Oligonucleotides Incorporating Palladacyclic Nucleobase Surrogates

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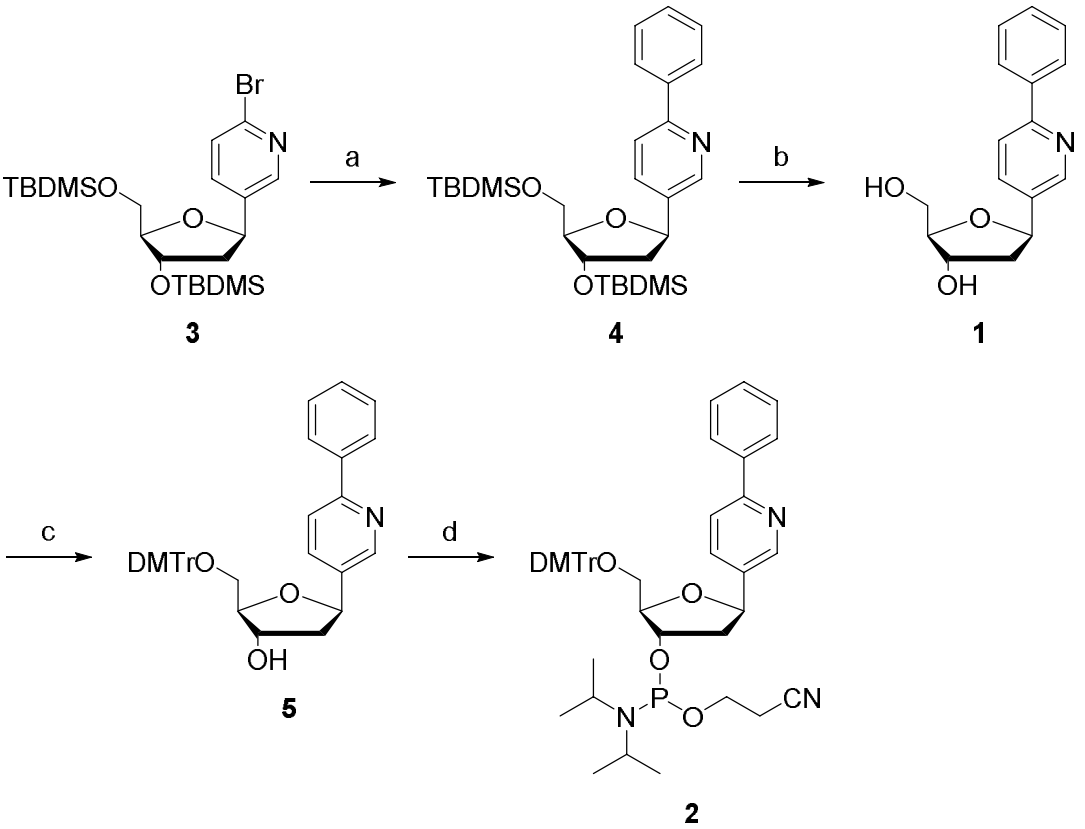
Abstract

An oligonucleotide incorporating a palladacyclic nucleobase has been prepared by ligand-directed metalation of a phenylpyridine moiety. This oligonucleotide hybridized with natural counterparts placing any of the canonical nucleobases opposite to the palladacyclic residue. The palladated duplexes had B-type conformation and melting temperatures comparable to those of respective unmodified duplexes with a single mismatch. In the duplexes placing C, G or T (but not A) opposite to the palladacyclic residue, greatly increased absorptivity suggested formation of a Pd^{II}-mediated base pair. Absorptivity and ellipticity of these duplexes persisted even at the highest temperatures applicable in T_m and CD experiments (90 °C). Evidently the Pd^{II}-mediated base pairs do not dissociate under the experimental conditions.

