Tetrahedron Letters, Vol.31, No.47, pp 6773-6776, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

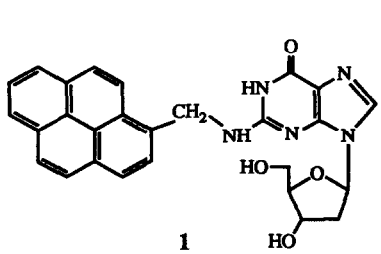
SYNTHESES OF POLYCYCLIC AROMATIC HYDROCARBON-NUCLEOSIDE AND OLIGONUCLEOTIDE ADDUCTS SPECIFICALLY ALKYLATED ON THE AMINO FUNCTIONS OF DEOXYGUANOSINE AND DEOXYADENOSINE

Hongmee Lee, Michael Hinz,† John J. Stezowski,† and Ronald G. Harvey*,
Ben May Institute, University of Chicago, Chicago, Illinois 60637
and †Institut fur Organische Chemie und Isotopenforschung,
der Universitat Stuttgart, Stuttgart, FRG

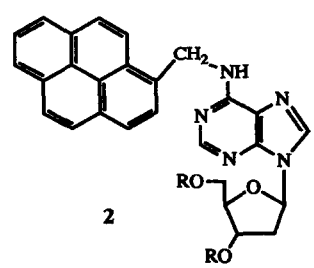
Summary: Efficient syntheses of 1-pyrenylmethyl-mononucleoside adducts with the hydrocarbon moiety attached to the exocyclic amino functions of deoxyguanosine and deoxyadenosine are described.

Carcinogenic polycyclic aromatic hydrocarbons (PAHs) are known to undergo metabolic activation to reactive diol epoxide intermediates that bind covalently to DNA in vivo.[1,2] There is now substantial evidence that this process leads initially to mutations and ultimately to induction of cancer. The major adduct formed with DNA by the diol epoxide metabolite of benzo[a]pyrene arises from trans addition of the exocyclic amino group of deoxyguanosine (dG) to the epoxide ring.[3] An analogous minor adduct in which the hydrocarbon moiety is covalently linked to the exocyclic amino group of deoxyadenosine (dA) is also detected. Other PAHs exhibit similar metabolic activation and interaction with nucleic acids, but variation in the extents of binding and the ratios of the adducts formed is observed. In particular, the diol epoxide metabolite of the highly potent carcinogen 7,12-dimethylbenz[a]anthracene binds much more extensively to dA sites.[4] It has been suggested that dA binding is more critical to cancer induction than dG binding.[5]

In order to further elucidate the mechanism of PAH carcinogenesis at the molecular-genetic level, we have undertaken to devise methods for the sythesis of oligonucleotide adducts covalently bound at specific base sites for site-directed mutagenesis and other studies. As the initial target, we have chosen the dG and dA adducts of 1-methylpyrene covalently linked to the exocyclic amino groups. 1-Methylpyrene was selected as the model PAH because of its close structural relationship to the adduct formed by the reaction of the benzo[a]pyrene diol epoxide and because it lacks the hydroxyl groups anticipated to complicate protection and deprotection in oligonucleotide assembly. We now report synthesis of the 1-pyrenylmethyl-substituted N^2-dG and N^6-dA adducts **1** and **2a** (R = H).



