Covalently Palladated Oligonucleotides Through Oxidative Addition of Pd(0)

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Abstract

An 11-mer oligonucleotide incorporating a central (2-iodobenzoylamino)methyl residue has been synthesized and palladated by oxidative addition of $Pd_2(dba)_3$. UV melting profiles of the duplexes formed by the palladated oligonucleotide with its natural complements were biphasic and the higher melting temperatures (T_m) exhibited considerable hysteresis. CD spectra, in turn, resembled those of canonical B-type double helices. Two-step denaturation, with the "low-T_m" melting involving only canonical base pairs and the "high-T_m" melting involving also dissociation of a Pd(II)-mediated base pair, appears the most likely explanation for the observed UV melting profiles. As the latter step in all cases takes place at a higher temperature than denaturation of natural duplexes of the same length, the putative Pd(II)-mediated base pairs are stabilizing.

Introduction

An increasing number of applications of metal-mediated base pairing have been proposed over the past decade, including sensors for metal ions, molecular wires, DNA-templated nanoparticles and, more recently, recognition of nucleic acid sequences.[1] To widen the scope of the latter application to biological systems, i.e. to overcome the scarcity of transition metals available for metal-mediated base pairing in the intracellular medium, we have explored the possibility of using oligonucleotides with organometallic nucleobase surrogates.[2, 3] We have successfully synthesized such structures by two approaches – either direct mercuration of electron-rich sites on aromatic rings[4] or ligand-directed cyclopalladation of benzylamine or phenylpyridine moieties.[5] Palladation of aromatic rings without the assistance of a directing ligand would also be desirable, as the resulting monodentate organopalladium complexes would leave the palladium center more exposed and amenable to pseudo-linear coordination (Figure 1).

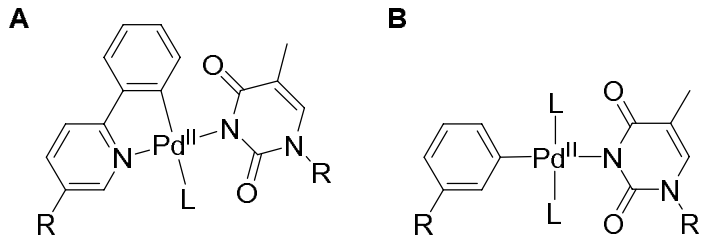


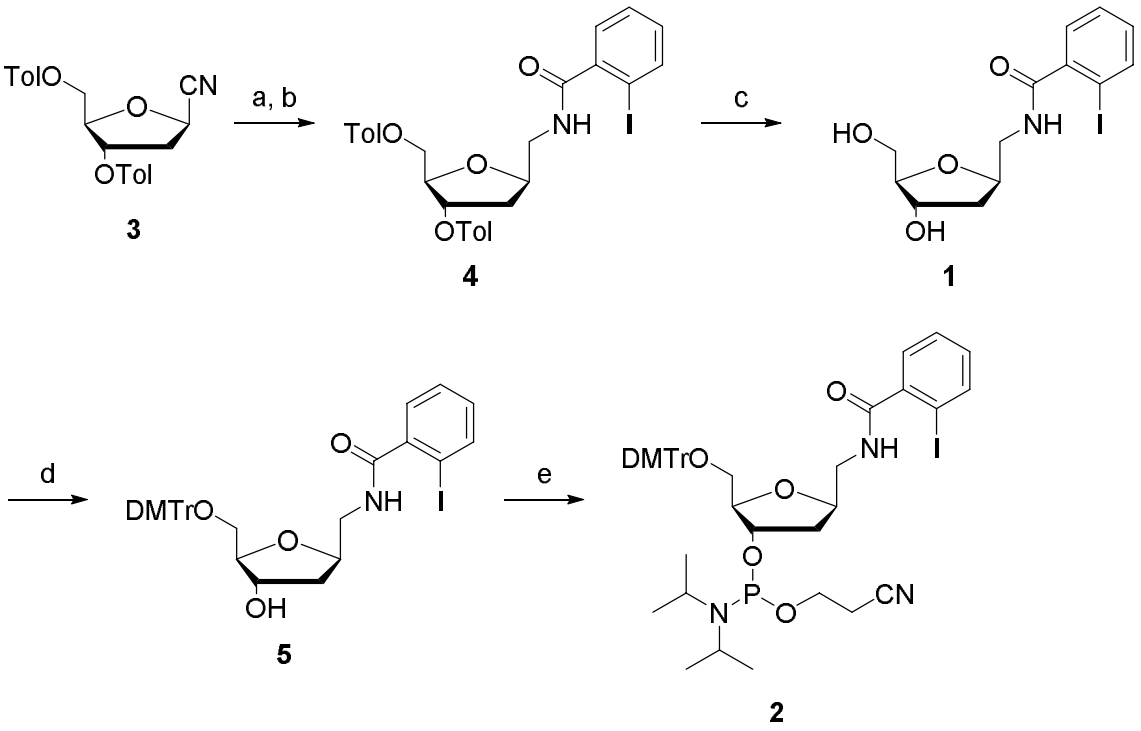
Figure 1. Pd(II)-mediated base pairs formed by A) a pallacyclic and B) a monodentate organopalladium nucleobase surrogate. The latter would be expected to be more easily accommodated within the base stack of a double helix.

