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Palladium-Catalyzed Synthesis of Carcinogenic Polycyclic Aromatic Hydrocarbon Epoxide-Nucleoside Adducts: The First Amination of a Chloro Nucleoside[1]

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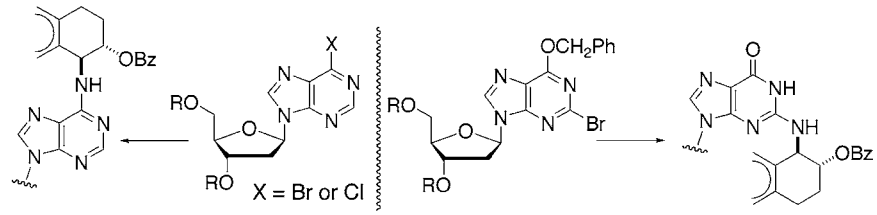
ABSTRACT



***N*^{6}-deoxyadenosine adducts** ***N*^{2}-deoxyguanosine adducts**

Pd-catalyzed coupling of the axially constrained, less reactive benzo[*a*]pyrene bay-region amino benzoates, derived from the tetrahydro and diol epoxides, with C-6 and C-2 halopurine deoxynucleosides offers an efficient approach to the synthesis of the corresponding nucleoside–epoxide adducts. Also reported are the first examples involving the coupling of a 6-chloropurine deoxynucleoside with these amines, a reaction that is difficult by direct halide displacement. Certain mechanistic aspects of this metal-catalyzed C–N bond formation are also discussed.

Polycyclic aromatic hydrocarbons (PAHs) are widely present in the environment, and several members of this class of compounds undergo metabolic activation to four isomeric diol epoxides.[2] These electrophiles alkylate cellular DNA producing covalent lesions,[2,3] which is considered to be the first step in the multistep cascade leading to tumorigenesis. DNA alkylation by diol epoxides occurs by protonation and ring opening of the oxirane, followed by the formation of a C−N bond between the amino groups of the nucleobases and the benzylic carbon of the PAH. Metabolism and DNA binding of any PAH results in the formation of two sets of 8 isomeric deoxyadenosine and deoxyguanosine adducts. For detailed studies into PAH-induced carcinogenesis substantial effort by us and others has led to the development of techniques for the site-specific modification of DNA by stereochemically defined PAH diol epoxide−nucleoside adducts.[4] The most generally applicable method involves the coupling of an amino derivative of a hydrocarbon with an electrophilic nucleoside. Due to conformational factors, bay-region amines of PAHs are not very nucleophilic and the

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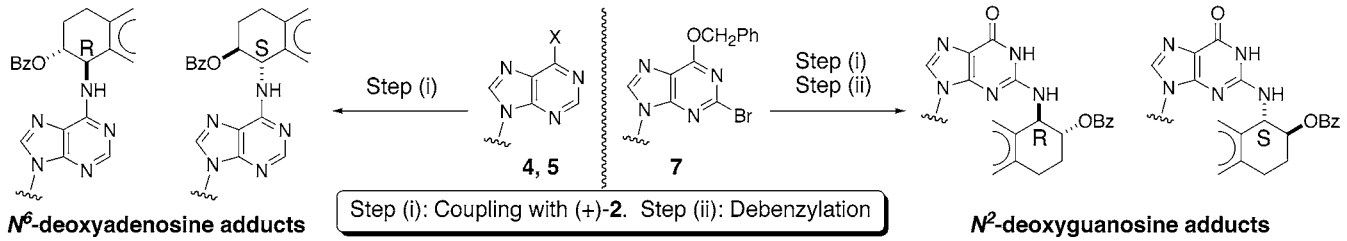
Scheme 1



highly reactive fluoro or triflate bearing nucleosides are required for the synthesis of the nucleoside adducts.[4a,c,d,5,6]

Recently, we[7] and others[8−10] have developed methods for Pd-catalyzed C−N bond formation of nucleosides based upon new catalysis methods.[11] Since the bromo nucleosides required for these reactions are simpler to prepare than the fluoro analogues, we reasoned that Pd-mediated C−N bond formation would offer a unique and more facile entry to the biologically important PAH epoxide−nucleoside adducts. This letter discloses our preliminary results on the use of Pd catalysis as well as an analysis of certain mechanistic aspects of the reactions leading to these adducts.

