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Synthesis of the C8-Deoxyguanosine Adduct of the Food Mutagen IQ

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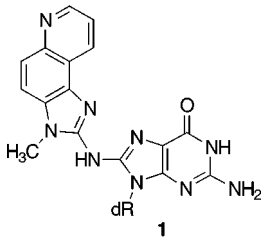
ABSTRACT



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The C8-2′-deoxyguanosine adduct of the food mutagen 2-amino-3-methylimadazo[4,5-f]-quinoline (IQ) has been synthesized. The key step is a palladium-catalyzed N-arylation of a suitably protected 8-bromo-2′-deoxygunaosine derivative.

Covalent modification of DNA by electrophiles is the initial step in chemical carcinogenesis.[1] If these modifications are not repaired, they compromise the fidelity of DNA replication, leading to mutations and possibly cancer. Many such electrophiles are generated only after metabolic activation of a procarcinogen. Examples of such procarcinogens include polycyclic aromatic hydrocarbons, vinyl chloride, and arylamines. To properly study the mutagenic effects, structure, and repair of these lesions, strategies for the site-specific incorporation of DNA−carcinogen adducts into oligonucleotides must be developed. The adducted nucleosides are of value as potential building blocks for modified oligonucleotides as well as for analytical standards.

A growing number of mutagenic compounds from cooked meats have been identified.[2] These compounds are believed to arise from the pyrolysis of amino acids and proteins. One class, shown in Figure 1, possesses a common 2-amino-3-methylimidazole subunit fused to a heteroaromatic ring system. These compounds are highly mutagenic in the Ames *Salmonella* test system. The most potent food mutagens are IQ (**2**) and MeIQ (**3**), which are 15 and 24 times more mutagenic than aflatoxin b1, respectively.[1b] The ultimate carcinogenic species is an arylnitrenium ion generated by cytochrome P450 oxidation to the corresponding hydroxylamine, followed by esterification and solvolysis. The predominant site of reaction is the C8-position of deoxyguanosine, although N^2-adducts have also been isolated as minor products (Figure 2).

Oligonucleotides containing a site-specific C8-dG adduct

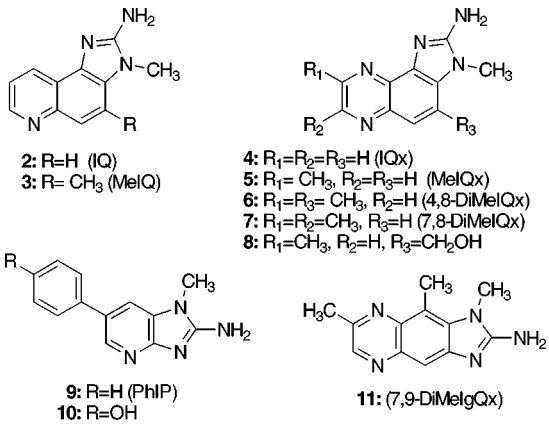

Figure 1. Aminoimidazoazaarene (AIA) food mutagens.

(1) Garner, R. C. *Mutat. Res.* **1998**, *402*, 67.
(2) (a) Schut, H. A. J.; Snyderwine, E. G. *Carcinogenesis* **1999**, *20*, 353. (b) Sugimura, T. *Mutat. Res.* **1997**, *376*, 211.

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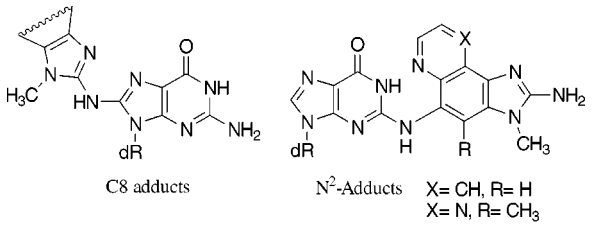
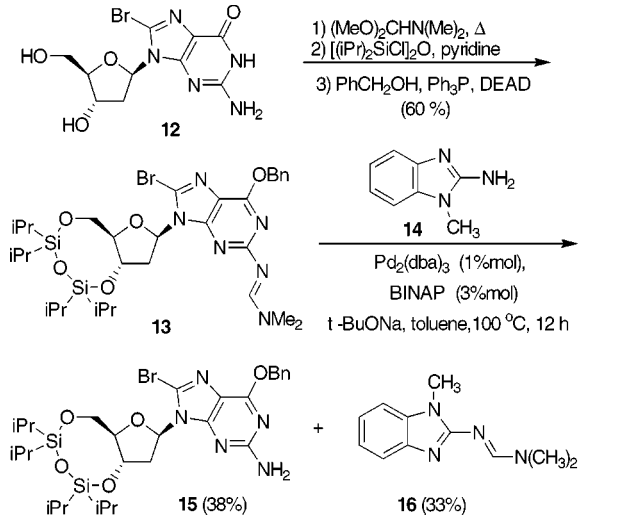


