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2,6-Bis(functionalized) purines as metal-ion-binding surrogate nucleobases that enhance hybridization with unmodified 2′-*O*-methyl oligoribonucleotides†

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The capacity of three different purine bases, *viz.* 2,6-bis(3,5-dimethylpyrazol-1-yl)purine, 2-(3,5-dimethylpyrazol-1-yl)adenine and 2,6-bis(2-acetyl-1-methylhydrazino)purine, to form metal-ion mediated base pairs with the native nucleobases has been examined. For this purpose, ribonucleosides derived from these bases were incorporated into an intrastrand or a 3′-terminal position of short 2′-*O*-methyl oligoribonucleotides and the hybridization properties of these base modified oligomers in the absence and presence of three different metal ions (Cu^{2+}, Zn^{2+} and Pd^{2+}) were studied by UV- and CD-spectrometry. The first two bases were found to stabilize short oligonucleotide duplexes when incorporated into the 3′-termini of both strands, even in the absence of divalent metal ions but especially in the presence of Cu^{2+}. The highest melting temperature determined for such a duplex was 71.8 °C, nearly 30 °C higher than the T_m of the respective solely Watson–Crick paired duplex. Despite the dramatic stabilizing effect of the terminal metallo-base pairs, these short modified oligonucleotides retained sequence-selectivity for the internal Watson–Crick base pairs: two internal mismatches dropped the melting temperature to 10–11 °C. In an internal position, only 2,6-bis(3,5-dimethylpyrazol-1-yl)purine, which in the absence of metal ions was destabilizing, exhibited metal-ion-dependent stabilization of duplex formation with unmodified 2′-*O*-methyl oligoribonucleotides. The melting temperature in the presence of Cu^{2+} was increased from 6 to 14 °C, depending on the identity of the opposite base.

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Introduction

Metal-ion-mediated base-pairing of nucleic acids has attracted considerable interest during the past decade, largely owing to the desire to expand the genetic code by artificial base-pairs, to create a predesigned molecular architecture by metal-ion-mediated inter-strand cross-links, and to impregnate DNA with metal ions for nanotechnological applications.[1–3] By contrast, recognition of natural nucleic acid sequences by base-modified oligonucleotides that tightly bind metal ions has not been much studied, with the exception of the formation of Hg^{2+}-mediated TT-base pairs.[4] High affinity binding to short sequences might, however, find useful biological applications, for instance in recognition of short non-coding RNAs.[5] The desired high affinity could, in principle, be achieved by coordination of a soft metal ion, such as Pd^{2+}, carried by an artificial nucleobase, to a ring nitrogen (N1 of purines or N3 of pyrimidines) of the natural nucleobase.[6] We have recently described such an effort, using the Pd^{2+} chelate of 2,6-bis(3,5-dimethylpyrazol-1-yl)purine as a non-natural complement for uracil (or thymine) (Fig. 1).[7] While formation of a stable base-pair was indeed observed on the monomeric level by ^{1}H NMR, only a modest increase in the T_m of oligonucleotide duplexes placing the artificial nucleoside against a thymidine could be seen.

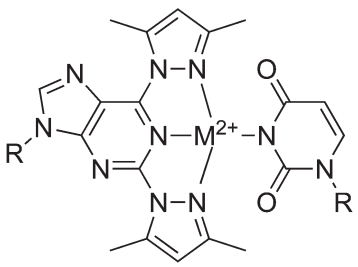

Fig. 1 Putative ternary complex between 2,6-bis(3,5-dimethylpyrazol-1-yl)-purine (**1**), a divalent metal ion and uridine.

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†Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR and mass spectra of compounds **3**, **6**, **7** and **14**, ^{1}H and ^{13}C NMR spectra of the product mixture of the ammonolysis of compound **1**, ^{31}P NMR spectrum of compound **3** and mass spectra of the modified oligonucleotides. See DOI: 10.1039/c2ob26885j

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One reason for this discrepancy probably lies in the steric bulk of the artificial nucleobase but strong off-target coordination of the kinetically inert Pd^{2+} ion may also play a role (it should be noted that only an equimolar amount of Pd^{2+} was used). To address these points, in the present study, the affinity at the oligonucleotide level has been investigated further using kinetically more labile, intracellularly occurring metal ions Cu^{2+} and Zn^{2+} and placing the artificial metal-ion-binding nucleoside in positions having different steric requirements. To compensate for the weaker coordination of these metal ions, a new nucleoside, 2,6-bis(2-acetyl-1-methylhydrazino)purine, with the potential for concomitant deprotonation upon chelation of the metal ion, has also been prepared and introduced into an oligonucleotide.

Results

Synthesis of the artificial nucleosides and their phosphoramidite building blocks

Preparation of 2,6-bis(3,5-dimethylpyrazol-1-yl)purine riboside (**1**) and its conversion into a protected phosphoramidite building block (**2**) have been described earlier.[7] A synthesis strategy for the corresponding phosphoramidite building block of 2,6-bis(1-methylhydrazino)purine riboside (**3**) is presented in Scheme 1. First, the 2,6-bis(1-methylhydrazino)purine riboside (**4**) was prepared by treating commercial 2′,3′,5′-tri-*O*-acetyl-6-chloro-2-iodopurine riboside with *N*-methylhydrazine at room temperature, as previously described for hydrazine.[7,8] The *N*-methylhydrazino groups at C2 and C6 were then protected as trifluoroacetohydrazides by treatment with ethyl trifluoroacetate and the 5′-OH as a 4,4′-dimethoxytrityl ether. Finally, the 2′-OH was silylated with TBDMSCl and the 3′-OH phosphitylated by conventional methods to yield the phosphoramidite building block **3**.

Oligonucleotide synthesis

The 9-mer 2′-*O*-methyl-RNA oligonucleotides (**8A**, **9U**, **9A**, **9G**, **9C**, **10U**, **10A**, **10G** and **10C** in Table 1) and the 6-mer 2′-*O*-methyl-RNA oligonucleotides (**11UA**, **11AA**, **11GA**, **11CA**, **12UA**, **12AA**, **12GA** and **12CA** in Table 2) were synthesized from commercial phosphoramidite building blocks by a conventional phosphoramidite strategy on an automated synthesizer. 2′-*O*-Methyl-RNA was chosen as a mimic of the biologically more interesting RNA targets because of its higher hydrolytic stability (the potential of the metal-ion-carrying oligonucleotides to act as artificial ribonucleases remains a topic of further studies). The lengths of the oligonucleotides used, on the other hand, were selected based on their estimated thermal stabilities so that in all cases the melting temperature would fall between 20 and 80 °C. Two terminal metallo-base pairs are expected to stabilize the duplex more than a single central one, which is why shorter oligonucleotides were used in the former case. Finally, the short sequences allow for sensitive probing of the effects of mismatches in the Watson–Crick part of the oligonucleotide even in the presence of the strongly stabilizing metal-ion-mediated base pairs.

The modified oligonucleotide **8Y** was synthesized by inserting the phosphoramidite monomer **3** in the middle of a 9-mer 2′-*O*-methyl-RNA sequence applying manual coupling and an exceptionally long coupling time (60 min). The coupling yield for building block **3** was 41% but the subsequent couplings proceeded with normal (approximately 99%) efficiency. During the capping steps, the 1-methyl-2-(trifluoroacetyl)hydrazino groups of the modified nucleobase were acetylated and the final ammonia treatment only removed the initial, more labile, trifluoroacetyl protections (Scheme 2).

