Published in final edited form as:

Curr Protoc Nucleic Acid Chem. ; 69: 4.73.1–4.73.15. doi:10.1002/cpnc.28.

Synthesis of Oligodeoxynucleotides Containing a C8-2'-Deoxyguanosine Adduct Formed by the Carcinogen 3-Nitrobenzanthrone

Arindom Chatterjee¹, Chanchal K. Malik^{1,2}, and Ashis K. Basu¹

¹Department of Chemistry, University of Connecticut, Storrs, Connecticut 06269

²Department of Chemistry, Vanderbilt University, College of Arts & Science, Nashville, Tennessee 37235

Abstract

This unit describes the detailed procedure for the synthesis of C8-2'-deoxyguanosine-3-aminobenzanthrone adduct located in a desired site in an oligonucleotide. The procedure has been described in five different parts. The synthesis of the protected 2'-deoxyguanosine, *O*⁶-benzyl-*N*²-DMTr-3'-5'-bisTBDMS-C8-Br-2'-deoxyguanosine, is described in the first part. The synthesis of the reduced carcinogen 3-aminobenzanthrone is detailed in part two. The third part outlines the key step of the adduct formation between the reduced carcinogen and the protected nucleoside by a palladium-catalyzed cross coupling reaction. The final two sections provide the phosphoramidite synthesis from the nucleoside-carcinogen adduct followed by its site-specific incorporation in DNA by solid-phase oligonucleotide synthesis. The adducted oligonucleotides are purified by reverse phase HPLC and characterized by mass spectrometry.

Keywords

DNA adduct; environmental carcinogen; palladium-catalyzed; cross coupling; C8 guanine adduct; DNA lesion bypass

INTRODUCTION

3-Nitrobenzanthrone (3-nitro-7*H*-benz-*[d,e]*anthracen-7-one, 3-NBA), a nitropolycyclic aromatic hydrocarbon (nitro PAH) compound, is found in exhausts from diesel and gasoline engines due to incomplete combustion of the fuel (Pearce, 1997). It is an environmental pollutant and a suspected human carcinogen (Arlt, 2005). It causes DNA damage and exhibits mutagenicity in bacterial and mammalian cells (Arlt et al., 2003, 2004, 2005; Bieler et al., 1999; Murata et al., 2006; Phousongphouang et al., 2000; Takamura-Enya et al., 2007). 3-NBA is one of the most potent mutagens in the Ames' *Salmonella* reversion assay (Enya et al., 1997). 3-NBA is approximately three times more mutagenic than benzo[*a*]pyrene (B[*a*]P), the most extensively studied PAH, or 1-nitropyrene, the most abundant nitroarene found in diesel exhaust. 3-NBA undergoes metabolic nitro-reduction to form three DNA-adducts, namely, *N*⁶-dA-ABA, *N*²-dG-ABA, and C8-dG-ABA (Figure 1). C8-dG-ABA and *N*²-dG-ABA are the predominant DNA lesions *in vivo* in experimental animals (Arlt et al., 2001; Bieler et al., 2005; Bieler et al., 1999). They are also the most

persistent DNA adducts in target tissues in rodents. Chemical synthesis of the DNA-carcinogen adducts and their incorporation in DNA are essential for investigating their biological effects (Basu and Essigmann, 1988; Delaney and Essigmann, 2008). Even though synthesis of various site-specifically adducted oligonucleotides has been reported, frequently one or more dissimilar challenges are encountered in the synthetic route of each adducted DNA fragment (Wang and Rizzo, 2001; Gillet and Scharer, 2002; Gillet et al., 2005). In addition to our continued interest of the synthesis of nitropyrene-derived and cyclopurine DNA adducts and their biological relevance (Purohit and Basu, 2000; Chakraborti et al., 2003; Huang et al., 2011), we have carried out the synthesis of C8-dG-ABA containing oligonucleotides to investigate its structural and biological effects (Godkari et al., 2014; Pande et al., 2014; Patra et al., 2016). Here we wish to report the detailed synthetic route to C8-dG-ABA phosphoramidite and its incorporation in DNA. The key step in the synthesis of C8-dG-ABA adduct **3** was achieved by the Buchwald–Hartwig aryl-amination reaction (Wolfe et al., 1998; Hartwig, 1998) between the aromatic amine (3-ABA) and 8-bromo-2'-deoxyguanosine derivative **2** using a palladium catalyst and phosphine ligand.

After the successful coupling reaction, the protected functional groups of the nucleoside–carcinogen adduct was selectively deprotected to generate the precursor of the phosphoramidite monomer. The synthesis begins from the commercially available 2′-deoxyguanosine monohydrate and the process has been divided into five parts (A, B, C, D and E). Part A describes the synthesis of a key intermediate, the protected C8-bromo-2′-deoxyguanosine. Part B, elucidated in Basic Protocol 2, describes the synthesis of 3-aminobenzanthrone. Part C describes the Buchwald–Hartwig coupling reaction (Basic Protocol 3) to generate C8-dG-ABA. Part D details the phosphoramidite monomer synthesis (Basic protocol 4) and finally part E (Basic Protocol 5) describes the solid phase oligonucleotide synthesis and its purification.

