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IMPROVED HIGH-YIELD SYNTHESIS OF POLYCYCLIC AROMATIC HYDROCARBON AMINO TRIBENZOATES, NUCLEOPHILIC COMPONENTS FOR SYNTHESIS OF DIOL EPOXIDE-NUCLEOSIDE ADDUCTS

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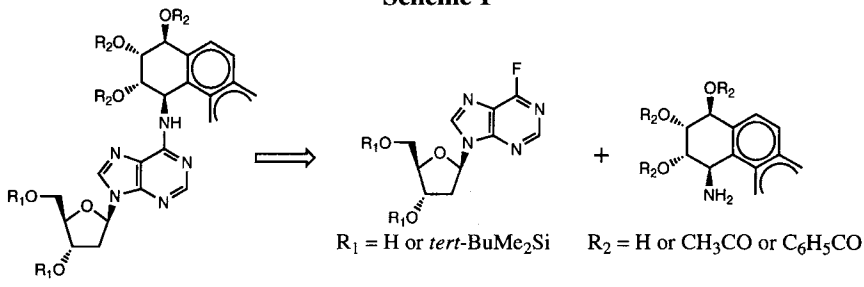
ABSTRACT: This report describes an improved high-yield synthesis of amino tribenzoates derived through a trans-ring opening of diol epoxides. A significant difference in the relative reactivities of benzo[*a*]pyrene series-1 and series-2 diol epoxide diastereomers with LiN_3 has been noted. Facile triacylation of the azido triols derived through this ring-opening with benzoyl cyanide and reduction using PtO_2 afforded the corresponding amines in high yields.

Binding of electrophiles to cellular DNA is thought to be the first step in the mutational event that could ultimately lead to neoplasia. Bay-region diol epoxides, which are ultimate carcinogens formed by metabolism of the environmentally prevalent polycyclic aromatic hydrocarbons, have been shown to alkylate DNA.[1,2] Although a substantial amount of information on the structures of individual diol epoxide-nucleoside adducts is available, an understanding of the relationship of individual adducts to cell transformation has not emerged.[2] A current approach to improved understanding entails developing strategies for the site-specific modification of DNA with stereochemically defined polycyclic aromatic hydrocarbon metabolites and evaluation of the effects upon biochemical processes such as mutation.[3,4] We have shown that 6-fluoro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (shown in Scheme 1) is sufficiently electrophilic to undergo coupling reactions with the hindered, axially constrained bay-region amines derived from hydrocarbon epoxides to provide access to 2'-deoxyadenosines alkylated at N^6.[5]

Scheme 1

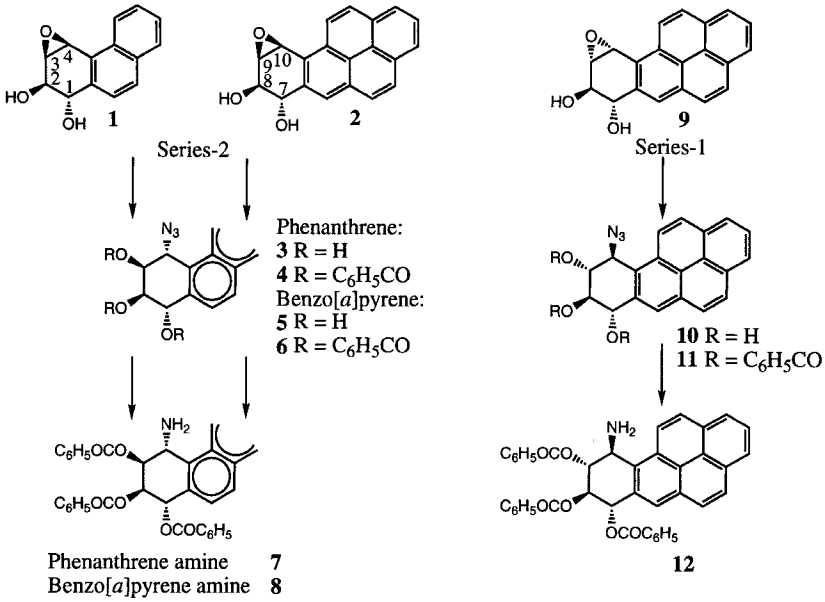


R_1 = H or *tert*-$BuMe_2Si$ R_2 = H or CH_3CO or C_6H_5CO

For use in oligonucleotide synthesis, since there are a total of five hydroxyl groups that need to be selectively protected and derivatized, two alternatives can be envisioned: (a) the nucleoside component can be protected and then coupled with the amino triols derived by a direct aminolysis of the diol epoxide,[3] or (b) a suitably protected hydrocarbon component can be directly coupled with the fluoro nucleoside. In the first approach, two additional steps (acetylation of the hydrocarbon hydroxyls and desilylation) are necessary subsequent to the convergent coupling step, before the nucleoside can be converted to the 5'-*O*-DMT 3'-*O*-phosphoramidite, the critical intermediate needed for oligonucleotide synthesis. In the latter approach, although three steps are required to convert the diol epoxide to the amine with protected hydroxyl groups, no additional steps are required after the critical coupling step. Hence, we have been interested in developing this alternative approach to carcinogen modified DNA.

