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Preparation of C8-Amine and Acetylamine Adducts of 2′-Deoxyguanosine Suitably Protected for DNA Synthesis

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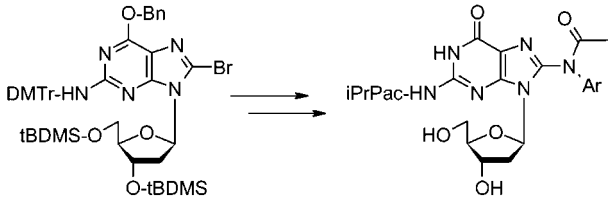
ABSTRACT



C8-Amine and acetylamine adducts of 2′-deoxyguanosine were synthesized. Our approach provides solutions for the coupling of aromatic amines to a protected 8-bromo-2′-deoxyguanosine derivative, for the selective acetylation of the coupled adduct at N^8 and for a protecting group scheme preserving the integrity of the base-labile N^8 acetyl group during DNA synthesis.

A number of carcinogenic aryl-amino and -nitro compounds are known to produce C8-arylamine adducts of 2′-deoxyguanosine (dG).[1] If not corrected, these covalent DNA modifications can trigger mutagenesis and eventually induce cancer. Among these adducts, 8-(*N*-acetyl-aminofluorene)-2′-deoxyguanosine (dG-AAF) (**1**) has been intensively used as a model compound (Figure 1) for studying mutagenesis, carcinogenesis, and DNA repair.[1a] Interestingly, although 8-(*N*-aminofluorene)-2′-deoxyguanosine (dG-AF) (**2**) and dG-AAF (**1**) only differ by one acetyl group, they exhibit very different physicochemical properties and biological effects: dG-AAF causes much more severe local distortion of DNA than dG-AF[1a,2] and is thus a much more potent block to replication and transcription,[1a,3] as well as a better substrate for DNA repair enzymes.[4] Despite its importance, a straightforward method for the site-specific incorporation of dG-AAF and related adducts into DNA using solid-phase synthesis is still not available. Two major issues need to be addressed to achieve this goal. (i) The base labile N^8 acetyl group of dG-AAF is known to be unstable upon the standard NH_4OH deprotection step after solid-phase DNA synthesis.[5] Therefore, protecting groups that can be removed under conditions compatible with the maintenance of the N^8 acetyl group are required. Zhou and Romano have reported a

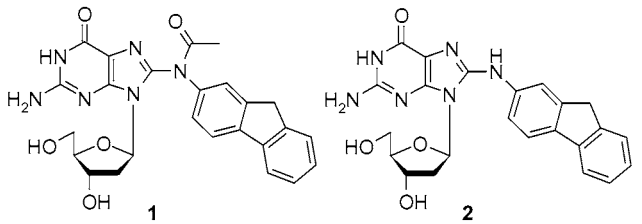


Figure 1. C8-Arylamine adducts of 2′-deoxyguanosine naturally formed by carcinogenic aminofluorene derivatives: **1**, *N*-acetyl-2-aminofluorene (AAF); **2**, 2-aminofluorene (AF).

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(2) O'Handley, S. F.; Sanford, D. G.; Xu, R.; Lester, C. C.; Hingerty, B. E.; Broyde, S.; Krugh, T. R. *Biochemistry* **1993**, *32*, 2481−2497.

