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Synthesis of N^2 2′-Deoxyguanosine Adducts Formed by 1-Nitropyrene

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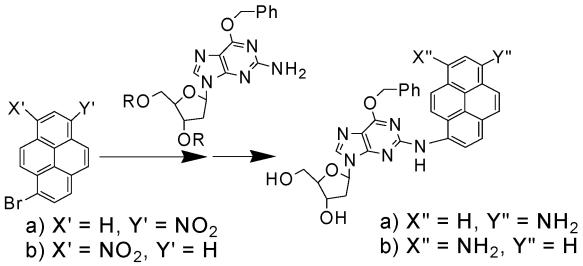
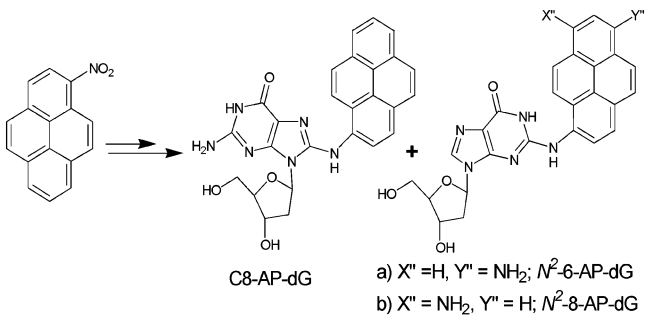
ABSTRACT



Synthesis of N^2 2′-deoxyguanosine adducts formed by the ubiquitous carcinogen, 1-nitropyrene, is reported. Various conditions of Buchwald–Hartwig palladium-catalyzed amination are examined. The most convenient synthetic approach involved a straightforward coupling between protected 2′-deoxyguanosine and bromonitropyrenes, which, upon reductive deprotection, provided excellent yield of the two 1-nitropyrene adducts.

1-Nitropyrene (1-NP), the most abundant nitroaromatic compound in the environment,[1] is mutagenic[2] and carcinogenic.[3] Reductively activated 1-NP forms a C8 and two N^2 2′-deoxyguanosine (dG) adducts (Scheme 1).[4] To study the

Scheme 1



structural and biological effects of these DNA adducts, synthesis of adducted nucleosides and oligonucleotides is a goal of our research. In prior work, we have successfully synthesized oligonucleotides containing *N*-(deoxyguanosin-8-yl)-1-aminopyrene (C8-AP-dG) by allowing the unmodified oligonucleotides in duplex form to react with *N*-hydroxy-1-aminopyrene generated in situ from 1-nitrosopyrene.[5] However, the N^2 dG adducts of 1-NP, 6-(deoxyguanosin-N^2-yl)-1-aminopyrene (N^2-6-AP-dG) and 8-(deoxyguanosin-N^2-yl)-1-aminopyrene (N^2-8-AP-dG), cannot be synthesized by this approach. The alternative and higher yielding method for synthesizing the modified oligonucleotides is a total synthesis strategy that involves preparation of the adducted

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nucleosides as the essential first step. In recent years, Buchwald−Hartwig palladium-catalyzed amination[6,7] has been extensively used to synthesize many carcinogen−DNA adducts.[8,9] In this approach a bromo derivative of dG, dI, or dA at the 8-, 2-, or 6-position, respectively, is coupled with an alkyl or aralkylamine.[8] DNA−DNA cross-links have also been synthesized by a slight modification of this strategy.[9] Significant success has been achieved in the synthesis of the C8 dG and dA adducts of aromatic amine carcinogens,[8d−g] and recently the C8 dG adduct of 1-NP (i.e., C8-AP-dG in Scheme 1) has been prepared in 51% yield.[8g] A general method for synthesizing N^2 dG adducts has been reported by Johnson and co-workers,[8b] which was utilized by others as well.[8c,9a] However, none of the arylamines contained a polycyclic ring system. An alternate strategy, in which the N^2 amino functionality of dG was coupled with 2-nitroaryl bromides or triflates, was also used successfully by Johnson.[10] However, it was unclear if this approach would be applicable to aryl or aralkyl halides that did not contain an activating group at the ortho or para position. Hopkins compared the coupling between phenyl bromide/protected dG and phenylamine/protected 2-Br-dI and found that the latter approach provides a better yield except when the phenyl ring contains a nitro group at the para position.[9a] It is noteworthy that, unlike the substrates used by Johnson[10] and Hopkins,[9a] the N^2 dG adducts of 1-NP contain an amino group on a distal phenyl ring of the pyrene moiety, which, in principle, can be easily derived by reduction of a nitro group. The objective of the current work is to evaluate the two approaches of Pd-catalyzed couplings under various conditions, and herein we report a high-yielding method of synthesis of these adducts.

