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SYNTHESIS OF POLYCYCLIC AROMATIC HYDROCARBON SUBSTITUTED 2'-DEOXYADENOSINE ANALOGS

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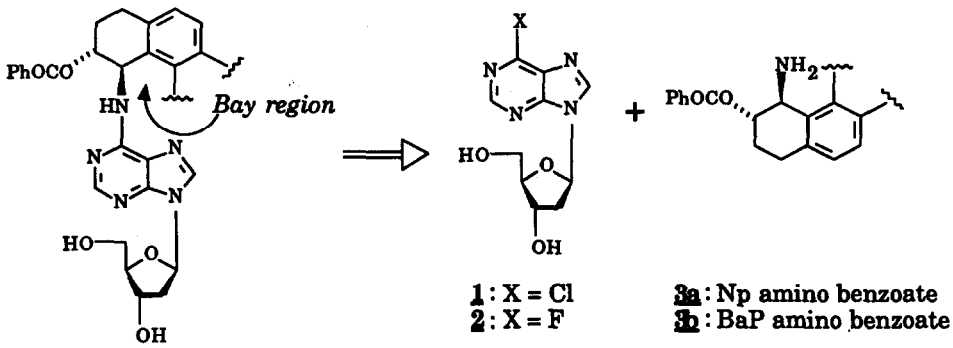
Abstract : The chemical synthesis of polycyclic aromatic hydrocarbon (PAH) modified 2'-deoxyadenosine analogs has been achieved. Two model adducts, incorporating a naphthalene (Np) and a benzo[a]pyrene (BaP) unit have been prepared.

"*Bay region*" diol epoxides are generally considered to be the ultimate carcinogenic forms of PAH.[1] These electrophilic diol epoxides react with the nucleophilic sites in DNA to form PAH-DNA adducts. It is quite likely that intercalation of the PAH diol epoxide into DNA preceeds covalent binding to form these adducts.[2] The major covalent DNA adducts result from a *trans* ring opening of the oxirane moiety with the exocyclic nitrogen of the purine base becoming attached to the benzylic carbon of the PAH. Activation of the H-*ras* protooncogene is believed to occur through mutations (effected by covalent binding of the PAH diol epoxide) in the codons that encode for amino acids 12 or 61 of the *ras*-protein.[3] Codon 12 has the base sequence GGA whereas codon 61 has the sequence CAA. It has recently been shown that PAH diol epoxides having the same absolute stereochemistry in the tetrahydrobenzo ring show different specificities for binding at codons 12 or 61.[3] Further, based on the binding affinities of the stronger carcinogens (diol epoxides of DMBA and BcPh) to dA, it has been suggested that dA binding is more critical to cancer induction than dG binding.[4,5,6] Even though research in the past has led to the identification of structural features of the PAH that are related to carcinogenic activity, the actual mechanism or mechanisms of carcinogenesis is as yet unknown. In order to understand better the steps involved in the induction of cancer, we have been interested in the synthesis of PAH substituted mononucleosides for studies such as site-directed mutagenesis. The synthesis of a 2'-deoxycytidine adduct of the Np diol epoxide has been reported recently.[7] Herein, we wish to report the synthesis of two model PAH substituted dA analogs.

As shown in Scheme 1, our strategy for the synthesis of these PAH derivatives was to couple an activated purine (chloro **1** or fluoro **2** substituted) deoxyriboside and an amino substituted PAH (**3a** or **3b**). These amino substituted PAH derivatives were synthesized from the corresponding tetrahydroepoxides and will be reported separately. Synthesis of the halo purine derivatives has been reported in the literature.[8] Tetrahydro derivatives



Scheme 1

of these PAH were chosen since they are easier to synthesize as compared to the corresponding diol epoxides and would serve as good models for reactions with the diol epoxide derivatives.

