

# Synthesis, Characterization, and Comparative Conformational Analysis of *N*-(Deoxyguanosin-8-yl)aminopyrene Adducts Derived from the Isomeric Carcinogens 1-, 2-, and 4-Nitropyrene

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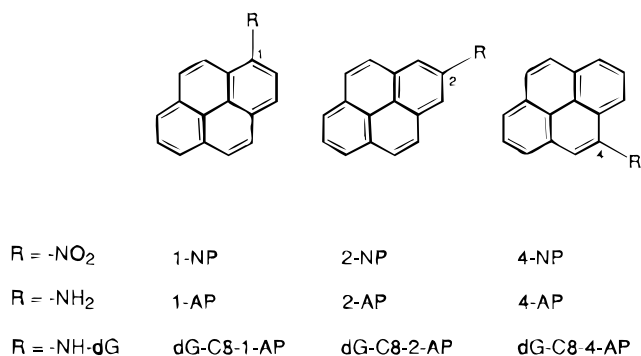
Nitrated polycyclic aromatic hydrocarbons are mutagens/carcinogens that undergo in vivo activation by ring-oxidation and nitro-reduction pathways. We report the syntheses and comparative conformational analyses of *N*-(deoxyguanosin-8-yl)-*n*-aminopyrene adducts (dG-C8-*n*-AP, *n* = 1, 2, 4) derived from the three isomeric mononitropyrenes (1-, 2-, and 4-NP). The C8-amine nitrogens of these adducts have been enriched with <sup>15</sup>N to examine the conformation about the pyrenyl–nitrogen and guanyl–nitrogen bonds that link the guanine and the pyrene moiety. These adducts are structurally isomeric, thus providing an interesting opportunity for systematic probing of the isomeric adduct conformations. Spectroscopic data indicated that the three isomeric aminopyrene adducts favor anti-glycosyl conformations, with C2'-*endo* (*S*) sugar puckering and a nearly planar conformation at the central amine nitrogen. The data further indicated differences in the extent of  $\pi$ -electron conjugations about the pyrenyl–nitrogen bond, depending on the location of aminopyrene substitution. Thus while the 1- and 4-isomers both have substitution adjacent to a fused aromatic ring, the 2-isomer is highly symmetric and less sterically hindered. The 2-isomer adopts the most planar conformation, thereby having the most efficient  $\pi$ -electron delocalization between the guanine and pyrene rings. The isomeric dG-C8-AP adducts and their nitro and amino precursors display physicochemical properties (HPLC retention time, UV pattern, <sup>1</sup>H NMR data, mass fragmentation, etc.) distinctly dependent on their structures (1- and 4-isomers versus 2-isomer).

## Introduction

Nitropyrenes are an important class of pollutants that exist in various environmental sources, including certain occupational environments (1). Some of these compounds are potent bacterial mutagens and rodent carcinogens, posing some concerns to human health (2, 3). 1-Nitropyrene (1-NP; Chart 1) is among the most abundant in the environment and has been studied extensively (2–12). There are two additional isomers of mononitropyrene: i.e., 2- and 4-nitropyrene (2-NP and 4-NP; Chart 1). Unlike the ubiquitous 1-NP, 2-NP is detected only in the atmospheric environment and is believed to be formed by a free radical process (13). 4-NP has been detected in both air (14) and diesel soot extracts (15), even though its level is much lower than those of 1- and 2-NP.

Despite the structural similarity, the three isomeric NPs differ markedly in their mutagenic and carcinogenic activities (16–24). While the relative order of bacterial mutagenicities in *Salmonella typhimurium* strains TA1538 and TA98 was 1-NP  $\ll$  2-NP  $<$  4-NP (18–20) in both the presence and absence of rat liver supernatant, 2-NP was found to be inactive without the supernatant in a TM677 forward mutation assay (16). 2-NP was also nonmutagenic in a forward mutation assay using a metabolically competent human cell line (MCL-5) (17). A similar order

Chart 1. Structures of *n*-Nitropyrene (*n*-NP), *n*-Aminopyrene (*n*-AP), and *N*-(Deoxyguanosin-8-yl)-*n*-aminopyrene (dG-C8-*n*-AP, *n* = 1, 2, 4)



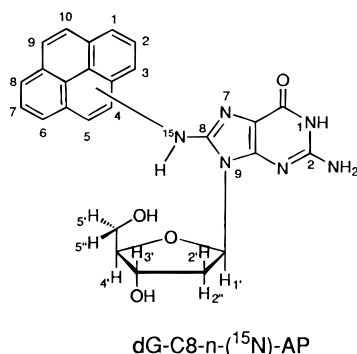
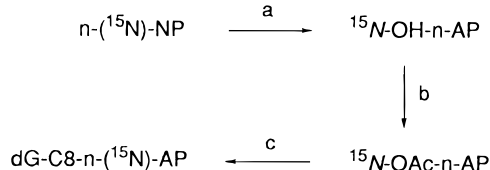
of carcinogenicities has been observed following direct administration of each isomer into the mammary pads of weanling female CD rats (20). 4-NP is more tumorigenic than 1-NP in newborn female rats (21) and in the livers and lungs of newborn male mice (24). The biological diversities observed for these NPs may presumably be due to differences in their structures and metabolism.

NPs undergo in vivo activation through either nitro reduction or ring oxidation, or a combination of both, generating a complex array of metabolites (2–6, 10, 25–31). Studies have shown that both 1- and 2-NP are metabolized, via the nitro-reduction route, into *N*-hy-

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**Scheme 1. Synthesis and Structure of [<sup>15</sup>N](Deoxyguanosin-8-yl)-*n*-aminopyrene [dG-C8-*n*-(<sup>15</sup>N)-AP, *n* = 1, 2, 4]<sup>a</sup>**



<sup>a</sup> (a)  $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ , Pd/C, THF, 0 °C; (b)  $\text{CH}_3\text{COCN}$ ,  $\text{NEt}_3$ , THF, -45 °C; (c) dG,  $\text{H}_2\text{O/THF}$ , 37 °C.

droxylamines, which after further enzymatic activation, produce C8-substituted (deoxyguanosinyl)aminopyrene (dG-C8-AP) derivatives as major adducts (4, 10, 26, 27). Some minor adducts, in which the pyrene ring is attached to either the N<sup>2</sup>-position of dG (10) or the C8-position of dA (26, 27), have also been isolated. Upadhyaya et al. (28) have shown that ring oxidation is the major metabolic route for the strong carcinogen 4-NP in rat liver microsomes (28). In vivo, however, 4-NP is metabolized by both ring oxidation and nitro reduction. They have also shown that excretion of 1- and 2-NP and their metabolites in rats occurs mainly via the intestinal tract, while the major portion (40%) of the 4-NP dose was found in the urine. While the patterns of fecal and urinary excretion and metabolism of the three NPs are similar, 4-NP exhibits a higher level of DNA binding affinity in mammary tissues in vivo than 1- or 2-NP (30, 31). These results may account for the higher carcinogenic activity of 4-NP. While no dG-C8-4-AP adduct has yet been isolated (30, 31), the possible existence of deoxyinosine-based adducts in rats treated with 4-NP has been suggested (30).

We report here the syntheses and comparative conformation analyses of three C8-deoxyguanosinyl adducts derived from the isomeric NPs (Scheme 1). The three dG-C8-AP adducts are structurally identical, except for the position (1, 2, and 4) of substitution in the pyrene ring, providing a unique opportunity for the systematic probing of isomeric adduct conformations. The C8-amine nitrogens of these adducts have been enriched with <sup>15</sup>N to facilitate the study of the conformation about the central nitrogen that links the guanine base and the pyrene moiety. Spectroscopic data (UV, <sup>1</sup>H NMR, mass) have been collected and analyzed in order to understand the conformational basis for their diverse mutagenic and carcinogenic activities.

## Experimental Procedures

**Warning:** Mononitropyrenes (NP) and their amino derivatives are potential carcinogens and therefore should be handled with care.

**Chemicals.** 1-Nitropyrene, 1-aminopyrene, 1,2,3,6,7,8-hexahydropyrene, and pyrene were purchased from Aldrich Chemical Co. (Milwaukee, WI). 2'-Deoxyguanosine was obtained from Sigma Chemical Co. (St. Louis, MO).  $\text{Na}^{15}\text{NO}_3$  was obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA). HPLC grade solvents and other reagent grade solvents were purchased from Fisher Scientific, Inc. (Pittsburgh, PA).

