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Synthesis of Adducts of *o*-Quinone Metabolites of Carcinogenic Polycyclic Aromatic Hydrocarbons with 2′-Deoxyribonucleosides

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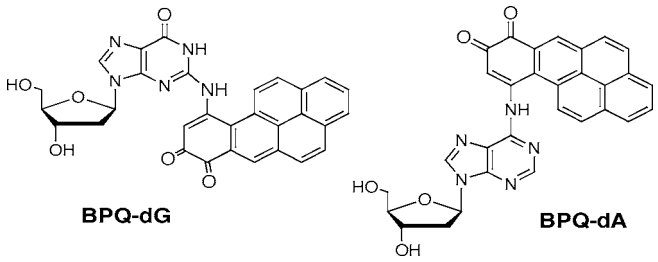
ABSTRACT



The first syntheses of the adducts formed in the reactions of *o*-quinone metabolites of carcinogenic polycyclic aromatic hydrocarbons (BPQ and BAQ) at 2′-deoxyadenosine and 2′-deoxyguanosine sites in DNA are reported. These syntheses entail Pd-catalyzed coupling of protected amine derivatives of catechols with suitably protected halopurine analogues of 2′-deoxyribonucleosides.

Polycyclic aromatic hydrocarbons (PAHs), some of which are potent carcinogens, are common environmental pollutants produced in the combustion of organic matter.[1,2] PAHs are activated enzymatically to reactive metabolites that attack DNA to form adducts that result in mutations and induction of tumors.[2−4] At least two mechanisms are operative. The most studied pathway involves activation by cytochrome P-450 (CYP) enzymes to diol epoxides, e.g., benzo[*a*]pyrene *anti*-diol epoxide (*anti*-BPDE) (Figure 1).[2,5] The alternative pathway involves activation by aldo-keto reductase (AKR) enzymes to catechols that enter into redox cycles with quinones, e.g., benzo[*a*]pyrene-7,8-dione (BPQ), thereby

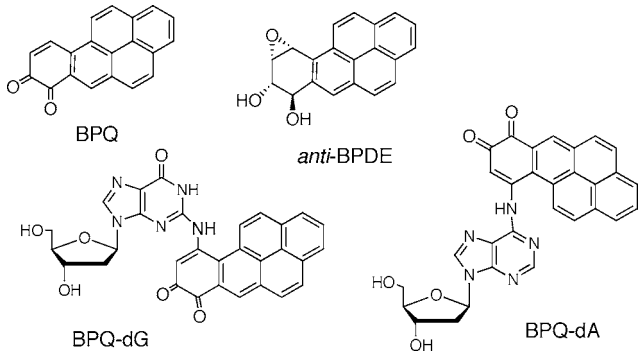


Figure 1. Active metabolites of benzo[*a*]pyrene (*anti*-BPDE, BPQ) and structures of the BPQ-dG and BPQ-dA adducts.

(1) International Agency for Research on Cancer. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. *Polynuclear Aromatic Compounds, Part 1*; IARC: Lyon, France, 1983; Vol. 32.

(2) Harvey, R. G. *Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity*; Cambridge University Press: Cambridge, U.K., 1991.

(3) Jeffrey, A. M.; Weinstein, I. B.; Jennette, K.; Grzeskowiak, K.; Nakanishi, K.; Harvey, R. G.; Autrup, H.; Harris, C. *Nature (London)* **1977**, *269*, 348−350. Jennette, K.; Jeffrey, A. M.; Blobstein, S. H.; Beland, F. A.; Harvey, R. G.; Weinstein, I. B. *Biochemistry* **1977**, *16*, 932−938.

(4) Dipple, A. In *DNA Adducts: Identification and Biological Significance*; Hemminki, K., Dipple, A., Segerbäck, D., Kadulbar, F. F., Shuker, D., Bartsch, H., Eds.; IARC: Lyon, France, 1994; Sci. Pub. no. 125, pp 107−129.

(5) Conney, A. H. *Cancer Res.* **1982**, *42*, 4875.