Although diol epoxides (**A** and **B**, Figure 1) are the metabolically activated forms of the carcinogen benzo[*a*]-pyrene (B*a*P), the tetrahydroepoxide (±)-**1** (Figure 1) was

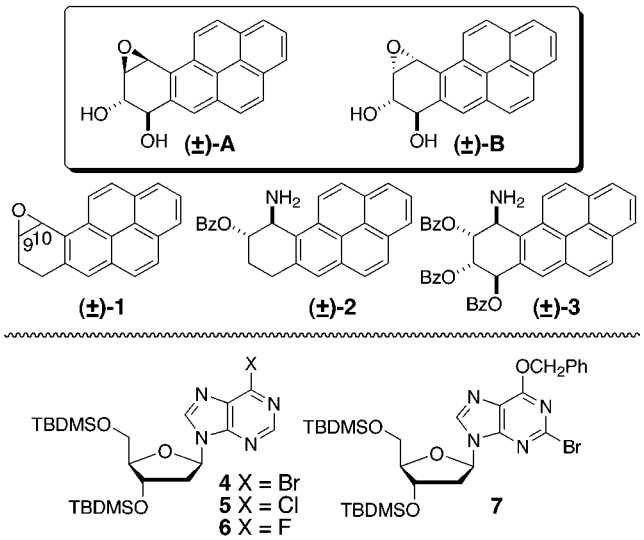


Figure 1. Structures of the B*a*P diol and tetrahydro epoxides, the amino benzoates derived by a trans ring opening of these as well as halo nucleosides utilized in this study.

chosen as the experimental prototype for several reasons. (a) Epoxide (±)-**1** is simpler to prepare compared to the diol epoxides. (b) In contrast to the four chiral centers in **A** and **B**, (±)-**1** has only two but this model is representative of both **A** and **B**. This feature simplifies assessment of chiral integrity. (c) Since **B** is carcinogenic but not **1**, comparative studies on DNA containing the tetrahydro and diol epoxides will be useful in understanding what, if any influence the two additional hydroxyl groups have on the local DNA structure that is ultimately reflected in the biological response. Amine (±)-**2** and nucleosides **4**−**7** required for these studies were prepared on the basis of known methods (see the Supporting Information).

Our earlier studies[7a,12] provided a reasonable starting point for determining whether (±)-**2** and the halo nucleosides could be coupled by Pd catalysts. Using the catalytic system $Pd_2(dba)_3$/2-(dicyclohexylphosphino)-2′-(*N*,*N*-dimethylamino)-1,1′-biphenyl (10:30 mol %) and Cs_2CO_3 (1.4 molar equiv) the cross-coupling of **4** with (±)-**2** [**4**:(±)-**2** 1:1.1 molar equiv] was conducted in PhMe at 80 °C. In contrast to arylamination reactions[7a] this reaction was slow reaching completion in 34 h and in low yield (25% of the N^6-deoxyadenosine adducts, Scheme 1). Replacement of Cs_2CO_3 with K_3PO_4 (1.5 molar equiv), under otherwise identical conditions, also resulted in a slow reaction and low yield (35 h, 27%). Changing the supporting ligand for Pd was then considered. Use of the $Pd_2(dba)_3$/(±)-BINAP/Cs_2CO_3 system

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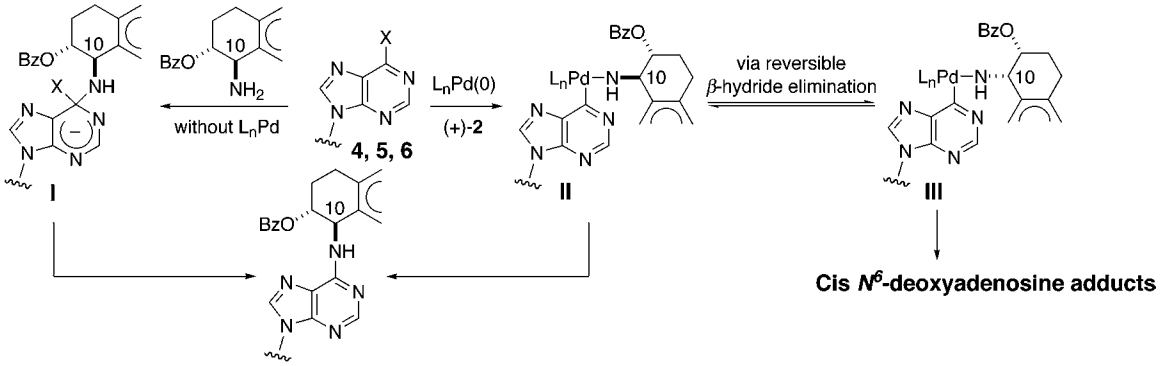
Scheme 2