The 6-mer 2′-*O*-methyl-RNA oligonucleotides bearing the modified nucleoside **1** at the 3′-terminus (**11UX**, **11AX**, **11GX**, **11CX**, **12UX**, **12AX**, **12GX** and **12CX** in Table 2) were assembled on a support obtained by immobilizing the 3′-*O*-TBDMS-protected derivative of **1** (**13**, obtained as a side product in the synthesis of the phosphoramidite building block **2**) to aminoalkyl-CPG by 2′-*O*-succinylation followed by conventional HBTU-promoted peptide coupling (Scheme 3). The couplings proceeded on this support with normal efficiency. During deprotection and release from support by the conventional ammonia treatment, one of the 3,5-dimethylpyrazolyl groups of the modified nucleoside **1** was displaced by ammonia (Scheme 4), yielding oligonucleotides **11UZ**, **11AZ**, **11GZ** and **11CZ** (Table 2). Oligonucleotides containing **1** in its original form (**11UX**, **11AX**, **11GX**, **11CX**, **12UX**, **12AX**, **12GX** and **12CX**) were, however, obtained in good yields by using a shorter ammonia treatment (2 h at 55 °C). To verify the site of ammonolysis, a sample of the modified nucleoside **1** was incubated for 5 h under the conditions used for the deprotection of the oligonucleotides (*i.e.* concentrated aq. ammonia at 55 °C), after which the product mixture was analyzed by NMR (Fig. 2). After 5 h, 50% of the starting material **1** had reacted to give essentially one product. The HMBC spectrum of this product revealed a coupling between the C5 atom of the purine ring and the hydrogen atoms of an exocyclic amino group, establishing that the 3,5-dimethylpyrazol-1-yl substituent at position 6, rather than position 2, had been displaced by ammonia. This reaction seems to be a problem only in the case of terminal nucleosides, as in the middle of the chain the same structure remained unaffected during the conventional (longer) ammonia treatment. The crude oligonucleotides were purified by RP-HPLC and characterized by electrospray ionization mass spectrometry (ESI-MS). The concentrations of the purified oligonucleotides were determined UV-spectrophotometrically using molar absorption coefficients calculated by an implementation of the nearest-neighbors method.[9–11]

Melting temperature measurements

The melting temperatures of the duplexes that the modified oligonucleotides incorporating 2,6-bis(3,5-dimethylpyrazol-1-yl)purine (**8X**) or 2,6-bis(2-acetyl-1-methylhydrazino)purine (**8Y**) formed with complementary 2′-*O*-methyl-RNA sequences (**9U**, **9A**, **9G** and **9C**) bearing U, A, G or C nucleoside opposite to the base-modified nucleoside were measured in

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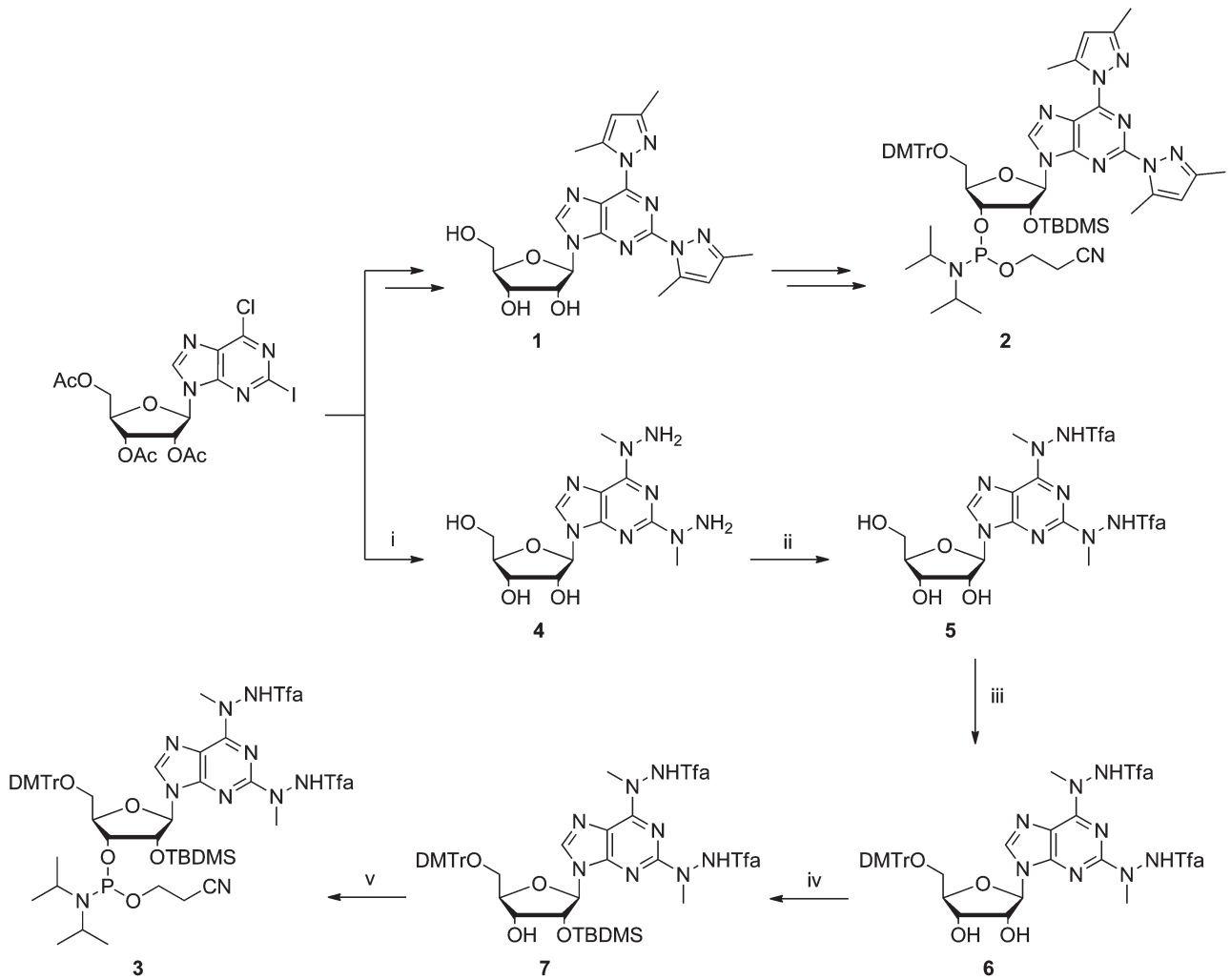
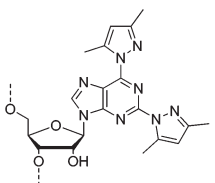


Scheme 1 Preparation of the modified nucleosides and their conversion to phosphoramidite building blocks. Reagents and conditions: (i) $CH_3NH_2NH_2$; (ii) ethyl trifluoroacetate, Et_3N, MeOH; (iii) DMTrCl, pyridine; (iv) TBDMSCl, DMF, imidazole; (v) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, Et_3N, CH_2Cl_2.

