H, OCH_3), 3.42 (dd, 1H, J_1 = 10.4 Hz, J_2 = 3.1 Hz, H5′), 3.36 (dd, J_1 = 10.4 Hz, J_2 = 4.5 Hz, H5″), 2.61 (s, 3H, pyrazole-CH_3), 2.21 (s, 3H, pyrazole-CH_3). ^{13}C NMR (δ_H)(100 MHz, $CDCl_3$): 158.5 (OAr), 158.5 (C6), 151.2 (pyrazole-C3), 148.8 (C4), 147.0 (C2), 144.6 (Ar), 143.1 (pyrazole-C5), 137.5 (C8), 135.7 (Ar), 135.7 (Ar), 130.1 (Ar), 128.2 (Ar), 127.8 (Ar), 126.8 (Ar), 121.4 (C5), 113.1 (Ar), 110.9 (pyrazole-C4), 88.7 (C1′), 86.4 (Ar_3C), 84.3 (C4′), 75.0 (C2′), 71.33 (C3′), 63.8 (C5′), 55.2 (OCH_3), 15.0 (pyrazole-CH_3), 13.5 (pyrazole-CH_3). HRMS (ESI-TOF-Q) *m*/*z*: $[M + H]^+$ Calcd for $C_{36}H_{37}N_6O_7$ 665.2718; Found 665.2714.

2′-*O*-*tert*-Butyldimethylsilyl-5′-*O*-(4,4′-dimethoxytrityl)-2-(3,5-dimethylpyrazol-1-yl)inosine (10). To a solution of compound **9** (1.55 g, 2.33 mmol) in dry DMF (10 mL), a solution of imidazole (1.52 g, 22.3 mmol) in DMF (25 mL) was added, followed by TBDMSCl (563 mg, 3.74 mmol). After being stirred at room temperature for 4 days, the reaction was quenched by adding MeOH (5.0 mL). Stirring was continued for 10 min, after which CH_2Cl_2 (50 mL) and water (100 mL) were added and the organic and aqueous phases separated. The organic phase was dried with Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel chromatography eluting with a mixture of CH_2Cl_2, MeOH and Et_3N (97:2:1, *v*/*v*). Yield: 154 mg (9%). ^{1}H NMR (δ_H) (400 MHz, $CDCl_3$): 7.95 (s, 1H, H8), 7.42 (m, 2H, Ar), 7.34−7.25 (m, 6H, Ar), 7.23 (m, 1H, Ar), 6.83 (d, 4H, *J* = 8.6 Hz, Ar), 6.06 (s, 1H, pyrazole), 5.96 (d, 1H, *J* = 6.3 Hz, H1′), 4.75 (dd, 1H, J_1 = 6.3 Hz, J_2 = 5.2 Hz, H2′), 4.29 (m, 2H, H3′ and H4′), 3.79 (s, 6H, OCH_3), 3.48 (dd, 1H, J_1 = 10.7 Hz, J_2 = 2.8 Hz, H5″), 3.39 (dd, 1H, J_1 = 10.7 Hz, J_2 = 3.3 Hz, H5″), 2.66 (s, 3H, pyrazole-CH_3), 2.27 (s, 3H, pyrazole-CH_3), 0.82 (s, 9H, $SiCCH_3$), −0.01 (s, 3H, $SiCH_3$), −0.21 (s, 3H, $SiCH_3$). ^{13}C NMR (δ_H) (100 MHz, $CDCl_3$): 158.7 (Ar), 156.4 (C6), 151.8 (pyrazole-C3), 148.8 (C4), 146.1 (C2), 144.3 (Ar), 143.0 (pyrazole-C5), 137.2 (C8), 135.4 (Ar), 135.3 (Ar), 130.1 (Ar), 130.0 (Ar), 128.0 (Ar), 128.0 (Ar), 127.1 (Ar), 121.8 (C5), 113.3 (Ar), 111.5 (pyrazole-C4), 86.9 (C1′ and Ar_3C), 84.4 (C4′), 77.2 (C2′), 72.0 (C3′), 63.7 (C5′), 55.2 (OCH_3), 25.4 ($SiC\underline{C}H_3$), 17.8 ($Si\underline{C}CH_3$), 15.0 (pyrazole-CH_3),

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13.6 (pyrazole-CH_3), −5.1 ($SiCH_3$), −5.2 ($SiCH_3$). HRMS (ESI-TOF-Q) *m/z*: $[M + H]^+$ Calcd for $C_{42}H_{51}N_6O_7Si$ 779.3583; Found 779.3571.