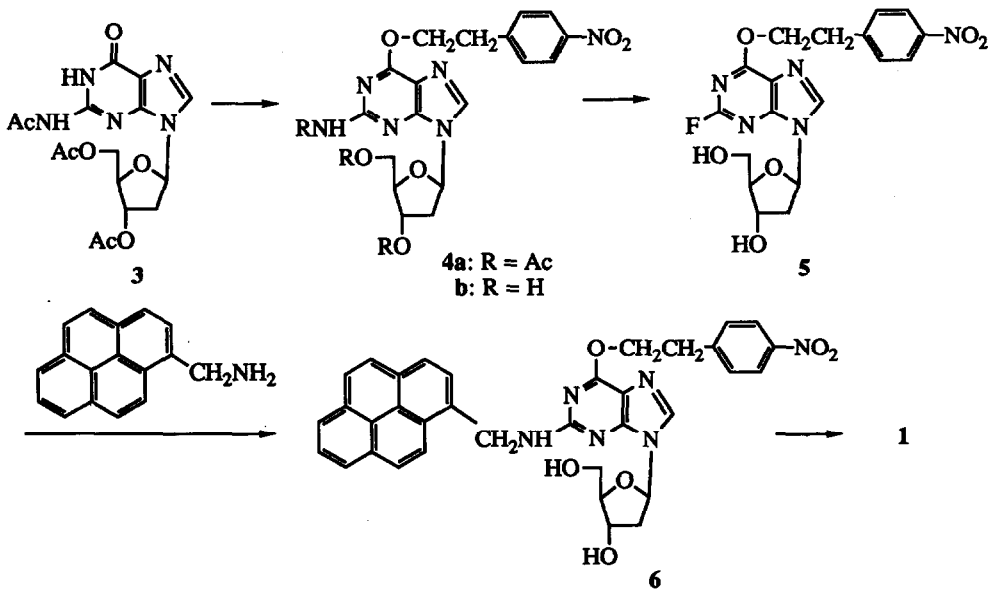
1



2

Our synthetic strategy entailed in the key step the coupling of an appropriately protected halopurine derivative with 1-aminomethylpyrene. The synthetic route to 2'-deoxy-N^2-(1-pyrenylmethyl)guanosine (**1**) is shown in Scheme 1. Treatment of 2'-deoxy-N^2,3',5'-triacetylguanosine (**3**) with 1.5 molar equivalents each of diethylazodicarboxylate, triphenylphosphine, and 2-(*p*-nitrophenyl)ethanol in dioxane at room temperature[6,7] led after 24 hrs directly to the corresponding O^6-*p*-nitrophenyl derivative (**4a**) in quantitative yield.[8] Deacetylation of **4a** took place smoothly on treatment with methanolic ammonia to furnish pure 2'-deoxy-O^6-(*p*-nitrophenylethyl)guanosine (**4b**) in 87% yield. Although deacetylation of the sugar hydroxyl groups was complete in 1 hr, deprotection of the amine group required 6 days for completion. Treatment of **4b** with *tert*-butyl nitrite (TBN) in 60% anhydrous HF/pyridine at -20°C[9] afforded 2-fluoro-O^6-(*p*-nitrophenylethyl)-9-(2'-deoxy-β-D-ribofuranosyl)purine (**5**) in 79% yield. Condensation of **5** with 1-aminomethylpyrene took place smoothly in dimethylformamide at room temperature to furnish N^2-(1-pyrenylmethyl)-O^6-(*p*-nitrophenylethyl)-2'-deoxyguanosine (**6**) in 70% yield. Cleavage of the *p*-nitrophenylethyl group by 0.5 M DBU in pyridine[10] provided **1** in quantitative yield. The NMR spectrum, mass spectrum and other properties of **1** were in good agreement with this structural assignment.[11]

Scheme 1

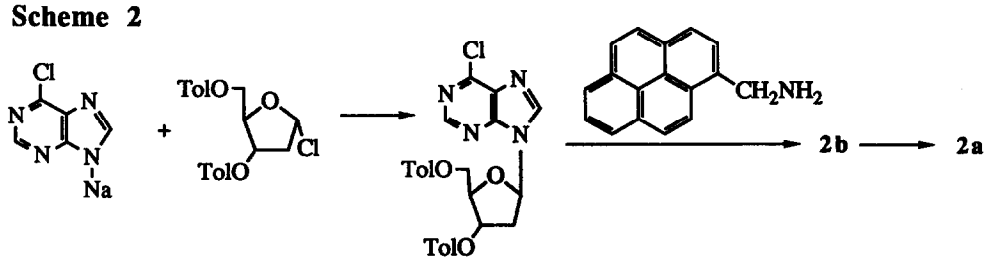


The 1-aminomethylpyrene used in the foregoing synthesis was synthesized from 1-pyrenecarboxaldehyde by conversion to the corresponding 1-pyrenyloxime by reaction with hydroxylamine followed by reduction with zinc in acetic acid in 90% overall yield.