led to improved coupling yield and reaction time (60%, 5 h). Next $Pd(OAc)_2$-based catalytic systems were tested. These reactions were conducted in toluene at 80 °C, with 10:30 mol % of $Pd(OAc)_2$:ligand in each case. A summary of these experiments is as follows. (a) 2-(Dicyclohexylphosphino)-1,1′-biphenyl, Cs_2CO_3 led to complete consumption of **4** within 23 h but an insignificant amount of product was observed. (b) 2-(Dicyclohexylphosphino)-2′-(*N*,*N*-dimethylamino)-1,1′-biphenyl, Cs_2CO_3 led to no appreciable product formation. (c) (±)-BINAP and K_3PO_4 resulted in a 73% yield of product in 12 h. (d) (±)-BINAP and Cs_2CO_3 produced a 5 h reaction with a product yield of 80%. (e) 1,1′-Bis(diphenylphosphino)ferrocene with Cs_2CO_3 produced a 10 h reaction time and a 44% product yield. These catalyst optimization experiments showed that the combination of $Pd(OAc)_2$/(±)-BINAP/Cs_2CO_3 in toluene at 80 °C gave the best results. The diastereomeric N^6-deoxyadenosine adducts could not be easily resolved, but they could be readily characterized as a mixture.

Next we turned to the investigation of Pd-mediated C−N bond formation using nucleoside **5**. Despite the fact that chloroaromatics and chloroheterocycles have been utilized for C−N bond formation,[13] the extension to chloro nucleosides is not necessarily obvious. We have recently shown that **5** is superior to **4** for C−C cross coupling,[12] but the utility of **5** for metal-catalyzed C−N bond formation is currently unknown. Coupling of **5** with (±)-**2** using the optimized catalytic system described above was very interesting. This reaction was complete in 5.5 h affording the N^6-deoxyadenosine adducts in 90% yield. Thus, it appears that chloro nucleoside **5** may be superior and at the least comparable to the bromo analogue **4** in its reactivity. It is noteworthy that bay region amines such as (±)-**2** react poorly with **5** via the S_NAr mechanism.[5,6] *This represents the first example of Pd-catalyzed C−N bond formation involving chloro nucleoside* ***5*** *and also the first efficient synthesis of bay region amine adducts from this substrate*.

At this point it was important to address some critical questions. The first, more obvious, was a determination that the reactions of **4** and **5** were mediated by Pd and that the reactions did not proceed by the well-known S_NAr displacement (Scheme 2). In control experiments with **4** and **5** the reactions were conducted under the optimized conditions except that $Pd(OAc)_2$ was omitted from the reaction mixtures. In these cases, no product formation was observed even after 24 h, clearly indicating the catalytic role of Pd. This implied that intermediate **I** (Scheme 2) was not responsible for product formation. The second and perhaps more important question was with regard to the chiral integrity in the reaction. As described above, since a catalytic role of Pd had been established, the putative intermediate **II** leads to the N^6 adduct by a reductive-elimination and regeneration of the Pd(0) catalyst. Intermediate **II** can also potentially undergo β-hydride elimination and such a process has been known to cause loss of chirality in aryl aminations.[14] In the present case, a β-hydride elimination would yield a highly conjugated 10-imino B*a*P derivative. In fact, facile oxidation at the C-10 position of tetrahydro B*a*P is known, such as the mild DDQ-mediated oxidation to the ketone as well as the benzylic acetate.[15] If the β-hydride elimination from intermediate **II** was reversible, then loss of stereochemical integrity at C-10 was possible. This could result in adducts with cis and trans stereochemistry. Although bis-coordinating ligands such as (±)-BINAP have been shown to prevent attrition of chirality,[14] whether loss of stereochemistry was a problem in the present case had to be unequivocally addressed. Fluoride displacement from **6** by (±)-**2** should occur via intermediate **I** without erosion of the relative stereochemistry. Thus, the N^6-deoxyadenosine mixture was synthesized via the S_NAr displacement.[4a] Comparison of the NMR data of the product from this reaction to those isolated

