Still Elusive – Pd(II)-Mediated Base Pairing by an Acetophenone Oxime Palladacycle within ^{15}N-Labelled Double-Helical Oligonucleotides

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Abstract

α and β anomers of an acetophenone *C*-nucleoside were synthesized and incorporated in the middle of short oligodeoxynucleotides. The ketone oligonucleotides were converted to ^{15}N-labelled oxime oligonucleotides by treatment with ^{15}N-hydroxylamine and, finally, cyclopalladated by treatment with lithium tetrachloropalladate. Comparison of the UV melting profiles of duplexes bearing the β anomer of either the palladacyclic or the metal-free oxime *C*-nucleoside suggested formation of a stable Pd(II)-mediated base pair, especially with adenine or thymine as the base pairing partner. Melting profiles of the corresponding duplexes bearing the α anomer were much more convoluted, precluding meaningful comparison. ^{15}N NMR spectra were obtained for the β anomeric oxime oligonucleotide as well as its palladacyclic derivative but the signals unfortunately diminished below detection limit when the latter was hybridized with a complementary strand placing a ^{15}N3-labelled thymine opposite to the palladacyclic residue.

Keywords

oligonucleotides; palladium; base pairing; isotopic labeling; NMR spectroscopy

Introduction

Metal-mediated base pairing has been studied for more than two decades[1–8] for potential applications ranging from molecular switches[9–11] and wires[12–16] to sequence recognition[17–21] to

expansion of the genetic code[22–26]. Of the numerous metal ions used in these studies, Ag(I), Cu(II) and Hg(II) have met with the greatest success. On the other hand, the metal ion employed in the very first artificial metal-mediated base pair[27], namely Pd(II), has proven challenging and elusive to the conventional experiments. Melting temperature measurements, for example, are complicated by the relatively slow ligand exchange at Pd(II), which manifests itself as multiphasicity and hysteresis between the denaturation and renaturation curves.[28–30] We have previously tried to address this problem by complementing melting temperature measurements with a FRET-based strand displacement assay, with moderate success.[30] However, strand displacement and melting temperature experiments both fundamentally quantify the same property, namely hybridization affinity of an oligonucleotide as a whole. Failure of the stability of a metal-mediated base pair to translate into duplex stability of a corresponding oligonucleotide has been reported not only with Pd(II)[31,32] but also with Ag(I)[33]. Such discrepancies are usually attributed to incompatible geometries of the metal-mediated base pair and the double helix. An independent method for monitoring the association and dissociation of a single metal-mediated base pair within a double-helical oligonucleotide would, hence, be highly desirable.

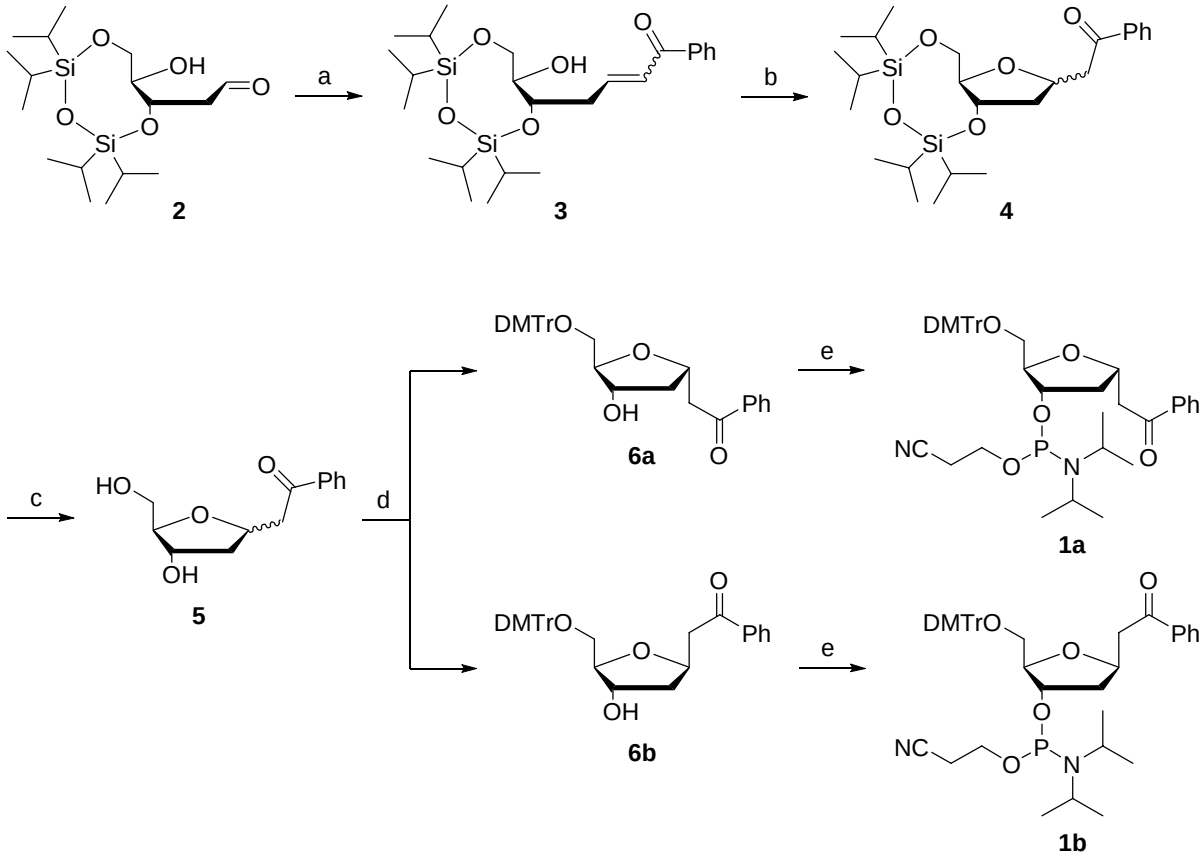
^{15}N NMR has already proven to be a valuable tool for studying Ag(I)-[34] and Hg(II)-[35,36] mediated base pairing, although the insensitivity of ^{15}N necessitates isotopic labeling of the ligands. In this paper we report the first attempt at ^{15}N NMR monitoring of Pd(II)-mediated base pairing within a double-helical oligonucleotide. Acetophenone oxime palladacycle, allowing easy post-synthetic ^{15}N-labeling as well as comparison of the hybridization data with those obtained on related oligonucleotides[29], was chosen as the Pd(II)-bearing nucleobase analogue. Both anomers of the corresponding *C*-nucleoside were synthesized and studied separately.

Results and Discussion

Building block synthesis

Synthesis of the phosphoramidite building blocks of the α and β anomers of the acetophenone *C*-nucleoside (1a and 1b, respectively) is outlined in Scheme 1. First, Wittig reaction between 2-deoxy-3,5-*O*-[1,1,3,3-tetraisopropyl-1,3-disiloxanediyl]-D-*erythro*-pentose (2)[37] and 2-(triphenylphosphoranylidene)acetophenone gave the enone intermediate 3 (a mixture of *E* and *Z* isomers), as previously reported for related ketones and esters[38]. As a significant fraction of compound 3 spontaneously cyclized to compound 4 (a mixture of α and β anomers), this reaction was driven to completion by the addition of potassium *tert*-butoxide, rather than attempting to isolate

compound 3. Removal of the Markiewicz protection by conventional fluoride treatment and introduction of a 4,4´-dimethoxytrityl protection to the 5´-OH function afforded chromatographically separable α and β anomers (6a and 6b, respectively) of the desired acetophenone *C*-nucleoside. Absolute configuration of the anomeric carbon atom of 6a and 6b was established based on NOESY couplings between the anomeric proton and either H3´ or H4´ (Figure 1). Finally, both anomers were converted into phosphoramidite building blocks by conventional treatment with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite.



Scheme 1. Synthesis of the α and β anomers of an acetophenone *C*-nucleoside and the corresponding protected phosphoramidite building blocks. Reagents and conditions: a) 2-(triphenylphosphoranylidene)acetophenone, benzene, 50 °C, 45 h; b) *t*-BuOK, THF, 25 °C, 5 min; c) $Et_3N \bullet 3HF$, THF, 25 °C, 18 h; d) DMTrCl, pyridine, N_2 atmosphere, 25 °C, 16 h; e) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, Et_3N, CH_2Cl_2, N_2 atmosphere, 25 °C, 1 h.