The synthetic route to 2'-deoxy-N^6-(1-pyrenylmethyl)adenosine (**2a**) is outlined in Scheme 2. The starting compound 6-chloro-9-(2'-deoxy-3',5'-di-O-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (**7**) was

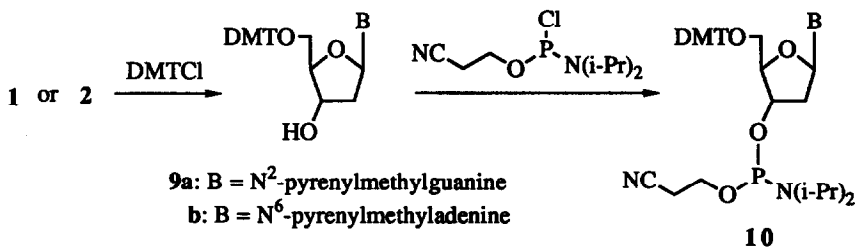
prepared by reaction of the protected 2'-deoxychloro sugar with the sodium salt of 6-chloropurine.[12] Condensation of **7** with 1-aminomethylpyrene in dimethylformamide at room temperature furnished **2b** (R = toluoyl) which was converted to **2a** 69% yield by treatment with sodium methoxide in MeOH/THF at room temperature for 4 hrs. The 500 MHz ^{1}H NMR spectrum of **2a** was entirely consistent with its structural assignment.[13]

Scheme 2



7

Incorporation of the mononucleoside adducts **1** and **2a** into oligonucleotides was also investigated. For this purpose, **1** and **2a** were converted to their respective 5'-O-(4,4'-dimethoxytrityl) derivatives **9a** and **9b** by standard methods.[14] These compounds were then reacted with 2-cyanoethyl-N,N'-diisopropyl-chloride phosphoramidite to give the corresponding 3'-[O-(2-cyanoethyl)diisopropylphosphoramidite derivatives (**10**). They were then incorporated in oligonucleotides with the sequences CGGCA*GCTCTC and CGGCG*GCTCTC, where G* and A* are N^2-pyrenylmethylguanosine and N^6-pyrenylmethyladenosine, respectively, using an Applied Biosystems DNA synthesizer.[15]



9a: B = N^2-pyrenylmethylguanine
b: B = N^6-pyrenylmethyladenine

10

DMTCl = 4,4'-dimethoxytrityl chloride

The foregoing procedures provide an efficient synthetic approach to the specifically alkylated PAH-mono- and oligonucleotide adducts urgently required for site-directed mutagenesis and other studies directed toward elucidation of the molecular mechanism of PAH carcinogenesis. Good yields were obtained in each step in the syntheses of **1** and **2a** and these methods are, in principle, readily adaptable to relatively large scale syntheses of a wide range of PAH-mono- and oligonucleotide adducts. This synthetic route offers significant advantages over the direct alkylation of oligonucleotides or nucleic acids in that it allows the synthesis of PAH-oligomers containing a PAH attached to a specific site in a single dG or dA in a sequence containing multiple dGs or dAs. It is complementary to the approach of Casale and McLaughlin which requires alkylation of a modified dG.[16]

Acknowledgement. This research was supported by grants from the American Cancer Society (CN-22-O) and the National Institute of Environmental Health Sciences (ES 04732) and in part by the Deutsche Forschungsgemeinschaft (Ste 230-4).