Herein we describe the synthesis and hybridization properties of an oligonucleotide incorporating a monodentate organopalladium nucleobase surrogate by oxidative addition of Pd(0) to a 2-iodobenzamide residue. Palladation of a 5-iodouracil residue by a similar method has been reported previously[6] but we found the resulting oligonucleotides to be only transiently stable. Hybridization of the palladated oligonucleotides with their natural counterparts exhibited considerable hysteresis, in all likelihood attributable to the relatively slow ligand exchange at Pd(II).

Results and Discussion

Building block synthesis

Synthesis of the (2-iodobenzoylamino)methyl nucleoside 1 and its phosphoramidite building block 2 is outlined in Scheme 1. The cyano group of 2,5-anhydro-3-deoxy-4,6-di-O-toluoyl-D-ribohexononitrile[7] (3) was first selectively reduced by treatment with sodium trifluoroacetoxyborohydride, after which the resulting primary amino group was acylated with 2-iodobenzamide to give the protected (2-iodobenzamido)methyl nucleoside 4. Sodium methoxide catalyzed methanolysis of the toluoyl protections afforded the unprotected nucleoside 1 and conventional 6-O-tritylation and 4-O-phosphitylation the phosphoramidite building block 2.



Scheme 1. Synthesis of the (2-iodobenzoylamino)methyl nucleoside phosphoramidite 2. Reagents and conditions: a) $NaBH_4$, TFA, THF, 25 °C, 2 h; b) 2-iodobenzoyl chloride, THF, 25 °C, 4 h; c) NaOMe, MeOH, 40 °C, 1h; d) 4,4´-dimethoxytrityl chloride, pyridine, 25 °C, 16 h; e) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, Et_3N, CH_2Cl_2, N_2 atmosphere, 25 °C, 1 h.

Oligonucleotide synthesis and palladation

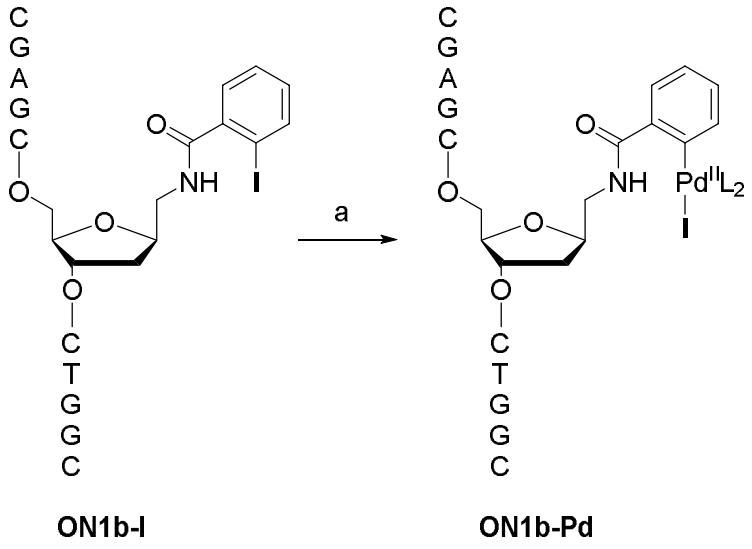
Table 1 summarizes the sequences of the oligonucleotides used in the present study. The modified oligonucleotide ON1b-I was synthesized on solid support by conventional phosphoramidite strategy. The unmodified regions flanking the modified residue were assembled by an automated DNA/RNA synthesizer. The modified building block 2, in turn, was coupled manually to avoid wasting it in priming the synthesizer. Coupling yields for the automated and manual couplings were approximately 99 and 61%, respectively. Removal of the base and phosphate protections and cleavage from the solid support were accomplished by conventional ammonolysis. Finally, the modified oligonucleotide was purified by RP HPLC, characterized mass spectrometrically and quantified UV spectrophotometrically.

Table 1. Sequences of the oligonucleotides used in this study.

	Sequence[a]
ON1b-I	5´-CGAGC$\underline{B^{I}}$CTGGC-3´
ON1b-Pd	5´-CGAGC$\underline{B^{Pd}}$CTGGC-3´
ON2a	5´-GCCAG$\underline{A}$GCTCG-3´
ON2c	5´-GCCAG$\underline{C}$GCTCG-3´
ON2g	5´-GCCAG$\underline{G}$GCTCG-3´
ON2t	5´-GCCAG$\underline{T}$GCTCG-3´
ON2s	5´-GCCAG$\underline{S}$GCTCG-3´

[a] B^{I} refers to (2-iodobenzoylamino)methyl and B^{Pd} to (2-palladabenzoylamino)methyl base surrogate and S to an abasic site (2-(hydroxymethyl)tetrahydrofuran-3-ol spacer). The residues varied in the hybridization studies have been underlined.