Metal mediated base pairing has attracted increasing attention over the past decade, mainly because of potential applications in DNA nanotechnology,[1] such as sensors for metal ions,[2] molecular wires[3] and DNA-templated nanoparticles.[4] The superior hybridization affinity of metal ion carrying oligonucleotides[5] could also make them valuable chemotherapeutic agents but intracellular concentrations of suitable transition metals are too low to support metal mediated base pairing. In other words, the bridging metal ion must be supplied with the therapeutic oligonucleotide and the oligonucleotide metal complex must resist dissociation under the metal-deficient conditions of the cell. The only way to achieve this with metals other than platinum[6] is by incorporating an organometallic nucleobase. Palladacyclic bases appear particularly attractive given their stability and similar coordination chemistry with platinum. Several palladacycles have, in fact, been reported as potential anticancer agents[7] but incorporation of such compounds into oligonucleotides has, to the best of our knowledge, yet to be demonstrated. Herein we describe for the first time synthesis and hybridization properties of an oligonucleotide incorporating an organopalladium base moiety.

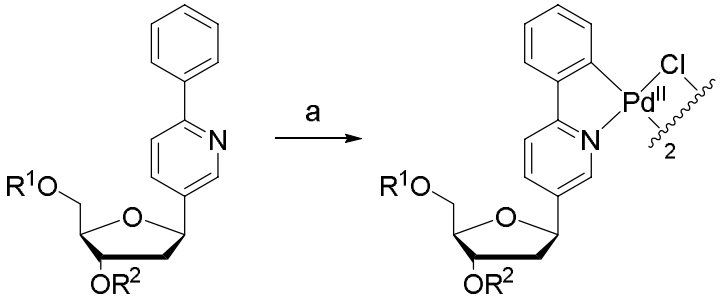
Synthesis of 2-phenylpyridine C-nucleoside 1 and its phosphoramidite building block 2 is depicted in Scheme 1. Suzuki-Miyaura coupling of compound 3[8] and phenylboronic acid afforded the persilylated 2-phenylpyridine nucleoside 4. Desilylation furnished compound 1 and subsequent 5´-

tritylation and 3´-phosphitylation the phosphoramidite 2. Preparation of compound 1 by a similar strategy but via 2-chloropyridine (rather than 2-bromopyridine) nucleoside has been described in the literature.[9]



Scheme 1 Synthesis of the 2-phenylpyridine C-nucleoside phosphoramidite 2. Reagents and conditions: a) $PhB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, toluene, Ar atmosphere, 110 °C, 3 h, 98%; b) $Et_3N{\bullet}3HF$, THF, N_2 atmosphere, 25 °C, 14 h, 99%; c) DMTrCl, pyridine, 25 °C, 12 h, 83%; d) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, Et_3N, CH_2Cl_2, 25 °C, 2 h, 99%.

Palladation was first explored with compound 1 (Scheme 2). Of the reagents tested ($Pd(OAc)_2$, $PdCl_2$, K_2PdCl_4 and Li_2PdCl_4), Li_2PdCl_4 proved the most efficient. With 1 eq. of Li_2PdCl_4 and 3 eq. of NaOAc, quantitative conversion was achieved in 4 h at 55 °C. ^1H and ^{13}C NMR spectra of the product showed loss of one ortho-proton of the phenyl ring compared to the starting material and a 37 ppm downfield shift of the respective carbon signal, consistent with proton-Pd^{II} exchange. Palladation was also evident in HRMS, with a main peak corresponding to $[C_{32}H_{32}N_2O_6ClPd_2]^+$. Metalation of 2-phenylpyridine and related compounds typically yields dimeric complexes with bridging halido or acetato ligands[10, 11] and formation of a similar complex (Scheme 2, compound 6) appears likely also in the present case.



1 : $R^1 = R^2 = H$ **6** : $R^1 = R^2 = H$

ON1p : R^1 = CGAGC-3´, **ON1p-Pd** : R^1 = CGAGC-3´,

R^2 = 5´-CTGGC R^2 = 5´-CTGGC

Scheme 2 Palladation of nucleoside 1 and the corresponding modified oligonucleotide ON1p. Reagents and conditions: a) Li_2PdCl_4, NaOAc, MeOH, H_2O, 55 °C.

