Synthetic and Oxidative Studies on 8-(Arylamino)-2'-deoxyguanosine and -guanosine Derivatives

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Facile aerial oxidation is a general feature of guanine ribo- and 2'-deoxyribonucleosides that are substituted at the 8-position by an aminoaryl group. In previous work, it had been suggested that two of the major oxidation products are a pair of diastereomers having a spiro structure. These were presumed to be related by a chiral difference at the spiro carbon atom. The pattern of the oxidative process involves a contraction of the pyrimidine ring. It was thought to be analogous to that suggested by other investigators for the oxidation of uric acid, but for which no really definitive evidence had been presented. We have been able now to isolate in a crystalline state one of the diastereomers produced by the aerial oxidation of 8-phenylaminoguanosine under alkaline conditions. Analysis by X-ray diffraction has now confirmed the type of spiro structure promulgated previously. These findings also imply that spiro compounds are likely to be produced during the aerial oxidation of any 8-arylaminoguanine nucleoside or 2'-deoxynucleoside. In addition, this work adds considerable weight to the results of Poje and Sokolic-Maravic who proposed that a spiro intermediate is produced during the aerial oxidation of uric acid. However, they found this compound to be unstable to base, in contrast to the arylaminoguanine oxidation products. In the course of the above work we showed that the 8-arylamino derivatives of guanosine can be converted by the Barton deoxygenation method to the corresponding 2'-deoxyribonucleosides. This makes available a number of the latter compounds, which are not easily prepared by other methods. — Environ Health Perspect 102(Suppl 6):143-149 (1994)

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Introduction

It is now well established that the treatment of DNA with the ultimate carcinogen 2-(N-acetoxy-N-acetyl)aminofluorene (AAAF) under solvolytic conditions leads principally to the formation of adducts in which aminoaylation has occurred at the C-8 position of deoxyguanosine residues.

The major adduct formed in DNA in vivo can be viewed as being derived from the 2'-deoxynucleoside (Structure 1) deoxyguanosine-aminofluorene (dGuo-AF), whereas under in vitro conditions the major adduct is derived from the acetyl derivative (Structure 2) deoxyguanosine-acetylaminofluorene (dGuo-AAF).

Structures 1 and 2

These reactions of AAAF with DNA may be regarded as prototypical of the way that most carcinogenic amines behave towards DNA after they have been metabolically activated.

What seems to have escaped general attention, however, is that all of these arylamine adducts, either as the free deoxynucleosides or as residues in DNA, are not stable in solution above pH 7. Nevertheless, the instability associated with AF-modified RNA and DNA, N-(guanosine-8-yl)-2-aminofluorene (Guo-AF) and dGuo-AF had been reported (1-3) more then 20 years ago, but its origin was unrecognized at that time. Two groups reported further studies around 1980. Spodheim-Maurizot et al. (4), conducted kinetic studies on the action of alkali on the AF adducts of guanosine (Guo), deoxyguanosine (dGuo), deoxyguanosine 5'-monophosphate and denatured DNA, whereas Kriek and Weistra (5,6) studied the formation...
directly of products obtained when dGuo-AAF was hydrolyzed by alkali. Both groups observed the formation of two products and these were identified (5) tentatively by the latter group as 2'-diastereomers of the 7,8-seco-imidazole derivative (Structures 3a and 3b).

Around the same time, Kadlubar et al. (7) presented data on the reaction of N-hydroxy-2-naphthylamine with DNA. In addition to two other adducts, they also found a degradation product in the alkaline enzymatic digests of the DNA. Although this was assigned as the alternate ring-opened structure, 8,9-seco-imidazole, in neither case was the spectroscopic evidence sufficient to make the identifications unambiguous (Structures 4 and 5).

Later reports by Stohrer and his associates (8) described the alkali instability of synthetic DNA containing a dGuo-AAF residue. They found, nevertheless, that the addition of a thiol at the ammonia deblocking step protected the DNA from degradation; this indicated, for the first time, that the degradation might be oxidative. They further suggested that the nature of the oxidation might involve an allantoin type of residue, but did not pursue the matter further.