Palladation of oligonucleotide ON1b-I (Scheme 2) was carried out by treatment with a large excess of $Pd_2(dba)_3$ in a mixture of argon-purged water and acetonitrile (1:1, v/v). Small molecular ortho-palladated benzamides have been synthesized previously under similar conditions.[8] After overnight incubation at 55 °C, the reaction mixture was fractioned by RP HPLC. The fraction containing the desired monopalladated product ON1b-Pd was identified mass spectrometrically and its concentration determined UV spectrophotometrically.



ON1b-I **ON1b-Pd**

Scheme 2. Palladation of oligonucleotide ON1b-I by oxidative addition. Reagents and conditions: $Pd_2(dba)_3$, H_2O, MeCN, Ar atmosphere, 55 °C, 18 h.

Hybridization studies

Hybridization affinity of the covalently palladated oligonucleotide ON1b-Pd for its unmodified counterparts ON2a, ON2c, ON2g, ON2t and ON2s was assessed by measuring the UV melting temperatures (T_m) of the corresponding duplexes. In these duplexes, either adenine, cytosine, guanine, thymine or an abasic site was placed opposite to the palladated residue. For reference, similar experiments were also carried out on the unmetalated oligonucleotide ON1b-I. The measurements were carried out at pH 7.4 (20 mM cacodylate buffer) and 0.10 M ionic strength (adjusted with $NaClO_4$), concentration of the oligonucleotides being 1.0 μM.

Denaturation and renaturation curves for duplexes formed by ON1b-Pd are presented in Figure 2A-E. In all cases, the denaturation curves were biphasic, with "low" T_m values ranging from 21 to 29 °C and "high" T_m values from 68 to 72 °C. The renaturation curves were also biphasic with similar "low" T_m values. The "high" T_m values, on the other hand, were considerably lower, ranging from 52 to 66 °C. In other words, considerable hysteresis was observed between denaturation and renaturation, especially with ON1b-Pd•ON2a, ON1b-Pd•ON2t and ON1b-Pd•ON2s. The differences between the denaturation and renaturation T_m values are illustrated in Figure 2F (all T_m values are tabulated in the Supporting Information). The equilibrium T_m values lie somewhere between these two extremes[9] but the overlapping of the two transitions on the biphasic curves precludes a more detailed analysis. It is worth pointing out, however, that even the lower limits for the "high" T_m are higher than the T_m of a respective fully matched natural duplex under the same conditions (approximately 50 °C).[2] Averages of the upper and lower limits would in all cases mean considerable stabilization. In contrast, melting profiles of duplexes formed by the unmetalated oligonucleotide ON1b-I were monophasic with T_m values of approximately 30 °C and did not exhibit appreciable hysteresis (renaturation curves are presented in the Supporting Information).

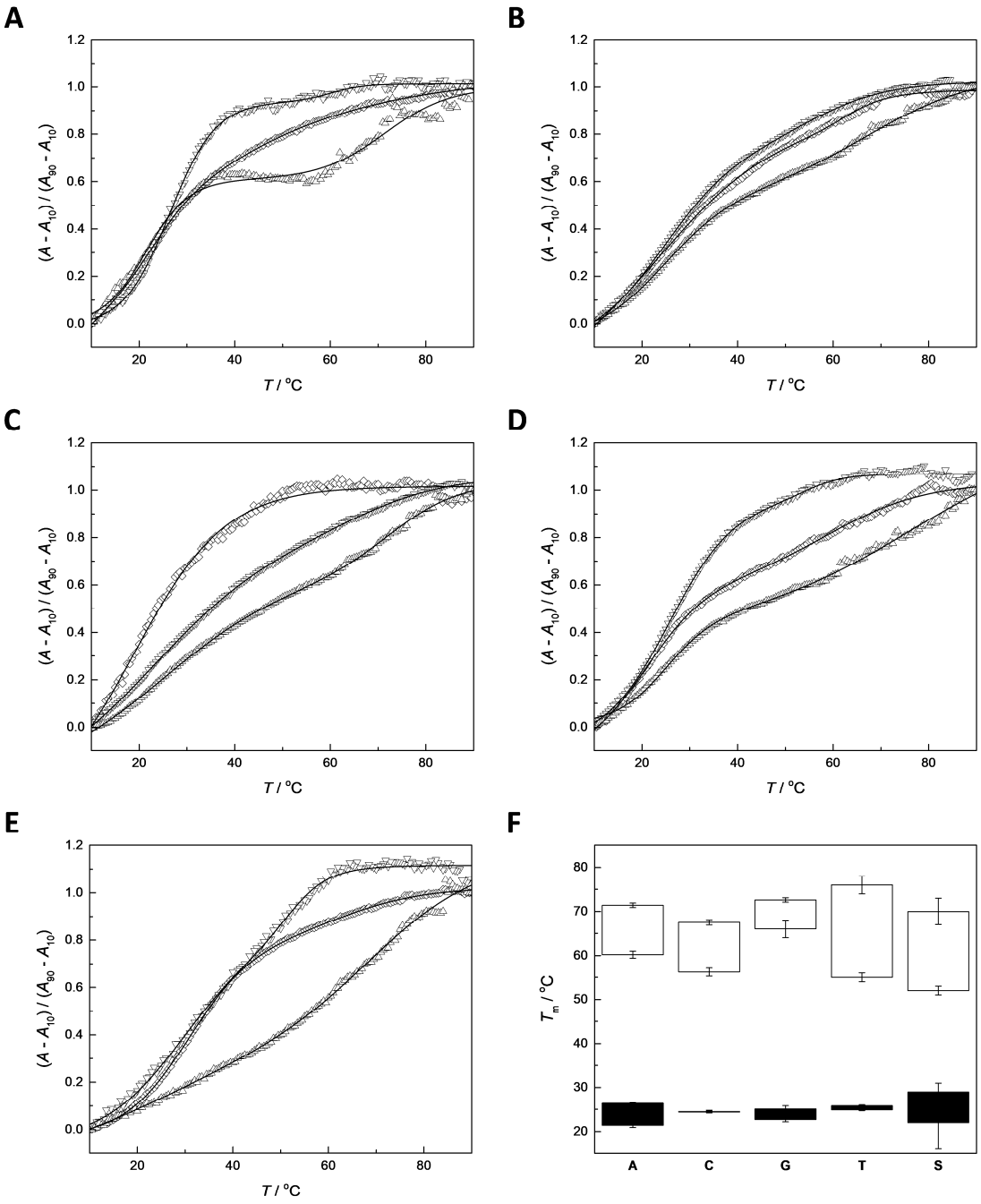
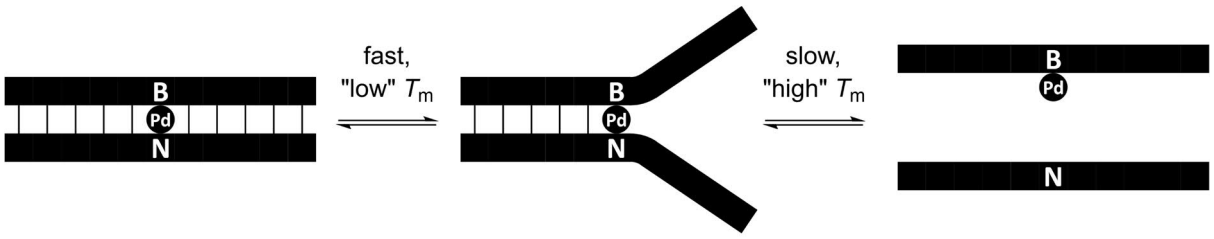