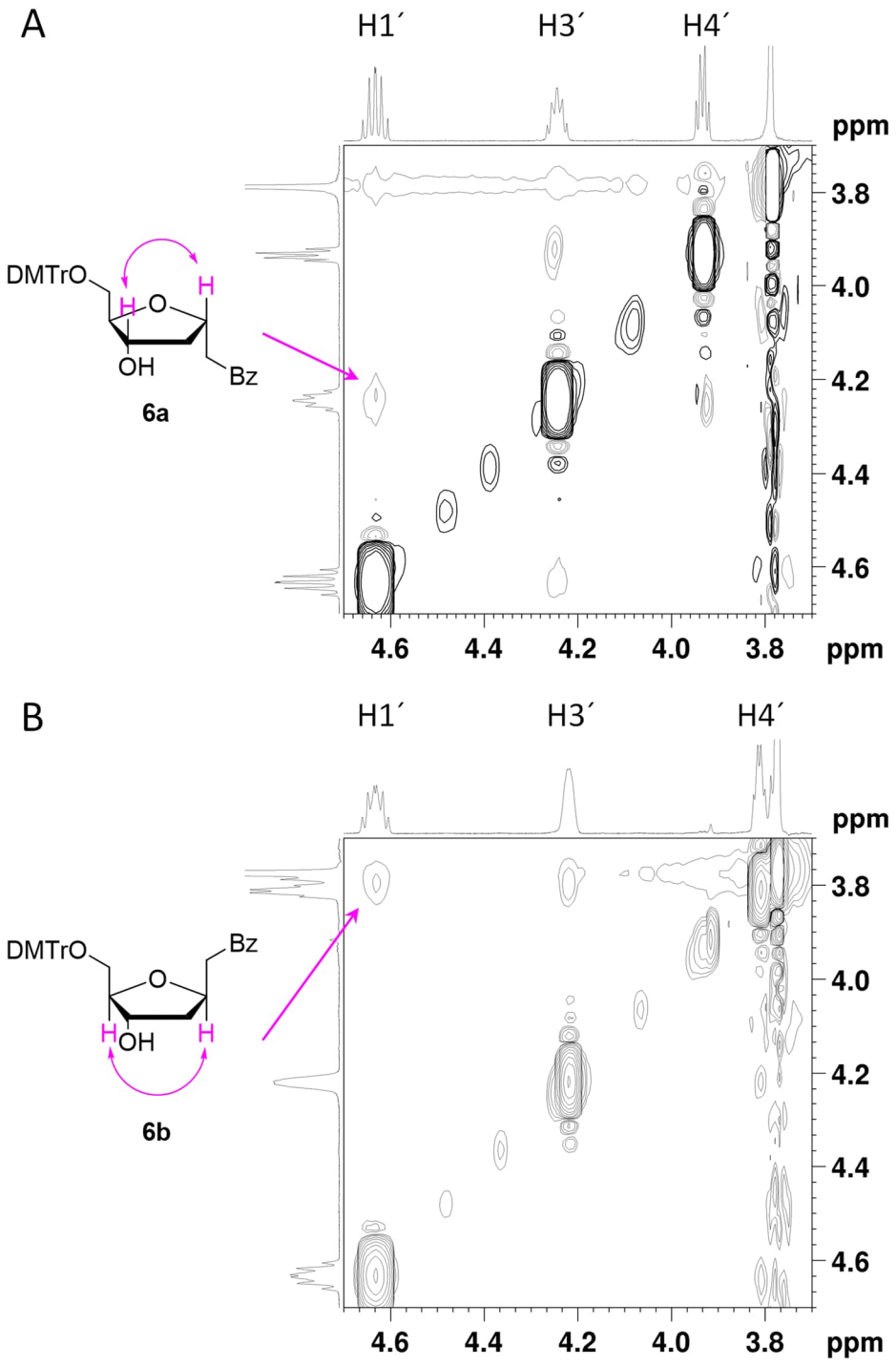


Figure 1. NOESY spectra of compounds 6a (A) and 6b (B), showing coupling of the anomeric proton with either H3´ or H4´, respectively.

Oligonucleotide synthesis

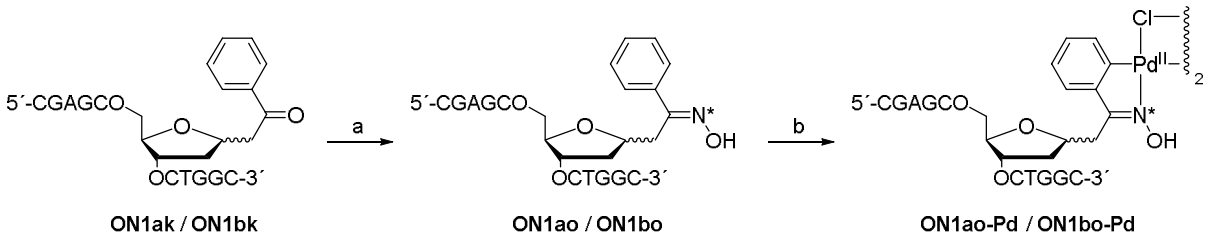
Sequences of the oligonucleotides used in this study are summarized in Table 1. The modified oligonucleotides ON1ak and ON1bk, incorporating either the α or the β anomer of the ketone *C*-nucleoside in the middle of the sequence, were synthesized on an automated DNA/RNA synthesizer by the conventional phosphoramidite strategy. According to trityl response, coupling yields were essentially quantitative throughout the sequence. On completion of chain assembly, the oligonucleotides were deprotected and released from the solid support by the conventional ammonolysis. The crude products were purified by RP-HPLC and characterized by ESI-TOF-MS. Curiously, the desired products ON1ak and ON1bk eluted as two distinct peaks, possibly owing to intramolecular imine formation between the ketone and an exocyclic amino function. In addition, two faster-eluting peaks of comparable height were collected and found to contain oligonucleotides with molecular weight 17 units higher than that of the desired products.

Table 1. Oligonucleotides used in this study.

Oligonucleotide	Sequence[a]
ON1ak	5´-CGAGC<u>aK</u>CTGGC-3´
ON1bk	5´-CGAGC<u>bK</u>CTGGC-3´
ON1ao	5´-CGAGC<u>aO</u>CTGGC-3´
ON1bo	5´-CGAGC<u>bO</u>CTGGC-3´
ON1ao-Pd	5´-CGAGC<u>aO^{Pd}</u>CTGGC-3´
ON1bo-Pd	5´-CGAGC<u>bO^{Pd}</u>CTGGC-3´
ON2a	5´-GCCAG<u>A</u>GCTCG-3´
ON2c	5´-GCCAG<u>C</u>GCTCG-3´
ON2g	5´-GCCAG<u>G</u>GCTCG-3´
ON2t	5´-GCCAG<u>T</u>GCTCG-3´
ON2t*	5´-GCCAG<u>T*</u>GCTCG-3´

[a] aK and bK refer to the α and β anomers of the benzoylmethyl *C*-nucleoside, aO and bO to the respective ^{15}N-labeled oximes and aO^{Pd} and bO^{Pd} to their palladacycles. In each sequence, the residue varied in the hybridization experiments has been underlined.

The purified ketone oligonucleotides ON1ak and ON1bk were converted into the respective oxime oligonucleotides ON1ao and ON1bo by treatment with aqueous ^{15}N-hydroxylammonium chloride (Scheme 2). On completion of the reaction, as verified by ESI-TOF-MS analysis, the product mixtures were subjected to RP-HPLC purification. Two peaks with nearly identical retention times were obtained, in all likelihood corresponding to *E* and *Z* isomers of the oxime. In contrast to ON1ak and ON1bk, the abovementioned side products did not react with ^{15}N-hydroxylammonium chloride, suggesting a modification at the ketone function. A mass increase of 17 units would be consistent with addition of ammonia, present in very high concentration during the final deprotection step. Tentatively the corresponding imine could be unexpectedly stable during RP-HPLC purification but ionize as the carbinolamine during MS analysis. It is, however, difficult to understand why the ammonia would not be eventually displaced by hydroxylamine. No further attempts were made to identify these side products.



ON1ak / ON1bk **ON1ao / ON1bo** **ON1ao-Pd / ON1bo-Pd**

Scheme 2. Oximation and cyclopalladation of the ketone oligonucleotides ON1ak and ON1bk. Reagents and conditions: a) $HO^{15}NH_3Cl$, H_2O, 25 °C, 96 h; Li_2PdCl_4, NaOAc, H_2O, 55 °C, 96 h.

