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Inhibitory Effects of the Guanine Moiety on Suzuki Couplings of Unprotected Halonucleosides in Aqueous Media

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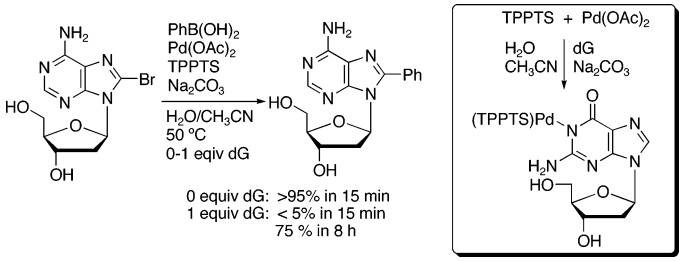
Received April 25, 2005



In the Suzuki arylations of unprotected halonucleosides in aqueous media, 8-bromo-2′-deoxyguanosine (8BrdG) couplings were slower to reach completion than the corresponding 8-bromo-2′-deoxyadenosine (8BrdA) couplings. The guanine moiety has an acidic proton, which under our Suzuki conditions (pH ≅ 10) may be deprotonated to give an anion that can coordinate to palladium. The possibility that guanine coordination was responsible for the observed slower rates was explored using additive experiments in which nonhalogenated nucleosides were added to the Suzuki coupling reaction of 8BrdA or 4-bromotoluene and $PhB(OH)_2$ and the reaction progress monitored by HPLC or GC. Adding dG slowed these reactions, and an induction period was observed. The addition of dA or 1-methyl-2′-deoxyguanosine (1MedG) to these couplings did not affect the rate of conversion to product. Guanine coordination was further explored using ^{13}C and ^{31}P NMR spectroscopy, which implies that guanine *is* coordinating to palladium through *N*-1 or *O*-6, or both. Furthermore, the presence of dG inhibited the formation of the active palladium(0) catalytic species, which may account for both the observed induction period and the sluggishness of reactions where guanine is involved.

Introduction

C-Modified nucleosides are interesting targets for antiviral[1−10] and anticancer[4,10−13] pharmaceuticals. Covalently modified nucleosides have also been applied as DNA and RNA structural probes by appending fluorescent,[14−17] protein recognition,[14,18,19] and spin label functionalities.[20−23] *C*-Modified nucleosides also play important

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10.1021/jo050832l CCC: $30.25
Published on Web 06/30/2005

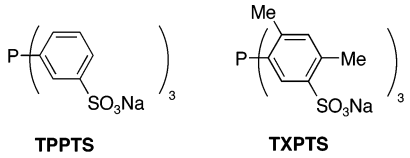

FIGURE 1. Water-soluble phosphine ligands.

roles as authentic standards and models of covalent adducts formed when carcinogens react with DNA.[24−34] Palladium-catalyzed cross-coupling reactions have proven useful in synthesizing these compounds.[35−37] Recently, we reported the efficient Suzuki arylation of unprotected halonucleosides using water-soluble arylphosphines in aqueous media, which allows for the modification of these hydrophilic biomolecules in a relatively cost-effective, environmentally benign solvent while eliminating protection/deprotection steps.[38] Burgess[17] has reported the use of a similar system for the Sonogashira coupling of a halonucleotide in water.

We were intrigued, however, by the difference in activities among the nucleoside substrates. Couplings involving 8-bromoguanosine (8BrG) or 8-bromo-2′-deoxyguanosine (8BrdG) occurred at much slower rates and with lower isolated yields than couplings with 8-bromoadenosine (8BrA), 8-bromo-2′-deoxyadenosine (8BrdA), and 5-iodo-2′-deoxyuridine (5IdU). The reactivity of the deoxyribonucleosides was compared by coupling halonucleosides 8BrdG, 8BrdA, and 5IdU with phenylboronic acid ($PhB(OH)_2$) in the presence of $Pd(OAc)_2$ (10 mol %), Na_2CO_3, and water-soluble arylphosphine ligands TPPTS or TXPTS (Figure 1) in 2:1 water−acetonitrile at room temperature. The reaction progress was followed by HPLC (Figure 2). Regardless of which ligand was used, 8BrdA and 5IdU gave greater rates of conversion than 8BrdG. With TXPTS, these substrates were converted

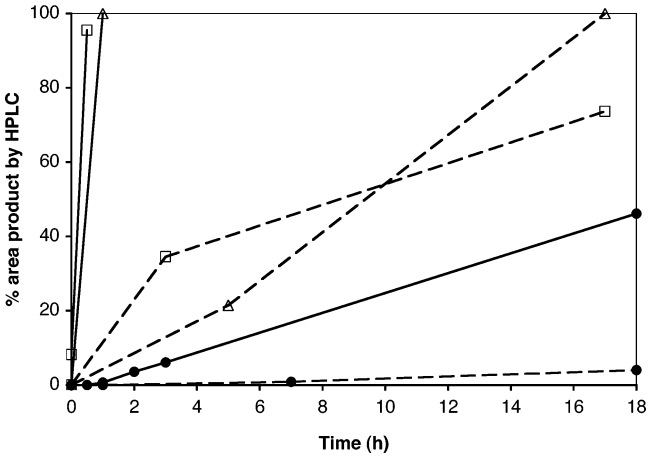

FIGURE 2. Comparison of reactivities of halonucleosides in Suzuki coupling with $PhB(OH)_2$ at room temperature with ligands TPPTS (dotted line) or TXPTS (solid line): □, 8PhdA; ●, 8PhdG; △, 5PhdU.

almost completely to the coupling product within 1 h, while with TPPTS, over 70% yield (by HPLC) was achieved, but only after 17 h. Suzuki coupling of 8BrdG under these conditions gave less than 50% yield in 18 h with both catalyst systems. The TXPTS system shows greater activity for all substrates than TPPTS. For both catalytic systems, the nucleosides exhibited the following trend in substrate reactivity: 8BrdA > 5IdU ≫ 8BrdG. The low activity of guanosine derivatives have been previously observed,[39] and most examples of palladium-catalyzed cross-couplings employ *N*-2- and *O*-6-protected substrates.[34,40−44] Similarly, 5-iodocytidine has shown low reactivity in Suzuki cross-coupling reactions.[45]

We hypothesized that guanine coordination to palladium was causing the 8BrdG couplings to be slower than those of 8BrdA or 5IdU. Purines are known to be potential ligands for metals, particularly platinum(II) and palladium(II). A variety of coordination modes have been proposed for the coordination of guanine derivatives to palladium(II) and platinum(II) complexes.[46−50] Coordination can occur through *N*-1 or *N*-7 depending on the choice of metal complex and the conditions.[51−54] At low

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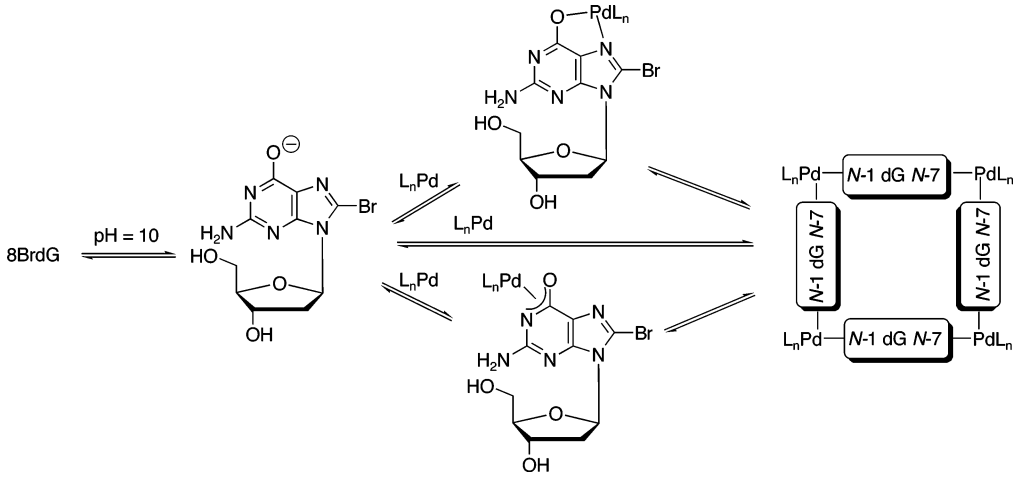
SCHEME 1. Possible Coordination of 8BrdG (pK_a(dG *N*-1) = 9.2−9.4)[62] to Palladium under Basic Conditions



pH, *N*-7 coordination is preferred, while at higher pH coordination to *N*-1 becomes more important.[55] Bidentate coordination has also been proposed with a variety of arrangements, including *N*-7/*O*-6 chelation,[56,57] polymeric *N*-1/*N*-7 complexes,[58,59] and cyclic tetrameric complexes with bridging guanine bases coordinated through *N*-1 and *N*-7.[55,60,61] Thus, under the basic conditions of the Suzuki coupling, the most likely coordination modes would be coordination to *N*-1, to *N*-7/*O*-6, or possibly a tetrameric *N*-1/*N*-7-bridged complex (Scheme 1).