Figure 2. C8- and N^2-deoxyguanosine AIA adducts.

of 2-aminofluorene (AF) and its *N*-acetyl analogue have been prepared, and their structures and mutagenic effects are well studied.[3] Recently, PhIP (**9**) has been site-specifically incorporated into oligonucleotides.[4] These oligonucleotides were prepared by a biomimetic approach in which *N*-acetoxy-PhIP was reacted with oligonucleotides containing a single guanosine; Johnson has previously commented on the drawbacks to this synthetic appraoch.[3c] Overall, the mutagenicity of the C8-PhIP adduct was similar to that of the corresponding AF adduct; however, the mutagenic frequency of PhIP was up to nine times higher depending on sequence. A computational study of an IQ adduct has been recently reported and suggests some structural differences from the corresponding AF adduct.[5] We report here the synthesis of the C8-2′-deoxyguanosine adduct of the food mutagen IQ (**2**). The synthesis features a Buchwald−Hartwig[6] palladium-catalyzed N-arylation of a suitably protected 8-bromo-2′-deoxyguanosine derivative with IQ as the key reaction.

The Buchwald−Hartwig reaction has been used recently for the preparation of nucleoside−carcinogen adducts by Lakshman[7] and later Johnson,[8a] for the preparation of N^6-2′-deoxyadensosine derivatives. Hopkins and Sigurdsson[9] and Johnson[4b−d] synthesized N^2-dG-N^2-dG and N^2-dG-N^6-dA nitrous acid cross-links as well as other N^2-dG aryl derivatives via an N-arylation reaction. It is worth noting that Johnson's N-arylation approach involved coupling of the exocyclic amino groups of a suitably protected dA and dG derivative with bromoarenes, while Lakshman and Hopkins and Sigurdsson employed the corresponding bromopurine

Scheme 1



with arylamines. To our knowledge, the synthesis of C8-dG adducts of arylamine using a palladium-catalyzed N-arylation reaction has not yet been reported.

Buchwald and others have reported the N-arylation of amides.[10] Thus, conventional amide protecting groups for N^2 of dG would be unsatisfactory. Initially, we employed a dimethyl formamidine group which is commonly used for N^2-dG protection (Scheme 1). The O^6-position was protected as a benzyl ether. Attempted palladium-catalyzed N-arylation of **13** with model substrate **14** gave only transfer of the N^2-protecting group to the amino group of **14**. No N-arylation of the C8-position was observed. The transfer of the formamidine group to other amines has been previously reported.[11]

We next examined the tetramethyldisilylazacyclopentane (STABASE) group, a base-stable protecting group for primary amines developed by Magnus.[12] The substrate for the N-arylation reaction (**17**) was readily prepared from 8-bromo-2′-deoxyguanosine according to Scheme 2. Buchwald−Hartwig reaction of **17** with model amine **14** under the conditions shown in Scheme 1 gave the desired product in 32% yield. We found that the prolonged reaction time led to decomposition of **17**. Other mild bases such Cs_2CO_3 or K_3PO_4 gave lower yields. The optimal conditions for the desired reaction involved increasing the catalyst loading to 10 mol % and using lithium hexamethyldisilazide as the base (Scheme 2). Under these conditions a 68% yield of the desired product (**18**) could be obtained in just 20 min. These conditions were also satisfactory for the coupling of IQ (**2**) with **17** to give **19** in 68% yield (Scheme 3). Treatment of

(3) (a) Zhou, Y.; Romano, L. J. *Biochemistry* **1993**, *32*, 14043. (b) Shibutani, S.; Gentles, R. G.; Iden, C. R.; Johnson, F. *J. Am. Chem. Soc.* **1990**, *112*, 5667. (c) Johnson, F.; Huang, C.-Y.; Yu, P.-L. *Environ. Healh Perspect.* **1994**, *102 Supp. 6*, 143. (d) Patel, D. J.; Mao, B.; Gu, Z.; Hingerty, B. E.; Gorin, A.; Basu, A. K.; Broyde, S. *Chem. Res. Toxicol.* **1998**, *11*, 391.

(4) Shibutani, S.; Fernandes, A.; Suzuki, N.; Zhou, L.; Johnson, F.; Grollman, A. P. *J. Biol. Chem.* **1999**, *274*, 27433.