Recent work by the author and his associates (9-11) has suggested that the degradation might be considerably more complicated than first imagined. Both at the level of the deoxynucleoside (dGuo-AAF) and the oligomers containing this residue, it was found that the decomposition could be completely suppressed even under highly alkaline conditions, provided either an antioxidant was present or the reaction was run under anaerobic conditions. Thus, the earlier views of Stohrer et al. (8) were confirmed. However, in the presence of air at 75°C (Kriek and Westra conditions (5)), the acetylated nucleoside 2 was found to disappear rapidly in a 0.2 N sodium hydroxide solution, being replaced by three new compounds (as determined by HPLC analysis), which were simply designated for convenience as ring-opened products (ROP-1, -2, and -3) (Structures 6a, 6b, and 7, respectively). Under these conditions, ROP-3 appeared only in the early stages of the reaction, as did a fourth peak representing the intermediate deacetylated nucleoside 2 (Figure 1). Treatment of ROP-1 under identical conditions also gave rise to the same ring-opened products. Although the 1H-NMR spectra of ROP-1 and ROP-2 indicated that they were identical to the two substances 3a and 3b isolated previously (5), careful scrutiny of both the positive-ion and negative-ion fast atom bombardment mass spectroscopy (FAB-MS) showed that each has a molecular ion at m/z 462 rather than at 464 as reported earlier (5). This led to doubt that these substances arise via an oxidation mechanism rather than by a simple alkaline hydrolysis. Based on the 1H-NMR and FAB-MS analyses, it was concluded (9-11) that instead of the diaminopyrimidine structures (3a,3b), most probably these substances are the spirodiastereomers 6a and 6b.

In assigning these structures we noted that there was a parallel with the case of uric acid whose oxidation in alkali had been studied most recently by Poje and Sokolic-Maravic (12,13). The authors concluded through an indirect proof that one of the degradation products was 8, but they noted that it underwent further rapid degradation in the alkaline medium to give noncyclic products. This stands in contrast to Structure 6a and 6b, both of which are stable to further oxidation by oxygen in alkali. Despite a wealth of spectroscopic evidence, Structures 6a, 6b, and 8 still remain speculative. Major difficulties in conducting further analytical studies were the unavailability of quantities of Structure 6a or 6b in greater than milligram amounts, and the lack of a general synthetic method for the preparation of 8-arylamino derivatives of deoxyguanosine derivatives. Thus, the latter problem had to be tackled before further structure studies were possible.

**Synthetic Studies**

Most published works that deal with the synthesis of 8-aminoaryl nucleotides use conventional methods that involve the solvolysis at acid pH, of an arylhydroxylamine derivative (Structure 10) in the presence of the nucleoside (Structure 9). The
former reputedly generates a nitrenium ion, the intermediate that attacks the guanine base, principally at C-8 to give the derived product (Structure 11) (14–16).

This approach works well (yields up to 35%) with compounds such as AAAF (14) that solvolysse easily. However, in cases where hydrolysis predominates (17,18), such as with phenylhydroxylamine and the corresponding biphenyl derivatives, or where the hydroxylamine is very unstable (19), as in the case of the food mutagen 2-amino-3-methyl-1H-imidazo[4,5-f]quinoxline (IQ), little or no product is obtained.

More recently, DeFrancq et al. (20,21) have described an elegant, intramolecular version of the solvolytic method for the elaboration of the dG-C8-AF; however, the reaction is quite pH-dependent, and the yield is not satisfactory for large-scale production. From the chemical point of view then, the solvolytic method is neither general nor reliable, especially for a multigram scale synthesis. In addition, it is hazardous, as it involves the ultimate carcinogen. Therefore, we decided to investigate an alternative route to the synthesis of arylamine-modified deoxyguanosines, which focuses on a nucleophilic rather than an electrophilic approach.

Although aliphatic amines are known to react with 8-haloguanosines to give the expected 8-alkylamino derivatives (22,23) only one report has described the use of an aromatic amine, and this was in the deoxy-ribonucleoside series (24). This involved an attempt to induce 2-aminofluorene to react directly with 8-bromo-2'-deoxyguanosine. However, because of the lability of the glycosidic linkage at the high temperatures used, the reaction was accompanied by complete depurination. We attempted to improve the nucleophilicity of the amino group by conversion to the cyanamide (Structure 12). However, reaction of the compound represented by Structure 12 with 13, even under forcing basic conditions, did not lead to the compound shown in Structure 14.