The slower-eluting isomers of the oxime oligonucleotides ON1ao and ON1bo were converted to the respective palladacyclic oligonucleotides ON1ao-Pd and ON1bo-Pd by incubation in an aqueous solution of lithium tetrachloropalladate and sodium acetate at 55 °C for 4 d (Scheme 2). Configuration of the oxime double bond of the starting materials was unknown but, according to previous reports[39], both *E* and *Z* isomers eventually react to give the same palladacycle, with the oxime-OH and the benzene ring *trans* to each other. RP-HPLC analysis of the product mixture revealed several new peaks (chromatograms presented in the Supporting Information). This phenomenon has been reported before[28–30,40,41] and attributed to relatively slow ligand exchange at Pd(II). The collected fractions were analyzed by ESI-TOF-MS and those showing addition of a single Pd(II) ion combined. In line with previous reports, the initially formed chlorido-bridged dimer could not be detected, suggesting dissociation during HPLC purification.

UV and CD melting studies

UV melting profiles of duplexes formed by the cyclopalladated oligonucleotides ON1ao-Pd and ON1bo-Pd with the unmodified complementary strands ON2a, ON2c, ON2g and ON2t were measured to assess the base pairing preferences of both anomers of the acetophenone oxime palladacycle *C*-nucleoside within a double helix. Corresponding unmetallated duplexes were also studied for reference. In the middle of each duplex, an artificial nucleobase surrogate (acetophenone oxime or the respective palladacycle) was paired with a canonical nucleobase (adenine, cytosine, guanine or thymine). The samples had an oligonucleotide concentration of 1.0 μM, pH of 7.4 (20 mM cacodylate buffer) and ionic strength of 0.10 M (adjusted with sodium perchlorate).

Melting profiles of duplexes formed by the faster- and slower-eluting isomers of the unmetallated oxime oligonucleotides ON1ao and ON1bo and the respective palladacyclic oligonucleotides ON1ao-Pd and ON1bo-Pd with the unmodified complementary strand ON2a are presented in Figure 2 and all melting profiles in the Supporting Informations (Figures S24—S47). Melting curves of all duplexes incorporating the alpha anomer of the acetophenone oxime *C*-nucleoside (Figure 2A) were multiphasic, making determination of melting temperatures somewhat ambiguous and in some cases impossible. Furthermore, with the possible exception of ON1ao-Pd•ON2a, hybridization was not complete even at the low end of the experimental temperature range (10 °C). Figure 3 summarizes the highest melting temperatures associated with a significant transition for all duplexes, while all melting temperatures are tabulated in Table 2. Overall, melting temperatures of the "alpha duplexes" were highly scattered, precluding reasonable comparison or interpretation in terms of probable binding modes.

In contrast to ON1ao, duplexes formed by both isomers of ON1bo showed typical monophasic sigmoidal melting curves (Figure 2B), allowing reliable determination of a single melting temperature for each duplex. These melting temperatures were remarkably similar irrespective of the isomer of ON1bo or the canonical nucleobase opposite to the acetophenone oxime residue, ranging from 27 to 32 °C (Figure 3). Melting curves of the respective palladacyclic duplexes, in turn, were biphasic although the higher melting temperature could only be quantified with ON1bo-Pd•ON2a and ON1bo-Pd•ON2t. Similar melting profiles have been previously reported for other duplexes incorporating palladacyclic nucleobase surrogates[29,30] and the higher melting temperature proposed to stem from dissociation of a Pd(II)-mediated base pair. A similar interpretation seems reasonable also in the present case. With thymine as the base pairing partner, Pd(II) would in all likelihood coordinate to N3, whereas with adenine both N1- and N7-coordination are possible.[42] At least duplexes ON1bo-Pd•ON2a and ON1bo-Pd•ON2t would appear to be more stable than the previously studied[29]

counterparts bearing either a more flexible or, especially, a more rigid analogue of the palladacyclic oxime residue. Unfortunately, difficulties in determining the higher melting temperature in the latter two cases preclude a more quantitative comparison.

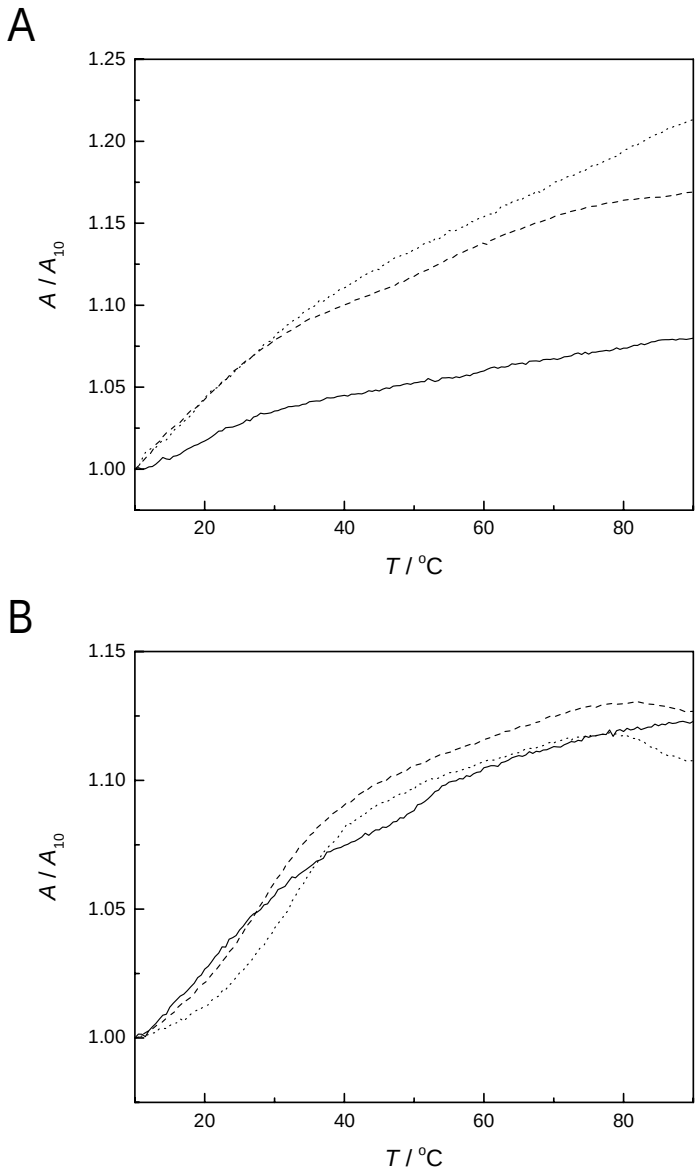


Figure 2. UV melting profiles of duplexes A) ON1ao•ON2a and ON1ao-Pd•ON2a and B) ON1bo•ON2a and ON1bo-Pd•ON2a; pH = 7.4 (20 mM cacodylate buffer); [oligonucleotides] = 1.0 μM; *I*($NaClO_4$) = 0.10 M. In both panels, dotted and dashed lines refer to the faster- and slower-eluting isomers of the unpalladated oxime oligonucleotide and solid line to the palladacyclic oligonucleotide.