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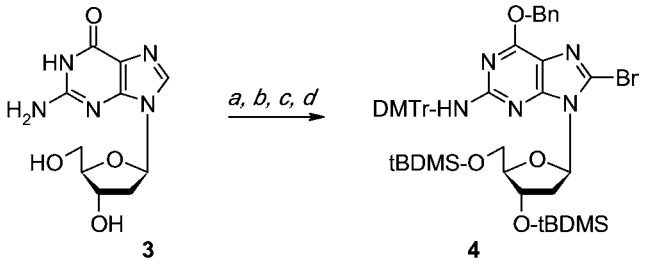
solution to this problem using an Fmoc protecting group strategy and milder deprotection conditions that ensured the stability of the dG-AAF.[6,7] A limitation of this strategy, however, is that Fmoc-protected nucleotide phosphoramidites are not commercially available. (ii) The syntheses of C8-arylamine and acetylarylamine adducts of dG have traditionally been based on the modification of nucleosides and oligonucleotides with the corresponding N-hydroxy or N-acetoxy arylamine derivates. This reaction is severely limited in yield and scope;[1b,6] in particular, a single site-specific modification cannot be introduced into oligonucleotides that contain multiple guanines, therefore restricting the sequence context in which dG-AAF can be introduced. An alternative strategy for obtaining these adducts is a Buchwald−Hartwig coupling reaction[8] of protected 8-bromo-2′-deoxyguanosine (Br-dG) derivates with aromatic amines. Indeed the Buchwald−Hartwig coupling reaction has been reported for the formation of N^6 adducts of dA,[9] N^2 adducts of dG,[10] and while this work was in progress, C8 adducts of dA[11] and dG.[12,13] Previous syntheses of C8-arylamine adducts of dG have shown that protections of the hydroxyl groups of the deoxyribose and of the N^2 and O^6 positions of the base of Br-dG were required for the palladium-mediated coupling reactions. Depending on the amine to be coupled, Wang and Rizzo used either bis-BOC or STABASE protection for the N^2 position,[12] while Meier and Gräsl reported that an N^2-isobutyryl protecting group allowed the coupling of a number of simple amines.[13] Although the isobutyryl group is the standard N^2 protecting group for dG in DNA synthesis, the conditions required for its removal are incompatible with the base sensitive N^8 acetyl group of dG-AAF.

Despite these advances, an efficient method for the synthesis of *N^8-acetyl* arylamine adducts of dG and a protecting group strategy compatible with their incorporation into DNA are still missing. Here, we disclose our approach to address these problems. The use of a transient dimethoxytrityl (DMTr) protecting group for the N^2 position of Br-dG derivatives allowed the efficient coupling of a wide variety of amines under Buchwald−Hartwig conditions. The products of this coupling reaction were subsequently selectively acetylated at the N^8 position, and the facile replacement of the *N^2*-DMTr with the *N^2*-isopropylphenoxyacetyl (^{i}PrPac) group yielded monomers suitable for solid-phase DNA synthesis in which the integrity of the base labile N^8 acetyl group should be preserved by using the commercial "ultra-mild" phosphoramidites.[14] Our approach thus provides a general strategy for the synthesis of C8-arylamine and acetyl arylamine adducts of dG suitably protected for incorporation into DNA.

Our synthesis starts by bromination of dG (**3**) with NBS[15] and precipitation in acetone as a convenient purification step. Silylation of the 5′- and 3′-hydroxyl groups and protection of the O^6 position as benzyl ether were achieved using standard methods (Scheme 1). In search for a generally applicable N^2 protection, we examined the DMTr group, which is stable under basic conditions and should thus survive the Buchwald−Hartwig coupling reaction. The fully protected Br-dG derivative (**4**) was obtained in good yields on a multigram scale, using chromatography on aluminum oxide for all compounds carrying the *N^2*-DMTr group to prevent loss of this acid-labile group on silica gel. Among the various conditions tested for the coupling reaction, we found that $Pd_2(dba)_3$/BINAP as a catalyst, NaOtBu as a base, and toluene as a solvent gave good coupling yields for a number of structurally diverse aromatic amines (Scheme 2).[8d,16] As expected, protection of both the N^2 and O^6 positions was required for successful coupling reactions. Our method allowed the coupling of 2-aminofluorene (**5a**) and the synthesis of several new C8 adducts of dG such as those with 2-amino-1-methylimidazo[4,5-f]-quinoline (isoIQ) (**5b**), 1-aminopyrene (**5c**), the electron-poor *p*-aminobenzophenone (**5d**) and its isomer *m*-aminobenzophenone (**5e**). To get direct access to dG-AAF, the acetylated form of **5a**, we attempted a direct coupling between **4** and AAF, but we only observed the formation of the deacetylated product in low yields.[17]

Scheme 1. Preparation of the Fully Protected Br-dG[a]



[a] Reaction conditions: (a) NBS, water, acetonitrile (80%); (b) tBDMS-Cl, imidazole, DMF (98%); (c) Bn-OH, PPh_3, DIAD, dioxane (78%); (d) DMTr-Cl, pyridine (94%).