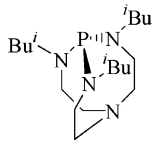
Our preliminary experiments involved coupling between 2-bromo-O^6-benzyl-3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxyinosine and 1-aminopyrene. This was compared with the coupling between O^6-benzyl-3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxyguanosine and 1-bromopyrene. To monitor the progress of the reaction, we followed disappearance of the pyrene derivative by reverse-phase HPLC. After 80−90% disappearance of the 1-bromopyrene or 1-aminopyrene, the coupled product was isolated to assess the actual yield. For the coupling between protected 2-Br-dI and 1-aminopyrene, we found that $Pd(OAc)_2/Cs_2CO_3$ was superior to $Pd_2(dba)_3$ (dba = *trans*,*trans*-dibenzylidenacetone) with either NaO*t*Bu or K_3PO_4, when BINAP was used as the ligand and the reaction was carried out in toluene at 80 °C. The catalytic system of 10 mol % $Pd(OAc)_2$, 15 mol % racemic BINAP, with 1.5 equiv of the base Cs_2CO_3 in toluene at 80 °C gave 88% conversion of 1-aminopyrene and 80% isolated yield of the coupled product in 16 h. For the opposite strategy of coupling between protected dG and 1-bromopyrene, we found that similar conditions with an increase in BINAP to 70 mol % gave 83% conversion of 1-bromopyrene, though the isolated yield was 57%.[11] Allowing the reaction to continue for a longer period and increasing the temperature up to 100 °C were not helpful and led to partial decomposition of the product. While the reaction of 2-bromo dG with 1-aminopyrene provided a much better yield, an attractive feature of the coupling between dG and 1-bromopyrene is that it reduces a step.

With these encouraging results, we attempted coupling of the protected dG with 1-bromo-8-nitropyrene (Table 1).

Table 1. Comparison of Coupling of Protected dG with 1-bromo-8-nitropyrene

	Pd reagent (mol %)	base	ligand (mol %)	temp (°C)	time (h)	yield (%)
1	$Pd_2(dba)_3$ (10)	K_3PO_4	L-2 (30)	80	16	<30
2	$Pd_2(dba)_3$ (10)	Cs_2CO_3	L-2 (30)	100	16	15
3	$Pd(OAc)_2$ (2)	^{t}BuONa	L-3 (4)	80	3	decomp
4	P-3 (0.5)	^{t}BuONa	L-4	60	6	<30
5	P-3 (0.5)	^{t}BuONa	L-5	60	6	<30
6	$Pd(OAc)_2$ (10)	Cs_2CO_3	L-1 (70)	80	8	88
7	$Pd_2(dba)_3$ (10)	^{t}BuONa	L-1 (70)	80	3	multiple products

[a] Key: L-1 = (±)-BINAP; L-2 = 2-(dicyclohexylphosphino)-2′-(*N*,*N*-dimethylamino)-1,1′-biphenyl;

L-3 = 

P-3 = [{PdBrL*}$_2$]; L* = L-4 or L-5; L-4 = P(1-Ad)^{t}Bu$_2$; L-5 = P^{t}Bu$_3$