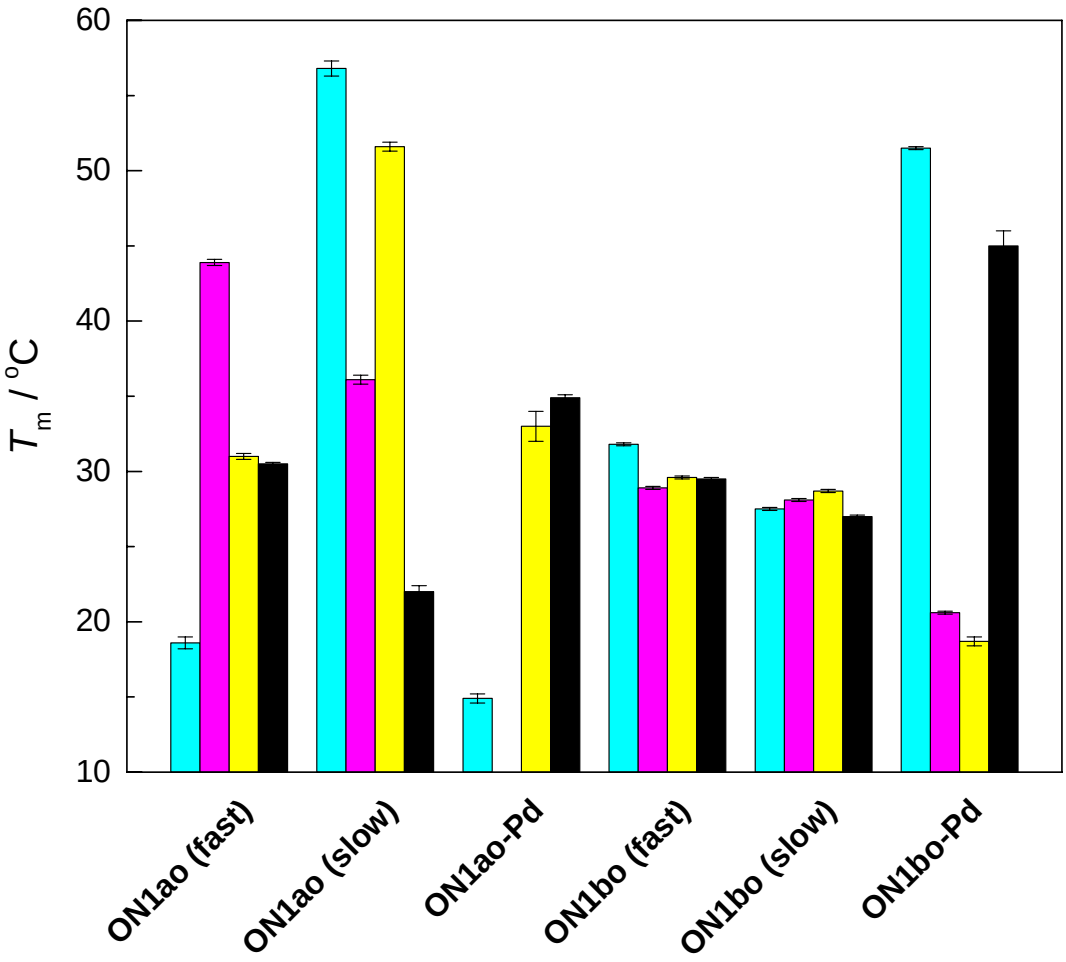


Figure 3. UV melting temperatures of duplexes formed by the modified oligonucleotides ON1ao, ON1ao-Pd, ON1bo and ON1bo-Pd with the natural oligonucleotides ON2a (cyan), ON2c (magenta), ON2g (yellow) and ON2t (black); pH = 7.4 (20 mM cacodylate buffer); [oligonucleotides] = 1.0 μM; I($NaClO_4$) = 0.10 M. For each duplex, the column represents the highest melting temperature that could be determined reliably. The error bars indicate standard errors of the average of three experiments.

Table 2. UV melting temperatures of the duplexes studied; pH = 7.4 (20 mM cacodylate buffer); [oligonucleotides] = 1.0 μM; $I(NaClO_4)$ = 0.10 M. The error limits represent standard errors of the average of three experiments.

	ON2a	ON2c	ON2g	ON2t
ON1ao (fast)	18.6 ± 0.4	43.9 ± 0.2	31.0 ± 0.2	30.5 ± 0.1
			59.2 ± 0.2	
ON1ao (slow)	16.5 ± 0.4	36.1 ± 0.3	25.5 ± 0.6	22.0 ± 0.4
	56.8 ± 0.4		51.6 ± 0.3	36.7 ± 0.3
				56.0 ± 0.2
				70.2 ± 0.2
ON1ao-Pd	19.7 ± 0.2	n.a.[a]	16.9 ± 0.3	12.6 ± 0.7
	60.1 ± 0.3		52 ± 5	34.9 ± 0.2
				55.2 ± 0.4
ON1bo (fast)	31.8 ± 0.1	28.9 ± 0.1	29.6 ± 0.2	29.5 ± 0.1
ON1bo (slow)	27.5 ± 0.2	28.1 ± 0.1	28.7 ± 0.1	27.0 ± 0.1
ON1bo-Pd	21.0 ± 0.2	20.6 ± 0.1	18.7 ± 0.3	12 ± 3
	51.5 ± 0.1			45 ± 1

[a] No sigmoidal melting curve observed.

After completion of the UV melting studies, the samples were analyzed by UPLC-ESI-TOF-MS to verify the stability of the palladacyclic oligonucleotides under the experimental conditions and to test whether the putative duplexes could be detected directly. Signals for single-stranded ON1ao-Pd or ON1bo-Pd and one of the complementary oligonucleotides were observed with each sample (Figure S48—S55 in the Supporting Information), indicating that the palladacyclic oligonucleotides persisted over several heating and cooling ramps. Duplexes, on the other hand, were not detected but this is not very surprising given the denaturing conditions inside the UPLC column (T = 60 °C and a significant fraction of methanol in the eluent).

Secondary structures of the oligonucleotide duplexes were studied by CD spectropolarimetric analysis of the same samples as used in the UV melting studies. The spectra were acquired at 10 °C intervals over a range of 10—90 °C (Figure S56—S79 in the Supporting Information). With most duplexes, the spectra obtained at 10 °C resembled those of B-type double helices, with positive and negative Cotton effects around 280 and 250 nm, respectively. With duplexes incorporating the α anomer of the acetophenone oxime residue, the latter extended further towards shorter wavelengths whereas with duplexes incorporating the β anomer, the two Cotton effects were more symmetric. Duplexes ON1ao(faster-eluting isomer)•ON2a, ON1ao(slower-eluting isomer)•ON2c, ON1ao-Pd•ON2c and ON1bo(slower-eluting isomer)•ON2t showed little ellipticity, suggesting a distorted secondary structure. In all cases, both Cotton effects diminished on increasing temperature, consistent with denaruration of the double helix.

For more quantitative comparison with the UV melting profiles, the molar ellipticity at 285 nm was plotted as a function of temperature for each duplex (Figure S80—S103 in the Supporting Information). In contrast to the UV melting profiles, the limited number of data points precluded smoothing and Gaussian fitting of the first-derivative curve. Instead, Equation 1 was fitted to the experimental data by a non-linear least-squares method, yielding a single melting temperature for each duplex.

$$[\theta]_{obs} = [\theta]_{ss} + \frac{[\theta]_{ds}-[\theta]_{ss}}{1+10^{(T_m-T)p}} \quad (1)$$

$[\theta]_{obs}$ is the observed molar ellipticity, $[\theta]_{ss}$ and $[\theta]_{ds}$ molar ellipticities of the single- and double-stranded oligonucleotides, T_m the melting temperature, T the measurement temperature and p the slope of the sigmoidal curve. With the "alpha duplexes", many of the CD melting temperatures were highly uncertain but the more reliable ones were in reasonable agreement with the UV melting temperatures corresponding to the most prominent transition. Curiously, CD melting temperatures of most of the "beta duplexes" were consistently higher than the UV melting temperatures, by 15—30 °C. A notable exception was duplex ON1bo-Pd•ON2t, with which the two values were identical within error limits.

^{15}N NMR spectrometric studies

For ^{15}N NMR spectrometric monitoring of the putative Pd(II)-mediated base pairing, ON1bo-Pd was paired with ON2t*, placing a ^{15}N3-labelled thymine opposite to the palladacyclic residue. ^{15}N3-labelled thymine was selected as the base pairing partner because of the synthetic availability of the

corresponding nucleoside phosphoramidite[43] and the unambiguous coordination preferences (exclusively N3-coordination) of thymidine[42]. ON1bo-Pd●ON2t was also one of the duplexes showing a distinct high UV melting temperature (45 ± 1 °C), tentatively assigned to Pd(II)-mediated base pairing. The measurements were carried out in D_2O at pD of 7.8 (20 mM cacodylate buffer) and ionic strength of 0.10 M (adjusted with $NaClO_4$).

^{15}N NMR spectrum of the slower-eluting isomer of the unpalladated oligonucleotide ON1bo (Figure 4A) showed a peak at 349.2 ppm, characteristic of an aromatic oxime nitrogen[44]. Likewise, the chemical shift of the sole peak in the spectrum of the complementary sequence ON2t* (Figure 4B, 156.0 ppm) was in good agreement with the previously reported values[35,36]. With the palladacyclic oligonucleotide ON1bo-Pd, two peaks of nearly equal intensity were observed (Figure 4C, 351.0 and 360.7), possibly reflecting differences in the Pd(II) coordination sphere.