(3) (a) van Oosterwijk, M. F.; Filon, R.; de Groot, A. J. L.; van Zeeland, A. A.; Mullenders, L. H. F. *J. Biol. Chem.* **1998**, *273*, 13599−13604. (b) Shibutani, S.; Suzuki, N.; Grollman, A. P. *Biochemistry* **1998**, *37*, 12034−12041.

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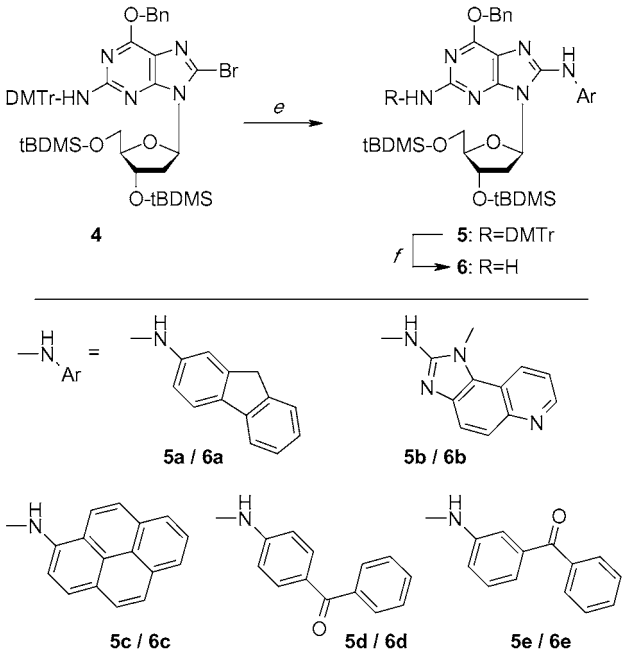
(13) Meier, C.; Gräsl, S. *Synlett* **2002**, 802−804.

(14) Schulhof, J. C.; Molko, D.; Teoule, R. *Nucleic Acids Res.* **1987**, *15*, 397−416.

(15) Gannett, P. M.; Sura, T. P. *Synth. Com.* **1993**, *23*, 1611−1615.

(16) We tested a number of other base and solvent systems for this reaction, including: K_3PO_4 in DME and Cs_2CO_3 in dioxane. The reactions using NaOtBu/toluene were complete after 1 h at 100 °C; reactions with weaker bases such as K_3PO_4 or Cs_2CO_3 yielded no detectable product or took several days to go to completion.

Scheme 2. Buchwald−Hartwig Coupling Reactions[a]



[a] Reaction conditions: (e) $Pd_2(dba)_3$, BINAP, toluene, NaOtBu and the corresponding amines 2-aminofluorene (**5a**, 72%), isoIQ (**5b**, 68%), 1-aminopyrene (**5c**, 51%), 4-aminobenzophenone (**5d**, 83%), or 3-aminobenzophenone (**5e**, 61%); (f) HCl, MeOH (89%).