Table 1 Structures of the 9-mer 2′-*O*-methyl-RNA oligonucleotides used in this study

	Sequence
8A	5′-GCGCACCGG-3′
8X	5′-GCGCXCCGG-3′
8Y	5′-GCGCYCCGG-3′
9U	5′-CCGGUGCGC-3′
9A	5′-CCGGAGCGC-3′
9G	5′-CCGGGGCGC-3′
9C	5′-CCGGCGCGC-3′
10U	5′-CCGAUACGC-3′
10A	5′-CCGAAACGC-3′
10G	5′-CCGAGACGC-3′
10C	5′-CCGACACGC-3′

X =



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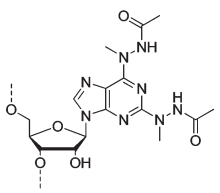
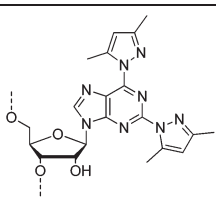


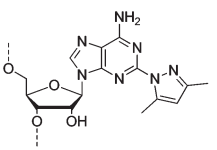
Table 2 Structures of the 6-mer 2′-*O*-methyl-RNA oligonucleotides used in this study

	Sequence
11UA	5′-UGCGCA-3′
11AA	5′-AGCGCA-3′
11GA	5′-GGCGCA-3′
11CA	5′-CGCGCA-3′
11UX	5′-UGCGCX-3′
11AX	5′-AGCGCX-3′
11GX	5′-GGCGCX-3′
11CX	5′-CGCGCX-3′
11UZ	5′-UGCGCZ-3′
11AZ	5′-AGCGCZ-3′
11GZ	5′-GGCGCZ-3′
11CZ	5′-CGCGCZ-3′
12UA	5′-UGCACA-3′
12AA	5′-AGCACA-3′
12GA	5′-GGCACA-3′
12CA	5′-CGCACA-3′
12UX	5′-UGCACX-3′
12AX	5′-AGCACX-3′
12GX	5′-GGCACX-3′
12CX	5′-CGCACX-3′

X =



Z =



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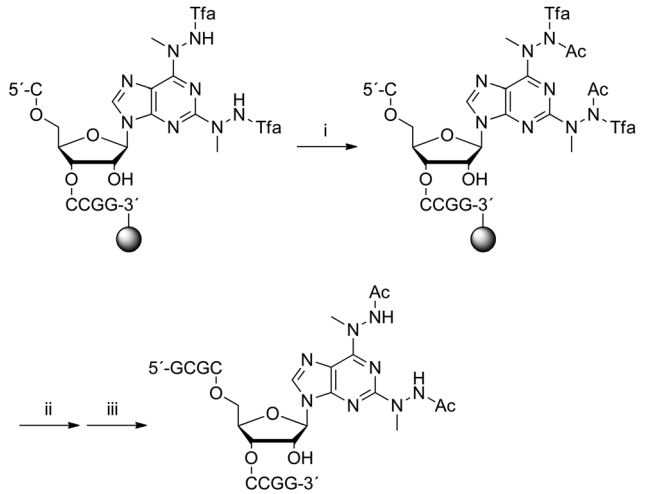
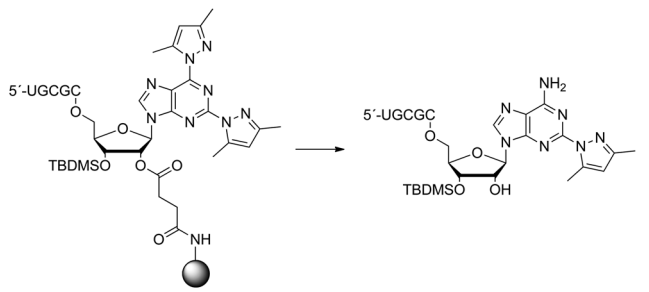
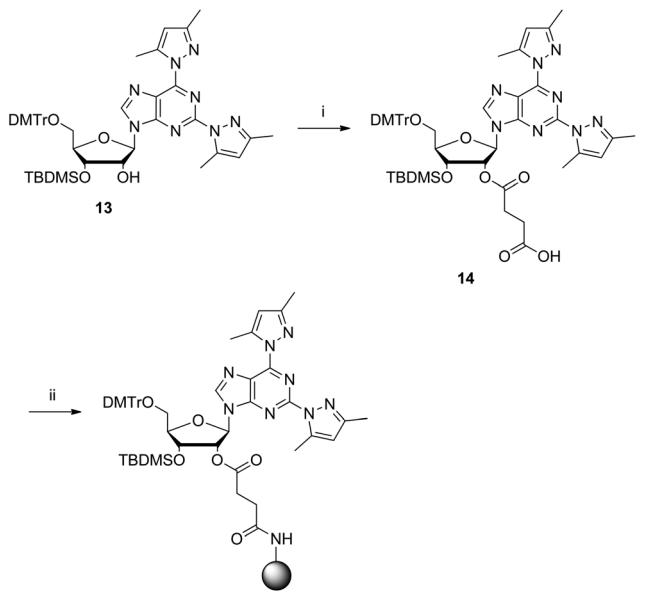


Scheme 2 Acetylation of the 1-methyl-2-(trifluoroacetyl)hydrazido groups of the modified nucleoside **5** during Ac_2O capping and removal of the trifluoroacetyl groups during ammonolysis. Reagents and conditions: (i) conventional Ac_2O capping; (ii) conventional phosphoramidite strategy; (iii) NH_3, H_2O.



Scheme 4 Displacement of a dimethylpyrazol-1-yl group by ammonia during deprotection and release of the oligonucleotide from the support.



Scheme 3 Preparation of a solid support for the synthesis of oligonucleotides incorporating a 3′-terminal modified nucleoside **1**. Reagents and conditions: (i) succinic anhydride, pyridine; (ii) long chain aminoalkyl-CPG, HBTU, DIPEA.

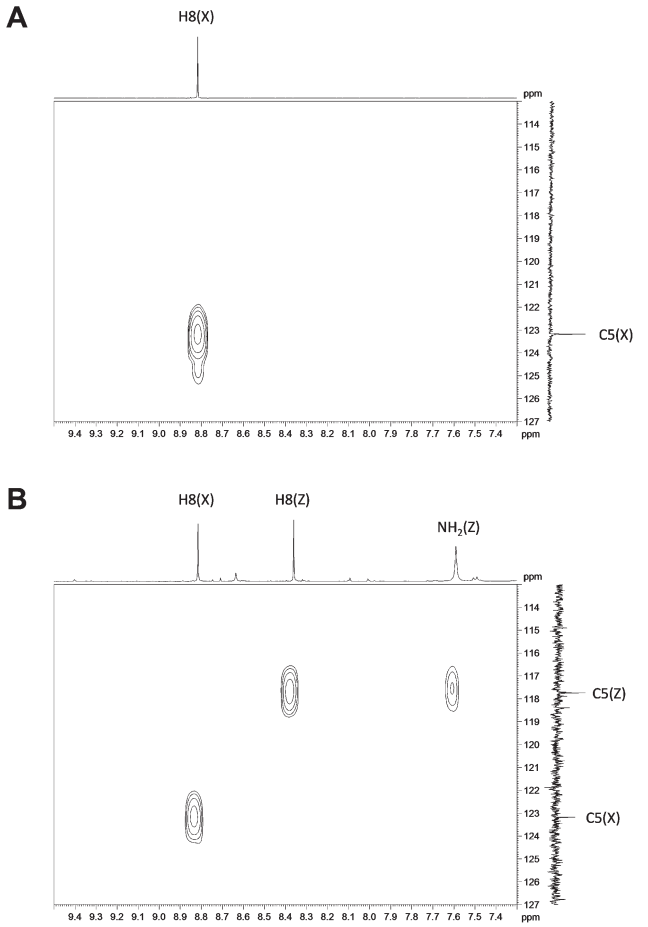


Fig. 2 HMBC spectra of the modified nucleoside **1** (A) before and (B) after 5 h incubation in concentrated aq. ammonia at 55 °C, showing correlations between the C5 and H8 atoms of the purine ring and, in the case of the ammonolysis product, the hydrogen atoms of an exocyclic amino group. The spectra were measured in DMSO-d_6.