**Instrumentation.** Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F<sub>254</sub> plastic plates (E. Merck, Darmstadt, Germany). Unless otherwise indicated, a 1:9 mixture of ethyl acetate and hexanes was used as a TLC eluent system. HPLC data were obtained on a Waters Associates system equipped with two 501 pumps, a U6K injector, a 680 automated gradient controller, and a Hitachi L-3000 photodiode array detector. HPLC separations were conducted using one of the following systems: system 1—*isocratic* 90% methanol/10% water (1 mL/min), Ultrasphere 5- $\mu\text{m}$  C<sub>18</sub> ODS analytical column (4.6 × 250 mm) (Beckman Instruments, Inc., San Ramon, CA); system 2—20-min linear gradient of 25–60% acetonitrile/0.1 M ammonium acetate (pH 7.0) (2 mL/min), Ultrasphere 5- $\mu\text{m}$  C<sub>18</sub> ODS semipreparative column (10.0 × 250 mm); system 3—20-min linear gradient of 60–90% methanol/water (2 mL/min), same semipreparative column. A model SK-14D Stir-Kool apparatus (Thermoelectrics Unlimited Inc., Wilmington, DE) was used as a cooling bath.

<sup>1</sup>H and <sup>15</sup>N NMR spectra were recorded on a Bruker DPX400 NMR spectrometer, operating at 400.1 and 40.6 MHz, respectively. The <sup>1</sup>H chemical shifts are reported in ppm downfield from internal tetramethylsilane (TMS). The <sup>15</sup>N chemical shifts are reported in ppm downfield from anhydrous ammonia, by assigning the external  $\text{Na}^{15}\text{NO}_3$  reference to 376.53 ppm. Electron impact mass spectra (EI-MS) were measured on a 70-VSE instrument at the Mass Spectrometry Laboratory, University of Illinois (Urbana–Champaign, IL). Electrospray mass spectra of the adducts were obtained on a Micromass VG Quattro triple quadrupole mass spectrometer equipped with a VG electrospray source (Department of Chemistry, Northeastern University, Boston, MA). UV spectra were recorded on a Hitachi U-2000 spectrophotometer. The IR spectrum was recorded on a Perkin-Elmer 1600 series FTIR instrument. Elemental analyses were performed by M-H-W Laboratories (Phoenix, AZ).

**Syntheses. 1-<sup>15</sup>NO<sub>2</sub>-pyrene.** Sodium nitrate ( $\text{Na}^{15}\text{NO}_3$ ) (0.172 g, 2 mmol) was added slowly over the period of 30 min to a solution of pyrene (0.404 g, 2 mmol) in trifluoroacetic acid (TFA; 15 mL) and acetic anhydride (25 mL) (32). The resulting dark-green solution was stirred at ambient temperature for 1.5 h. The reaction mixture was treated with crushed ice and 2 mL of  $\text{H}_2\text{SO}_4$  and stirred overnight to destroy excess acetic anhydride. The crude product was filtered, washed with water, and air-dried. Column chromatography on silica gel using ethyl acetate/hexanes as eluent afforded the pure product as a yellow solid (0.18 g, 36%): TLC  $R_f$  0.33; HPLC  $t_R$  6.59 min (system 1); UV  $\lambda_{\text{max}}$  235, 290 nm; EI-MS  $m/z$  248 ( $\text{M}^+$ , 99), 201 ( $\text{Py}^+$ , 100), 189 (43), 100 (33); <sup>1</sup>H NMR ( $\text{CDCl}_3$ )  $\delta$  8.97 (d,  $J_{9,10} = 9.5$  Hz, 1H, H10), 8.72 (dd,  $J_{2,3} = 8.5$  Hz,  $J_{1^5\text{N},\text{H}2} = 2.0$  Hz, 1H, H2), 8.38 (m, 3H, H6,8,9), 8.30 (d,  $J_{4,5} = 8.9$  Hz, 1H, H5), 8.23 (d, 1H, H4), 8.17 (m, 2H, H3,7).

**2-<sup>15</sup>NO<sub>2</sub>-pyrene.** This was prepared by the general literature procedure (32–34). 4,5,9,10-Tetrahydropyrene was obtained by catalytic hydrogenation of pyrene with 10% Pd/C in ethyl acetate (34). The hydrogenation was repeated until <sup>1</sup>H NMR of the mixture indicated the presence of <5% 4,5-dihydropyrene (– $\text{CH}_2\text{CH}_2$ –, 3.35 ppm). Column chromatography on silica gel using a mixture of hexanes as eluent yielded the pure product as a white solid (yield ~50%): <sup>1</sup>H NMR  $\delta$  2.90 (s, 8H, H4,5,9,10), 7.05–7.15 (m, 6H, H1,2,3,6,7,8); TLC (hexane)  $R_f$  0.39; HPLC (methanol, *isocratic*)  $t_R$  5.41 min (tetrahydropyrene), 5.58 min (dihydropyrene), 6.44 min (pyrene); UV  $\lambda_{\text{max}}$  (tetrahydro)

280 nm, (dihydro) 300 nm. The nitration of 4,5,9,10-tetrahydropyrene with  $\text{Na}^{15}\text{NO}_3/\text{TFA}$ -acetic anhydride was complete in 3 h with stirring at room temperature. The excess acetic anhydride in the reaction mixture was hydrolyzed by stirring overnight with crushed ice and  $\text{H}_2\text{SO}_4$ . The product was extracted with dichloromethane. The combined organic extracts were sequentially washed with 1 N NaOH, saturated  $\text{NaHCO}_3$ , and brine and dried over anhydrous  $\text{MgSO}_4$ : TLC  $R_f$  0.44. The crude 2-nitro-4,5,9,10-tetrahydropyrene was refluxed with 4 equiv of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dry benzene for 20 h. The hot mixture was poured onto a neutral aluminum oxide column, which, after recrystallization from dichloromethane and hexanes, afforded pure 2- $^{15}\text{NO}_2$ -pyrene as yellow needles (59%): TLC  $R_f$  0.37; HPLC (system 1)  $t_R$  8.01 min; UV  $\lambda_{\text{max}}$  293 nm; EI-MS  $m/z$  248 ( $\text{M}^+$ , 80), 201 ( $\text{Py}^+$ , 100), 189 (13), 100 (25);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  9.01 (d,  $J_{\text{N,H1}} = J_{\text{N,H3}} = 2.0$  Hz, 2H, H1,3), 8.31 (d,  $J_{6,7} = J_{7,8} = 7.6$  Hz, 2H, H6,8), 8.20 (m, 5H, H4,5,7,9,10). Anal. Calcd for  $\text{C}_{16}\text{H}_9^{15}\text{NO}_2$ : C, 77.41; H, 3.65;  $^{15}\text{N}$ , 6.04. Found: C, 77.20; H, 4.00;  $^{15}\text{N}$ , 5.89.

**4- $^{15}\text{NO}_2$ -pyrene.** This was synthesized by nitration of 1,2,3,6,7,8-hexahydropyrene with  $\text{Na}^{15}\text{NO}_3/\text{TFA}$ -acetic anhydride followed by DDQ aromatization (33). Recrystallization from dichloromethane and hexanes gave the product as yellow crystals (40%): TLC  $R_f$  0.24; HPLC  $t_R$  6.95 min (system 1); UV  $\lambda_{\text{max}}$  235, 313 nm; EI-MS  $m/z$  248 ( $\text{M}^+$ , 93), 201 ( $\text{Py}^+$ , 100), 189 (54), 100 (31);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.94 (d,  $J_{2,3} = 8.43$  Hz, 1H, H3), 8.93 (d,  $J_{\text{N,H5}} = 2.8$  Hz, 1H, H5), 8.39 (d,  $J_{6,7} = J_{7,8} = \sim 7.8$  Hz, 2H, H6,8), 8.35 (d,  $J_{1,2} = 7.6$  Hz, 1H, H1), 8.19 (d, 1H, H2), 8.14 (m, 3H, H7,9,10). Anal. Calcd for  $\text{C}_{16}\text{H}_9^{15}\text{NO}_2$ : C, 77.41; H, 3.65;  $^{15}\text{N}$ , 6.04. Found C, 77.60; H, 4.00;  $^{15}\text{N}$ , 5.84.

**$n$ - $^{15}\text{NH}_2$ -pyrene ( $n = 1, 2, 4$ ).** The three isomeric aminopyrenes were prepared by hydrazine reduction of the corresponding  $n$ - $^{15}\text{NO}_2$ -pyrene ( $n = 1, 2, 4$ ) in average yields of 80–90%:  $n$ -Nitropyrene (40 mg) was dissolved in 15 mL of 95% ethanol containing hydrazine hydrate (50  $\mu\text{L}$ ) and 10% Pd/C (30 mg) and refluxed for 1 h. The reaction mixture was filtered through a Celite bed and evaporated to dryness. The residue was suspended in water and extracted with ether. The combined ether extracts were washed with water and dried over anhydrous  $\text{MgSO}_4$ . Silica gel column chromatography (ethyl acetate/hexanes) afforded the pure product: HPLC (system 1)  $t_R$  4.29 min (1-AP), 3.80 min (2-AP), 4.29 min (4-AP); UV  $\lambda_{\text{max}}$  243, 283 nm (1-AP), 263 nm (2-AP), 240, 285 nm (4-AP). The three APs exhibited essentially the same EI-MS fragmentation:  $m/z$  218 ( $\text{M}^+$ , 100), 189, 109.

