Synthesis of *N*-Acetoxy-*N*-benzoyl-2-aminofluorene, an Ultimate Carcinogen by LTA Oxidation of α-Phenyl-*N*-(2-aminofluorenyl)nitrone, and *N*-(2′-Deoxyguanosin-8-yl)-2-aminofluorene

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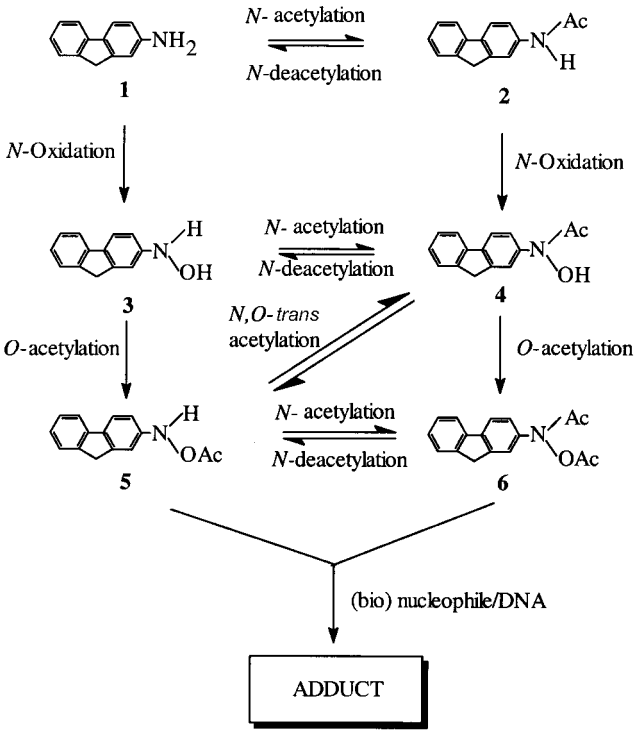
Abstract: The rearrangement of a new α-phenyl-*N*-(2-aminofluorenyl)nitrone (**8**) to a new ultimate carcinogen, *N*-acetoxy-*N*-benzoyl-2-aminofluorene (**9**) is achieved in a lead tetraacetate (LTA) oxidation reaction. Compound **9** reacts with deoxyguanosine (dG) at pH 7.0 to give *N*-(benzoyl)-*N*-(deoxyguanosin-8-yl)-2-aminofluorene (**10**). Subsequent debenzoylation with the heterogeneous system (sodium carbonate/methanol) leads to the C8-adduct, *N*-(2′-deoxyguanosin-8-yl)-2-aminofluorene (**11**).

Key words: N-acetoxy compound, LTA oxidation, α-phenyl-*N*-(2-aminofluorene)nitrone, dG adduct, *N*-(2′-deoxyguanosin-8-yl)-2-aminofluorene

2-Aminofluorene (**1**), 2-acetylaminofluorene (**2**), *N*-hydroxy-2-aminofluorene (**3**), and *N*-hydroxy-2-acetylaminofluorene (**4**) are known to be potent carcinogens,[1] particularly effective in inducing cancer in the kidney, liver, and bladder[2] in humans. Compounds **1**–**4** are referred to as "precarcinogens" since they induce cancer in tissues distant from the site of entry. Essentially, in vivo or in vitro metabolic activation is required to produce the reactive metabolites *N*-acetoxy-2-aminofluorene (**5**) or *N*-acetoxy-2-aminofluorenylacetamide (**6**); different established pathways[3–9] are shown in Scheme 1. The metabolites **5** and **6** are putative reactive species (ultimate carcinogens), they react with (bio)nucleophiles[10] to give adducts. These ultimate carcinogens are directly responsible for the induction of cancer.[11,12] Several unknown reactive metabolites of 2-aminofluorene are also suspected. These are emphasized in the present work.

