JOC*Note*

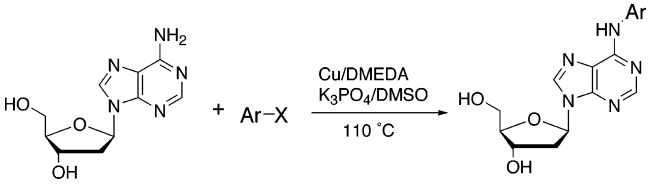
N^6-Arylation of 2′-Deoxyadenosine via Copper-Catalyzed Direct Coupling with Aryl Halides

Chongzhao Ran, Qing Dai, and Ronald G. Harvey*

Ben May Institute for Cancer Research, University of Chicago, Chicago, Illinois 60637

rharvey@ben-may.bsd.uchicago.edu

Received November 18, 2004



A general method for efficient N^6-arylation of 2′-deoxyadenosine via copper-catalyzed direct coupling with aryl iodides and bromides is described. The method is useful for aryl halides with either electron-donating or electron-withdrawing groups.

Polycyclic aromatic hydrocarbons (PAHs) and nitrosamines are strongly implicated as the principal cancer-causative agents in tobacco smoke,[1] and smoking of cigarettes has been related directly to 30% of *all cancers*.[2,3] PAH carcinogens formed by combustion of organic matter, e.g., fossil fuels, are also widespread contaminants of urban environments.[4,5]

PAHs are activated by CYP enzymes to form diol epoxide metabolites that react with the amino groups of 2′-deoxyadenosine (dA) and 2′-deoxyguanosine (dG) in DNA to form adducts that lead to mutations and tumor induction.[4,6,7] More recent studies have shown that the PAH dihydrodiol precursors of the diol epoxide metabolites are oxidized by aldo-keto reductase enzymes to catechols that enter into redox cycles with O_2 to generate quinones and reactive oxygen species.[8] The PAH quinones react with DNA to form stable and depurinating adducts.[8−10] However, the structures of the adducts are not established with certainty due to insufficiency of the amounts available and the failure of attempts to develop satisfactory methods for their synthesis.[11,12]

In connection with studies aimed at devising practical methods for synthesis of these adducts, we investigated copper-catalyzed coupling of aryl halides with dA as a synthetic route to N^6-PAH-dA adducts (Scheme 1).[13] Synthesis of N^6-aryl-dA adducts by Buchwald−Hartwig Pd-catalyzed coupling of a suitably protected derivative of dA with an *o*-nitroaryl bromide has been reported.[14a] However, the method is limited to aryl halides with strong electron-withdrawing groups, and attempted analogous coupling of aryl halides with electron-donating groups was not successful.[14b] The classic copper-catalyzed Ulmann reaction of aryl halides with arylamines[15] was impractical because of the relatively severe conditions required. However, it has been shown more recently that copper-mediated coupling can take place under milder conditions in the presence of various ligands.[15,16] Although palladium-catalyzed coupling of arylamines is known to proceed under mild conditions,[17] the choice of copper was dictated by its lower cost, relative insensitivity to air and moisture, anticipated broader substrate specificity, and the likelihood that protection−deprotection of the hydroxyl groups of the 2′-deoxyribose component would be unnecessary.

Preliminary experiments were conducted to assess the effectiveness of various ligands for the CuI-catalyzed reaction of 4-iodotoluene with dA (Table 1). Among the ligands most frequently employed for copper-mediated

* To whom correspondence should be addressed. Fax: (773) 702-6260.

(1) Review: Pfiefer, G. P.; Denissenko, M. F.; Olivier, M.; Tretyakova, N.; Hecht, S.; Hainaut, P. *Oncogene* **2002**, *21*, 7435−7451.

(2) International Agency for Research on Cancer. Monographs on the Evaluation of the Carcinogenic Risks to Humans. *Tobacco Smoke and Involuntary Smoking*; IARC: Lyon, France, 2004; Vol. 83.

(3) World Health Organization. *Tobacco or Health: A Global Status Report*; WHO: Geneva, 1997; pp 10−48.

(4) Harvey, R. G. *Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity*; Cambridge University Press: Cambridge, U.K., 1991.

(5) International Agency for Research on Cancer. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. *Polynuclear Aromatic Compounds, Part 1, Chemical, Environmental and Experimental Data*; IARC: Lyon, France, 1983; Vol. 32.