**Synthesis of the  $N$ -(Deoxyguanosin-8-yl)- $n$ -aminopyrene (dG-C8- $n$ -AP,  $n = 1, 2, 4$ ) Adducts.**  $n$ -NP  $\rightarrow$   $N$ -OH- $n$ -AP. [ $^{15}\text{N}$ ]Hydroxylaminopyrenes were prepared by the general procedure of Westra et al. (35): Typically,  $n$ -nitropyrene (50 mg) in 25 mL of dry THF was cooled to 0  $^\circ\text{C}$  under argon atmosphere, and 30 mg of 10% Pd/C was added with stirring. Hydrazine monohydrate (98%) (50  $\mu\text{L}$ ) was added in a dropwise fashion and the mixture stirred for 2–4 h at the same temperature. It was necessary to add additional amounts of hydrazine monohydrate (10–30  $\mu\text{L}$ ) to complete the reduction. When the reduction was complete as judged by HPLC, the reaction mixture was filtered through a Celite bed under argon atmosphere and the solvent evaporated in a rotary evaporator while maintaining the bath temperature below 30  $^\circ\text{C}$ . The residue was then partitioned between water and ether. The ether layer was washed with cold water and dried over anhydrous  $\text{MgSO}_4$ . While [ $^{15}\text{N}$ ]hydroxy-2-aminopyrene could be isolated via a regular aqueous workup procedure, its 1- and 4-isomers were too unstable to be isolated under the same conditions. Therefore, the filtered THF solutions of the 1- and 4-isomers were used without isolation for the subsequent adduction step.

**$N$ -OH- $n$ -AP  $\rightarrow$   $N$ -OAc- $n$ -AP.** The [ $^{15}\text{N}$ ]hydroxylaminopyrenes were converted to their  $O$ -acetyl derivatives by the general method of Lobo et al. (36): A 1.1 equiv of acetyl cyanide was added via a syringe to a dry THF solution containing [ $^{15}\text{N}$ ]hydroxy- $n$ -aminopyrene and 1.1 equiv of triethylamine at  $-45$   $^\circ\text{C}$ . The reaction was complete within 15–30 min as indicated

by HPLC. The unstable  $O$ -acetyl derivatives were kept in solution at  $-45$   $^\circ\text{C}$  and used immediately for the adduct syntheses. The 2-isomer, however, was stable enough to be isolated and revealed a carbonyl stretching at 1796  $\text{cm}^{-1}$  in the IR spectrum, which is evidence for the  $O$ -acetyl structure.

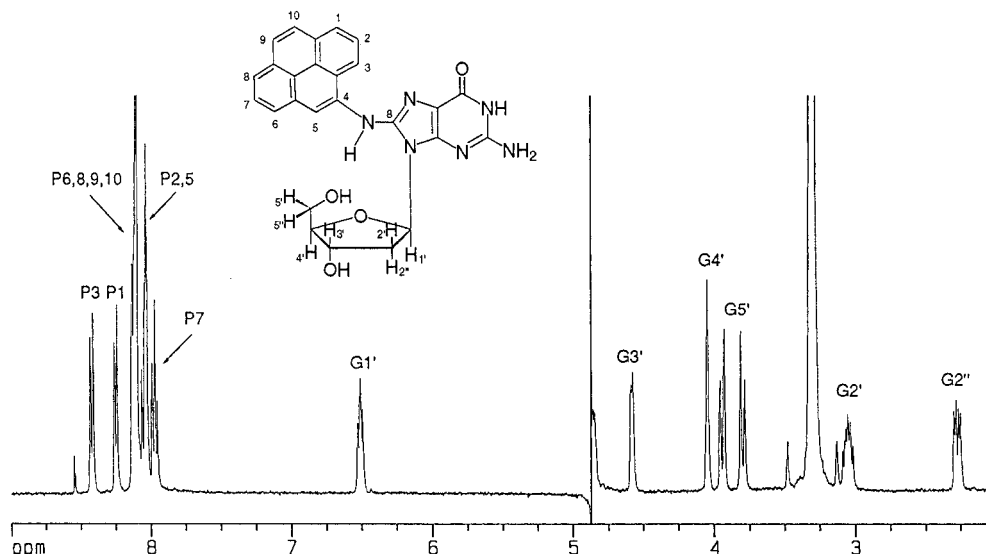
**$N$ -OAc- $n$ -AP  $\rightarrow$  dG-C8- $n$ -AP.** A solution of dG (40 mg) in 5 mL of water was added to a THF solution of  $N$ -acetoxy- $n$ -aminopyrene, prepared as described above, at  $-45$   $^\circ\text{C}$  under argon atmosphere. The reaction mixture was stirred at 37  $^\circ\text{C}$  for 15–20 h. After evaporation to dryness, the residue was partitioned between water and ether. The aqueous layer was extracted with ether several times. The aqueous layer contained only unreacted dG, while the adduct and other pyrene derivatives were found in the ether layer. The pure dG-C8- $n$ -AP adducts were isolated from reversed-phase HPLC using system 2 followed by system 3. The average overall yields from the corresponding NP were 1–3%. The structures of the adducts were established on the basis of their HPLC, UV, mass, and NMR spectra (see Results and Discussion: Characterization of the dG-C8-AP Adducts). dG-C8-( $^{15}\text{N}$ )- $n$ -AP: HPLC  $t_R$  (system 2) 16.80 min ( $n = 1$ ), 18.61 min ( $n = 2$ ), 16.87 min ( $n = 4$ ); UV  $\lambda_{\text{max}}$  240, 273 nm ( $n = 1$ ), 300 nm ( $n = 2$ ), 240, 273 nm ( $n = 4$ ); electrospray MS  $m/z$  484 ( $[\text{M} + \text{H}]^+$ ), 368 ( $[\text{BH}_2]^+$ ), 201 ( $\text{Py}^+$ ). See Figures 1 and 2c for representative  $^1\text{H}$  NMR and UV spectra, respectively. See Table 1 for the detailed  $^1\text{H}$  NMR data.

Nonlabeled dG-C8- $n$ -AP adducts were prepared similarly starting from the corresponding NPs and characterized on the basis of HPLC, UV, mass, and NMR spectral data.

## Results and Discussion

**Synthesis of the dG-C8-AP Adducts.** The C8-substituted dG adducts (dG-C8-1-AP and dG-C8-2-AP; Scheme 1) from 1- and 2-NP have previously been prepared by reaction of calf thymus DNA with the corresponding  $N$ -hydroxylamine followed by enzymatic hydrolysis of the modified DNA and subsequent HPLC purification (4, 26, 27). The synthesis of the 4-isomer (dG-C8-4-AP), which has not been reported, is described below.

Although several synthetic approaches are available in the literature, the strategies involving the reaction of dG with  $N$ -acetoxy- $N$ -(trifluoroacetyl) (37) or  $N$ -acetoxy derivatives of arylamines (36) have been used most extensively for the preparation of dG-C8-arylamine adducts (36–45). Examples include those derived from the strong carcinogens 4-aminobiphenyl (ABP) (37, 38, 40) and 2-aminofluorene (AF) (38, 41) at both the monomer and oligomer levels. We concentrated on the  $N$ -acetoxy procedure, in view of a report by Vyas et al. (46) that the  $N$ -acetoxy- $N$ -(trifluoroacetyl) derivative of 1-aminopyrene is too unstable to be employed in the adduction step. The  $N$ -acetoxy procedure (36) entails a reduction of the nitro precursor to the hydroxylamine followed by a selective  $O$ -acetylation and reaction with dG (Scheme 1). We prepared [ $^{15}\text{N}$ ]hydroxy-4-AP by reduction of 4-( $^{15}\text{N}$ )NP with hydrazine and converted it to [ $^{15}\text{N}$ ]acetoxy-4-AP by the addition of 1.1 equiv of acetyl cyanide in the presence of triethylamine in THF at  $-45$   $^\circ\text{C}$  (Scheme 1). This conversion took place smoothly within 20 min, as monitored by HPLC. Due to its instability, the freshly prepared  $N$ -acetoxy-4-AP was reacted immediately with dG. After partitioning between water and ether, the desired adduct was found mostly in the ether phase, along with solvolysis products of  $N$ -acetoxy-4-AP, which reflects the hydrophobic nature of the adduct. Pure dG-C8-4-AP was obtained after extensive purification on reversed-phase HPLC.



**Figure 1.**  $^1\text{H}$  NMR spectrum (400.1 MHz) of dG-C8-4-AP in methanol- $d_4$ . The HOD resonance at 4.9 ppm was presaturated with a weak 1-s pulse during the recycle delay. P, pyrene; G, 2'-deoxyguanosine.