3′-*O*-[(2-cyanoethoxy)(*N,N*-diisoproylamino)phosphinyl]-2′-*O-tert*-butyldimethylsilyl-5′-*O*-(4,4′-dimethoxytrityl)-2-(3,5-dimethylpyrazol-1-yl)inosine (11). To solution of compound **10** (154 mg, 0.198 mmol) in dry CH_2Cl_2 (2.0 mL), dry Et_3N (750 μL, 5.38 mmol) and 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (198 μL, 0.891 mmol) were added. The resulting mixture was stirred at room temperature under nitrogen atmosphere for 48 h, after which the reaction was quenched with MeOH (1.0 mL). Stirring was continued for 10 min, after which CH_2Cl_2 (60 mL) was added and the resulting solution washed with saturated aq. $NaHCO_3$ (100 mL). The organic phase was dried with Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel chromatography eluting first with a mixture of EtOAc, CH_2Cl_2, and TEA (29:70:1, *v/v*) and finally with a mixture of CH_2Cl_2, MeOH, and TEA (94:5:1, *v/v*). Yield: 72.5 mg (37%) as a mixture of two diastereomers. ^{31}P NMR (δ_P) (162 MHz, $CDCl_3$, major diastereomer): 148.7; (δ_P) (162 MHz, $CDCl_3$, minor diastereomer) 152.3. ^1H NMR (δ_H) (400 MHz, $CDCl_3$, major diastereomer) 8.00 (s, 1H, H8), 7.47−7.41 (m, 2H, Ar), 7.37−7.27 (m, 6H, Ar), 7.26−7.20 (m, 2H, Ar), 6.88−6.80 (m, 4H, Ar), 6.06 (s, 1H, pyrazole), 6.03 (d, 1H, *J* = 7.3 Hz, H1′), 4.73 (dd, 1H, J_1 = 7.3 Hz, J_2 = 4.8 Hz, H2′), 4.36 (br dd, 1H, J_1 = 13.3, J_2 = 4.8 Hz, H3′), 4.32 (m, 1H, H4′), 3.91−3.84 (m, 2H, $POCH_2$), 3.79 (s, 6H, OCH_3), 3.62 (m, 2H, $NC\underline{H}CH_3$), 3.44 (dd, 21, J_1 = 10.7 Hz, J_2 = 3.0 Hz, H5′), 3.34 (dd, 1H, J_1 = 10.7 Hz, J_2 = 2.9 Hz, H5″), 2.68 (s, 3H, pyrazole-CH_3), 2.63−2.55 (m, 2H, CH_2CN), 2.27 (s, 3H, pyrazole-CH_3), 1.19 (d, 6H, *J* = 6.8 Hz, $NCHC\underline{H}_3$), 1.07 (d, 6H, *J* = 6.8 Hz, $NCHC\underline{H}_3$), 0.75 (s, 9H, $SiCCH_3$), −0.02 (s, 3H, $SiCH_3$), −0.21 (s, 3H, $SiCH_3$); ^1H NMR (400 MHz, $CDCl_3$, minor diastereomer): 8.05 (s, 1H, H8), 7.47−7.41 (m, 2H, Ar), 7.37−7.27 (m, 6H, Ar), 7.26−7.20 (m, 2H, Ar), 6.88−6.80 (m, 4H, Ar), 6.04 (s, 1H, pyrazole), 6.02 (d, 1H, *J* = 7.7 Hz, H1′), 4.62 (dd, 1H, J_1 = 7.7 Hz, J_2 = 5.0 