

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

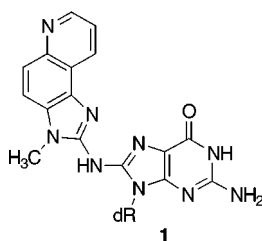
Zhiwei Wang and Carmelo J. Rizzo*

Department of Chemistry, Vanderbilt University, VU Station B 351822,
Nashville, Tennessee 37235-1822

c.j.rizzo@vanderbilt.edu

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ABSTRACT



The C8-2'-deoxyguanosine adduct of the food mutagen 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) has been synthesized. The key step is a palladium-catalyzed N-arylation of a suitably protected 8-bromo-2'-deoxyguanosine 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 N²-adducts have also been isolated as minor products (Figure 2).

Oligonucleotides containing a site-specific C8-dG adduct

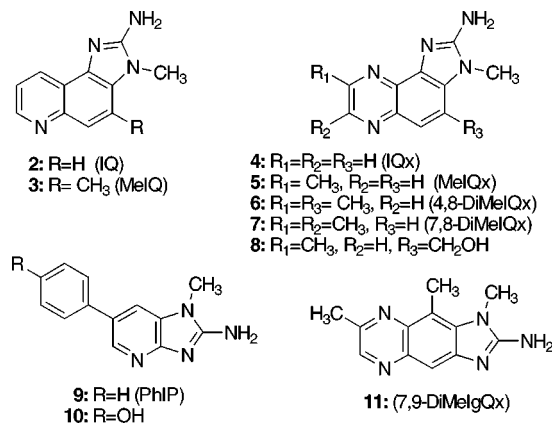


Figure 1. Aminoimidazoazaarene (AIA) food mutagens.

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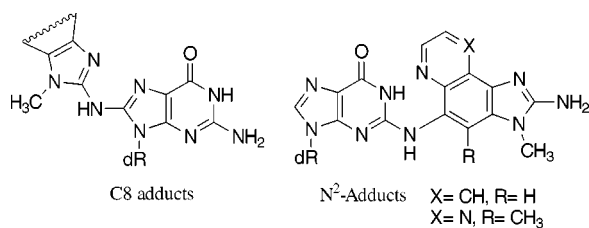
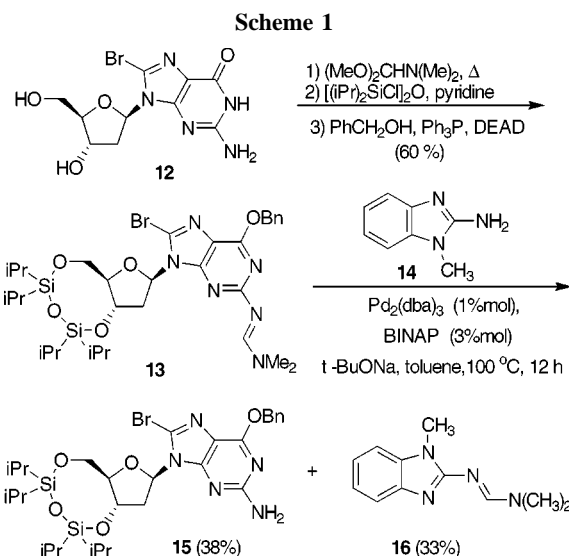


Figure 2. C8- and N²-deoxyguanosine AIA adducts.

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 approach.^{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–Hartwig⁶ 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 Lakshman⁷ and later Johnson,^{8a} for the preparation of N⁶-2'-deoxyadenosine derivatives. Hopkins and Sigurdsson⁹ and Johnson^{4b–d} synthesized N²-dG–N²-dG and N²-dG–N⁶-dA nitrous acid cross-links as well as other N²-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



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 N² of dG would be unsatisfactory. Initially, we employed a dimethyl formamidinium group which is commonly used for N²-dG protection (Scheme 1). The O⁶-position was protected as a benzyl ether. Attempted palladium-catalyzed N-arylation of **13** with model substrate **14** gave only transfer of the N²-protecting group to the amino group of **14**. No N-arylation of the C8-position was observed. The transfer of the formamidinium 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 Cs₂CO₃ or K₃PO₄ 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

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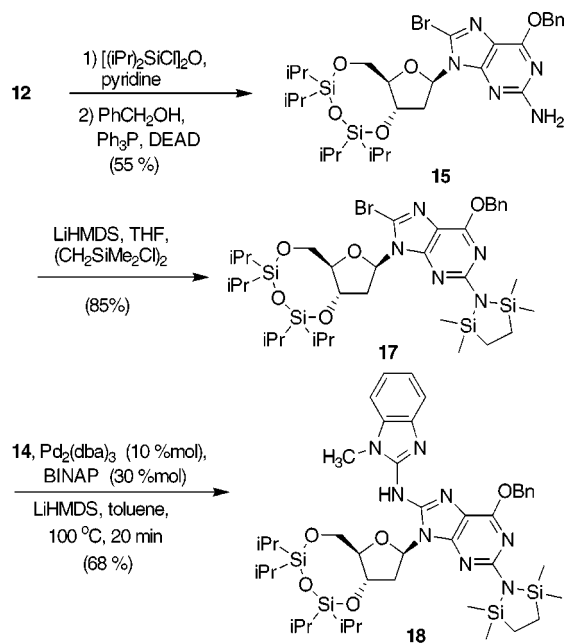
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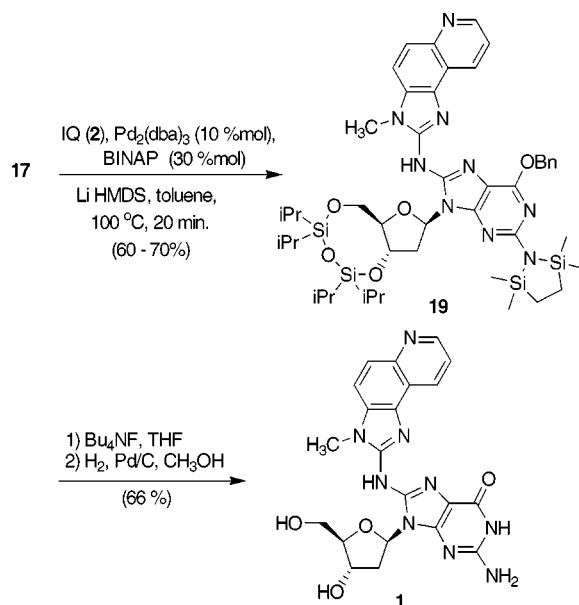
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Scheme 2

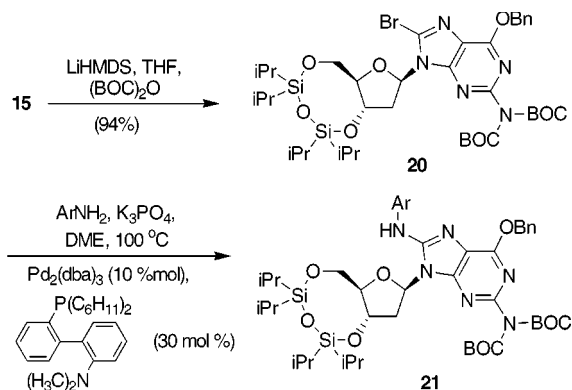


19 with fluoride followed by hydrogenolysis of the O⁶-benzyl group gave the desired C8–IQ adduct of dG (**1**) in 66% overall yield. The synthesis of **1** required six steps from commercially available 8-bromo-2'-deoxyguanosine and proceeded in 32% overall yield. The key to the successful coupling of **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 N²-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 N² 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.

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

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