The  $^{15}\text{N}$ -enriched 1- and 2-isomer adducts were prepared similarly, starting from the corresponding nitro precursors (Scheme 1). After HPLC, the overall average yields of dG-C8-AP via the *N*-acetoxy procedure after HPLC purification were in the range of 1–3%. The use of pivaloyl cyanide (45) or acetylsalicylic acid (47) as selective *O*-acetylation reagents did not improve the yields.

**Characterization of the dG-C8-AP Adducts.** The structure of dG-C8-4-AP was established on the basis of the chromatographic, NMR, UV, and MS data. The HPLC retention time (system 2, see Experimental Procedures) observed for dG-C8-4-AP (16.87 min) was very similar to that of the 1-isomer (16.80 min) but is significantly different from that of the 2-isomer (18.61 min). These results reflect a structural similarity between the 4- and 1-isomers (vide infra). The  $^1\text{H}$  NMR spectrum of dG-C8-4-AP, recorded in methanol- $d_4$  (Figure 1), showed seven proton signals at 2.3–6.5 ppm which is characteristic of the deoxyribose ring. All the aromatic resonances of the 4-substituted pyrene moiety were accounted for, while the guanine H8 was missing, thus indicating a C8-substitution (Table 1). The pyrene H5 proton was shifted downfield considerably (0.84 ppm) relative to that of 4-aminopyrene, which is in good agreement with the position (C4) of amino attachment. Upon similar conversions, deshieldings of comparable magnitude (0.70 and 1.09 ppm) were noted for the H2 and H1,3 protons of the 1- and 2-isomers, respectively (Table 1). The UV spectral pattern of dG-C8-4-AP resembled closely that of the 1-isomer but differed considerably from that of the 2-isomer (Figure 2c), reflecting differences in their  $\pi$ -electronic structure (vide infra). The structural identities of the corresponding 1- and 2-isomers (dG-C8-1-AP and dG-C8-2-AP) were confirmed by matching their UV and  $^1\text{H}$  NMR data with those of the nonlabeled analogues reported previously (Table 1) (4, 26).

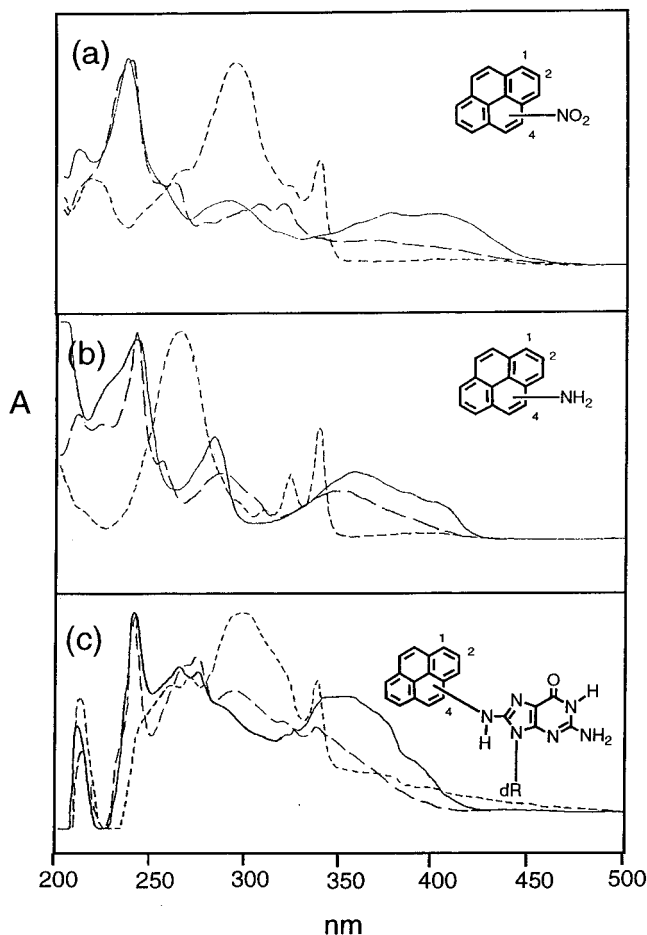
The  $^1\text{H}$  NMR spectra of the three dG-C8-AP adducts in DMSO- $d_6$  revealed the splitting ( $^1J_{\text{N-H}} = -90.3$  to  $-92.3$  Hz) of the  $\text{N}^{\text{H}}$  proton, expected from  $^{15}\text{N}$ -enrichment at the central nitrogen (Table 1). Figure 3 shows a typical example of such coupling, measured from both

**Table 1.**  $^1\text{H}$  NMR Data: Chemical Shifts (ppm) and Coupling Constants (Hz) for the dG-C8-*n*-AP Adducts and the Corresponding *n*-APs<sup>a</sup>

assignment	dG-C8-1-AP <sup>b</sup>	1-AP <sup>c</sup>	dG-C8-2-AP <sup>b</sup>	2-AP <sup>c</sup>	dG-C8-4-AP <sup>b</sup>	4-AP <sup>c</sup>	dG <sup>b</sup>
G1'	6.56		6.55		6.53		6.27
G2'	3.02		2.82		3.07		2.69
G2''	2.29		2.21		2.29		2.35
G3'	4.59		4.65		4.60		4.52
G4'	4.09		4.07		4.06		4.00
G5' <sup>d</sup>	3.95		4.06		3.96		3.80
G5'' <sup>d</sup>	3.81		3.98		3.82		3.72
$J_{1',2'}$	9.1		9.2		9.1		
$J_{1',2''}$	5.9		6.0		5.9		
$J_{2',2''}$	-13.4		-13.2		-13.5		
$J_{2',3'}$	6.1		6.2		6.3		
$J_{2',3''}$	2.0		1.7		1.8		
$J_{3',4'}$	2.4		3.1		2.4		
$J_{4',5'}$	2.8		2.8		2.6		
$J_{4',5''}$	2.1		1.7		1.9		
$J_{5',5''}$	-11.6		-11.8		-11.7		
P1			8.54	7.43	8.27	8.26	
P2	8.05	7.35			8.06	8.03	
P3	8.17	7.71	8.54	7.43	8.44	8.48	
P4	8.04	7.89	8.04	7.98			
P5	8.00	7.89	8.04	7.88	8.05	7.21	
P6	8.15	7.97	8.15	8.10	8.15	8.09	
P7	7.99	7.89	7.92	7.82	7.99	7.88	
P8	8.17	7.97	8.15	8.10	8.13	8.09	
P9	8.07	7.97	8.04	7.88	8.12	7.90	
P10	8.25	8.25	8.04	7.98	8.12	7.90	
$J_{1,2}$					7.9		
$J_{2,3}$	8.1				7.9		
$J_{1,3}$			2.0				
$J_{4,5}$	8.9		9.0				
$J_{6,7}$	7.7		7.6		7.7		
$J_{7,8}$	7.7		7.6		7.7		
$J_{9,10}$	9.3		9.0		7.6		
$^{15}\text{N-H}^c$	8.97	6.33	9.17	5.70	8.88	6.07	
$^1J_{\text{N-H}}^c$	-90.8	-85.3	-92.3	-83.8	-90.3	-83.6	

<sup>a</sup> The  $^1\text{H}$  NMR spectra data for dG-C8-1-AP and dG-C8-2-AP in methanol- $d_4$  were in accord with published data (4, 26). <sup>b</sup> In methanol- $d_4$ . <sup>c</sup> In DMSO- $d_6$ . <sup>d</sup> Assignments may be reversed.

$^1\text{H}$  and  $^{15}\text{N}$  NMR spectra of dG-C8-( $^{15}\text{N}$ )-2-AP. Electro-spray mass spectral data (48) were also in agreement with the assigned structures. Briefly, the spectra exhibited fragmentation at  $m/z$  484 and 368, which is consistent with  $[\text{M} + \text{H}]^+$  and loss of the deoxyribose moiety, respectively. The nonlabeled analogues displayed essentially the same fragmentations, except that they were 1 mass unit lower, again confirming the  $^{15}\text{N}$ -enrichment at the central nitrogen (48).



**Figure 2.** UV spectra of (a) *n*-[<sup>15</sup>N]nitropyrene (*n*-NP), (b) *n*-[<sup>15</sup>N]aminopyrene (*n*-AP), and (c) [<sup>15</sup>N](deoxyguanosin-8-yl)-*n*-aminopyrene (dG-C8-*n*-AP, *n* = 1, 2, 4) measured in methanol (—, 1-isomer; ---, 2-isomer; - · -, 4-isomer).

**Conformational Analysis. 1. Glycosyl Conformation.** Among the NMR approaches for determining the dynamic *anti-syn*-glycosyl conformation of C8-substituted purine nucleosides or nucleotides, the chemical shift of the sugar H2' is regarded as a simple indicator (49, 50). In general, deshielding of the H2' resonance relative to dG is considered as stemming from an increased population of *syn* conformers. The close proximity of the H2' sugar proton to the guanine N3 atom in the *syn* conformation is believed to be responsible for the downfield shift.