Figure 2. UV denaturation (△) and renaturation (▽) curves in the absence of competing ligands and averaged denaturation and renaturation curves (◇) in the presence of 2-mercaptoethanol for duplexes ON1b-Pd•ON2a (A), ON1b-Pd•ON2c (B), ON1b-Pd•ON2g (C), ON1b-Pd•ON2t (D) and ON1b-Pd•ON2s (E); differences between the "low" (black) and "high" (white) T_m values obtained from the denaturation and renaturation curves of duplexes ON1b-Pd•ON2a, ON1b-Pd•ON2c, ON1b-Pd•ON2g, ON1b-Pd•ON2t and ON1b-Pd•ON2s in the absence of competing ligands; pH = 7.4 (20 mM cacodylate buffer); I($NaClO_4$) = 0.10 M; [oligonucleotides] = 1.0 μM; [2-mercaptoethanol] = 0 / 100 μM.

Hybridization of two short oligonucleotides composed entirely of canonical nucleobases is very rapid so the observed hysteresis and, hence, the “high” melting temperature should be related to the palladated residue and most likely to ligand exchange at Pd(II). This hypothesis was tested by repeating the UV melting experiments in the presence of 2-mercaptoethanol as a competing ligand. Under these conditions, hysteresis was not observed and the “high” melting temperatures could not be determined reliably in most cases. The “low” melting temperatures were largely unaffected with ON1b-Pd•ON2a, ON1b-Pd•ON2c and ON1b-Pd•ON2s and approximately 5 °C lower than in the absence of 2-mercaptoethanol with ON1b-Pd•ON2g and ON1b-Pd•ON2t. As expected, introduction of 2-mercaptoethanol had no effect on the melting temperatures of duplexes formed by the unmetalated oligonucleotide ON1b-I.

The sensitivity of the “high-T_m” transition and the relative insensitivity of the “low-T_m” transition to the presence of 2-mercaptoethanol strongly suggest that the former involves ligand exchange at the Pd(II) center and the latter does not. Presumably one of the flanking double-helical stems opens at a low temperature without dissociation of the putative Pd(II)-mediated base pair (Scheme 3). The low melting temperature of this first transition could indicate strain caused by suboptimal geometry of the Pd(II)-mediated base pair. Opening of the remaining stem, on the other hand, would only take place with concomitant dissociation of the Pd(II)-mediated base pair, resulting in a much higher melting temperature. The observed hysteresis is the result of this process (and its reversal) being relatively slow. It is also worth pointing out that any difficulties in accommodating the Pd(II)-mediated base pair in the base stack would be largely alleviated on opening of either of the flanking double helices. In ON1b-Pd•ON2s the palladated residue is placed opposite to an abasic site and thus lacks an obvious base pairing partner. Observation of hysteresis even with this duplex suggests, hence, Pd(II)-mediated base pairing with one of the bases flanking the abasic site.