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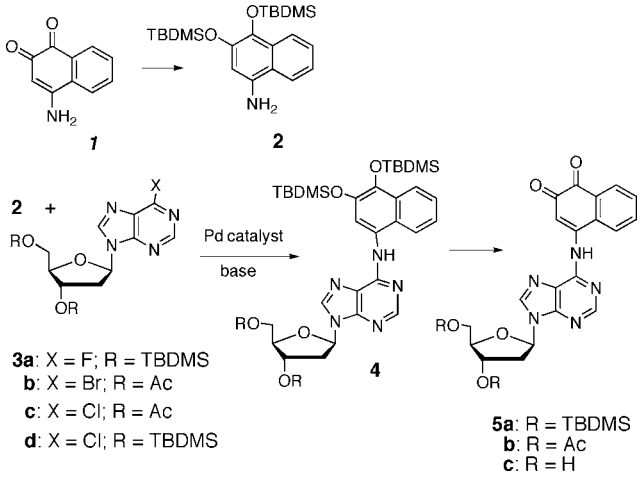
generating reactive oxygen species that attack DNA.[6,7] The quinones combine with DNA to form stable and depurinating adducts.[8,9] This mechanism parallels AKR-mediated activation of estrogens.[10]

The PAH diol epoxides react at 2′-deoxyguanosine (dG) and 2′-deoxyadenosine (dA) sites in DNA to afford adducts whose structures are well-established.[2−4,11] The stable adducts of the PAH quinones are presumed to arise via 1,4-Michael addition of the purines in DNA to the quinones followed by auto-oxidation of the air-sensitive catechol intermediates. However, the structures of the stable BPQ-dG and BPQ-dA adducts (Figure 1)[8b] were not confirmed by independent synthesis.

The aim of this investigation was to devise methods for the synthesis of adducts of PAH quinones with dA and dG. These adducts are urgently required for studies of the mechanisms of PAH carcinogenesis. Syntheses of similar adducts of a noncarcinogenic quinone, 1,2-naphthoquinone (NQ), were reported,[8,12] but attempted extension of the methodology to the BPQ adducts was not successful.

Our synthetic approach entailed Pd-catalyzed coupling of an amine derivative of a PAH quinone (or catechol) with a halopurine analogue of a 2′-deoxyribonucleoside (Scheme 1). Initial studies were conducted with 4-amino-NQ (**1**),[13]

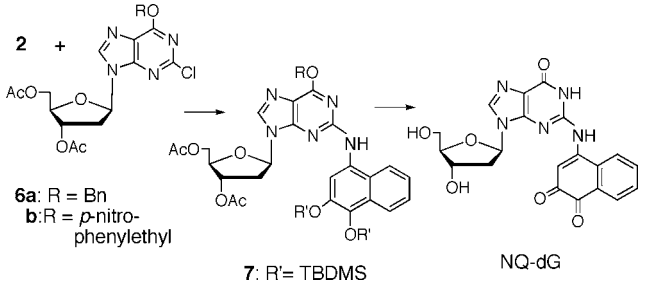
Scheme 1. Synthesis of NQ-dA Adduct



but coupling of **1** with **3a** failed to take place under various conditions.[14,15] Since this was likely a consequence of the strong electron-withdrawing character of the carbonyl groups, a protected catechol derivative (**2**) was prepared by reduction of **1** with $NaBH_4$ and reaction of the air-sensitive catechol with TBDMS-Cl.[16−18] Compound **2** reacted smoothly with the bromo-dA analogue **3b** in the presence of $Pd(OAc)_2$, BINAP, and Cs_2CO_3 at 80 °C overnight to furnish adduct **4** (47%).[19] Similar reaction of the chloro-dA analogue **3c** required only 1 h for completion at 60 °C and gave **4** in higher yield (85%). Treatment of **4** with TBAF in CH_3CN furnished the deprotected catechol, which underwent auto-oxidation to the quinone and subsequent deacetylation with TMG to yield NQ-dA (**5c**).