Table 1 summarizes the sequences of the oligonucleotides used. ON1p and ON2s were assembled on an automated DNA synthesizer by the standard phosphoramidite protocol with an extended coupling time (300 s) for incorporation of building block 2. ON1p was palladated under the same conditions as the monomer 1 except that 3 eq. of Li_2PdCl_4 was used. To rule out off-target palladation, ON1c, having cytosine in place of 2-phenylpyridine, was subjected to the same conditions and found to be unreactive (data presented in the Supporting Information). All modified oligonucleotides were purified by RP-HPLC, characterized by ESI-TOF-MS and quantified UV spectrophotometrically. With ON1p-Pd, MS analysis revealed collapse of the initial dimeric product into a monopalladated single strand, in all likelihood during chromatographic purification.

Table 1 Oligonucleotides used in this study.

	Sequence[a]
ON1p	5´-CGAGC<u>P</u>CTGGC-3´
ON1p-Pd	5´-CGAGC<u>P^{Pd}</u>CTGGC-3´
ON1c	5´-CGAGC<u>C</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´
ON2s	5´-GCCAG<u>S</u>GCTCG-3´

[a] P refers to 2-phenylpyridine, P^{Pd} to palladated 2-phenylpyridine and S to an abasic site (2-(hydroxymethyl)tetrahydrofuran-3-ol spacer). The residue varied in the hybridization studies has been underlined.

Hybridization of ON1p and ON1p-Pd was studied at pH 7.4 by recording the melting profiles of duplexes formed with the complementary strands ON2a, ON2c, ON2g, ON2t and ON2s, placing either A, C, G, T or an abasic site opposite to the modified base. Each sample was annealed by incubating at 90 °C for several minutes and then allowing to gradually cool down before the measurement. Duplexes formed by ON1p exhibited sigmoidal melting curves (Fig. 1 for ON1p:ON2g and Supporting Information for the other duplexes) with T_m values ranging from 34 to 39 °C (Fig. 2), typical for an 11-mer DNA double helix with a single mismatch. A similar T_m was observed for the duplex ON1p:ON2s where phenylpyridine is paired with an abasic site. Evidently there is little, if any, hydrogen bonding between phenylpyridine and the canonical nucleobases.

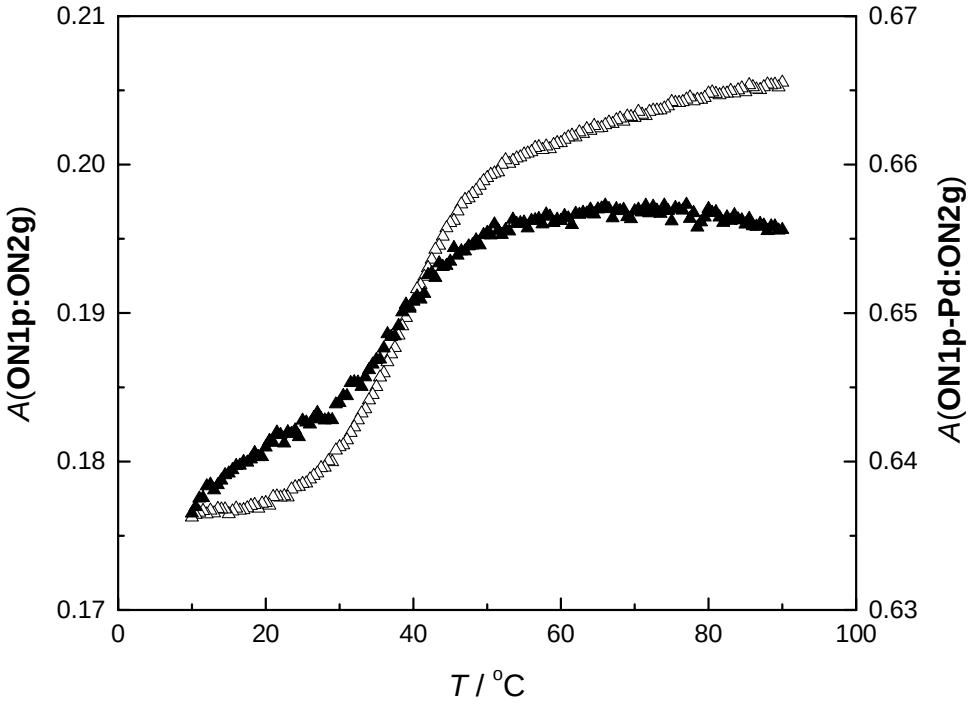


Figure 1 UV melting profile of ON1p:ON2g (△) and ON1p-Pd:ON2g (▲); pH = 7.4 (20 mM cacodylate buffer); [oligonucleotides] = 1.0 μM; I($NaClO_4$) = 80 mM. The left and right vertical axes refer to absorbance of the unpalladated and palladated duplex, respectively. The two axes have the same length but are offset to account for the different absorbance of the duplexes at 10 °C.