Scheme 3. Schematic presentation of the two-step denaturation of duplexes formed by the covalently palladated oligonucleotide ON1b-Pd.

The palladated duplexes were also studied by circular dichroism (CD) spectropolarimetry to gain further insight into their structure. The CD spectra were recorded at 10 °C intervals over a temperature range of 10 – 90 °C under otherwise the same conditions as used in the UV melting experiments. At each temperature, the samples were allowed to equilibrate for 2000 s before acquisition. The spectra obtained at the low end of the temperature range were consistent with formation of somewhat distorted B-type double helices, with minima at approximately 250 nm and maxima at approximately 280 nm (spectra presented in the Supporting Information). Increasing temperature led to diminution of both signals in all cases although with ON1b-Pd•ON2g, ON1b-

Pd•ON2t and ON1b-Pd•ON2s some ellipticity persisted even at 90 °C. The general trend expected for thermal denaturation of the duplexes was, hence, evident but the data were unfortunately too scattered to allow a meaningful comparison with the UV melting profiles.

Pd(II) coordination sites on canonical nucleobases include the N3 of cytosine and thymine and N1 and N7 of adenine and guanine.[10] With the latter, N7 coordination is usually favored by sterically demanding Pd(II) complexes,[11] such as the ortho-palladated benzamide of the present study. Coordination of the nucleobase can in principle take place either cis or trans to the carbon donor and both geometries are feasible given the flexibility provided by the methylene bridge between the anomeric carbon and the amide nitrogen (Figure 3). Trans coordination would require some shearing of the Pd(II)-mediated base pair but no more than with the relatively stable guanine-uracil or hypoxanthine-uracil wobble base pairs. Cis coordination would result in a more natural orientation of the glycosidic bonds but also more steric crowding. We consider, hence, trans coordination to be more likely.

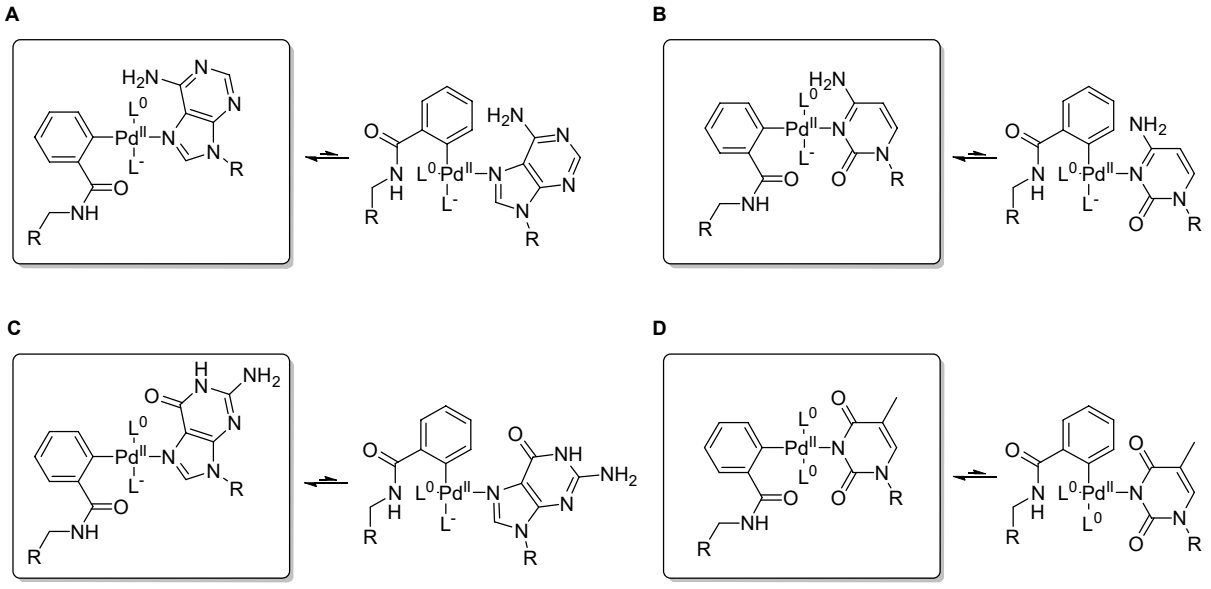


Figure 3. Proposed Pd(II)-mediated base pairs formed in duplexes ON1b-Pd•ON2a (A), ON1b-Pd•ON2c (B), ON1b-Pd•ON2g (C) and ON1b-Pd•ON2t (D). The trans isomers, deemed to be the more likely structures, have been framed. L^0 refers to a neutral and L^- to an anionic ligand.

