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Novel trifluoroethanol mediated synthesis of benzo[*a*]pyrene 7,8-diol 9,10-epoxide adducts at the N^2-position of deoxyguanosine and the N^6-position of deoxyadenosine

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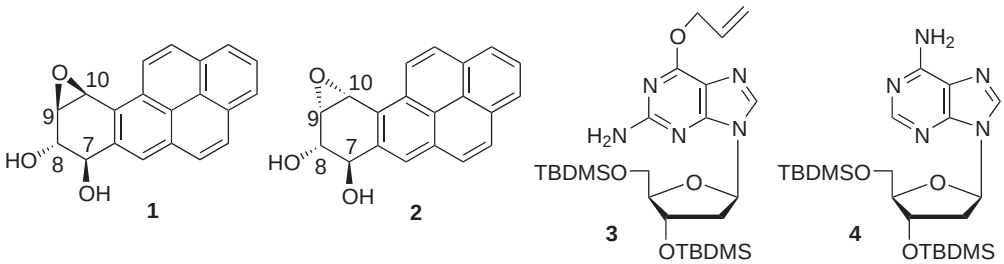
Abstract—The exocyclic amino groups of deoxyadenosine and deoxyguanosine readily add to C-10 of the benzo[*a*]pyrene 7,8-diol 9,10-epoxides at room temperature overnight in trifluoroethanol. Whereas the dG adducts are obtained as a mixture of *cis*- and *trans*-opened diastereomers, the dA adducts arise exclusively by *cis*-opening. Published by Elsevier Science Ltd.

Benzo[*a*]pyrene (B[*a*]P), one of the most studied carcinogenic polycyclic aromatic hydrocarbons (PAH), is metabolically activated to form bay-region diol epoxides (DE) in which the benzylic hydroxyl group and the oxiranyl ring are either *cis* (DE-1) **1** or *trans* (DE-2) **2**.[1] It is believed that DE of the PAH exert their carcinogenic properties by forming stable DNA adducts, primarily at the exocyclic amino groups of deoxyguanosine (dG) and deoxyadenosine (dA).[2,3] In order to elucidate the role of these adducts in carcinogenesis, there has been a continued need for oligonucleotide containing site-specific as well as stereospecific adducts from B[*a*]P and other PAH.

Most reports of the synthesis of dA and dG DE adducts involve a nucleophilic replacement reaction on protected deoxyinosine derivatives with either fluoro-[4–6] or sulfonate leaving groups[7–10] by diol epoxide derived aminotriols and their subsequent incorporation into oligonucleotides. All these approaches involve multistep syntheses of the appropriate deoxyinosine derivatives[4,6,9,10] and aminotriols. Although the synthesis of 9,10-*trans* aminotriols by direct opening of **1** and **2** with ammonia in a Parr reactor[4,11] is straightforward, synthesis of the corresponding 9,10-*cis* aminotriols of **1** and **2** requires several steps.[12,13]

Recently, our group made significant breakthroughs in the synthesis of *cis*-opened N^6-dA adducts of **2** employing the Sharpless aminohydroxylation reaction[13] and in the preparation of *cis*- and *trans*-opened N^2-dG adducts of **1** and **2** on heating in dimethylacetamide.[14] We now report a new method for the synthesis of *cis*- and *trans*-opened N^2-dG and *cis*-opened N^6-dA



Scheme 1.

Keywords: benzo[*a*]pyrene; diol epoxides; dA and dG adducts; trifluoroethanol.

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Table 1. Trifluoroethanol mediated synthesis of *cis*- and *trans*-opened N^2-dG and N^6-dA adducts

Entry	Diol epoxide	Nucleoside	Yield[a] (%)	Adduct ratio (*cis*:*trans*)
1	**1**	**3**	65	85:15 (**5**:**6**)
2	**2**	**3**	43 (65)[b]	40:60 (**7**:**8**)
3	**1**	**4**	22	100:0 (**9**)
4	**2**	**4**	33	100:0 (**10**)

