A Novel Synthetic Method for Cis-Opened Benzo[*a*]pyrene 7,8-Diol 9,10-Epoxide Adducts at the Exocyclic N^6-Amino Group of Deoxyadenosine

Anthony S. Pilcher, Haruhiko Yagi, and Donald M. Jerina*

Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases The National Institutes of Health, Bethesda, Maryland 20892

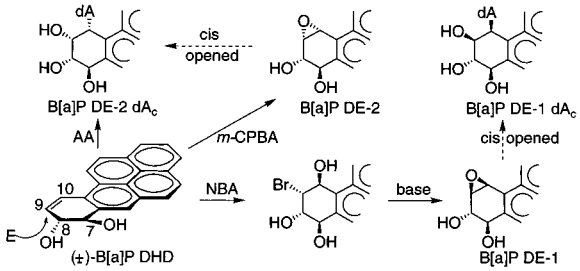
Received January 16, 1998

Polycyclic aromatic hydrocarbon (PAH) diol epoxide (DE) adducts at the exocyclic amino groups of the purine bases in DNA have been implicated in the transformation of normal cells to cancer cells.[1] Metabolism of benzo-ring trans dihydrodiols (Scheme 1; cf. (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene, (±)-B[*a*]P DHD) produces diastereomeric DEs in which the benzylic 7-hydroxyl group and the epoxide oxygen are either cis (DE-1) or trans (DE-2). Solution structures of oligonucleotide duplexes containing DE adducts, as determined by 2-D NMR,[2] have been of substantial interest in that such conformational analysis should lead to an understanding of their enzymatic processing. Most of these conformational studies[2] have focused on adducts derived from trans-opened DEs since cis-opened adducts have not been readily accessible. We here describe an extremely facile synthesis of such cis-opened DE-2 adducts based on the asymmetric aminohydroxylation (AA) procedure of Sharpless and co-workers.[3] Synthesis of the corresponding cis-opened DE-1 adducts is also described.

Previously, we had documented that attack of electrophiles on the double bond of benzo-ring trans dihydrodiols of PAHs occurs from the same face of the molecule as the adjacent allylic hydroxyl group, provided these hydroxyl groups prefer an equatorial conformation.[4,5] Thus, epoxidation of B[*a*]P DHD (**1**) produces B[*a*]P DE-2 whereas intermediate halohydrin formation (same facial attack) followed by cyclization produces B[*a*]P DE-1 (Scheme 1). We reasoned that substitution of 3′,5′-di-*O*-*tert*-butyldimethylsilyl (TBDMS) protected dA for the alkyl carbamates used as the nitrogen source in the AA reaction would produce amino triols corresponding to those which result from cis opening of DE-2 by the exocyclic amino group of the purine bases. Coordination of osmium with the 8-hydroxyl group adjacent to the double bond in the DHD may also be a factor in enhancing this facial selectivity. Known regioselectivity of the Sharpless AA reaction[3] would require nitrogen substitution at C-10 of the DHD for electronic reasons. Silyl protection of the sugar hydroxyl groups allows differentiation from the hydrocarbon hydroxyl groups in subsequent synthetic steps.

Reaction of (±)-**1** with a 3-fold excess of 3′,5′-di-*O*-(TBDMS)-2′-deoxyadenosine (**2**) and *tert*-butyl hypochlorite in the presence of catalytic amounts of $(DHQD)_2PHAL$ and $K_2OsO_2(OH)_4$ (3 h at room temperature in aqueous propanol, see the Supporting Information for all experimental details) provided a 1:1 mixture

Scheme 1[a]



[a] Products shown for the (7*R*,8*R*)-dihydrodiol

Scheme 2

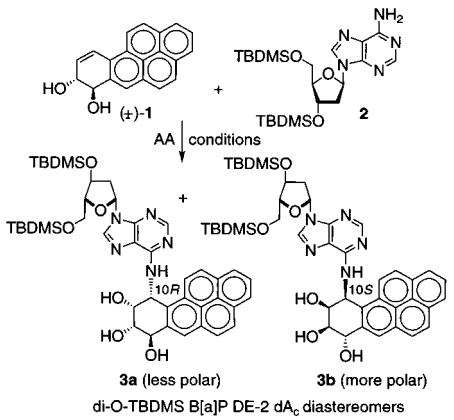


Table 1. Comparison of Benzo-ring ^{1}H NMR Data for Tetraol Tetraacetates (220 MHz, $CDCl_3$)[a] with Those for the Blocked Adducts (300 MHz, acetone-d_6)[b]