(6) Jeffrey, A. M.; Weinstein, I. B.; Jennette, K.; Grzeskowiak, K.; Nakanishi, K.; Harvey, R. G.; Autrup, H.; Harris, C. *Nature (London)* **1977**, *269*, 348−350. Jennette, K.; Jeffrey, A. M.; Blobstein, S. H.; Beland, F. A.; Harvey, R. G.; Weinstein, I. B. *Biochemistry* **1977**, *16*, 932−938.

(7) Dipple, A. In *DNA Adducts: Identification and Biological Significance*; Hemminki, K., Dipple, A., Segerbäck, D., Kadulbar, F. F., Shuker, D., Bartsch, H., Eds.; IARC Scientific Publication No. 125; IARC: Lyon, France, 1994; pp 107−129.

(8) Smithgall, T. E.; Harvey, R. G.; Penning, T. M. *J. Biol. Chem.* **1986**, *261*, 6184−6191; *Cancer Res.* **1988**, *48*, 1227−1232; *J. Biol. Chem.* **1988**, *263*, 1814−1820.

(9) Penning, T. M.; Onishi, S. T.; Onishi, T.; Harvey, R. G.; *Chem. Res. Toxicol.* **1996**, *9*, 84−92. Flowers, L.; Onishi, S. T.; Penning, T. M. *Biochemistry* **1997**, *36*, 8640. Shou, M.; Harvey, R. G.; Penning, T. M. *Carcinogenesis* **1993**, *14*, 475−482.

(10) A third mechanistic pathway that involves activation of PAHs by CYP peroxidase to generate PAH radical-cations that combine with DNA to form unstable adducts lost by depurination has also been proposed: Cavalieri, E. L.; Rogan, E. *Xenobiotica* **1995**, *25*, 677. The relevance of this mechanism is disputed: Melendez-Colon, V.; Luch, A.; Seidel, A.; Baird, W. *Carcinogenesis* **1999**, *20*, 1885.

(11) Gopishetty, S. R.; Harvey, R. G.; Lee, S.-H.; Blair, I. A.; Penning, T. M. In *Aldo-Keto Reductases and Toxicant Metabolism*; Penning, T. M., Petrash, J. M., Eds.; ACS Symposium Series No. 865: American Chemical Society: Washington, DC, 2003; Chapter 9, pp 127−137.

(12) McCoull, K. D.; Rindgen, D.; Blair, I. A.; Penning, T. M. *Chem. Res. Toxicol.* **1999**, *12*, 237−246.

(13) The synthetic route to the PAH quinone adducts of 2′-deoxyribonucleosides entails coupling of protected halogen-substituted derivatives of the PAH quinones (or the corresponding catechols) with dA or dG, or their derivatives, followed by deprotection (and in the case of the catechols reoxidation back to quinones).

(14) (a) De Riccardis, F.; Bonala, R. R.; Johnson, F. *J. Am. Chem. Soc.* **1999**, *121*, 10453−10460. (b) Lakshman, M. K.; Keeler, J. C.; Hilmer, J. H.; Martin, J. Q. *J. Am. Chem. Soc.* **1999**, *121*, 6090−6091.

(15) Hassan, J.; Sevignon, M.; Gozzi, C.; Schulz, E.; Lemaire, M. *Chem. Rev.* **2002**, *102*, 1359−1470.

(16) (a) Ley, S. V.; Thomas, A. W. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 5400−5449. (b) Gujadhur, R.; Venkataraman, D.; Kintigh, J. T. *Tetrahedron Lett.* **2001**, *42*, 4791−4793. (c) Kelkar, A. A.; Patil, N. M.; Chaudhari, *Tetrahedron Lett.* **2002**, *42*, 7143−7146. (d) Ma, D.; Cai, Q.; Zhang, H. *Org. Lett.* **2002**, *5*, 2453−2455. (e) Klapars, A.; Huang, X.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 7421−7428.

(17) Yang, B. H.; Buchwald, S. L. *J. Organomet. Chem.* **1999**, *576*, 125−146. Wolfe, J. P.; Wagaw, S.; Marcoux, J.-F.; Buchwald, S. L. *Acc. Chem. Res.* **1998**, *31*, 805−818. Hartwig, J. F. *Angew. Chem., Int. Ed.* **1998**, *37*, 2046−2067.

