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Solvent-Free Synthesis of Benzo[*a*]pyrene 7,8-Diol 9,10-Epoxide Adducts at the N^2-Position of Deoxyguanosine

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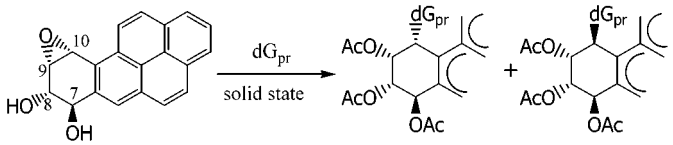
ABSTRACT



2 **6** **7**

The first solid-state (or solvent-free) synthesis of protected deoxyguanosine (dG) adducts of benzo[*a*]pyrene diol epoxides at room temperature is reported. Whereas dG adducts derived from *cis*- and *trans*-opening of (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (DE-1 1) are formed as a 1:1 mixture, the direct opening of the diastereomeric (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]-pyrene (DE-2, 2) produced a 15:85 ratio favoring the *trans*-opened dG adduct 7.

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous environmental pollutants which are formed during incomplete combustion processes and exert their mutagenic and carcinogenic activity upon metabolic activation to electrophilic reactive metabolites.[1] Benzo[*a*]pyrene (B[*a*]P), a typical and widely studied PAH, is metabolized to bay-region diol epoxides (DE) which account for most if not all of its carcinogenic activity.[2,3] These DE are metabolically formed as a pair of diastereomers in which the benzylic hydroxyl group and epoxide oxygen are either *cis* (DE-1) or *trans* (DE-2). In the case of B[*a*]P, the (*R*,*S*,*S*,*R*)-7,8-diol 9,10-epoxide-2 enantiomer is highly tumorigenic[4] and selectively binds to the exocyclic amino group of dG residues in DNA to form stable, *trans*-opened N^2-dG adducts.[5] Because of the importance of these DNA adducts in understanding how the PAH induce cancer, there has been considerable interest in the synthesis of oligonucleotides containing N^2-dG adducts for the study of their conformational[6] and biological properties.[7−9]

The synthesis of oligonucleotides containing N^2-dG adducts of the PAH has been achieved by direct reaction[10] of

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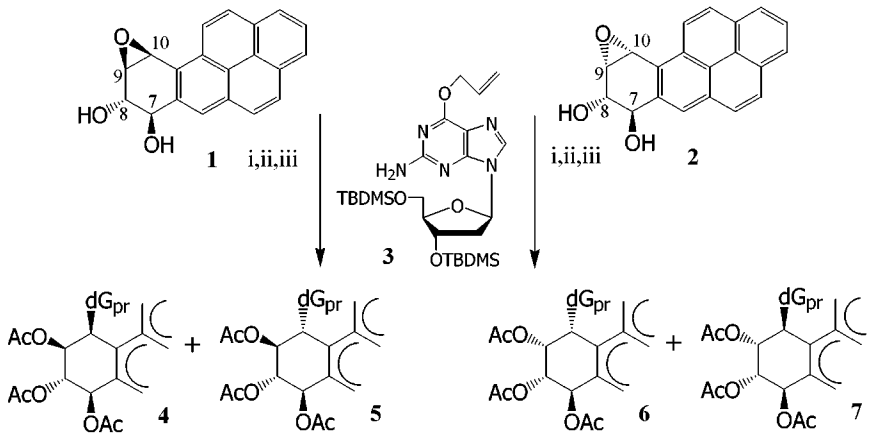
Scheme 1[a]



[a] (i) Solid state; (ii) HPLC; (iii) Ac_2O, DMAP, pyridine.

the DE with a short oligonucleotide typically containing a single dG, by postoligomerization modification of an oligonucleotide containing a reactive dG derivative,[11] or by a total synthetic approach employing an adduct phosphoramidite.[7,12] Although the total synthetic approach requires more steps, it generally provides a more easily purified product mixture and is more flexible in terms of sequence.

Solvent-free reactions so far have been predominately used in industrial gas-phase processes or polymerizations. However, a recent review[13] showed that a variety of chemical reactions can be performed under solvent-free conditions. Encouraged by this review, by a recent report of solvent-free addition of Me_3SiN_3 to epoxides,[14] and by our present observation that dodecylamine undergoes *cis*-addition to 9,10-epoxy-7,8,9,10-tetrahydro B[*a*]P at C-10 (40% yield) in the absence of solvent (^{1}H NMR [$CDCl_3$, 300 MHz] H_{10} δ 5.71 and H_9 4.16 with $J_{9a,10e} = 4.7$, $J_{8e,9a} = 3.3$, and $J_{8a,9a} = 12.1$ Hz), we investigated the use of solid-state conditions to prepare dG adducts. In the present report, we describe for the first time an efficient *solvent-free* synthesis of protected N^2-dG adducts of B[*a*]P, which are formed by *cis*- and *trans*-opening of both B[*a*]P DE-1[15] (**1**) and B[*a*]P DE-2[15] (**2**) at C-10.