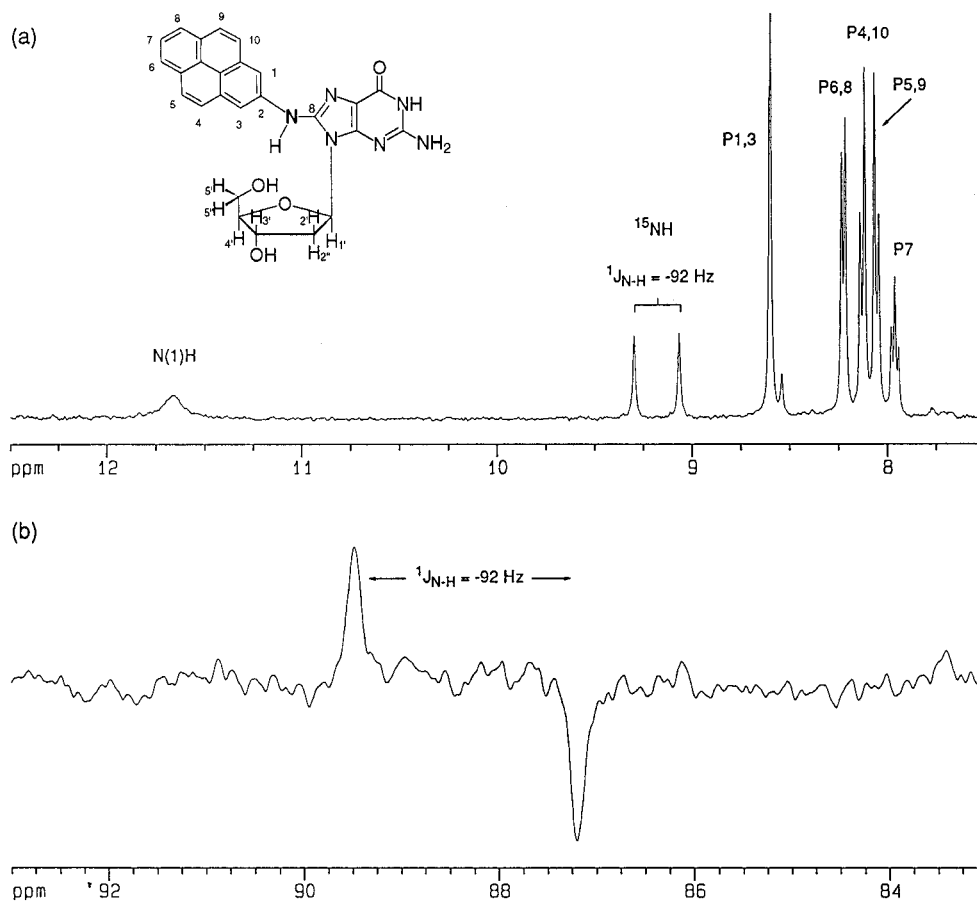
Compared to dG, the H2' resonances of the AP adducts were generally deshielded in both methanol (0.13–0.38 ppm) (Table 1) and DMSO (0.06–0.37 ppm) solutions. Downfield shifts of similar magnitude have been observed in DMSO solutions of the *N*-(deoxyguanosin-8-yl)-2-(acetylamino)fluorene adduct (dG-C8-AAF; 0.47 ppm) (51) and other dG-C8-arylamine adducts and associated with a higher *syn* population (44, 45, 52). This observation suggests that the AP adducts may favor *syn* conformations. However, it has been shown that dG-C8-AF, a structural analogue of dG-C8-AP, adopts an *anti* conformation by forming an intramolecular hydrogen bond involving N<sup>8</sup>H and O5' (51). The occurrence of a similar intramolecular interaction has been noted in a number of this type of dG-C8-arylamine adducts (44, 45, 52). Since both the AF and AP adducts have the free amine proton function (N<sup>8</sup>H), the possibility of an *anti* conformation for the AP adducts should be sought. Inspection

of the dG-C8-AP adducts in an *anti* arrangement (Figure 4) indicated that their H2' sugar protons may reside in a deshielding region of the strongly anisotropic pyrene ring. Curiously, the H2' resonances of the 1- and 4-isomers were deshielded considerably more (0.33 and 0.38 ppm, respectively) than that of the 2-isomer (0.13 ppm). This observation is consistent with the position of substitution on the pyrene ring, i.e., the 1- and 4-isomers both have amino substitution adjacent to a fused aromatic ring, whereas the 2-isomer does not. As a result, the pyrene ring of dG-C8-2-AP is shifted upward, away from the sugar moiety, such that its H2' proton becomes less susceptible to the ring current effects. Nolan et al. (12) have shown that the CD spectral transition profiles of dG-C8-1-AP from dG are quite similar to those of 8-(methylamino)guanosine, which adopts a higher *anti* conformation (53). Taken together, the available data are consistent with high *anti* conformations for the dG-C8-AP adducts.

The preferred *anti* conformation of dG-C8-AP is in good agreement with other structurally related dG-C8-arylamine adducts, such as those derived from 2-aminofluorene (51), 4-aminobiphenyl (52), and methylated anilines (44, 45). Our NMR measurements were made in polar organic solvents (DMSO and methanol); therefore, the relative *anti/syn* population can change in aqueous solution where the hydrogen-bonding patterns may be quite different. Mao et al. (11) have shown that the dG-C8-1-AP adduct opposite dC in an 11-mer DNA duplex adopts a *syn* conformation with the aminopyrene ring intercalated into the helix. This base displacement structure appears to be accommodated by a strong pyrene–base stacking at the duplex level. It is also worth mentioning that the H2' proton of *syn*-dG-C8-1-AP:dC of the above-mentioned duplex is unusually deshielded (3.51 ppm) relative to the analogous resonances (2.1–2.6 ppm) of the unsubstituted dGs in aqueous solution (11). Downfield shifts of similar magnitude have also been detected at the H2' protons (3.11–3.68 ppm) of the AF-modified dG adducts, regardless of the glycosyl conformation: *syn*-dG-C8-AF opposite dA (54), dI (55), dG (55), and –1 (56) and –2 (57) deletion sites or *anti*-dG-C8-AF opposite dC (58). In this regard, it should also be noted that the DNA-containing dG-C8-arylamine adducts [i.e., dG-C8-ABP (59), dG-C8-AF (58, 60)] adopt both *anti* and *syn* conformations and that their populations are modulated by the nature of the carcinogen (61) as well as the local DNA sequence surrounding the adduct site.

**2. Sugar Conformation.** Analysis of the Karplus dependence of the  $J_{1',2'}$  (9.1–9.2 Hz) and  $J_{3',4'}$  (2.4–3.1 Hz) coupling constants observed for the three isomeric AP adducts (Table 1) indicated a strong preference for the C2'-*endo* (*S*) sugar puckering (49, 62). A similar analysis of  $J_{4',5'}$  and  $J_{4',5''}$  (48, 50) showed a preference (81–85%) of *gauche-gauche* populations (*gg*) of the exocyclic C4'–C5' bond. A slightly higher *gg* population (>90%) has been observed for the low-energy conformer of dG-C8-AF, whose N<sup>8</sup>H forms a hydrogen bond with O5' (51).

**3. Conformation about the N<sup>8</sup>–C<sub>(py)</sub> Bond.** The nature of the carcinogen in dG-C8-arylamine adducts has been shown to be a key factor for determining the orientations of the guanine and aromatic rings with respect to the pyrenyl–nitrogen bond that connects the two ring systems (44, 45, 63). Three general conforma-



**Figure 3.** (a)  $^1\text{H}$  (400.1 MHz) and (b)  $^{15}\text{N}$  (40.6 MHz) NMR spectra of [ $^{15}\text{N}$ ](deoxyguanosin-8-yl)-2-aminopyrene (dG-C8-2-AP) in  $\text{DMSO}-d_6$ , showing the one-bond N–H splitting ( $^1J_{\text{N-H}} = -92.3$  Hz). The  $^{15}\text{N}$  NMR spectrum was acquired using refocused-INEPT pulse sequence. See Table 1 for details.

