Synthesis of the *Fjord*-region *cis*- and *trans*-Amino Triol Derivatives of the Carcinogenic Hydrocarbon Benzo[*g*]chrysene and Utilization for the Synthesis of a Deoxyadenosine Adduct Linked to the N6-Amino Group

Alexander S. Kiselyov, Thomas Steinbrecher, and Ronald G. Harvey*

Ben May Institute, University of Chicago, Chicago, Illinois 60637

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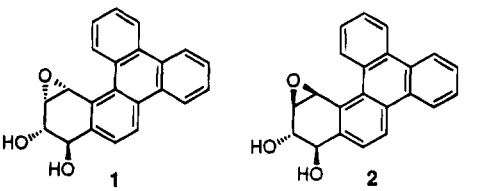
Efficient syntheses of the complete set of four diastereomeric *fjord*-region amino triol derivatives of benzo[*g*]chrysene in which the amino group in the 14-position and the adjacent 13-hydroxyl group are *trans* or *cis* to one another (*trans*- and *cis*-**5** and **6**) is described. This is the first description of the syntheses of the bay- or *fjord*-region *cis*-amino triol derivatives of any carcinogenic polycyclic aromatic hydrocarbon (PAH). The amino triols are key synthetic precursors of PAH–oligonucleotide adducts in which the PAH moiety is covalently linked to the exocyclic amino groups of deoxyadenosine or deoxyguanosine. Formation of adducts of this type via reaction of a PAH diol epoxide metabolite with DNA is believed to be a critical step in the mechanism of PAH carcinogenesis. The synthetic amino triol isomers may be used to synthesize PAH–oligonucleotides needed for site-directed mutagenesis studies to relate isomer structural differences to their effects on DNA replication.

Introduction

As summarized in the introduction to the preceding paper, bay- and *fjord*-region diol epoxide metabolites are implicated as the principal active forms of carcinogenic polycyclic aromatic hydrocarbons (PAHs).[1] A major objective of current investigations is to identify specific target sites on DNA, alkylation of which results in tumorigenesis. Recent evidence suggests that these may be sites in *ras* oncogenes in which the PAH moiety is covalently linked to the exocyclic amino function of a deoxyadenosine base.[2,3]

In order to gain more definitive evidence, we have undertaken the synthesis of PAH–oligonucleotide adducts in which the isomeric *fjord*-region diol epoxide metabolites of the carcinogenic hydrocarbon benzo[*g*]-chrysene are covalently bound to specific purine base sites, particularly deoxyadenosine (dA) sites in *ras* gene sequences. Direct alkylation of oligonucleotides by *anti*- and *syn*-diol epoxide metabolites of carcinogenic PAHs[4] is of limited practical utility[5] because of the multiplicity of potential reaction sites coupled with the inherent greater reactivity of the deoxyguanosine nucleotides. In principle, synthetic accessibility of PAH–oligonucleotide adducts specifically alkylated at predetermined sites makes possible correlation between molecular structure determined by physical methods (NMR and X-ray crystallography) and the mutations induced in replication of the adducted oligonucleotides incorporated in DNA. The diol epoxides of benzo[*g*]chrysene (**1** and **2**) were chosen as models because of their relative stabilities and demonstrated affinity for binding to dA.[3e,6] Efficient synthetic



approaches to **1** and **2** which are adaptable to relatively large scale preparation are described in the accompanying manuscript. This paper reports conversion of these diol epoxides to the corresponding *fjord*-region *cis*- and *trans*-amino triols, key intermediates in the synthesis of the desired oligonucleotide adducts. The use of the *trans*-amino triol adduct of benzo[*g*]chrysene for the synthesis of *trans*-adduct of the *anti*-diol epoxide of benzo[*g*]-chrysene (**1**) bound to the N6 of dA is also described.

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(4) The *anti* diol epoxide is defined as the isomer in which the epoxide function is on the face opposite the benzylic hydroxyl group; the *syn* isomer has these groups on the same face. For a discussion of PAH nomenclature see ref 1, Chapter 1.

(5) The direct synthetic route to PAH–oligonucleotide adducts is limited by the inherent greater reactivity of dG relative to dA which in turn is considerably more reactive than dC or dT. As a consequence, it is applicable mainly to preparation of PAH–oligonucleotides with a PAH component linked to dG in a sequence containing few dGs: Cosman, M.; Ibanez, V.; Geacintov, N. E.; Harvey, R. G. *Carcinogenesis* **1990**, *11*, 1667. Cosman, M.; de los Santos, C.; Fiala, R.; Hingerty, B. E.; Ibanez, V.; Margulis, L. A.; Live, D.; Geacintov, N. E.; Broyde, S.; Patel, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 1914. M. Cosman, M.; de los Santos, C.; Fiala, R.; Hingerty, B. E.; Ibanez, V.; Luna, E.; Harvey, R. G.; Geacintov, N. E.; Broyde, S.; Patel, D. J. *Biochemistry* **1993**, *32*, 41. Cosman, M.; Fiala, R.; Hingerty, B. E.; Laryea, A.; Lee, H.; Harvey, R. G.; Amin, S.; Geacintov, N. Broyde, S.; Patel, D. J. *Biochemistry* **1993**, *32*, 12488. Mao, B.; Margulis, L. A.; Li, B.; Ibanez, V.; Lee, H.; Harvey, R. G.; Geacintov, N. E. *Chem. Res. Toxicol.* **1992**, *5*, 773.

