Supplementary Material Available: Experimental details for the preparation of compounds **2b, 9b, 11, 12a, 12b, 14, 15,** and **16** (9 pages). Ordering information is given on any current masthead page.

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[26] An invention disclosure has been filed to cover the use of *n*-pentenyl glycosides as glycosyl donors.

Facile Aerial Oxidation of the DNA-Base Adduct *N*-(2′-Deoxyguanosin-8-yl)-2-aminofluorene [dG(C8)AF]

S. Shibutani, R. G. Gentles, C. R. Iden, and
Francis Johnson*

Department of Pharmacological Sciences and Chemistry
State University of New York at Stony Brook
Stony Brook, New York 11794

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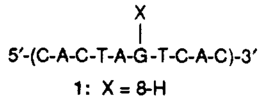
Recently, in connection with our studies on the synthesis of DNA oligomers containing mutagenic adducts, we were interested in obtaining an oligodeoxynucleotide (**2**) having a deoxyguanosine residue substituted at the C-8 position by an *N*-2-fluorenylamino group.

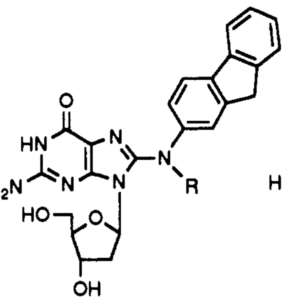


$$5'\text{-(C-A-C-T-A-}\overset{\overset{X}{|}}{\text{G}}\text{-T-C-A-C)-}3'$$

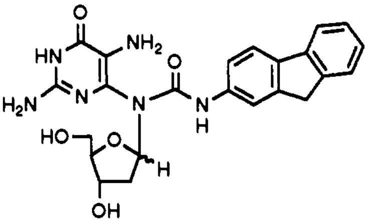
1: X = 8-H
2: X = 8-AF
3: X = 8-AAF
AF = 2-aminofluorene; AAF = *N*-acetyl-2-aminofluorene

Although **3** was easily prepared[1] by the reaction of **1** with *N*-acetoxy-*N*-acetyl-2-aminofluorene, attempts to remove the acetyl group from **3** to obtain **2** using base, invariably led to degraded products. However, the inclusion of a thiol totally prevented[2] the degradation and allowed the isolation of **2** in excellent yield. This result indicated that the degradation is oxidative in nature. Earlier work by Kriek et al.[3,4] had claimed, however, that this degradation of the modified nucleoside *N*-acetyl-*N*-(2′-deoxyguanosin-8-yl)-2-aminofluorene (**4a**) which is present in **3** is solely hydrolytic at alkaline pH. The two products that they isolated were assigned structures **5a** and **5b**, on purely spectroscopic evidence. These conflicting findings led us to reinvestigate this problem both at the level of the oligomers **2** and **3** and at the level of the modified nucleoside **4a**. Although we report studies on **4a** only, in this communication work with both oligomers **2** and **3** has revealed that the corresponding nucleoside residue within the oligomer **3** behaves similarly.[5]

In aqueous solution, in the presence of either a thiol or ascorbic acid or under anaerobic conditions, **4a** is cleanly deacetylated to **4b**, and no degradation could be detected (pH 7–13). This clearly indicates that previously observed transformations are oxidative in nature. Our investigations now show that *the degradative pathway parallels mechanistically the much-studied*[5-8] *oxidation of uric acid in alkali*. In 0.2 N NaOH, in the presence of air at 75 °C (Kriek and Westra conditions),[4] **4a** rapidly disappears and by HPLC[9] three new compounds arise, which we have designated as ring-opened products (ROP-1, -2, and -3). Under these conditions ROP-3 appears only in the early stages of the reaction as does a fourth peak representing the intermediate deacetylated nucleoside **4b**. Treatment of **4b** under identical conditions also gives rise to the same ring-opened products.