The charge on the canonical nucleobase may determine which ligand it displaces. The most stabilizing metal-mediated base pairs reported typically have no net charge.[12] Adenine and cytosine are neutral N donors that could form a charge-neutral Pd(II)-mediated base pair by displacing a neutral ligand, such as acetonitrile, triethylamine or water. Similarly, thymine-N3, on concomitant deprotonation, would displace a negatively charged ligand, such as iodido, acetato or hydroxo. With guanine, the situation is more complicated – N1 coordination always involves deprotonation whereas N7 coordination does not. Finally, the possibility of the amide oxygen occupying one of the coordination sites cannot be verified or discounted based on the data at hand.

Conclusion

Post-synthetic palladation of an oligonucleotide featuring an iodoaryl base moiety has been accomplished by oxidative addition of a Pd(0) reagent. The method described allows the synthesis of covalently palladated oligonucleotides without the assistance of an appropriately positioned guiding ligand. Hybridization of the covalently palladated oligonucleotide with natural complementary sequences exhibited considerable hysteresis, in all likelihood attributable to relatively slow dissociation and association of the putative Pd(II)-mediated base pairs. In all cases, this transition took place at a higher temperature than the dissociation of natural duplexes of the same length, indicating that the Pd(II)-mediated base pairs are stabilizing.

Experimental

General methods

Organic solvents were of reagent grade and, when necessary, dried over 3Å molecular sieves. Freshly distilled triethylamine was used for preparation of HPLC elution buffers. All other chemicals, including unmodified oligonucleotides, were commercial products and used as received. NMR spectra were recorded on Bruker Biospin 500 and 600 MHz NMR spectrometers and the chemical shifts (δ, ppm) are given relative to the residual solvent peak. Mass spectra were recorded on a Bruker Daltonics micrOTOF-Q ESI mass spectrometer.

4,6-Di-O-toluoyl-1-(2-Iodobenzoylamino)-2,5-anhydro-1,3-dideoxy-D-ribohexitol (4)

2,5-Anhydro-3-deoxy-4,6-di-O-toluoyl-D-ribohexononitrile (3, 500 mg, 1.32 mmol) was dissolved in THF (25 mL). $NaBH_4$ (0.150 g, 3.96 mmol) and TFA (0.150 mL, 1.96 mmol) were added and the resulting mixture stirred at 25 °C for 2 h. 2-Iodobenzoyl chloride (0.350 g, 1.31 mmol) was added and stirring at 25 °C continued for 4 h. The reaction mixture was evaporated to dryness and the residue dissolved in CH_2Cl_2 (50 mL) and washed with saturared aq. $NaHCO_3$ (50 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified on a silica gel column eluting with a mixture of MeOH and CH_2Cl_2 (1:99, v/v) to yield 466 mg (56%) of the desired product 4. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.95 (d, J = 8.2 Hz, 2H), 7.86 (d, J = 8.2 Hz, 2H), 7.85 (dd, J_1 = 7.9 Hz, J_2 = 1.1 Hz, 1H), 7.40 (dd, J_1 = 7.7 Hz, J_2 = 1.8 Hz, 1H), 7.34 (ddd, J_1 = 7.6 Hz, J_2 = 7.4 Hz, J_3 = 1.1 Hz, 1H), 7.27 (d, J = 8.2 Hz, 2H), 7.20 (d, J = 8.2 Hz, 2H), 7.09 (ddd, J_1 = 7.9 Hz, J_2 = 7.4 Hz, J_3 = 1.8 Hz, 1H), 6.48 (dd, J_1 = 5.7 Hz, J_2 = 5.6 Hz, 1H), 5.51 (m, 1H), 4.59 – 4.49 (m, 3H), 4.41 (m, 1H), 3.86 (ddd, J_1 = 14.1 Hz, J_2 = 5.7 Hz, J_3 = 3.2 Hz, 1H), 3.61 (ddd, J_1 = 14.1 Hz, J_2 = 5.9 Hz, J_3 = 5.8 Hz, 1H), 2.44 (s, 3H), 2.42 (s, 3H), 2.31 (ddd, J_1 = 13.9 Hz, J_2 = 5.4 Hz, J_3 = 1.4 Hz, 1H), 2.23 (ddd, J_1 = 13.9 Hz, J_2 = 10.5 Hz, J_3 = 6.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 169.7, 166.5, 166.2, 144.2, 143.9, 142.0, 139.9, 131.1, 129.7, 129.6, 129.22, 129.21, 128.2, 128.1, 92.6, 83.1, 77.9, 76.7, 64.5, 42.5, 35.3, 21.72, 21.70. HRMS (ESI^+) m/z calcd 636.0854 obsd 636.0815 $[M + Na]^+$.