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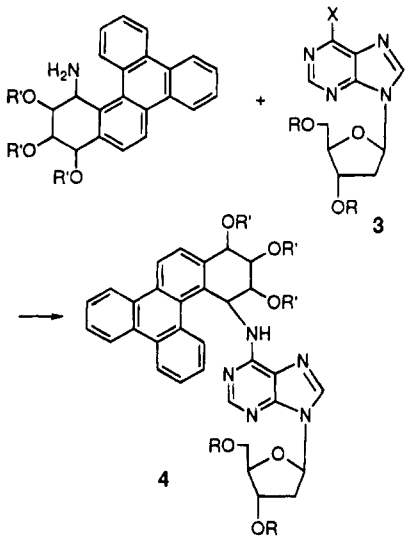
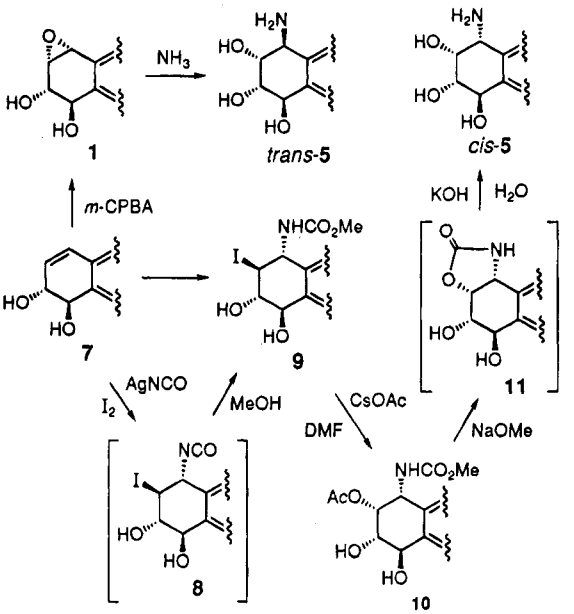
Scheme 1

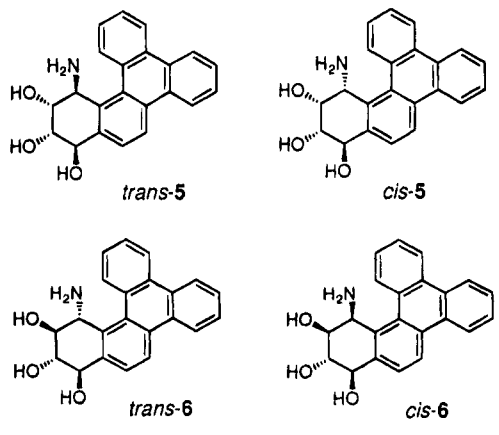


Scheme 2



Results

Prior studies by ourselves[7] and others[8] have shown that the most efficient synthetic route to PAH diol epoxide–oligonucleotide adducts of the type sought is an inverse synthetic method. This entails reaction of amino triol derivatives of the PAH diol epoxides with purine base analogs in which the amino function is replaced by a halogen atom or other appropriate leaving group. In the present example, an amino triol derivative of benzo[*g*]chrysene in which the amino group is in the sterically crowded *fjord*-region 14-position is required to react with an inosine derivative (**3**) in which X is a halogen or other appropriate leaving group to form a deoxyadenosine adduct (**4**) in which the PAH moiety is linked to the 6-amino function of the nucleoside (Scheme 1). The *anti*- and *syn*-diol epoxide isomers (**1** and **2**) can each give rise to a pair of diastereomeric amino triol derivatives in which the relation between the amino group and the adjacent hydroxyl group is either trans or cis. As a consequence, four amino triol diastereomers are possible, *trans*- or *cis*-**5** and *trans*- or *cis*-**6**. Each of these can



potentially exist as a pair of enantiomers, making a total of eight possible amino triol isomers. All of these isomers must be considered, since studies of the metabolism of polycyclic hydrocarbons show detectable amounts of all possible stereoisomeric nucleoside adducts, the ratios of which vary considerably from one PAH to another.[1,3,9,10] Although metabolic activation by mammalian cells and subsequent DNA binding often favors formation of particular adduct isomers, there is no cogent reason to assume that the major isomers are more important in tumorigenesis than the minor isomers.

Synthesis of the *trans*-**5** amino triol was accomplished by direct ammonolysis of the *anti*-diol epoxide derivative of benzo[*g*]chrysene (**1**) (Scheme 2). Reaction of **1** with aqueous ammonia in acetonitrile took place smoothly to yield the product of *trans*-stereospecific addition of ammonia to the epoxide ring, 14β-amino-11β,12α,13α-trihydroxy-11,12,13,14-tetrahydrobenzo[*g*]chrysene (*trans*-**5**). The corresponding *cis* isomer could not be detected. The observed stereospecificity is in agreement with previous findings for other diol epoxides,[7] although formation of a small amount of a second isomer was observed in one case.[11]

The starting compound for the synthesis of the *cis*-amino triol (*cis*-**5**) was the *trans*-7,8-dihydro diol of benzo[*g*]chrysene (**7**). Reaction of **7** with iodine and silver isocyanate in THF at 0 °C furnished a *trans* adduct (**8**) which underwent methanolysis to yield a *trans*-β-iodocarbamate product (**9**).[12,13] Reaction of **9** with cesium

