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Synthesis of the N^2-Deoxyguanosine Adduct of the Potent Dietary Mutagen IQ [1]

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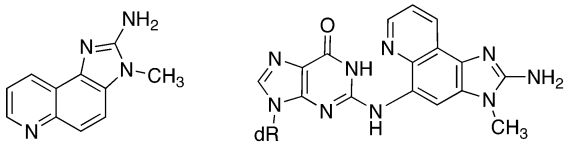
ABSTRACT



IQ N^2-IQ Adduct of Deoxyguanosine

After bioactivation, the potent dietary mutagen 2-amino-3-methylimidazo[4,5-*f*]-quinoline (IQ) reacts with DNA to give two regioisomeric adducts of deoxyguanosine. The synthesis of the minor N^2-adduct has been achieved utilizing the Buchwald–Hartwig palladium-catalyzed N-arylation reaction as the key step.

In the early 1940s, the simple aromatic amines 2-amino-fluorene (AF) and its *N*-acetyl analogue (AAF) were developed as pesticides. They were subsequently found to be potent animal carcinogens and never used as intended.[2] Although there has never been significant environmental exposure to AF or AAF, exposure to other aromatic amines is of concern. For example, 2,6-dimethylaniline, a widely used industrial chemical and a constituent of cigarette smoke, has been identified as a rodent carcinogen.[3] Recently, a strong link has been demonstrated between bladder cancer and exposure to 4-aminobiphenyl, a common component of permanent hair dyes.[4,5] The process of cooking meats produces a wide array of highly mutagenic heterocyclic amines, including 2-amino-3-methyl-imidazo[4,5-*f*]quinoline (IQ, **1**).[6] Because of their source, a wide cross-section of the population is exposed to these dietary mutagens and there is significant interest in their chemistry and biology. Some members of this family are among the most potent agents tested in the Ames assay.[7]

Aromatic amines are procarcinogens and require metabolic activation in order to covalently modify DNA.[8,9] This involves the initial cytochrome P450 *N*-oxidation to the corresponding hydroxylamine followed by *O*-esterification by *N*-acetyl transferase (NAT) or a related enzyme. Solvolysis then gives the arylnitrenium ion, which is the ultimate carcinogenic species. Arylnitrenium ions are ambident electrophiles and react with nucleophiles at both nitrogen and carbon. Their reaction with DNA is highly selective for deoxyguanosine. Furthermore, reaction of the arylnitrenium ion at nitrogen gives the C8-adduct, while reaction at carbon gives the N^2-modified deoxyguanosine. For IQ, both the C8- and N^2-deoxyguanosine adducts have been characterized (Scheme 1).[10,11]

(1) This manuscript is dedicated to Professor Amos B. Smith, III, in celebration of his 60th birthday.

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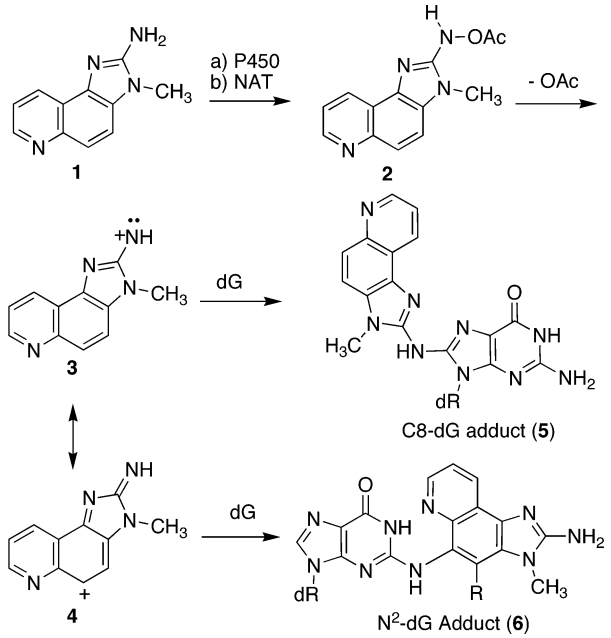
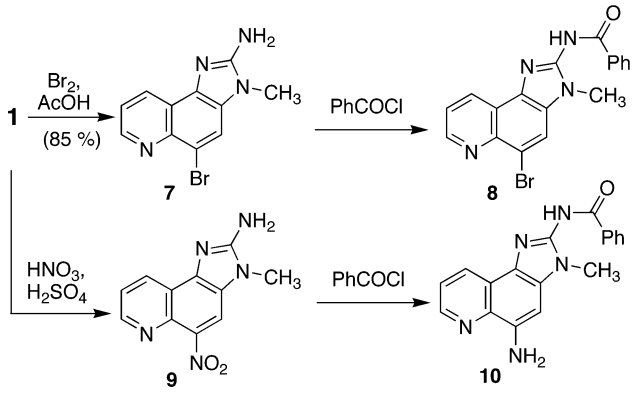
Scheme 1