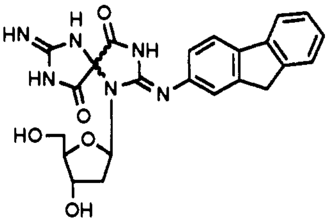


4a: R = Ac; dG(C8)AAF
b: R = H; dG(C8)AF



5a, 5b

The first products isolated in ~12% yield by HPLC (ROP-1 and ROP-2) occur in a 2:3 ratio[10] and spectroscopically appear to be identical with the two substances **5a** and **5b** first isolated by Kriek and his associates.[4] However, from our own spectroscopic analysis we conclude that most probably these substances are the spirodiastereomers **6a** and **6b**. The ^{1}H NMR and ^{13}C NMR data[11] unfortunately are not definitive because of the polyaza nature of the substances. Nevertheless the mass spectral results revealed that a good correlation exists between the FAB-MS positive- and negative-ion modes for **6a** and **6b**. Both positive-ion spectra show a peak at m/z 463 corresponding to the ion $(M + 1)^+$ whereas the negative-ion spectra show a peak at m/z 461 attributable to the ion $(M - 1)^-$. This clearly indicates that the molecular weight of both compounds is 462 daltons (Da), a result that is at variance with the value of 464 found by Kriek and Westra[4] using field-desorption mass spectrometry. The fragmentation patterns in the positive-ion mass spectra are also more easily interpreted in terms of structures **6a** and **6b**. Most significantly, the peak at m/z 207 represents the protonated fluorenyl cyanamide (or carbodiimide) ion $\text{FlN{=}C{=}NH}_2^+$ rather than the protonated isocyanate ion, $\text{FlN{=}C{=}OH}^+$. These new structural assignments make it easy to understand the origin of the diastereoisomeric relationship of **6a** and **6b**, which was assigned originally[4] to (improbable) differences at the anomeric 1′-carbon. It now appears that **6a** and **6b** are (cyclic) reaction path analogues of **7**, a skeletal-rearrangement intermediate postulated to occur along the uric acid–allantoin–uroxanate oxidative pathway.[6] Both



6a (ROP-1), **6b** (ROP-2)

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[1] Bases, R.; Mendez, F.; Mendez, L. *Carcinogenesis* (*London*) **1983**, *4*, 1445–1450.

[2] Stohrer, G.; Osband, J. A.; Alvarado-Urbina, G. *Nucleic Acids Res.* **1983**, *11*, 5093–5102. Stohrer, G.; O'Connor, D. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2325–2329. We found that the quantity of thiol recommended by Stohrer and his associates was insufficient to avoid some oxidation of the aminofluorene adduct. By raising the concentration of mercaptoethanol to 0.25 N, oxidation was completely inhibited at both the monomer and the oligomer levels.

[3] Kriek, E. *Chem.-Biol. Interact.* **1969**, *1*, 3–17. See also: Spodheim-Maurizot, M.; Dreux, M.; Saint-Ruf, G.; Leng, M. *Nucleic Acids Res.* **1979**, *7*, 2347–2356.

[4] Kriek, E.; Westra, J. G. *Carcinogenesis* (*London*) **1980**, *1*, 459–468.

[5] Brandenberger, H. *Biochim. Biophys. Acta* **1952**, *15*, 108; *Experientia* **1956**, *12*, 208–210; *Helv. Chim. Acta* **1954**, *37*, 641–644.

[6] Brandenberger, H.; Brandenberger, R. H. *Helv. Chim. Acta* **1954**, *37*, 2207–2220.

[7] Poje, M.; Sokolic-Maravic, L. *Tetrahedron* **1986**, *42*, 747–751.

[8] Poje, M.; Sokolic-Maravic, L. *Tetrahedron* **1988**, *44*, 6723–6728.

[9] The degraded monomeric ring-opened products were separated by a reverse-phase column, Bondapak C18 (0.39 × 30 cm, Waters), with a linear gradient of 0.05 M triethylamine acetate, at a flow rate of 1.0 mL/min. Under these conditions the retention times, in minutes, of the relevant compounds, in order of elution, were as follows: dG, 2.7; ROP-2, 11.1; ROP-1, 11.6; ROP-3, 12.6; dG(C8)AAF, 19.9; dG(C8)AF, 23.4.

[10] Neither ROP-1 (**6a**) nor ROP-2 (**6b**) is convertible to ROP-3 on treatment with base, as might be expected, on the basis of their assigned structures.