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Scheme 3

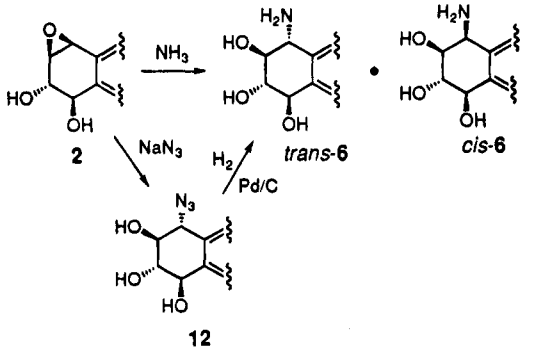


Table 1. ^{1}H NMR Data for the Amino Triols of Benzo[*g*]chrysene

compd	H_{11}	H_{12}	H_{13}	H_{14}
trans-**5**	4.58	4.34	3.98	4.81
	$J_{11,12}$ = 6.0; $J_{12,13}$ = 2.5; $J_{13,14}$ = 3.0 Hz			
cis-**5**	4.94	4.44	3.91	5.18
	$J_{11,12}$ = 2.50; $J_{12,13}$ = 2.5; $J_{13,14}$ = 2.5 Hz			
trans-**6**	5.16	3.38	3.82	4.76
	$J_{11,12}$ = 8.0; $J_{12,13}$ = NA;[a] $J_{13,14}$ = 2.5 Hz			
cis-**6**	4.41	3.21	4.06	5.44
	$J_{11,12}$ = 9.0; $J_{12,13}$ = NA; $J_{13,14}$ = NA			

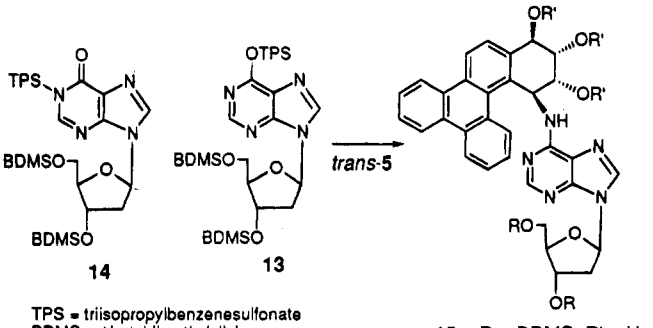
[a] NA = not assigned.

acetate in DMF at 80 °C took place with inversion to provide the *N*-carbomethoxy monoacetate derivative of the *cis*-amino triol (**10**).[13] Hydrolysis of **10** by methanolic KOH afforded initially a *cis*-fused 2-oxazolidone intermediate (**11**) which underwent further conversion to the *cis*-amino triol, 14α-amino-11β,12α,13α-trihydroxy-11,-12,13,14-tetrahydrobenzo[*g*]chrysene (*cis*-**5**).

The synthetic routes to the *trans*-**6** and *cis*-**6** amino triol derivatives of benzo[*g*]chrysene are based on the *syn*-diol epoxide derivative of benzo[*g*]chrysene (**2**) (Scheme 3). Addition of ammonia to **2** took place regiospecifically, but nonstereoselectively, to afford a mixture of the products of both *trans*- and *cis*-addition to the epoxide ring, 14α-amino-11β,12α,13β-trihydroxy-11,12,13,14-tetrahydrobenzo[*g*]chrysene (*trans*-**6**) and 14β-amino-11β,-12α,13β-trihydroxy-11,12,13,14-tetrahydrobenzo[*g*]chrysene (*cis*-**6**) in approximately equal ratio. A more efficient synthetic route to the *trans*-stereoisomer was through reaction of **2** with sodium azide which proceeded stereospecifically to provide the corresponding *trans*-azidotriol (**12**). Hydrogenation of **12** over a 10% palladium-charcoal catalyst took place with retention of stereochemistry to furnish *trans*-**6** free of the *cis*- isomer within the limit of detectability.

The structural assignments of the amino triols were entirely consistent with the NMR spectral data summarized in Table 1. In particular, the small values of the coupling constants for the *fjord*-region benzylic protons ($J_{13,14}$ = 2.5 Hz) for the two trans isomers *trans*-**5** and *trans*-**6** are consistent with a trans-diaxial relation and the prior generalization that substituents in bay or *fjord* regions are strongly disposed to adopt an axial conformation to minimize the expected strong steric interaction with the aryl hydrogen atom of the adjacent aromatic ring.[14,15] The values of the coupling constants

Scheme 4



TPS = triisopropylbenzenesulfonate
BDMS = *t*-butyldimethylsilyl

for the $H_{11,12}$ protons of *cis*- and *trans*-**6** ($J_{11,12}$ = 9.0 and 8.0 Hz, respectively) are consistent with a normal diequatorial conformation for the hydroxyl groups in these positions. In contrast, the coupling constants for the $H_{11,12}$ protons of *cis*- and *trans*-**5** are unusually low ($J_{11,12}$ = 2.5 and 6.0 Hz, respectively), more consistent with a diaxial than a diequatorial configuration. This is apparently a consequence of the steric interaction between the *cis*-hydroxyl groups in the 12,13-positions which causes further flattening of the ring structure. Additional confirmation for the assigned structures of the amino triols as well as information on their conformations is provided by NOE experiments. The assumption that H_1 resonates furthest downfield because of its repulsive van der Waals interactions was confirmed. Irradiation of H_1 with the low power radiofrequency gave an NOE enhanced signal assigned as H_{14}. A series of subsequent irradiations of H_{14} followed by H_{11}-H_{13} allowed easy assignment of the signals corresponding to cyclohexene portion of the molecule.