Aside from coordination to palladium, another possible explanation for the varied reactivities of the nucleosides includes differences in the ability of the halonucleosides to oxidatively add to palladium(0). dG is more electron rich than dA based on the lower oxidation potential of guanine compared to adenine.[63] More electron-deficient halonucleosides should be more susceptible to oxidative addition, while more electron-rich substrates should undergo this step much more slowly.

Herein, we report our efforts to understand the differences in reactivity between the purine nucleosides 8BrdG and 8BrdA (Figure 3). We find that guanine inhibits cross-coupling reactions of both halonucleosides and simple aryl bromides. Through multinuclear NMR spectroscopic studies, we show that this inhibition appears to be due to coordination of guanine to palladium(II), which prevents formation of the active catalyst species.

Results and Discussion

Effect of dG on Product Formation. To determine whether 8BrdG is a lesser competitor for oxidative addition or a conspirator in a less active catalyst system, a competition experiment was devised. The purines 8BrdA and 8BrdG were chosen for comparison because they can both be observed at 263 nm, whereas 5IdU is observable only at longer wavelengths. 8BrdA and 8BrdG (0.10 mmol each) were added to a solution containing 0.06 mmol $PhB(OH)_2$ under general Suzuki conditions (eq 1), and the reaction was monitored by HPLC (Figure 4). After 8 h, 8-phenyl-2′-deoxyguanosine (8PhdG) accounted for <1% of the total area by HPLC, while 8-phenyl-2′-deoxyadenosine (8PhdA) accounted for slightly more than

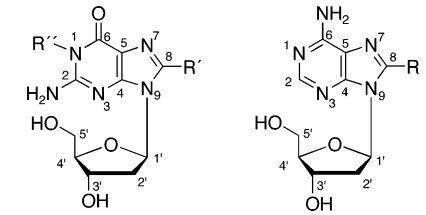


FIGURE 3. Abbreviations used for nucleosides studied.

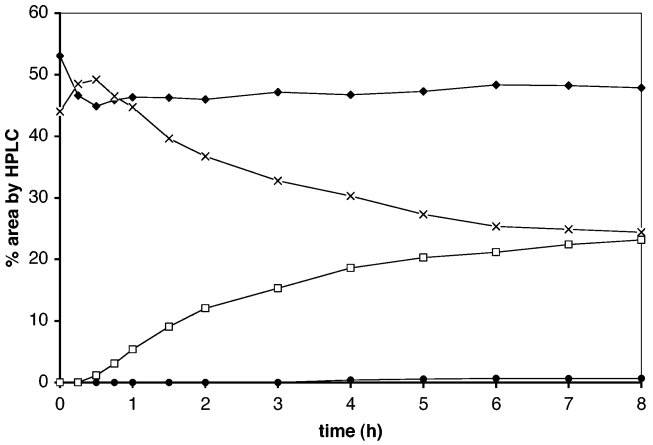


FIGURE 4. Results of HPLC competition experiment between 8BrdA and 8BrdG at 50 °C: ◆, 8BrdG; ●, 8PhdG; ×, 8BrdA; □, 8PhdA (eq 1).

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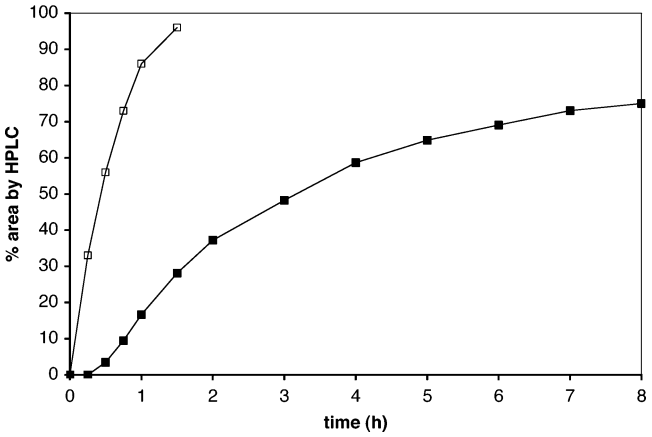
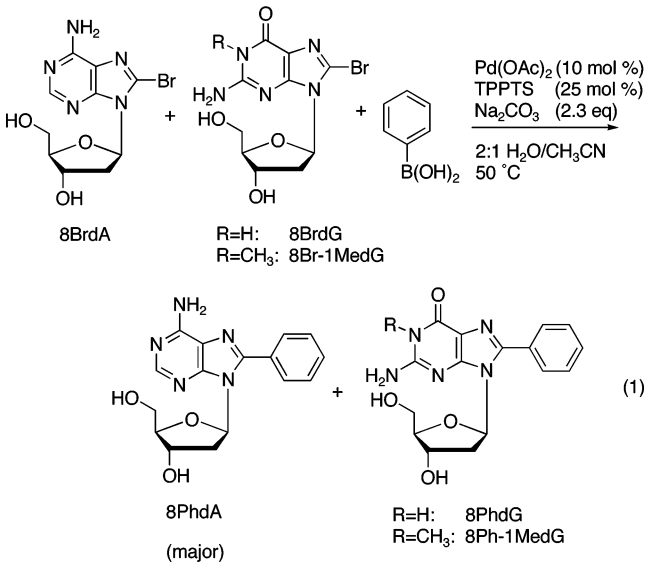


FIGURE 5. Comparison of formation of 8PhdA from 8BrdA and $PhB(OH)_2$ at 50 °C alone (□) and in the presence of 8BrdG (■). For coupling in the presence of 8BrdG, percent area was normalized by $[(A_{8PhdA}/(A_{total} - (A_{8BrdG} + A_{8PhdG}))100)]/0.6$, where A = raw peak area in HPLC chromatograph and dividing by 0.6 corrects for the fact that a full equivalent of $PhB(OH)_2$ was not present to completely consume the substrate 8BrdA.

20%, which roughly corresponds to complete consumption of $PhB(OH)_2$.



8BrdA was obviously favored over 8BrdG in this coupling reaction, but the presence of 8BrdG also severely decreased the rate of formation of 8PhdA. In the absence of 8BrdG, but under otherwise identical conditions, 8BrdA was coupled to $PhB(OH)_2$ in 96% yield in 90 min (Figure 5). Furthermore, the presence of 8BrdG resulted in an induction period of about 15 min, during which time essentially no conversion was observed.