Recently, we have synthesized a new class of model carcinogens, *N*-acetoxy-*N*-benzoylarylamines[13] from arylnitrones of phenyl moiety, and showed that they are also reactive metabolites like *N*-acetoxyaniline.[14] We have extended the same with polynuclear nitrone **8** to test its reactivity and justify the earlier reactions. Herein, we report the synthesis of **9** by LTA oxidation of **8**, the reaction of **9** with dG, and finally the debenzoylation of *N*-(benzoyl)-*N*-(deoxyguanosin-8-yl)-2-aminofluorene (**10**) with a heterogeneous system into C8 adduct, *N*-(2′-deoxyguanosin-8-yl)-2-aminofluorene (**11**) (Scheme 2).



Scheme 1

Compound **3**, precursor for the synthesis of **8** was prepared by the reduction of 2-nitrofluorene (**7**) using the modified procedure of Patrick[15] using zinc–ammonium chloride and catalytic amount of histidine[16] in distilled water. Condensation of **3** with freshly distilled benzaldehyde in ethanol gave polynuclear nitrone **8**. Compound **8** was found to be acid sensitive[17] and light sensitive,[18] and decomposes to aldehyde, amine, nitroso, imine, azo compound, etc. Hence, it was stored in the freezer under dark until further use.

A new *N*-benzoylated ultimate carcinogen **9** was prepared by the lead tetraacetate (LTA) oxidation[19,20] rearrangement reaction of **8** in benzene. The exothermic reaction was temperature dependent and thus maintained between –8 °C to 0 °C. The reaction is believed to proceed through an intramolecular 1,4-acetyl transfer.[13]. After purification, compound **9** was obtained as a crystalline white solid, stable for a week at –10 °C. IR and ^{1}H NMR studies confirmed its structure. IR absorption peak at 1788 cm^{-1} was assigned to ester C=O, and that at 1670 cm^{-1} was assigned

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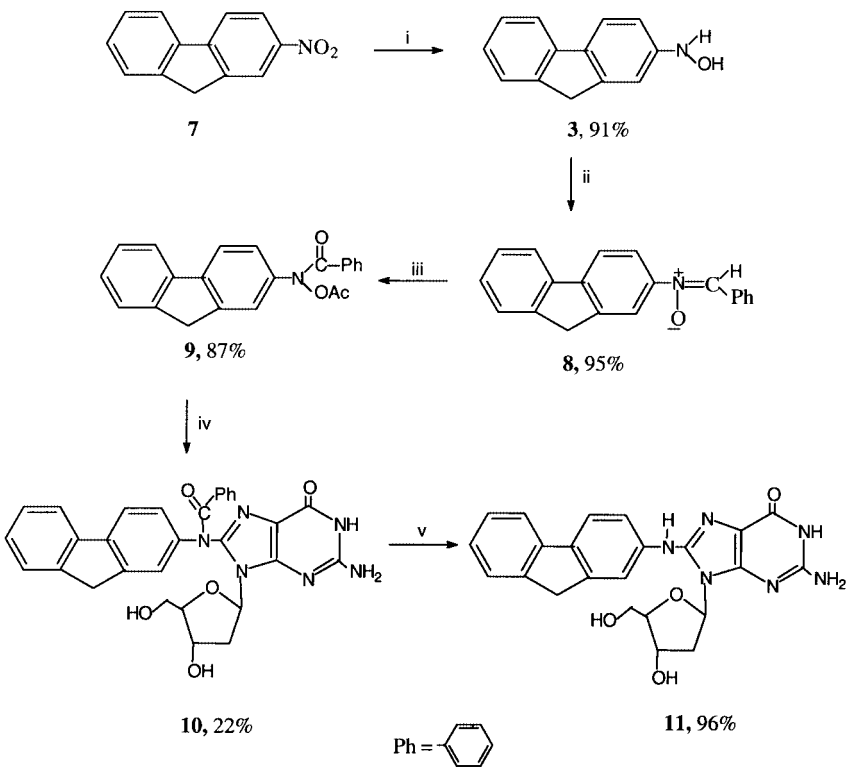
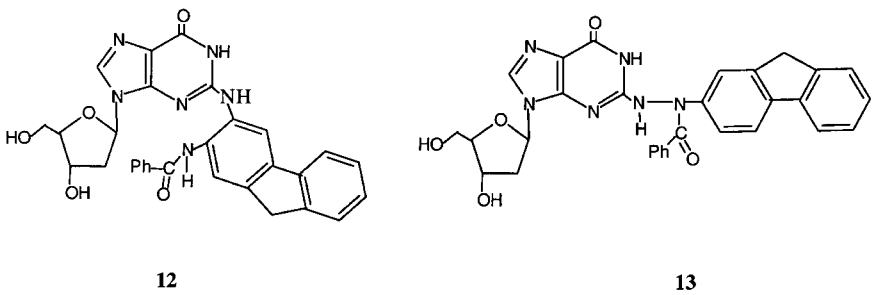