A similar strategy was adopted for synthesis of NQ-dG (Scheme 2). Pd-catalyzed coupling of **2** with the benzyl ether

Scheme 2. Synthesis of NQ-dG Adduct



derivative of the chloropurine analogue of dG (**6a**) at 60 °C for 1 h furnished **7** (88%). Hydrogenolysis of **7** over a Pd catalyst followed by consecutive deacetylation with TMG and removal of the TBDMS groups by treatment with TBAF furnished NQ-dG.

Synthesis of the analogous BPQ-dG and BPQ-dA adducts was carried out by similar methods (Scheme 3). Reaction of BPQ[20] with Me_3SiN_3 in DMF gave 10-amino-BPQ. This was transformed to the aminocatechol derivative (**8a**) by reductive hydrogenation over a Pd catalyst and treatment of the aminocatechol product with *N*-methyl-*N*-TBDMS trifluoroacetamide. Pd-catalyzed coupling of **8a** with **3c** by the procedure employed for synthesis of **5** furnished a bis-adduct

(6) Smithgall, T. E.; Harvey, R. G.; Penning, T. M. *J. Biol. Chem.* **1986**, *261*, 6184−6191; *Cancer Res.* **1988**, *48*, 1227−1232; *J. Biol. Chem.* **1988**, *263*, 1814−1820.

(7) Penning, T. M.; Onishi, S. T.; Onishi, T.; Harvey, R. G. *Chem. Res. Toxicol.* **1996**, *9*, 84−92. Flowers, L.; Onishi, S. T.; Penning, T. M. *Biochemistry* **1997**, *36*, 8640.

(8) (a) Shou, M.; Harvey, R. G.; Penning, T. M. *Carcinogenesis* **1993**, *14*, 475−482. (b) McCoull, K. D.; Rindgen, D.; Blair, I. A.; Penning, T. M. *Chem. Res. Toxicol.* **1999**, *12*, 237−246.

(9) A third mechanism has been proposed that involves activation of PAHs by CYP peroxidase to generate PAH radical-cations that combine with DNA to form depurinated adducts: Cavalieri, E. L.; Rogan, E. *Xenobiotica* **1995**, *25*, 677. Its relevance is disputed: Melendez-Colon, V.; Luch, A.; Seidel, A.; Baird, W. *Carcinogenesis* **1999**, *20*, 1885.

(10) Bolton, J. I.; Trush, M. A.; Penning, T. M.; Dryhurst, G.; Monks, T. J. *Chem. Res. Toxicol.* **2000**, *13*, 135−160.

(11) Lee, H.; Luna, E.; Hinz, M.; Stezowski, J. J.; Kiselyov, A. S.; Harvey, R. G. *J. Org. Chem.* **1995**, *60*, 5604−5613.

(12) Gopishetty, S. R.; Harvey, R. G.; Lee, S.-H.; Blair, I. A.; Penning, T. M. In *Aldo-Keto Reductases and Toxicant Metabolism*; Penning, T. M., Petrash, J. M., Eds.; ACS Symposium Series 865; American Chemical Society: Washington, DC, 2004; Chapter 9, pp 127−137.

(13) Husu, B.; Kafka, S.; Kadunc, Z.; Tisler, M. *Monat. Chem.* **1988**, *119*, 215−222.

(14) Fluoropurine **3a** is known to react more readily with arylamines, such as 1-aminopyrene, than its bromo- or chloro analogues.[11]

(15) Coupling of **1** with **3a** also failed to take place in the presence of the Pd catalyst system successfully employed for coupling **2** and **3b**.

(16) Although PAH catechols are highly susceptible to air oxidation, their diester or diether derivatives are relatively stable in air.