(5) Wu, X.; Shapiro, R.; Broyde, S. *Chem. Res. Toxicol.* **1999**, *12*, 895.

(6) (a) Wolfe, J. P.; Wagaw, S.; Marcoux, J.-F.; Buchwald, S. L. *Acc. Chem. Res.* **1998**, *31*, 805. (b) Hartwig, J. F. *Acc. Chem. Res.* **1998**, *31*, 852. (c) Hartwig, J. F. *Angew. Chem., Int. Ed.***1998**, *37*, 2046. (d) Yang, B. H.; Buchwald, S. L. *J. Organomet. Chem.* **1999**, *576*, 125.

(7) Lakshman, M. K.; Keeler, J. C.; Hilmer, J. H.; Martin, J. Q. *J. Am. Chem. Soc.* **1999**, *121*, 6090.

(8) (a) De Riccardis, F.; Bonala, R. R.; Johnson, F. *J. Am. Chem. Soc.* **1999**, *121*, 10453. (b) Bonala, R. R.; Yu, P.-L.; Johnson, F. *Tetrahedron Lett.* **1999**, *40*, 597. (c) De Riccardis, F.; Johnson, F. *Org. Lett.* **2000**, *2*, 293. (d) Bonala, R. R.; Shishkina, I. G.; Johnson, F. *Tetrahedron Lett.* **2000**, *41*, 7281.

(9) (a) Harwood, E. A.; Hopkins, P. B.; Sigurdsson, S. Th. *J. Org. Chem.* **2000**, *65*, 2959. (b) Harwood, E. A.; Sigurdsson, S. Th.; Edfeldt, N. B. F.; Reid, B. R.; Hopkins, P. B. *J. Am. Chem. Soc.* **1999**, *121*, 5081.

(10) Yin J.; Buchwald, S. L. *Org. Lett.* **2000**, *2*, 1101 and references therein.

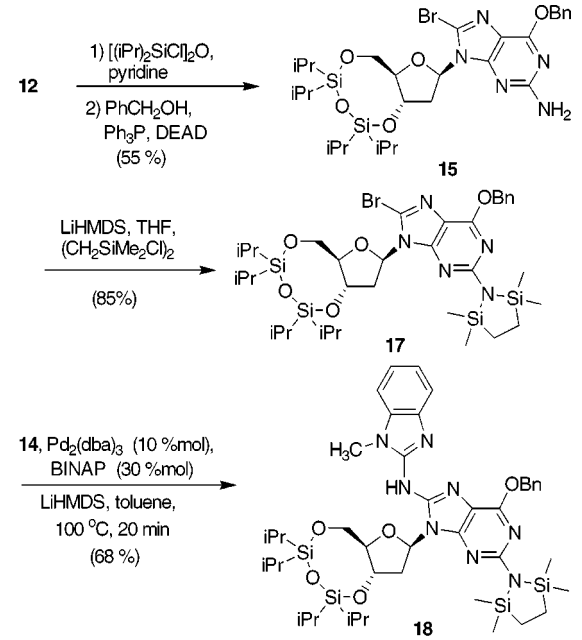
(11) (a) Vincent, S.; Mons, S.; Lebeau, L.; Mioskowski, C. *Tetrahedron Lett.* **1997**, *38*, 7527. (b) Theisen, P.; McCollum, C.; Andrus, A. *Nucleosides Nucleotides* **1993**, *12*, 1033.

(12) Djuric, S.; Venit, J.; Magnus, P. *Tetrahedron Lett.* **1981**, *22*, 1787.

(13) (a) Louie, J.; Hartwig, J. F.; Fry, A. J. *J. Am. Chem. Soc.* **1997**, *119*, 11695. (b) Louie, J.; Hartwig, J. F. *Tetrahedron Lett.* **1995**, *36*, 3609.

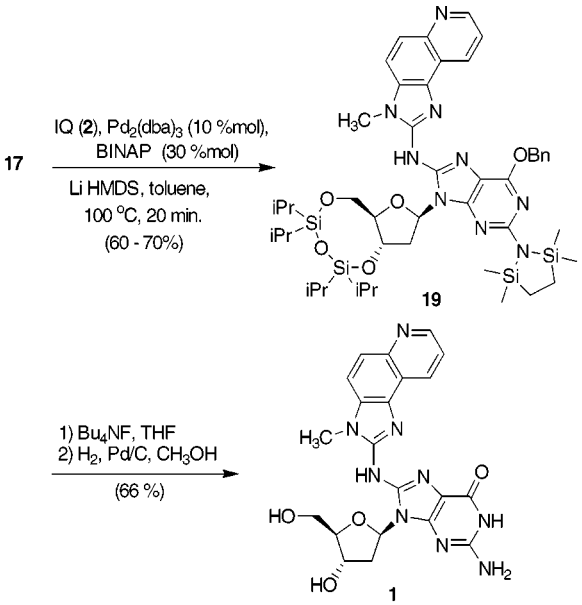
(14) Kelly, T. A.; McNeil, D. W. *Tetrahedron Lett.* **1994**, *35*, 9003.