In the more stable ribonucleoside series, it has been reported by Jacobson et al. (25) that 8-bromo-2',3',5'-tris-O-acetylguanosine (Structure 15) under controlled conditions reacts with aniline to give the 8-phenylamino derivative (Structure 16), which on base hydrolysis gives 8-phenylaminoguanosine. Taking this approach, we used 2-aminofluorene in place of aniline and the reaction afforded 8-(2-amino-fluorenyl)-2',3',5'-tris-O-acetylguanosine (Structure 17) in better than 60% yield.

Despite the fact that the substitution reaction involves an amine nucleophile, the mechanism of the reaction is driven by acid catalysis (20). In the absence of added amine hydrobromide, the reaction is extremely slow, whereas the addition of the proton sponge (1,8-diaminonaphthalene) completely inhibits both the substitution and the depurination reactions (26). Under the conditions, even after 60 hr at 110°C, both starting materials were the only substances present.

Although this substitution reaction appears to work well with the amines of aromatic hydrocarbons, unfortunately we could not obtain any reaction with the heterocyclic amine IQ. Neither substitution nor depurination was observed. The lack of substitution probably is related to the poor nucleophilicity associated with 2-aminimidazoles, a property that likely is diminished even further by electron delocalization into the pyridine ring.

To obtain the corresponding deoxyribonucleosides, compounds 16 and 17 were first deacetylated under mildly basic conditions to give compounds 19 and 18, respectively (95% yield), and the latter after suitable protection were subjected to the
Although the overall yields from guanosine are only in the region of 10 to 15%, the reactions are easy to carry out on a large scale. Now, with substantial amounts of both the nucleosides 18 and 19, and the corresponding 2'-deoxy compounds 1 and 23 on hand, we were able to examine in detail their aerial oxidation reactions. However, in this article we present only the work that confirms the structure of the spiro derivatives.

**Further Oxidation and Structure Studies**

The major objective at this point in the study was to obtain for X-ray analysis a crystal of one of the spiro-derivatives 6a or 6b. However, all attempts to obtain satisfactory crystals from these oxidation products of dGuo-AF were unsuccessful. Nevertheless, because it had already been demonstrated (4) that the alkaline (aerobic) degradation of Guo-AF and dGuo-AF led to the formation of what appeared to be parallel products (5), the data indicated that the substitution pattern of the sugar residue probably has little influence on the oxidative pathway. In order to confirm these results, we treated Guo-AF under the aerobic alkaline conditions (70°C), that we had used previously (9–11) with dGuo-AF. The results were quite analogous (Figure 2). Three major products can be observed by HPLC analysis (which we trivially designate GOP-1, -2 and -3), and their behavior is very similar to what was observed in the dGuo-AF series. Levels of GOP-1 and GOP-2 rise steadily with time, arriving at roughly a ratio of 2:3. On the other hand, GOP-3 is produced only in low concentration and rapidly vanishes from the system. It likely is the guanosine analog of the compound represented by Structure 7, but structure proof is lacking.

![Scheme 1](image)

Scheme 1. General approach for the removal of 2'-OH of arylamine-modified guanosines.

The product 23, when deprotected by tetrabutylammonium fluoride, afforded the desired 2'-deoxynucleoside 1 in the case where Ar = fluorenyl, and product 22 where Ar = phenyl.

In the former case, the spectral data of the product were identical to those previously published (14). Analytical methods including 'H-NMR, 13C-NMR, and FAB-MS confirmed that the compounds obtained in the phenylamine series had the designated corresponding structures.

![Graph](image)

Figure 2. A sample of Guo-AF (1 mg) was incubated with 1 N NaOH at 70°C for 1.25 hr.
The positive FAB mass spectrum of GOP-1 shows a parent ion at m/z 479 [M+H]⁺, whereas that of 6a (ROP-1) is known to be at m/z 463 [M+H]⁺; a mass difference of 16 corresponding exactly to one oxygen atom (9,11). Thus, it appears likely that the oxidation products from Guo-AF and dGuo-AF are quite homologous.