The key step in our synthesis is the direct opening of the DE's with O^6-allyl-3′,5′-di-*O*-(*tert*-butyldimethysilyl)-2′-deoxyguanosine (**3**)[16] in a solid-state reaction at rt overnight to yield a mixture of *cis*- and *trans*-opened N^2-dG adducts (Scheme 1).[17] The reaction products and yields are summarized in Table 1. Whereas the reaction of **1** with **3** resulted in a 1:1 mixture of the *cis*- and *trans*-opened N^2-dG adducts **4** and **5** (45% combined yield after acetylation), the reaction of **2** with **3** led to the formation of the corresponding *cis*- and *trans*-opened N^2-dG adducts **6** and **7** in a ratio of 15:85 (54% combined yield after acetylation). In contrast to the solvent-free reaction, direct opening of **1** or **2** by **3** in DMA[12a] required heating at 90−100 °C for 2 h. Although both procedures gave comparable overall yields for each of the DE's, product ratios differed dramatically. Under both reaction conditions, **1** produced an aproximately 1:1 ratio of *cis*:*trans* adducts **4** and **5**, respectively. In contrast, the

Table 1. Solvent-Free Synthesis of *cis*- and *trans*-N^2-dG Adducts as Acetates

epoxide	nucleoside	% yield	*cis*:*trans* ratio
1	**3**	45	50:50 (**4**:**5**)
2	**3**	54	15:85 (**6**:**7**)

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(17) In a typical experiment 30 mg (100 μmol) **2** was reacted with a 4.0 molar excess of protected dG **3** by mixing the solids in a 50 mL porcelain mortar. The mixture of solids was thoroughly ground with a pestle for 5 min. Then a few drops of CH_2Cl_2 were added, and the mixture was again ground for 5 min and left overnight in the mortar. The mixture was diluted with CH_2Cl_2−MeOH (95:5) and purified by chromatography (column: 30 × 2 cm) on silica (CH_2Cl_2−MeOH, 95:5) to afford a mixture of *cis*- and *trans*-N^2-dG adducts. This mixture of diastereomeric *cis*- and *trans*-N^2-dG adducts was separated by HPLC (ratio 15:85) on an Axxiom silica column (10 × 250 mm, 5 μm) and eluted at a flow rate of 5 mL/min with an isocratic mixture of ethyl acetate/hexane (75:25 for DE-2 adducts and 70:30 for DE-1 adducts) as described.[12a] The individual *cis*- and *trans*-N^2-dG adducts were then acetylated overnight with acetic anhydride in pyridine containing catalytic amounts of DMAP. Evaporation of the solvent followed by chromatography on silica using CH_2Cl_2−MeOH (98:2) afforded the acetylated *cis*- and *trans*-N^2-dG adducts **6** (6.8 mg) and **7** (38.4 mg) (54% combined yield). All compounds gave satisfactory ^{1}H NMR spectral and high-resolution mass spectral data which were in accord with the published data.[12a] All yields are based on isolated, purified materials.

relative percentage of *trans* adduct **7** increased from 40% in DMA to 85% in the absence of solvent. The remarkable *trans*-selectivity for the solid-state reaction of B[*a*]P DE-2 is reminiscent of the high percentage of *trans*-opened tetraol product obtained from **2** relative to **1** on acid-catalyzed solvolysis.[18] Clearly, the 7- and 8-hydroxyl groups in **1** or **2** play a dominant role since the tetrahydro B[*a*]P 9,10-epoxide gave all *cis*-addition with a simple alkylamine. Under both reaction conditions, **1** and **2** failed to react with 3′,5′-di-*O*-(*tert*-butyldimethysilyl)-2′-deoxyguanosine. The O^6-allyl protecting group presumably enhances the nucleophilicity of the N^2-amino group of the dG building block **3**. Our attempts to prepare the corresponding *cis*- and *trans*-opened N^6-dA adducts of B[*a*]P DE-1 and DE-2 in solid-state reactions of 3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine with the DE's resulted in very poor yields (∼5%) of the desired products as was also the case in solvent.[12a]

The N^2-dG adducts obtained after the adduct coupling step were separated into their diastereomeric mixtures of *cis*- and *trans*-opened isomers prior to acetylation as described.[12a] After blocking of the secondary hydroxyl groups by acetylation, the diastereomeric N^2-dG adducts **4**, **5**, **6**, and **7** can be easily transformed into their corresponding phosphoramidites by standard procedures for incorporation into oligonucleotides.[19]

In conclusion, we have found the solvent-free synthesis of the *cis*- and *trans*-opened N^2-dG adducts of **1** and **2** to be more convenient than heating in DMA and to proceed in comparable yields. The high *trans*-selectivity of the solvent-free reaction for opening of **2** makes this reaction very attractive for the large scale synthesis of the corresponding *trans*-N^2-dG adducts needed for structural and biological studies.

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