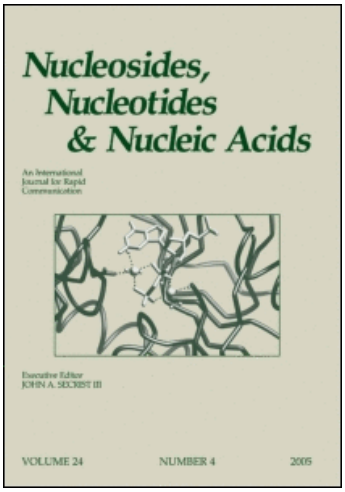

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SYNTHESIS AND CHARACTERIZATION OF NUCLEOSIDE DERIVATIVES, *N*-(BENZOYL)-*N*-(DEOXYGUANOSIN-8-YL)-4-AMINOBIPHENYL AND *N*-(2'-DEOXYGUANOSIN-8-YL)-4-AMINOBIPHENYL VIA α-PHENYL-*N*-(4-BIPHENYL)NITRONE

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SYNTHESIS AND CHARACTERIZATION OF NUCLEOSIDE DERIVATIVES, *N*-(BENZOYL)-*N*-(DEOXYGUANOSIN-8-YL)-4-AMINOBIPHENYL AND *N*-(2′-DEOXYGUANOSIN-8-YL)-4-AMINOBIPHENYL VIA α-PHENYL-*N*-(4-BIPHENYL)NITRONE

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ABSTRACT

Lead tetraacetate (LTA) oxidation of α-Phenyl-*N*-(4-biphenyl)nitrone (8) to give a new ultimate carcinogen, *N*-acetoxy-*N*-benzoyl-4-aminobiphenyl (9) which was reacted with deoxyguanosine (dG) at pH 6.9 to give nucleoside derivative, *N*-(benzoyl)-*N*-(deoxyguanosin-8-yl)-4-aminobiphenyl (10). Following debenzoylation with sodium carbonate-methanol leads to *N*-(2′-deoxyguanosin-8-yl)-4-aminobiphenyl (11).

Key Words: Nucleoside derivatives; LTA oxidation; α-Phenyl-*N*-(4-biphenyl)nitrone; *N*-(benzoyl)-*N*-(deoxyguanosin-8-yl)-4-aminobiphenyl

4-Aminobiphenyl **(1)**, 4-acetylaminobiphenyl **(2)**, *N*-hydroxy-4-aminobiphenyl **(3)** and *N*-hydroxy-4-acetylaminobiphenyl **(4)** form a class of compounds induce tumours in a wide variety of tissues in a number of species.[1–6] The carcinogenicity of **1**, **2**, **3** and **4** depend on their activation to become reactive metabolites **5** or **6** or *N*-hydroxy derivatives.[7–16] Different pathways have