[11] Sufficient quantities of ROP-3 have not been available for a ^{13}C NMR spectrum determination.

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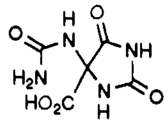
6a and **6b** are quite stable to basic reaction conditions.



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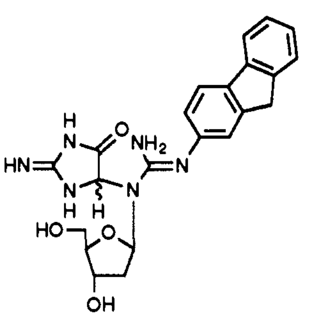
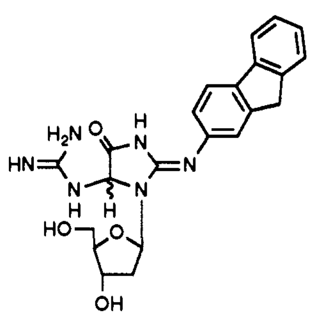
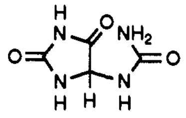
The third compound (ROP-3) isolated by HPLC, although transient at 75 °C, becomes the major product (~60% yield) in 1 N NaOH at 10 °C. This, by ^{1}H NMR spectroscopy, appears to be a 4:1 mixture of inseparable isomers of **8a** and **8b**, although structures **9a** and **9b** cannot be completely excluded. The epimeric hydrogen atoms at C-5 of the imidazolone ring appear as non-exchangeable (D_2O) singlets at δ 5.44 and 5.48 respectively. The positive- and negative-ion FAB-MS again show good correlation, exhibiting parent ions at *m/z* 437 and 435 respectively, thus identifying the molecular weights as 436 Da. Significantly there is a positive-ion peak at *m/z* 340 (negative-ion peak at *m/z* 338) corresponding to the loss of the imidazolone ring. Subsequent loss of the sugar residue to give the fluorenylguanidine ion is indicated by peaks at *m/z* 207 (positive ion) and 205 (negative ion). The structure postulated for ROP-3 is analogous to allantoin (**10**), again a well-established oxidative degradation product of uric acid.[7,8]



8a, 8b (ROP-3)



9a, 9b



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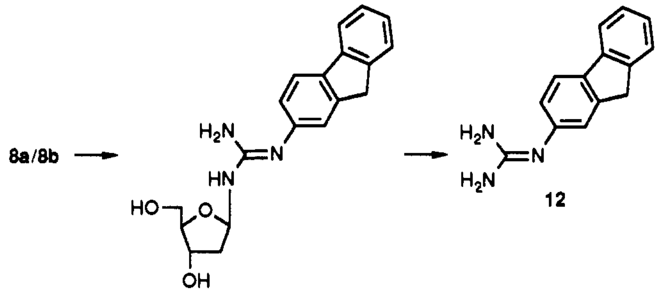

The further action of base on ROP-3 causes a rapid conversion at pH 13 to *N*-(2-fluorenyl)guanidine (**12**) identical with a synthetic sample.[12] At neutral pH, however, the dominant product becomes the deoxyribofuranoside intermediate **11**. We have also found that **11** can be obtained from **4b** directly by allowing the latter to stand in aqueous buffer at neutral pH (half-life of **4b**: 6.9 days).

Finally the oxidative pathway that leads to the destruction of **4a**/**4b** provides a complete mechanistic explanation for the strand scission and depurination observed by Johnson et al.[13] when dG(C8)AF- or dG(C8)AAF-modified oligomers were treated with 1 M piperidine at 90 °C. It now appears that an *abasic site* is therefore an intermediate in this strand scission process.

Given the sensitivity to aerial oxidation of dG(C8)AF (**4b**), it seems highly likely that many of the related analogues derived from different carcinogenic amines will be equally susceptible to oxidative degradation. *These findings may have significant implications for the mutagenic profile of* ***4a*** (and for dG(C8) adducts derived from other carcinogenic amines) when present as a residue in DNA. Further studies in this area are being pursued.