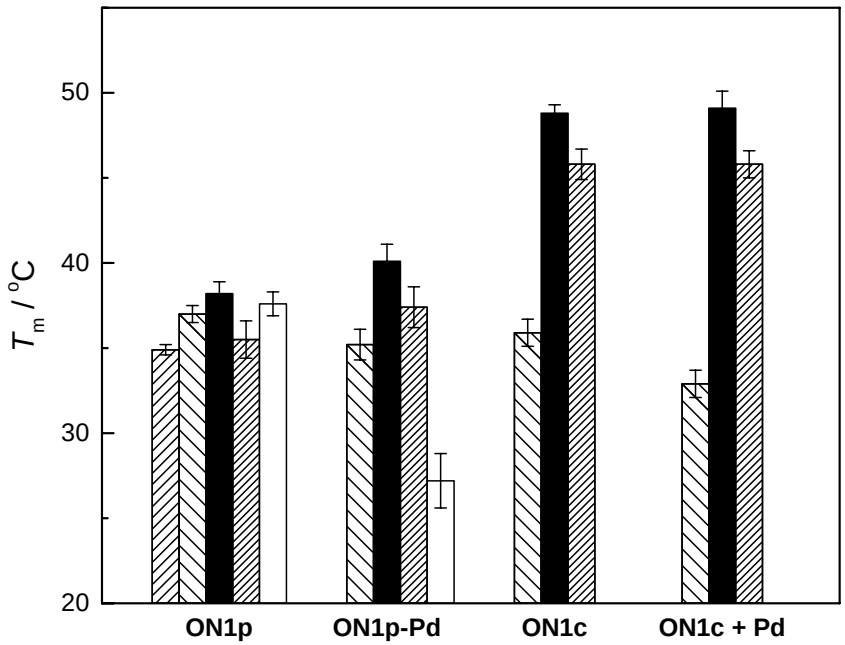


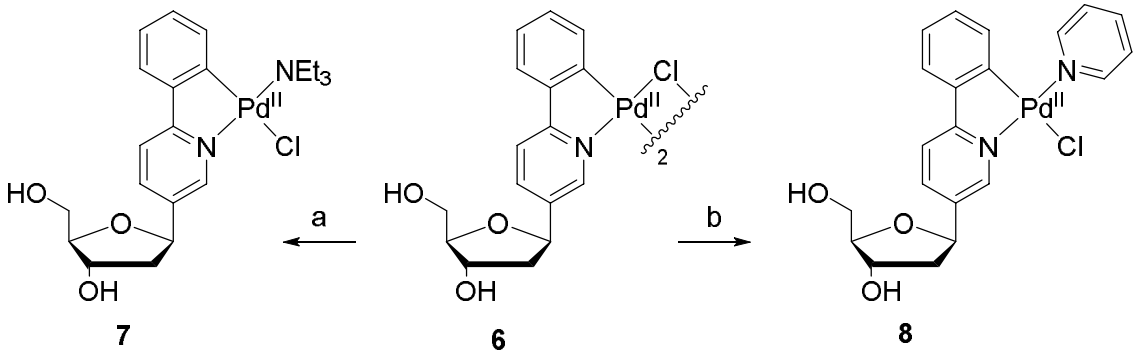
Figure 2 Melting temperatures of the duplexes formed by ON1p, ON1p-Pd and ON1c with ON2a (medium hash), ON2c (sparse hash), ON2g (black), ON2t (dense hash) and ON2s (white); pH = 7.4 (20 mM cacodylate buffer); [oligonucleotides] = 1.0 μM; I($NaClO_4$) = 80 mM. In the case of ON1c, the measurements were also carried out in the presence of 1.0 μM Li_2PdCl_4.