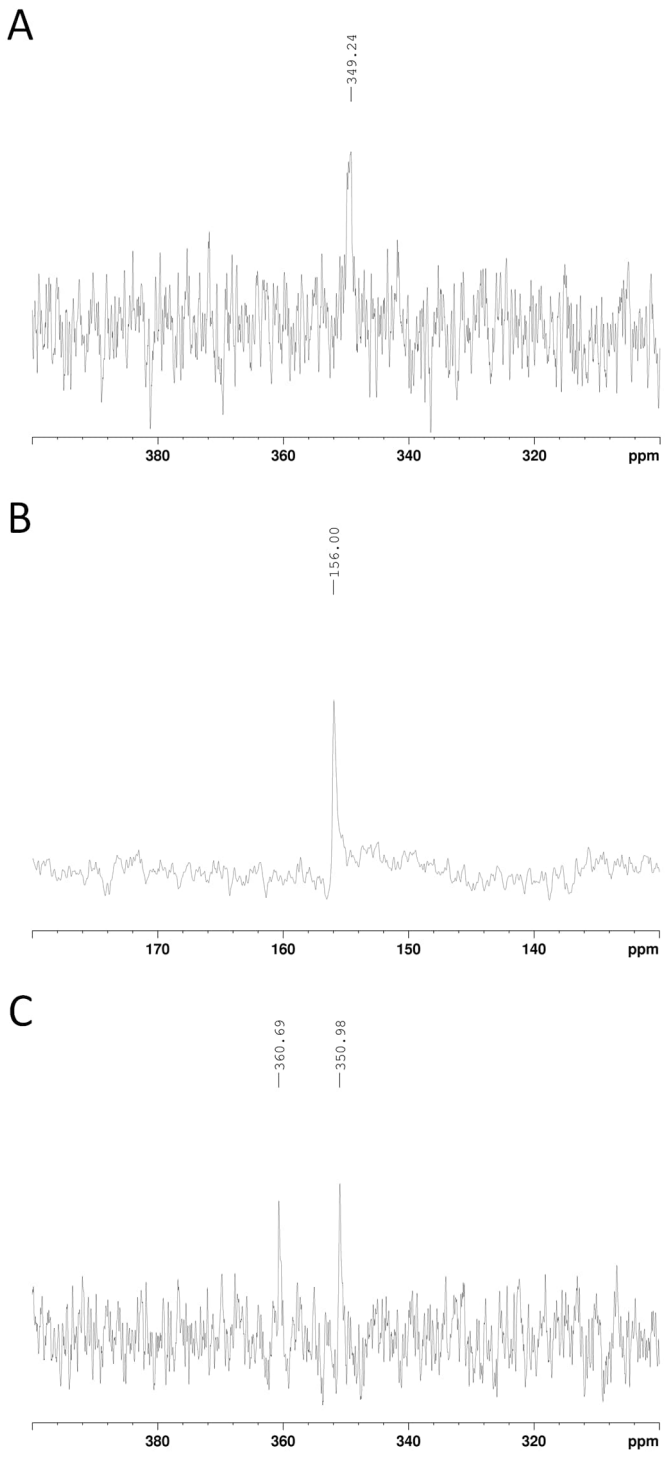


Figure 4. ^{15}N NMR spectra of oligonucleotides A) ON1bo, B) ON2T* and C) ON1bo-Pd; T = 298 K; pD = 7.8 (20 mM cacodylate buffer in D_2O); [oligonucleotides] = 150 µM; *I*($NaClO_4$) = 0.10 M.

Curiously, hybridization of equimolar amounts of ON1bo-Pd and ON2t* resulted in disappearance of the ^{15}N NMR signals of both oligonucleotides (data not shown). In the case of the complementary

strand ON2t*, this observation would be at least in qualitative agreement with signal broadening on coordination of the paramagnetic Pd(II). Disappearance of the signals of ON1bo-Pd, on the other hand, is more difficult to explain as in all likelihood the oxime nitrogen is coordinated to Pd(II) even before hybridization. ^{15}N—Pd(II)—^{15}N coupling would also decrease the signal to noise ratio, possibly below detection limit. ^{15}N NMR spectrum of ON1bo-Pd•ON2t* was recorded at 10 °C intervals over a range of 10—70 °C to find out if the signals would reappear on dissociation of the duplex, but without success (data not shown). It would be tempting to interpret these results as very high thermal stability of the putative Pd(II)-mediated base pair but we feel that such a conclusion would be premature.

Conclusion

A short DNA oligonucleotide incorporating the β anomer of an acetophenone oxime *C*-nucleoside in the middle of the sequence hybridized equally well with complementary oligonucleotides placing any of the canonical nucleobases opposite to the modified residue, yielding a single well-defined UV melting temperature. The respective palladacyclic duplexes, on the other hand, showed biphasic melting curves, with one melting temperature lower and one higher than that of the unpalladated duplex. Similar behavior has been reported previously for related duplexes and attributed to sequential dissociation of the Watson—Crick and metal mediated parts. Duplexes bearing the α anomer of the acetophenone oxime *C*-nucleoside, either in metal-free or palladacyclic form, gave much more convoluted melting curves, in line with the "unnatural" orientation of the nucleobase surrogate. The β anomer of the palladacyclic oligonucleotide gave two ^{15}N NMR signals, presumably corresponding to differences in the Pd(II) coordination sphere. Lamentably, these signals, as well as that of the ^{15}N3-labelled complementary sequence, diminished below detection limit on hybridization of the two strands. While this result is consistent with a stable Pd(II)-mediated base pair, direct and compelling NMR evidence still remains elusive.

Experimental

General methods

Unless otherwise stated, all chemicals, including the unmodified oligonucleotides, were commercial products and used as received. Et_3N was dried over CaH_2 and solvents over activated 4 Å molecular sieves. Freshly distilled Et_3N was used for the preparation of HPLC elution buffers. NMR spectra were recorded on a Bruker Biospin 500 MHz NMR spectrometer. The ^{1}H and ^{13}C chemical shifts (δ, ppm)

were referenced to the corresponding residual signal of the solvent and the ^{15}N and ^{31}P chemical shifts to external liquid ammonia and phosphoric acid, respectively. Mass spectra were recorded on a Bruker Daltonics micrOTOF-Q mass spectrometer.

2-[2-Deoxy-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-D-ribofuranosyl]acetophenone (4): 2-Deoxy-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-D-ribofuranose (2, 1.250 g, 3.319 mmol) was coevaporated from anhydrous benzene (2 × 5 mL). The residue and 2-(triphenylphosphoranylidene)acetophenone (2.500 g, 6.572 mmol) were dissolved in anhydrous benzene (100 mL) and the resulting mixture stirred at 50 °C for 45 h. Hexane (20 mL) was added, the white precipitate removed by filtration and the filtrate evaporated to dryness. The crude intermediate 3 thus obtained was dissolved in THF (8.9 mL) and KO^{t}Bu (50.0 mg, 0.446 mmol) was added. The reaction mixture was stirred at 25 °C for 5 min, after which saturated aqueous NH_4Cl (100 mL) was added. The resulting solution was extracted with a mixture EtOAc (30 mL) and hexane (70 mL), the organic phase washed with brine (100 mL), dried over Na_2SO_4 and evaporated to dryness. The residue was purified on a silica gel column eluting with a mixture of EtOAc and hexane (1:4, *v*/*v*), affording 0.9487 g (59%) of compound 4 (colorless glass) as a mixture of two anomers. ^1H NMR (500 MHz, $CDCl_3$, major anomer) δ 7.98 (d, *J* = 7.8 Hz, 2H, Bz-H2 and H6), 7.59 (t, *J* = 7.4 Hz, 1H, Bz-H4), 7.48 (t, *J* = 7.6 Hz, 2H, Bz-H3 and H5), 4.66 (m, 1H, H1′), 4.45 (m, 1H, H3′), 4.05 (m, 1H, H5′), 3.81—3.72 (m, 2H, H4′ and H5′′), 3.45 (dd, *J* = 5.5, 21.3 Hz, 1H, $BzCH_2$), 3.05 (dd, *J* = 7.1, 16.5 Hz, 1H, $BzCH_2$), 2.29 (ddd, *J* = 4.9, 6.7, 12.7 Hz, 1H, H2′), 1.89 (m, 1H, H2′′), 1.09 (m, 28H, SiCH and SiCH(CH_3)$_2$). ^{13}C NMR (126 MHz, $CDCl_3$, major anomer) δ 198.2 (Bz-CO), 136.9 (Bz-C1), 133.2 (Bz-C4), 128.6 (Bz-C3 and C5), 128.21 (Bz-C2 and C6), 85.8 (C4′), 74.0 (C1′), 73.4 (C3′), 63.64 (C5′), 44.7 ($BzCH_2$), 40.6 (C2′), 17.7—16.8 (SiCH(CH_3)$_2$), 13.6—12.5 (SiCH). ^1H NMR (500 MHz, $CDCl_3$, minor anomer) δ 7.98 (d, *J* = 7.8 Hz, 2H, Bz-H2 and H6), 7.59 (t, *J* = 7.4 Hz, 1H, Bz-H4), 7.48 (t, *J* = 7.6 Hz, 2H, Bz-H3 and H5), 4.65 (m, 1H, H1′), 4.50 (q, *J* = 7.0 Hz, 1H, H3′), 4.01 (dd, *J* = 3.2, 11.4 Hz, 1H, H5′), 3.84 (m, 1H, H4′), 3.77 (m, 1H, H5′′), 3.48 (dd, *J* = 5.9, 20.9 Hz, 1H, $BzCH_2$), 3.23 (dd, *J* = 7.4, 16.5 Hz, 1H, $BzCH_2$), 2.56 (m, 1H, H2′), 1.89 (m, 1H, H2′′), 1.09 (m, 28H, SiCH and SiCH(CH_3)$_2$). ^{13}C NMR (126 MHz, $CDCl_3$, major anomer) δ 197.8 (Bz-CO), 137.0 (Bz-C1), 133.2 (Bz-C4), 128.6 (Bz-C3 and C5), 128.18 (Bz-C2 and C6), 83.6 (C4′), 73.9 (C1′), 73.8 (C3′), 63.56 (C5′), 44.8 ($BzCH_2$), 40.7 (C2′), 17.7—16.8 (SiCH(CH_3)$_2$), 13.6—12.5 (SiCH). HRMS (ESI$^+$-TOF): *m*/*z* calcd for [$C_{25}H_{42}NaO_5Si_2$]: 501.2463; found: 501.2451 [M + Na]$^+$.