CAUTION: Carry out all the reactions and purifications involving organic solvents and reagents in a well-ventilated fume hood and wearing personal protective equipment.

BASIC PROTOCOL 1: SYNTHESIS OF PROTECTED 8-BROMO-2'-DEOXYGUANOSINE

The scheme in Part I is displayed in Figure 2. Starting with 10 g (35.08 mmol) of commercially available 2'-deoxyguanosine monohydrate **1**, synthesize 18.2 g of **5** (~51 % overall yield). The first two steps involve replacement of the C8-hydrogen of 2'-deoxyguanosine with a bromine followed by silylation of both 3'-OH and 5'-OH groups. The next step is selective protection of *O*⁶-carbonyl as benzyl ether via Mitsunobu reaction followed by the dimethoxytrityl (DMTr) protection of *N*²-amine to form a fully protected C8-bromo-2'-deoxyguanosine derivative to be used as a coupling partner for the Buchwald-Hartwig aryl amination reaction.

Materials

2'-deoxyguanosine monohydrate (**1**) (Berry & Associates)

Argon gas

Acetonitrile (Sigma Aldrich Chemical)

Acetone (Sigma Aldrich Chemical)

Distilled water (DI water)

N-Bromosuccinimide (freshly recrystallized from hot DI water)

Anhydrous *N,N'*-dimethylformamide (Agros Organics)

tert-Butylchlorodimethylsilane (Agros Organics)

Imidazole (Acros Organics)

Benzyl alcohol (PhCH₂OH, Sigma Aldrich Chemical)

Triphenylphosphine (PPh₃, 99%; Acros Organics)

Diisopropylazodicarboxylate (DIAD, 94%; Acros Organics)

Anhydrous 1, 4-Dioxane (Acros Organics)

Anhydrous Pyridine (Acros Organics)

4, 4'-Dimethoxytrityl chloride (DMTr-Cl, 98%, Chem-Impex Int'l Inc)

Silica gel (63 –200 µm, 65 to 250 mesh; Sorbent Technologies)

Aluminium oxide, neutral (Brockmann I, 50±200 µm; Acros Organics)

n-Hexane for chromatography

Ethyl acetate (EtOAc) for chromatography

Dichloromethane (CH₂Cl₂) for chromatography

Methanol (MeOH) for chromatography

Round bottom flasks, 500 mL, 100 mL, 50 mL

Magnetic stir plate and magnetic stir bar

Vacuum apparatus for filtration

Oil bath

Chromatography columns

Fume hood

Thin layer Chromatography (TLC) equipment

Perform Bromination of 2'-deoxyguanosine monohydrate

- 1 Suspend 2'-deoxyguanosine monohydrate **1** (2 g, 7.5 mmol) in 20 mL mixture of acetonitrile and water (80:20) in a 50 mL round bottom flask.
- 2 To the suspended mixture, add *N*-bromosuccinimide (2 g, 11.2 mmol) slowly and stir the mixture for 45 min at room temperature. The solution should turn light yellow/orange upon addition of *N*-bromosuccinimide.

- 3** Remove the solvent by filtration, suspend the precipitate in acetone (40 mL), and stir for 2 h at room temperature, after which cool it overnight at -20°C . Collect the precipitate by filtration, wash it with cold acetone, and dry under vacuum to obtain a light purple powder of 8-bromo-2'-deoxyguanosine **2** (2.2 g, 85%).

It is not necessary to purify this derivative. But check purity using ^1H -NMR and ^{13}C -NMR. Either use the compound immediately for the subsequent step or store overnight at $0-4^{\circ}\text{C}$ to be used in a day or two.

^1H -NMR (DMSO- d_6): δ 2.10 (1H, m), 3.15 (1H, m), 3.51 (1H, dd, $J = 5.8, 11.6$ Hz), 3.62 (1H, dd, $J = 5.3, 11.6$ Hz), 3.80 (1H, br s), 4.40 (1H, br s), 4.84 (1H, s), 5.24 (1H, s), 6.15 (1H, br s), 6.47 (2H, s), 10.78 (1H, s); ^{13}C -NMR (DMSO- d_6): δ 37.0, 62.5, 71.5, 85.6, 88.4, 118.0, 121.0, 152.5, 153.8, 155.9; MS (ESI): m/z : calcd for $[\text{M}]^+ \text{C}_{10}\text{H}_{12}\text{BrN}_5\text{O}_4$ 345.0073 and observed 345.1.

Perform *tert*-butyldimethylsilane protection of alcohols

- 4** To a 50 mL round bottom flask equipped with magnetic stir bar and argon balloon, dissolve 8-bromo-2'-deoxyguanosine **2** (630 mg, 1.82 mmol) in anhydrous DMF (10 mL).
- 5** To the mixture, add imidazole (590 mg, 8.7 mmol) and *tert*-butyldimethylsilyl chloride (754 mg, 5 mmol).
- 6** The initial turbid mixture should become a clear solution after 15 min, but continue stirring it for an additional 12 h at room temperature.
- 7** Quench the reaction with 10 mL methanol and concentrate under vacuum. Purify the crude by flash column chromatography over silica gel (MeOH/DCM: 10/90) to provide a white powder compound **3** (860 mg, 82%).