To eliminate the possible involvement of oxidative addition and therefore focus on the coordinating ability of the nucleoside, the experiment was repeated using nonhalogenated dG in place of 8BrdG (eq 2). Two experiments were carried out, both at 55 °C (Figure 6): with 0.025 mmol dG added (equimolar to TPPTS) and with 0.104 mmol dG added (equimolar to 8BrdA). In the absence of dG, 95% HPLC yield of the coupling product (8PhdA) was achieved in 15 min. When a ligand amount

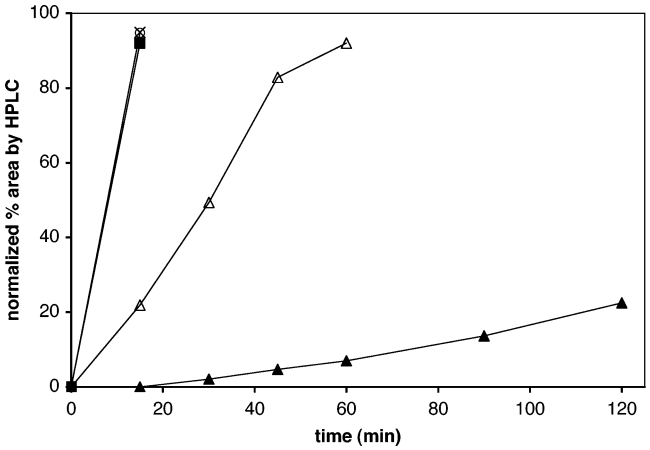
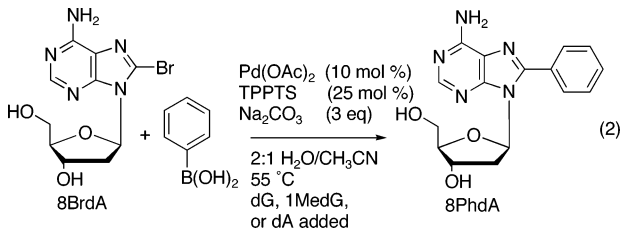


FIGURE 6. Comparison of formation of 8PhdA in the presence of the following: △, dG (0.025 mmol); ▲, dG (0.104 mmol); □, 1MedG (0.029 mmol); ■, 1MedG (0.10 mmol); ×, dA (0.10 mmol); or ○, no additive at 55 °C. □, ■, ×, and ○ are overlapping as these reactions were all complete within 15 min (eq 2).

of dG was added (0.025 mmol), only 22% yield 8PhdA was achieved in 15 min with a final yield of 92% once the substrate (8BrdA) was consumed (1 h). A more dramatic inhibition was observed when 0.104 mmol dG was added. No detectable amount of product was present in 15 min and only a 23% yield of 8PhdA was achieved after 2 h. The induction period seen with dG was similar to that seen with 8BrdG.



If coordinating ability to the metal center accounts for the retardation of reaction rates, the addition of 2′-deoxyadenosine (dA), which has no acidic proton to promote coordination, to the same coupling reaction should have no effect on the reaction rate. dA (0.10 mmol) was therefore added to the 8BrdA/$PhB(OH)_2$ coupling reaction at 55 °C and 95% 8PhdA formed in 15 min (Figure 6). *Addition of dA did not decrease the rate of coupling*.

To confirm that deprotonation at the acidic *N*-1 site is required for inhibition by dG, 1MedG was synthesized.[64] Methylating at *N*-1 removes the acidic proton and therefore should block coordination through the oxyanion. 1MedG was added to the coupling reaction at 55 °C in similar amounts as the dG experiment: 0.029 and 0.10 mmol. In both cases, 8BrdA was completely converted to 92% 8PhdA in 15 min (Figure 6). *Addition of 1-MedG did not decrease the rate of the coupling either.*

This additive experiment was expanded to include the Suzuki coupling of 4-bromotoluene and $PhB(OH)_2$ (eq 3). The reactions were carried out at 50 °C and analyzed by

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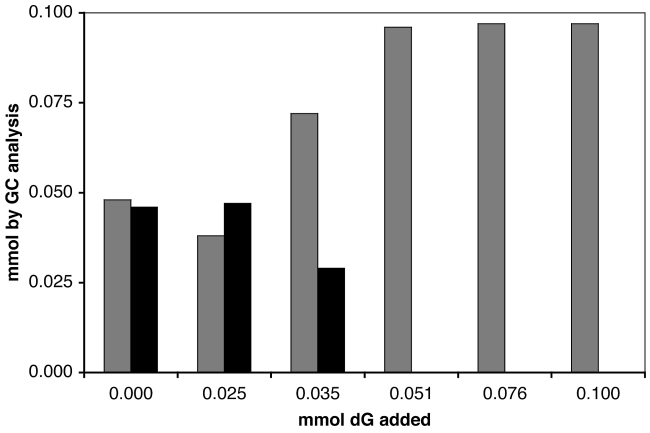
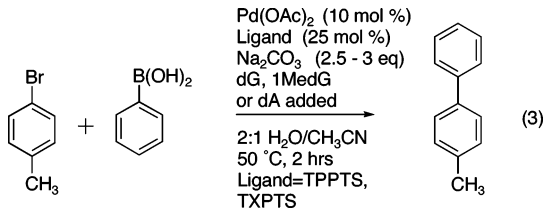


FIGURE 7. Results of GC experiment with TPPTS as ligand and dG as additive at 50 °C (eq 3): 4-bromotoluene (gray), 4-methylbiphenyl (black).

GC after 2 h by quenching and adding mesitylene as an internal standard. As seen in the HPLC experiment (vide supra), adding up to 0.025 mmol dG to the reaction did not significantly change the reaction rate, while more than 0.025 mmol dG severely retarded the reaction rate (Figure 7). In fact, if the dG amount added exceeded 0.05 mmol, no product formation was observed at all within the 2 h time frame.



When the ligand TPPTS was replaced with TXPTS, a similar trend for aryl halides was obtained: increasing the concentration of dG slowed the reaction (Figure 8). However, even with 0.10 mmol dG added, there was still modest product formation. The halonucleoside HPLC experiment could not be carried out with the TXPTS system because the reaction is too fast to effectively monitor by HPLC, even at room temperature.

Since methylation at *N*-1 of dG blocks the inhibitory effect of guanine, we compared the reaction rates of

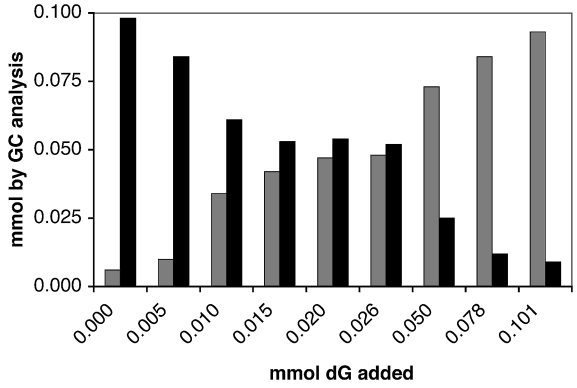


FIGURE 8. Results of GC experiment with TXPTS as ligand and dG as additive at 50 °C (eq 3): 4-bromotoluene (gray), 4-methylbiphenyl (black).

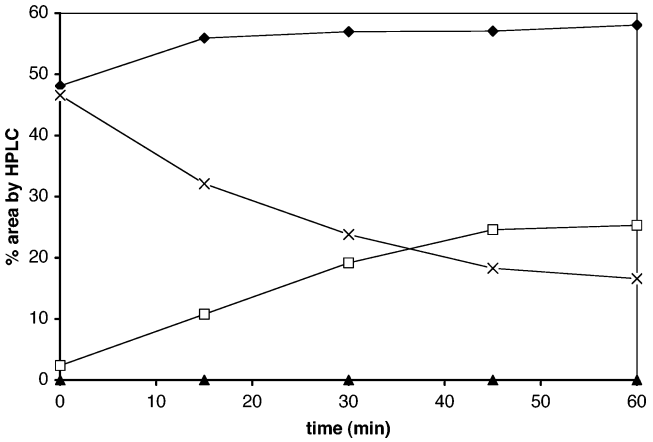


FIGURE 9. Results of HPLC competition experiment between 8BrdA and 8Br-1MedG (see Scheme 2): ×, 8BrdA; □, 8PhdA; ◆, 8Br-1MedG; ▲, 8Ph-1MedG (eq 1).

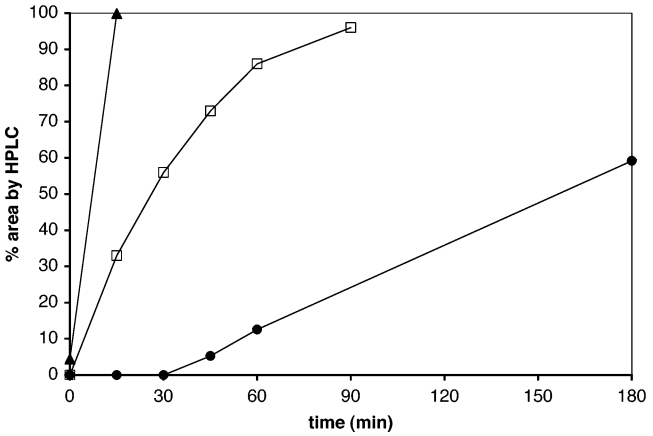


FIGURE 10. HPLC comparison of coupling reactions at 50 °C: ●, 8PhdG; ▲, 8Ph-1MedG; □, 8BrdA.