a 20 mmol L^{-1} cacodylate buffer at pH 7.4 in the presence and absence of 1 equiv. of Cu^{2+}, Zn^{2+} or Pd^{2+} ions. For comparison, the same measurements were carried out on the respective duplexes formed by the unmodified oligonucleotide **8A**, containing an adenine residue in place of the modified base. The concentration of the oligonucleotides was 3 μmol L^{-1} in each experiment and the ionic strength was adjusted to 0.1 mol L^{-1} with $NaClO_4$. The results of these measurements are summarized in Table 3. As expected, in the absence of divalent metal ions, the most stable duplex was formed between the unmodified oligonucleotides **8A** and **9U**, with a Watson–Crick A:U base pair in the central intrachain position (T_m = 75.1 °C). Under these conditions, neither of the modified nucleobases exhibited clear preference for any of the four canonical nucleobases. The duplexes formed by **8X** were of similar stability as the mismatched ones formed by **8A**, whereas the duplexes formed by **8Y** were much less stable. In the presence of an equimolar amount of Cu^{2+}, the situation became quite different: while the stabilities of the duplexes formed by unmodified **8A** remained largely unaffected, the ones formed by **8X** experienced marked stabilization. Furthermore, this

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Table 3 Melting temperatures for the duplexes formed between the 2′-*O*-methyl-RNA oligonucleotides **8** and **9** with various combinations of the central nucleosides N^1 (in **9**) and N^2 (in **8**); pH = 7.4 (20 mM cacodylate buffer); [oligonucleotides] = 3.0 μM; [metal ions] = 0 or 3.0 μM; I(NaClO$_4$) = 0.10 M)

5′—G C G C N^2 C C G G—3′
3′—C G C G N^1 G G C C—5′

	N^2,[b] no metal			N^2, Cu^{2+}		N^2, Zn^{2+}		N^2, Pd^{2+}	
N^1[a]	**A**	**X**[c]	**Y**[c]	**A**	**X**	**A**	**X**	**A**	**X**	**Y**
U	75.1 ± 0.3	61.8 ± 0.4	42.5 ± 0.4	75.8 ± 0.1	68.3 ± 0.2	43.2 ± 0.8	76.0 ± 0.3	65.0 ± 1.0	42.3 ± 0.4	73.7 ± 0.1	60.6 ± 0.6	42.4 ± 0.5
A	56.3 ± 0.2	61.4 ± 0.4	44.9 ± 0.2	58.8 ± 0.3	70.9 ± 0.7	44.5 ± 0.5	56.4 ± 0.2	69.9 ± 3.0	45.7 ± 0.2	56.3 ± 0.1	61.6 ± 0.1	47.5 ± 1.6
G	61.2 ± 0.3	61.6 ± 0.3	45.4 ± 0.2	62.5 ± 0.1	64.4 ± 1.6	45.3 ± 0.3	62.3 ± 0.1	63.6 ± 0.8	45.7 ± 0.6	60.4 ± 0.1	60.1 ± 0.6	45.7 ± 0.9
C	59.0 ± 0.2	60.7 ± 0.2	47.2 ± 0.3	61.7 ± 0.2	74.1 ± 0.1	50.7 ± 3.0	61.4 ± 0.1	68.3 ± 2.0	45.7 ± 0.3	58.8 ± 0.2	59.9 ± 0.4	50.7 ± 3.2

[a] The central nucleoside of the unmodified oligonucleotide **9**. [b] The central nucleoside of the modified oligonucleotide **8**. [c] For the structures of **X** and **Y**, see Table 1.

stabilization was base-selective, duplexes **8X:9A** and **8X:9C** exhibiting the highest melting temperatures (70.9 and 74.1 °C, respectively). Similar, but lesser, stabilization was observed with Zn^{2+}, whereas Pd^{2+} had essentially no effect. The hybridization of **8Y**, in turn, was hardly affected by the presence of any of the three metal ions studied, with the possible exception of a modest increase in T_m by Cu^{2+} and Pd^{2+} in the case of the **8Y:9C** duplex.

Our previous results suggest that the relatively bulky metal-ion-mediated base pairs formed by a purine base derivative, such as **1**, are not easily accommodated within the rigid base-stacked A-type duplex formed between two fully complementary 2′-*O*-methyl-RNA oligonucleotides.[7] To study the effect of making the structure more flexible, mismatches were introduced to both sides of the metallo-base pair and the melting temperatures were, hence, measured for the duplexes that oligonucleotides **8A** and **8X** formed with their mismatched target oligonucleotides **10U**, **10A**, **10G** and **10C** under the same conditions as used above for hybridization with **9U**, **9A**, **9G** and **9C**. The results of these measurements are summarized in Table 4. Introduction of mismatches on both sides of the central base pair dramatically destabilized hybridization with the unmodified oligonucleotide **8A** in the absence of metal ions. In contrast, no change in stability was observed with **8X**. Addition of Cu^{2+} did not restore the stability of the duplexes formed by **8A**, whereas the duplexes formed by **8X** experienced marked stabilization. With the mismatched target oligonucleotides **10U**, **10A**, **10G** and **10C**, however, the sequence-selectivity of **8X** was lost: all of the duplexes were almost as stable as the most stable ones between **8X** and the matched target oligonucleotides **9U**, **9A**, **9G** and **9C** (T_m = 71.9–72.6 °C). Zn^{2+} modestly stabilized all of the duplexes formed by **8X**, whereas Pd^{2+} had no effect.

To study the impact of a metal-ion-binding nucleoside in a terminal, rather than an internal, position, melting temperatures of duplexes formed by the self-complementary 6-mer 2′-*O*-methyl-RNA-oligonucleotides (**11UX**, **11AX**, **11GX**, **11CX**, **11UZ**, **11AZ**, **11GZ** and **11CZ**) and, for comparison, the corresponding unmodified oligonucleotides (**11UA**, **11AA**, **11GA** and **11CA**) were measured under the same conditions as used above for the 9-mer oligonucleotides. The results of these measurements are summarized in Table 5. In contrast to the results obtained with the 9-mer oligonucleotides, the duplexes formed by the modified self-complementary 6-mer oligonucleotides are actually more stable than the ones formed by their unmodified counterparts even in the absence of divalent metal ions. Curiously, oligonucleotides **11UZ**, **11AZ**, **11GZ** and **11CZ** having the 3,5-dimethylpyrazol-1-yl group at position 6 displaced by an amino group form stronger duplexes than their counterparts (**11UX**, **11AX**, **11GX** or **11CX**) with both of the 3,5-dimethylpyrazole moieties intact. Neither of these series of oligonucleotides shows any marked selectivity toward the nucleoside opposite to the modified base. The duplex formed by the fully self-complementary oligonucleotide **11UA** is modestly stabilized in the presence of Cu^{2+}, whereas the other unmodified duplexes remain largely unaffected. The modified duplexes incorporating the 2,6-bis(3,5-dimethylpyrazol-1-yl)purine base (**11UX**, **11AX**, **11GX** or **11CX**) enjoy, in

Table 4 Melting temperatures for the duplexes formed between the 2′-*O*-methyl-RNA oligonucleotides **8** and **10** with various combinations of the central nucleosides N^1 (in **10**) and N^2 (in **8**); pH = 7.4 (20 mM cacodylate buffer); [oligonucleotides] = 3.0 μM; [metal ions] = 0 or 3.0 μM; *I*($NaClO_4$ = 0.10 M)

5′–G C G C N^2 C C G G–3′
3′–C G C A N^1 A G C C–5′

	N^2,[b] no metal		N^2, Cu^{2+}		N^2, Zn^{2+}		N^2, Pd^{2+}	
N^1[a]	**A**	**X**[c]	**A**	**X**	**A**	**X**	**A**	**X**
U	26.9 ± 0.3	60.4 ± 0.3	28.6 ± 0.1	72.6 ± 0.2	26.7 ± 0.4	64.4 ± 0.3	27.6 ± 0.1	60.4 ± 0.1
A	43.6 ± 0.7	61.0 ± 0.9	43.8 ± 0.5	71.9 ± 0.9	41.7 ± 0.3	63.8 ± 0.6	44.3 ± 0.6	61.7 ± 0.9
G	44.3 ± 0.3	61.8 ± 0.4	43.8 ± 0.5	72.6 ± 0.6	51.2 ± 0.2	62.8 ± 0.2	43.5 ± 0.9	60.1 ± 0.2
C	45.7 ± 0.8	60.3 ± 0.5	41.3 ± 0.5	72.4 ± 0.3	42.8 ± 0.2	63.9 ± 0.8	43.9 ± 0.7	61.1 ± 0.3

[a] The central nucleoside of the unmodified oligonucleotide **10**. [b] The central nucleoside of the modified oligonucleotide **8**. [c] For the structure of **X**, see Table 1.