TLC: R_f 0.51; 1:10 MeOH: CH_2Cl_2 , UV (254 nm)

^1H -NMR (DMSO- d_6): δ -0.02 (3H, s), -0.01 (3H, s), 0.11 (6H, s), 0.83 (9H, s), 0.89 (9H, s), 2.15 (1H, ddd, $J = 3.6, 7.0, 13.2$ Hz), 3.38 (1H, m), 3.66 (1H, m), 3.76 (2H, m), 4.58 (1H, m), 6.14 (1H, t, $J = 7.0$ Hz), 6.39 (2H, br s), 10.79 (s, 1H); ^{13}C -NMR (DMSO- d_6): δ -4.93, -4.38, -4.20, 18.20, 18.44, 26.19, 26.23, 36.54, 63.33, 72.88, 85.21, 87.63, 117.93, 121.12, 152.59, 153.80, 155.92; MS (ESI): m/z : calcd for $[\text{M}]^+ \text{C}_{22}\text{H}_{40}\text{BrN}_5\text{O}_4\text{Si}_2$ 573.1802 and observed 573.2

Perform O^6 -benzyl protection of 8-bromo-3',5'-O-bis(*tert*-butyldimethylsilane)-2'-deoxyguanosine **4**

- 8** To a 50 mL oven dried round bottom flask equipped with a magnetic stir bar, combine 8-bromo-3',5'-O-bis(*tert*-butyldimethylsilyl)-2'-deoxyguanosine **3** (1 g, 1.74 mmol) and triphenylphosphine (685 mg, 2.6 mmol) and dry the flask under vacuum for 12 h. Subsequently, add anhydrous 1,4-dioxane (20 mL) via a syringe and stir the mixture under Ar-atmosphere for 5 min.
- 9** To the suspension of solid mixture, add benzyl alcohol (275 μL , 2.6 mmol), followed by dropwise addition of diisopropyl azodicarboxylate (410 μL , 2.6

mmol). The mixture should turn clear after 15 min, but continue stirring for 12 h at room temperature.

- 10** Add a few drops of methanol to the above solution to quench the reaction and concentrate the mixture under vacuum. Purify the crude residue by flash column chromatography on silica gel (EtOAc/Hexane:20/80) to afford *O*⁶-benzyl-8-bromo-3',5'-*O*-bis(*tert*-butyldimethylsilyl)-2'-deoxyguanosine **4** as yellow oil (924 mg, 80 %).

TLC: R_f 0.34; 2: 8 EtOAc: Hexane, UV 254 nm

¹H-NMR (400 MHz, DMSO-*d*₆): δ -0.08 (3H, s), -0.06 (3H, s), 0.10 (6H, s), 0.77 (9H, s), 0.88 (9H, s), 2.17(1H, ddd, J = 4.0, 7.0, 13.2 Hz), 3.50 (1H, m), 3.62 (1H, m), 3.77 (2H, m), 4.67(1H, m), 5.46 (2H, m), 6.17(1H, t, J = 6.8 Hz), 6.40 (2H, br s), 7.3 (3H, m), 7.45 (2H, m); ¹³C-NMR (100 MHz, DMSO-*d*₆): δ -5.3, -4.8, -4.6, 18.0, 18.5, 25.8, 40.9, 62.9, 68.0, 72.0, 83.7, 87.7, 115.96, 127.9, 128.3, 128.4, 136.5, 137.6, 153.7, 159.2, 160.96; MS (ESI): m/z: calcd for [M]⁺ C₂₉H₄₆BrN₅O₄Si₂ 663.2272 and observed 663.8

Perform N²-protection using DMTr-Cl of *O*⁶-benzyl-8-bromo-3',5'-*O*-bis(*tert*-butyldimethylsilyl)-2'-deoxyguanosine **5**

- 11** To a 50 mL oven dried flask equipped with a magnetic stir bar, dissolve *O*⁶-benzyl-8-bromo-3',5'-*O*-bis(*tert*-butyldimethylsilyl)-2'-deoxyguanosine **4** (2 g, 3.013 mmol) in anhydrous pyridine (15 mL) under argon atmosphere.
- 12** Add 4,4'-dimethoxytrityl chloride (1.53 g, 4.52 mmol) to the mixture and stir the reaction for 12 h at room temperature.
- 13** Quench the reaction with methanol, concentrate under vacuo, and purify by column chromatography (EtOAc/Hexane:10/90) over neutral aluminum oxide to afford compound **5** (2.62 g, 90%) as white foamy solid.