1-(2-Iodobenzoylamino)-2,5-anhydro-1,3-dideoxy-D-ribohexitol (1)

Compound 4 (466 mg, 0.759 mmol) was dissolved in MeOH (7.6 mL). NaOMe (4.1 mg, 0.076 mmol) was added and the resulting mixture stirred at 40 °C for 1 h, after which it was neutralized with Dowex 50WX8 (H^+) cation exchange resin. The resin was removed by flitration and the filtrate evaporated to dryness. The residue was purified on a silica gel column eluting with a mixture of MeOH and CH_2Cl_2 (1:99, v/v) to yield 100 mg (34%) of the desired product 1. ^{1}H NMR (CD_3CN, 500 MHz): δ = 7.90 (dd, J_1 = 8.0 Hz, J_2 = 0.9 Hz, 1H), 7.43 (ddd, J_1 = J_2 = 7.5 Hz, J_3 = 1.1 Hz, 1H), 7.34 (dd, J_1 = 7.6 Hz, J_2 = 1.7 Hz, 1H), 7.21 (br, 1H), 7.16 (ddd, J_1 = J_2 = 7.6 Hz, J_3 = 1.7 Hz, 1H), 4.29 (m, 1H), 4.21 (m, 1H), 3.72 (m, 1H), 3.57 – 3.36 (m, 4H), 1.99 – 1.86 (m, 2H). ^{13}C NMR (CD_3CN, 125 MHz): δ = 169.9, 142.8, 139.6, 130.9, 128.2, 128.0, 92.3, 87.1, 76.9, 72.4, 62.4, 43.3, 37.8. HRMS (ESI^+) m/z calcd 400.0016 obsd 400.0013 $[M + Na]^+$.

4-O-[(2-Cyanoethoxy)(N,N-diisopropylamino)phosphinyl]-6-O-(4,4´-dimethoxytrityl)-1-(2-iodobenzoylamino)-2,5-anhydro-1,3-dideoxy-D-ribohexitol (2)

Compound 1 (100 mg, 0.265 mmol) was coevaporated from anhydrous pyridine (3 $\times$ 10 mL) and the residue was dissolved in anhydrous pyridine (10 mL). 4,4´-Dimethoxytrityl chloride (98.8 mg, 0.292 mmol) was added and the resulting mixture stirred at 25 °C for 16 h. The reaction mixture was concentrated under reduced pressure, diluted with CH_2Cl_2 (50 mL) and washed with saturared aq. $NaHCO_3$ (50 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness and the residue passed through a silica gel column eluting with a mixture of MeOH and CH_2Cl_2 (1:99, v/v). The purified intermediate 5 (18.0 mg, 0.0265 mmol) was dissolved in anhydrous CH_2Cl_2 (1.0 mL). Et_3N (100 μL, 0.717 mmol) and 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite (9.0 μL, 0.0403 mmol) were added and the resulting mixture stirred at 25 °C under N_2 atmosphere for 1 h, after which it was diluted with CH_2Cl_2 (20 mL) and washed with saturared aq. $NaHCO_3$ (20 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness to afford 20.0 mg (85%) of the desired product 2 as a mixture of R_P and S_P diastereomers. ^{1}H NMR ($CDCl_3$, 500 MHz, mixture of two diastereomers): δ = 7.86 (d, J = 8.0 Hz, 1H), 7.45 – 7.38 (m, 2H), 7.36 – 7.22 (m, 9H), 7.07 (m, 1H), 6.80 (m, 4H), 6.16 (br, 1H), 4.48 (m, 1H), 4.41 (m, 1H), 4.27 – 4.13 (m, 2H), 4.12 (m, 1H), 3.88 (m, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.64 – 3.42 (m, 3H), 3.17 (m, 1H), 3.14 (m, 1H), 2.62 (dd, J_1 = 6.4 Hz, J_2 = 6.3 Hz, 1H), 2.46 (dd, J_1 = J_2 = 6.4 Hz, 1H), 2.22 – 1.92 (m, 2H), 1.23 – 1.07 (m, 12H). ^{13}C NMR ($CDCl_3$, 125 MHz, mixture of two diastereomers): δ = 169.5, 158.6, 158.5, 144.8, 144.7, 142.14, 142.09, 139.9, 139.8, 136.0, 135.9, 131.1, 131.0, 130.1, 130.0, 129.1, 128.2, 128.1, 127.85, 127.78, 126.82, 126.78, 117.5, 116.7, 113.2, 113.1, 92.6, 92.4, 86.1, 85.7 (d, J = 4.3 Hz), 85.5 (d, J = 5.7 Hz), 77.2, 77.1, 75.8 (d, J = 16.6 Hz), 75.4 (d, J = 17.5 Hz), 64.2, 64.1, 58.3 (d, J = 19.2 Hz), 55.3, 55.2, 45.3 (d, J = 6.4 Hz), 43.3 (d, J = 6.9 Hz), 43.2, 43.1, 37.3 (d, J = 3.8 Hz), 37.2 (d, J = 4.5 Hz), 24.63 (d, J = 2.9 Hz), 24.57 (d, J = 2.7 Hz), 24.54 (d, J = 3.5 Hz), 24.48 (d, J = 3.6 Hz), 23.0 (d, J = 2.7 Hz), 22.9 (d, J = 2.8 Hz). ^{31}P NMR ($CDCl_3$, 202 MHz, mixture of two diastereomers): δ = 148.4, 147.9. HRMS (ESI^+) m/z calcd 902.2402 obsd 902.2429 $[M + Na]^+$.