8BrdA and 8-bromo-1-methyl-2′-deoxyguanosine (8Br-1MedG) to determine the inherent reactivity of guanine. 8BrdA and 8Br-1MedG (0.10 mmol each) were added to a solution containing $PhB(OH)_2$ (0.06 mmol) under the same general Suzuki conditions mentioned previously (eq 2). After 1 h, 8Ph-1MedG accounted for less than one percent of the total area by HPLC, while 8PhdA accounted for approximately 25% (Figure 9). There were no significant changes by HPLC after 1 h. 8BrdA was again favored over the dG analogue for coupling under these conditions. However, unlike the 8BrdA/8BrdG competition experiment, there was no induction period or inhibition of the 8BrdA coupling.

Two reactions were carried out concurrently with this competition experiment: the couplings of 8BrdG and 8Br-1MedG individually with $PhB(OH)_2$ (Figure 10). A snapshot of the reactions after 15 min showed *no* conversion of 8BrdG to 8PhdG and *full* conversion of 8Br-1MedG to 8Ph-1MedG. In a similar experiment, 8BrdA was coupled with $PhB(OH)_2$ in approximately 33% yield after 15 min (Figures 5 and 10). Therefore, the preference of this catalytic system for oxidatively adding 8BrdA over 8Br-1MedG does not necessarily correspond to the relative coupling rates.

^{13}C NMR Spectroscopic Analysis of Palladium−Nucleoside Interactions. To determine the existence

TABLE 1. ^{13}C NMR Chemical Shifts of Nucleoside Peaks in 2:1 Water−Acetonitrile and Na_2CO_3 (3 Equiv)

	dG (ppm)	1MedG (ppm)	dA (ppm)
C-6	167.93	158.47	156.23
C-2	160.85	154.67	152.96
C-4	151.19	149.02	148.96
C-8	136.63	137.78	140.93
C-5	118.88	116.38	119.75
C-4′	88.07	87.33	88.29
C-1′	85.47	77.98	85.61
C-3′	72.29	71.42	71.95
C-5′	62.73	62.02	62.52
C-2′	39.60	38.97	40.04
CH_3		28.69	
Na_2CO_3	166.40	167.68	168.38

and extent of nucleoside coordination to palladium, multinuclear NMR spectroscopy was employed. Solutions of $Pd(OAc)_2$, Na_2CO_3, ligand (TPPTS or TXPTS), nucleoside (dA, 1MedG, or dG), and $PhB(OH)_2$ were analyzed by ^{13}C and ^{31}P NMR spectroscopy. Each compound was analyzed in 2:1 water−acetonitrile by itself and with other compounds by strategic addition in order to tentatively assign peaks. A solution of nucleoside and Na_2CO_3 was used as the standard for the nucleoside chemical shifts in ^{13}C NMR spectra (Table 1). ^{13}C NMR resonances for $Pd(OAc)_2$ appeared at 177.2 and 21.6 ppm while free acetate (NaOAc) was observed at 181.2 and 23.9 ppm. $PhB(OH)_2$ alone was observed at 134.4, 131.4, and 128.5 ppm, but in the presence of Na_2CO_3 these peaks were shifted upfield to 132.3, 127.7, and 127.1 ppm. The *ipso* carbon was not observed.

The Na_2CO_3 resonance appeared at 168.6 ppm in 2:1 water−acetonitrile. However, in solution with other species its chemical shift depended on its relative concentration to $Pd(OAc)_2$, suggesting that carbonate was coordinating to palladium in certain cases. With 1.3 equiv of Na_2CO_3, resonances were observed at 181.0, 160.3, and 23.9 ppm. Therefore, the carbonate displaced the acetate ligands and the 160.3 ppm resonance corresponded to palladium-bound carbonate. With 2.1 equiv of Na_2CO_3, resonances were observed at 181.0, 177.3, 160.3, 23.9, and 21.2 ppm (five-line pattern). The acetate ligands were still displaced, and acetamide-d_3 (177.3 and 21.2 ppm peaks) was produced from hydrolysis of acetonitrile-d_3. With 6.4 and 10.4 equiv of Na_2CO_3, the same results were observed except the palladium-bound carbonate peak at 160.3 ppm shifted to 165.7 ppm (6.4 equiv) and 166.7 ppm (10.4 equiv). This new peak is likely an average of the rapidly exchanging free carbonate and palladium-bound carbonate species.

Solutions of $Pd(OAc)_2$ (usually 0.017 M in 2:1 water−acetonitrile), nucleoside (2 equiv), Na_2CO_3 (6−8 equiv), and ligand (2.5 equiv) were prepared, and the nucleoside ^{13}C NMR chemical shifts were compared with those of solutions where $Pd(OAc)_2$ was not present (Table 1).[65] Finally, solutions with $Pd(OAc)_2$, Na_2CO_3, nucleoside, ligand (TPPTS or TXPTS), and $PhB(OH)_2$ (3−4 equiv) present were analyzed. The dA and 1MedG carbon peaks appeared at the same chemical shift regardless of the ligand used or the presence or absence of $PhB(OH)_2$. The ^{13}C NMR resonances for dG, however, *did* shift, depending on the experimental conditions. When TXPTS was used as the ligand, the largest shift observed was that of *C*-2. Its resonance shifted from 160.9 ppm (with just nucleoside and base) to 155.2 ppm (with TXPTS and $Pd(OAc)_2$ added). The resonances for *C*-6 and *C*-5 also shifted upfield from 167.9 to 165.9 ppm and 118.9 to 117.5 ppm, respectively. The only downfield shift observed was for the *C*-8 resonance, which shifted from 136.6 to 137.9 ppm. These shifts were not dependent on the boronic acid, as the chemical shifts were identical in the presence or absence of $PhB(OH)_2$, as long as $Pd(OAc)_2$ and TXPTS were present along with the nucleoside and base. All other dG carbon peaks did not shift significantly. The *C*-2 shift is the most dramatic, followed by the *C*-6 shift, which implies that dG is coordinating to palladium through *N*-1 of the nucleobase rather than *N*-7. *N*-1 coordination is known to occur under basic conditions.[52]

A similar trend was observed when TPPTS was used as the ligand; however,only the dG *C*-6 and *C*-2 resonances were observed to shift and the change was not as dramatic as in the TXPTS system. Unlike the TXPTS system, the shifts *did* depend on the presence or absence of the boronic acid. Without $PhB(OH)_2$ present, the *C*-6 peak was shifted about 0.7 ppm upfield to 167.2 ppm while the *C*-2 peak was shifted by only about 0.5 ppm upfield to 160.4 ppm. When $PhB(OH)_2$ was present, *C*-6 and *C*-2 were both shifted upfield by about 1 ppm to 166.8 and 160.1 ppm, respectively. For the TPPTS system, none of the other dG peaks shifted significantly, implying that *N*-1 is involved in coordination.

^{31}P NMR Spectroscopic Analysis of Palladium−Nucleoside Interactions. In the ^{31}P NMR spectrum of TPPTS in 2:1 water−acetonitrile, the free ligand peak was observed around −5.1 ppm, while the phosphine oxide peak appeared at 32.3 ppm. Amatore and coworkers have shown that in 3:1 acetonitrile−water, $Pd(OAc)_2$ and TPPTS (4 equiv) quickly formed a $Pd^{II}(OAc)_2(TPPTS)_2$ complex which appeared at 29.7 ppm and was slowly reduced to the palladium(0) species at 13.5 ppm.[66] During the reduction, the free ligand peak (−4.8 ppm) decreased while the TPPTS oxide resonance (31.6 ppm) increased. Given the difference in solvent and relative amounts of ligand, our observations are in agreement. In the systems studied here, a peak at 23.3 ppm could be generated with $Pd(OAc)_2$ (0.017 M), Na_2CO_3 (6.0 equiv), and TPPTS (2.5 equiv) both in the presence and absence of $PhB(OH)_2$ (3.5 equiv). Treatment of this solution with phenyl iodide (5 equiv) resulted in the 23.3 ppm peak diminishing and a new resonance appearing at 23.6 ppm. The 23.3 ppm resonance was therefore assigned as a palladium(0) species.