In the past, we have used the benzoyl rather than the acetyl functionality as a protecting group due to its higher stability. In addition, in our hands, the amino acetates have been elusive due to O→N acyl migration.[6,7] In our original report on the synthesis of amines from hydrocarbon epoxides,[7] we used the N_3^- form of Amberlite resin for the ring-opening. The azido benzoates were prepared via an acylation of the trialkoxide produced by NaH in pyridine, and the reduction of the azide functionality was performed using Lindlar catalyst. The first two reactions had been yield limiting and although the reduction proceeds in good yield, this reaction is very sluggish. Compound **8** (shown in Scheme 2) has been prepared in 18% overall yield (55% for the ring opening, 44% for the acylation and 77% for the reduction) from the diol epoxide.[7] In this report, we describe a markedly improved, general procedure for the preparation of these amines. This report also documents the first synthesis of the nucleophilic component from a series-1 diol epoxide, namely the amino tribenzoate **12**.

Scheme 2



Initially, we decided to explore the methodology using the non-carcinogenic phenanthrene diol epoxide **1**. Although **1** can be easily converted to the azido triol **3** by NaN_3 in DMF at 65 C, the same transformation can be accomplished in 6-8 h at room temperature through the use of the more soluble LiN_3, in comparable yields (55-60%). Facile benzoylation of hydroxyl groups with benzoyl cyanide in DMF, in the presence of Et_3N has been reported.[8] Under these conditions, in contrast to the previously described approach,[7] the azido triol **3** was very smoothly converted to the tribenzoate within 0.5 h, and **4** was isolated in 96% yield after chromatography. Catalytic reduction of the azide functionality using PtO_2 (Adams catalyst) in 1:1 THF-EtOH, under a hydrogen balloon for 0.5 h afforded the amine **7** in quantitative yield after chromatography. The overall yield for conversion of the epoxide to the amine was 57%. Next, we investigated the corresponding transformation on the tumorigenic BaP diol epoxide **2**. In this case the ring opening by LiN_3 was allowed to proceed overnight, since diol epoxide was present after 8 h at room temperature. The isolated yield of **5** was 69%. Acylation as above (97%) and reduction (92%) gave the amino tribenzoate **8** in 61% overall yield. This represents a ***three-fold improvement*** in the yield for this compound. It should also be noted that the

azido triols obtained from the reaction of the series-2 diol epoxides with LiN_3 generally require no purification and can be used as such in the subsequent step. For typical reaction conditions see experimental section.

With this data in hand, we investigated the ring opening of diol epoxide **9** by LiN_3. The ring opening was complete within 1 h, which is consistent with the significantly higher reactivity of the series-1 diol epoxide under S_N2 conditions.[9] The enhanced reactivity in the case of **9** may be due to the possible intramolecular hydrogen bonding between the C-7 hydroxyl proton and the oxirane, which could then lead to the facile C-O bond scission. It should be noted that the hydroxyl moieties in the series-1 diastereomers are quasi-diaxial (facilitating this hydrogen bond), whereas the series-2 isomers have quasi-diequatorial hydroxyls. Azido triol **10**, purified by chromatography (79% yield), was triacylated as described above (92%). Finally, catalytic reduction (quantitative), gave the amino tribenzoate **12** in 73% overall yield. A tabulation of the chemical shifts and coupling constants for the tetrahydrobenzo-ring protons in the various products described, is given below.