Melting profiles of the palladated duplexes (Fig. 1 for ON1p:ON2g and Supporting Information for the other duplexes) were more convoluted than those of their unpalladated counterparts and in most cases did not allow determination of a single T_m. Comparison of T_m of the most prominent transition with T_m of the respective unpalladated duplex revealed modest stabilization with G and T and destabilization with C as the base pairing partner (Fig. 2). Curiously, ON1p-Pd:ON2s, pairing palladated phenylpyridine with an abasic site, was 10 °C less stable than its unpalladated counterpart ON1p:ON2s. The possibility that the differences in the melting profiles of ON1p and ON1p-Pd were caused by contamination of the latter by free Pd^{II} was ruled out by control experiments on duplexes formed by ON1c in the absence and presence of 1.0 μM Li_2PdCl_4. The impact of Li_2PdCl_4 was modestly destabilizing with ON1c:ON2c and negligible with ON1c:ON2g and ON1c:ON2t (Fig. 2), the shapes of the melting profiles (presented in the Supporting Information) being unaffected.

The most striking difference between duplexes formed by ON1p and ON1p-Pd was observed in their absorptivities. While the values of all duplexes formed by ON1p were typical for 11-mer oligonucleotides (1.8×10^5 M^{-1} cm^{-1} at 10 °C), the respective values for ON1p-Pd:ON2c, ON1p-

Pd:ON2g and ON1p-Pd:ON2c were much higher (6 – 7 × 10^5 M^{-1} cm^{-1}). Interestingly, absorptivities of ON1p-Pd:ON2a and ON1p-Pd:ON2s were similar to those of the unpalladated duplexes (2.4 and 1.8 × 10^5 M^{-1} cm^{-1}, respectively), indicating that the high values recorded for the other palladated duplexes are not inherent to ON1p-Pd alone.

The high absorptivity of ON1p-Pd:ON2c, ON1p-Pd:ON2g and ON1p-Pd:ON2t could stem from expanded π electron system of a Pd^{II}-mediated base pair between cyclopalladated phenylpyridine and C, G or T. The effect could be amplified if base stacking forced planarity on the base pair. This hypothesis was tested by recording UV spectra of two model compounds derived from dimer 6. Treatment of 6 with aq. triethylammonium acetate (Scheme 3, Route a) mimicked the conditions of the HPLC purification of ON1p-Pd, presumably affording the triethylamine derivative 7. Treatment with pyridine (Scheme 3, Route b), in turn, lead to the pyridine derivative 8, serving as a model for a Pd^{II}-mediated base pair. Pyridine was used instead of the canonical nucleosides because the excess could be removed easily and quantitatively by simple evaporation.



Scheme 3 Conversion of compound 6 into triethylamine and pyridine derivatives. Reagents and conditions: a) Et_3NH^{+}-OAc, H_2O; b) pyridine.

UV spectra of 1, 6, 7 and 8 are presented in Fig. 3. The spectrum of 1 closely resembles the one previously reported for 2-phenylpyridine,[12] with peaks of 27400 and 22300 M^{-1} cm^{-1} at 247 and 280 nm, respectively. The palladacyclic dimer 6, in turn, exhibited strong peaks at 250 and 262 nm (ε = 28100 and 32600 M^{-1} cm^{-1}, respectively) and weaker ones at 306 and 312 nm (ε = 11700 and 11600 M^{-1} cm^{-1}, respectively). The spectrum of the triethylamine derivative 7 had peaks at similar wavelengths (249, 261, 304 and 313 nm) but with much lower absorptivities (3100, 3300, 1200 and 1200 M^{-1} cm^{-1}, respectively). Finally, with the pyridine derivative 8 the corresponding wavelengths were 250, 260, 304 and 314 nm and absorptivities 21600, 24100, 7500 and 7400 M^{-1} cm^{-1}. Previous studies on cyclopalladated derivatives of 2-phenylpyridine reveal a similar pattern: while all complexes had peaks at similar wavelengths, highest absorptivities were observed with aromatic ligands.[10] The

results, hence, support the idea of Pd^{II}-mediated base pairing as the origin of the high absorptivity of ON1p-Pd:ON2c, ON1p-Pd:ON2g and ON1p-Pd:ON2t.