The *trans*-amino triol *trans*-**5** was employed as the starting compound for the synthesis of the *trans*-adduct of the *anti*-diol epoxide of benzo[*g*]chrysene (**1**) covalently linked to deoxyadenosine (Scheme 4). The protected purine nucleoside employed for this purpose was the 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl) derivative of the *O*-6-triisopropylbenzenesulfonate of deoxyinosine (**13**). The latter was prepared from 2′-deoxyinosine in two steps. Reaction of the sugar with *tert*-butyldimethylsilyl chloride and imidazole took place in dry DMF to furnish 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyinosine. It was necessary to dry the 2′-deoxyinosine thoroughly prior to use to ensure a high yield of the product. Reaction of the disilylated inosine with 2,4,6-triisopropylbenzenesulfonyl chloride in the presence of triethylamine and 4-(dimethylamino)pyridine in CH_2Cl_2 gave **13** in moderate yield (22%). The major product was the N-substituted compound **14** (42%). The reaction of *trans*-**5** with **13** was quite sensitive to the purity of the components, and anhydrous conditions were essential. With pure reactants under appropriately controlled conditions, reaction took place smoothly to furnish the desired adduct (**15a**). Formation of tarry side products made purification difficult, so that it was more convenient to acetylate the free hydroxyl groups of the polyarene component and desilyate the sugar component of the partially purified product with *n*-Bu_4NF to form the triacetate derivative (**15b**). Chromatographic purification of **15b** gave a

(13) Steinbrecher, T. Ph.D dissertation, Johannes Gutenburg-Universität Mainz, Mainz, Germany, 1991. Syntheses of the *cis*-amino triol derivatives of benzo[c]phenanthrene by the method employed herein is described.

(14) Reference 1, Chapter 13.

(15) Harvey, R. G. In *The Conformational Analysis of Cyclohexenes, Cyclohexadienes, and Related Hydroaromatics*; Rabideau, P. W., Ed.; VCH: New York, 1989; pp 267–298.

mixture of diastereomers resolvable by preparative reversed-phase chromatography to provide the optically pure (+)- and (−)-isomers of **15b**.

Discussion

This paper reports efficient syntheses of the complete set of four diastereomeric *fjord*-region amino triol derivatives of benzo[*g*]chrysene in which the 14-amino group and the adjacent 13-hydroxyl group are *trans* or *cis* to one another (*trans*- and *cis*-**5** and -**6**). Prior investigations of the synthesis of the amino triols of other PAH carcinogens have focused virtually exclusively on the synthesis of the *trans*-amino triol isomer derived from the *anti*-diol epoxide diastereomer or analogs with fewer hydroxyl groups.[7-9] This choice derived mainly from the fact that the *anti*-diol epoxide of benzo[*a*]pyrene was the first active PAH diol epoxide metabolite to be identified,[1,9] and it happens to form predominantly trans adducts with nucleic acids. Subsequently, it has been found that other PAH diol epoxides afford significantly larger amounts of cis adducts.[3,10] Insofar as far as we are aware, this report is the first description of the syntheses of the bay- or *fjord*-region *cis*-amino triol derivatives of any PAH carcinogen.[13]

As indicated above, amino triols are key synthetic precursors of oligonucleotide adducts having the PAH moiety covalently linked to the exocyclic amino groups of deoxyadenosine or deoxyguanosine. In principle, the availability of a complete set of amino triol isomers permits the synthesis of all possible PAH-dA and -dG adduct isomers which in turn may be utilized to compare the effects of isomer differences on PAH−oligonucleotide structure and the consequences of these differences for DNA replication. It should also be pointed out that the synthetic amino triol isomers are potentially useful precursors for the synthesis of PAH−oligonucleotide adducts not only by the inverse synthetic route utilized herein but also by the modified inverse synthetic method in which the halopurine compound is incorporated into an oligonucleotide prior to reacting it with an amino triol.[8b,c]

Experimental Section

Materials and Methods. The *anti*- and *syn*-diol epoxides of benzo[*g*]chrysene (**1** and **2**) and benzo[*g*]chrysene *trans*-7,8-dihydrodiol (**7**) were synthesized as described in the preceding paper. *N*-Bromosuccinimide was crystallized from water prior to use. Amberlite IRA-400 resin was purchased from Aldrich and activated by the following procedure: 5 g of the resin was washed with 5 × 50 mL of 30% KOH, 10 × 50 mL of distilled water until neutral pH, 2 × 25 mL of THF followed by 2 × 25 mL of dry THF, 3 × 25 mL of ether, and dried for 24 h under high vacuum at 30 °C. THF was distilled from sodium benzophenone ketyl prior to use. Melting points are uncorrected. The proton ^{1}H NMR spectra were obtained on the University of Chicago 300- or 500-MHz ^{1}H NMR spectrometers in $CDCl_3$ with tetramethylsilane as internal standard unless stated otherwise.

14β-Amino-11β,12α,13α-trihydroxy-11,12,13,14-tetrahydrobenzo[*g*]chrysene (*trans*-5). To a 25% aqueous solution of ammonia (10 mL) was added a solution of 164 mg (0.5 mM) of the *anti*-diol epoxide **2** in 30 mL of acetonitrile, and the resulting heterogeneous mixture was warmed to 40 °C and stirred at this temperature for 15 min. The reaction mixture was maintained at reflux for 3 h; TLC showed the absence of **2**. Concentration of the mixture under vacuum gave a solid residue which was coevaporated with 3 × 5 mL of toluene to remove traces of water and washed with 2 × 5 mL of ether to afford pure *trans*-**5** (128 mg, 74%) as a white solid, mp 232−233 °C dec: ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O) δ 3.98 (dd, 1, J = 3.0, 2.5 Hz), 4.34 (dd, 1, J = 6.0, 2.5 Hz), 4.58 (d, 1, J = 6.0 Hz), 4.72 (d, 1, J = 3.0 Hz), 7.52−7.64 (m, 4), 7.78 (d, 1, J = 8.5 Hz), 8.60−8.85 (m, 4), 9.78 (d, 1, J = 8.5 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 51.7, 62.9, 69.4, 76.0, 123.3, 123.9, 123.9, 124.6, 125.4, 127.3, 128.2, 128.4, 128.8, 129.5, 129.8, 130.0, 131.3, 131.4, 131.4, 131.8, 132.9, 133..6, 151.4; UV (MeOH) λ_{max} 200 (ε = 43 000), 262 (50 100); HRMS (CI): calcd for $C_{22}H_{19}NO_3$ 345.136 49, found 345.136 05. Anal. Calcd for $C_{22}H_{19}NO_3$ + 1/4H_2O: C, 75.52; H, 5.61. Found: C, 74.38; H, 5.67. *trans*-**5** and other amino triol isomers were hygroscopic, and removal of residual traces of water proved difficult.