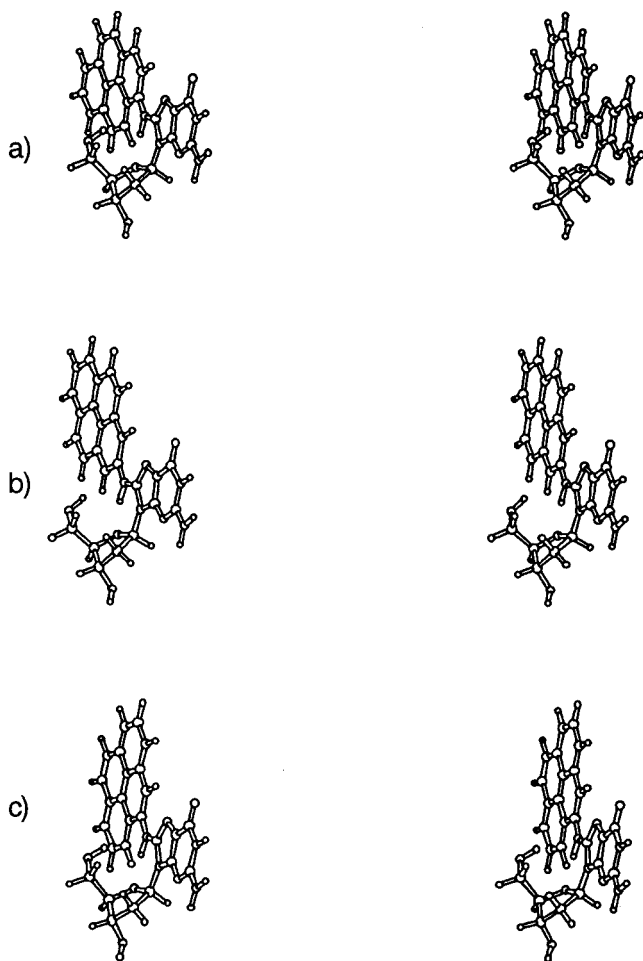
tions are possible, depending on the dihedral torsion angle  $\beta$  [ $-\text{C8}_{\text{dG}}-\text{N}^8-\text{C1}_{(\text{py})}-\text{C2}_{(\text{py})}$ ]: a perpendicular conformation ( $\beta = 90^\circ$  or  $270^\circ$ ), a planar conformation ( $\beta = 0^\circ$  or  $180^\circ$ ), and a helical conformation in which the  $\beta$  values are between the two extremes. The dG-C8-arylamine adducts derived from 2-aminofluorene and substituted aniline derivatives have been shown to adopt a helical conformation with a wide range of  $\beta$  values ( $20^\circ < \beta < 90^\circ$ ) (44, 45, 63).

Several lines of spectroscopic evidence support a nearly planar conformation of the dG-C8-AP adducts. The one-bond N–H coupling constants ( $^1J_{\text{N-H}} = -90.8$  to  $-92.3$  Hz, Table 1) observed for the AP adducts are similar to that ( $-90.4$  Hz) of diphenylamine but are significantly larger than those of the amino precursors ( $-83.1$  to  $-85.3$  Hz) (Table 1). These results strongly indicate a nonpyramidal configuration at the central amino nitrogen (63). The slightly larger  $^1J_{\text{N-H}}$  value ( $-92.3$  Hz) observed for the 2-isomer (Figure 3) is an indication of an additional flattening of the central nitrogen, possibly leading to more efficient  $\pi$ -electron delocalization. Support for this interpretation is also obtained from the comparison of chemical shift changes of the  $\text{N}^8\text{H}$  protons relative to those of the corresponding aminopyrene precursors. Such chemical shift changes can be regarded as an indicator for the relative extent of  $\pi$ -conjugation between the two aryl systems of the adducts. The 2-isomer exhibited an unusually large ( $\Delta 3.47$  ppm) deshielding of the  $\text{N}^8\text{H}$  proton as compared to the 1- and 4-isomers ( $\Delta 2.64$  and  $\Delta 2.81$  ppm, respectively). This can be interpreted as indicating that the  $\pi$ -electron conjugation in

the 2-isomer is the most efficient. In line with this argument, electrospray mass spectral studies showed that dG-C8-1-AP is the most susceptible to fragmentation followed by dG-C8-4-AP (48). dG-C8-2-AP was the most difficult adduct to be fragmented. It appears that the efficient  $\pi$ -electron interaction between the two ring systems is responsible for the relative resistance of the 2-isomer toward mass fragmentation.

The planar conformation [ $\beta' = \sim 180^\circ = \angle \text{C8}_{\text{dG}}-\text{N}^8-\text{C1}_{(\text{py})}-\text{C10A}_{(\text{py})}$ ] about the  $\text{N}^8-\text{C1}_{(\text{py})}$  bond of the monomeric dG-C8-1-AP adduct is not consistent with the analogous torsion angle ( $\beta' = 141^\circ$ ) observed in the same adduct opposite dC embedded in an 11-mer DNA duplex (11). This apparent discrepancy is probably due to a base displacement structure at the duplex, in which the aminopyrene ring is intercalated into the double helix.

**4. Structural Consequences of the Physicochemical Properties.** As mentioned in the previous section, the conformational characteristics of the dG-C8-AP adducts are in good agreement with their structural differences, namely, the existence of substituents adjacent to a fused aromatic ring in the 1- and 4-isomers and the highly symmetric and lower steric hindrance of the 2-isomer. The physicochemical properties of the dG-C8-AP adducts and their nitro, amino, and hydroxylamino precursors can also be grouped along the same way (i.e., 1- and 4-isomers versus 2-isomer). For example, 2-NP was shown to be unusually stable toward light as compared to 1- and 4-NP (64). While the [ $^{15}\text{N}$ ]hydroxy-2-AP could be isolated and purified using regular aqueous



**Figure 4.** Stereoviews of the *N*-(deoxyguanosin-8-yl)-*n*-aminopyrene adducts: (a) dG-C8-1-AP, (b) dG-C8-2-AP, and (c) dG-C8-4-AP, shown in anti conformations.

workup conditions, the corresponding 1- (2) and 4-isomers were too unstable to be treated by the same procedure and had to be used immediately for the subsequent adduction step. Similarly, by contrast with other isomers, [<sup>15</sup>N]acetoxy-2-AP was stable enough to allow recording of an IR spectrum, which provided a characteristic *O*-acetyl carbonyl stretching band at 1796 cm<sup>-1</sup>. Other physicochemical properties that fit into this classification include the HPLC retention times (see Experimental Procedures), the patterns of UV spectra (Figure 2), and the electrospray mass fragmentation (vide supra) (48).

### Conclusions

Spectroscopic data showed that the three isomeric AP adducts favor high *anti*-glycosyl conformations, with C2'-*endo* (*S*) sugar puckering and a nearly planar conformation at the central amine nitrogen. Among the three AP adducts, the 2-isomer exhibited the most efficient  $\pi$ -electron delocalization between the guanine and pyrene rings. The data further indicate that both the conformational and physicochemical properties of the AP adducts are consistent with their structural difference; i.e., while the 1- and 4-isomers both have substitution adjacent to a fused aromatic ring, the 2-isomer is highly symmetric and less sterically hindered (Figure 4).

The present study indicates that the nucleoside adduct structure per se cannot account for the diverse mu-

tagenic/carcinogenic activities of the three isomeric NPs. This is analogous to what has been observed with 1-NP and 1,6- and 1,8-dinitropyrene; the extreme mutagenicity (and carcinogenicity) of the dinitropyrenes does not appear to be due to their electron structure (65) or to unique properties of their adducts (2, 3) but rather to the very efficient activation of hydroxyamino intermediates to reactive electrophiles (i.e., acetylation by *O*-acetylases) (29, 66). Other possible factors for the difference in their carcinogenic potential include the modes of administration, excretion, metabolism, tissue susceptibility, and DNA binding affinities.

