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Synthesis of C8-Adenosine Adducts of Arylamines Using Palladium Catalysis

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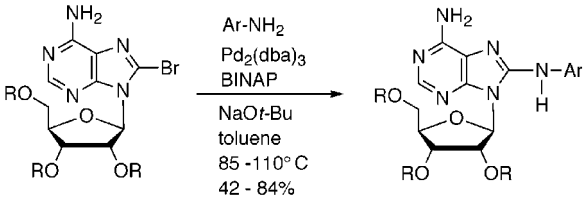
ABSTRACT



R = *tert*-butyldimethylsilyl

We employed palladium-catalyzed coupling procedures for the synthesis of new C8-adenosine adducts of various arylamines (aniline, benzidine, 4-aminobiphenyl, and 2-aminofluorene).

Any electrophile can become a potent chemical carcinogen by covalently modifying DNA.[1] For example, metabolic activation of polycyclic aromatic hydrocarbons and arylamines causes DNA mutations that may ultimately lead to cancer. To study mutagenic effects of chemicals, there has been a strong interest in the synthesis of artificial nucleosides such as altered adenosine and guanosine adducts. Their availability is necessary to identify and quantify structures of modified nucleosides.[2,3] Attention has been focused on lesions involving C8-, N2-, and N6-adducts of guanosine and adenosine derivatives. However, modified nucleosides have been difficult to study because they cannot be prepared in high yield by traditional methods. Nucleoside derivatives are also needed as building blocks for synthetic oligonucleotides[4] and to study DNA recognition[5] and repair mechanisms.[6] We now describe a facile, high-yield synthesis of nucleoside arylamine adducts using palladium catalysis.

Analytical tools continue to be critical for animal modeling of carcinogen exposure as a new strategy for cancer prevention.[7] To address the toxicity of environmental hazards such as arylamines, we needed a reliable synthesis of authentic and deuterated C8-purine adducts that could be used as analytical standards. We wanted to identify and quantify nucleotide adducts occurring in animals, that are exposed to arylamines, for dose response studies employing a new isotope dilution LC/MS/MS method. While some adducts of amines such as 2-aminofluorene[8] and 4-amino-

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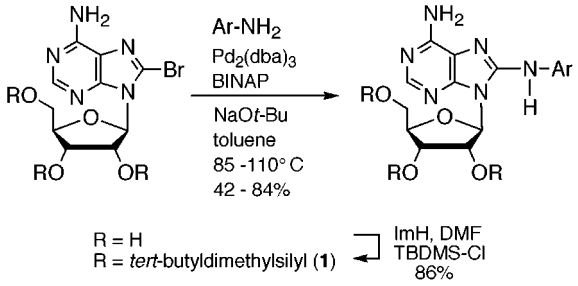
biphenyl[9] have been prepared before, the procedures tend to be difficult and low yields are obtained. This is primarily because traditional syntheses mimic biological activation and employ solvolysis of *O*-acetylamines[10] to generate nitrenium ions that react with nucleosides. Such electron-deficient species are highly reactive and unreliable as synthetic intermediates due to competing reactions, thus compromising yields and product isolation. To overcome these shortcomings, coupling procedures that use palladium catalysts have been developed recently.[11] Johnson et al.[12] prepared N2-deoxyguanosine and N6-deoxyadenosine adducts of *o*-nitroaryl bromides or triflates with protected purine nucleosides employing the Buchwald−Hartwig[13] coupling method as the key step. Additional reports by the same researchers[14] described N2-coupled derivatives of guanosine using 2-bromo-2′-deoxyinosine. A similar reciprocal approach was reported by Lakshman and co-workers[15] who substituted the N6-amine of adenosine with bromide and coupled it directly with arylamines in the presence of $Pd_2(dba)_3$, K_3PO_4, and the ligand 2-(dicyclohexylphosphino)-2′-(*N*,*N*-dimethyl-amino)-1,1′-biphenyl.[16]

On the basis of recent reports, we considered developing a catalytic method for the preparation of C8-purine adducts. Purine−arylamine adduct samples without a ribose unit were needed as references for comparisons with hydrolyzed DNA. Therefore, either ribose or deoxyribose could be used as a quasi-protecting group. More abundant C8-guanine adducts have dominated carcinogenesis studies; however, the significance of minor C8-adenine adducts is less studied and not well understood. 8-Bromoadenosine is commercially available and served as an inexpensive starting material. To avoid side reactions of the ribose hydroxyl groups, the starting material was silylated and easily purified using a modified literature procedure,[17] affording 8-bromo-2′,3′,5′-tris(*tert*-butyldimethylsilyl)-adenosine (**1**) in 86% yield. Typically, protection was complete after 24 h and formed only small amounts of mono- and diprotected products that were separated by flash column chromatography.