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striking contrast, marked stabilization by Cu^{2+}, the highest increase in T_m being nearly 28 °C (or 14 °C for one metallo-base pair). The stabilization is base-selective but, in contrast to the internal base pair of the 9-mer duplexes, U rather than C is the favoured complement. The oligomers containing a 2-(3,5-dimethylpyrazol-1-yl)adenine base (**11UZ**, **11AZ**, **11GZ** and **11CZ**) form in the presence of Cu^{2+} somewhat weaker duplexes than **11UX**, **11AX**, **11GX** or **11CX** but with higher U-selectivity. As described above for the 9-mer duplexes, the stabilizing effect of Zn^{2+} is modest and that of Pd^{2+} almost non-existent. The sole exception is the duplex formed by **11UX**, showing a 10 °C increase in T_m upon addition of 1 equiv. of Pd^{2+}, consistent with the previous finding of modest T-selectivity in an intrachain position.[7]

The immense stabilization of the 6-mer duplexes by the terminal metallo-base pairs could lead to diminished base-selectivity in other parts of the molecule. In other words, the stability of the duplexes could be dictated solely by the metal-ion-mediated base pairs, with the Watson–Crick base pairs playing only a minor role, if any. To rule out this possibility, melting temperatures of a series of mismatched 6-mer 2′-*O*-methyl-RNA oligonucleotides (**12UX**, **12AX**, **12GX**, **12GX**, **12UA**, **12AA**, **12GA** and **12CA**) were measured under the same conditions as used above for the other oligonucleotides. The results of these measurements are summarized in Table 6. In all of the cases studied, regardless of the presence of divalent metal ions, introduction of the two consecutive mismatches in the middle of the sequence results in a dramatic loss of stability, with all the duplexes exhibiting T_m values of approximately 10 °C. Evidently, the short metallo-oligonucleotides retain sensitivity to mismatches despite the strong stabilizing contribution of the two terminal metallo-base pairs.

CD spectropolarimetric measurements

To verify the secondary structure of the oligonucleotide duplexes formed in the presence of the divalent metal ions (Cu^{2+}, Zn^{2+} and Pd^{2+}), CD spectra of the assemblies were measured over a wide temperature range (6–94 °C) under the same conditions as the ones used for the T_m measurements. In all cases, the CD spectra are at low temperatures characteristic of an A-type duplex.[12–15] When the temperature is increased, a gradual decrease of the CD signals is observed, consistent with thermal denaturation of the duplexes (Fig. 3). Furthermore, the thermal loss of ellipticity is in good agreement with the thermal hyperchromicity of the T_m studies, exhibiting sigmoidal dependence with similar inflection points (Fig. 4).

Discussion

As discussed in our previous study,[7] the ability of 2,6-bis(3,5-dimethylpyrazol-1-yl)purine riboside (**1**) to stabilize inherently weak oligonucleotide duplexes (such as short, mismatched or RNA/DNA heteroduplexes) even in the absence of bridging metal ions is probably due to high polarizability of the π-electron system that enforces stacking interactions. The influence is most prominent in a terminal position, whereas within the more confined environment of the relatively rigid base-stack of an A-form double helix, this stabilization is largely cancelled out by the steric bulk of the 2,6-bis(3,5-dimethylpyrazol-1-yl) purine base. The acetylated derivative of the 2,6-bis(1-methylhydrazino)purine riboside (**4**), being less bulky but also having a smaller surface for stacking, is even more destabilizing, leading to weaker duplexes than any of the mispairs between native nucleobases in the middle of a sequence. 2-(3,5-Dimethylpyrazol-1-yl)adenosine was only investigated in terminal positions of short duplexes where, unexpectedly, it was found to be more stabilizing than **1** in the absence of divalent metal ions. Perhaps this modified nucleobase represents a good compromise between a large stacking surface and a reasonable size. Hydrogen bonding interactions of the amino group at position 6 seem unlikely given the non-specific nature of the observed stabilization.

With all the model systems studied, the greatest stabilizing effect is observed with oligonucleotides incorporating the 2,6-bis(3,5-dimethylpyrazol-1-yl)purine base in the presence of Cu^{2+}. In the case of the 9-mer oligonucleotides having a single intrachain modification, this means merely overcoming the destabilizing effects of the bulky artificial nucleobase and/or

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Table 5 Melting temperatures for the duplexes formed by the self-complementary 2′-*O*-methyl-RNA oligonucleotides **11** with various combinations of the 5′- and 3′-terminal nucleosides N^1 and N^2, respectively; pH = 7.4 (20 mM cacodylate buffer); [oligonucleotides] = 6.0 μM; [metal ions] = 0 or 6.0 μM; /($NaClO_4$ = 0.10 M)

5′—N^1 G C G C N^2—3′
3′—N^2 C G C G N^1—5′

	N^2,[b] no metal			N^2, Cu^{2+}			N^2, Zn^{2+}			N^2, Pd^{2+}		
N^1[a]	**A**	**X**[c]	**Z**[c]	**A**	**X**	**Z**	**A**	**X**	**Z**	**A**	**X**	**Z**
U	39.2 ± 0.6	44.2 ± 0.6	53.5 ± 0.1	43.0 ± 0.3	71.8 ± 0.5	68.7 ± 0.3	40.0 ± 0.3	51.8 ± 1.0	54.3 ± 1.2	39.4 ± 0.3	54.3 ± 0.7	56.2 ± 0.8
A	36.8 ± 0.5	43.9 ± 1.5	54.0 ± 0.3	38.9 ± 0.6	62.5 ± 0.3	59.2 ± 0.7	36.6 ± 0.3	50.3 ± 1.8	54.4 ± 1.0	36.1 ± 0.3	44.5 ± 0.8	56.8 ± 0.2
G	37.8 ± 0.2	46.9 ± 0.2	55.7 ± 0.3	38.3 ± 0.4	69.4 ± 0.3	64.4 ± 0.1	37.0 ± 0.4	52.4 ± 0.1	55.2 ± 0.4	35.8 ± 0.6	41.0 ± 0.4	57.0 ± 2.0
C	36.8 ± 0.1	44.1 ± 0.5	51.8 ± 0.2	36.7 ± 0.8	62.7 ± 0.2	62.0 ± 0.5	37.1 ± 0.4	48.1 ± 0.5	52.9 ± 0.3	35.1 ± 1.2	38.6 ± 1.6	51.5 ± 0.4

[a] The 5′-terminal nucleoside. [b] The 3′-terminal nucleoside. [c] For the structures of **X** and **Z**, see Table 2.

mismatches in the middle of the sequence (Tables 3 and 4). The 6-mer duplexes with terminal Cu^{2+}-mediated base pairs, on the other hand, exhibit melting temperatures well beyond those achievable by Watson–Crick base-pairing (Tables 5 and 6). The effect of Zn^{2+} is similar but weaker, consistent with the lower affinity of Zn^{2+} for the ring nitrogens of nucleobases.[6] The fact that the metal-ion-mediated base pairs are most stabilizing in terminal positions or when flanked by mismatches is consistent with difficulties in accommodating these relatively large structures within the base-stack of fully complementary duplexes. Various Cu^{2+}-mediated base pairs embedded in double-stranded oligonucleotides have been described in the literature,[16–19] both between two bidentate ligands[20] and between one tridentate and one monodentate ligand[21] (as in the present study). Increases in T_m comparable to the ones reported here have been observed but only when both ligands have been specifically designed to form a metallo-base pair. For example, pyridine-2,6-dicarboxamide forms with pyridine a Cu^{2+}-mediated base pair of somewhat higher stability than either of the Watson–Crick base pairs but the respective metallo-base pairs formed with the native nucleobases are much weaker, despite the relatively high (15 μM) concentration of Cu^{2+} employed.[21]