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### References

- (1) IARC. (1989) *Diesel and Gasoline Engine Exhausts and Some Nitroarenes*, Monograph on the Evaluation of the Carcinogenic Risks of Chemicals to Humans, IARC, Lyon, France.
- (2) Beland, F. A., and Marques, M. M. (1994) DNA adducts of nitropolycyclic aromatic hydrocarbons. In *DNA Adducts: Identification and Biological Significance* (Hemminki, K., Dipple, A., Shuker, D. E. G., Kadlubar, F. F., Segerback, D., and Bartsch, H., Eds.) pp 229–244, IARC Scientific Publications No. 125, IARC, Lyon, France.
- (3) Fu, P. P. (1990) Metabolism of nitro-polycyclic aromatic hydrocarbons. *Drug Metab. Rev.* **22**, 209–268.
- (4) Howard, P. C., Heflich, R. H., Evans, F. E., and Beland, F. A. (1983) Formation of DNA adducts in vitro and in *Salmonella typhimurium* upon metabolic reduction of the environmental mutagen 1-nitropyrene. *Cancer Res.* **43**, 2052–2058.
- (5) Howard, P. C., Beland, F. A., and Cerniglia, C. E. (1983) Reduction of the carcinogen 1-nitropyrene to 1-aminopyrene by rat intestinal bacteria. *Carcinogenesis* **4**, 985–990.
- (6) Heflich, R. H., Howard, P. C., and Beland, F. A. (1985) 1-Nitrosopyrene: An intermediate in the metabolic activation of 1-nitropyrene to a mutagen in *Salmonella typhimurium* TA1538. *Mutat. Res.* **149**, 25–32.
- (7) Malia, S. A., and Basu, A. K. (1995) Mutagenic specificity of reductively activated 1-nitropyrene in *Escherichia coli*. *Biochemistry* **34**, 96–104.
- (8) Vyas, R. R., and Basu, A. K. (1995) DNA polymerase action on an oligonucleotide containing a site-specifically located *N*-(deoxyguanosin-8-yl)-1-aminopyrene. *Carcinogenesis* **16**, 811–816.
- (9) Malia, S. A., Vyas, R. R., and Basu, A. K. (1996) Site-specific frame-shift mutagenesis by the 1-nitropyrene-DNA adduct *N*-(deoxyguanosin-8-yl)-1-aminopyrene located in the (CG)<sub>3</sub> sequence: Effects of SOS, proofreading, and mismatch repair. *Biochemistry* **35**, 4568–4577.
- (10) Herreno-Saenz, D., Evans, F. E., Beland, F. A., and Fu, P. P. (1995) Identification of two *N*<sup>2</sup>-deoxyguanosinyl DNA adducts upon nitroreduction of the environmental mutagen 1-nitropyrene. *Chem. Res. Toxicol.* **8**, 269–277.
- (11) Mao, B., Vyas, R. R., Hingerty, B. E., Broyde, S., Basu, A. K., and Patel, D. J. (1996) Solution conformation of the *N*-(deoxyguanosin-8-yl)-1-aminopyrene ([AP]dG) adduct opposite dC in a DNA duplex. *Biochemistry* **35**, 12659–12670.
- (12) Nolan, S. J., Vyas, R. R., Hingerty, B. E., Ellis, S., Broyde, S., Shapiro, R., and Basu, A. K. (1996) Solution properties and computational analysis of an oligodeoxynucleotide containing *N*-(deoxyguanosin-8-yl)-1-aminopyrene. *Carcinogenesis* **17**, 133–144.
- (13) Ramdhal, T., Zielinska, B., Arey, J. A., Atkinson, R., Winer, A. M., and Pitts, J. N., Jr. (1986) Ubiquitous occurrence of 2-nitrofluoranthene and 2-nitropyrene in air. *Nature* **321**, 425–427.
- (14) Korfmacher, W. A., Rushing, L. G., Arey, J., Zielinska, B., and Pitts, J. N., Jr. (1987) Identification of mononitropyrenes and mononitrofluoranthenes in air particulate matter via fused silica gas chromatography combined with negative ion atmospheric pressure ionization mass spectrometry. *J. High-Resolut. Chromatogr., Chromatogr. Commun.* **10**, 641–646.

- (15) Gallagher, J., Helnrich, U., George, M., Hendee, L., Phillips, D. H., and Lewtas, J. (1994) Formation of DNA adducts in rat lung following chronic inhalation of diesel emissions, carbon black and titanium dioxide particles. *Carcinogenesis* **15**, 1291–1299.
- (16) Busby, W. F., Jr., Smith, H., Bishop, W. W., and Thilly, W. G. (1994) Mutagenicity of mono- and dinitropyrenes in the *Salmonella typhimurium* TM677 forward mutation assay. *Mutat. Res.* **322**, 221–232.
- (17) Busby, W. F., Jr. Penman, B. W., and Crespi (1994) Human cell mutagenicity of mono- and dinitropyrenes in metabolically competent MCL-5 cells. *Mutat. Res.* **322**, 233–242.
- (18) Fu, P. P., Chou, M. W., Miller, D. W., White, G. L., Heflich, R. H., and Beland, F. A. (1985) The orientation of the nitro substituent predicts the direct-acting bacterial mutagenicity of nitrated polycyclic aromatic hydrocarbons. *Mutat. Res.* **143**, 173–181.
- (19) Yu, S., Heflich, R. H., Von Tungeln, L. S., El-Bayoumy, K., Kadlubar, F. F., and Fu, P. P. (1991) Comparative direct-acting mutagenicity of 1- and 2-nitropyrene: Evidence for 2-nitropyrene mutagenesis by both guanine and adenine adducts. *Mutat. Res.* **250**, 145–152.
- (20) Imaida, K., Hirose, M., Tay, L., Lee, M., Wang, C. Y., and King, C. M. (1991) Comparative carcinogenicities of 1-, 2-, and 4-nitropyrene and structurally related compounds in the female CD rat. *Cancer Res.* **51**, 2902–2907.
- (21) Imaida, K., Lee, M.-S., Land, S. J., Wang, C. Y., and King, C. M. (1995) Carcinogenicity of nitropyrenes in the newborn female rat. *Carcinogenesis* **16**, 3027–3030.
- (22) Hirose, M., Lee, M.-S., Wang, C. Y., and King, C. M. (1984) Induction of rat mammary gland tumors by 1-nitropyrene, a recently recognized environmental mutagen. *Cancer Res.* **44**, 1158–1162.
- (23) El-Bayoumy, K., Rivenson, A., Johnson, B., DiBello, J., Little, P., and Hecht, S. S. (1988) Comparative tumorigenicity of 1-nitropyrene, 1-nitrosopyrene, and 1-aminopyrene administered by gavage to Sprague–Dawley rats. *Cancer Res.* **48**, 4256–4260.
- (24) Wislocki, P. G., Bagan, E. S., Lu, A. Y. H., Dooley, K. L., Fu, P. P., Han-Hsu, H., Beland, F. A., and Kadlubar, F. F. (1986) Tumorigenicity of nitrated derivatives of pyrene, benz[a]anthracene, chrysene and benzo[a]pyrene in the newborn mouse assay. *Carcinogenesis* **7**, 1317–1322.
- (25) Upadhyaya, P., Roy, A. K., Fu, P. P., and El-Bayoumy, K. (1992) Metabolism and DNA binding of 2-nitropyrene in the rat. *Cancer Res.* **52**, 1176–1181.
- (26) Fu, P. P., Miller, D. W., Von Tungeln, L. S., Bryant, M. S., Lay, J. O., Jr., Huang, K., Jones, L., and Evans, F. E. (1991) Formation of C8-modified deoxyguanosine and C8-modified deoxyadenosine as major DNA adducts from 2-nitropyrene metabolism mediated by rat and mouse liver microsomes and cytosols. *Carcinogenesis* **12**, 609–616.
- (27) Roy, A. K., Upadhyaya, P., Fu, P. P., and El-Bayoumy, K. (1991) Identification of the major metabolites and DNA adducts formed from 2-nitropyrene in vitro. *Carcinogenesis* **12**, 475–479.
- (28) Upadhyaya, P., Von Tungeln, L. S., Fu, P. P., and El-Bayoumy, K. (1994) In vitro and in vivo metabolism of the carcinogen 4-nitropyrene. *Chem. Res. Toxicol.* **7**, 690–695.
- (29) Rosser, P. F., Ramachandran, P., Sangaiah, R., Austin, R. N., Gold, A., and Ball, L. M. (1996) Role of *O*-acetyltransferase in activation of oxidized metabolites of the genotoxic environmental pollutant 1-nitropyrene. *Mutat. Res.* **369**, 209–220.
- (30) Chae, Y.-H., Ji, B.-Y., Upadhyaya, P., Lin, J.-M., and El-Bayoumy, K. (1996) On the identification of DNA adducts derived from the environmental mammary carcinogen 4-nitropyrene in rats. *Proc. Am. Assoc. Cancer Res.* **37**, 120.
- (31) Chae, Y.-H., Upadhyaya, P., Ji, B.-Y., Fu, P. P., and El-Bayoumy, K. (1997) Comparative metabolism and DNA binding of 1-, 2-, and 4-nitropyrene in rats. *Mutat. Res.* **376**, 21–28.
- (32) Cho, B. P. (1995) Recent progress in the synthesis of nitropol-yrenes. A review. *Org. Prep. Proc. Int.* **27**, 243–272.
- (33) Miller, D. W., Herreno-Saenz, D., Huang, K. H., Heinze, T. M., and Fu, P. P. (1992) Synthesis of nitropolycyclic aromatic hydrocarbons with the substituent at the longest axis. *J. Org. Chem.* **57**, 3746–3748.
- (34) Fu, P. P., Lee, H. M., and Harvey, R. G. (1980) Regioselective catalytic hydrogenation of polycyclic aromatic hydrocarbons under mild conditions. *J. Org. Chem.* **45**, 2797–2803.
- (35) Westra, J. G. (1981) A rapid and simple synthesis of reactive metabolites of carcinogenic aromatic amines in high yield. *Carcinogenesis* **2**, 355–357.
- (36) Lobo, A. M., Marques, M. M., and Prabhakar, S. (1985) Tetrahedral intermediates formed during acyl transfer. Reaction of acetyl cyanide. *J. Chem. Soc., Chem. Commun.* 1113–1115.
- (37) Lee, M.-S., and King, C. M. (1981) New syntheses of *N*-(guanosin-8-yl)-4-aminobiphenyl and its 5'-monophosphate. *Chem.-Biol. Interact.* **34**, 239–248.
- (38) Marques, M. M., and Beland, F. A. (1990) Synthesis, characterization, and solution properties of *ras* sequences modified by arylamine carcinogens at the first base of codon 61. *Chem. Res. Toxicol.* **3**, 559–565.
- (39) Famulok, M., Bosold, F., and Boche, G. (1989) Synthesis of *N*-acetoxy-2-aminonaphthalene, an ultimate carcinogen of the carcinogenic 2-naphthylamine, and its in vitro reactions with (bio)nucleophiles. *Tetrahedron Lett.* **30**, 321–324.
- (40) Famulok, M., Bosold, F., and Boche, G. (1989) Synthesis of *O*-acetyl-*N*-(4-biphenyl)hydroxylamine ("*N*-acetoxy-4-aminobiphenyl"), an ultimate metabolite of carcinogenic 4-aminobiphenyl, and its reaction with deoxyguanosine. *Angew. Chem., Int. Ed. Engl.* **28**, 337–338.
- (41) Famulok, M., and Boche, G. (1989) Formation of *N*-(deoxyguanosin-8-yl)aniline in the in vitro reaction of *N*-acetoxyaniline with deoxyguanosine and DNA. *Angew. Chem., Int. Ed. Engl.* **28**, 468–469.
- (42) Bosold, F., and Boche, G. (1990) The ultimate carcinogen, *O*-acetyl-*N*-(2-fluorenyl)hydroxylamine ("*N*-acetoxy-2-aminofluorene"), and its reaction in vitro to form 2-[*N*-(deoxyguanosin-8-yl)amino]fluorene. *Angew. Chem., Int. Ed. Engl.* **29**, 63–64.
- (43) Meier, C., and Boche, G. (1990) *N*-Acetoxy-4-methoxyaniline, a model compound for the ultimate carcinogen of the phenacetin related 4-ethoxyaniline. *Tetrahedron Lett.* **31**, 1693–1696.
- (44) Meier, C., and Boche, G. (1991) The modification of guanine nucleosides and nucleotides by the borderline arylamine carcinogens 4-methyl- and 4-methoxyaniline: Chemistry and structural characterization. *Carcinogenesis* **12**, 1633–1640.
- (45) Marques, M. M., Mourato, L. L. G., Santos, M. A., and Beland, F. A. (1996) Synthesis, characterization, and conformational analysis of DNA adducts from methylated anilines present in tobacco smoke. *Chem. Res. Toxicol.* **9**, 99–108.
- (46) Vyas, R. R., Nolan, S. J., and Basu, A. K. (1993) Synthesis and characterization of oligodeoxynucleotides containing *N*-(deoxyguanosin-8-yl)-1-aminopyrene. *Tetrahedron Lett.* **34**, 2247–2250.
- (47) Minchin, R. F., Ilett, K. F., Teitel, C. H., Reeves, P. T., and Kadlubar, F. F. (1992) Direct *O*-acetylation of *N*-hydroxy arylamines by acetylsalicylic acid to form carcinogen-DNA adducts. *Carcinogenesis* **13**, 663–667.
- (48) Ding, J., Zhou, L., Cho, B. P., and Vouros, P. (1997) Structural characterization of nitro-PAH-DNA adducts by triple quadrupole and ion trap ESI/MS/MS and detection limit study by  $\mu$ HPLC- $\mu$ ESI/SRM MS/MS method. *Proceedings of the 45th ASMS Conference on Mass Spectrometry and Allied Topics*, MP265, Palm Spring, CA.
- (49) Davies, D. B. (1978) Conformations of nucleosides and nucleotides. *Prog. Nucl. Magn. Reson. Spectrosc.* **12**, 135–225.
- (50) Cho, B. P., and Evans, F. E. (1991) Correlation between NMR spectral parameters of nucleosides and its implication to the conformation about the glycosyl bond. *Biochem. Biophys. Res. Commun.* **180**, 273–278.
- (51) Evans, F. E., Miller, D. W., and Beland, F. A. (1980) Sensitivity of the conformation of deoxyguanosine to binding at the C-8 position by *N*-acetylated and unacetylated 2-aminofluorene. *Carcinogenesis* **1**, 955–959.
- (52) Roberts, D. W., Benson, R. W., Groopman, J. D., Flammig, T. J., Nagle, N. A., Moss, A. J., and Kadlubar, F. F. (1988) Immunochemical quantification of DNA-adducts derived from the human bladder carcinogen 4-aminobiphenyl. *Cancer Res.* **48**, 6336–6343.
- (53) Miles, D. W., Townsend, L. B., Robins, M. J., Robins, R. K., Inskeep, W. H., and Eyring, H. (1971) Circular dichroism of nucleoside derivatives. X. Influence of solvents and substituents upon the Cotton effects of guanosine derivatives. *J. Am. Chem. Soc.* **93**, 1600–1608.
- (54) Norman, D., Abuaf, P., Hingerty, B. E., Live, D., Grunberger, D., Broyde, S., and Patel, D. J. (1989) NMR and Computational characterization of the *N*-(deoxyguanosin-8-yl)-amino-fluorene adduct [(AF)G] opposite adenosine in DNA: (AF)G[*syn*]·A[*anti*] pair formation and its pH dependence. *Biochemistry* **28**, 7462–7476.
- (55) Abuaf, P., Hingerty, B. E., Broyde, S., and Grunberger, D. (1995) Solution conformation of the *N*-(deoxyguanosin-8-yl)aminofluorene adduct opposite deoxyinosine and deoxyguanosine in DNA by NMR and computational characterization. *Chem. Res. Toxicol.* **8**, 369–378.
- (56) Mao, B., Cosman, M., Hingerty, B. E., Broyde, S., and Patel, D. J. (1995) Solution conformation of [AF]dG opposite a -1 deletion site in a DNA duplex: Intercalation of the covalently attached