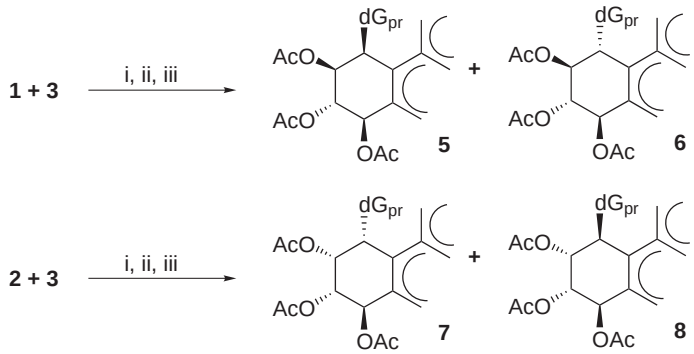
[a] After acetylation.
[b] Reaction in either hexafluoro-2-propanol or perfluoro-*tert*-butanol at rt was complete in 10–15 min.

adducts, employing O^6-allyl-3′,5′-di-TBDMS protected dG[14] **3** and 3′,5′-di-TBDMS protected dA[13] **4** for the direct opening of B[*a*]P DE-1 and DE-2 (Scheme 1) at room temperature in the high dielectric solvent trifluoroethanol (TFE).

Table 1 summarizes our results[15] for the direct opening reactions of DE **1** and **2** by the dG and dA derivatives **3** and **4** used in this study. Reaction of **1** with an excess of protected dG **3** (Scheme 2; entry 1) in TFE afforded a mixture (ratio 85:15) of *cis*- and *trans*-opened N^2-dG adducts as their acetates **5** and **6**, respectively (65% combined yield after acetylation). In contrast, reaction of **2** (Scheme 2; entry 2) with **3** resulted in a 40:60 mixture of the corresponding *cis*- and *trans*-opened N^2-dG adducts as their acetates **7** and **8**, respectively (43% combined yield after acetylation). No product was obtained when the O^6-position of dG was unprotected. This demonstrates the importance for protection of the O^6-position in order to increase the normally very weak nucleophilicity of the exocyclic N^2-amino group of dG.[16]

The *cis*- and *trans*-opened N^2-dG adducts of DE-1 and DE-2 were separated by HPLC prior to acetylation[14,15] (Fig. 1). Since the starting diol epoxides are racemic and the protected dG **3** is optically pure, the *cis*-opened adducts (**5** and **7** as their acetates) as well as the *trans*-opened adducts (**6** and **8** as their acetates) each consist of a mixture of two diastereomers. Interestingly, only the *cis*-N^2-dG adducts of DE-1 were separable before acetylation (Fig. 1). However, the diastereomeric pairs of adducts could be separated in a second HPLC step as their acetates[14] **6**, **7**, and **8** obtained from the individual *cis*- and *trans*-N^2-dG adducts (from the first HPLC separation of the unacetylated adducts, Fig. 1). This two-step chromatographic procedure was used for diastereomer separation, since mixtures containing the diastereomeric acetates from both *cis*- and *trans*-opened N^2-dG-adducts of DE-1 and DE-2 were not readily separable by HPLC due to overlapping peaks. In practice, separation of the diastereomers at this point of synthesis is unnecessary since the resultant pairs of adducted oligonucleotides (10*R*- and 10*S*-adducts) generally separate on HPLC.

Interestingly, reaction of the sugar silylated dA **4** with either **1** or **2** in TFE resulted in the exclusive formation



Scheme 2. (i) CF_3CH_2OH; (ii) HPLC; (iii) Ac_2O, DMAP, pyridine.