compound	H_7	H_8	H_9	H_{10}
DE-1 cis tetraol tetraacetate	6.64 ($J_{7,8}$ 8.0)	6.02 ($J_{8,9}$ 11.5)	5.55 ($J_{9,10}$ 3.5)	7.34
triacetate of **9a** ((10*R*)-di-*O*-TBDMS B[*a*]P DE-1 dA$_c$)	6.60 ($J_{7,8}$ 8.0)	6.27 ($J_{8,9}$ 11.5)	5.73 ($J_{9,10}$ 4.5)	7.29
DE-1 trans tetraol tetraacetate	6.80 ($J_{7,8}$ 8.0)	5.42 ($J_{8,9}$ 5.0)	5.68 ($J_{9,10}$ 3.5)	7.70
DE-2 cis tetraol tetraacetate	6.65 ($J_{7,8}$ 3.5)	5.66 ($J_{8,9}$ 2.5)	5.95 ($J_{9,10}$ 4.6)	7.33
triacetate of **3a** ((10*R*)-di-*O*-TBDMS B[*a*]P DE-2 dA$_c$)	6.65 ($J_{7,8}$ 3.2)	5.77 ($J_{8,9}$ 2.2)	5.98 ($J_{9,10}$ 5.4)	7.16
DE-2 trans tetraol tetraacetate	6.96 ($J_{7,8}$ 8.8)	5.89 ($J_{8,9}$ 2.5)	5.95 ($J_{9,10}$ 3.6)	7.12

[a] Data from ref 13. [b] Members of the (10*R*-)/(10*S*)-diastereomer pairs have nearly identical NMR spectra.

of the desired diastereomers (less polar (10*R*)-di-*O*-TBDMS B[*a*]P DE-2 dA$_c$ (**3a**) and more polar (10*S*)-di-*O*-TBDMS B[*a*]P DE-2 dA$_c$ (**3b**), Scheme 2) in 85% yield (CD spectra, Figure 1) after purification by HPLC (EtOAc in hexane on silica). Compared to alternate procedures in which preformed amino triols are coupled with protected, fluorinated,[8] or sulfonated[9] purines, the present approach is *much more* efficient.

We have also prepared the diastereomeric more polar (10*R*)-B[*a*]P DE-1 dA$_c$ (**9a**) and less polar (10*S*)-B[*a*]P DE-1 dA$_c$ (**9b**) isomers as disilyl ethers (TLC on silica gel with THF in EtOAc) which correspond to cis opening of B[*a*]P DE-1 at C-10 by the exocyclic amino group of dA (Scheme 3). This was achieved in

(1) For reviews, see: (a) Jerina, D. M.; Chadha, A.; Cheh, A. M.; Schurdak, M. E.; Wood, A. W.; Sayer, J. M. In *Biological Reactive Intermediates IV*; Witmer, C. M., Snyder, R., Jollow, D. J., Kalf, G. S., Kocsis, J. J., Sipes, I. G., Eds.; Plenum Press: New York, 1991; pp 533−553. (b) Dipple, A. In *DNA Adducts: Identification and Biological Significance*; Hemminki, K., Dipple, A., Shuker, D. E. G., Kadlubar, F. F., Segerback, D., Bartsch, H., Eds.; IARC Publications No. 125: Lyon, 1994; pp 107−129.

(2) (a) Geacintov, N. E.; Cosman, M.; Hingerty, B. E.; Amin, S.; Broyde, S.; Patel, D. J. *Chem. Res. Toxicol.* **1997**, *10*, 111−146. (b) Jerina, D. M.; Sayer, J. M.; Yeh, H. J. C.; Liu, X.; Yagi, H.; Schurter, E.; Gorenstein, D. *Polycyclic Aromat. Compd.* **1996**, *10*, 145−152.

(3) Li, G.; Angert, H. H.; Sharpless, K. B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2813−2817.

(4) Yagi, H.; Hernandez, O.; Jerina, D. M. *J. Am. Chem. Soc.* **1975**, *97*, 6881−6883.

(5) Yagi, H.; Sayer, J. M.; Thakker, D. R.; Levin, W.; Jerina, D. M. *J. Am. Chem. Soc.* **1987**, *109*, 838−846.

(6) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.