Initially, the Np adduct (4) was synthesized.[10] Both purine derivatives 1 and 2 were reacted with 3a separately, under identical conditions. As anticipated, the fluoro derivative was more reactive than the chloro

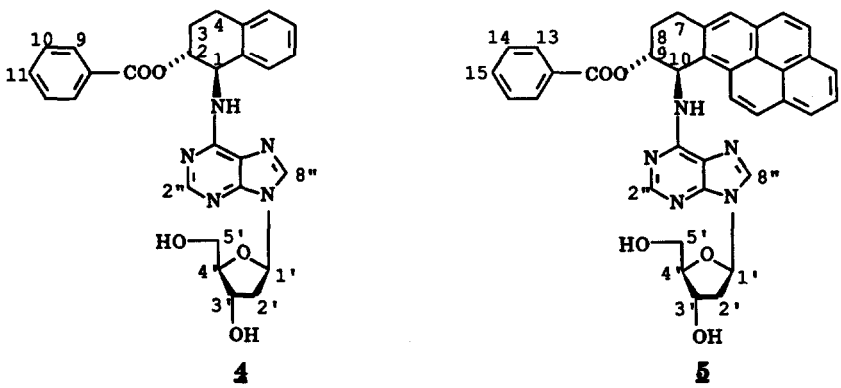


Figure 1

derivative: 2 reacted with 3a in 1¾ hours at room temperature, whereas 1, reacted very little with 3a in 35 hours (as observed by thin layer chromatography). The Np amino benzoate 3a [(±)-*trans*-1-amino-2-benzoyloxy-1,2,3,4-tetrahydronaphthalene] was expected to be more reactive than the BaP amino benzoate 3b [(±)-*trans*-10-amino-9-benzoyloxy-7,8,9,10-tetrahydrobenzo[a]pyrene], due to the presence of an equatorial amino group. In 3b the substituents are axial since the amino group lies in the bay region. Reaction of 3b with 2 revealed the anticipated lower reactivity of 3b relative to 3a: higher temperature of 40 °C was required to enable completion of the reaction in 34 hours. Figures 2(a) and 2(b) show the proton NMR spectra of 4 and 5, respectively. The variable

temperature proton NMR spectra of 4 in the 19-45 °C range show some interesting features. Warming of the

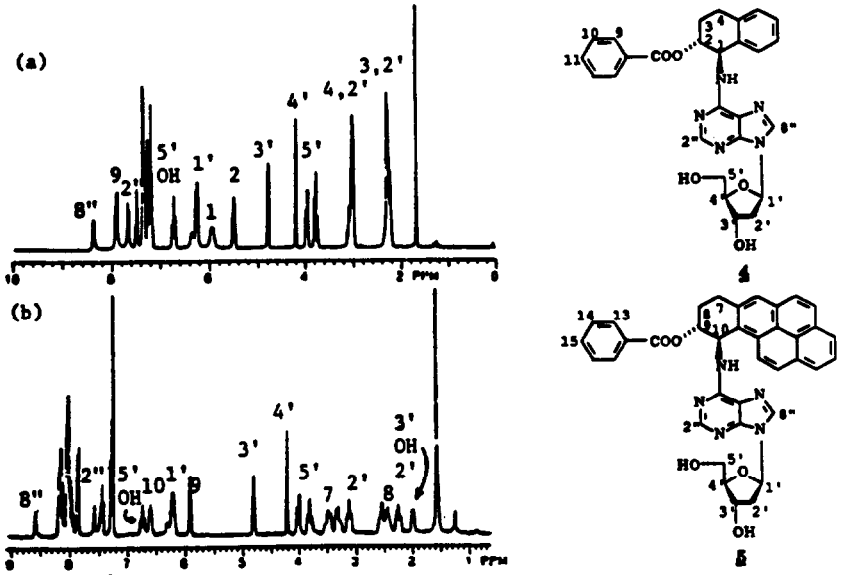


Figure 2 (a) ^{1}H NMR spectrum of 4 in $CDCl_3$ (b) ^{1}H NMR spectrum of 5 in $CDCl_3$.[11]