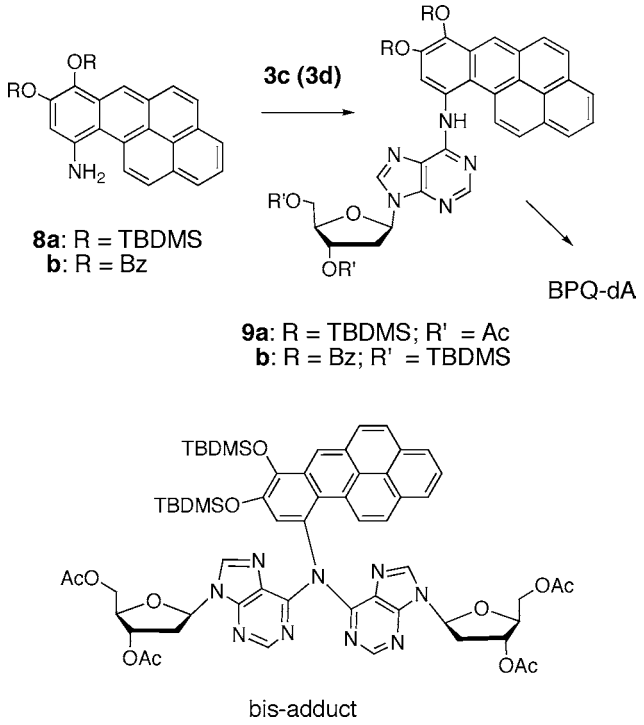
(17) Reagent abbreviations: TBDMS-Cl = *tert*-butyldimethylsilyl chloride; BINAP = 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl; TBAF = tetrabutylammonium fluoride; TMG = *N*,*N*,*N*′,*N*′-tetramethylguanidine.

(18) Cho, H.; Harvey, R. G. *J. Chem. Soc., Perkin I* **1976**, 836−839.

(19) A bis-adduct with two 2′-deoxyribonucleoside groups attached to the nitrogen atom of the aminocatechol was a minor product.

(20) Harvey, R. G.; Dai, Q.; Ran, C.; Penning, T. M. *J. Org. Chem.* **2004**, *64*, 2024−2032.

Scheme 3. Synthesis of BPQ-dA Adduct

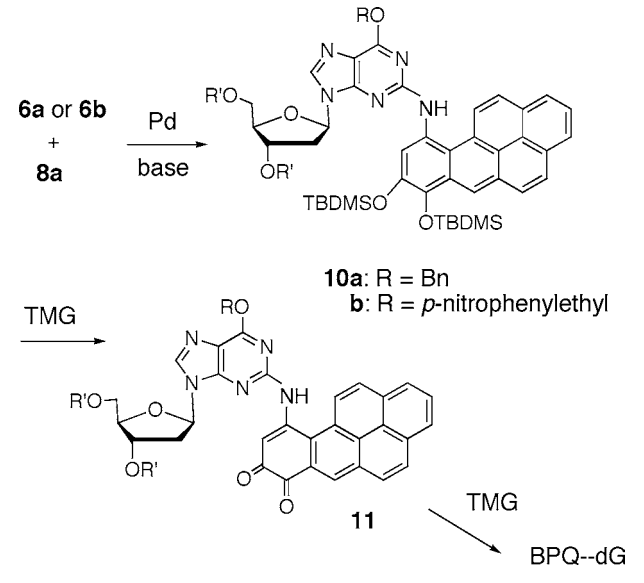


plus a small amount of the desired mono-adduct (**9a**). A more favorable product ratio resulted from use of the dibenzoate ester (**8b**) in place of **8a**. Pd-catalyzed reaction of **8b** with **3d** afforded **9b** and a bis-adduct in 2:1 ratio. Debenzoylation of **9b** with TMG provided the corresponding catechol, which underwent oxidation in air to yield BPQ-dA as its TBDMS derivative (**9a**). Treatment of **9a** with TBAF furnished BPQ-dA.

Synthesis of BPQ-dG was accomplished by modification of the procedure for preparation of NQ-dG (Scheme 4). Pd-catalyzed coupling of **8a** with **6a** took place smoothly at 60 °C to furnish an adduct (**10a**) (82%). Removal of the benzyl groups of **10a** by Pd-catalyzed hydrogenolysis was accompanied by partial reduction of the aromatic ring system. To obviate this problem, the *p*-nitrophenylethyl ether analogue (**6b**) was substituted for **6a**.[11,21] Analogous coupling of **8a** with **6b** gave (**10b**) in good yield (88%). Deacetylation of **10b** with TMG at room temperature, followed by removal of solvents under vacuum and allowing the solution to stand overnight, afforded an equal mixture of BPQ-dG and its *p*-nitrophenylethyl ether (**11**). Transformation of **11** to BPQ-dG was complete with longer reaction time. It is convenient that all three protecting groups may be removed by treatment with a single reagent.