Scheme 2 *Reagents and conditions*: i) Zn/NH_4Cl, Cat. histidine, distilled H_2O, 20–30 °C, 90 min, pH 7.4–7.5; ii) PhCHO/EtOH, 10–15 °C, 4 h, overnight 0 °C; iii) LTA/anhyd benzene, 0 °C, 10 min; iv) 95% EtOH, dG/2 mM sodium citrate buffer (pH 7.0), 55 °C, 2 h, then 60 °C, 2 h; v) anhyd Na_2CO_3/MeOH, r.t., 8 h

to amide C=O stretching frequency confirming the presence of $OCOCH_3$ and COPh groups. In the ^1H NMR spectrum, peaks at δ = 2.28 (3 H), 3.86 (2 H) and 7.34−7.82 (12 H) were assigned to $OCOCH_3$, CH_2 amd Ar-H′s respectively. Other spectral data were consistent with structure **9**. Satisfactory results were obtained by elemental analysis. All attempts to synthesize **9** by O-acetylation to **8** with acetyl chloride, acetic anhydride and benzoyl chloride failed.

Reaction of **9** with deoxyguanosine (dG) in sodium citrate buffer (pH 7.0) at 55 °C gave the new *N*-benzoylated C8 adduct **10**. It structure was confirmed by ^1H NMR spectral studies. The absence of a peak at δ = 8.01 ppm, attributed to the C8-H proton in dG,[21] indicated that substitution had taken place at C8 of the dG ring. The absence of a peak at δ = 8.76 ppm in **10**, assigned for NH proton in **5**, confirmed the C8 adduct formation through the nitrogen atom of **10**. The presence of twelve aromatic protons at δ = 7.24–8.12 ppm indicated that none of the aromatic protons on the fluorenyl moiety and benzoyl ring were substituted. Moreover, these signals strongly support the point of link at C8 of dG through the fluorenyl nitrogen. The peaks at δ = 2.04 (2 H), 3.74 (2 H), 3.98 (1 H), 4.41 (1 H), 5.38 (OH), 6.04 (OH) and 6.37 (1 H), integrating for nine protons, confirmed the presence of sugar moiety. The NH_2 and NH protons of the dG moiety resonated at δ = 6.52 and 10.59 ppm, respectively. The D_2O exchangeable protons of NH and OH indicated that these sites were free from substituents. Other spectral data were consistent with structure **10**.

The set of data assigned for **10** ruled out the other possible N2 adducts **12** (since C8-H proton was absent, it would have eight aromatic protons instead of nine), and **13** (since both NH-NH protons[22] were absent) (Figure).



12 **13**

Figure

Compound **10** was debenzoylated[23] to give product **11**. The ^{1}H NMR peak at δ = 8.76 ppm was attributed to the NH proton; and its disappearance upon addition of D_2O, confirmed the structure **11**.