R_f: 0.56; 3:7 EtOAc: Hexane, Alumina TLC plate, 254 nm

¹H-NMR (400 MHz, DMSO-*d*₆): δ -0.01 (3H, s), 0.02 (3H, s), 0.15 (6H, s), 0.87 (9H, s), 0.94 (9H, s), 2.03 (1H, m), 3.73–3.81 (11H, m), 4.85 (2H, br s), 6.13 (1H, m), 6.78–6.80 (4H, m), 7.28–7.20 (15H, m); ¹³C-NMR (100 MHz, DMSO-*d*₆): δ -5.4, -5.3, -4.6, -4.6, 18.1, 18.4, 25.3, 25.8, 25.9, 34.7, 55.2, 62.9, 67.9, 70.3, 72.6, 85.8, 87.4, 113.0, 116.0, 125.9, 126.7, 127.7, 127.8, 128.2, 128.3, 128.9, 130.11, 130.13, 136.3, 138.2, 146.0, 154.3, 157.4, 158.2; HRMS (ESI): m/z: calcd for [M]⁺ C₅₀H₆₄BrN₅O₆Si₂ 965.3579 and observed 965.7

BASIC PROTOCOL 2: SYNTHESIS OF THE REDUCED CARCINOGEN 3-AMINOBENZANTHRONE, A PRECURSOR OF THE BUCHWALD-HARTWIG CROSS-COUPPLING

The scheme of this part is shown in Figure 3. Starting from commercially available benzanthrone (1.1 g, 4.78 mmol), 3-aminobenzanthrone is prepared in two steps (~825mg, 77%). Nitration of benzanthrone furnishes 3-nitrobenzanthrone that can be reduced to 3-aminobenzanthrone, to be used as a cross coupling partner with fully protected C-8-bromo-deoxyguanosine derivative. (see Basic Protocol 1).

Materials

7H-Benz[d,e]anthracene-7-one (Benzanthrone, 99%, Alfa Aesar Chemical)

Nitrobenzene (Alfa Aesar Chemical)

Concentrated Nitric acid

Ethanol

Hydrazine monohydrate (Alfa Aesar Chemical)

Palladium, 10% on activated carbon (Alfa Aesar Chemical)

Dimorth condenser

Oil bath

Perform Nitration of Benzanthrone

- 1 In a 25 mL round bottom flask, add dropwise conc. HNO₃ (0.1 mL) to benzanthrone **6** (1 g, 4.35 mmol) dissolved in nitrobenzene (10 mL).
- 2 Heat the reaction mixture at 80° C for 24 h until all the starting material is consumed.
- 3 Filter the yellow-brown precipitated solid and wash several times with *n*-hexane to afford 3-nitrobenzanthrone **7** (1.1 gm 90 %) as bright yellow solid.

No further attempt is required to purify this derivative. Purity can be checked using ¹H-NMR and ¹³C NMR. It can either be used immediately or stored overnight at room temperature to be used for the next step.

¹H-NMR (400 MHz, CDCl₃): δ 7.66 (1H, t, J = 8.0 Hz), 7.81 (1H, t, J = 8.0), 7.97 (1H, t, J = 8.0 Hz), 8.33 (1H, d, J = 8 Hz), 8.38 (1H, d, J = 8 Hz), 8.56–8.61 (2H, m), 8.82 (1H, d, J = 8.4 Hz), 8.93 (1H, d, J = 8.8 Hz); ¹³C NMR (100 MHz, CDCl₃): δ 122.2, 124.1, 124.3, 124.8, 128.5, 128.6, 128.6, 129.6, 130.2, 130.3, 130.9, 131.0, 132.9, 134.1, 134.3, 147.1, 183.2; HRMS (ESI): m/z: calcd for [M]⁺ C₁₇H₉NO₃ 275.0582 and observed 275.1

Perform nitro reduction of 3-nitrobenzanthrone to synthesize 3-aminobenzanthrone **8**

- 4** In a 25 mL round bottom flask, add ethanol (30 mL) to 3-nitrobenzanthrone **7** (1 g, 1.3 mmol) and purge the mixture with argon for 30 min.
- 5** To this solution, add dropwise hydrazine monohydrate (1 mL). The reaction mixture should turn dark green.
- 6** Attach a condenser with the flask under argon atmosphere.
- 7** Then add Pd-C (10%) (10 mg) to it under argon.
- 8** Raise the temperature to 80° C in an oil bath and reflux the reaction mixture for 12 h.
- 9** Allow the reaction mixture to cool, and filter and dry the residue under vacuum to afford 3-aminobenzanthrone as a dark red solid **8** (750 mg, 85%).

No further attempt to purify this derivative is required. Purity can be checked using ¹H-NMR and ¹³C-NMR. It should either be used immediately or be stored at room temperature to be used for the Buchwald-Hartwig cross coupling reaction.

TLC: R_f : 0.66; 2 : MeOH: CH₂Cl₂, red visible spot

¹H-NMR (400 MHz, CDCl₃): δ 4.60 (2H, s), 6.92 (1H, d, J = 8.0 Hz), 7.43 (1H, t, J = 8.0 Hz), 7.68 (1H, t, J = 8.0 Hz), 7.75 (1H, t, J = 8.0 Hz), 8.22 (2H, m), 8.27 (1H, d, J = 8.0 Hz), 8.46 (1H, d, J = 8.0 Hz), 8.84 (1H, d, J = 8.8); ¹³C-NMR (100 MHz, CDCl₃): δ 109.9, 117.4, 118.0, 122.0, 125.1, 126.3, 126.5, 127.6, 128.0, 129.8, 130.2, 132.0, 133.2, 136.1, 185.1; MS (ESI): m/z: calcd for [M]⁺ C₁₇H₁₁NO 245.0841 and observed 245.1

BASIC PROTOCOL 3: BUCHWALD-HARTWIG CROSS-COUPLING REACTION

This part of the synthetic scheme is shown in Figure 4 in which the formation of C-N bond between 3-aminobenzanthrone and protected 2'-deoxyguanosine is generated via Buchwald-Hartwig cross coupling reaction. Though several ligands, bases, and catalysts were screened, BINAP, dibenzylidene acetone and sodium *tert*-butoxide turned out to be the best combination to perform this reaction to achieve moderate-to-good yields in our hand.