Unfortunately, neither GOP-1 nor GOP-2 could be induced to give crystals large enough for X-ray analysis. We turned, therefore, to an examination of the aniline derivative of guanosine—namely compound 19 (Guo-Anil). Surprisingly, when 19 is stirred under air in 1 N sodium hydroxide solution at room temperature, only two products are observed in about equal quantity (designated AOP-1 and AOP-2). At 70°C (Figure 3), AOP-2 vanishes after about 2 hr, while the concentration of AOP-1 rises slowly as that of 19 falls. In the presence of marcaptoethanol, the aerial oxidation of Guo-Anil is completely suppressed, in keeping with all previous studies on analogous compounds.

The positive ion FAB mass spectrum (Figure 4) of AOP-1 shows a parent ion at m/z 391 [(M+H)⁺; C_{16}H_{19}N_{6}O_{6}] corresponding to what should be expected in the phenyl series, if it is assumed that the oxidation pattern follows that of the higher homologs. Fragments present at m/z 259 (C_{11}H_{17}N_{5}O_{3}) and at m/z 181 (C_{9}H_{15}N_{4}O_{2}) represent, respectively, ions that have lost the ribose moiety [M-ribose +2H]⁺ and both the ribose and phenyl moieties [M-ribose-phenyl +H]⁺. The ¹H-NMR spectrum of AOP-1 is not very informative. However, the protons on the phenyl ring and the ribose moiety are easily discernible, and in addition to the three exchangeable hydrogens of the sugar hydroxyls, four other signals vanish in Figure 5. The carbon spectrum of AOP-1 (Guo-Anil) in DMSO at 150 MHz, 303°K.
deuterium oxide (D₂O) solution, as would be expected for a compound having the arylamino spiro system related to 6. The 13C-NMR spectrum (Figure 5) of AOP-1 provides evidence for six carbon atoms that have no attached hydrogen, and eight that bear hydrogen. The low field resonances at 180.56 and 172.66 ppm correspond to carbonyl carbons, whereas the absorption at 82.24 ppm is in the range where aliphatic quaternary carbon atoms absorb, thus providing evidence for the spiro carbon atom.

In contrast to the work with corresponding alkali stable derivatives of dGuo-AF and Guo-AF, AOP-1 derived from Guo-Anil (Structure 19) gave crystals from an aqueous solution that were sufficiently stable in the X-ray beam to provide sufficient data for structure determination. Positional and equivalent isotropic thermal parameters were obtained and give rise to the structure (a trihydrate) shown in Figure 6.

A conventional three-dimensional view of AOP-1 is presented in Figure 7. These figures clearly show the spiro structure of the oxidation product, the spiro center having the S-configuration. The bond lengths of N(2)-C(3) and C(5)-N(6) are 1.29 (1), 1.31 (1)Å, respectively, and show that the guanidine groups on both of the 5-membered rings exist in the exocyclic imino, rather than the more normal amino tautomeric form that is found in a wide variety of guanidine derivatives (29,30). This may be related to the ring strain energy associated with 5-membered rings in which an exocyclic imine is more stable than one that is endocyclic. In Figure 6, the torsion angle O(3)-C(12)-N(5)-C(5)X = -54(1)° indicates that AOP-1 adopts the anti conformation about the glycosidic bond in the crystalline state, and that the ribose adopts the C' 2 endo conformation (torsion angle O(6)-C(16)-C(15)-C(14): X = -58°). Both the imine C(5)-N(6) and the 5'-O(6)H of the ribose bisect the sugar ring, and the distance between N(6) to O(6) is 2.75Å, which suggests that there is a hydrogen bond between them, thus possibly stabilizing the anti-conformation of the glycosidic bond. Also in Figure 6 the torsion angle N(5)-C(5)-(N(6)-C(6): X = 179.1(9)°, indicates that the N-phenyl substituted imine prefers the E-configuration, which is obviously related to the steric hindrance that would exist between the ribose and the phenyl group were they in the Z-related positions.