S0002-7863(98)00176-0 CCC: $15.00
Published on Web 04/15/1998

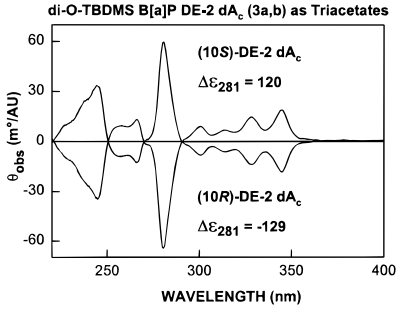
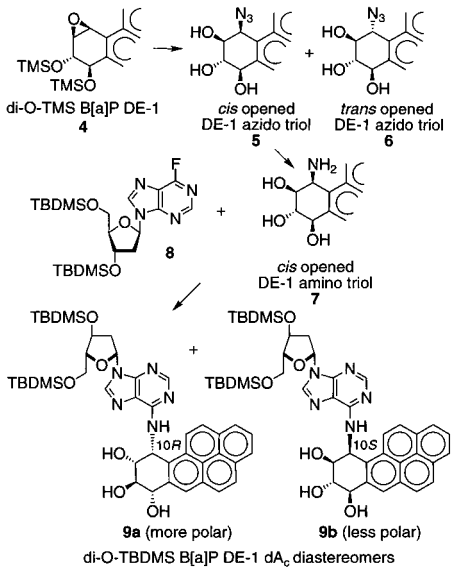


Figure 1. CD spectra (normalized to 1 absorbance unit at 280 nm, methanol) of the triacetates of the diastereomeric (10*R*)- and (10*S*)-di-*O*-TBDMS B[*a*]P DE-2 dA$_c$ adducts **3a** and **3b** (Scheme 2). Notably, the order of elution reverses on acetylation of these DE-2 derivatives, but not of the DE-1 adducts which have almost identical CD spectra. The strong, negative exciton band[6] at 280 nm (short wavelength portion obscured) is indicative of (10*R*) absolute configuration.[7] CD spectra of all four adducts as their disilyl derivatives and as their disilyl triacetates (ϵ_{280} 66 400) are given in the Supporting Information. Acetylation enhances the 280 nm CD band by >30%.

Scheme 3



74% yield by coupling (7 h at 90 °C, DMSO) the amino triol that corresponds to cis-opening of DE-1 by ammonia at C-10 with a 2-fold excess of 6-fluoro-9-(2′-deoxy-3′,5′-di-*O*-TBDMS-*β*-D-*erythro*-pentofuranosyl)purine in the presence of 2,6-lutidine and hexamethyldisiloxane.[8a] Previously, the amino triol that corresponds to cis-opening of DE-1 by ammonia at C-10 was prepared by reaction of the bistrimethylsilyl ether of DE-2 with trimethylsilyl azide (90% yield of the C-10 cis-opened azide) followed by catalytic hydrogenation to the cis-opened DE-2 amino triol.[10] When we repeated this procedure (trimethylsilyl azide, catalytic Ti(OiPr)$_4$ in THF for 3 h at room temperature), but now using the bis-trimethylsilyl ether of DE-1 (di-*O*-TMS DE-1, **4**, Scheme 3), stereoselectivity was lost in that a 60:40 mixture of cis:trans azido triols **5** and **6** was produced. The desired cis azido triol **5** was isolated by HPLC and catalytically reduced (H_2, 10% Pd/C) to the cis-opened amino triol **7** of DE-1, which was coupled with 6-fluoro-9-(2′-deoxy-3′,5′-di-*O*-TBDMS-*β*-D-*erythro*-pentofuranosyl)purine **8**.

All compounds gave the requisite mass spectra. ^{1}H NMR spectra of the separated (10*R*) and (10*S*) adducts, compared either as the disilyl derivatives with free hydroxyl groups on the hydrocarbon or as the disilyl triacetates, were practically identical. Saturated benzo-ring signals of the DE-1 dA$_c$ and DE-2 dA$_c$ isomers as their disilyl triacetates are compared to those from the tetraacetates of the tetraols formed by cis and trans opening of DE-1 and DE-2 at C-10 by water in Table 1. Assignment of relative stereochemistry by comparison is evident. Notably, only products derived from cis opening of DE-1 have a large value for $J_{8,9}$ (11.5 Hz compared to 5.0 Hz for trans-opened DE-1). In contrast, only products derived from cis opening of DE-2 have a small value for $J_{7,8}$ (~3.4 Hz compared to 8.8 Hz for trans-opened DE-2). The coupling constants for the adducts indicate that both prefer a twisted half chair conformation in which the three acetoxy groups are equatorial and the purine is axial in the DE-1 dA$_c$ isomers and in which the 7- and 8-acetoxy groups and the purine are axial and the 9-acetoxy group is equatorial in the DE-2 dA$_c$ isomers. Hindrance in the bay region dictates that the large C-10 purine substituent be axial. The ^{1}H NMR spectra of the present blocked dA$_c$ isomers differ significantly from the disilyl triacetates of the DE-1 dA$_t$ and DE-2 dA$_t$ trans-opened epoxide isomers prepared previously in this laboratory for NMR studies.[11,12] Removal of the TBDMS groups (tetrabutylammonium fluoride/THF) from the initial AA products (**3a** and **3b**, Scheme 2) and the products **9a** and **9b** obtained from coupling of amino triol **7** with fluoropurine nucleoside **8** (Scheme 3) provided diastereomeric pairs of free nucleoside adducts that were cochromatographic with authentic B[*a*]P DE-2 dA$_c$ and B[*a*]P DE-1 dA$_c$ adducts, respectively, on reverse phase HPLC. After removal of the silyl groups from the disilyl triacetates, the diastereomeric mixture of B[*a*]P DE-2 dA$_c$ triacetates was converted into 5′-DMT-3′-diisopropylcyanoethylphosphoramidites ready for incorporation into oligonucleotides.