14α-(Carbomethoxyamino)-11β,12α-dihydroxy-13β-iodo-11,12,13,14-tetrahydrobenzo[*g*]chrysene (9). A solution of iodine (127 mg, 0.5 mmol) in 1 mL of dry THF was added by syringe to a vigorously stirred mixture of the dihydro diol **7** (312 mg, 1 mmol) and silver isocyanate (900 mg, 6 mmol) in 60 mL of dry THF at 0 °C under argon. To avoid decomposition of silver salts the mixture was kept in the dark. After being stirred for 2 h at 0 °C, the resulting pale yellow suspension was allowed to warm to ambient temperature, and stirring was continued for 3 more h until TLC (EtOAc/hexanes, 2:1) indicated consumption of **7** to be complete. The mixture was filtered in the dark and concentrated *in vacuo* using a dry ice condenser. The pale yellow solid residue was washed with 3 × 10 mL of cold ether to furnish the *trans*-iodoisocyanate adduct **8** as a white solid. Dry MeOH was added, and the resulting suspension was heated at reflux for 3 h. During this time formation of a beige colored suspension of the *cis*-iodocarbamate (**9**) was observed. The precipitate was collected and washed with 2 × 10 mL of cold MeOH and 2 × 20 mL of ether to furnish pure **9** (437 mg, 85%) as a white solid, mp 174−175 °C: ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O) δ 3.35 (s, 3), 4.17 (dd, 1, J = 3.0, 8.0 Hz), 5.12 (d, 1, J = 8.0 Hz), 5.38 (dd, 1, J = 3.0, 2.5 Hz), 6.29 (d, 1, J = 3.0 Hz), 7.59−7.82 (m, 4), 8.22 (d, 1, J = 8.5 Hz), 8.65−8.85 (m, 4), 8.91 (d, 1, J = 8.5 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.6, 51.6, 62.9, 69.3, 76.1, 123.8, 123.9, 124.6, 125.4, 127.3, 128.2, 128.5, 128.8, 129.5, 130.0, 130.0, 131.3, 131.4, 131.6, 131.8, 132.9, 133.7, 151.4, 168.9; UV (MeOH) λ_{max} 200 (ε = 19 400), 260 (49 200). Anal. Calcd for $C_{24}H_{20}INO_4$: C, 56.15; H, 3.93; N, 2.73. Found: C, 55.92; H, 4.07; N, 2.55.

13α-Acetoxy-14α-(carbomethoxyamino)-11β,12α-dihydroxy-11,12,13,14-tetrahydrobenzo[*g*]chrysene (10). The success of this step depends on observation of precautions for anhydrous conditions. DMF was dried over molecular sieves (3 Å) before use, CsOAc was dried by heating in a vacuum oven at 35 °C for at least 12 h, **9** was dried by coevaporation with 3 × 5 mL of dry toluene, and all operations were conducted in a dry box under an inert atmosphere. A suspension of finely ground CsOAc (576 mg, 3 mmol) in 2 mL of dry THF was added to a vigorously stirred solution of **9** (513 mg, 1 mmol) in 5 mL of dry DMF. The resulting dark yellow mixture was slowly warmed to 80 °C over 30 min and allowed to stir at this temperature for an additional 8 h until TLC (on silica gel eluted with EtOAc) showed the absence of **9**. This mixture was poured into 50 mL of water, and the suspension was extracted with 3 × 25 mL of EtOAc. The EtOAc extract was washed with 2 × 15 mL of water, dried over $MgSO_4$, concentrated to dryness, and purified by flash chromatography on silica eluted with EtOAc−hexanes 3:1 to provide pure **10** (271 mg, 61%) as a white solid, mp 190−192 °C: ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O) δ 1.85 (s, 3), 3.36 (s, 3), 4.53 (br s, 1), 4.87 (dd, 1, J = 3.0 Hz, J = 2.5 Hz), 5.36 (d, 1, J = 2.5 Hz), 6.92 (d, 1, J = 2.5 Hz), 7.63−7.85 (m, 4), 8.25 (d, 1, J = 8.5 Hz), 8.72−8.83 (m, 4), 8.85 (d, 1, J = 8.5 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.6, 26.8, 62.9, 69.3, 76.1, 123.8, 123.9, 124.6, 125.4, 127.3, 128.2, 128.5, 128.8, 129.9, 130.0, 131.3, 131.4, 131.5, 131.8, 132.9, 133.7, 151.4, 168.9, 182.4; UV (MeOH) λ_{max} 202 (ε = 18 700), 258 (48 600). Anal. Calcd for $C_{26}H_{23}NO_6$: C, 70.10; H, 5.20; N, 3.14. Found: C, 69.88; H, 5.26; N, 2.91.