A solution of $Pd(OAc)_2$ (0.017 M), Na_2CO_3 (6.0 equiv), and TPPTS (2.5 equiv) in 2:1 water−acetonitrile gave resonances at 32.3 (integration = 1) and 23.7 and 23.4 ppm (0.57 together). Several very small, broad resonances were observed in this spectrum around 33.3 and 33.0 ppm (0.13), in a region between 20 and 18 ppm (0.20), 14.2 (0.10) and 13 ppm (0.08) (Figure 11A). A very similar spectrum was observed when the experiment was repeated in the presence of dA (2.0 equiv, Figure 11B). The only difference in the spectra of solutions with and without dA was the absence of a peak at 23.7 ppm in the

(65) See the Supporting Information for NMR spectra.

(66) Amatore, C.; Blart, E.; Genêt, J. P.; Jutand, A.; Lemaire-Audoire, S.; Savignac, M. *J. Org. Chem.* **1995**, *60*, 6829−6839.

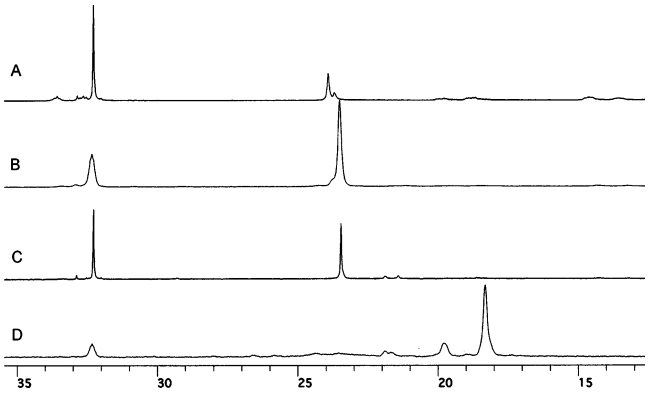


FIGURE 11. ^{31}P NMR spectra for $Pd(OAc)_2$ (0.017 M), Na_2CO_3 (6.4 equiv), and TPPTS (2.5 equiv) and (A) no nucleoside, (B) dA (2.0 equiv), (C) 1MedG (2.0 equiv), or (D) dG (2.0 equiv) in 2:1 water−acetonitrile.

dA solution. However, the 23.4 ppm peak present in the dA solution was very broad. When 1MedG (2.0 equiv, Figure 11C) was present, the major resonances were 32.3 (1), 23.4 (1), 21.8 and 21.4 ppm (0.25 together). Minor peaks were found at 70.8 (0.14), 32.8 (0.07), and 29.2 (0.07), with a region of small, broad peaks from about 20 to 16 ppm (0.71 together). Therefore, the only differences in the peaks observed in this spectrum and the solution with no nucleoside were the peaks at 29.2, 21.8, and 21.4 ppm. In all three cases, the major resonances observed were TPPTS oxide at 32.3 ppm and a peak at 23.3 ppm. The large TPPTS oxide peak is consistent with phosphine-mediated reduction of $Pd(OAc)_2$ to $(TPPTS)_nPd(0)$.

When dG (2.0 equiv) was used, however, the spectrum was quite different (Figure 11D). The major resonances observed were 32.3 (integration = 1), 21.9 and 21.7 (~1 together), 19.7 (1.7) and 18.3 ppm (6), with a small, broad region of peaks from about 27 to 22 ppm. Notably, the TPPTS oxide was significantly smaller than in Figure 11A−C, which means that phosphine-mediated reduction of $Pd(OAc)_2$ was inhibited by the presence of dG. The very large resonance at 18.3 ppm was not observed for solutions of dA, 1MedG, or without nucleoside. After 24 h, however, this 18.3 ppm resonance diminished. The major resonances observed at that time were 32.3 (1) and 23.5 ppm (1.6), with smaller peaks located at 32.4 (0.02), 28.3 (0.03), 24.6 (0.02), 24.1 (0.04), 19.6 (0.02), 18.6 and 18.2 (0.23 together), and 17.3 ppm (0.09).[65]

In the presence of $PhB(OH)_2$, the spectra were simplified compared to those without (Figure 12). The TPPTS oxide peaks were smaller due to $PhB(OH)_2$ acting as the primary reducing agent. The major resonances in the spectrum without nucleoside present were the phosphine oxide resonance at 32.2 ppm and the Pd(0) species at 23.4 ppm (Figure 12A). A solution with dA (2.8 equiv) added gave an identical spectrum (Figure 12B). In the presence of 1MedG (2.1 equiv), two new resonances emerged at 21.8 (2.3) and 19.1 ppm (0.50) (Figure 12C). The dG (2.0 equiv) solution deviated the most with new resonances observed at 21.9 (0.41), 19.7 (0.45) and 18.3 ppm (4.7) (Figure 12D). The most notable resonance of the dG solution is the rather large peak at 18.3 ppm, which did not appear in spectra for dA or 1MedG or without nucleoside, but *does* appear in the dG solution without $PhB(OH)_2$ (vide supra). It is interesting to note, however,

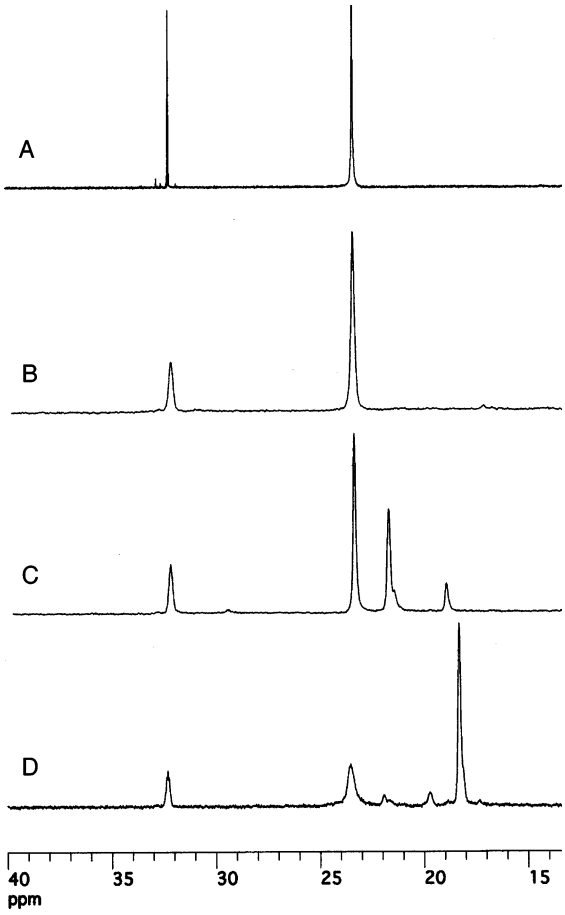


FIGURE 12. ^{31}P NMR spectra for $Pd(OAc)_2$ (0.017 M), Na_2CO_3 (6.0 equiv), TPPTS (2.5 equiv), and $PhB(OH)_2$ (3.5 equiv) and (A) no nucleoside, (B) dA (2.8 equiv), (C) 1MedG (2.1 equiv), or (D) dG (2.0 equiv) in 2:1 water−acetonitrile.

that in 24 h, the species at 18.3 ppm was completely converted to the Pd(0) complex at 23 ppm.

When only 1 equiv of dG was added to a solution of $Pd(OAc)_2$ (0.01 M), TPPTS (2.5 equiv), Na_2CO_3 (34 equiv), and $PhB(OH)_2$ (~15 equiv) in 2:1 water−acetonitrile, the two major peaks observed were the TPPTS oxide peak at 32.3 ppm (integration = 1) and the palladium(0) resonance at 23.3 ppm (3.7) (Figure 13C). When 2 equiv of dG was present in an otherwise identical solution, the 23.3 ppm resonance was broadened and diminished (~0.75) and new peaks at 21.7 (~1), 19.5 (3.4), and 18.1 ppm (2.6) were observed (Figure 13B). When the stoichiometry was further increased to 5 equiv of dG, no palladium(0) peak at 23.3 ppm was observed (Figure 13A). The major resonances observed were TPPTS oxide (integration = 1) and peaks at 19.5 (6.2) and 18.2 ppm (19.5). Also, a small, broad peak around the expected resonance for free TPPTS began to emerge (not shown). Since the peaks around 19.5 and 18.2 ppm were not observed with dA, 1MedG, or in the absence of nucleoside, these resonances presumably correspond to palladium species in which dG and TPPTS are both coordinated to palladium. Neither of these resonances seems to correspond to a palladium(0) species because addition of iodobenzene (4 equiv) to the solution had no effect on the spectra. Therefore, these resonances must be due to $Pd^{II}(TPPTS)_n(dG^-)_n$ species (dG^- = deprotonated dG).