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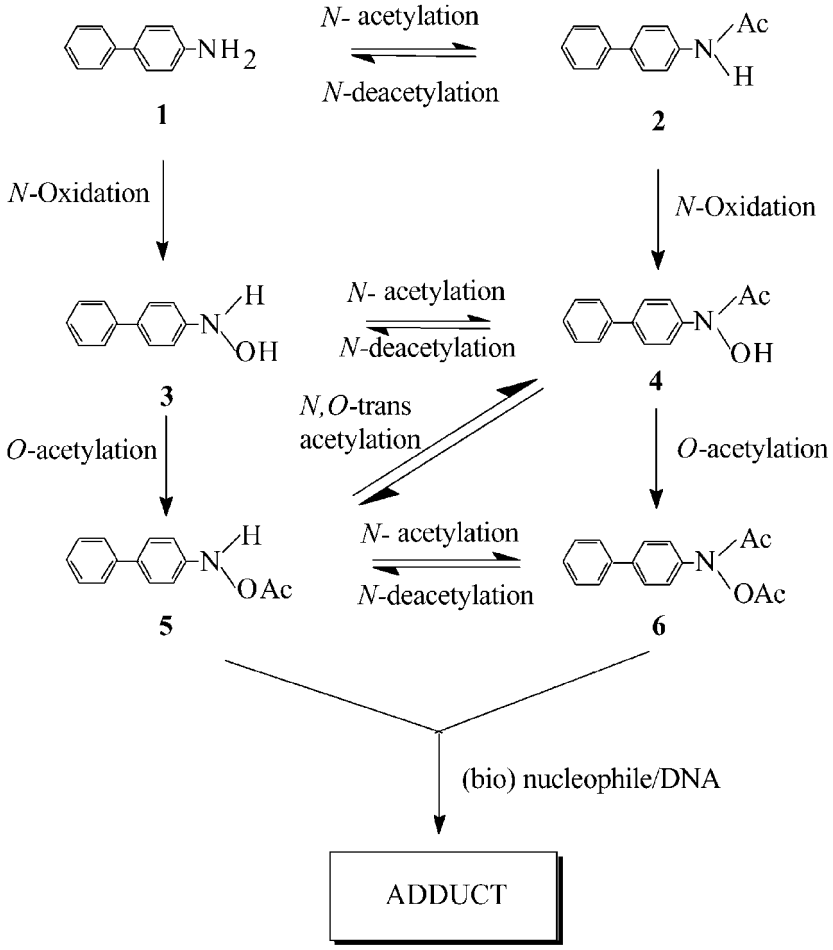
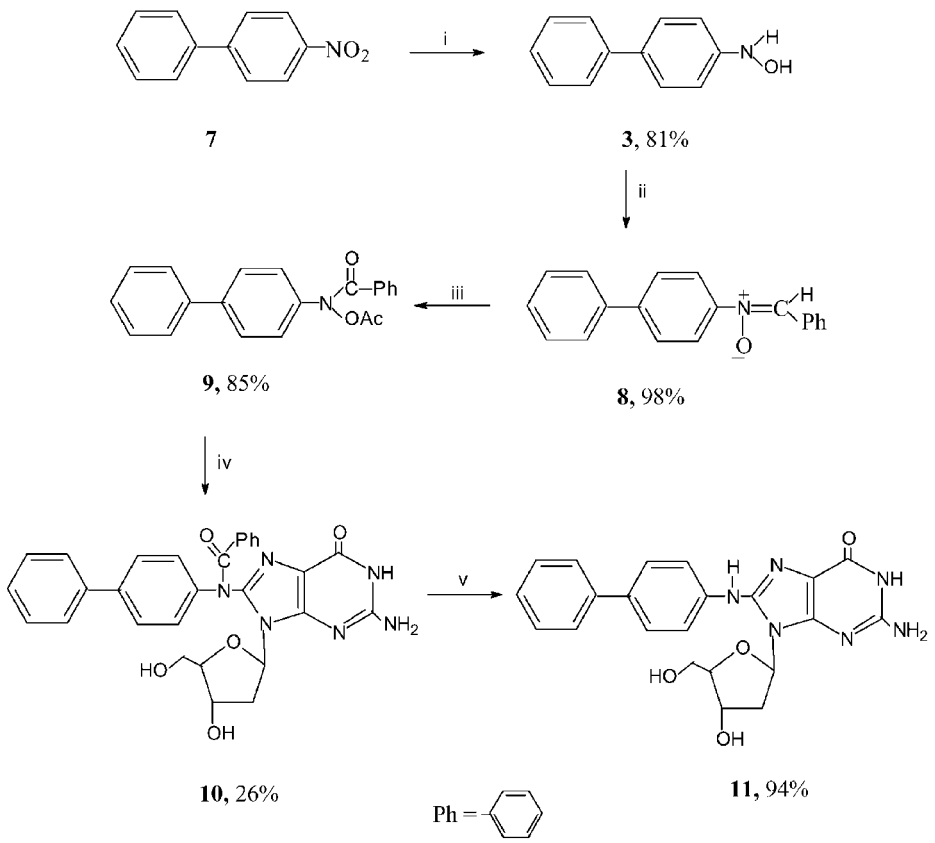


Scheme 1.

been developed in both in vivo and in vitro for the activation of **1**, **2**, **3** and **4** into their reactive metabolites **5** and **6**,[10–16] as shown in Sch. 1. The metabolites **5** and **6** are putative reactive species, react with (bio)nucleophiles.[11,17–21] to form adducts and are directly responsible for the induction of cancer.[17–21]