Hz, H2′), 4.40 (m, 1H, H4′), 4.25 (dd, 1H, J_1 = 9.0 Hz, J_2 = 5.0 Hz, H3′), 3.97−3.88 (m, 2H, $POCH_2$), 3.80 (s, 6H, OCH_3), 3.68−3.52 (m, 3H, $NC\underline{H}CH_3$ and H5′), 3.28−3.24 (m, 1H, H5″), 2.64 (s, 3H, pyrazole-CH_3), 2.63−2.55 (m, 2H, CH_2CN), 2.27 (s, 3H, pyrazole-CH_3), 1.22 (d, 2H, *J* = 7.2 Hz, 6H, $NCHC\underline{H}_3$), 1.18 (d, 2H, *J* = 7.3 Hz, 6H, $NCHC\underline{H}_3$), 0.71 (s, 9H, $SiCCH_3$), −0.05 (s, 3H, $SiCH_3$), −0.26 (s, 3H, $SiCH_3$). ^{13}C NMR (δ_H) (100 MHz, $CDCl_3$, major diastereomer): 158.7 (Ar), 155.8 (C6), 152.1 (pyrazole-C3), 148.9 (C4), 145.6 (C2), 144.2 (Ar), 143.0 (pyrazole-C5), 137.1 (C8), 135.2 (Ar), 135.1 (Ar), 130.1 (Ar), 130.0 (Ar), 128.2 (Ar), 127.9 (Ar), 127.1 (Ar), 121.4 (C5), 117.4 (CN), 113.4 (Ar), 111.7 (pyrazole-C4), 87.1 (Ar_3C), 86.4 (C1′), 84.6 (d, *J* = 3.2 Hz, C4′), 77.6 (d, *J* = 2.7 Hz, C2′), 73.0 (d, *J* = 12.8 Hz, C3′), 63.6 (C5′), 58.8 (d, *J* = 16.4 Hz, $POCH_2$), 55.3 (OCH_3), 43.0 ($N\underline{C}HCH_3$), 42.9 ($N\underline{C}HCH_3$), 25.5 ($SiC\underline{C}H_3$), 24.7 ($NCH\underline{C}H_3$), 24.6 ($NCH\underline{C}H_3$), 20.5 (d, *J* = 6.1 Hz, $\underline{C}H_2CN$), 17.9 ($Si\underline{C}CH_3$), 15.0 (pyrazole-CH_3), 13.6 (pyrazole-CH_3), −4.7 ($SiCH_3$), −5.3 ($SiCH_3$). ^{13}C NMR (δ_H)(100 MHz, $CDCl_3$, minor diastereomer): 158.8 (Ar), 155.9 (C6), 152.0 (pyrazole-C3), 149.0 (C4), 145.5 (C2), 144.4 (Ar), 143.2 (pyrazole-C5), 137.0 (C8), 135.5 (Ar), 135.4 (Ar), 130.0 (Ar), 129.9 (Ar), 128.0 (Ar), 127.9 (Ar), 127.3 (Ar), 121.7 (C5), 117.2 (CN), 113.4 (Ar), 111.6 (pyrazole-C4), 86.9 (Ar_3C), 86.5 (C1′), 85.1 (C4′), 77.3 (C2′), 74.4 (d, *J* = 10.1 Hz, C3′), 63.4 (C5′), 57.4 (d, *J* = 18.4 Hz, $POCH_2$), 55.3 (OCH_3), 43.6 ($N\underline{C}HCH_3$), 43.4 ($N\underline{C}HCH_3$), 25.4 ($SiC\underline{C}H_3$), 24.7 ($NCH\underline{C}H_3$), 24.6 ($NCH\underline{C}H_3$), 20.1 (d, *J* = 7.4 Hz, $\underline{C}H_2CN$), 17.9 ($Si\underline{C}CH_3$), 14.9 (pyrazole-CH_3), 13.6 (pyrazole-CH_3), −4.8 ($SiCH_3$), −5.4 ($SiCH_3$). HRMS (ESI-TOF-Q) *m/z*: $[M + H]^+$ Calcd for $C_{51}H_{68}N_8O_8$ 979.4661; Found 969.4658.