We began with the reaction of protected adenosine with aniline as a simplified model for C8-coupling. However, conditions reported for a similar coupling[15] of adenosine at N6 were unsuccessful for our system, even when reagent concentration was increased (10 mol % $Pd_2(dba)_3$, 30 mol % ligand). Senanayake and colleagues published a method for the Pd-mediated amination of 2-chlorobenzimidazole and other 1,3-azole derivatives and observed that protection at N3 was crucial.[18] Their conditions accomplished selective coupling of a primary amine in the presence of a secondary amine, and their protocol was selected for additional studies (Scheme 1). We tested coupling conditions for aniline in

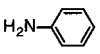
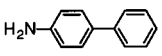
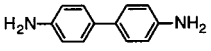
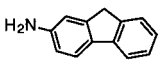
Scheme 1



toluene or *N*-methylpyrrolidinone. The latter solvent was considered to improve the solubility of all reactants, but even though some product formed, isolation was unsuccessful. Reactions succeeded with the use of 1.25 mol % $Pd_2(dba)_3$, 3.75 mol % racemic BINAP, and 1.4 equiv NaO*t*Bu in anhydrous toluene at 80 °C for 6 h, affording the corresponding C8-adduct in 84% isolated yield. This yield is much higher than any previously reported in the synthesis of C8-purine adducts. A control reaction without catalyst formed no product within the same period of time.

Reactions of polycyclic arylamines were more capricious, slower (8−24 h), and required more catalyst (30 mol % $Pd_2(dba)_3$, 50 mol % BINAP). We observed shorter reaction times (6 h) and comparable yields when $Pd(OAc)_2$ was employed, premixed with the ligand. All products were purified by column chromatography, which caused some depurination. Isolated yields are reported in Table 1.

Table 1. C8-Coupling of Adenosine Derivative **1** with Arylamines (Ar-NH_2)

Ar-NH_2	yield of **2**
	84%
	56%
	*60%
	42%

*Adduct ratio = 1:1.

For all new compounds prepared, each pair of diastereotopic methyl and *tert*-butyl groups was well resolved in

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^{1}H and ^{13}C NMR spectra. The ribose group in bromide **1** was beneficial for both protection and product characterization; NMR spectra showed distinct chemical shift changes upon C−N bond formation. Proton resonances for the 5′-methylene groups were shifted downfield and became well-resolved double AB quartets. Moreover, the C_1-ribose carbons became more shielded in the ^{13}C NMR spectra and they shifted upfield by ca. 15 ppm.

It is noteworthy that no additional N6-protection was necessary in our procedures. We detected no N6 mono- or disubstituted side products as reported by Johnson et al. in the coupling of equimolar amounts of 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine and *o*-nitroaryl triflates or bromides.[12] In the reaction with benzidine, we observed no bisadduct that could result from 2-fold coupling, nor was a dimer formed arising from the reaction of two molecules of adenosine derivative **1**.

The palladium-catalyzed reactions were carried out with racemic BINAP. To determine if the configuration of the catalyst has any effect on coupling, aniline and protected adenosine were treated with either (*R*)- or (*S*)-BINAP and the results were compared to results obtained with a racemic catalyst. Yields were 77% for (*R*)-BINAP and 87% for (*S*)-BINAP. While the yield was slightly higher for the (*S*)-catalyst than for the racemic one (84%), it appears that the diastereomeric relationship between substrate and catalyst is not a significant factor.

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To our knowledge, this is the first report on the preparation of C8-adenosine arylamine adducts using palladium catalysis. We are currently investigating the synthesis of additional C8-modified adenosine and guanosine derivatives. While our work was in progress, Rizzo and Wang[2] reported the coupling of nucleosides with methyl imidazole-type food mutagens and other arylamines. Their route used Lakshman-type conditions[15] for palladium-catalyzed N-arylation of a suitably protected 8-bromo-2′-deoxy-guanosine derivative in the presence of LHMDS as a stronger base. Under these conditions, two of the same arylamines, aminobiphenyl and 2-aminofluorene, were coupled in similar yields as reported here.

In conclusion, we have accomplished the synthesis of new C8-adenosine arylamine adducts by employing palladium-catalyzed N-arylation methods that gave much higher isolated yields than previously reported. Our approach allows for easy access to adenosine adducts without additional purine protection. Compounds **2** were hydrolyzed and used as standards for analytical methods that will be instrumental for dose response studies of known and suspected carcinogens and for elucidation of their mechanism of action.

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Supporting Information Available: Detailed descriptions of experimental procedures and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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