Recently, we have synthesized a new class of carcinogens, *N*-acetoxy-*N*-benzoylarylamines from arylnitrones[22,23] of phenyl moiety to form C8 adduct with dG and they are also shown to be reactive metabolites like *N*-acetoxyanilines.[14] Now, we have extended this study with polynuclear nitrone **8** to check its reactivity and justification for the earlier reactions made. Hence, we report the synthesis of a new ultimate carcinogen *N*-acetoxy-*N*-benzoyl-4-aminobiphenyl **9** by LTA oxidation[24–26] of α-Phenyl-*N*-(4-biphe-nyl)nitrone **8** and the reaction of **9** with the dG, finally the debenzoylation of *N*-(benzoyl)-



Scheme 2. *Reagents and conditions*: i, Zn/NH_4Cl, Cat histidine, distilled H_2O-DMF, 20–30°C, 90 min, pH 7.4–7.5; ii, PhCHO/EtOH, 5–10°C, 3 h, overnight 0°C; iii, LTA/anhyd benzene, 0°C, 20 min; iv, 95% EtOH, dG/2 mM sodium citrate buffer of pH 6.9, 55°C, 3 h, then 60°C, 2 h; v, anhyd Na_2CO_3/MeOH, r.t. 6–7 h.

N-(deoxyguanosin-8-yl)-4-aminobiphenyl **10** with heterogeneous system into C8 adduct, *N*-(2′-Deoxyguanosin-8-yl)-4-aminobiphenyl **11** (Sch. 2).

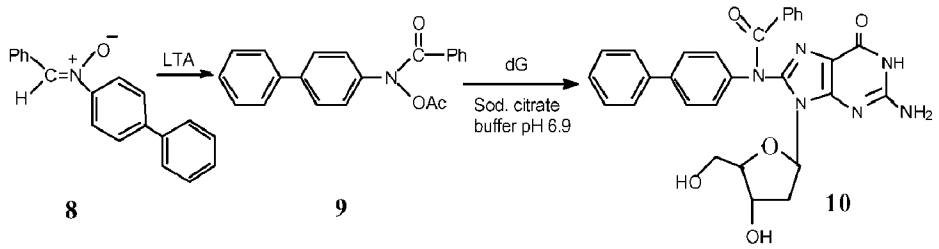
RESULTS AND DISCUSSION

Compound **3**, precursor for the synthesis of **8** was prepared by the reduction of 4-nitrobiphenyl **(7)** using modified procedure of patric[27] method with zinc-ammonium chloride and catalytic amount of histidine[28] in a solvent mixture of water-*N*, *N*-dimethyformamide (1:1). Condensation of **3** with freshly distilled benzaldehyde in ethanol gave nitrone **8**. IR absorption at 1540 cm^{-1} and ^1H NMR singlet peak at δ 8.04 confirmed the presence of CH=N group in **8**. Compound **8** was found to be acid sensitive[29] and light sensitive,[30] decomposes to aldehyde, amine, nitroso, imine, azo compound

etc. Hence, it was stored in the freeze under dark until further use. ^{1}H NMR, IR and elemental analysis studies are confirmed the structure of **8**.

A new *N*-benzoylated ultimate carcinogen **9** was prepared by LTA oxidative rearrangment reaction[31] in benzene. The reaction was found to be exothermic, temperature dependent and maintained at 0°C. The reaction appears to proceed through the intramolecular acetyl transfer,[22] leads to the formation of **9**. Compound **9** was obtained as crystalline white solid after purification and found to be stable for a week at -10°C. ^{1}H NMR and IR studies are made to confirm the product **9**. IR absorption peaks at δ 1678 and 1790 cm^{-1} are assigned to amide, C=O and ester C=O stretching frequency respectively, confirmed the presence of $OCOCH_3$ and COPh groups. In the ^{1}H NMR spectrum, a singlet peak at δ 2.26 is assigned to $OCOCH_3$. Other spectral data are consistent with the structure **9**. Satisfactory results are obtained by elemental analysis. All attempts to synthesize **9** by O-acetyl-ation to **8** with acetyl chloride, acetic anhydride and benzoyl choloride are failed.