Materials

5 (See Basic Protocol 1)

8 (See Basic Protocol 2)

Tris (dibenzylideneacetone)-dipalladium (0) [(Pd₂(dba)₃]; (Aldrich Chemical)

Rac. -2, 2'-Bis(diphenylphosphino)-1,1'-binaphthyl (rac. BINAP, 98%, Acros Organics)

Sodium *tert*-butoxide (98%, Acros Organics)
 Toluene (anhydrous, kept dry with molecular sieves)
 Aluminium oxide, neutral (Brockmann I, 50 –200 µm; Acros Organics)
n-Hexane
 Ethyl acetate (EtOAc)
 Argon Balloon
 Oil bath

Perform Buchwald-Hartwig coupling for preparing benzanthrone-2'-deoxyguanosines adduct

- 1 Combine 5 g (5.08 mmol) of *O*⁶-benzyl-8-bromo-3', 5'-O-bis(tert-butyltrimethylsilyl)-*N*²-dimethoxytrityl-2'-deoxyguanosine **5** and 1.02 g (4.14 mmol) of 3-aminobenzanthrone **8** in a 50 mL round bottom flask.
- 2 Evacuate the flask back fill with argon 2 times.
- 3 Add anhydrous toluene (10 mL) to it.
- 4 Degas the reaction mixture using argon for 30 min.
- 5 Combine Tris (dibenzylidenacetone)-dipalladium (Pd₂(dba)₃) (380 mg) and *rac*-2, 2'- Bis(diphenylphosphino)-1,1'-binaphthyl (64 mg) to the degassed reaction mixture with stirring and heat the reaction mixture at 90° C for 40 min.
- 6 Add sodium *tert*-butoxide (621 mg, 6.5 mmol) and continue stirring the reaction mixture for an additional 1 h at 100°C.
- 7 After that the reaction is cooled to room temperature, filter through a celite bed and concentrate the filtrate under vacuum and purify by chromatography (EtOAc / Hexane: 2/ 8) over neutral aluminum oxide to afford the compound **9** (4.97g, 85%) as red solid.

Note: Degassing the solvent with argon and maintaining inert atmosphere is critical for the Buchwald-Hartwig cross coupling reaction.

TLC: R_f 0.45, 2:8 EtOAc: Hexane, UV at 254 nm

¹H-NMR (400 MHz, DMSO-d₆) δ -0.27 (3H, s), -0.15 (3H, s), 0.14 (6H, s), 0.66 (9H, s), 0.95(9H, s), 1.64 (1H, br s), 2.22 (1H, br s), 3.80 (7H, br s), 4.14 (3H, m), 4.50 (1H, br s), 5.08 (2H, br s), 6.14 (1H, br s), 6.80 (4H, m), 7.28–7.72 (19H, m), 8.24 (2H, m), 8.38 (2H, m), 8.49 (1H, d, J = 7.1 Hz), 8.76 (1H, d, J = 7.1 Hz); ¹³C-NMR (100 MHz, DMSO-d₆):, -5.7, -4.7, 1.1, 14.2, 18.0, 18.2, 25.7, 25.8, 25.9, 36.7, 39.9, 39.9, 55.2, 60.4, 63.1, 67.5, 70.2, 72.6, 86.2, 88.5, 112.0, 112.8, 112.9, 112.9, 117.1, 121.0, 122.8, 125.9, 125.9, 126.5, 128.6, 129.1, 129.1, 133.4, 136.7, 137.1, 137.7, 146.3, 147.2, 156.2, 158.1, 184.1; MS (ESI): m/z: calcd for [M+H]⁺ C₆₇H₇₅N₆O₇Si₂ 1131.5236 and observed 1131.5

BASIC PROTOCOL 4: DEPROTECTION OF *N*²-DMT FOLLOWED BY *N*²-AMINO PROTECTION, DIMETHOXYTRITYLATION OF 5'-HYDROXYL AND CONVERSION TO PHOSPHORAMIDITE

This segment (Figure 5) illustrates the stepwise deprotection of the protected nucleoside-carcinogen adduct followed by subsequent *N*²-amino and 5'-hydroxyl protection and its conversion to the corresponding phosphoramidite in good yield.