2-(2-Deoxy-D-ribofuranosyl)acetophenone (5): 2-[2-Deoxy-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-D-ribofuranosyl]acetophenone (4, 0.9702 g, 2.026 mmol) was dissolved in THF (24.3 mL). $Et_3N{\bullet}3HF$ (1.32 mL, 8.11 mmol) was added and the resulting mixture stirred at 25 °C for 72 h. The product mixture was purified on a silica gel column eluting with a mixture of MeOH and CH_2Cl_2 (8:92, *v*/*v*), affording 0.3972 g (82%) of compound 5 (colorless glass) as a mixture of two anomers. ^{1}H NMR (500 MHz, $CDCl_3$, major anomer) δ 7.95 (m, 2H, Bz-H2 and H6), 7.56 (m, 1H, Bz-H4), 7.45 (m, 2H, Bz-H3 and H5), 4.69 (m, 1H, H1′), 4.35 (m, 1H, H3′), 3.89 (q, *J* = 4.0 Hz, 1H, H4′), 3.68 (m, 1H, H5′), 3.62 (dd, *J* = 4.9, 11.8 Hz, 1H, H5′′), 3.34 (dd, *J* = 6.4, 16.4 Hz, 1H, $BzCH_2$), 3.19 (dd, *J* = 5.9, 16.6 Hz, 1H, $BzCH_2$), 2.18 (ddd, *J* = 2.4, 5.7, 13.1 Hz, 1H, H2′), 1.86 (ddd, *J* = 9.5, 6.5, 13.2 Hz, 1H, H2′′). ^{13}C NMR (126 MHz, $CDCl_3$, major anomer) δ 198.5 (Bz-CO), 136.7 (Bz-C1), 133.44 (Bz-C4), 128.68 (Bz-C3 and C5), 128.3 (Bz-C2 and C6), 87.1 (C4′), 74.7 (C1′), 73.2 (C3′), 63.1 (C5′), 44.2 ($BzCH_2$), 41.2 (C2′). ^{1}H NMR (500 MHz, $CDCl_3$, minor anomer) δ 7.95 (m, 2H, Bz-H2 and H6), 7.56 (m, 1H, Bz-H4), 7.45 (m, 2H, Bz-H3 and H5), 4.69 (m, 1H, H1′), 4.36 (m, 1H, H3′), 3.95 (q, *J* = 4.6 Hz, 1H, H4′), 3.68 (m, 1H, H5′), 3.62 (dd, *J* = 4.9, 11.8 Hz, 1H, H5′′), 3.50 (dd, *J* = 7.0, 17.0 Hz, 1H, $BzCH_2$), 3.22 (dd, *J* = 5.8, 17.5 Hz, 1H, $BzCH_2$), 2.49 (m, 1H, H2′), 1.81 (m, 1H, H2′′). ^{13}C NMR (126 MHz, $CDCl_3$, minor anomer) δ 198.9 (Bz-CO), 136.8 (Bz-C1), 133.40 (Bz-C4), 128.65 (Bz-C3 and C5), 128.2 (Bz-C2 and C6), 85.5 (C4′), 74.4 (C1′), 72.6 (C3′), 62.4 (C5′), 44.9 ($BzCH_2$), 40.4 (C2′). HRMS (ESI^+-TOF): *m*/*z* calcd for $[C_{13}H_{16}NaO_4]$: 259.0941; found: 259.0943 $[M + Na]^+$.

2-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-**α**-D-ribofuranosyl]acetophenone (6a) and 2-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-**β**-D-ribofuranosyl]acetophenone (6b): 2-(2-Deoxy-D-ribofuranosyl)acetophenone (5) was coevaporated from anhydrous pyridine (3 × 10 mL) and the residue was dissolved in anhydrous pyridine (5 mL) under N_2 atmosphere. A solution of DMTrCl (0.6220 g, 1.849 mmol) in anhydrous CH_2Cl_2 (1 mL) was added and the resulting mixture stirred at 25 °C under N_2 atmosphere for 5 h, after which it was concentrated to approximately 33% volume. The remaining solution was diluted with CH_2Cl_2 (100 mL), washed with saturated aqueous $NaHCO_3$ (100 mL), dried over Na_2SO_4 and evaporated to dryness. The residue was purified on a silica gel column eluting with a mixture of Et_3N, MeOH and CH_2Cl_2 (1:4:95, *v*/*v*), affording 0.2319 g (25%) of the α anomer 6a and 0.3614 g (39%) of the β anomer 6b (yellowish foam). ^{1}H NMR (500 MHz, CD_3CN, 6a) δ 8.04 (m, 2H, Bz-H2 and H6), 7.64 (m, 1H, Bz-H4), 7.54 (m, 2H, Bz-H3 and H5), 7.45 (m, 2H, Ph-H2 and H6), 7.31 (m, 6H, MeOPh-H2 and H6 and Ph-H3 and H5), 7.24 (m, 1H, Ph-H4), 6.87 (m, 4H, MeOPh-H3 and H5), 4.63 (m, 1H, H1′), 4.24 (m, 1H, H3′), 3.93 (q, *J* = 4.5 Hz, 1H, H4′), 3.79 (s, 6H, $ArOCH_3$), 3.50 (dd, *J* = 7.3, 16.0 Hz, 1H, $BzCH_2$), 3.21 (dd, *J* = 5.7, 16.0 Hz, 1H, $BzCH_2$), 3.17 (d, *J* = 4.6 Hz, 3′-OH), 3.06

(dd, *J* = 4.0, 10.0 Hz, 1H, H5´), 3.02 (dd, *J* = 5.4, 10.0 Hz, 1H, H5´´), 2.44 (m, 1H, H2´), 1.70 (ddd, *J* = 7.1, 5.6, 12.7 Hz, 1H, H2´´). ^{13}C NMR (126 MHz, CD_3CN, 6a) δ 198.8 (Bz-CO), 158.6 (MeOPh-C4), 145.3 (Ph-C1), 136.17 (Bz-C1), 136.16 (MeOPh-C1), 133.1 (Bz-C4), 130.0 (MeOPh-C2 and C6), 128.7 (Bz-C3 and C5), 128.2 (Bz-C2 and C6), 128.1 (Ph-C3 and C5), 127.8 (Ph-C2 and C6), 126.7 (Ph-C4), 113.0 (MeOPh-C3 and C5), 85.8 (Ar_3C), 84.7 (C4´), 75.1 (C1´), 73.0 (C3´), 64.5 (C5´), 54.9 ($ArOCH_3$), 45.1 ($BzCH_2$), 40.5 (C2´). ^{1}H NMR (500 MHz, CD_3CN, 6b) δ 7.98 (m, 2H, Bz-H2 and H6), 7.62 (m, 1H, Bz-H4), 7.50 (m, 2H, Bz-H3 and H5), 7.46 (m, 2H, Ph-H2 and H6), 7.32 (m, 6H, MeOPh-H2 and H6 and Ph-H3 and H5), 7.24 (m, 1H, Ph-H4), 6.85 (m, 4H, MeOPh-H3 and H5), 4.63 (m, 1H, H1´), 4.22 (m, 1H, H3´), 3.81 (m, 1H, H4´), 3.77 (s, 6H, $ArOCH_3$), 3.41 (dd, *J* = 7.2, 16.3 Hz, 1H, $BzCH_2$), 3.15 (dd, *J* = 5.6, 16.4 Hz, 1H, $BzCH_2$), 3.13 (br, 1H, 3´-OH), 3.07 (dd, *J* = 4.3, 10.0 Hz, 1H, H5´), 3.01 (dd, *J* = 5.0, 9.9 Hz, 1H, H5´´), 2.06 (ddd, *J* = 2.0, 5.5, 12.9 Hz, 1H, H2´), 1.84 (ddd, *J* = 6.2, 9.7, 13.1 Hz, 1H, H2´´). ^{13}C NMR (126 MHz, CD_3CN, 6b) δ 198.4 (Bz-CO), 158.6 (MeOPh-C4), 145.3 (Ph-C1), 136.16 (Bz-C1), 136.15 (MeOPh-C1), 133.1 (Bz-C4), 130.0 (MeOPh-C2 and C6), 128.7 (Bz-C3 and C5), 128.1 (Bz-C2 and C6 and Ph-C3 and C5), 127.8 (Ph-C2 and C6), 126.8 (Ph-C4), 113.0 (MeOPh-C3 and C5), 85.9 (C4´), 85.8 (Ar_3C), 74.8 (C1´), 73.1 (C3´), 64.6 (C5´), 54.9 ($ArOCH_3$), 44.5 ($BzCH_2$), 40.8 (C2´). HRMS (ESI$^+$-TOF): *m*/*z* calcd for [$C_{34}H_{34}NaO_6$]: 561.2248; found: 561.2259 [M + Na]$^+$.