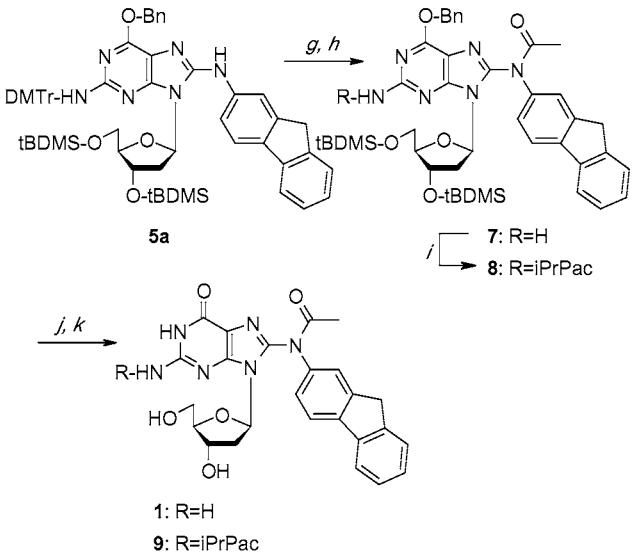
More recently developed catalysts described by Buchwald and co-workers might be used for this purpose.[18] dG-AAF could, however, readily be obtained from **5a** by selective acetylation of the N^8 position (Scheme 3). The bulky DMTr group apparently prevented further acylation reaction at the N^2 position successfully. The product of acetylation turned out to be rather acid- and base-sensitive, and the position of the acetyl group was verified by extensive NMR analysis after removal of the DMTr group using very dilute hydrochloric acid in methanol. The silyl protecting groups of **7** were removed using TBAF buffered with acetic acid to preserve the base-sensitive N^8 acetyl group and benzyl group deprotection was achieved smoothly by transfer hydrogenolysis to yield the AAF modified nucleoside **1**. For purposes of oligonucleotide synthesis, the N^2 position of **7** could also be selectively protected by reaction with ^{i}PrPac-Cl to yield **8**. The removal of the silyl and benzyl groups was achieved using the same conditions employed for the unprotected compound (**7**) and yielded the ^{i}PrPac protected form of the AAF modified nucleoside **9** (Scheme 3).[19] This ^{i}PrPac group of **9** could be deprotected under very mild basic conditions: treatment with $^i Pr_2NH$ in MeOH (1:20) at room temperature for 1−12 h led to complete deprotection of the

(17) Failure of efficient coupling of acetyl arylamines under similar conditions has been reported previously (Wolfe, J. P.; Rennels, R. A.; Buchwald, S. L. *Tetrahedron* **1996**, *52*, 7525−7546. Yang, B. H.; Buchwald, S. L. *Org. Lett.* **1999**, *1*, 35−37).

(18) Klaspars, A.; Huang, X.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 7421−7428.

(19) Compound **9** was further successfully converted to its 5′-*O*-DMTr-3′-*O*-phosphoramidite derivative using standard methods.

Scheme 3. Selective Acetylation and Deprotection of the DG-AF Adduct[a]



[a] Reaction conditions: (g) Ac_2O, Et_3N, DMAP, pyridine; (h) HCl 0.01 M, MeOH (78% for g−h); (i) ^{i}PrPac-Cl, pyridine (97%); (j) CH_3COOH, TBAF, THF (79%); (k) Pd/C, cyclohexene, EtOH (88%).

N^2-^{i}PrPac without compromising the integrity of the N^8 acetyl group (Figure 2).[20] Only after 24−48 h under these conditions did we observe the appearance of the deacetylated product (**2**) in noticeable amounts. We furthermore confirmed that both the N^2-^{i}PrPac and N^8 acetyl groups of **9** were compatible with conditions used in DNA synthesis as shown by Zhou and Romano with N^2-Fmoc-dG-AAF.[6]

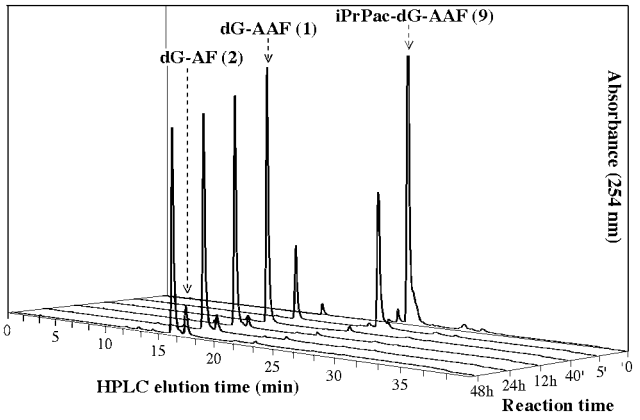
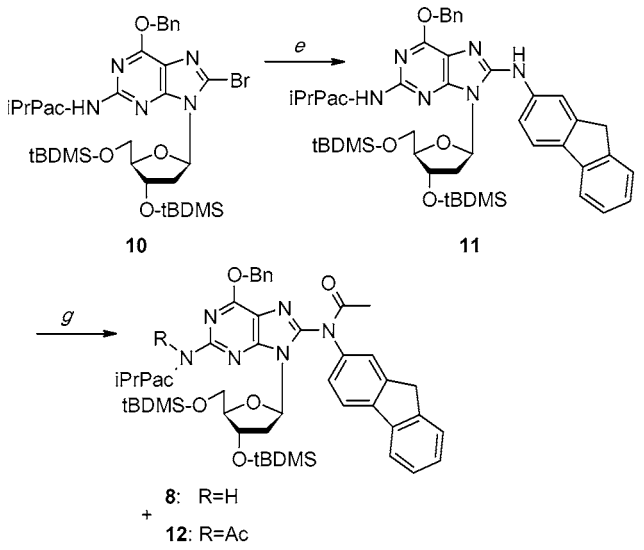