In conclusion, we have developed a facile activation pathway to *N*-benzoylated ultimate carcinogen **9** from the "precarcinogen" polynuclear nitrone **8**. The activation[13] of **8** which is required to produce the reactive metabolite **9** was achieved using lead tetraacetate (LTA), an excellent oxidant. Compound **9** belongs to a new type of reactive metabolites like its *N*-deacetylated and *N*-acetylated analogues **5** and **6,**[3–9] respectively.

Silica gel (E-Merck GF_{254}, 0.2 mm) with fluorescent indication was used for TLC. The mobile phases used for TLC: $CHCl_3$, benzene, $CHCl_3$–hexane (9:2), Et_2O–hexane (1:1), MeOH–H_2O (7:3) and (9:1), EtOAc–hexane (1:1), MeOH, MeCN–MeOH–H_2O (4:3:1), and benzene–Et_2O (1:1). HPLC was performed with one of the following mobile phases: H_2O–MeCN (5:3), (7:3) and (7:1). Reagents were obtained from commercial sources as indicated: dG (Aldrich), 2-nitrofluorene (E-Merck), LTA (Aldrich), benzaldehyde (E-Merck), silica gel-923 (Aldrich), saphadex G-15 (Aldrich), sodium citrate (Aldrich), ammonia (s.d. fine). Mps (uncorrected) were recorded on a SELACO 605 melting point apparatus. ^{1}H NMR spectra were recorded on Bruker AMX-400 (400 MHz) spectrometer using $CDCl_3$ or DMSO-d_6 as solvent with TMS as internal standard. IR spectra were recorded on a Bio-Rad Win-IR spectrometer. Elemental analyses were obtained on Vario-EL instrument. Low temperature reactions were carried out using cryostat model MRP 700. HPLC was performed with a Lachrom-2000 Merck-Hitachi L7100 pump with RP18.250-4 mm column, and UV Detector-UV-VIS L7400.

N-(2-Fluorenyl)hydroxylamine (3)

Significant modifications were made to the method of Patrick.[15]

Zn dust (1.31 g, 0.02 mol) was added in portions over 45 min to a stirred, cooled (20–30 °C) mixture of 2-nitrofluorene (**7**, 2.11 g, 0.01 mol), NH_4Cl (2.12 g, 0.04 mol), and catalytic amount of histidine[16] in H_2O (50 mL). The pH was kept at 7.4–7.5 by adding small portions of ammonia. The reaction temperature was kept at 20–30 °C. The reaction mixture was stirred for an additional 45 min and the mixture was filtered by suction at r.t. The filtrate was saturated with common salt, and cooled in an ice bath for 2 h to ensure maximum crystallization of product **3**. The yellow solid was filtered and dried. Crude product was washed with cyclohexane and recrystallised from benzene to give **3**, as a colourless solid (1.8 g, 91%); mp 168–170 °C (Lit.[15] mp 170 °C).

α-Phenyl-*N*-(2-aminofluorenyl)nitrone (8)

Equimolar solutions of *N*-(2-fluorenyl)hydroxylamine (**3**) and benzaldehyde in a minimum volume of EtOH was kept at 10–15 °C and set aside in the dark for 4 h. The reaction mixture was stored overnight at 0 °C. The crude nitrone was separated and crystallised from EtOH to give **8**, as a white crystalline solid (95%).

Mp 117–118 °C (dec.).

IR (paraffin): 1552 (C=N), 1088 (NO) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.98 (s, 2 H, -CH_2-), 7.32–7.99 (m, 8 H, Ar-H), 8.12 (s, 1 H, CH=N), 8.31 (d, 2 H, Ar-H), 8.44 (d, 2 H, Ar-H).

Anal. Calcd for $C_{20}H_{15}NO$: C, 84.21; H, 5.26; N, 4.91. Found: C, 84.23; H, 5.25; N, 5.04.