Lakshman successfully applied the electron-rich ligand, 2-(dicyclohexylphosphino)-2′-(*N*,*N*-dimethylamino)-1,1′-biphenyl), L2, with $Pd_2(dba)_3$ and K_3PO_4 in 1,2-DME for the synthesis of N^6-aryl dA adducts,[8a] whereas Rizzo achieved a decent yield of the C8-dG arylamine adducts using the same.[8d] However, coupling of protected dG with 1-bromo-8-nitropyrene proceeded very slowly to ∼30% in 16 h (entry 1, Table 1), whereas changing the base to Cs_2CO_3 and increasing the temperature to 100 °C decreased the yield to 15% (entry 2, Table 1). The bicyclic triaminophosphine ligand L3, used successfully by Verkade and co-workers,[12]

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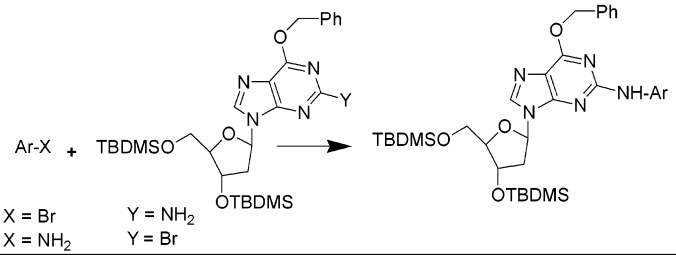
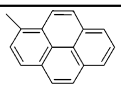
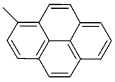
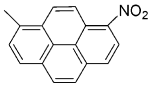
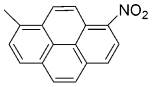
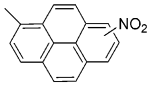
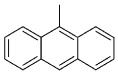
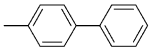
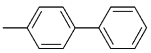
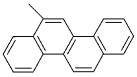
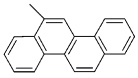
(10) De Riccardis, F.; Bonala, R. R.; Johnson, F. *J. Am. Chem. Soc.* **1999**, *121*, 10453.

(11) After completion of this work but before submission of our manuscript, we became aware of a preprint (Lakshman, M. K.; Ngassa, F. N.; Bae, S.; Buchanan, D. G.; Hahn, H.-G.; Mah, H. *J. Org. Chem.*, in press) in which the coupling efficiency between 1-bromopyrene/protected dG and 1-aminopyrene/protected 2-Br-dI had been compared. The yield

Table 2. Aryl Donors/Acceptors and N^2-Adduct of Protected dG[a]



	Ar	X	Y	time (h)	conversion (%) [b]	yield (%) [c]
1		Br	NH_2	16	83	57
2		NH_2	Br	16	88	80
3		Br	NH_2	8	90	88
4		NH_2	Br	16	91	76
5	Mixture[d] of crude 	Br	NH_2	8	80	60[e]
6		Br	NH_2	16	82	19
7		Br	NH_2	16	83	19
8		Br	NH_2	16	87	28
9		NH_2	Br	16	90	86
10		Br	NH_2	16	87	40
11		NH_2	Br	16	90	76

[a] Each reaction was carried out for the specified time at 80 °C in toluene with $Pd(OAc)_2$, BINAP, and Cs_2CO_3. [b] Reactions were monitored by reverse-phase HPLC, and conversion indicates the % Ar-X used up at the end of the reaction. [c] Yield (%) refers to isolated purified product. [d] Mixture of bromonitropyrenes was used. [e] Combined yield of only 6-(deoxyguanosin-N^2-yl)-1-nitropyrene and 8-(deoxyguanosin-N^2-yl)-1-nitropyrene derivatives in approximately 1:1 ratio.

was disappointing and led to decomposition of the starting material (entry 3, Table 1). In addition to $Pd(OAc)_2$ and $Pd_2(dba)_3$, we also evaluated dimeric [{PdBrL*}$_2$] (where L* = P(1-Ad)*t*Bu$_2$ or P*t*Bu$_3$), recently reported by Hartwig and co-workers,[13] which are formally Pd^I complexes and are active as catalysts for coupling of various aryl chlorides or bromides and amines. The most attractive feature of the latter catalysts is that the reaction proceeds at room temperature. Unfortunately, in the present case, we were unable to detect any reaction at room temperature, and warming to 60 °C allowed the isolation of only 30% product after 6 h (entry 4 and 5, Table 1). To our delight, however, a combination of $Pd(OAc)_2$, BINAP, and Cs_2CO_3 in toluene at 80 °C gave 88% yield in 8 h. Evidently, the 8-nitro group played a role in activating this aryl system, since the parent compound, 1-bromopyrene, provided only 57% yield of the coupled product after 16 h (Table 2). The coupling yield (76%) of