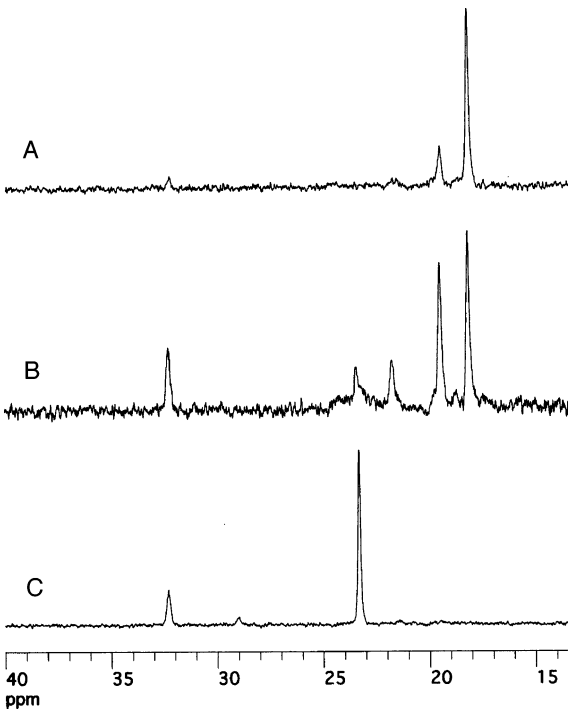


FIGURE 13. ^{31}P NMR spectra for $Pd(OAc)_2$ (0.01 M), Na_2CO_3 (~35 equiv), TPPTS (2.5 equiv), $PhB(OH)_2$ (15.6−18.6 equiv) and (A) 5 equiv of dG, (B) 2 equiv of dG, and (C) 1 equiv of dG in 2:1 water−acetonitrile.

These results suggest that dG coordination to palladium(II) inhibits reduction to palladium(0). This observation is consistent with both the induction period and the decreased coupling rate in the presence of dG.

For ^{31}P NMR spectra of the TXPTS system, the free ligand resonance appeared between −30 and −33 ppm while the TXPTS oxide peak was observed at 37.6 ppm. A peak at −10 ppm could be generated when a solution of $Pd(OAc)_2$ (0.010 M), Na_2CO_3 (6.6 equiv), TXPTS (2.4 equiv), and $PhB(OH)_2$ (16.4 equiv) was allowed to sit for 24 h. Upon addition of iodobenzene (4 equiv), this peak disappeared and several peaks grew between 14 and 28 ppm. Therefore, the −10 ppm resonance in the TXPTS system spectra was assigned as a palladium(0) species. A solution of $Pd(OAc)_2$ (0.010 M), Na_2CO_3 (6.0 equiv), and TXPTS (2.6 equiv) was then analyzed by ^{31}P NMR spectroscopy and used as a comparison for spectra of solutions with nucleosides. The major resonances in this spectrum were 37.5 (integration = 1), 19.3 (2.7), 17.6 (0.30), and −32.4 ppm (5.2). After 24 h, the 17.6 ppm resonance had disappeared and the 19.2 peak was more intense relative to the free TXPTS peak than the day before. Significantly, no reduction to the −10 ppm peak was seen in the absence of $PhB(OH)_2$. In contrast, TPPTS can reduce palladium(II) to give palladium(0) and TPPTS oxide in the absence of $PhB(OH)_2$.[66,67]

A solution of $Pd(OAc)_2$ (0.017 M), Na_2CO_3 (7.3 equiv), TXPTS (2.5 equiv), and dA (2.0 equiv) was analyzed and compared to the spectrum without nucleoside present. The major resonances observed were 37.6 (integration = 1), 19.2 (1.5), 17.7 (0.13), −31.8 (7.1) and −32.2 ppm (0.47).[65] In 24 h, the 19.2 ppm peak increased in intensity relative to the free TXPTS resonance. The dA spectrum was identical to the spectrum that had no nucleoside, except for resolution of the −32.3 ppm resonance into −31.8 and −32.2 ppm. After 9 h, the free TXPTS resonance (then 4.9) had grown into the 19.3 ppm resonance. The 1MedG spectrum was also very similar except for a small resonance observed at 18.3 ppm (0.16). No significant change was observed for this solution over a period of 24 h except that the broad resonance around −32 ppm (5.6) resolved to −31.9 (4.6) and −32.3 ppm (0.27). The corresponding dG solution was also analyzed for comparison. The major difference in this spectrum compared to the previous spectra mentioned was that the free TXPTS resonance integration (9.3) was much greater in relation to the oxide resonance (1) and all other peaks in the spectrum were diminished. Unlike the spectra without nucleoside or with dA or 1MedG, the spectrum changed significantly in 24 h. The 19.3 and 16.3 ppm resonances grew in intensity relative to the free TXPTS resonance and new peaks at 18.2 and 17.8 ppm emerged. The −32.1 ppm resonance also resolved into two peaks: −32.0 (6.6) and −32.4 ppm (0.37).

When $PhB(OH)_2$ was present, a more dramatic difference in the spectra was observed (Figure 14). The "standard" solution used for comparison included Pd(OAc) (0.018 M), Na_2CO_3 (6.0 equiv), TXPTS (2.7 equiv) and $PhB(OH)_2$ (3.3 equiv). The resonances observed for this solution were 40.6 (0.25), 37.6 (integration = 1), 27.5 (2.6, broad), 19.9 (0.4), 19.2 (7.6), 18.2 (3.0), 16.4 (0.26), −9.9 (0.15), and −32.3 ppm (17.3) (Figure 14A). When iodobenzene (4.9 equiv) was added to this solution, the resonances at 40.6 and −9.9 ppm disappeared. Furthermore, two peaks were present in the free TXPTS range: −31.2 and −31.6 ppm. The three largest peaks in the dA (2.0 equiv) solution spectrum (Figure 14B), aside from the oxide peak (integration = 1), were 19.2 (10.5), 18.2 (1.7) and −32.2 ppm (16.3), which correspond nicely with the "standard" spectrum. However, there were minor differences in the small peaks in the range of 22 to 14 ppm. After 13 h, the 18.2 ppm resonance had disappeared and the free TXPTS resonance had shifted to −32.0 ppm. Upon addition of iodobenzene (6.4 equiv), the 40.7 ppm resonance disappeared and the free TXPTS resonance (22.9) grew in intensity relative to all other peaks.

The 1MedG (2.0 equiv) solution gave a similar spectrum (Figure 14C); the three major peaks were 19.3 (8.0), 18.4 and 18.3 (4.3 together), and −32.3 ppm (18.5), and there were some differences in the minor peaks between 22 and 14 ppm. After 10 h, the free TXPTS resonance had decreased in intensity and shifted slightly downfield to −32.2 ppm. Upon addition of iodobenzene (6.4 equiv), the 40.6 ppm resonance disappeared while the 18.3 resonance diminished and the free TXPTS resonance (−32.1 ppm) had increased in intensity. There were more significant differences in the dG (2.0 equiv) spectrum, however (Figure 14D). The 19.3 (2.9) and 18.3 ppm (2.5) resonances were much smaller relative to the phosphine oxide than the other solutions. Furthermore, the free TXPTS resonance appeared to be sharper and more downfield (−31.9 ppm) than the corresponding peaks in the other spectra and was also larger (23.9) relative to oxide than the other solutions. Upon addition of iodobenzene (6.4 equiv) after 6 h, essentially no change was

(67) Amatore, C.; Jutand, A. *J. Organomet. Chem.* **1999**, *576*, 254−278.