2-{2-Deoxy-5-*O*-(4,4´-dimethoxytrityl)-3-*O*-[(2-cyanoethoxy)-(*N*,*N*-diisopropylamino)phosphinyl]-**α**-D-ribofuranosyl}acetophenone (1a): 2-[2-Deoxy-5-*O*-(4,4´-dimethoxytrityl)-α-D-ribofuranosyl]acetophenone (6a, 0.2319 g, 0.4305 mmol) was dissolved in anhydrous CH_2Cl_2 (4.5 mL) under N_2 atmosphere. Et_3N (300 µL, 2.15 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (116 µL, 0.520 mmol) were added and the resulting mixture stirred at 25 °C under N_2 atmosphere for 1 h. The product mixture was diluted with CH_2Cl_2 (20 mL), washed with saturated aqueous $NaHCO_3$ (20 mL), dried over Na_2SO_4 (20 mL) and evaporated to afford 0.2327 g (73%) of compound 1a (white foam) in sufficient purity for oligonucleotide synthesis. ^{1}H NMR (500 MHz, CD_3CN) δ 8.03 (m, 2H, Bz-H2 and H6), 7.64 (m, 1H, Bz-H4), 7.54 (m, 2H, Bz-H3 and H5), 7.45 (m, 2H, Ph-H2 and H6), 7.32 (m, 6H, MeOPh-H2 and H6 and Ph-H3 and H5), 7.24 (m, 1H, Ph-H4), 6.87 (m, 4H, MeOPh-H3 and H5), 4.72 (m, 1H, H1´), 4.48 (m, 1H, H3´), 4.11 (m, 1H, H4´), 3.79 (s, 3H, $ArOCH_3$), 3.78 (s, 3H, $ArOCH_3$), 3.77—3.63 (m, 2H, $POCH_2$), 3.63—3.47 (m, 3H, PNCH and $BzCH_2$), 3.23 (m, 1H, $BzCH_2$), 3.13 (m, 1H, H5´), 3.04 (m, 1H, H5´´), 2.61 (t, *J* = 6.0 Hz, 1H, $NCCH_2$), 2.52 (m, 2H, $NCCH_2$ and H2´), 1.95—1-81 (m, 1H, H2´´), 1.17 (d, *J* = 6.5 Hz, 3H, $NCH(CH_3)_2$), 1.16 (d, *J* = 6.4 Hz, 3H, $NCH(CH_3)_2$), 1.13 (d, *J* = 6.8 Hz, 3H, $NCH(CH_3)_2$), 1.06 (d, *J* = 6.8 Hz, 3H, $NCH(CH_3)_2$). ^{13}C NMR (126 MHz, CD_3CN) δ 198.7 (Bz-CO), 158.6 (MeOPh-C4), 145.3 (Ph-C1), 137.39 (Bz-C1), 137.35 (Bz-C1), 136.10 (MeOPh-C1),

136.05 (MeOPh-C1), 136.04 (MeOPh-C1), 136.00 (MeOPh-C1), 133.1 (Bz-C4), 130.0 (MeOPh-C2 and C6), 128.7 (Bz-C3 and C5), 128.14 (Bz-C2 and C6), 128.13 (Bz-C2 and C6), 128.07 (Ph-C3 and C5), 128.0 (Ph-C3 and C5), 127.8 (Ph-C2 and C6), 126.77 (Ph-C4), 126.75 (Ph-C4), 118.5 (CN), 118.4 (CN), 113.0 (MeOPh-C3 and C5), 85.9 (Ar_3C), 84.2 (d, *J* = 3.8 Hz, C4´), 84.0 (d, *J* = 6.2 Hz, C4´), 75.54 (C1´), 75.47 (d, *J* = 16.3 Hz, C3´), 75.1 (d, *J* = 17.1 Hz, C3´), 64.3 (C5´), 64.2 (C5´), 58.41 (d, *J* = 18.4 Hz, $POCH_2$), 58.38 (d, *J* = 19.0 Hz, $POCH_2$), 54.92 ($ArOCH_3$), 54.90 ($ArOCH_3$), 44.87 ($BzCH_2$), 44.85 ($BzCH_2$), 42.9 (d, *J* = 12.4 Hz, PNCH), 39.50 (d, *J* = 3.6 Hz, C2´), 39.47 (d, *J* = 4.6 Hz, C2´), 23.92 ($NCH(CH_3)_2$), 23.88 ($NCH(CH_3)_2$), 23.86 ($NCH(CH_3)_2$), 23.83 ($NCH(CH_3)_2$), 20.03 (d, *J* = 7.2 Hz, $NCCH_2$), 19.97 (d, *J* = 7.1 Hz, $NCCH_2$). ^{31}P NMR (202 MHz, CD_3CN) δ 147.6. HRMS (ESI^+-TOF): *m*/*z* calcd for [$C_{34}H_{34}NaO_6$]: 739.3507; found: 739.3541 [M + H]$^+$.

2-{2-Deoxy-5-*O*-(4,4´-dimethoxytrityl)-3-*O*-[(2-cyanoethoxy)-(*N*,*N*-diisopropylamino)phosphinyl]-**α**-D-ribofuranosyl}acetophenone (1b): 2-[2-Deoxy-5-*O*-(4,4´-dimethoxytrityl)-α-D-ribofuranosyl]acetophenone (6b, 0.1566 g, 0.2907 mmol) was dissolved in anhydrous CH_2Cl_2 (3.0 mL) under N_2 atmosphere. Et_3N (202 µL, 1.45 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (78 µL, 0.351 mmol) were added and the resulting mixture stirred at 25 °C under N_2 atmosphere for 1 h. The product mixture was diluted with CH_2Cl_2 (20 mL), washed with saturated aqueous $NaHCO_3$ (20 mL), dried over Na_2SO_4 (20 mL) and evaporated to afford 0.1826 g (85%) of compound 1b (white foam) in sufficient purity for oligonucleotide synthesis. ^1H NMR (500 MHz, CD_3CN) δ 7.99 (m, 2H, Bz-H2 and H6), 7.63 (m, 1H, Bz-H4), 7.51 (m, 2H, Bz-H3 and H5), 7.46 (m, 2H, Ph-H2 and H6), 7.31 (m, 6H, MeOPh-H2 and H6 and Ph-H3 and H5), 7.23 (m, 1H, Ph-H4), 6.85 (m, 4H, MeOPh-H3 and H5), 4.64 (m, 1H, H1´), 4.45 (m, 1H, H3´), 3.98 (m, 1H, H4´), 3.80 (m, 1H, $POCH_2$), 3.777 (s, 1.5H, $ArOCH_3$), 3.774 (s, 1.5H, $ArOCH_3$), 3.772 (s, 1.5H, $ArOCH_3$), 3.769 (s, 1.5H, $ArOCH_3$), 3.69 (m, 1H, $POCH_2$), 3.59 (m, 2H, PNCH), 3.46 (m, 1H, $BzCH_2$), 3.19 (dd, *J* = 5.3, 16.5 Hz, 1H, $BzCH_2$), 3.14 (m, 1H, H5´), 3.02 (m, 1H, H5´´), 2.65 (t, *J* = 6.0 Hz, 1H, $NCCH_2$), 2.54 (t, *J* = 6.0 Hz, 1H, $NCCH_2$), 2.33—2.19 (m, 1H, H2´), 1.92 (ddd, *J* = 6.2, 9.7, 13.1 Hz, 1H, H2´´), 1.19 (d, *J* = 6.9 Hz, 3H, $NCH(CH_3)_2$), 1.17 (d, *J* = 5.7 Hz, 3H, $NCH(CH_3)_2$), 1.16 (d, *J* = 5.6 Hz, 3H, $NCH(CH_3)_2$), 1.07 (d, *J* = 6.8 Hz, 3H, $NCH(CH_3)_2$). ^{13}C NMR (126 MHz, CD_3CN) δ 198.2 (Bz-CO), 158.6 (MeOPh-C4), 145.3 (Ph-C1), 137.3 (Bz-C1), 136.10 (MeOPh-C1), 136.04 (MeOPh-C1), 135.99 (MeOPh-C1), 133.1 (Bz-C4), 130.06 (MeOPh-C2 and C6), 130.04 (MeOPh-C2 and C6), 130.02 (MeOPh-C2 and C6), 128.7 (Bz-C3 and C5), 128.2 (Bz-C2 and C6), 128.10 (Bz-C2 and C6), 128.09 (Ph-C3 and C5), 128.04 (Ph-C3 and C5), 127.8 (Ph-C2 and C6), 126.77 (Ph-C4), 126.75 (Ph-C4), 118.6 (CN), 118.4 (CN), 113.0 (MeOPh-C3 and C5), 85.87 (Ar_3C), 85.86 (Ar_3C), 85.20 (d, *J* = 4.4 Hz, C4´), 84.95 (d, *J* = 5.5 Hz, C4´), 75.4 (d, *J* = 17.2 Hz, C3´), 75.2 (d, *J* = 16.9 Hz, C3´),