sample resulted in the sharpening of the purine resonances at δ 8.4 and 7.65 ppm, and the doublet (H-9) at δ 7.95 ppm. These effects are possibly due to the presence of a barrier to the inversion of the nitrogen at C-6" and hindered rotation of the benzoate group, respectively. Of the diastereotopic C-5' protons, one appeared as a doublet at δ 4.0 ppm whereas the other appeared as a pseudo-triplet at δ 3.8 ppm (addition of MeOH-d_4 converted the pseudo-triplet to a doublet). This observation is consistent with an intramolecular hydrogen bond between the C-5' hydroxyl proton and N-3 of the purine ring. This type of hydrogen bonding has been observed by us in the NMR spectra of other purine deoxyribosides in $CDCl_3$ and in the case of the benz[a]anthracene derivatives of dA in the solid state.[9]

Current efforts on our laboratory are directed towards the synthesis of diol epoxide derivatives of dA and incorporation of these adducts into oligonucleotides.

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References and footnotes

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4. For the sake of brevity, 2'-deoxyadenosine, 2'-deoxyguanosine, 7,12-dimethylbenz[a]anthracene and benzo[c]-phenanthrene have been termed dA, dG, DMBA and BcPh, respectively.
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10. For the synthesis of **4** and **5**, 5 mg of the amine (**3a** or **3b**) was stirred with 2 equivalents of **2** in dry DMF, with a pinch of solid $NaHCO_3$. The DMF was removed under vacuum and the residue was dissolved in dry THF and loaded on a silica gel preparative TLC plate. Chromatography using MeOH/$CHCl_3$ (6% MeOH in $CHCl_3$ for **4** and 5% MeOH in $CHCl_3$ for **5**) gave two fractions of which the non-polar fraction was the desired adduct and the polar fraction was unreacted **2**. Compounds **4** and **5** were obtained as white waxy solids in 73 and 38% yield, respectively.
11. 300 MHz ^1H NMR data for **4** (in $CDCl_3$): δ ppm 2.1-2.4 (m, $2H_3$, $1H_{2'}$), 3.0-3.13 (m, $2H_4$, $1H_{2'}$), 3.75 (t, $1H_{5'}$ J_{app}= 12.9), 3.98 (d, $1H_{5'}$ J = 12.9), 4.21 (s, $1H_{4'}$), 4.7 (d, $1H_{3'}$, J = 4.2), 5.5 (m, $1H_2$), 5.95 (br, $1H_1$), 6.25 (dd, $1H_{1'}$, J = 9.6, 5.5), 7.49 (t, $1H_{11}$, J = 7.1), 7.71 (br s, $1H_{2''}$), 7.9 (br d, $2H_9$), 8.38 (br s, $1H_{8''}$), remaining aromatic resonances appear at 7.1-7.4 ppm. The 5'-OH appears as a triplet at 6.72 (J_{app}= 10.5).
FAB mass spectrum for **4** shows: 524 (M^++Na) 502 (M^++1), 329, 274, 258, 176.
300 MHz ^1H NMR data for **5** (in $CDCl_3$): δ ppm 2.25 (m, $1H_{2'}$), 2.5 (m, $1H_8$), 2.55 (m, $1H_8$), 3.1 (m, $1H_{2'}$), 3.3 (m, $1H_7$), 3.5 (m, $1H_7$), 3.8 (t, $1H_{5'}$, J_{app}= 12.7), 4.0 (d, $1H_{5'}$, J = 12.7), 4.21 (s, $1H_{4'}$), 4.81 (s, $1H_{3'}$), 5.91 (br s, $1H_9$), 6.19 (m, $1H_{1'}$), 6.59 (br s, $1H_{10}$), 7.28 (t, $2H_{14}$, J = 7.5), 7.45 (t, $1H_{15}$, J = 7.5), 7.55 (br s, $1H_{2''}$), 7.87 (d, $2H_{13}$, J = 7.5), 8.58 (s, $1H_{8''}$), remaining aromatic resonances appear at 7.95-8.2 ppm. The 5'-OH appears as a triplet at 6.74 ppm (J_{app}= 11.8).
FAB mass spectrum for **5** shows: 648 (M^++Na) 626 (M^++1), 503, 307, 252.

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