Scheme 2



There are multiple lines of evidence suggesting that the C8- and N^2-deoxyguanosine adducts of IQ are repaired at different efficiencies.[6] In feeding studies with rats and monkeys, it was found that the major DNA lesion 24 h after a single dose of IQ was the C8-adduct.[12] The relative ratio of the C8- to N^2-deoxyguanosine adducts was in the range of 6:1, which roughly parallels the natural reactivity of the IQ nitrenium ion toward DNA. However, in chronically treated monkeys undergoing long-term carcinogen bioassays, a sharp increase in the proportion of N^2-adduct was observed after 3.6 years. This increase was found in slowly dividing tissue such as the liver, pancreas, and kidney. There was no increased persistence of the N^2-adduct in colon tissue, which divides at a high rate. These observations suggest that the C8-adduct (**5**) is repaired more efficiently in slow growing tissue. This has led to speculation that the minor N^2-deoxyguanosine adduct (**6**) plays a more significant role in the tumorigenic properties of IQ and other aromatic amines.

The Buchwald−Hartwig palladium-catalyzed *N*-arylation reaction has been widely used for the synthesis of modified nucleosides.[13−15] Chida and co-workers used this reaction as the key step in the total synthesis of the nucleoside antibiotic spicamycin.[16,17] Shortly thereafter, reports by Lakshman, Johnson, Hopkins and Sigurdsson, and others demonstrated the utility of the Buchwald−Hartwig reaction for the synthesis of nucleoside adducts of carcinogens.[13,18−32] We have previously reported the synthesis of the C8-deoxyguanosine adduct of IQ and its subsequent incorporation into oligonucleotides via phosphoramidite-based solid-phase synthesis.[28,33] The key step was a Buchwald−Hartwig reaction between a suitably protected 8-bromo-2′-deoxyguanosine derivative with IQ. We report here the synthesis of the N^2-deoxyguanosine adduct of IQ, which also uses the Buchwald−Hartwig reaction as the key step.

Two strategies have been previously employed for the synthesis of N^2-modified deoxyguanosine derivatives utilizing the Buchwald−Hartwig reaction. The first involved the reaction of a suitably protected 2-bromopurine riboside with an arylamine,[23−27] while the second strategy utilized the coupling of the N^2-amino group of deoxyguanosine with haloarenes.[19,26,27] To examine both strategies, we synthesized the protected 5-bromo- and 5-amino-IQ derivatives as shown in Scheme 2. The electrophilic bromination of IQ was previously reported to give the desired 5-bromo-IQ (**7**).[34] Protection of the amine as the corresponding benzamide gave

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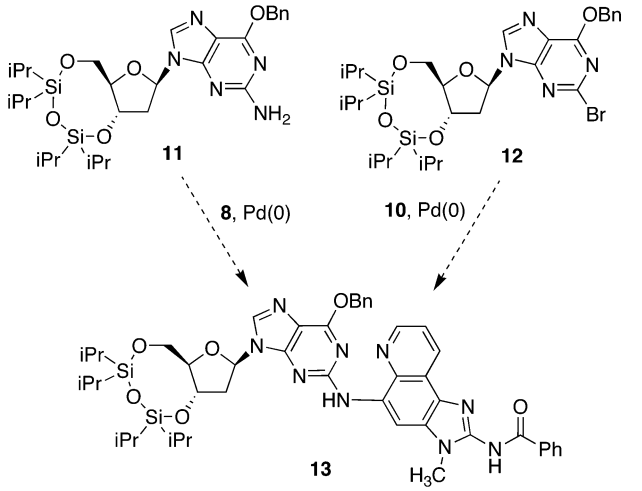
Scheme 3



8 in good overall yield. Likewise, nitration of IQ gave 5-nitro-IQ (**9**). Protection of the primary amine and reduction of the nitro group gave **10**.

The Buchwald−Hartwig cross-couplings of protected 5-bromo-IQ (**8**) with protected guanosine derivative **11** as well as protected 5-amino-IQ (**10**) with protected 2-bromopurine (**12**) were attempted under a number of conditions (Scheme 3). Unfortunately, we were never able to achieve a successful cross-coupling to give **13**. Other protecting groups for the primary amine of **7** and **9** were examined but were also unsuccessful.

Previous synthetic studies that utilized the Buchwald−Hartwig reaction for the synthesis of N^2-arylamine derivatives of deoxyguanosine revealed two important reactivity trends.[13,19,26] The cross-coupling of a haloarene with the N^2-amino group of a suitably protected deoxyguanosine derivative gave the highest yields when electron-deficient haloarenes were used. When a 2-halopurine was used as the reactant, electron-rich arylamines gave the best yield. With this in mind, we decided to attempt the cross-coupling of 5-bromo-2-nitro-IQ (**14**) with protected deoxyguanosine **11**. The nitro group would significantly increase the electron-deficient nature of the halide (**14**) and also serve as a masked amino group.[19]

Oxidation of 5-bromo-IQ with sodium nitrite gave **14** in 71% yield.[35] Buchwald−Hartwig cross-coupling of **14** and **11** using $Pd_2(dba)_3$/BINAP and NaOtBu in toluene at 80 °C