Oligonucleotide Synthesis. The 9-mer 2′-*O*-methyl oligoribonucleotides **ON1p** and **ON1q** were assembled on a CPG-supported succinyl linker at a loading of 26 μmol g^{-1}. For the synthesis of the 5-mer oligonucleotide **ON3x**, the solid support incorporating the modified nucleoside **1** was prepared as previously described, resulting in a loading of 54 μmol g^{-1}.[8] For the commercial 2′-*O*-methylated building blocks, standard phosphoramidite strategy with 600 s coupling time was used throughout the sequences. The modified building blocks **8** and **11**, in turn, were coupled manually using a 60 min coupling time. Based on the trityl response, coupling yields were 40 and 44% for **8** and **11**, respectively. The automated couplings, including the ones immediately following the incorporation of the modified building blocks, proceeded with normal efficiency. The products were released from support and the base and phosphate protections removed by treatment with 33% aq. NH_3 (4 h at 55 °C).

Oligonucleotide **ON1z** was prepared in approximately 40% yield from the previously synthesized oligonucleotide **ON1x** by treatment with 33% aq. NH_3 (6 h at 55 °C). This treatment converted the 3,5-dimethylpyrazol-1-yl group at position 6 of the modified nucleoside **1** to an amino group without appreciable loss of the 2′-*O*-TBDMS protection.

In all cases, the TBDMS protection was removed by treatment with 1.5 M triethylamine trihydrofluoride in DMSO (2 h at 55 °C). Finally, the crude oligonucleotides were purified by RP HPLC on a Hypersil ODS C18 column (250 × 4.6 mm, 5 μm), eluting with mixture of acetonitrile (linear gradient from 5 to 30% during 30 min for **ON1q** and 10 to 40% during 25 min for the other products) and 0.1 mol L^{-1} aq. triethylammonium acetate, the flow rate being 1.0 mL min^{-1}. The purified oligonucleotides were characterized by ESI-MS analysis and their concentrations determined UV-spectrophotometrically using molar absorptivities calculated by an implementation of the nearest-neighbors method.[9,10]

Kinetic Measurements. Hydrolytic reactions of oligonucleotide **ON3x** were carried out in sealed glass tubes in a thermostated water bath at 37.0 °C. The initial concentration of the oligonucleotide and the metal ions (Cu^{2+} or Zn^{2+}) was 2.0 μmol L^{-1}. The pH of the reaction solutions was adjusted to 7.4 with 20 mmol L^{-1} cacodylic acid buffer and the ionic strength to 0.1 mol L^{-1} with $NaClO_4$. The composition of the samples withdrawn at appropriate time intervals were analyzed by RP HPLC on a Hypersil ODS C18 column (250 × 4.6 mm, 5 μm), eluting with mixture of acetonitrile (linear gradient from 10 to 40% during 25 min) and 0.1 mol L^{-1} aq. triethylammonium acetate, the flow rate being 1.0 mL min^{-1}. Detection of the eluting components was by UV absorption at 260 nm. The observed retention times were 17.1 and 10.8 min for **ON3x** and its hydrolysis product **ON3q**, respectively. The identity of the product was characterized by ESI-MS. The signal area for the starting material was normalized by dividing with the signal area for potassium 4-nitrobenzenesulfonate, used as an internal standard. The pseudo-first-order rate constants for the hydrolysis of **ON3x** were then calculated by applying the integrated first-order rate law to the time-dependent decrease of the relative signal area of **ON3x** thus obtained.

■ ASSOCIATED CONTENT

Supporting Information

^1H, ^{13}C, and (where applicable) ^{31}P NMR spectra of compounds **2**, **3**. and **5−11** and HPLC traces and mass spectra of oligonucleotides **ON1p**, **ON1q**, **ON1z**, and **ON3x**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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