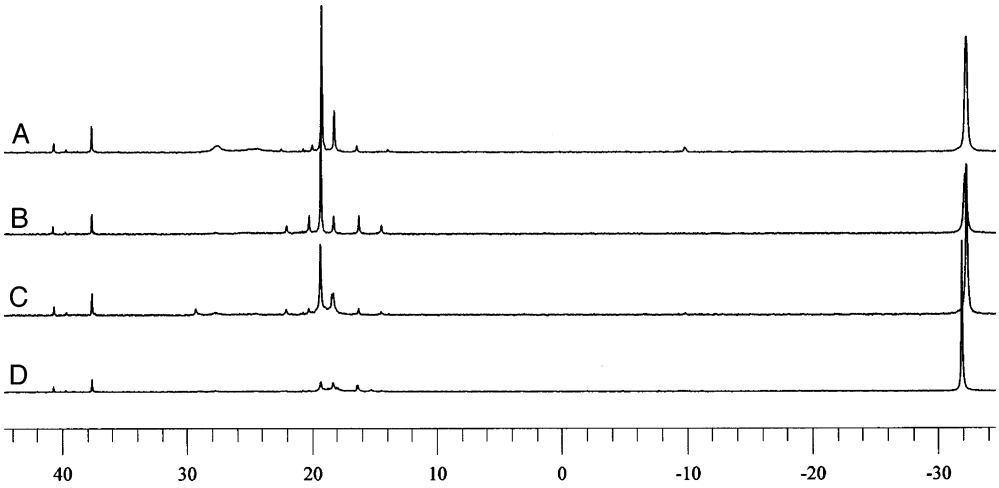


FIGURE 14. ^{31}P NMR spectra of $Pd(OAc)_2$ (0.018 M), Na_2CO_3 (6.1 equiv), TXPTS (2.5−2.7 equiv), and $PhB(OH)_2$ (3.3−3.6 equiv) and (A) no nucleoside, (B) dA (2.0 equiv), (C) 1MedG (2.0 equiv), or (D) dG (2.0 equiv).

observed except for the disappearance of the 40.7 ppm resonance. Although the TXPTS active species is unclear at this time, it is obvious that dG changes the species present in solution to a much greater degree than do dA or 1MedG.

Discussion

Reactivity Studies. In the competition experiment between 8BrdA and 8BrdG, 8BrdG was not only significantly less reactive than 8BrdA but also inhibited the rate of the Suzuki coupling of 8BrdA. Furthermore, an induction period of approximately 15 min was observed in this reaction before 8BrdA began to be converted to 8PhdA. We have never observed induction periods for the arylation of 8BrdA in the absence of dG. Therefore, the slower cross-coupling of 8BrdG compared to 8BrdA was not simply due to a lower inherent reactivity of 8BrdG. If 8BrdG were simply less reactive than 8BrdA, but had no other effect on the catalyst system, then 8BrdG should have had no effect on the coupling of 8BrdA in the competition experiment.

We considered two possible scenarios to account for the observed inhibition of 8BrdA coupling caused by 8BrdG. The decrease in rate could be attributed to coordination of 8BrdG to palladium to give a less active catalyst. Guanine is known to be a good ligand for palladium(II) and platinum(II).[46−61] Alternatively, oxidative addition of 8BrdG could be facile, but be followed by a slow step in the catalytic cycle, thereby trapping out the active catalyst as the 8BrdG oxidative addition product. To discount this latter possibility, the arylation of 8BrdA was repeated in the presence of an equimolar amount of dG. The coupling of 8BrdA occurred at a similar rate in the presence of dG as it did in the presence of 8BrdG. Since oxidative addition of dG was not possible, we concluded that the inhibition was most likely due to coordination of the guanine base to palladium.

In contrast to dG, dA had no effect on the cross-coupling of 8BrdA. The most significant difference between the purine nucleosides is that dG has an appreciably acidic proton on *N*-1 (pK_a = 9.2−9.4).[62] 1MedG, which lacks this acidic proton, also had no effect on the Suzuki coupling of 8BrdA. Cross-coupling reactions were carried out with 8Br-1MedG in an effort to determine the inherent reactivity of the guanine base when deprotonation was not possible. In a competition experiment with 8BrdA and 8Br-1MedG, 8BrdA was found to react exclusively. In contrast to 8BrdG, 8Br-1MedG did not inhibit the coupling of 8BrdA. Interestingly, when the cross-coupling of 8BrdA and 8Br-1MedG was performed independently, 8Br-1MedG was coupled at a slightly faster rate than 8BrdA.

The fact that 8BrdA reacts exclusively in a competition with 8Br-1MedG, yet the individual reaction rates are similar, can be explained if the substrate-selection step in the competition experiment, oxidative addition, is irreversible and precedes the rate-limiting step. It is possible that 8BrdA would undergo oxidative addition faster than 8Br-1MedG due to adenine's electron deficiency compared to guanine.[63] Since the individual coupling rates for 8BrdA and 8Br-1MedG are similar, there must be a step after oxidative addition that is rate-limiting, but for which the rates for 8BrdA and 8Br-1MedG are similar. This scenario would account for the preference of the catalyst for 8BrdA in the competition experiments while also explaining the similar individual coupling rates. Therefore, we believe that the coordinating ability of dG rather than differences in the inherent reactivity of 8BrdG is largely responsible for the observed differences in the relative reactivities of the halonucleosides studied.

The results with 1MedG and 8Br-1MedG show that the *N*-1 proton plays a critical role in the effect of guanine on the Suzuki coupling reaction. Therefore, it is likely the conjugate base of dG that coordinates to palladium resulting in the observed inhibition. Possible coordination modes would include chelation between *N*-7 and *O*-6, coordination to *N*-1 and/or *O*-6, or formation of bridged structure with different palladium atoms bonded to *N*-1 and *N*-7. Under the basic conditions used in the Suzuki coupling, coordination to *N*-1 or a bridging coordination between *N*-1 and *N*-7 have been previously reported (Scheme 1).[55]

NMR Spectroscopic Studies. In an effort to characterize the nature of the interaction of nucleosides with palladium under Suzuki coupling conditions, ^{13}C NMR

spectra of mixtures of nucleosides, $Pd(OAc)_2$, ligand (TPPTS or TXPTS), and sodium carbonate in the presence or absence of phenylboronic acid were obtained. In the cases of dA and 1MedG, there were no changes in the chemical shifts of the purine base when either TPPTS or TXPTS were used as the ligand. In contrast, the guanine carbons were affected by the presence of palladium. When TPPTS was used as the ligand, small upfield shifts were observed in *C*-2 and *C*-6. With TXPTS, larger magnitude upfield shifts were seen for *C*-2 and *C*-6, in addition to a smaller upfield shift by *C*-5 and a small downfield shift in *C*-8. The fact that the largest magnitude shifts were seen for the carbons flanking *N*-1 suggests that the largest change in environment upon adding the palladium complex occurs at this site. Therefore, we propose that the conjugate base of guanine coordinates to palladium through *N*-1 and/or *O*-6 under these conditions. The larger shifts observed when TXPTS was used as the ligand suggests that dG coordinates more strongly to palladium under these conditions, than when TPPTS is used.

^{31}P NMR spectra of mixtures of nucleosides, $Pd(OAc)_2$, ligand (TPPTS or TXPTS), and sodium carbonate in the presence or absence of phenylboronic acid gave a number of species, of which only some could be unambiguously assigned. Despite the complexity of these spectra, significant differences were seen in the major resonances upon adding dG to the mixture. With TPPTS, the major species present in the absence of nucleoside or in the presence of dA or 1MedG were TPPTS oxide and the $(TPPTS)_nPd(0)$ complex (23 ppm). When dG was present, the Pd(0) species at 23 ppm was not observed. Instead, a major resonance is seen at 18.3 ppm, which has been assigned to a Pd(II) species based on the fact that it was unaffected by addition of phenyl iodide. The resonance at 18.3 most likely represents $(TPPTS)_nPd^{II}(dG^-)_m$.

The formation of the Pd(0) complex at 23 ppm was inhibited by the presence of dG. When only 1 equiv of dG relative to palladium was present, the Pd(0) complex formed within 15 min (Figure 13). When the amount of dG was increased to 2 equiv, the Pd(0) complex was a minor component initially, but it became the major species after 24 h. A further increase to 5 equiv of dG relative to Pd resulted in the 18.3 ppm species being the predominant complex in solution. Since reduction to Pd(0) is necessary for initiation of the catalytic cycle, the ability of dG to inhibit the formation of the Pd(0) complex is consistent with our reactivity data. In the presence of dG, we observed a decrease in catalyst activity as well as an induction period. In the early stages of the reaction, all of the palladium is tied up as a Pd(II)dG complex, resulting in low activity. Over time, a sufficient amount of Pd(0) active species is formed to provide a measurable rate of reaction. The concentration of active catalyst is lowered due to the competitive coordination by dG, however, resulting in the observed decrease in cross-coupling rate.

