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Epoxide and Diol Epoxide Adducts of Polycyclic Aromatic Hydrocarbons at the Exocyclic Amino Group of Deoxyguanosine

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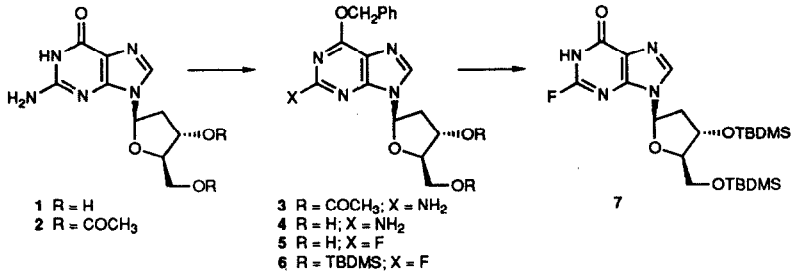
Abstract: **Synthesis and separation of the diastereomeric trans N^2-2'-deoxyguanosine adducts of tetrahydrophenanthrene 3,4-epoxide and benzo[a]pyrene 7,8-diol 9,10-epoxide (benzylic hydroxyl group and epoxide oxygen trans), as well as the incorporation of the former into the pentanucleotide TpApG*pApT, are described.**

The carcinogenic activity of polycyclic aromatic hydrocarbons is mediated by the metabolic formation of bay-region diol epoxides,[1] their ultimate carcinogenic forms.[2] These electrophilic species are thought to produce their carcinogenic and other genotoxic effects through covalent bonding to DNA.[3] The principal targets of these diol epoxides in DNA are the exocyclic amino groups of the purine bases adenine and guanine which react by both cis and trans opening of the epoxides at their benzylic positions. A relationship between these DNA adducts and cellular transformation has yet to be established. In order to probe the mechanism(s) by which diol epoxides transform cells, specifically modified DNA oligomers are required for both biochemical and biological studies. To this end, we have described synthetic approaches useable for the synthesis of DNA oligomers containing a 2'-deoxyadenosine which has been modified at N^6 by either trans or cis opening of a benzylic epoxide,[4a,b] as well as trans opening of a bay-region diol epoxide.[4c] Synthesis of oligonucleotides containing a trans opened naphthalene 1,2-diol 3,4-epoxide adduct of dC has also been reported.[5] In the present study, we describe the site-specific incorporation of an N^2-adducted dG into an oligomer, in which the exocyclic amino group has opened 1,2,3,4-tetrahydrophenanthrene 3,4-epoxide at the benzylic, bay-region position in a trans fashion. The method represents a prototype for the synthesis of oligonucleotides containing dG residues alkylated by carcinogenic, bay-region diol epoxides of polycyclic aromatic hydrocarbons.

In our earlier reports, synthesis of dA adducts consisted of coupling an amino derivative of the hydrocarbon (*cis*- or *trans*-4-amino-3-hydroxy-1,2,3,4-tetrahydrophenanthrene) with a 6-fluoro analog of dA in which the sugar hydroxyl groups were blocked to distinguish them from hydroxyl groups on the hydrocarbon, for subsequent oligonucleotide synthesis.[4] Recently, the synthesis of 2-fluoro-2'-deoxyinosine and its ability to couple with simple amines has been demonstrated.[6,7] However, the viability of this precursor for the generation of adducts from sterically hindered bay-region amines is as yet unknown. To demonstrate the feasibility of such an approach for the generation of bay-region diol epoxide-dG adducts, our synthetic strategy was to couple *trans*-4-amino-3-hydroxy-1,2,3,4-tetrahydrophenanthrene[4a] **8** with the disilyl compound **7**. For our purposes (Scheme 1), it was unnecessary to protect the C-2 amino group of dG prior to the Mitsunobu reaction for the introduction of the O^6 protecting group.[6-8] Thus, the time consuming N-deacetylation[7] is eliminated and only 3',5'-O-deacetylation is required. Therefore, dG was converted to the