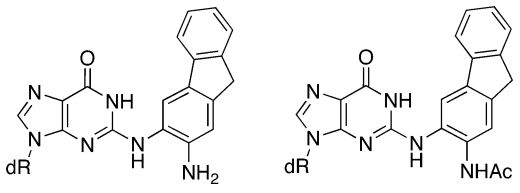
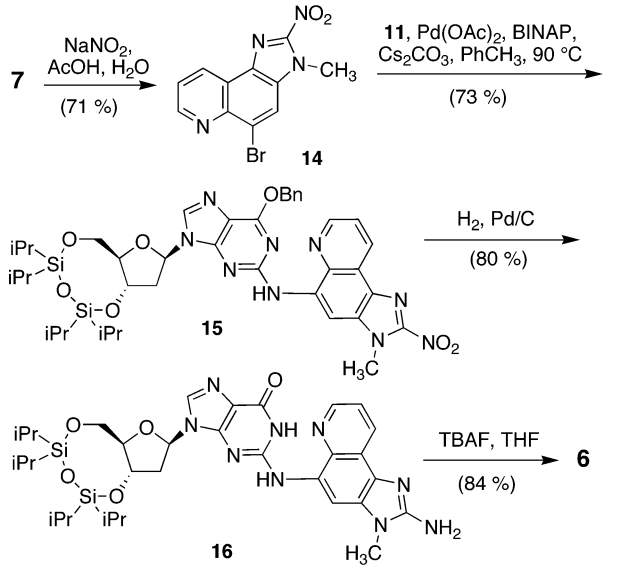


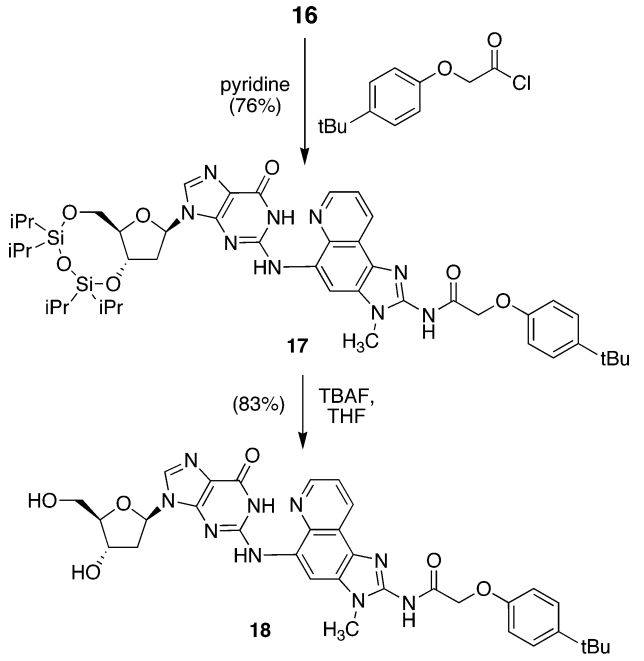
Figure 1. N^2-Deoxygunaosine adduct of a simple arylamine.

Scheme 4



gave the desired product **15** in 30% yield. When $Pd(OAc)_2$/BINAP was used as the catalyst and Cs_2CO_3 as the base, complete consumption of **11** and a 73% isolated yield of **15** was observed. Catalytic hydrogenation removed the O^6-benzyl group and simultaneously reduced the nitro group to give **16** in 80% yield. Desilylation of **16** gave the N^2-deoxyguanosine adduct of IQ (**6**). The ^{1}H NMR spectrum of **6** was identical to that previously published.[10] In Johnson's report on the synthesis of N^2-deoxyguanosine adducts derived from simple arylamines, it was noted that the products were sensitive to air oxidation and characterized as their *N*-acetyl

Scheme 5



derivatives (Figure 1).[19] We have observed no such sensitivity for the N^2-IQ adduct (**6**). The products obtained by the Johnson group were *o*-phenylenediamine derivatives and are likely to be more sensitive to oxidation than our example (**6**).

The amino group of the IQ moiety must be protected for incorporation into oligonucleotides by solid-phase synthesis. This amino group was protected as its *p-tert*-butylphenoxyacetyl derivitive by the reaction of **16** with the proper acid chloride (Scheme 5). Removal of the silyl protecting group with fluoride gave *N*-protected nucleoside **18**. This intermediate is ideal for the preparation of the corresponding phosphoramidite.

In conclusion, we have developed an efficient strategy for the synthesis of the N^2-deoxyguanosine adduct of the dietary mutagen IQ by employing palladium-catalyzed N-arylation methods. The synthesis proceeds in five linear steps from IQ in 29% overall yield. The synthesis of the phosphoramidite reagent and the incorporation of the N^2-IQ adducts into oligonucleotides is currently underway in our laboratory. This will allow for conformational studies of the adducted oligonucleotide and assessment of differential mutagenicity and repair efficiency of the C8- and N^2-adducts.

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Supporting Information Available: Experimental procedures for the preparation of **6** and **18** and copies of all ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

OL047851M

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