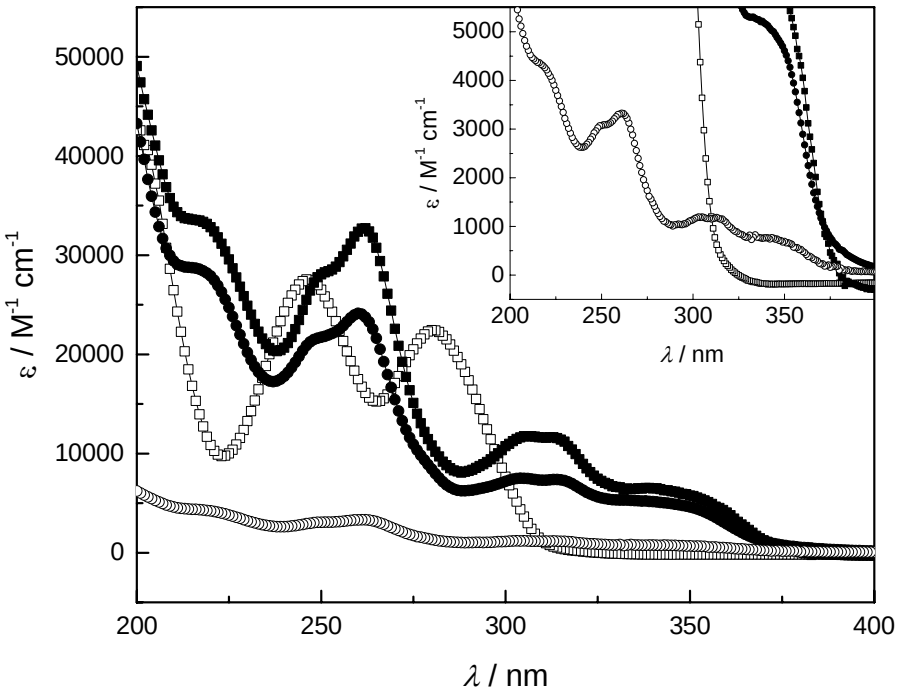


Figure 3 UV spectra of compounds 1 (□), 6 (■), 7 (○) and 8 (●).

Pd^{II}-mediated base pairing most likely involves coordination of N3 of cytosine or thymine or N1 of guanine trans to the pyridine nitrogen, with a chlorido ligand completing the square planar coordination sphere (Fig. 4).[13] With G and T, coordination is accompanied by deprotonation. Persistence of the unusually high absorptivity over the temperature range of the T_m measurements (10 – 90 °C) suggests that the putative base pairs do not dissociate appreciably while the thermal hyperchromicity is explained by unwinding of the flanking Watson-Crick regions. Pd^{II}-mediated base pairing with adenine cannot be confirmed or discounted based on the data at hand.

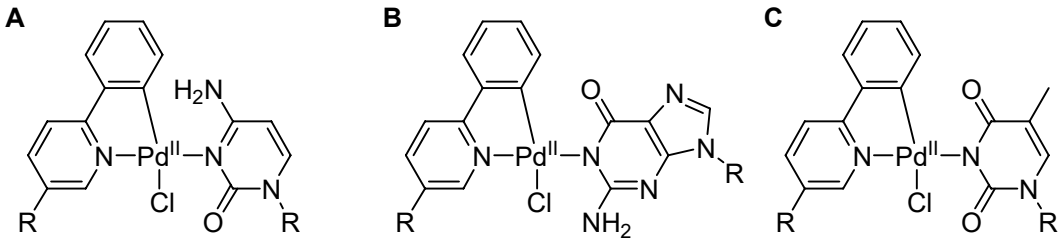


Figure 4 Proposed structures of Pd^{II}-mediated base pairs between cyclopalladated phenylpyridine and A) cytosine, B) guanine and C) thymine.

Secondary structure of the modified duplexes was studied CD spectropolarimetrically over a temperature range of 10 – 90 °C at 10 °C intervals, with an equilibration time of 600 s before each acquisition (Fig. 5 for ON1p:ON2g and ON1p-Pd:ON2g and Supporting Information for the other duplexes). All spectra were typical for B-type double helices, with positive and negative peaks at 280 and 250 nm, respectively. Thermal diminution of the peaks, consistent with unwinding of the double helix, was also observed with all duplexes but with the palladated ones considerable ellipticity persisted even at 90 °C. Plotting the ellipticity at 250 nm as a function of temperature largely corroborated the results of the UV melting experiments with the unpalladated duplexes but resulted in systematically higher (albeit also less accurate) melting temperatures for the palladated duplexes (T_m values obtained from UV and CD experiments are summarized in Table S1 of the Supporting Information).