Figure 2. Time course of the reaction of ^{i}PrPac-dG-AAF in 20/1 MeOH/$^i Pr_2NH$. Elution times on reverse-phase HPLC are ^{i}PrPac-dG-AAF (**9**) t = 23.2′; dG-AAF (**1**) t = 15.9′; and dG-AF (**2**) t = 17.2′.

While we were finishing up this work, the Buchwald−Hartwig reaction of N^2-isobutyryl-protected Br-dG using K_2CO_3 as a base was reported.[13] We therefore investigated

Scheme 4. Coupling and Acetylation of the N^2-^{i}PrPac Derivative[a]



[a] Reaction conditions: (e) $Pd_2(dba)_3$, BINAP, toluene, NaOtBu, 2-aminofluorene (**11**, 70%); (g) Ac_2O, Et_3N, DMAP, pyridine (**8**, 5%; **12**, 95%) *or* Ac_2O, Et_3N, pyridine (**8**, 20%; **12**, 80%) *or* Ac_2O, pyridine (**8**, 80%; **12**, 20%).

whether an analogous coupling reaction could be carried out with N^2-^{i}PrPac-protected Br-dG (**10**), which might provide more direct access to the acetyl arylamine-modified building block for DNA synthesis. We thus investigated the Buchwald−Hartwig coupling reaction of the 2-aminofluorene and N^2-^{i}PrPac protected **10** (Scheme 4). Reaction of **10** with 2-aminofluorene, the palladium catalyst, and K_2CO_3 in dioxane yielded the coupled product **11** after 5 days at 80 °C without affecting the ^{i}PrPac group.[21] The N^8-acetylation reaction, however, turned out to be problematic (Scheme 4): under a variety of conditions, we observed the formation of a byproduct (**12**), doubly acetylated at the N^2 and N^8 positions, that we were unable to separate from the desired N^8 acetylated product **8**.[22] This approach is apparently not suited for the generation of C8 aromatic acetylamine adducts of dG. The N^2-DMTr is a preferable protecting group both for efficient Buchwald−Hartwig coupling reaction and subsequent acetylation of the N^8 position.

In conclusion, we expanded the scope of the Buchwald−Hartwig reaction to generate C8-arylamination adducts of 2′-deoxyguanosine through improved protecting group strategy and coupling reactions.[23] Our strategy furthermore allows selective N^8-acetylation of the dG adducts and a facile N^2 reprotection with an ^{i}PrPac group, yielding building blocks suitable for ultra-mild DNA synthesis compatible with the maintenance of the base-labile N^8 acetyl group. The incorporation of these adducts into DNA and the study of the repair mechanisms of such DNA lesions are presently underway in our laboratory.

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

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(20) These new deprotection conditions have been developed in our laboratory to release oligonucleotides from the ultra-mild Q-supports and to remove all the protecting groups, including the cyanoethyl groups on the phosphates and the ultra-mild exocyclic amino protections on the bases, for oligomers that contained very base labile modifications (Alzeer, J.; Gillet, L. C. J.; Schärer, O. D. Manuscript in preparation).

(21) Reaction of **10** using the previously established conditions with NaOtBu in toluene led to complete loss of the ^{i}PrPac group in less than 1 h.

(22) Such transamidation on an N^2-^{i}PrPac-protected dG was previously observed during the capping step of standard oligonucleotide synthesis (Zhu, Q.; Delaney, M. O.; Greenberg, M. M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1105−1107).

(23) Our conditions allow the coupling of amines such as *p*-aminobenzophenone that we found to be too unstable to be activated to their *N*-hydroxylamine for direct coupling with dG.