The Cu^{2+} chelate of 2,6-bis(3,5-dimethylpyrazol-1-yl)purine exhibits some discrimination towards its native complement, but in an environment-dependent manner. In a terminal position, U and G are favored over A and C, the difference in T_m being 7 °C (or 3.5 °C per bp). For comparison, terminal Watson–Crick base pairs are known to be weak[22,23] and, accordingly, only small differences in T_m values were observed between the unmodified oligonucleotides **11UA**, **11AA**, **11GA** and **11CA**. The preference of the Cu^{2+} chelate of 2,6-bis(3,5-dimethylpyrazol-1-yl)purine for U and G over C and A is understandable since among the four canonical nucleobases, they are the ones that can easily undergo deprotonation to serve as anionic nitrogen ligands. When the metallo-base pair is placed in the middle of a fully complementary double helix, the situation becomes quite different: C is favored over the other nucleobases and with G, the increase in T_m upon introduction of Cu^{2+} is very modest. While the latter observation may be understood in terms steric crowding, as discussed above, the preference of C and, in particular, A to U is not easily explained without further studies.

In the light of the high stability of the Pd^{2+}-mediated base pairs formed by the modified nucleoside **1** at the monomeric level,[7] the inability of Pd^{2+} to stabilize oligonucleotide duplexes incorporating the same residue appears enigmatic. This peculiar finding is, however, not unprecedented: while a number of coplanar Pd^{2+}-mediated base pairs have been reported,[7,24–26] we are not aware of a study that would unambiguously demonstrate the successful incorporation of such a metallo-base pair within an oligonucleotide structure. Possibly the slow ligand-exchange reactions of Pd^{2+} present too high a barrier for efficient hybridization, leaving the Pd^{2+} chelate kinetically trapped in binding to an undesired ligand.

Table 6 Melting temperatures for the duplexes formed by the mismatched 2′-*O*-methyl-RNA oligonucleotides **12** with various combinations of the 5′- and 3′-terminal nucleosides N^1 and N^2; pH = 7.4 (20 mM cacodylate buffer); [oligonucleotides] = 6.0 μM; [metal ions] = 0 or 6.0 μM; $I(NaClO_4 = 0.10$ M)

5′—N^1 G C A C N^2—3′
3′—N^2 C A C G N^1—5′

	N^2,[b] no metal		N^2, Cu^{2+}		N^2, Zn^{2+}		N^2, Pd^{2+}	
N^1[a]	**A**	**X**[c]	**A**	**X**	**A**	**X**	**A**	**X**
U	10.9 ± 0.1	11.2 ± 0.2	10.3 ± 0.1	10.6 ± 0.3	10.3 ± 0.1	10.4 ± 0.2	10.4 ± 0.1	10.6 ± 0.3
A	10.3 ± 0.1	14.3 ± 0.3	10.4 ± 0.1	11.7 ± 1.2	10.3 ± 0.1	10.7 ± 0.5	10.2 ± 0.1	10.5 ± 0.2
G	12.0 ± 0.5	[d]	12.3 ± 1.3	10.7 ± 0.5	10.5 ± 0.1	10.1 ± 0.1	10.3 ± 0.2	10.7 ± 0.4
C	12.4 ± 0.4	[d]	13.7 ± 0.8	10.3 ± 0.1	10.6 ± 0.3	10.2 ± 0.2	10.5 ± 0.4	10.9 ± 0.5

[a] The 5′-terminal nucleoside. [b] The 3′-terminal nucleoside. [c] For the structure of **X**, see Table 2. [d] Not determined.

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The inability of the acetylated derivative of the 2,6-bis-(1-methylhydrazino)purine to serve as a metallo-nucleobase, although unexpected, may be reasoned as follows. Despite the superficial similarity, 2,6-bis(2-acetyl-1-methylhydrazino) purine and 2,6-bis(3,5-dimethylpyrazol-1-yl)purine are electronically rather different as tridentate ligands. In the former, the lone electron pair of N2 is involved in resonance with the carbonyl group and, hence, binding of the metal ion may be largely impeded.

Conclusions

2,6-Bis(3,5-dimethylpyrazol-1-yl)purine nucleosides present in the 3′-terminus of both strands of a double-stranded 6-mer 2′-*O*-methyl-RNA oligonucleotide increase the melting temperature of this duplex relative to the respective canonical duplex by more than 30 °C in the presence of 1 equiv. of Cu^{2+}. The Cu^{2+}-mediated base pairing exhibits some preference for U and G, with the central Watson–Crick base pairs conferring most of the sequence-specificity. Two consecutive mismatches in the middle of the short duplex result in a 60 °C drop in T_m (from 71.8 to 10.6 °C). In other words, the metallo-nucleobases presented in this study offer a way to dramatically stabilize short oligonucleotide duplexes without compromising sequence-specificity. Potential applications include recognition of short biologically relevant RNA molecules, such as miRNA.

Experimental

General methods

6-Chloro-2-iodo-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine was a commercial product that was used as received. The NMR spectra were recorded on a Bruker Avance 500 NMR spectrometer and the chemical shifts are given in ppm. The mass spectra were recorded on a Bruker micrOTOF-Q ESI-MS system and the CD spectra on an Applied Photophysics Chirascan spectropolarimeter. Oligonucleotides were prepared by an Applied Biosystems 3400 DNA/RNA synthesizer.

9-[5-*O*-(4,4′-Dimethoxytrityl)-β-D-ribofuranosyl]-*N*,*N*′-bis-(2,2,2-trifluoroacetyl)-2,6-bis(1-methylhydrazino)purine (6). To neat *N*-methylhydrazine hydrate (20 mL, 0.38 mol) 6-chloro-2-iodo-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (1.0 g, 1.86 mmol) was added and the bright yellowish mixture was stirred for 4 days at room temperature. Water was added and the mixture was stirred for 5 min, after which all volatiles were evaporated. The residue was diluted with 2-propanol (20 mL) and stirred for 20 min, after which the precipitate was collected by filtration and washed with cold 2-propanol (4 × 100 mL). Finally, the precipitate was dried under vacuum to yield 2,6-bis(1-methylhydrazino)-9-(β-D-ribofuranosyl)purine (**4**) as a white powder. The product (0.464 g, 1.36 mmol) was dissolved in MeOH (10 mL) and 4 equiv. of freshly distilled dry Et_3N (0.76 mL, 5.46 mmol) and 4 equiv. of ethyl trifluoroacetate (0.65 mL, 5.46 mmol) were added. The reaction was left overnight at room temperature with stirring, after which the volatiles were removed under reduced pressure. The crude 2,6-bis(1-methyl-2-trifluoroacetylhydrazino)-9-(β-D-ribofuranosyl)-purine (**5**, 0.726 g, 1.36 mmol) obtained was dried by coevaporation from dry pyridine and dissolved in dry pyridine (17 mL). DMTrCl (0.462 g, 1.36 mmol) was added and the red-brown colored oily reaction mixture left overnight at room temperature with stirring, after which it was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (40 mL) and extracted with saturated aq. $NaHCO_3$ (2 × 40 mL). The organic phase was dried with Na_2SO_4 and evaporated to dryness. The residual red oil was purified by silica gel chromatography eluting with a mixture of MeOH, CH_2Cl_2, and Et_3N (6 : 93.8 : 0.2, v/v). Yield 0.626 g (55.0%). ^{19}F NMR (δ_F) (470 MHz, $CDCl_3$): −76.72. ^1H NMR (δ_H)(500 MHz, $CDCl_3$): 8.58 (s, 1H, H8), 7.26 (m, 9H, Ar), 6.75 (m, 4H, Ar), 5.80 (d, 1H, H1′, J = 5.8 Hz), 4.78 (m, 1H, H2′), 4.34 (m, 2H, H3′& H4′), 3.77 (s, 6H, OCH_3), 3.39 (m, 1H, H5′), 3.34 (m, 1H, H5″), 3.30 (s, 3H, $NNCH_3$), 3.28 (s, 3H, $NNCH_3$). ^{13}C NMR (δ_C) (125 MHz, $CDCl_3$): 170.2, 158.4, 149.6, 144.3, 136.1, 135.6, 135.5, 130.1, 130.0, 128.1, 127.9, 126.9, 123.8, 113.3, 113.2, 86.5, 85.5, 72.3, 63.8, 55.3, 55.2, 45.7. HRMS (ESI^-): m/z calcd 833.2488 obsd 833.2534 $[M - H]^-$.