Acknowledgment. We thank Mr. Robert Rieger for his expert assistance in obtaining the mass spectral data. We are pleased also to acknowledge the interest and helpful discussions provided by Drs. A. P. Grollman and M. Takeshita. This research was supported by a grant (No ES04068) from the National Institutes of Health, National Institute of Environmental Health Sciences.

Supplementary Material Available: Schemes depicting the synthesis of **13**, **6a,b**, **9a,b**, and **12** and the base-catalyzed equilibration of **8** and **9** (3 pages). Ordering information is given on any current masthead page.

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(12) The isolation of *N*-(2-fluorenyl)guanidine as the end product of the degradation of **4a** by alkali in air confirms completely that an oxidative mechanism is involved; otherwise the end product should have been the corresponding fluorenylurea, no trace of which could be discerned in the reaction mixture.

(13) Johnson, D. L.; Reid, T. M.; Lee, M.-S.; King, C. M.; Romano, L. J. *Carcinogenesis (London)* **1987**, *8*, 619–623.

Reactions of Dimethylamine with Multiply Charged Ions of Cytochrome *c*[†]

Scott A. McLuckey,* Gary J. Van Berkel, and Gary L. Glish

Analytical Chemistry Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37831-6365

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It has recently been demonstrated, first by Fenn and co-workers[1] and subsequently by other groups,[2,3] that multiply charged ions from high-mass molecules can be formed from electrospray ionization. It is particularly noteworthy that proteins and peptides show a strong tendency for multiple cationization. Thus far, all of the proteins studied are characterized by multiple cationization to the extent that the observed mass/charge ratio is less than 3000. Therefore, these unusual ions, despite masses sometimes in excess of 10^5 daltons (Da),[2c] fall within the mass/charge range accessible to many modern mass spectrometers. We have recently coupled electrospray with a three-dimensional quadrupole[4] (i.e., a Paul trap[5]). This type of mass spectrometer is particularly well-suited for kinetic studies due to its ion-trapping and ion-isolation capabilities.[6] We describe here results of the first systematic study

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[†] Research sponsored by the U.S. Department of Energy, Office of Energy Research, under Contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

(1) (a) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64. (b) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F. *Mass Spectrom. Rev.* **1990**, *9*, 37. (c) Meng, C. K.; Mann, M.; Fenn, J. B. *Z. Phys. D* **1988**, *10*, 361. (d) Wong, S. F.; Meng, C. K.; Fenn, J. B. *J. Phys. Chem.* **1988**, *92*, 546.

(2) Loo, J. A.; Udseth, H. R.; Smith, R. D. *Biomed. Environ. Mass Spectrom.* **1988**, *17*, 411. (b) Loo, J. A.; Udseth, H. R.; Smith, R. D. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 207. (c) Loo, J. A.; Udseth, H. R.; Smith, R. D. *Anal. Biochem.* **1989**, *179*, 404.

(3) Covey, T. R.; Bonner, R. F.; Shushan, B. I.; Henion, J. D. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 249.

(4) A 4 × 10^{-6} M solution of cytochrome *c* was prepared in a solvent mixture of HPLC grade water, methanol, and glacial acetic acid in relative proportions of 20%, 75%, and 5% by volume, respectively. This solution was passed at a flow rate of 1.0 μL/min through a 120 μm i.d. stainless steel capillary needle held at a potential of +3.5 kV. The outlet of the needle was positioned about 1 cm from a 100-μm inlet aperture into the mass spectrometer. For details of this system, see: Van Berkel, G. J.; Glish, G. L.; McLuckey, S. A. *Anal. Chem.*, in press.

(5) (a) March, R. E.; Hughes, R. J. *Quadrupole Storage Mass Spectrometry*; John Wiley and Sons: New York, 1989. (b) Stafford, G. C.; Kelley, P. E.; Syka, J. E. P.; Reynolds, W. E.; Todd, J. F. J. *Int. J. Mass Spectrom. Ion Processes* **1984**, *60*, 85. (c) Louris, J. N.; Cooks, R. G.; Syka, J. E. P.; Kelley, P. E.; Stafford, G. C., Jr.; Todd, J. F. J. *Anal. Chem.* **1987**, *59*, 1677.

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