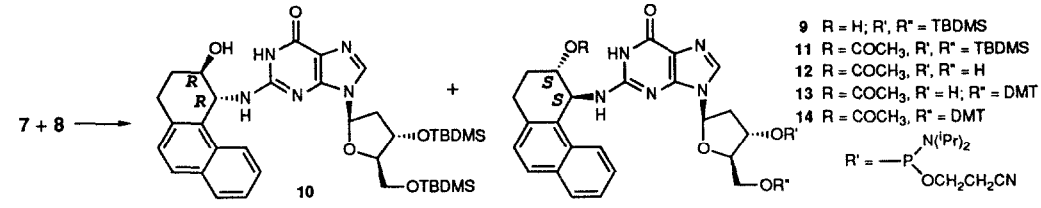
Scheme 1



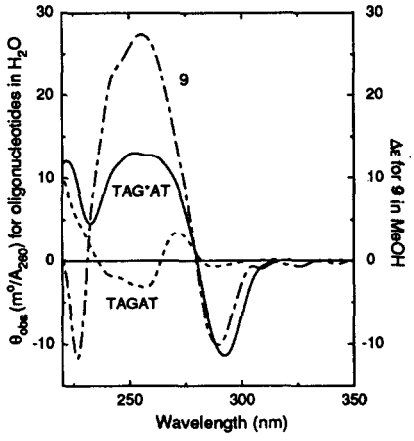
diacetate **2**, followed by O^6 protection to yield the C-6 benzyloxy derivative **3**. Deacetylation of **3** afforded the deblocked nucleoside **4**. Normally, the conversion of **1** to **4** could be effected without complete purification of the intermediates and **4** was obtained in 49% yield.[9] Diazotization and fluorination as described (-25 °C, 10 min)[6] gave a mixture of **5** and the depurinated product. However, a minor modification of this procedure (-50 °C, 20 min) afforded **5** cleanly (90% after crystallization from CH_2Cl_2/*n*-hexane). Protection of 3',5'-hydroxyl groups (quant) was accomplished as previously published.[4] Finally, debenzylation of **6** by catalytic hydrogenation (5% Pd/C, 1 atm, 35 min; 88%) afforded **7** (~40% overall). The present route represents a significant improvement over existing procedures.

Coupling of (±)-*trans*-4-amino-3-hydroxy-1,2,3,4-tetrahydrophenanthrene **8** with **7** was accomplished by heating the mixture at 90 °C in DMSO/2,6-lutidine/hexamethyldisiloxane for 6 h (Scheme 2, 55%).

Scheme 2



Partitioning of the reaction mixture between EtOAc and brine followed by conventional workup yielded a mixture of diastereomeric adducts **9** and **10**, which were subsequently separated by HPLC.[10] Unequivocal assignments of the absolute configurations of **9** and **10** were accomplished by comparison of the HPLC retention times and CD spectra of desilylated **9** and **10** with those of the corresponding trans adduct generated by the reaction of optically active (-)-(3*R*,4*S*)-tetrahydrophenanthrene epoxide and 2'-deoxyguanosine-5'-monophosphate (dGMP) under standard conditions.[4a,11] The *early* eluting (trans) adduct formed in the dGMP reaction was cochromatographic with the desilylated product from **10** (4.5 min) on reverse phase HPLC (Beckman Ultrasphere, 250 X 4.6 mm, 60% MeOH: 40% H_2O at 1.2 mL/min) and had an identical CD spectrum. The product formed by desilylation of **9** (4.9 min) had an almost mirror image CD spectrum. Based upon these observations, **9**, which has a positive CD band at 250-260 nm (see Figure), was assigned (3*S*,4*S*)-configuration. This assignment is also consistent with the observation that dG adducts of diol epoxides at the exocyclic amino group show three bands with the intense, central band (260-280 nm) being positive for a benzylic *S*-configuration.[12]



The *early* adduct **9** was acetylated and the silyl groups were cleaved as reported.[4] Conversion of **12** to the 5'-DMT derivative **13** was accomplished through the use of the reactive $DMT^+BF_4^-$ (1.4 eq) in 2,6-lutidine and Li_2CO_3 (1.9 eq).[4b] Finally, **13** was converted to the phosphoramidite by standard procedures. ^{31}P NMR (acetone-d_6, 0.1 M H_3PO_4 external standard) showed two resonances at 149.31 and 149.51 ppm, corresponding to the two diastereomeric phosphoramidites. After incorporation into the pentamer TpApG*pApT (where G* is the adducted nucleoside) using modified solid phase DNA synthesizer methodology,[4b] the adducted pentamer was purified on a Hamilton PRP-1 column (10μ, 7 X 305 mm) with solvent A: 0.1 M $(NH_4)_2CO_3$ and solvent B: 50% CH_3CN in solvent A, both adjusted to pH 7.5. The gradient was ramped from 20% B to 100% B over 15 min and maintained at 100% B for 5 min, at a flow rate of 2.5 mL/min. Retention time of the adducted DMT-pentamer was 13.2 min. After removal of the DMT group,[4] both oligomers were chromatographed as before, except that the gradient was ramped from 0% B to 35% B over 20 min and then to 100% B over the next 10

pentamers differ substantially (Figure). The spectrum of the adducted oligomer shows marked similarity to that of **9** in that it has a strong negative band at ~290 nm and a strong positive band at ~250 nm, both of which are absent in the unadducted pentamer.