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from the Pd-mediated reactions of **4** and **5** revealed these were identical. This clearly indicated that no detectable loss of stereochemistry had occurred in the metal-catalyzed reaction, and that β-hydride elimination was perhaps not competitive with reductive-elimination. Another factor also became clear in this comparison. Although dimeric products are known to form in Pd-catalyzed aminations of nucleosides, particularly at the N6,[8a,16] such a product was not obvious in the present case.

At this point we were interested in testing the method for the synthesis of the N^2-deoxyguanosine adducts, for which compound **7** was a suitable substrate. Thus, we tested the coupling of (±)-**2** with **7** using the optimized conditions [Pd(OAc)$_2$/(±)-BINAP/Cs_2CO_3 in toluene at 80 °C]. This reaction was complete within 3.5 h producing the O^6-benzyl-N^2-deoxyguanosine adducts in 79% yield. This yield is significantly better than that reported for the synthesis of similar N^2-deoxyguanosine adducts by fluoride displacement.[17] Debenzylation of the adduct mixture (1:1 THF−MeOH, 1 atm of H_2, 5 h at room temperature) yielded the N^2-deoxyguanosine adducts (Scheme 1) in 97% yield. Whereas the O^6-benzyl derivatives were not separated on TLC, a clear separation of the two diastereomers was evident after debenzylation. Partial resolution of the diastereomers was therefore performed by chromatography. Chirality assignment to the two diastereomers was done by CD

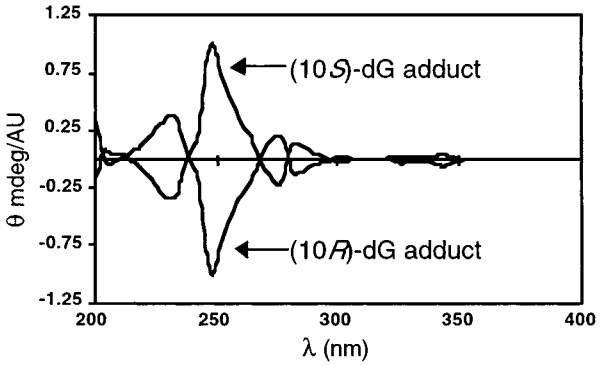

Figure 2. CD spectra of the diastereomeric N^2-deoxyguanosine adducts in MeOH, normalized to 1 OD at λ_{max}.

analysis (Figure 2) and comparison to the CD spectra of deoxyguanosine adducts arising from a trans ring opening of B*a*P diol epoxides.[18] Thus, the *more mobile* diastereomer on silica gel was assigned a 10*S* absolute configuration at the point of attachment of the hydrocarbon to the nucleoside and in the *less mobile* isomer this was 10*R*.

Finally, the method was evaluated for synthesis of diol epoxide adducts. In a single unoptimized reaction (±)-**3**, derived from the carcinogen (±)-**B**,[19] was coupled with **5** using the optimized catalytic system. This reaction was complete within 4 h and produced a 71% yield of the N^6-diol epoxide adducts. For comparison, this adduct mixture was also synthesized from a reaction of (±)-**3** with **6**, and the products from the two methods proved identical.

This study demonstrates that Pd-catalyzed C−N bond formation between the axially constrained PAH bay-region amines and halo nucleosides offers a facile approach to the biologically important PAH epoxide−nucleoside adducts. It is also shown, for the first time, that chloro nucleosides are good partners in these reactions, and this metal-mediated synthesis of such adducts appears superior to conventional ones.[20] These adducts can be converted to phosphoramidites for DNA assembly. Alternatively, Pd-mediated amination of DNA oligomers containing **4**, **5**, and **7** may provide a facile method for site-specific modification. These and other related aspects are being studied in our laboratories.

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Supporting Information Available: Synthetic details and ^{1}H NMR spectra of the adducts. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Coupling of (±)-**3** and the 6-fluoro nucleoside was complete in 18 h at 85 °C (Chaturvedi, S. Ph.D. Dissertation, University of Oklahoma, Norman, OK, 1993), comparable to our experience.