The TXPTS system also showed a significant effect upon addition of dG. Spectra obtained in the absence of nucleoside or with dA or 1MedG present were qualitatively similar, although there were differences in the minor peaks. The major species present were a Pd(II) complex at 19.2 ppm and free TXPTS at −32.2 ppm (Figure 14). Interestingly, TXPTS does not appear to coordinate strongly to $Pd(OAc)_2$. In addition, the reduction to the Pd(0) complex at −10 ppm is very slow, even in the presence of phenylboronic acid. In the presence of dG, the (TXPTS)Pd(II) complex at 19.2 is greatly diminished, and the predominant peak is at −31.9 ppm, which is slightly downfield from free TXPTS.

These results suggest TXPTS is largely displaced from the palladium center by dG. This result is consistent with the observation of a stronger interaction of dG with Pd(II) when TXPTS was used as the ligand in the ^{13}C NMR spectroscopic studies. It is likely that TXPTS is too sterically demanding to allow for formation of a stable $(TXPTS)Pd(dG^-)$ complex, while such a complex is possible for the smaller TPPTS ligand. Under these conditions, the major palladium complex in solution appears to be $Pd(dG^-)_n$.

The fact that TXPTS appears to interact weakly with palladium in the presence of dG is surprising in light of our catalytic results. TXPTS gave a more active catalyst for the Suzuki arylation of 8BrdG than TPPTS.[38] TXPTS also gave a catalyst that was more resistant to inhibition by dG in the coupling of 4-bromotoluene and phenylboronic acid than TPPTS (Figures 7 and 8). Thus, it is unclear why the TXPTS/Pd system gave a more effective catalyst in the presence of dG relative to the TPPTS/Pd system, when it appears to interact with palladium to a lesser extent than TPPTS. These results suggest that the TXPTS system may be catalyzed by a different active species than the TPPTS system. In the case of TPPTS, the active species that we have observed is likely the $(TPPTS)_nPd(0)$ complex, which agrees with Amatore and co-workers' findings.[66,67] Given the weak interaction of TXPTS with Pd, it appears possible that the active species may actually be noncoordinated palladium clusters or nanoparticles.[68,69] Further investigations into the nature of the active species for the TXPTS/Pd catalyst system are currently underway in our laboratory.

Conclusions

In an effort to understand the low reactivity of unprotected 8BrdG in the Suzuki reaction compared to other halonucleosides, we carried out a series of reactivity and NMR spectroscopic studies. Our results show that guanine nucleosides coordinate to Pd(II) inhibiting reduction to the Pd(0) active species. These results provide useful insights into how nucleosides affect commonly used palladium-catalyzed cross-coupling reactions. The competitive coordination can be avoided by replacement of the acidic *N*-1 proton. Thus, to achieve optimal reaction rates under mild conditions, *N*-1 or *O*-6 protected dG substrates should be used. Protection of dG is not always practical or desirable, however. We originally envisioned the use of water-soluble catalysts to avoid atom inefficient protection/deprotection sequences. Protection would also not be practical in the cross-coupling of halogen-containing oligonucleotides, where multiple guanine bases may be present. Thus identification of catalyst systems that are not inhibited by dG will be desirable for future applications. The TXPTS/Pd system is less strongly inhibited by dG than the TPPTS system, but the nature

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of this effect is unclear. Further understanding of the active species in the TXPTS/Pd system will hopefully lead to improved catalyst designs that are not inhibited by dG.

Experimental Section

General Procedure for HPLC Experiments. $Pd(OAc)_2$ (2.3 mg, 0.01 mmol), ligand (TPPTS or TXPTS, 0.027 mmol), Na_2CO_3 (24.2 mg, 0.23 mmol), nucleoside (8BrdA, 8BrdG, or 8Br-1MedG, 0.11 mmol), and $PhB(OH)_2$ (18.2 mg, 0.15 mmol) were assembled in a nitrogen drybox in vials closed with screw-cap septa. Once the vials were removed from the drybox, solvent (1 mL 2:1 water−acetonitrile) was added via syringe and reactions were allowed to stir in an oil bath. Aliquots (ca. 20 μL) were removed periodically via syringe, diluted with methanol (ca. 2 mL), and placed in a container packed with ice. Reaction progress was monitored by RP-HPLC (C-18 column, eluted with gradient ranging from 10% MeOH in H_2O to 80% MeOH in H_2O, with detection at 263 nm). In general, under these elution conditions the retention times were as follows: 8BrdA (15.2 min), 8PhdA (17.3 min), 8BrdG (13.4 min), 8PhdG (16.3 min), 8Br-1MedG (14.4 min), and 8Ph-1MedG (16.9 min).

HPLC Competition Experiments. Reactions were set up and monitored according to the general procedure using $Pd(OAc)_2$ (2.3 mg, 0.01 mmol), TPPTS (15.4 mg, 0.027 mmol), Na_2CO_3 (24.2 mg, 0.23 mmol), nucleoside (8BrdA, 8BrdG, or 8Br-1MedG, 0.11 mmol), and $PhB(OH)_2$ (7.3 mg, 0.06 mmol). Once the vials were removed from the drybox, solvent (2:1 water−acetonitrile) was added via syringe and the reaction vials were placed in a 50 °C oil bath.

HPLC Additive Experiments. Reactions were set up and monitored according to the general procedure. In general, $Pd(OAc)_2$ (2.2 mg, 0.01 mmol), TPPTS (14.2 mg, 0.025 mmol), Na_2CO_3 (21−32 mg, 0.2−0.3 mmol), 8BrdA (33 mg, 0.1 mmol), and $PhB(OH)_2$ (18.2 mg, 0.15 mmol) along with the additive (dA, dG, or 1MedG) in varying amounts were collected in a nitrogen drybox in vials closed with screw-cap septa. Once the vials were removed from the drybox, solvent (2:1 water-acetonitrile) was added via syringe and the reaction vials were placed in a 50 °C oil bath.

General Procedure for GC Experiments. $Pd(OAc)_2$ (2.2 mg, 0.01 mmol), ligand (TPPTS or TXPTS, 0.025 mmol), Na_2CO_3 (27.5−28.7 mg, 0.26−0.27 mmol), and $PhB(OH)_2$ (18.9 mg, 0.156 mmol) were assembled in a nitrogen drybox in vials closed with screw-cap septa. Once the vials were removed from the drybox, 4-bromotoluene (12.5 μL, 0.1 mmol) and solvent (1 mL 2:1 water−acetonitrile) were added via syringe and reactions were allowed to stir in a 50 °C oil bath. Aliquots (ca. 20 μL) were removed periodically via syringe. These aliquots were diluted with ethyl acetate (ca. 2 mL) and the internal standard mesitylene (10 μL) added. Samples were then analyzed by GC (Alltech EC-WAX capillary column, FID detector). Product yields were calculated based on calibrations obtained using authentic compounds and mesitylene as internal standard.

General Procedure for NMR Experiments. $Pd(OAc)_2$, Na_2CO_3, TXPTS, TPPTS, dG, 1MedG, dA, and $PhB(OH)_2$ were collected in varying amounts depending on the system being studied in a nitrogen drybox in vials closed with screw-cap septa. The vials were brought out of the drybox and solvent added via syringe. A portion of this solution (2.5 mL for the 10 mm tubes or 0.5 mL for the 5 mm tubes) was transferred via syringe to a septum-sealed NMR tube under nitrogen. In most cases, samples were analyzed immediately after solvent was added and then analyzed at intervals as indicated after that.

Acknowledgment. The National Science Foundation (CHE-0124255) provided funding for this work. E.C.W. was partially funded by a University of Alabama Graduate Council Research and Creative Activity Fellowship. Dr. Ken Belmore and Prof. Russell Timkovich provided assistance with the NMR results. We thank Prof. Peter M. Gannett and Jonathan Daft for helpful discussions involving nucleosides.

Supporting Information Available: Experimental details, characterization data for 8Br-1MedG, and copies of ^{13}C and ^{31}P NMR spectra from coordination studies. This material is available free of charge via the Internet at http://pubs.acs.org.

JO050832L