reported here for the latter combination is identical (i.e., 80%) to what we noted (entry 2, Table 2). However, for the former, Lakshman et al. reported 86% yield of the impure product, whereas we were able to isolate the pure product with lower yield (57%) (entry 1, Table 2). There are some notable differences in the conditions of these experiments, and an important issue may be that we used a lower temperature for a longer time (80 °C for 16 h compared to 90 °C for 3.5 h by Lakshman). Despite the differences in conditions and yields, we agree with Lakshman et al. that the coupling of 1-aminopyrene with protected 2-Br-dI is a more efficient reaction than 1-bromopyrene with protected dG.

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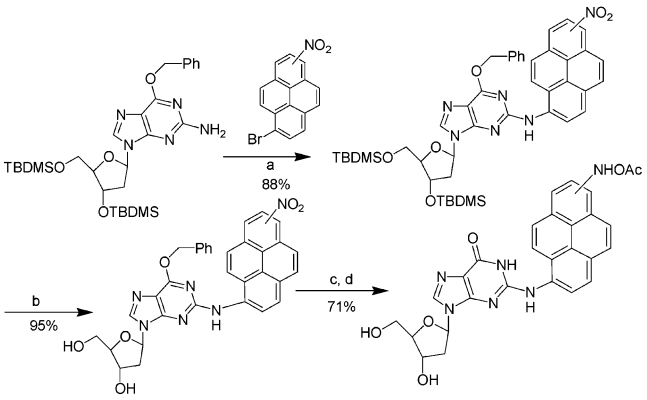
(13)) Stambuli, J. P.; Kuwano, R.; Hartwig, J. F. *Angew. Chem., Int. Ed.* **2002**, *41*, 4746.

1-amino-8-nitropyrene with 2-bromo dI (entry 4, Table 2) was also lower than the reaction of bromonitropyrene with protected dG. It appears that increasing the number of rings does not activate the aryl system, but introduction of a nitro group even at a remote position from the bromo substituent activates the aryl bromides for efficient cross-coupling reactions.

Nitration of 1-bromopyrene generates 1-bromo-3-nitropyrene, 1-bromo-6-nitropyrene, and 1-bromo-8-nitropyrene in approximately 1:2:2 ratio, but separation of the three isomers is tedious.[14] To develop a more convenient method, we evaluated a reaction of the mixture of the crude bromonitropyrenes with protected dG and found that, following the Pd-catalyzed coupling, the N^2-6-AP-dG and N^2-8-AP-dG derivatives can be cleanly separated from the rest by preparative silica gel TLC. This not only was a simple approach to making the two desired isomeric adducts but also provided a satisfactory combined yield of 60% (entry 5, Table 2). Deprotection of the coupled products gave the dG adducts (Scheme 2).

Scheme 2 [a]



[a] Reaction conditions: (a) 10 mol% $Pd(OAc)_2$, 70 mol% BINAP, Cs_2CO_3, toluene, 80 °C, 8 h; (b) TBAF, THF, 0 °C to rt, 2 h; (c) 5% Pd−C, MeOH−THF (1:1), H_2, rt, 5 h; (d) Ac_2O, DCM, rt, 5 h [Note: the yields indicated are for 1-bromo-8-nitropyrene].

Even though coupling of protected dG with the pyrene derivatives worked well, the coupling yields for the bromides of several other polycyclic aromatic hydrocarbons were modest (entries 6−8 and 10, Table 2). In comparison, when some of the corresponding polycyclic aromatic amines were coupled with 2-Br-dI, the yields of the isolated products were much higher (entries 9 and 11, Table 2), suggesting that in the absence of an activating group in the ring system, this should be the method of choice.

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

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