Table: Chemical shifts and coupling constants for the tetrahydrobenzo-ring protons. (Data obtained at 300 MHz in $CDCl_3$ unless indicated otherwise.[a])

Cpd.	H-7 (1)	H-8 (2)	H-9 (3)	H-10 (4)	J (Hz)
3[b]	d 5.24	app t 4.39	dd 3.99	d 4.90	1,2 = 8.3; 2,3 = 2.5; 3,4 = 3.5
4	d 5.59	m 6.11-6.15		d 7.15	1,2 = 8.5; 3,4 = 3.4
5[b]	d 5.21	dd 4.15	app t 4.53	d 5.65	7,8 = 8.6; 8,9 = 2.3; 9,10 = 3.5
6	*c*	m 6.27-6.31		d 5.93	9,10 = 3.5
7	d 5.15	br m 5.96	dd 6.43	d 7.19	1,2 = 9.1; 2,3 = 2.3; 3,4 = 2.8
8	*c*	dd 6.58	br s 6.09	d 5.45	7,8 = 9.8; 8,9 = 2.0; 9,10 = 3.0
10[b]	d 5.03	t 3.77	dd 4.32	d 5.51	7,8 = 8.1; 8,9 = 7.9; 9,10 = 5.4
11	d 7.16	t 6.02	dd 6.11	d 5.90	7,8 = 6.3; 8,9 = 5.7; 9,10 = 3.5
12	*c*	t 6.04	dd 5.87	d 5.44	7,8 ≃ 8,9 ~ 6.0; 9,10 = 2.6

[a]Numbers in parentheses indicate the protons in the phenanthrene skeleton, where the tetrahydro-ring is numbered 1 through 4. [b]Spectra were acquired in acetone-d_6, hydroxyl protons have been exchanged. [c]Appears below 7.30 ppm along with the aromatic resonances.

We have also investigated the adduct forming reaction of amine **8** with 6-fluoro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine. The adduct formation proceeds in good yield (68%) and the diastereomeric adducts can be resolved by HPLC. Conversion of the individual adducts to the 5'-*O*-DMT 3'-*O*-phosphoramidites and incorporation into DNA oligomers has also been accomplished. The synthetic sequence leading to the adducted oligonucleotides and physical measurements on the DNA fragments will be reported separately. With this substantial improvement in yields by the presently described methodology, the strategy involving protection of the hydrocarbon residue prior to the synthesis of the adducted nucleoside should be an equally viable approach to the ones described in the literature[4,5] for the synthesis of DNA oligomers containing diol epoxide adducts at defined sites.

EXPERIMENTAL SECTION

Given below is a description for the preparation of the amino tribenzoate **12** which is a typical procedure for the synthesis of any of the amines described.

(±)-10β-Azido-7α,8β,9α-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (10):
To a stirred solution of diol epoxide **9** (30 mg, 0.1 mmol) in anhydrous DMF (1 mL), LiN_3 (48.6 mg, 1 mmol) was added and the mixture was stirred at rt, under argon, until the diol epoxide was completely consumed (within 1 h in this case). The mixture was diluted with EtOAc and washed sequentially with 5% aq. NH_4Cl (2 x 5 mL) and water. Evaporation of the organic layer after drying over Na_2SO_4 gave a yellow oil. Chromatography of the crude product on a silica gel column using EtOAc gave **10** as a white solid (26.7 mg, 79%).

(±)-10β-Azido-7α,8β,9α-tris(benzoyloxy)-7,8,9,10-tetrahydrobenzo[*a*]pyrene (11):
The azido triol (26.7 mg, 0.077 mmol) was dissolved in anhydrous DMF (1 mL), Et_3N (100 μL) and benzoyl cyanide (0.101 mg, 0.77 mmol) were added and the mixture was stirred at rt for 0.5 h. The reaction was quenched by addition of a few drops of MeOH and diluted with EtOAc. The organic layer, after extraction twice with brine, was dried over Na_2SO_4 and evaporated to yield a reddish oil. Chromatography of this product on a silica gel column using 20% hexane in CH_2Cl_2 gave pure **11** (46.5 mg, 92%) as a pale yellow solid.

(±)-10β-Amino-7α,8β,9α-tris(benzoyloxy)-7,8,9,10-tetrahydrobenzo[*a*]pyrene (12): Adams catalyst (10 mg) was stirred in EtOH (1 mL). A solution of the azido tribenzoate (31.4 mg, 0.048 mmol) in THF (1 mL) was added and the mixture was stirred for 0.5 h under a hydrogen balloon, at which time the azide was completely consumed. The mixture was filtered through Celite and the filtrate was evaporated. Chromatography of the crude amine on a silica gel column using 1% MeOH in CH_2Cl_2 gave pure **12** (30.1 mg, quantitative) as a white solid.

High Resolution Mass spectral data for the amino tribenzoates (Positive FAB, M+1 observed):

7	Calculated 558.1917	Found 558.1942
8	Calculated 632.2073	Found 632.2076
12	Calculated 632.2073	Found 632.2064

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