The sequence of reactions described above is equally applicable for dG adducts resulting from trans ring opening of 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene. Adduct formation between **7** and (±)-10-amino-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene[4a,13] was complete in 24 h. CD spectra of the acetylated diastereomers (HPLC as in ref. 10 using 4.5% MeOH:30% CH_2Cl_2:65.5% *n*-hexane at 6 mL/min; $k'_{early} = 0.84$, $k'_{late} = 1.09$) allow assignment[14] of (10*S*)-absolute configuration to the *early* eluting adduct. Incorporation of these adducts into DNA oligomers will be reported separately.

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9. 2'-Deoxyguanosine **1** (1 g, 3.7 mmol), Et_3N (10 mL), Ac_2O (10 mL) and DMAP (46 mg, 0.37 mmol) were stirred in CH_3CN (56 mL) at rt for 20 min. The product was filtered, washed with CH_3CN and dioxane (80% isolated yield), and sonicated in distilled dioxane (94 mL). Triphenylphosphine (1.96 g, 7.5 mmol) and benzyl alcohol (775 μL, 7.5 mmol) were added. The suspension was heated at 101 °C, followed by careful addition of diethyl azodicarboxylate (1.18 mL, 7.5 mmol; the suspension turned homogeneous before complete addition). The mixture was heated at 101 °C a further 15 min. Column chromatography and deacetylation (NH_3/MeOH) yielded the O^6-benzyl compound **4** (0.65 g, 49%).
10. HPLC on an Axxiom silica column (5μ, 10 X 250 mm) using 3.5% MeOH in CH_2Cl_2 at a flow rate of 6 mL/min (k'_{early} = 2.5, k'_{late} = 2.9). ^1H NMR (500 MHz, DMSO-d_6): *Early*-diastereomer **9**: 9.9 (br s, N*H*, purine); 7.95 (br s, $1H_{8''}$); 7.90-7.28 (6H, aromatic); 6.34 (t, $1H_{1'}$, J = 6.6); 5.42 (br s, $1H_4$); 4.58 (m, $1H_{3'}$); 4.24 (br s, $1H_3$); 3.86 (q, $1H_{4'}$, J ~ 4.7); 3.79 (dd, $1H_{5a'}$, J = 4.8, 11.4); 3.72 (dd, $1H_{5b'}$, J = 4.8, 11.4); 3.12 (m, $1H_1$); 2.82 (m, $2H_{1,2a'}$); 2.37 (m, $1H_{2b'}$); 1.95 (m, $2H_2$); 0.89-0.84 (18H, *t*-Bu); 0.1-0.01 (12H, Me). *Late*-diastereomer **10**: 9.9 (br s, N*H*, purine); 7.96 (br s, $1H_{8''}$); 7.90-7.28 (6H, aromatic); 6.32 (t, $1H_{1'}$, J = 6.6); 5.42 (br s, $1H_4$); 4.55 (m, $1H_{3'}$); 4.21 (br s, $1H_3$); 3.86 (m, $1H_{4'}$); 3.80 (dd, $1H_{5a'}$, J = 6.2, 11.0); 3.71 (dd, $1H_{5b'}$, J = 4.4, 11.0); 3.11 (ddd, $1H_1$, J = 7.3, 10.6, 18.0); 2.91 (quint, $1H_{2a'}$, J_{app} = 6.2); 2.80 (m, $1H_1$); 2.34 (m, $1H_{2b'}$); 1.95 (m, $2H_2$); 0.80-0.88 (18H, *t*-Bu); 0.12-0.00 (12H, Me). UV (MeOH): λ 259 (ϵ 19,000), λ 226 (ϵ 88,000). HRMS calcd for $C_{36}H_{54}N_5O_5Si_2$ (M^+ + 1): 692.3664, found for **10**: 692.3693.
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