10.1021/jo040284w CCC: $30.25
Published on Web 03/30/2005

SCHEME 1

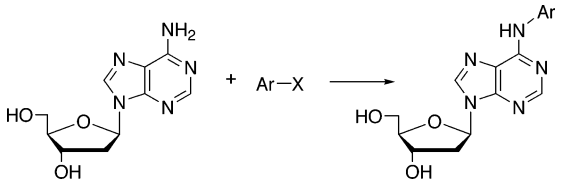


TABLE 1. CuI-Catalyzed Arylation of dA by 4-Iodotoluene

ligand	time (h)	N^6-aryl-dA[a] yield (%)
N,*N*′-dimethylethylene diamine	2.5	88
1,10-phenanthroline	36	81
1,10-phenanthroline, dba	36	81
N-methylglycine	24	68
L-proline	24	70

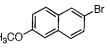
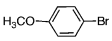
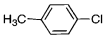
[a] Aryl = 4-methylphenyl.

coupling of aryl halides with amines and amides are ethylene glycols,[18a] salicylamides,[18b] amino acids,[19] ethylenediamines,[16e] and 1,10-phenanthrolines.[20] Ethylene glycols and salicylamides have been used mainly with alkylamines, while amino acids, ethylenediamines, and 1,10-phenanthrolines have been used principally for coupling arylamines. Ligands of the latter three types were used in these preliminary experiments. Copper(I) iodide was employed as the catalyst, and equimolar ratios of CuI, the ligand, the reactants, and the base were used. The yields of the adduct N^6-(4-methylphenyl)-2′-deoxyadenosine (N^6-aryl-dA) and the approximate times for completion of reaction were determined (Table 1).

N,*N*′-Dimethylethylenediamine (DMEDA) was the most effective ligand based on either time for completion of reaction (2.5 h vs 24−36 h for other ligands) or adduct yield (88%). 1,10-Phenanthroline, with or without dba, gave a slightly lower yield of adduct (80%), but required longer time (36 h) for completion of reaction. The ratio of catalyst to reactants also affected the rate. With a catalyst ratio of 100%, reaction was complete in 2−6 h. With a ratio of 10%, 36 h were required. The effectiveness of the bases was $K_2CO_3 < K_3PO_4 = Cs_2CO_3$ in accord with previous observations.[16e]

A series of experiments were also conducted to compare the reactions of various substituted aryl halides in the CuI-catalyzed arylation of dA (Table 2). Reactions were carried out in DMSO in the presence of DMEDA with 1 equiv each of CuI and K_3PO_4 at 110 °C. The reactions of the aryl iodides with dA (entries 1−10) proceeded smoothly to provide good yields of N^6-aryl-dA aducts. In most cases, minor amounts of bis-arylated adducts were also detected as products. The electronic character of the substituents in the aryl halide had remarkably little effect on the facility of reaction. Thus, arylation of dA by aryl halides

TABLE 2. Coupling of Aryl Halides with 2′-Deoxyadenosine

entries	Ar-X	N^6-Ar-dA adduct	time (h)	yield (%)[a,b]
1		**1**	2.5	88
2		**2**	2.5	85
3		**3**	2.5	84
4		**4**	2.5	80
5		**5**	2.5	78
6		–	1.5	complex[c]
7		**6**	1.5	70
8		**7**	1.5	73
9		**8**	48	65[d]
10		–	2.5	complex
11		**9**	72.0[e], 3.0[f]	20[e], 82[f]
12		**1**	3.5[f]	80
13		**3**	3.5[f]	84
14		**1**	24[f]	no reaction
15		**10**	6.0[e]	75
16		**11**	6.0[e]	70

[a] Yields are the average of two runs. [b] Reactions were carried out in DMSO at 110 °C, with equal ratios of ArX, dA monohydrate, CuI, DMEDA, and K_3PO_4. [c] It was not attempted to identify the components of the complex mixture of products formed. [d] Reaction was incomplete after 48 h. [e] K_2CO_3 was used as the base, and reaction was incomplete after 72 h. [f] One equivalent of NaI was added, and K_3PO_4 was used as the base.

with electron-donating substituents (methyl or methoxy) (entries 1, 3, 4, and 5) took place readily. Substrates with electron-withdrawing groups (entries 6−8) also reacted readily with dA but required shorter time for completion (∼1.5 h).

As expected, steric hindrance strongly inhibited coupling. The hindered aryl halide 2,6-dimethyliodobenzene entered into reaction with dA to form an adduct (entry 9), but reaction was slow, failing to attain completion in 48 h. In this case, a bis-arylated adduct was not formed.