In the light of the determination of the structure of AOP-1, it would now appear that all of the other derivatives (ROP-1, ROP-2, GOP-1, and GOP-2) likely have the same general structure, given the close chemical relationships of their production. In addition, ROP-1 and ROP-2 almost certainly are related in having opposite chiralities at the spiro-center, a kinship that probably also is shared by GOP-1 and GOP-2. On the other hand, the lack of observation of two diastereomers in the case of ROP-1 is a mystery that still awaits resolution. Finally, the current work strongly supports the postulated spiro-nature of the alkali labile intermediate [8] observed in the oxidation of uric acid under alkaline conditions (12,13).

Further investigation of the oxidation chemistry of the 8-arylamino-guanosine derivatives is being pursued.

REFERENCES

1. Irving CC. Enzymatic deacetylation of N-hydroxy-2-acetylaminofluorene by liver microsomes. Cancer Res 26:1390-1396 (1966).
2. Kriek E. On the mechanism of action of carcinogenic aromatic amines. I. Binding of 2-acetylaminofluorene and N-hydroxy-2-acetaminofluorene to rat-liver nucleic acids in vivo. Chem Biol Interact 1:3-17 (1969).
3. King CM, Phillips B. N-Hydroxy-2-fluorenyl-acetamide: reac-
tion of the carcinogen with guanosine, ribonucleic acid, deoxyribonucleic acid and protein, following enzymatic deacetylation or esterification. J Biol Chem 244:6209–6216 (1969).

4. Spodheim-Maurizot M, Dreux M, Saint-Ruf G, Leng M. Alkaline stability of guanosine and some of its derivatives modified by the carcinogen N-acetoxyacetaminofluorene. Nucleic Acids Res 8:2347–2356 (1979).

5. Kriek E, Westra JG. Structural identification of the pyrimidine formed from N-(deoxyguanosine-8-yl)2-aminofluorene in aqueous solution at alkaline pH. Carcinogenesis 1:459–469 (1980).

6. Kriek E, Westra JG. Formation of N-2-fluorenyldihydroxylamine adducts of DNA in vivo and in vitro and some of their properties. Natl Cancer Inst Monogr 58:139–142 (1981).

7. Kadlubar FF, Urruh LE, Beland FA, Straub KM, Evans FE. In vitro reaction of the carcinogen, N-hydroxy-2-naphthylamine, with DNA at the C-8 and N2 atoms of guanine and at the N6 atom of adenine. Carcinogenesis 1:139–150 (1980).

8. Stohrer G, Osband JA, Alvarado-Urbino G. Site-specific modification of the lactose operator with acetylaminofluorene. Nucleic Acids Res 11:5093–5102 (1983).

9. Shibutani S, Gentles R, Johnson F. Aerial oxidation of acetylaminofluorene-derived DNA adducts. In: Nitroarenes (Howard, PC et al., eds). New York:Plenum Press, 1990; 135–147.

10. Shibutani S, Gentles R, Johnson F, Grollman AP. Isolation and characterization of oligonucleotides containing dG-N2-AAF and oxidation products of dG-C8-AF. Carcinogenesis 12:813–818 (1991).

11. Shibutani S, Gentles RG, Iden CR, Johnson F. Facile aerial oxidation of the DNA-base adduct N-(2′-deoxyguanosine-8-yl)-2-aminofluorene [dG(C8)AF]. J Am Chem Soc 112:5667–5668 (1990).

12. Poje M, Sokolic-Maravic L. The mechanism for the conversion of uric acid into allantoin and dehydro-allantoin. Tetrahedron 42:747 (1986).

13. Poje M, Sokolic-Maravic L. The mechanism for the conversion of uric acid into uroxanate and allantoin. A new base-induced 1,2-carboxylate shift. Tetrahedron 44:6723 (1988).

14. Elfara AA, Hanna PE. Substituent effects on the bioactivation of 2-(N-hydroxycetamide)fluorones by N-arylhydroxamic acid N,O-acyltransferase. J Med Chem 28:1453–1460 (1985).