Materials

9 (see Basic Protocol 3)

Isopropanol (*i*PrOH; Aldrich Chemical)

Anhydrous Dichloromethane (CH₂Cl₂, Acros Organics)

Zinc Bromide (ZnBr₂; Acros Organics)

Sodium Bicarbonate (NaHCO₃; saturated aqueous solution)

Triethylamine trihydrofluoride (Et₃N.3HF; 98%, Aldrich Chemical)

Triethyl amine (Et₃N; Aldrich Chemical)

Anhydrous Tetrahydrofuran (THF, Acros Organics)

Anhydrous Methanol (MeOH, Acros Organics)

Palladium – Black (99.9% metal basis, Alfa –Aesar Chemical)

H₂ gas cylinder or balloon

Anhydrous *N*, *N*'-Dimethylformamide dimethylacetal (Acros Organics)

Anhydrous Pyridine (Acros Organics)

4-*N*, *N*'-dimethylaminopyridine (DMAP, Chem-Impex Int'l Inc)

4, 4'-Dimethoxytrityl chloride (DMTr-Cl, 98%, Chem-Impex Int'l Inc)

N, *N*'-diisopropylethyl amine (DIPEA, Aldrich chemical)

2- Cyanoethyl diisopropylchlorophosphoramidite [*i*PrNP(Cl)O(CH₂)₂CN; Aldrich Chemical]

Oil Bath

Magnetic stir plate and stir bar

n-Hexane for chromatography

Ethyl acetate (EtOAc) for chromatography

Methanol for column chromatography

Dichloromethane for column chromatography

Vacuum apparatus for filtration

Fume hood

Perform DMTr-Cl deprotection of the nucleoside adduct

- 1 Dissolve the Buchwald–Hartwig coupling adduct **9** (100 mg, 0.088 mmol) in dichloromethane (10 mL).
- 2 Add 1(M) zinc bromide (ZnBr_2) solution in isopropanol: dichloromethane (1:1) (2 mL) to the mixture and stirred the reaction mixture for 20 min.
- 3 Quench the reaction with saturated aqueous solution of sodium bicarbonate (NaHCO_3).
- 4 Extract the compound with dichloromethane and dry the organic layer under reduced pressure.
- 5 Purify the crude residue by using column chromatography (silica gel, EtOAc – Hexane: 2:8) to afford compound **10** (44 mg, 60%) as dark red foamy solid.
 ^1H -NMR (300 MHz, CDCl_3) δ –0.33 (3H, s), –0.19 (3H, s), 0.16 (3H, s), 0.18 (3H, s), 0.60 (9H, s), 0.97 (9H, s), 2.43 (1H, m), 3.08 (1H, m), 3.82 (2H, m), 3.96–3.98 (1H, m), 4.21 (1H, s), 4.59 (1H, s), 4.71 (2H, br s), 5.59 (2H, s), 6.42 (1H, t, $J = 7.1$ Hz), 7.20 (1H, m), 7.34 – 7.36 (4H, m), 7.50–7.55 (3H, m), 7.76–7.81 (2H, m), 8.34 – 8.37 (2H, m), 8.50 (3H, m), 8.71 (1H, s), 8.82 (1H, m); ^{13}C -NMR (100 MHz, CDCl_3) δ –5.65, –4.66, –4.51, 14.2, 18.1, 18.2, 24.7, 36.8, 39.2, 53.5, 60.4, 63.1, 67.8, 72.5, 85.4, 85.4, 88.1, 117.7, 122.8, 125.5, 125.6, 127.8, 127.9, 128.2, 128.3, 130.1, 133.2, 136.5, 136.9, 147.5, 153.6, 157.5, 183.9; MS (ESI): m/z : calcd for $[\text{M}+\text{H}]^+$ $\text{C}_{49}\text{H}_{57}\text{N}_6\text{O}_5\text{Si}_2$ 829.3929 and observed 829.0

Perform deprotection of tert-butyldimethylsilyl (TBDMS) groups of the nucleoside-benzanthrone adduct **10**

- 6 Transfer detritylated compound **10** (200 mg, 0.242 mmol) in a 25 mL round bottom flask and dissolve in anhydrous THF (5 mL) under argon.
- 7 Add dropwise triethylamine trihydrogenfluoride (100 μL) into the mixture and allow the reaction to continue for 12 h at room temperature.
- 8 After that, add triethylamine (88 μL) to quench the reaction. Evaporate the solvent under reduced pressure and purify the crude mixture by silica gel column chromatography (MeOH/ DCM: 5/95) to afford the diol **11** (122 mg, 84%) as reddish-brown solid.
 ^1H -NMR (300 MHz, $\text{DMSO}-d_6$) δ : 2.22 (1H, m), 2.89 (1H, m), 3.68 (1H, m), 3.76 (1H, m), 3.99 (1H, br s), 4.46 (1H, br s), 5.35 (1H, s), 5.44 (2H, br s), 5.53 (1H, m), 6.30 (2H, s), 6.50 (1H, m), 7.46–7.30 (5H, s), 7.58 (1H, m), 7.83 (1H, m), 7.90 (1H, m), 7.93 (1H, m), 8.52 (1H, m), 8.60 (1H, m), 8.68 (1H, m), 8.70 (1H, d, $J = 8.0$ Hz), 9.33 (1H, s); ^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$) δ : 9.0, 47.0, 62.0, 67.1, 71.8, 84.1, 84.5, 88.1, 118.9, 124.0, 126.40, 126.42, 126.7, 126.8, 127.7, 128.0, 128.1, 128.4, 128.4, 128.8, 129.0, 129.9, 130.1, 130.9, 134.3,