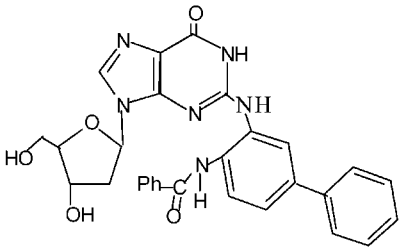
Reaction of **9** with deoxyguanosine (dG) in sodium citrate buffer of pH 6.9 at 55°C gave a new N-benzoylated nucleoside derivative **10**. The yield of the product **10** is 26%, which is much improved yield compared to its analogous.[10,12,14] The ^{1}H NMR, IR and elemental analysis data confirmed the structure of **10**. The absence of C8-H proton of dG[32] in the region δ 8.01 indicates that substitution had taken place at C8 position of dG ring. The NH_2 and NH protons of dG moiety appeared at δ 6.37 and 10.57 respectively. All the active hydrogens of NH and OH disappeared upon addition of D_2O indicate that NH and OH sites are free from substituent. Other spectral data are also consistent with the structure **10**.



8 **9** **10**

The compound **10** was debenzoylated[10] to form an authenticated product[12] **11**. Appearance of NH proton at δ 8.68 and disappearance of this active NH proton upon addition of D_2O confirmed the product **11**.

The spectral data assigned for **10** ruled out the other possible N2 adduct **12**, since C8-H proton was absent, it would have eight aromatic protons instead of nine, and **13**, since both NH-NH protons were absent. Thus, the presence of nine aromatic protons at δ 7.06–7.79 indicated that none of the aromatic protons in the biphenyl moiety are substituted. Moreover, these signals strongly support the point of link at C8 of dG through the biphenyl nitrogen.



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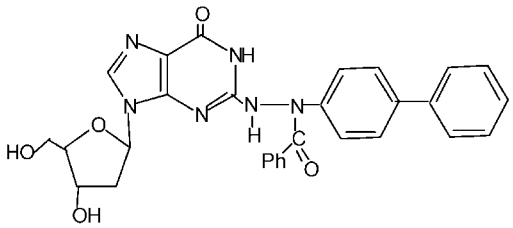

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LTA oxidation of **8** to give **9**, inturn reacted with dG to form C8 adduct **10**. This concludes: (i) **9** is also a new reactive metabolite like other N-acetoxyarylamines and amides reported earlier,[10–15,22,23] (ii) nitrone **8** is also a precarcinogen i.e., it is essentially require activation to produce the reactive metabolite **9**. Therefore, LTA is used as an excellent oxidant[24,25] to activate **8** into reactive metabolite **9**. This is a simple activation pathway to **9**, a new reactive metabolite via nitrone **8**.

EXPERIMENTAL TLC was performed with 0.2 mm silica gel GF254 (E-merck) with fluorescent indication. Melting points were recorded on SELACO 605 melting point apparatus and were uncorrected. ^{1}H NMR Spectra were recorded on Bruker AMX-400, 400 Mz, NMR spectrophotometer using $CDCl_3$ and DMSO-d_6 as solvent with TMS as internal standard. IR spectra are recorded on a Bio-Rad Win-IR spectrometer. Low temperature reactions are carried out using cryostat model MRP 700. All HPLC analysis are performed with Lachrom-2000 Merck-Hitachi L7100 pump with RP18.250-4 mm column and UV Detector-UV-VIS L7400.

α-Phenyl-N-(4-biphenyl)nitrone (8). Equimolar solutions of *N*-4-(biphenyl)hydroxylamine **3** and benzaldehyde in minimum volume of ethanol-ether (1:1) was kept at 5–10°C and set aside in the dark for 3 h. Ether was removed under reduced pressure. Resulting mixture was freezed overnight in dark. Separated needles were recrystallized from ethanol to give **8** as white crystalline solid. Yield (98%); mp 105–107°C; ^{1}H NMR ($CDCl_3$): δ 7.52–7.98 (m, 10 H, Ar-H), 8.04 (s, 1H, CH=N), 8.25–8.62 (m, 4 H, Ar-H); IR (Paraffin):1540 (C=N), 1090 cm^{-1} (NO). Anal. Calcd. for $C_{19}H_{15}NO$: C, 83.51; H, 5.49; N, 5.31. Found: C, 83.48; H, 5.48; N, 5.16.