75.1 (C1´), 75.0 (C1´), 64.14 (C5´), 64.10 (C5´), 58.4 (d, J = 19.6 Hz, $POCH_2$), 54.9 ($ArOCH_3$), 44.27 ($BzCH_2$), 44.25 ($BzCH_2$), 43.0 (d, J = 12.3 Hz, PNCH), 39.9 (d, J = 3.6 Hz, C2´), 39.8 (d, J = 4.7 Hz, C2´), 23.93 (d, J = 3.6 Hz, $NCH(CH_3)_2$), 23.89 (d, J = 3.6 Hz, $NCH(CH_3)_2$), 23.87 (d, J = 3.6 Hz, $NCH(CH_3)_2$), 23.83 (d, J = 3.6 Hz, $NCH(CH_3)_2$), 20.06 (d, J = 7.3 Hz, $NCCH_2$), 19.97 (d, J = 6.6 Hz, $NCCH_2$). ^{31}P NMR (202 MHz, CD_3CN) δ 147.3, 147.2. HRMS (ESI$^+$-TOF): m/z calcd for [$C_{34}H_{34}NaO_6$]: 739.3507; found: 739.3541 [M + H]$^+$.

Oligonucleotide synthesis

Oligonucleotides ON1ak and ON1bk were assembled in 40 µmol scale on polystyrene support by an ÄKTA Oligopilot plus 10 DNA/RNA synthesizer. Conventional phosphoramidite strategy was followed, with 5-(benzylthio)-1*H*-tetrazole as the activator. A recycling time of 180 s was employed for the commercially available building blocks as well as the acetophenone *C*-nucleoside phosphoramidites 1a and 1b. The coupling yields, based on trityl response, were essentially quantitative throughout the syntheses. After chain assembly, the oligonucleotide products were released from the solid supports and the base and phosphate protections removed by incubation in 25 % aqueous NH_3 at 55 °C for 16 h. The crude products were purified by RP-HPLC on a Hypersil ODS C18 column (250 × 10 mm, 5 µm) eluting with a linear gradient (10—40% over 25 min) of MeCN in aqueous triethylammonium acetate, the flow rate being 3.0 mL min^{-1} and the detection wavelength 260 or 290 nm (for the largest injections, a wavelength offset from the absorption maximum of the oligonucleotides was used to avoid saturation of the detector). HPLC traces are presented in Figures S15A and S16A of the Supporting Information. The identity of the purified oligonucleotides was established by ESI-TOF-MS and the quantity by UV spectrophotometry. The UV spectra were recorded over a range of 200—400 nm with a Jenway Genova Nano micro-volume spectrophotometer and the molar absortivities of the oligonucleotides were calculated by an implementation of the nearest-neighbors method, assuming the absorptivity of the acetophenone residue to be negligible.

The ketone oligonucleotides ON1ak and ON1bk were converted to the corresponding ^{15}N-labelled oxime oligonucleotides ON1ao and ON1bo by incubation in 50 mM aqueous ^{15}N-hydroxylammonium chloride at 25 °C for 96 h. The product mixtures were fractioned by RP-HPLC (Figures S15B and S16B of the Supporting Information) and the purified products characterized and quantified as described above. Two peaks of nearly equal retention times and identical mass spectra were obtained for both ON1ao and ON1bo, most likely corresponding to *E* and *Z* isomers of the oxime moiety.

Cyclopalladation of the oxime oligonucleotides ON1ao and ON1bo was carried out by incubation in an aqueous solution of Li_2PdCl_4 and NaOAc at 55 °C for 96 h. Concentrations of the oligonucleotide, Li_2PdCl_4 and NaOAc were 4.0 mM, 6.0 mM and 24 mM, respectively. The product mixtures were fractioned by RP-HPLC (Figure S17 of the Supporting Information) and the purified products characterized and quantified as described above. Several peaks showing the expected mass spectrum were observed, in line with previous reports on other palladacyclic oligonucleotides.

UV and CD melting studies

Sample preparation for the UV and CD melting studies was identical. Equimolar (1.0 μM) mixtures of a modified (ON1ao, ON1bo, ON1ao-Pd or ON1bo-Pd) and a natural (ON2a, ON2c, ON2g or ON2t) oligonucleotide were prepared in 20 mM cacodylate buffer (pH = 7.40), the ionic strength of which was adjusted to 0.10 M with $NaClO_4$. For the measurement, the samples were placed in quartz cuvettes with optical path length of 10 mm.

UV melting profiles were acquired by a PerkinElmer Lambda 35 UV/vis spectrophotometer equipped with a Peltier temperature control unit. Three heating and cooling ramps (10—90 °C, 0.5 °C min^{-1}) were carried out on each sample and absorbance at 260 nm recorded at 0.5 °C intervals. UV melting temperatures were determined by Gaussian fitting of the first derivative curves of the UV melting profiles.

CD spectra were acquired by an Applied Photophysics Chirascan spectropolarimeter equipped with a Peltier temperature control unit. For each sample, nine spectra at 10 °C intervals over a temperature range of 10—90 °C were recorded. At each temperature, the samples were allowed to equilibrate for either 120 s (unmetallated duplexes) or 1800 s (metallated duplexes) before measurement. The wavelength range employed was 220—360 nm. CD melting temperatures were determined by non-linear least-squares fitting of Equation 1 to plots of molar ellipticity at 285 nm as a function of temperature.

^{15}N NMR spectrometric studies

Samples for the ^{15}N NMR spectrometric studies were prepared by lyophilizing appropriate amounts of the aqueous stock solutions of oligonucleotides ON1bo, ON1bo-Pd and ON2t* and redissolving the residue in D_2O, the pD of which was adjusted to 7.8 (20 mM cacodylate buffer) and the ionic strength

to 0.10 M ($NaClO_4$). The oligonucleotide concentration of the samples was 150 µM. The spectra were acquired on a Bruker Biospin 500 MHz instrument equipped with a BBO cryoprobe. For single-stranded ON1bo, ON1bo-Pd and ON2t*, a single spectrum was recorded at 25 °C. With the duplex ON1bo-Pd•ON2t*, seven spectra were recorded at 10 °C intervals over a range of 10—70 °C. 22000 scans spanning 25252 Hz (spectral width) were averaged for a typical spectrum. Default parameters for a 1D ^{15}N NMR measurement were used, with the exception of a longer pre-scan delay (30 µs). The spectra were processed with an exponential window function, resulting in a line broadening of 2.0 Hz.

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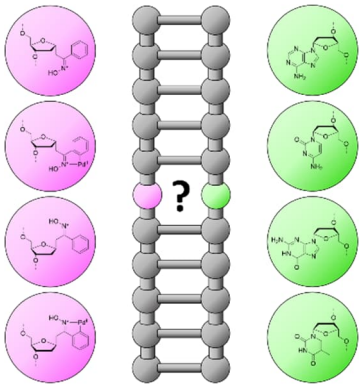
Entry for the Table of Contents



Oligodeoxynucleotides incorporating either an α or a β anomer of an ^{15}N-labelled palladacyclic acetophenone oxime *C*-nucleoside were synthesized and hybridized with various natural counterparts. UV, CD and ^{15}N NMR data were consistent with a stable Pd(II)-mediated base pair in the middle of the duplex but failed to prove it conclusively.

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