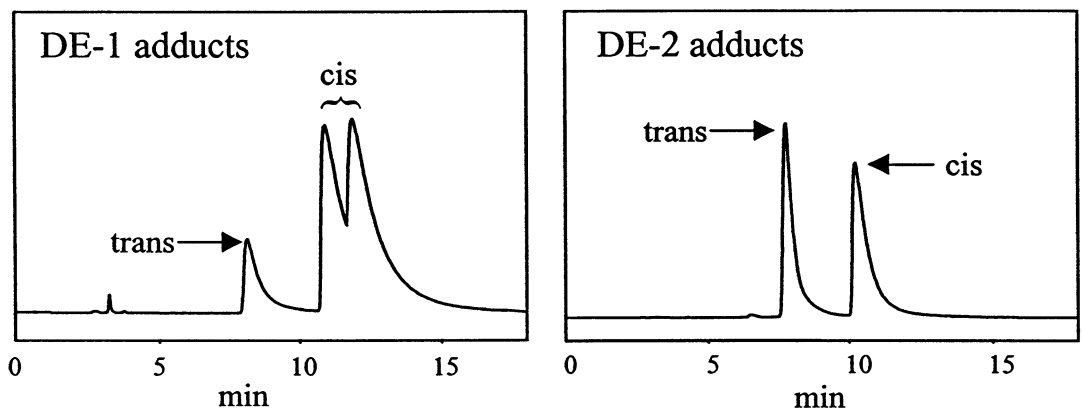
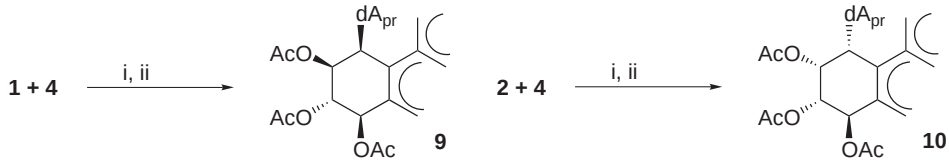


Figure 1. HPLC separation of the unacetylated, O^6-protected N^2-dG adducts.



Scheme 3. (i) CF_3CH_2OH; (ii) Ac_2O, DMAP, pyridine.

of *cis*-opened N^6-dA adducts **9** and **10** (Scheme 3; Table 1, entries 3 and 4) in 22 and 33% yield, respectively, after acetylation. This result is particularly significant since **9** has not been accessible by aminohydroxylation.[13] The major side product in these dA reactions (~50%) consists of *trans*-addition of TFE at C-10 to either **1** or **2**. We explored the use of the sterically hindered but also more acidic hexafluoro-2-propanol and perfluoro-*tert*-butanol. Although the yield improved for the reaction of the dG-derivative **3** with **2** from 43 to 65% (Table 1), there was little improvement for reaction with **1**. Furthermore, no reaction occurred between the protected dA **4** and either **1** or **2** in these solvents.

In conclusion, we report a novel TFE mediated synthesis of *cis*-opened N^6-dA and *cis*- and *trans*-opened N^2-dG adducts of B[*a*]P DE-1 and DE-2. This enables for the first time a convenient access to the elusive *cis*-opened N^6-dA and N^2-dG adducts of B[*a*]P DE-1. We attribute our finding that this reaction takes place at room temperature to an enhanced nucleophilicity of the exocyclic amino groups of both dA and dG in the presence of TFE as well as potential acid catalysis by the solvent. The methodology reported here should have high applicability especially for the synthesis of *cis*-N^6-dA and N^2-dG DE adducts of other PAH. Recently, cyclohexene and styrene oxides have been found to react efficiently with amines in fluoroalkyl alcohols.[17] In addition, TFE compared to other solvents has dramatic effects on the positions at which dG and its anion are alkylated.[18]

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15. In a typical experiment 20 mg (66 μmol) of **1** was reacted with a 4.0 molar excess of protected dG **3** in 5 mL of TFE (passed through aluminum oxide prior to use) overnight at 25°C. After evaporation of the solvent, the residue was purified by chromatography (column: 30×2 cm) on silica (CH_2Cl_2–MeOH, 95:5) to afford the mixture of *cis*- and *trans*-opened N^2-dG adducts. This mixture of adducts was separated by HPLC (ratio 85:15) on an Axxiom silica column (10×250 mm, 5 μm), eluted isocratically at a flow rate of 5 mL/min with a mixture of ethyl acetate/hexane (75:25 for DE-2 adducts and 70:30 for DE-1 adducts). The individual *cis*- and *trans*-opened N^2-dG adducts were then acetylated overnight with acetic anhydride in pyridine containing a catalytic amount of DMAP.[14] Evaporation of the solvent followed by chromatography on silica (CH_2Cl_2–MeOH, 98:2) afforded the acetylated N^2-dG adducts **5** (35.2 mg) and **6** (6.1 mg) (65% combined yield). All compounds gave satisfactory ^{1}H NMR and high-resolution mass spectroscopy data and were in accord with the published data.[13,14] All yields are based on isolated, purified materials.
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