14α-Amino-11β,12α,13α-trihydroxy-11,12,13,14-tetrahydrobenzo[*g*]chrysene (*cis*-5). A solution of NaOMe (freshly prepared from 23 mg of Na dissolved in 0.5 mL of dry MeOH)

was added by syringe to a vigorously stirred suspension of **10** (223 mg, 0.5 mmol) in 7 mL of dry MeOH under argon at room temperature. The resulting mixture was stirred at room temperature for 4 h to produce a yellow homogeneous mixture, TLC of which (silica gel eluted with EtOAc) revealed absence of **10** and the presence of a product with R_f 0.35–0.37 presumed to be **11**. A solution of KOH (56 mg, 1 mmol) in 1 mL of MeOH–H_2O 4:1 was added to this mixture, and stirring was continued for an additional 8 h until TLC indicated complete conversion of the intermediate with R_f 0.35–0.37 to the major product. The mixture was evaporated to dryness and the residue treated with 2 × 3 mL of ice-cold water and purified by repeated preparative HPLC on a Zorbax ODS column (21.2 mm × 25 cm eluted with MeOH–water, 70:30, flow rate 10 mL/min) to give pure *cis*-**5** (98 mg, 57%) as a white solid, mp 212–213 °C dec: ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O) δ 3.91 (dd, 1, J = 3.0, 2.5 Hz), 4.44 (dd, 1, J = 2.5 Hz), 4.94 (d, 1, J = 2.5 Hz), 5.18 (d, 1, J = 2.5 Hz), 7.40–7.52 (m, 4), 7.58 (d, 1, J = 8.5 Hz), 8.45–8.54 (m, 2), 8.58 (d, 1, J = 8.5 Hz), 8.67 (d, 1, J = 8.5 Hz), 9.13 (d, 1, J = 8.5 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 51.7, 62.9, 69.4, 76.0, 123.8, 124.0, 124.6, 125.4, 127.3, 128.2, 128.4, 128.8, 129.5, 129.8, 129.9, 131.3, 131.4, 131.5, 131.8, 132.9, 133.6, 151.4; HRMS (CI) calcd for $C_{22}H_{19}NO_3$ 345.136 49, found 345.136 33; UV (MeOH) λ_{max} 200 (ϵ = 42 900), 262 (49 800). Anal. Calcd for $C_{22}H_{19}NO_3$ + 1/2H_2O: C, 74.56; H, 5.69. Found: C, 74.81; H, 5.69.

14α-Azido-11β,12α,13β-trihydroxy-11,12,13,14-tetrahydrobenzo[*g*]chrysene (12). A solution of the *syn*-diol epoxide **2** (164 mg, 0.5 mmol) in 5 mL of acetone was added to a suspension of NaN_3 (650 mg, 10 mmol) in 30 mL of acetone–water (2:1), and the resulting mixture was heated at reflux for 3 h. TLC showed absence of the starting diol epoxide. The mixture was concentrated to dryness, and the yellow residue was purified by chromatography on a column of silica gel. Elution with CH_2Cl_2–MeOH (5:1) gave the pure azidotriol **12** (108 mg, 58%) as a white solid, mp 178–179 °C dec: ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O) δ 3.75 (dd, 1, J = 8.5, 6.5 Hz), 4.04 (dd, 1, J = 4.5, 6.5 Hz), 4.98 (d, 1, J = 8.5 Hz), 5.85 (d, 1, J = 6.5 Hz), 7.66–7.84 (m, 4), 7.80 (d, 1, J = 8.5 Hz), 8.84–8.96 (m, 4), 8.70 (d, 1, J = 8.5 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 51.6, 63.1, 69.4, 76.6, 123.8, 123.8, 123.8, 124.6, 125.5, 127.3, 128.2, 128.4, 128.9, 129.5, 129.9, 129.9, 131.2, 131.4, 131.4, 131.8, 133.0, 133.6, 151.4; UV (MeOH) λ_{max} 202 (ϵ = 37 800), 262 (49 600). Anal. Calcd for $C_{22}H_{17}N_3O_3$: C, 71.15; H, 4.62. Found: C, 70.88; H, 4.75.

14α-Amino-11β,12α,13β-trihydroxy-11,12,13,14-tetrahydrobenzo[*g*]chrysene (*trans*-6). Hydrogenation of a solution of **12** (74 mg, 0.2 mmol) in 10 mL of absolute ethanol was carried out in the presence of 400 mg of a 10% Pd/C catalyst for 45 min. The resulting solution was filtered and concentrated to dryness, and the solid residue was washed with 3 × 5 mL of dry ether to furnish the crude *trans*-amino triol *trans*-**6**. Purification by HPLC on a Zorbax ODS column (21.2 mm × 25 cm eluted with MeOH–water, 70:30, flow rate 10 mL/min) afforded pure *trans*-**6** (39 mg, 56%) as a white solid, mp 226–227 °C dec: ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O) δ 3.38 (d, 1, J = 8.0 Hz), 3.82 (br s, 1), 4.76 (d, 1, J = 2.5 Hz), 5.16 (d, 1, J = 8.0 Hz), 7.61–7.80 (m, 4), 7.82 (d, 1, J = 8.5 Hz), 8.74–8.88 (m, 4), 9.11 (d, 1, J = 8.5 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 51.6, 63.0, 69.4, 76.1, 123.8, 123.9, 123.9, 124.6, 125.5, 127.3, 128.3, 128.4, 128.9, 129.5, 129.8, 130.0, 131.2, 131.3, 131.4, 131.8, 133.0, 133.6, 151.4; UV (MeOH) λ_{max} 200 (ϵ = 42 800), 262 (50 100); HRMS (CI) calcd for $C_{22}H_{19}NO_3$ 345.1365, found 345.1360. Anal. Calcd for $C_{22}H_{19}NO_3$ + 1/4H_2O: C, 75.52; H, 5.61. Found: C, 74.59; H, 5.66.