References

1. R. G. Harvey. Polycyclic Hydrocarbons and Carcinogenesis. American Chemistry Society: Washington, D. C., 1985.
2. R. G. Harvey, and N. E. Geacintov. Acc. Chem. Res., **21**, 66 (1988).
3. I. B. Weinstein, A. M. Jeffrey, K. Jennette, S. Blobstein, R. G. Harvey, C. Harris, H. Autrup, H. Kasai, and K. Nakanishi. Science., **193**, 592 (1976). A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, I. B. Weinstein, F. A. Beland, R. G. Harvey, H. Kasai, I. Miura, and K. Nakanishi. J. Am. Chem. Soc., **98**, 5714 (1976).
4. A. Dipple, M. Pigott, R. C. Moschel & N. Costantino. Cancer Res., **43**, 4232 (1983). S. C. Cheng, A. S. Prakash, M. A. Pigott, B. D. Hilton, J. M. Roman, H. Lee, R. G. Harvey, and A. Dipple. Chem. Res. Toxicol., **1**, 216 (1988).
5. C. A. H. Bigger, J. T. Sawicki, D. M. Blake, L. G. Raymond, and A. Dipple. Cancer Res., **43**, 5647.
6. O. Mitsunobu. Synthesis, 1-28 (1981).
7. F. Himmelsbach, B. S. Schulz, T. Trichtinger, R. Charubala, and W. Pfleiderer. Tetrahedron, **40**, 59-72 (1984).
8. In preliminary studies the benzyl rather than the p-nitrophenyl derivative was employed, but it was found that later removal of this protecting group by hydrogenation was complicated by competitive hydrogen addition to the pyrene ring.
9. M. J. Robins and B. Uznanski. Can. J. Chem., **59**, 2608 (1981).
10. B. L. Gaffney and R. A. Jones. Tetrahedron Lett., **23**, 2257 (1982).
11. 500 MHz ^{1}H NMR of **1** (in DMSO-d_6): δ 2.27 (m, 1, $H_{2'}$), 2.65 (m, 1, $H_{2'}$), 3.55 and 3.58 (m, 2, $H_{5'}$), 3.86 (m, 1, $H_{4'}$), 4.38 (s, 1, $H_{3'}$), 4.91 (t, 1, OH), 5.27 (d, 2, -CH_2NH, J = 2.8 Hz), 5.30 (s, 1, OH), 6.28 (t, 1, $H_{1'}$, $J_{1',2'}$ = 6.7 Hz), 7.30 (br s, 1, H_2), 7.95 (s, 1, H_8), 8.10 (t, 1, H_7), 8.17 (d, 1, H_2, $J_{2,3}$ = 8.1 Hz), 8.19 (s, 2, $H_{4,5}$), 8.29-8.34 (m, 4, $H_{3,6,8,9}$), 8.50 (d, 1, H_{10}, $J_{9,10}$ = 9.3 Hz), 10.6 (br s, 1, H_1).
12. Z. Kazimierczuk, H. B. Cottam, G. R. Revankar, and R. K. Robins. J. Am. Chem. Soc., **106**, 6379 (1984).
13. 500 MHz ^{1}H NMR of **2a** (in DMSO-d_6): δ 2.10 (d of d, 1, $H_{2'}$), 2.95 (m, 1, $H_{2'}$), 3.69 (t, 1, $H_{5'}$), 3.90 (d, 1, $H_{5'}$, $J_{5'\alpha,\beta}$ = 12.8 Hz), 4.07 (s, 1, $H_{4'}$), 4.68 (d, 1, $H_{3'}$), 5.48 (br s, 2, CH_2), 6.03 (t, 1, $H_{1'}$), 7.44 (s, 1, H_8), 7.94-8.14 (m, 8, aryl), 8.25 (d, 1, $H_{10''}$, $J_{9'',10''}$= 9.2 Hz), 8.39 (br s, 1, H_2).
14. M. J. Gait. Oligonucleotide Synthesis a Practical Approach Oxford, England: IRL Press, 1984.
15. In the synthesis of oligonucleotides incorporating **10**, standard procedures of olignucleotide synthesis were employed except that the reaction times were increased from 1 min to 5 mins in the addition of the PAH-adducted mononucleotide and the subsequent base-addition step. The UV spectra of the purified adducts **1** and **2a** showed the presence of the pyrene chromophore in confirmation of their structures.
16. R. Casale and L. W. McLaughlin. J. Am. Chem. Soc., **112**, 5264 (1990).

(Received in USA 6 August 1990)