This synthetic approach was extended successfully to synthesis of the analogous adducts of benz[*a*]anthracene-3,4-dione (BAQ) with both dA and dG (Figure 2). The adducts (BAQ-dA and BAQ-dG) were obtained in excellent overall yields. It is worthy of note that in the synthesis of BAQ-dA a bis-adduct was not detected as a significant product.[22]

Scheme 4. Synthesis of BPQ-dG Adduct



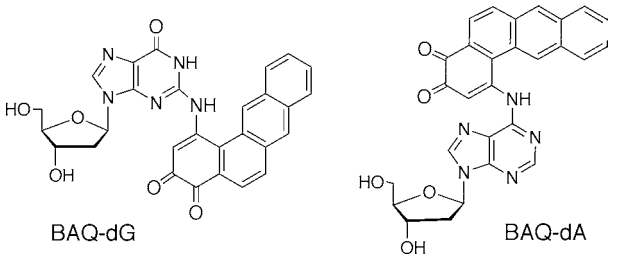


Figure 2. Structures of the BAQ-dG and BAQ-dA adducts.

In related studies, Balu et al.[23] have recently identified several adducts formed in low yields in the reactions of BPQ with dG and dA in DMF−phosphate buffer solutions (pH 7.5) at 50−60 °C.[5] Reaction of BPQ with dG gave a mixture of two pairs of diastereomeric adducts in 2.5−3.5% yield each. One of these pairs of adducts was assigned a structure corresponding to a product of hydration of BPQ-dG. There is currently no evidence that supports formation of these types of adducts in vivo.

In summary, we have reported the first syntheses of the dA and dG adducts of *o*-quinone metabolites of carcinogenic PAHs (BPQ and BAQ) (Figures 1 and 2). The structures of these adducts correspond to those proposed[8,10,24] for the stable adducts formed by the reactions of PAH quinones at dA and

(21) Lee, H.; Hinz, M.; Stezowski, J. J.; Harvey, R. G. *Tetrahedron Lett.* **1990**, *31*, 6773−6776.

(22) Full details of the syntheses of BAQ-dA and BAQ-dG will be reported separately.

(23) Balu, N.; Padgett, W. T.; Lambert, G. R.; Swank, A. E.; Richard, A. M.; Nesnow, S. *Chem. Res. Toxicol.* **2004**, *17*, 827−838.

(24) Penning, T. M.; Palackal, N. T.; Lee, S.-L.; Blair, I.; Yu, D.; Berlin, J. A.; Field, J. M.; Harvey, R. G. In *Aldo-Keto Reductases and Toxicant Metabolism*; Penning, T. M., Petrash, J. M., Eds.; ACS Symposium Series 865; American Chemical Society: Washington, DC, 2004; Chapter 6, pp 83−99.

dG sites in DNA in vivo.[6] In principle, this general methodology is applicable to synthesis of similar adducts of other carcinogenic and noncarcinogenic PAH quinones, as well as to the synthesis of analogous adducts of estrogenic quinones.[10] The BPQ-dG adduct may also be expected to serve as a convenient synthetic precursor of the hydrated BPQ-dG adducts formed by reaction of BPQ with dG in phosphate buffer solution.[23]

The BPQ-dA and BPQ-dG adducts have been provided to Dr. Trevor Penning and Dr. Ian Blair at the University of Pennsylvania for investigations to elucidate the role of these adducts in the mechanism(s) of PAH carcinogenesis. Syntheses of the N^{15}-labeled analogues of the BPQ-dA and BPQ-dG adducts have also been accomplished, and these compounds have been furnished to Dr. Blair as standards for LC-MS-MS studies to develop methods for the screening and detection of these types of adducts in human tissues.

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Supporting Information Available: Experimental details and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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