- aminofluorene ring into the helix with base displacement of the C8-modified *syn* guanine into the major groove. *Biochemistry* **34**, 6226–6238.
- (57) Mao, B., Hingerty, B. E., Broyde, S., and Patel, D. J. (1995) Solution conformation of [AF]dG opposite a -2 deletion site in a DNA duplex: Intercalation of the covalently attached aminofluorene ring into the helix with base displacement of the C8-modified *syn* guanine and adjacent unpaired 3'-adenine into the major groove. *Biochemistry* **34**, 16641–16653.
- (58) Eckel, E., and Krugh, T. R. (1994) Structural characterization of two interchangeable conformations of a 2-aminofluorene-modified DNA oligomer by NMR and energy minimization. *Biochemistry* **33**, 13611–13624.
- (59) Cho, B. P., Beland, F. A., and Marques, M. M. (1992) NMR structural studies of a 15-mer DNA sequence from a *ras* protooncogene, modified at the first base of codon 61 with the carcinogen 4-aminobiphenyl. *Biochemistry* **31**, 9587–9602.
- (60) Cho, B. P., Beland, F. A., and Marques, M. M. (1994) NMR structural studies of a 15-mer DNA duplex from a *ras* protooncogene, modified with the carcinogen 2-aminofluorene: Conformational heterogeneity. *Biochemistry* **33**, 1373–1384.
- (61) Zhou, L., Rajabzadeh, M., Traficante, D. D., and Cho, B. P. (1997) Conformational heterogeneity of arylamine-modified DNA:  $^{19}\text{F}$  NMR evidence. *J. Am. Chem. Soc.* **119**, 5384–5389.
- (62) Sarma, R. H., Lee, C.-H., Evans, F. E., Yathindra, N., and Sundaralingam, M. (1974) Probing the interrelation between the glycosyl torsion, sugar pucker, and the backbone conformation in C(8) substituted adenine nucleotides by  $^1\text{H}$  and  $^1\text{H}\{-^{31}\text{P}\}$  fast Fourier transform nuclear magnetic resonance methods and conformational energy calculations. *J. Am. Chem. Soc.* **96**, 7337–7348.
- (63) Evans, F. E., and Levine, R. A. (1986) Conformation and configuration at the central amine nitrogen of a nucleotide adduct of the carcinogen 2-(acetylamino)fluorene as studied by  $^{13}\text{C}$  and  $^{15}\text{N}$  NMR spectroscopy. *J. Biomol. Struct. Dyn.* **3**, 923–934.
- (64) van den Braken-van Leersum, A. M., Tintel, C., van't Zelfde, M., Cornelisse, J., and Lugtenburg, J. (1987) Spectroscopic and photochemical properties of mononitropyrenes. *Recl. Trav. Chim. Pays-Bas* **106**, 120–128.
- (65) Combariza, J. E., Hajos, A. K. D., and Winston, G. W. (1995) Semiempirical calculations of nitrated pyrenes and their reduced metabolites: Relationship to characteristics of bioactivation. *J. Phys. Chem.* **99**, 14539–14543.
- (66) King, C. M., Land, S. J., Jones, R. F., Debiec-Rychter, M., Lee, M.-S., and Wang, C. Y. (1997) Role of acetyltransferases in the metabolism and carcinogenicity of aromatic amines. *Mutat. Res.* **376**, 123–128.

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