Ammonolysis of the Benzo[*g*]chrysene *syn*-Diol Epoxide (2). Reaction of **2** (328 mg, 1 mmol) with ammonia was carried out by the procedure employed for the ammonolysis of **1**. Purification of the crude product by preparative HPLC on a Zorbax ODS column (21.2 mm × 25 cm eluted with MeOH–water, 70:30, flow rate 10 mL/min) provided 14β-amino-11β,12α,13β-trihydroxy-11,12,13,14-tetrahydrobenzo[*g*]chrysene (*cis*-**6**) (105 mg) and 14α-amino-11β,12α,13β-trihydroxy-11,12,13,14-tetrahydrobenzo[*g*]chrysene (*trans*-**6**) (116 mg) in 64% overall yield. The latter was identical in its physical and spectral properties with authentic *trans*-**6** prepared via the sodium azide route. The *cis*-**6** isomer was obtained as a white solid, mp 238–239 °C dec: ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O) δ 3.21 (d, 1, J = 9.0 Hz), 4.06 (br s, 1), 4.41 (d, 1, J = 9.0 Hz), 5.44 (br s, 1), 7.65–7.86 (m, 4), 7.89 (d, 1, J = 8.5 Hz), 8.77–8.89 (m, 4), 8.93 (d, 1, J = 8.5 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 51.6, 63.0, 69.4, 76.1, 123.8, 123.9, 123.9, 123.9, 124.7, 125.5, 127.3, 128.2, 128.4, 128.8, 129.5, 129.9, 130.0, 131.2, 131.4, 131.4, 131.8, 133.0, 133.6, 151.4; UV (MeOH) λ_{max} 200 (ϵ = 42 400), 262 (49 900). Anal. Calcd for $C_{22}H_{19}NO_3$ + 1H_2O: C, 72.71; H, 5.27. Found: C, 72.64; H, 5.39.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-2′-deoxyinosine. 2′-Deoxyinosine is available from commercial sources, but it is expensive and the relatively large amounts required as the starting material for this synthesis may be conveniently prepared by the following procedure. A solution of 100 mg of adenosine deaminase type II in 5 mL of deionized water was added to a stirred suspension of 20 g (80 mmol) of 2′-deoxyadenosine in 10 mL of 0.02 M KH_2PO_4. After being stirred for 10 h, the solution became completely transparent. It was allowed to stir for an additional 14 h and concentrated *in vacuo* to 150 mL and the precipitate removed by filtration. The solid product was recrystallized from EtOH/H_2O 4:1 to give 2′-deoxyinosine (19.1 g, 91%) identical by NMR with an authentic sample. It was necessary to dry the 2′-deoxyinosine thoroughly before use (by 3-fold evaporation with pyridine at 70 °C under high vacuum) to ensure a high yield in the following reaction.

2′-Deoxyinosine (6 g, 24 mmol), imidazole (12 g, 176 mmol), and *tert*-butyldimethylsilyl chloride (9 g, 60 mmol) were dissolved in 100 mL of dry degassed DMF under argon, and the solution was stirred at room temperature. After 8 h, formation of a precipitate was observed. Stirring was continued for an additional 10 h, then the resulting mixture was concentrated at 50 °C under vacuum and filtered. The solid residue was washed with 2 × 5 mL of DMF, 3 × 15 mL of EtOAc, and 2 × 50 mL of dry ether to give pure 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxyinosine (9.97 g, 87%), mp 206 °C dec: ^{1}H NMR (500 MHz, $CDCl_3$ + D_2O) δ 0.04 (s, 6), 0.06 (s, 6), 0.86 (s, 9), 0.88 (s, 9), 2.48 (m, 1), 2.51 (m, 1), 3.73 (dd, 1, J = 11.5, 2.5 Hz), 3.82 (dd, 1, J = 11.5, 4.0 Hz), 3.98 (m, 1), 4.58 (m, 1), 6.37 (t, 1, J = 6.0 Hz), 8.17 (s, 1), 8.28 (s, 1).

***O*⁶-((2,4,6-Triisopropylphenyl)sulfonyl)-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyinosine (13).** A solution of 2,4,6-triisopropylphenylsulfonyl chloride (5 g, 16.6 mmol) in 7 mL of dry CH_2Cl_2 (freshly distilled from CaH_2) was added to a vigorously stirred mixture of 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyinosine (4.0 g, 8.3 mmol), freshly distilled triethylamine (3.2 g, 32 mmol), and 4-(dimethylamino)pyridine (0.1 g) in 30 mL of dry CH_2Cl_2 at room temperature under argon. After 35 min the reaction was essentially complete (TLC, silica gel, CH_2Cl_2). The resulting mixture was concentrated at 30 °C under vacuum to 10 mL, and the resulting concentrate was purified by chromatography on column of silica gel eluted with CH_2Cl_2 to afford **13** (1.42 g, 22%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.06 (s, 3), 0.09 (s, 3), 0.11 (s, 6), 0.88 (s, 9), 0.91 (s, 9), 1.22–1.35 (m, 18), 2.44 (m,1), 2.62 (m,1), 2.92 (m,1), 3.77 (dd, 1, J = 10.5, 1.2 Hz), 3.86 (dd, 1, J = 10.5, 1.2 Hz), 4.04 (m, 1), 4.37 (m, 2), 4.61 (m, 1), 6.49 (t, 1, J = 6.5 Hz), 7.21 (s, 2), 8.38 (s, 1), 8.56 (s, 1); HRMS calcd for $C_{37}H_{62}N_4O_6SSi_2$ 746.3928, found 746.3932. Further elution gave the N-substituted product **14** (2.71 g, 42%) as an oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.05 (s, 3), 0.09 (s, 3), 0.14 (s, 6), 0.89 (s, 9), 0.93 (s, 9), 1.22–1.36 (m, 18), 2.43 (m, 1), 2.62 (m, 1), 2.94 (m, 1), 3.77 (dd, 1, J = 10.5, 1.2 Hz), 3.83 (dd, 1, J = 10.5, 1.2 Hz), 4.08 (m, 1), 4.39 (m, 2), 4.60 (m, 1), 6.44 (t, 1, J = 6.5 Hz), 7.18 (s, 2), 8.11 (s, 1), 8.82 (s, 1); HRMS calcd for $C_{37}H_{62}N_4O_6SSi_2$ 746.3928, found 746.3922.