Although our present synthesis of B[*a*]P DE-1 dA$_c$ derivatives is conventional in that it parallels earlier studies,[8] use of the Sharpless AA procedure[3] to prepare B[*a*]P DE-2 dA$_c$ derivatives constitutes a significant advance in overall yield from the dihydrodiol and ease of preparation: one step to di-*O*-TBDMS B[*a*]P DE-2 dA$_c$ diastereomers **3a** and **3b** (85%) compared to six steps from dihydrodiol for pathways involving amino triols. In addition, the five-step synthesis of the blocked fluoropurine nucleoside **8** (Scheme 3) from deoxyinosine[14] is avoided. Attempts to change the facial selectivity of the AA reaction through use of either the diacetate or dibenzoate of the B[*a*]P DHD failed to produce any product. We are presently attempting to extend the scope of this reaction to other dihydrodiols with more hindered fjord regions instead of bay regions as found in B[*a*]P and to produce dG adducts. The only apparent requirement for the amine in the AA reaction is that the intermediate chloramine should have a relatively low pK_a.

Acknowledgment. We are grateful to Dr. K. Barry Sharpless for providing preprints describing advances in the Sharpless asymmetric aminohydroxylation reaction and to Dr. Jane M. Sayer for numerous helpful suggestions during the course of these studies.

Supporting Information Available: Complete experimental details are provided including NMR and CD spectra as well as HPLC separations of nucleoside adducts (14 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA980176P

(7) Sayer, J. M.; Chadha, A.; Agarwal, S. K.; Yeh, H. J. C.; Yagi, H.; Jerina, D. M. *J. Org. Chem.* **1991**, *56*, 20−29. Agarwal, S. K.; Sayer, J. M.; Yeh, H. J. C.; Pannell, L. K.; Hilton, B. D.; Pigott, M. A.; Dipple, A.; Yagi, H.; Jerina, D. M. *J. Am. Chem. Soc.* **1987**, *109*, 2497−2504.

(8) (a) Lakshman, M. K.; Sayer, J. M.; Yagi, H.; Jerina, D. M. *J. Org. Chem.* **1992**, *57*, 4585−4590. (b) Lakshman, M.; Lehr, R. E. *Tetrahedron Lett.* **1990**, *31*, 1547−1550. (c) Kim, S. J.; Stone, S. J.; Harris, C. M.; Harris, T. M. *J. Am. Chem. Soc.* **1992**, *114*, 5480−5481.

(9) Steinbrecher, T.; Becker, A.; Stezowski, J. J.; Oesch, F.; Seidel, A. *Tetrahedron Lett.* **1993**, *34*, 1773−1774.

(10) Jhingan, A. K.; Meehan, T. *J. Chem. Res. (S)* **1991**, 122−123.

(11) Schurter, E. J.; Yeh, H. J. C.; Sayer, J. M.; Lakshman, M. K.; Yagi, H.; Jerina, D. M.; Gorenstein, D. G. *Biochemistry* **1995**, *34*, 1364−1375.

(12) Schurter, E. J.; Oh-hara, T.; Sayer, J. M.; Yagi, H.; Luxon, B. A.; Jerina, D. M.; Gorenstein, D. G. *Biochemistry* **1995**, *34*, 9009−9020.

(13) Yagi, H.; Thakker, D. R.; Hernandez, O.; Koreeda, M.; Jerina, D. M. *J. Am. Chem. Soc.* **1977**, *99*, 1604−1611.

(14) Robins, M. J.; Basom, G. L. *Can. J. Chem.* **1973**, *51*, 3161−3169.