The aryl bromides were less reactive than the aryl iodides, requiring longer time (3.5 h vs 2.5 h), and the

(18) (a) Kwong, F. Y.; Klapars, A.; Buchwald, S. L. *Org. Lett*. **2002**, *4*, 581−584. (b) Kwong, F. Y.; Buchwald, S. L. *Org. Lett*. **2003**, *5*, 793−796;

(19) (a) Ma, D.; Zhang, Y.; Yao, J.; Wu, S.; Tao, F. *J. Am. Chem. Soc.* **1998**, *120*, 12459. (b) Ma, D.; Xia, C. *Org. Lett*. **2001**, *3*, 2583−2586. (c) Ma, D.; Cia, Q.; Zhang, H. *Org. Lett*. **2003**, *5*, 2453−2455. (d) Clement, J.-B.; Hayes, J. F.; Sheldrake, H. M.; Sheldrake, P. W.; Wells, A. S. *Synlett* **2001**, 1423−1427.

(20) (a) Kiyomori, A.; Marcoux, J.-F.; Buchwald, S. L. *Tetrahedron Lett.* **1999**, *40*, 2657−2660. (b) Gujadhur, R.; Bates, C. G.; Venkataraman, D. *Org. Lett.* **2001**, *3*, 4315−4317. (c) Goodbrand, H. B.; Hu, N.-X. *J. Org. Chem.* **1999**, *64*, 670−674.

sole aryl chloride, 4-chlorotoluene (entry 14), failed to enter into reaction. These findings accord with previous observations on the relative reactivities of aryl halides (I > Br > Cl) in copper-catalyzed arylation of arylamines.[16e] It is also worthy of mention that 4-bromotoluene (entry 12) reacted smoothly to give a good yield of N^6-aryl-dA adduct (80%), despite its previous reported failure[14b] to undergo similar reaction with a Pd catalyst. It was also of interest to compare the relative reactivities of two different halogen atoms in the same molecule. Reaction of 4-chloro-1-iodobenzene with dA took place predominantly on the iodo function to furnish up to 70% of the expected product, N^6-(4-chlorophenyl)-dA. Analogous reaction of 4-bromo-1-iodobenzene exhibited less selectivity, affording a complex mixture of products. Although methoxy groups were well tolerated (entries 3, 11, and 13), the presence of a free phenolic hydroxy group (entry 10) resulted in formation of a complex mixture of products.

Another significant finding was that the rates of reaction of the aryl bromides were increased by addition of sodium iodide (entries 11−13, 15, 16). Thus, reaction of 2-bromo-6-methoxynaphthalene with dA and K_2CO_3 was slow in the absence of NaI, affording the N^6-aryl-dA adduct in low yield (20%) after 72 h. In contrast, analogous reaction of the same aryl halide conducted in the presence of 1 equiv of NaI with K_3PO_4 as the base gave the N^6-aryl-dA adduct in 82% yield in 3 h. This rate enhancement is likely due to conversion of the aryl bromide to an aryl iodide. Aryl bromides are known to be converted to aryl iodides on treatment with CuI and KI or NaI.[21] To verify this hypothesis, we carried out a control reaction using the same reactants and conditions in the absence of dA. Under these conditions, 2-bromo-6-methoxynaphthalene underwent smooth conversion to 2-iodo-6-methoxynaphthalene, confirming that halogen exchange is the basis of the rate enhancement effect. On the other hand, 4-chlorotoluene failed to react with dA in the presence of NaI and K_3PO_4, indicating that aryl chlorides do not participate in halogen exchange under these conditions.

Because a longer range aim of this investigation was to develop a practical synthetic route to adducts of PAH carcinogens with 2′-deoxyribonucleosides, these studies were extended to include two examples of larger PAHs (entries 15 and 16). Copper-catalyzed coupling of 9-bromophenanthrene and 1-bromopyrene with dA under the usual conditions took place smoothly to furnish the corresponding N^6-aryl-dA adducts in yields of 75% and 70%, respectively.

The potential applicability of the copper-catalyzed coupling method to synthesis of analogous N^2-aryl adducts of 2′-deoxyguanosine was also briefly examined. However, dG failed to enter into reaction with 4-iodoanisole in the presence of CuI, DMEDA and K_3PO_4 in DMSO under the usual conditions employed for reaction of dA. The most likely reason for the failure of dG to participate in this reaction was the relatively weak nucleophilic character of the N^2-amino group due to the nearby presence of the strongly electron-withdrawing carbonyl group. To counteract this effect, dG was converted to its O^6-benzyl ether derivative, effectively converting the carbonyl group into an electron-donating group. Reaction of O^6-benzyl-dG with 4-iodoanisole under the standard conditions took place smoothly to afford the N^2-aryl-dG adduct in good yield (79%).