(±)-*N*⁶-β-(11β,12α,13α-Triacetoxy-11,12,13,14-tetrahydrobenzo[*g*]chrysenyl)-2′-deoxyadenosine (15c). *All reagents and solvents must be dried thoroughly before use.* Traces of water were removed from the hygroscopic *trans*-**5** amino triol (500 mg, 1.4 mmol) by coevaporation with toluene (3 × 7 mL) at 80 °C (0.5 mmHg). The residue was cooled to room temperature in an argon stream, and a solution of the *O*⁶-TIPS-inosine derivative **13** (1.9 g, 2.5 mmol) in 10 mL of

dry degassed DMF was added. The yellow mixture under argon was slowly brought to 85 °C by heating in an oil bath, resulting in formation of a dark yellow solution. This solution was stirred at 80 °C until HPLC analysis (Zorbax Sil, MeOH/H_2O, 70:30) indicated absence of **13** (72 h). Although some *trans*-**5** was still present, attempts to react it with additional amounts of **13** were unsuccessful. The resulting dark orange-colored solution was concentrated under vacuum (75 °C, 0.1 mmHg), and the residue was dissolved in 5 mL of EtOAc plus 1 drop of EtOH and purified by flash chromatography to remove polar components and tar. ^{1}H NMR analysis of the crude product confirmed the presence of the **15a**, but further purification by either plate or column chromatography were unsuccessful.

The crude **15a** was dissolved in 5 mL of freshly distilled Ac_2O plus 10 mL of freshly distilled pyridine to give a dark red solution which was stirred at room temperature for 24 h. Concentration of the solution *in vacuo* at 60 °C gave a residue which was dissolved in THF (10 mL) and treated with 5 mL of a 1 M solution of *n*-Bu_4NF in THF for 30 min; TLC analysis (silica gel, CH_2Cl_2) indicated the reaction to be complete. Stirring was continued for 30 min, and then the solution was concentrated to ~5 mL and purified by chromatography on a column of silica gel eluted with EtOAc to give a mixture of diastereomers of **15b** (146 mg, 14.3%). The mixture was successfully resolved by preparative reversed-phase HPLC chromatography (C-8-RP, MeOH/H_2O, 70:30) to furnish 64 mg of the (+)-isomer and 67 mg of the (−)-isomer as analytically pure compounds. The (−)-isomer had mp 212 °C dec: ^{1}H NMR (500 MHz, $CDCl_3$ + D_2O) δ 1.79 (s, 3), 2.01 (s, 3), 2.08 (s, 3), 2.22 (m, 1), 3.05 (m, 1), 3.78 (dd, 1, J = 2.0, 1.5 Hz), 3.97 (dd, 1, J = 13.0, 1.5 Hz), 4.16 (br s, 1), 4.73 (dd, 1, J = 5.0, 1.5 Hz), 5.95 (dd, 1, J = 7.5, 1.5 Hz), 6.12 (t, 1, J = 4.0 Hz), 6.21 (dd, 1, J = 9.5, 6.0 Hz), 6.24 (d, 1, J = 7.5 Hz), 6.52 (d, 1, J = 4.0 Hz), 7.12 (d, 1, J = 8.0 Hz), 7.19 (t, 1, J = 8.0 Hz), 7.22 (t, 1, J = 7.5 Hz), 7.54 (t, 1, J = 7.5 Hz), 7.58 (d, 1, J = 7.5 Hz), 7.60−7,75 (m, 3), 8.38 (d, 1, J = 7.5 Hz), 8.50−8.62 (m, 2), 8.74 (d, 1, J = 8.5 Hz); HRMS calcd for $C_{38}H_{35}N_5O_9$ 705.24348, found 705.2451. Anal. Calcd for $C_{38}H_{35}N_5O_9$ − $1H_2O$ 687.2329, found 687.2310. Anal. Calcd for $C_{38}H_{35}N_5O_9$ +1/2H_2O: C, 63.85; H, 5.08. Found: C, 63.88; H, 5.39. The (+)-isomer had mp 210 °C dec: ^{1}H NMR (500 MHz, $CDCl_3$ + D_2O) δ 1.81 (s, 3), 2.04 (s, 3), 2.09 (s, 3), 2.23 (m, 1), 3.03 (m, 1), 3.74 (dd, 1, J = 2.0, 1.5 Hz), 3.95 (dd, 1, J = 13.0, 1.5 Hz), 4.14 (br s, 1), 4.72 (dd, 1, J = 5.0, 1.5 Hz), 5.95 (dd, 1, J = 7.5, 1.5 Hz), 6.11 (t, 1, J = 4.0 Hz), 6.20 (dd, 1, J = 9.5, 6.0 Hz), 6.23 (d, 1, J = 7.5 Hz), 6.52 (d, 1, J = 4.0 Hz), 7.12 (d, 1, J = 8.0 Hz), 7.19 (t, 1, J = 8.0 Hz), 7.22 (t, 1, J = 7.5 Hz), 7.54 (t, 1, J = 7.5 Hz), 7.58 (d, 1, J = 7.5 Hz), 7.60−7,75 (m, 3), 8.38 (d, 1, J = 7.5 Hz), 8.50−8.63 (m, 2), 8.74 (d, 1, J = 8.5 Hz). For the mixture of isomers: HRMS calcd for $C_{38}H_{35}N_5O_9$ 705.243 48, found: 705.2451; calcd for $C_{38}H_{35}N_5O_9$ − $1H_2O$ 687.2329, found 687.2310. Anal. Calcd for $C_{38}H_{35}N_5O_9$ + 1/2H_2O: C, 63.85; H, 5.08. Found: C, 63.88; H, 5.39.

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