N-Acetoxy-N-benzoyl-4-aminobiphenyl (9). LTA (800 mg, 1.80 mmol) was added to nitrone **8** (500 mg, 1.83 mmol) in 10 ml benzene at 0°C and the mixture was stirred in cold condition for 10 min. After 10 min, white lead diacetate was filtered at room temperature and evaporation of the solvent at reduced pressure afforded **9** as solid. It was repeatedly washed with cold (-20°C) ether. Finally it was recrystallised from ether-petroleum mixture to give crystals of **9**. Yield was 508 mg (85%); mp 97–99°C; ^{1}H NMR ($CDCl_3$):

δ 2.26 (s, 3H, OCOCH3), 7.34–7.40 (d, 2H, Ar-H), 7.72–7.86 (m, 10 H, Ar-H), 7.90–8.01 (d, 2 H, Ar-H); IR (KBr): 1678 (C=O str, PhCON), 1790 (C=O str, $OCOCH_3$), 1480, 1486 (C−N str), 1217 cm^{-1} (C-O str, $OCOCH_3$). Anal. Calcd. for $C_{21}H_{17}NO3$: C, 76.13; H, 5.13; N, 4.23. Found: C, 76.15; H, 5.13; N, 4.26.

N-(2′-Deoxyguanosin-8-yl)-4-benzoylaminobiphenyl (10). Compound **9** (281 mg, 0.85 mmol) in 15 ml of 95% ethanol was added to dG (49 mg, 0.17 mmol) in 30 ml of 2 mM sodium citrate buffer[33] of pH 6.9 at 55°C over 2 h and the mixture was stirred further for 3 h at 60°C. The reaction mixture was diluted with 60 ml of water and filtered. The ethanol was evaporated and the aqueous phase was extracted with ether (3×10 ml) and ethyl acetate (3×15 ml). The combined extracts were dried over anhydrous sodium sulfate and evaporated to give crude solid product **10**. Compound **10** was washed over a silica gel column with ether-benzene (1:1) and eluted with methanol-chloroform (7:4). Second fraction was re-chromatographed over a sephadex G-15 with ethanol-chloroform (7:4) to give pure solid product **10** (24 mg, 26%), which was stable in neutral aqueous solution for several weeks at 0°C. Analysis of the aqueous solution with HPLC using water-acetonitrile (6:1) showed that the product was 98.8% pure. ^{1}H NMR (DMSO-d6): δ 2.06 (m, $2H_2{}^1$), 3.75 (m, $2H_5{}^1$), 4.04 (m, $1H_4{}^1$), 4.46 (m, $1H_3{}^1$), 5.36 (s, $1H_3{}^1$, OH), 6.02 (s, $1H_5{}^1$, OH), 6.20 (m, $1H_1{}^1$), 6.37 (s, 2H, Gu-NH_2), 7.06–7.38 (m, 5H, CO−Ar-H), δ 7.41 (d, 2H, BP), 7.52(t, 1H, BP), 7.63 (t, 2H, BP), 7.79 (dd, 4H, BP), 10.57 (s, 1H, Gu-NH); IR (KBr): 3330, 2928, 1680, 1644, 1562, 1355, 1052, 1025, 1013,960 cm^{-1}. Anal. Calcd. for $C_{29}H_{26}N_6O_5$: C, 64.68; H, 4.83; N, 15.61. Found: C, 64.66; H, 4.87; N, 15.65.

N-(2′-Deoxyguanosin-8-yl)-4-aminobiphenyl (11). This was prepared by using Underwood procedure.[10] Compound **10** was debenzoylated with heterogeneous system, sodium carbonate-methanol to give **11**. Spectral data of **11** is in good agreement with the data of authentic sample.[12,34]

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