In summary, we report a remarkably convenient method for N^6-arylation of 2′-deoxyadenosine. The method entails copper-catalyzed direct coupling of unprotected dA with aryl iodides and bromides. It is broad in scope, allowing the use of aryl halides with either electron-withdrawing or electron-donation groups. It also has the practical advantage that protection-deprotection of the OH groups of the sugar is not necessary. Although these investigations focused primarily on arylation of dA, the preliminary findings indicate that the method is readily adaptable to synthesis of analogous aryl adducts of dG and other deoxyribonucleosides. The findings also indicate, on the basis of two examples (entries 15, 16), that the method is equally applicable to the synthesis of analogous adducts of larger polycyclic aromatic compounds, presumably including the DNA adducts formed by PAH carcinogens. Application of the method to synthesis of the adducts formed by carcinogenic PAH quinones, such as benzo[*a*]pyrene-7,8-dione, at dA and dG sites in DNA is currently under investigation.[13]

Experimental Section

General Methods. The starting compounds, reagents, and solvents were commercial grade and were used without further purification. NMR (^{1}H and ^{13} C) spectra were recorded on 400 or 500 MHz spectrometers, and samples were dissolved in CD_3OD-d_4 or DMSO-d_6. Chemical shifts are reported in δ ppm relative to TMS for the proton spectra and to the deuterated solvent for the carbon spectra. TLC was performed on silica gel sheets containing fluorescent indicator. Chromatographic separations were carried out on silica gel 60 M (230−400 mesh). Melting points are uncorrected.

Typical Procedure for N^6-Arylation of 2′-Deoxyadenosine: Preparation of N^6-(4-Methylphenyl)-2′-deoxyadenosine (1). 4-Iodotoluene (0.11 g, 0.5 mmol) and 2′-deoxyadenosine monohydrate (0.14 g, 0.5 mmol) were added to a flask containing DMSO (5.0 mL). This was followed by K_3PO_4 (0.11 g, 0.5 mmol), CuI (0.1 g, 0.5 mmol), and DMEDA (44.0 mg, 0.5 mmol). The resulting mixture was heated to 100−110 °C and stirred for 2.5 h. The solution was cooled to rt and poured into cold water (50 mL). Then 0.5 mL of a 28−30% aqueous solution of ammonia hydroxide was added. The product was extracted with EtOAc (3 × 30 mL), dried over Na_2SO_4, and purified by flash chromatography on a silica gel column eluted with CH_2Cl_2/EtOAc/CH_3OH (6:6:1) to furnish the N^6-aryl-dA adduct (**1**) (0.143 g, 84.0%): mp 201−202 °C; ^{1}H NMR (DMSO) δ 2.27 (s, 3), 2.31 (m, 1), 2.75 (m, 1), 3.53 (m, 1), 3.63 (m, 1), 3.89 (dd, 1, J = 4.0, 7.0 Hz), 4.43 (m, 1), 5.13 (t, 1, J = 6.5 Hz), 5.32 (d, 1, J = 4.0 Hz), 6.40 (dd, 1, J = 6.0, 6.0 Hz), 7.11 (d, 2, J = 8.5 Hz), 7.80 (d, 2, J = 8.5 Hz), 8.35 (s, 1), 8.49 (br, 1), 9.78 (s, 1); ^{13} C NMR (DMSO) 22.4, 41.3, 63.7, 72.8, 85.8, 89.9, 122.1, 122.8, 130.7, 133.5, 138.8, 142.1, 150.9, 153.8, 154.0; HRMS $(M + 1)^+$ calcd 342.1566, found 342.1575.

Acknowledgment. This investigation was supported by Grant No. P01 CA-92537 from the National Cancer Institute, DHHS.

Supporting Information Available: Details of the synthetic procedures and the ^{1}H NMR, ^{13}C NMR, and HRMS spectra of the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO040284W

(21) Klapars, A.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 14844−14845. Xu, G.; Sygula, A.; Marcinow, Z.; Rabideau, P. W. *Tetrahedron Lett.* **2000**, *41*, 9931−9934.