9-[2-*O*-*tert*-Butyldimethylsilyl-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-2,6-bis(1-methyl-2-trifluoroacetylhydrazino)-

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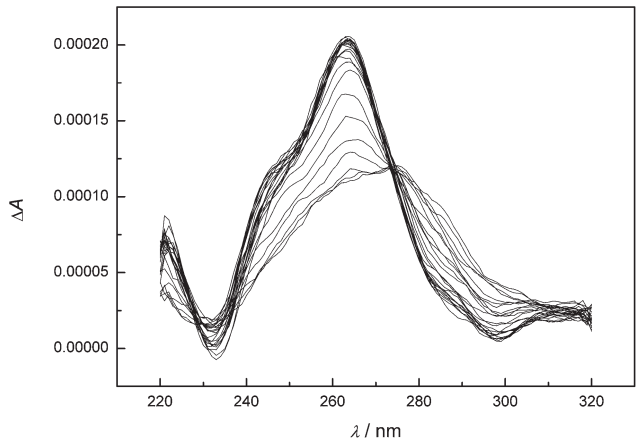

Fig. 3 CD spectra of the duplex formed by the self-complementary modified oligonucleotide **11UX** in the presence of Cu^{2+}, recorded at 4 °C intervals between 6 and 94 °C; [**11UX**] = [Cu^{2+}] = 6.0 μM; pH = 7.4 (20 mM cacodylate buffer); $I(NaClO_4)$ = 0.10 M.

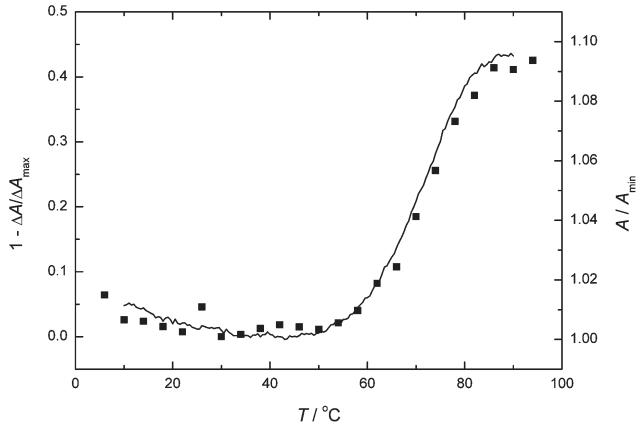

Fig. 4 Loss of ellipticity (1 − $\Delta A/\Delta A_{max}$, ■) and increase of UV absorbance at 260 nm (A/A_{min}, solid line) of the duplex formed by the self-complementary modified oligonucleotide **11UX** in the presence of Cu^{2+} as functions of temperature; [**11UX**] = [Cu^{2+}] = 6.0 μM; pH = 7.4 (20 mM cacodylate buffer); $I(NaClO_4)$ = 0.10 M.

purine (7). To a solution of dry compound **6** (0.549 g, 0.658 mmol) in dry DMF (10 mL), a solution of dry imidazole (0.45 g, 6.58 mmol) in dry DMF (10 mL), followed by 1.81 equiv. of TBDMSCl (0.18 g, 1.19 mmol), was added. The resulting mixture was stirred for 16 h at room temperature, after which the reaction mixture was quenched by the addition of MeOH (3.5 mL). The stirring was continued for 10 min, after which EtOAc (50 mL) and water (50 mL) were added and the organic and aqueous phases separated. The organic phase was dried with Na_2SO_4 and evaporated to dryness. The desired 2′-*O*-TBDMS isomer was separated from the 3′-*O* and disubstituted isomers in the residual red oil by silica gel chromatography eluting with a mixture of EtOAc, CH_2Cl_2, and Et_3N (10 : 89 : 1, v/v). Yield 0.067 g (10.7%). ^{1}H NMR (δ_H) (500 MHz, $CDCl_3$): 7.79 (s, 1H, H8), 7.29 (m, 9H, Ar), 6.79 (m, 4H, Ar), 5.91(d, 1H, H1′, J = 5.2 Hz), 4.83 (dd, 1H, H2′, $J_1 = J_2$ = 5.1 Hz), 4.29 (dd, 1H, H3′, J_1 = 4.4 Hz, J_2 = 8.9 Hz), 4.25 (dd, 1H, H4′, J_1 = 3.8 Hz, J_2 = 7.5 Hz), 3.78 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.42 (m, 2H, H5′ & H5″), 3.30 (s, 3H, $NNCH_3$), 2.73 (d, 1H, OH, J = 4.3 Hz), 0.84 (s, 9H, $SiCCH_3$), −0.03 (s, 3H, $SiCH_3$), −0.17 (s, 3H, $SiCH_3$). ^{13}C NMR (δ_C) (125 MHz, $CDCl_3$): 171.2, 158.6, 158.5, 157.5, 144.6, 137.9, 135.7, 135.5, 130.2, 130.1, 128.1, 127.9, 127.0, 114.6, 114.5, 113.2, 88.1, 86.6, 84.0, 75.3, 71.5, 63.7, 55.3, 37.9, 25.5, 17.9, 14.2, −5.0, −5.3. HRMS (ESI^+): m/z calcd 971.3317 obsd 971.3345 $[M + Na]^+$.