135.8, 136.6, 136.8, 137.3, 147.0, 158.7, 183.2; MS (ESI): m/z: calcd for M⁺ C₃₄H₂₈N₆O₅ 600.2121 and observed 600.7

Perform deprotection of O-benzyl (OBn) group of the nucleoside-benzanthrone adduct

- 9 Dissolve diol **11** (200 mg, 0.330 mmol) in 20 mL THF/MeOH (1:1) mixture under argon atmosphere in a 25 mL round bottom flask.
- 10 Purge the reaction mixture with argon for 30 min to deoxygenate the solution. Add Pd-black (15 mg) to the reaction mixture and purge H₂ gas for 2 h, after which allow the reaction to be stirred for an additional 12 h under H₂ atmosphere.
- 11 Filter the solid that separates out. The solid compound is highly pure and there is no need for further purification.
- 12 Concentrate the filtrate under reduced pressure and purify the crude by using a reversed phase C18 column (MeOH/ H₂O: 4/6) to obtain the additional 50 mg of product **12**. Typical combined of the product is (153 mg, 90% yield).
¹H-NMR (400 MHz, DMSO-d₆): δ 2.09–2.16 (1H, m), 2.86 (1H, br s), 3.61–3.72 (2H, m), 3.90 (1H, br s), 4.39 (1H, s), 5.27 (1H, br s), 5.36 (1H, br s), 6.38 (3H, br s), 7.59 (1H, s), 7.75 (1H, s), 7.84–7.91 (2H, m), 8.34 (1H, s), 8.55 (1H, br s), 8.72 (3H, br s), 9.13 (1H, s), 10.63 (1H, s); ¹³C-NMR (100 MHz, DMSO-d₆): δ 9.6, 46.1, 62.0, 71.6, 84.0, 88.1, 116.4, 119.8, 123.8, 125.8, 126.2, 127.0, 127.6, 127.8, 128.3, 128.4, 129.7, 130.1, 130.7, 134.2, 136.7, 144.0, 150.5, 153.5, 156.2, 183.2; MS (ESI): m/z: calcd for M⁺ C₂₇H₂₂N₆O₅ 510.1652 and observed 510.8

Perform synthesis of N²-dimethylformamidyl-2'-deoxyguanosine adduct

- 13 Suspend compound **12** (150 mg, 0.294 mmol) in dry MeOH (10 mL) under Ar-atmosphere in a 25 mL round bottom flask.
- 14 Add *N, N'*-Dimethylformamide dimethyl acetal (1 mL) dropwise to the solution and stir the mixture for 3 days at room temperature.
- 15 Filter the solid precipitate formed to afford pure compound **13** (157 mg, 95 % yield) as dark brown solid.
¹H-NMR (400 MHz, DMSO-d₆) δ 2.20–2.23 (1H, m), 2.87–2.9 (1H, m), 2.93 (3H, s), 3.05 (3H, s), 3.64 (1H, d, J = 12 Hz), 3.75 (1H, d, J = 12 Hz), 3.91 (1H, br s), 4.46 (1H, br s), 5.33 (2H, s), 6.27 (1H, s), 6.44 (1H, s), 7.57–7.60 (1H, m), 7.76–7.78 (1H, m), 7.85 (1H, t, J = 8 Hz), 7.92 (1H, t, J = 8 Hz), 8.34 (1H, d, J = 8 Hz), 8.55 (2H, s), 8.70–8.72 (3H, m), 9.24 (1H, s); ¹³C-NMR (75 MHz, DMSO-d₆) δ 38.60, 41.6, 44.64, 65.44, 74.97, 87.4, 91.5, 120.27, 120.28, 123.6, 127.3, 129.42, 129.83, 130.44, 131.25, 131.67, 131.87, 133.25, 133.67, 134.24, 137.84, 140.17, 144.97, 148.57, 152.83, 160.30, 160.72, 161.74, 186.76; HRMS (ESI) m/z calcd for [M+H], C₃₀H₂₈N₇O₅ 566.2152 and observed 566.2183.

Perform dimethoxytrityl – protection of 5'OH of nucleoside-benzanthrone adduct

- 16** Dissolve *N*²-dimethylformamidyl-2'-deoxyguanosine **13** (100 mg, 0.177 mmol) in anhydrous pyridine (5 mL) under Ar-atmosphere in a 50 mL round bottom flask.
- 17** Evaporate pyridine three times with *N*²-dimethylformamidyl-2'-deoxyguanosine and dry each time under high vacuum.
- 18** Add DMAP (1 mg) and DMTr-Cl (71 mg) in to the flask. Evacuated and back fill the flask with argon and then add anhydrous pyridine (10 mL). Heat the reaction mixture at 50°C for 90 min.
- 19** Evaporate the solvent under reduced pressure and purify the crude by column chromatography (silica gel, MeOH: DCM: Et₃N/ 5: 94: 1) to afford compound **14** (107 mg, 70%) as dark red solid.