N-Acetoxy-*N*-benzoyl-2-fluorenylamine (9)

A solution of nitrone **8** (500 mg, 1.76 mmol) in anhyd benzene (10 mL) was kept at –7 °C to 0 °C. LTA (800 mg, 2.46 mmol) was added in portions (exothermic reaction took place immediately), and the reaction mixture was stirred for 10 min at 0 °C. Filtration of white lead diacetate, and evaporation of the solvent at reduced pressure afforded the crude solid product **9**. Crude product was successively washed with cold (–20 °C) Et_2O and CH_2Cl_2. The white solid obtained was recrystallised several times from Et_2O–hexane at 0 °C to give pure **9**, as a white crystalline solid (700 mg, 87%).

Mp 143–145 °C.

IR (KBr): 1670 (s, C=O, PhCON), 1788 (s, C=O, $OCOCH_3$), 1482, 1490 (s, C-N), 1218 (s, C-O, $OCOCH_3$) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.28 (s, 3 H, $OCOCH_3$), 3.86 (s, 2 H, -CH_2-), 7.34–7.82 (m, 12 H, Ar-H).

Anal. Calcd for $C_{22}H_{17}NO_3$: C, 76.96; H, 4.95; N, 4.08. Found: C, 76.84; H, 4.94; N, 4.11.

N-(Benzoyl)-*N*-(deoxyguanosin-8-yl)-2-aminofluorene (10)

Significant modifications were made to the procedure used by Kriek et al.[24] in the synthesis of Gu-adducts.

Compound **9** (291 mg, 0.89 mmol) in 95% EtOH (15 mL) was added to dG (49 mg, 0.17 mmol) in sodium citrate buffer (pH 7.0, 2 mM, 30 mL) at 55 °C over 2 h, and the mixture was stirred further for 12 h at 60 °C. The reaction mixture was diluted with H_2O (60 mL) and the EtOH was evaporated. The aqueous phase was extracted with Et_2O (5 × 10 mL) and EtOAc (5 × 15 mL). The Et_2O extract was discarded; the EtOAc extract was dried (Na_2SO_4) and evaporated to give crude solid **10**. The crude product was first purified over a silica gel column (MeOH–$CHCl_3$, 9:2); then chromatographed on sephadex G-15 (EtOH–$CHCl_3$, 7:4) to give the product **10** (107 mg, 22%), which was stable in neutral aqueous solution for several weeks at 0 °C. HPLC analysis (H_2O–MeCN, 7:1) of the aqueous solution of **10** indicated 98.9% purity.

IR (KBr): 3340, 3032, 2920, 1676, 1645, 1560, 1400, 1064, 1030, 1011, 891 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.04 (m, 2 $H_2{}^1$, sug), 3.74 (m, 2 $H_5{}^1$, sug), 3.92 (s, 2 H_9, AF), 3.98 (m, 1 $H_4{}^1$, sug), 4.41 (m, 1 $H_3{}^1$, sug), 5.38 (s, 1 $H_3{}^1$, OH), 6.04 (s, 1 $H_5{}^1$, OH, sug-OH), 6.37 (m, 1 $H_1{}^1$, sug), 6.52 (s, 2 H, Gu-NH_2, D_2O exchangeable), 7.24 (t, 1 H_7, AF), 7.36 (t, 1 H_6, AF), 7.52–7.69 (m, 1 H_8, AF and 5 H, Ar-H), 7.71 (d, 1 H_3, AF), 7.78 (d, 1 H_4, AF), 7.81 (d, 1 H_5, AF), 8.12 (s, 1 H_1, AF), 10.59 (s, 1 H, Gu-NH, D_2O exchangeable).

Anal. Calcd for $C_{30}H_{26}N_6O_5$: C, 65.45; H, 4.72; N, 15.27. Found: C, 65.10; H, 4.71; N, 15.67.

N-(2′-Deoxyguanosin-8-yl)-2-aminofluorene (11)

Compound **11** was prepared by the method of Underwood et al.[23] using the heterogeneous system (Na_2CO_3–MeOH) for debenzoylation of **10**. Spectral data are in good agreement with those of an authentic sample.[25]

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