15. Bosold F, Boche G. The ultimate carcinogen, O-acetyl-N-(2-fluorenlyl)-hydroxylamine (N-acetoxy-2-aminofluorene), and its reaction in vitro to form 2-(N-(deoxyguanosine-8-yl) amino)fluorene. Angew Chem (Int Ed Engl) 29:63–64 (1990).

16. Scribner JD, Scribner NK. Acetylation of nucleosides by N-acetoxy-N-arylacetamides: dependence on base, aryl group and buffer composition. J Org Chem 47:3143–3145 (1982).

17. Underwood GR, Kirsch RB. The solvolysis of N-acetoxy-2-acetaminofluorene and N-acetoxy-4-acetaminobiphenyl: delicate balance between nitrenium ion formation and hydrolysis. J Chem Soc Chem Commun:136–138 (1985).

18. Underwood GR, Gallahan RJ. The solvolysis of N-acetoxy-acetaminolines: the elucidation of two major reaction pathways for model ultimate carcinogens under neutral conditions. Tetrahedron Lett 28:5427–5430 (1987).

19. Synderwine EG, Roller PP, Wirth PJ, Adamson RH, Sato S, Thorgersson SS. Synthesis, purification and mutagenicity of 2-hydroxyamino-3-methyl-imidazol[4,5-f]quinoline. Carcinogenesis 8:1017–1020 (1987).

20. DeFranco E, Pelloux N, Lettera A, Lhomme M-F, Lhomme J. Interaction and reactivity of carcinogenic N-acetyl-N-(acetoxy)-2-aminofluorene with deoxyguanosine. An intramolecular approach. J Org Chem 56:4817–4819 (1991).

21. DeFranco E, Lettera A, Pelloux N, Lhomme M-F, Lhomme J. Arylamidation of the guanosine by a cancerogen, le 2-aminofluorenone approche intramoluculaire. Tetrahedron Lett 47:5725–5736 (1991).

22. Long RA, Robins RK, Townsend LB. Purine nucleosides XV. The synthesis of 8-amino and 8-substituted aminopurine nucleosides. J Org Chem 32:2751–2756 (1967).

23. Lin TS, Cheng JC, Ishigaku K, Sartorelli AC. 8-Substituted guanosine and 2′-deoxyguanosine derivatives as potential inducers of the differentiation of friend erythroleukemia cells. J Med Chem 28:1194–1198 (1985).

24. Kriek E, Miller JA, Juul U, Miller EC. 8-(N′-2-fluorenylacetamido)guanosine, an arylation reaction product of guanosine and the carcinogen N-acetoxy-N′-2′-fluorenylacetamide in neutral solution. Biochemistry 6:177–182 (1967).

25. Jacobson MD, Shapiro R, Underwood GR, Bryde S, Verna L, Hingerry BE. Synthesis and conformation of a dinucleoside monophosphate modified by anilines. Chem Res Toxicol 1:152–159 (1988).

26. Staab HA, Sauer T. "Proton sponges" and the geometry of hydrogen bonds: aromatic nitrogen bases with exceptional basicities. Angew Chem (Int Ed Engl) 27:865–879 (1988).

27. Barton DHR, McCombie SW. A new method for the deoxy- genation of secondary alcohols. J Chem Soc Perkin Trans 1:1574 (1975).

28. Robins MJ, Wilson JS, Hanske F. Nucleic acid related compounds. 42. A general procedure for the efficient deoxygenation of secondary alcohols. Regiospecific and stereoselective conversion of ribonucleosides to 2′-deoxyribonucleosides. J Am Chem Soc 105:4059–4065 (1983).

29. Jackman LM, Jen T. 14H and 13C nuclear magnetic resonance studies on the tautomeration and conformation of some cyclic amidines, guanidines and related systems. J Am Chem Soc 97:2811–2818 (1975).

30. Smith RL, Cochran DW, Gund P, Craigie EJ. Proton, carbon-13, and nitrogen-15 nuclear magnetic resonance and CNDO/2 studies on the tautomeration and conformation of amidine, a novel acylguanidine. J Am Chem Soc 101:191–201 (1979).