9-{2-*O*-*tert*-Butyldimethylsilyl-3-*O*-[(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphinyl]-β-D-ribofuranosyl}-2,6-bis-(1-methyl-2-trifluoroacetylhydrazino)purine (3). To a solution of dry compound **7** (67 mg, 0.071 mmol) in dry CH_2Cl_2 (4 mL), 9 equiv. of dry Et_3N (88.5 μL, 0.636 mmol) and 4.7 equiv. of 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (74.1 μL, 0.332 mmol) were added under nitrogen. The mixture was stirred under nitrogen for 4 days, after which CH_2Cl_2 (40 mL) was added and the solution was washed with saturated aq. $NaHCO_3$ (40 mL). The organic phase was dried with Na_2SO_4 and evaporated to dryness. The residual yellowish oil was purified by silica gel chromatography eluting with a mixture of EtOAc, CH_2Cl_2, and Et_3N (stepwise gradient from 5 : 94 : 1 to 20 : 79 : 1, v/v). Yield 49.2 mg (61%) as a nearly equimolar mixture of R_P and S_P diastereomers. ^{31}P NMR (δ_P) (202 MHz, $CDCl_3$, diastereomer A): 151.1; (δ_P) (202 MHz, $CDCl_3$, diastereomer B): 149.4. ^{1}H NMR (δ_H) (500 MHz, $CDCl_3$, diastereomer A): 7.88 (s, 1H, H8), 7.46–7.18 (m, 9H, Ar), 6.80 (m, 4H, Ar), 5.94 (d, 1H, H1′, J = 6.3 Hz), 4.82 (m, 1H, H2′), 4.41 (m, 1H, H4′), 4.32 (m, 1H, H3′), 3.94 (m, 1H, $POCH_2$), 3.87 (m, 1H, $POCH_2$), 3.77 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.59 (m, 1H, PNCH), 3.55–3.29 (m, 2H, H5′ & H5″), 3.31 (s, 3H, $NNCH_3$), 2.63 (m, 2H, CH_2CN), 1.18 (m, 6H, $NCCH_3$), 1.02 (d, 6H, $NCCH_3$, J = 6.8 Hz), 0.77 (s, 9H, $SiCCH_3$), −0.05 (s, 3H, $SiCH_3$), −0.20 (s, 3H, $SiCH_3$); (δ_H) (500 MHz, $CDCl_3$, diastereomer B): 7.84 (s, 1H, H8) 7.46–7.18 (m, 9H, Ar), 6.80 (m, 4H, Ar), 5.98 (d, 1H, H1′, J = 6.2 Hz), 4.82 (m, 1H, H2′), 4.32 (m, 1H, H4′), 4.28 (m, 1H, H3′), 3.77 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.64 (m, 1H, $POCH_2$), 3.59 (m, 1H, PNCH), 3.59 (m, 1H, $POCH_2$), 3.55–3.29 (m, 2H, H5′ & H5″), 3.31 (s, 3H, $NNCH_3$), 2.28 (m, 2H, CH_2CN), 1.18 (m, 12H, $NCCH_3$), 0.76 (s, 9H, $SiCCH_3$), −0.06 (s, 3H, $SiCH_3$), −0.23 (s, 3H, $SiCH_3$). ^{13}C NMR (δ_C) (125 MHz, $CDCl_3$, diastereomer A): 158.6, 158.5, 157.5, 144.5, 137.6, 135.7, 135.4, 130.2, 130.1, 128.2, 128.0, 127.0, 117.3, 113.3, 87.4, 86.7, 84.0, 75.7, 72.8 (d, J = 14.8 Hz), 63.4, 58.7 (d, J = 16.2 Hz), 55.3, 55.2, 43.5, 43.4, 37.8, 20.4 (d, J = 6.2 Hz), 25.5, 24.7, 24.6, −4.9, −5.2; (δ_C) (125 MHz, $CDCl_3$, diastereomer B): 158.6, 158.5, 157.5, 144.4, 137.5, 135.5, 135.3, 130.2, 130.0, 128.2, 127.9, 127.0, 117.6, 113.3, 87.5, 86.6, 83.7, 74.8, 73.5 (d, J = 9.3 Hz), 63.6, 57.6 (d, J = 20.4 Hz), 55.3, 55.2, 43.0, 42.9, 37.8, 20.0 (d, J = 6.8 Hz), 25.6, 24.7, 24.6, −4.9, −5.1. HRMS (ESI^+): m/z calcd 1149.4504 obsd 1149.4400 $[M + Na]^+$.

9-[3-*O*-*tert*-Butyldimethylsilyl-5-*O*-(4,4′-dimethoxytrityl)-2-*O*-succinoyl-β-D-ribofuranosyl]-2,6-bis(3,5-dimethylpyrazol-1-yl)-purine (14). Compound **13** (113.7 mg, 0.133 mmol), separated from the same product mixture as **7**, was dissolved in dry pyridine (7 mL) and 7.45 equiv. of succinic anhydride (98.9 mg, 0.988 mmol) was added. The reaction mixture was heated

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between 40 and 50 °C for 8 days, after which it was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (40 mL) and extracted with saturated aq. $NaHCO_3$ (2 × 40 mL). The organic phase was dried with Na_2SO_4 and evaporated to dryness. The crude product (a foamy yellowish residue) was dried under vacuum to yield 0.123 g (97%) of **14**. ^{1}H NMR (δ_H) (500 MHz, $CDCl_3$): 8.4 (s, 1H, H8), 7.32 (m, 9H, Ar), 6.84 (m, 4H, Ar), 6.57 (d, 1H, H1′, J = 6.1 Hz), 6.10 (s, 1H, pyrazole), 6.05 (s, 1H, pyrazole), 5.59 (dd, 1H, H2′, J_1 = 5.7 Hz, J_2 = 5.9 Hz), 4.60 (m, 1H, H3′), 4.17 (m, 1H, H4′), 3.79 (s, 6H, OCH_3), 3.53 (dd, 1H, H5′, J_1 = 1.7 Hz, J_2 = 10.9 Hz), 3.36 (dd, 1H, H5″, J_1 = 3.1 Hz, J_2 = 10.8 Hz), 2.68 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.69 (m, 2H, CH_2), 2.40 (m, 2H, CH_2), 0.86 (s, 9H, $SiCCH_3$), −0.04 (s, 3H, $SiCH_3$), −0.06 (s, 3H, $SiCH_3$). ^{13}C NMR (δ_C) (125 MHz, $CDCl_3$): 174.9, 171.2, 158.6, 155.2, 154.7, 153.1, 151.0, 149.6, 146.1, 144.1, 143.3, 143.2, 142.5, 136.1, 135.5, 135.3, 128.2, 128.0, 127.0, 123.8, 113.3, 110.4, 109.7, 106.4, 86.9, 85.3, 85.1, 76.0, 70.7, 62.9, 55.2, 39.2, 30.0, 25.6, 25.3, 17.9, 20.5, 14.6, 14.3, 14.2, 13.5, −4.8, −5.0. HRMS (ESI^+): m/z calcd 957.4325 obsd 957.4344 $[M + H]^+$.

Immobilization of compound 14 on solid support

To a solution of dry compound **14** (19.14 mg, 20 μmol) in dry DMF (0.5 mL), HBTU (7.6 mg, 20 μmol) and DIPEA (7.0 μL, 41 μmol) were added. The bright yellowish solution was transferred to a microcentrifuge tube containing the long chain alkylamine-CPG (102 mg). The reaction mixture was shaken for 24 h at room temperature, after which the support was transferred to a synthesizer column and washed with dry DMF (15 mL) and dry MeCN (15 mL). Finally, the support was capped manually by the conventional Ac_2O treatment and washed with dry MeCN (15 mL) and dry CH_2Cl_2 (15 mL). Based on the trityl response upon treatment with 3% DCA in CH_2Cl_2, loading of the functionalized CPG thus obtained was determined as 54 μmol g^{-1}.

Oligonucleotide synthesis

The 2′-*O*-methyl oligoribonucleotides were assembled on a CPG-supported succinyl linker at a loading of 27 μmol g^{-1}. A standard phosphoramidite strategy (600 s coupling time) was used throughout the sequences, except for the modified phosphoramidite building block **2**, which was coupled manually using an elongated coupling time (60 min). Based on the trityl response, the coupling yield for building block **2** was 41%, while the other couplings proceeded with normal efficiency. The products were released from the support and deprotected by conventional treatment with 33% aq. NH_3 (2 h for modified RNA and 5 h for unmodified RNA at 55 °C). Oligonucleotides were purified by RP-HPLC on a Hypersil ODS C18 column (250 × 4.6 mm, 5 μm) eluting with a mixture of the triethylammonium acetate and MeCN, the flow rate being 1.0 mL min^{-1}. The amount of MeCN was increased linearly from 10 to 40% during 25 minutes, kept at 40% MeCN for 5 minutes. The purified products were characterized by ESI-MS analysis (Tables 1 and 2).

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