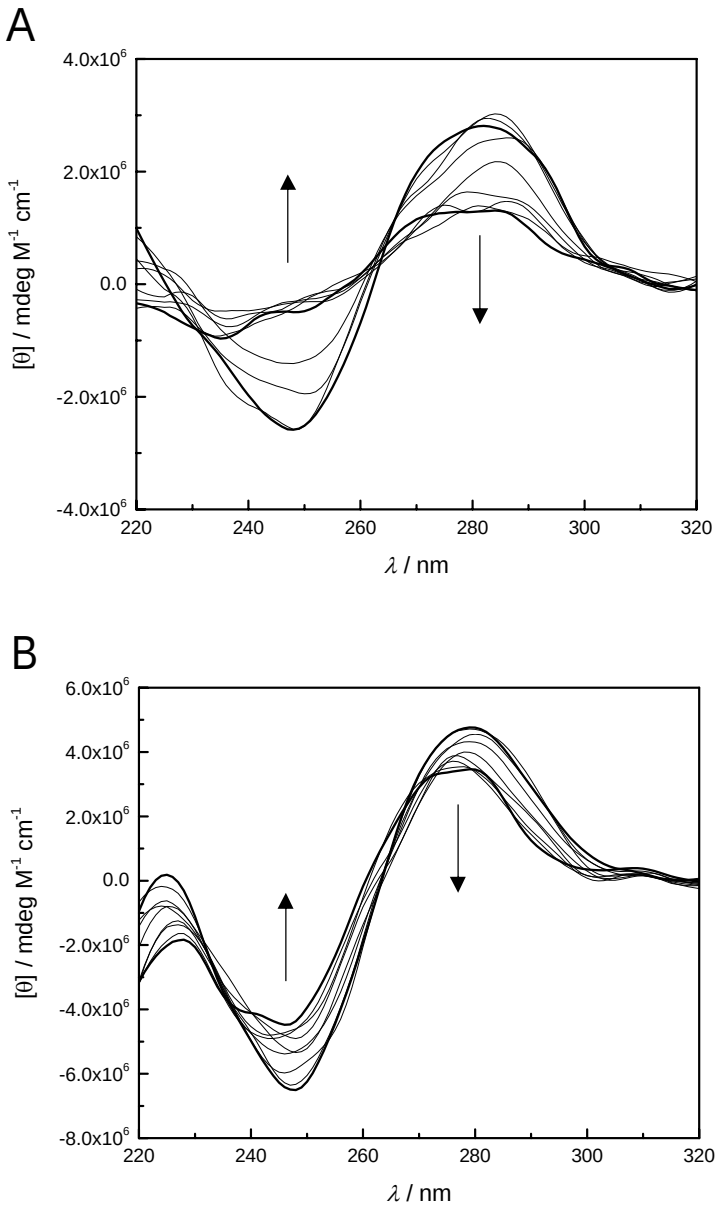


Figure 5 CD spectra of A) ON1p:ON2g and B) ON1p-Pd:ON2g at 10 °C intervals between 10 and 90 °C; pH = 7.4 (20 mM cacodylate buffer); [oligonucleotides] = 1.0 μM; I($NaClO_4$) = 80 mM. The spectra acquired at the extreme temperatures are indicated by thicker lines and the thermal loss of ellipticity by arrows.

Oligonucleotides incorporating palladacyclic nucleobases are readily available by ligand-directed metalation of appropriate “hot spots”, such as 2-phenylpyridine residues, under conditions where other parts of the oligonucleotide remain intact. Within double-helical oligonucleotides, cyclopalladated phenylpyridine forms highly stable Pd^{II}-mediated base pairs with canonical nucleobases. Dependence of the photophysical properties of the base pair on the ligand coordinated

trans to the pyridine nitrogen allows direct monitoring of Pd^{II}-mediated base pairing and, possibly, identification of the base pairing partner.

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