Oligonucleotide synthesis

The modified oligonucleotide ON1b was assembled by conventional phosphoramidite strategy on a CPG-supported succinyl linker. Couplings of the first four unmodified building blocks were performed by an automated DNA/RNA synthesizer. The modified building block 2 (20.0 mg, 22.7 μmol) in turn, was coupled manually by suspending it and the solid-supported pentamer in a 0.25 M solution of 5-(benzylthio)-1H-tetrazole in MeCN (200 μL) under N_2 atmosphere. The suspension was shaken at 25 °C for 30 min and transferred to a synthesizer column. After conventional Ac_2O capping and I_2 oxidation, the final five unmodified building blocks were coupled by synthesizer. Based on trityl response, yield of the manual coupling was estimated as 61%. The automated couplings, including the one immediately following the manual coupling, proceeded with normal efficiency. After chain assembly, the oligonucleotide was released from solid support and the protecting groups removed by treatment with 33% aq. NH_3 at 55 °C for 16 h. Finally, oligonucleotide ON1b was purified by RP HPLC on a Hypersil ODS C18 column (250 × 4.6 mm, 5 μm) eluting with a linear gradient (5 to 30% over 25 min) of MeCN in 50 mM triethylammonium acetate. A flow rate of 1.0 mL min^{-1} and a detection wavelength of 260 nm were employed. Purified ON1b was characterized mass spectrometrically and quantified UV spectrophotometrically using molar absorptivity calculated by an implementation of the nearest-neighbors method.[13] Molar absorptivity of the modified residue was assumed to be identical to that of benzamide (630 M^{-1} cm^{-1} at 260 nm).[14]

Oligonucleotide palladation

Oligonucleotide ON1b (25 nmol) was dissolved in Ar-purged H_2O (90 μL). A suspension of $Pd_2(dba)_3$ (2.0 mg, 2.2 μmol) in Ar-purged MeCN (90 μL) was added and the resulting mixture incubated at 55 °C for 18 h. The product mixture was diluted with H_2O (500 μL) and the precipitate removed by centrifugation. The supernatant was fractioned by RP HPLC under the conditions used for the purification of ON1b. The product peak was broad and convoluted, consistent with previous reports on covalently palladated oligonucleotides.[2] Mass spectrometric analysis revealed presence of the desired product ON1b-Pd in all of the fractions collected but only the one of the highest purity (eluting at 10.7 min) was used in the hybridization experiments. The concentration of this fraction was determined UV spectrophotometrically as described above. HRMS (ESI^-) m/z calcd for $C_{109}H_{134}IN_{40}O_{64}P_{10}Pd$ 1190.1347 obsd 1190.1563 $[M + I - 3H]^{3-}$.

Melting temperature measurements

UV melting profiles were acquired on a PerkinElmer Lambda 35 UV-vis spectrophotometer equipped with a Peltier temperature control unit. The samples were prepared in quartz glass cuvettes with 10 mm optical path length by mixing appropriate oligonucleotides (1.0 μM) in 20 mM cacodylate buffer (pH = 7.4), the ionic strength of which was adjusted to 0.10 mM with $NaClO_4$. The concentration of 2-mercaptoethanol, when applicable, was 100 μM. The samples were annealed by heating to 90 °C and allowing to gradually cool down to ambient temperature before each experiment. Denaturation and renaturation curves were acquired by recording the absorbance at λ = 260 nm at 0.5 °C intervals over a temperature range of 10 – 90 °C. Three heating and cooling cycles were carried out with each sample to exclude the possibility of chemical decomposition of the palladated oligonucleotide ON1b-Pd. No systematic change in the melting profiles from one cycle to the next was observed (renaturation

curves of ON1b-Pd•ON2a, ON1b-Pd•ON2c, ON1b-Pd•ON2g and ON1b-Pd•ON2t are presented in the Supporting Information). The melting temperatures were determined as inflection points on the averaged UV melting curves.

CD measurements

CD spectra were recorded on an Applied Photophysics Chirascan spectropolarimeter equipped with a Peltier temperature control unit. The sample preparation and the cuvettes were identical to the UV melting experiments. Spectra were acquired between 200 and 400 nm at 10 °C intervals over a temperature range of 10 – 90 °C. At each temperature, the samples were allowed to equilibrate for 2000 s before acquisition.

Acknowledgements

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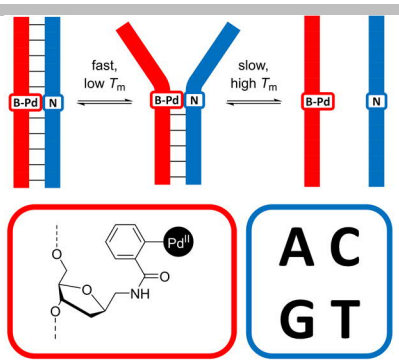
Entry for the Table of Contents (Please choose one layout)

FULL PAPER

An oligonucleotide incorporating a covalently palladated benzoylaminomethyl nucleobase surrogate has been synthesized by oxidative addition of Pd(0) to the corresponding iodoaryl residue. Melting profiles of duplexes formed by this oligonucleotide were biphasic and the higher melting temperatures exhibited considerable hysteresis, consistent with slow dissociation and association of the Pd(II)-mediated base pairs.



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Covalently Palladated Oligonucleotides Through Oxidative Addition of Pd(0)