Scheme 2

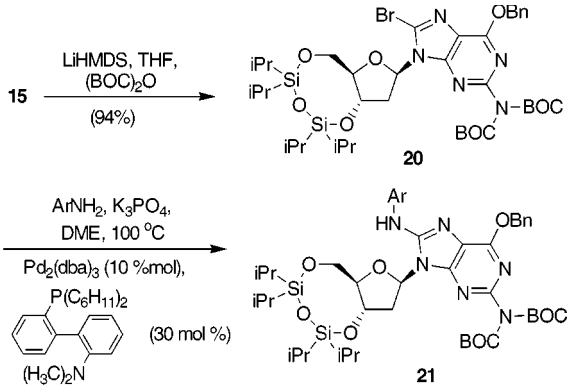


19 with flouride followed by hydrogenolysis of the O^6-benzyl group gave the desired C8−IQ adduct of dG (**1**) in 66% overall yield. The synthesis of **1** required six steps from commercially available 8-bromo-2′-deoxyguanosine and proceeded in 32% overall yield. The key to the successful coupling of **17** with **14** or **2** is the use of lithium hexamethyldisilazide (LiHMDS) as the base, which is much stronger than is typically used for the N-arylation reaction. Hartwig has reported the use of lithium amides or amines with LiHMDS in the cross-coupling with bromoarenes in high yield and short reaction times.[13] It is possible

Scheme 3



Scheme 4

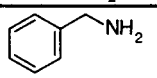
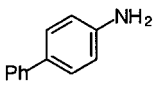
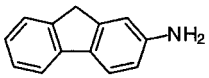
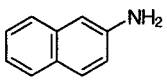


that LiHMDS is generating an appreciable concentration of the corresponding lithium amide of **14** or **2** which is the reactive substrate.

To examine the generality of this approach for the synthesis of C8-dG arylamine adducts, the N-arylation of **17** with benzylamine, 4-aminobiphenyl, 2-aminofluorene, and 2-naphthylamine was attempted. However, the optimal conditions for the Buchwald−Hartwig reaction of **17** with **14** and **2** shown Schemes 2 and 3 gave largely decomposition with these simple arylamines; less than 10% of the desired product was observed. We concluded that the STABASE group was not satisfactory for the N-arylation of other amines. Protection of the N^2-position as a bis-BOC derivative (**20**) improved the results.[14] The bis-BOC group is sensitive to strong base. When the coupling was attempted with LiHMDS or sodium *tert*-butoxide, the desired product could be obtained in 30−40% yields as a mixture of di-BOC and mono-BOC protected products. The optimal conditions for N-arylation of **20** are shown in Scheme 4, providing the C8−arylamine products (**21**) in 50−60% yields (Table 1). These conditions were as described by Lakshman for the N-arylation of 6-bromopurine. Comparable yields were obtained when 2-dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl and BINAP were used as the catalyst.

In conclusion, we have demonstrated the feasibility of the Buchwald−Hartwig palladium-catalyzed N-arylation reaction

Table 1. N-Arylation of **20** with Mutagenic Arylamines

Ar-NH_2	Yield of **21**
	56 %
	54 %
	61 %
	56 %

for the synthesis of C8-dG amine adducts. Using this method, we synthesized the C8-dG adduct of the food mutagen IQ. In the process, we introduced the use of the STABASE and bis-BOC protecting groups for N^2 of dG. This strategy appears to be general and should be applicable to the synthesis of other C8-dG food mutagen adducts. Work on the conversion of **1** into a phosphoramidite reagent suitable for solid-phase oligonucleotide synthesis as well as the synthesis of other C8-adducts of food mutagens is currently underway.

Acknowledgment. This work was supported by Grant RPG-96-061-04-CDD from the American Cancer Society. Mr. C. Eric Elmquist is gratefully acknowledged for the preparation of IQ.

Supporting Information Available: Experimental procedures for the preparation of **1** and copies of all ^1H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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