NOTE: Drying the compound by removing the absorbed moisture via co-evaporation of pyridine from the starting material before addition of DMAP and DMTr-Cl is critical. Also before charging the crude residue on the column, silica gel should be pretreated with 3% triethylamine (Et₃N) and then column chromatography can be performed using dichloromethane-methanol with 1% Et₃N to purify the product. Reaction can be monitored using TLC.

Stain used for TLC: Freshly prepared Vanillin stain [in 250 mL ethanol (EtOH) 15g vanillin was dissolved followed by dropwise addition of 2.5 mL conc. H₂SO₄]. After running the TLC in MeOH: DCM: Et₃N, the TLC plate is dried and dipped into the stain; air dried and heat the plate using the heat gun to see the developed color.

Perform phosphoramidation of the nucleoside **14**

- 20** Dissolve compound **14** (100 mg, 0.115mmol) in dry CH₂Cl₂ (10 mL) under Ar-atmosphere in a 25 mL round bottom flask and add DIEA (0.1 mL) to it.
- 21** Add 2-cyanoethyl diisopropylchlorophosphoramidite NC(CH₂)₂OP(Cl)N(iPr)₂ (50 µL)-dropwise into the flask and stir the mixture for 30 min.
- 22** Evaporate the solvent under reduced pressure and purify the crude by column chromatography (silica gel, MeOH: DCM: Et₃N/ 1: 98: 1) to afford compound **15** (74 mg, 60%) as a mixture of two diastereomers.

NOTE: Before charging the crude residue on to the column, silica gel should be pretreated with 3% triethylamine (Et₃N) and then column chromatography can be performed using dichloromethane-methanol with 1% Et₃N to purify the product.

¹H-NMR (400 MHz, CDCl₃) (mixture of two diastereomers) δ 1.07–1.34 (2H, m), 1.99 (1H, br s), 2.42–2.45 (1H, m), 2.59–2.65 (2H, m), 2.69–2.76 (2H, m), 3.09 (3H, br s), 3.15–3.16 (3H, m), 3.29–3.34 (1H, m), 3.39–3.40 (1H, m), 3.44–3.50 (2H, m), 3.56–3.57 (6H, br s), 3.57–3.91 (4H, m), 4.44–4.45 (1H, m),

4.63–4.76 (1H, m), 6.40–6.45 (1H, m), 6.50–6.55 (3H, m), 7.07–7.13 (6H, m), 7.23–7.24 (2H, m), 7.49–7.53 (2H, m), 7.72–7.76 (1H, m), 8.07–8.17 (1H, m), 8.28 (1H, br s), 8.35–8.39 (1H, m), 8.48–8.54 (3H, m), 8.73–8.74 (1H, m); ¹³C-NMR (100 MHz, CDCl₃) (mixture of two diastereomers) δ 20.42, 20.49, 20.66, 20.73, 21.72, 24.80, 35.4, 38.75, 41.47, 43.52, 43.64, 46.28, 53.05, 55.23, 58.13, 58.5, 58.7, 63.26, 63.64, 73.68, 73.85, 74.19, 74.37, 85.9, 86.14, 86.79, 86.82, 113.18, 115.52, 115.77, 117.68, 117.88, 122.96, 120.59, 122.96, 124.97, 126.00, 126.35, 127.09, 127.13, 127.37, 127.96, 128.13, 128.22, 128.25, 128.64, 129.18, 129.77, 130.07, 130.16, 133.64, 135.18, 135.25, 135.37, 136.91, 138.24, 144.3, 145.88, 146.01, 148.84, 148.89, 155.39, 155.45, 156.99, 157.67, 157.79, 158.62, 183.0. ³¹P-NMR (121.4 MHz, CDCl₃): 148.65, 148.71. HRMS (ESI) *m/z* calcd for [M+H], C₆₀H₆₃N₉O₈P 1068.4537 and observed 1068.4524.

BASIC PROTOCOL 5: SOLID PHASE AUTOMATED SYNTHESIS OF OLIGONUCLEOTIDE CONTAINING MODIFIED 2'-DEOXYGUANOSINE AND ITS PURIFICATION

A conventional DNA synthesizer equipped with the phosphoramidite **14** in Basic protocol 4 enable one to prepare the required oligonucleotide using 'DMTr-of' setting. After releasing from the solid support, the oligomer can be deprotected with the normal condition for DNA synthesis, except that 0.25 M 2-mercaptoethanol should be added to avoid oxidative degradation. Finally, the completely deprotected oligonucleotide can be purified by reverse phase HPLC.

Materials

Reversed phase HPLC

HPLC column (Phenomenex Gemini –NX 5μ C18 (250 × 10 mm) semi – prep column)

Acetonitrile (CAN; HPLC grade, Aldrich Chemical)

Ammonium acetate (NH₄OAc)

Nano –puro HPLC grade water (HPLC –H₂O)

1.5 mL microcentrifuge tubes

Membrane filters (Amicon ultracel 3K centrifugal filters, 0.5 mL, Merck Millipore Ltd.)

Perform HPLC purification of adducted oligonucleotides

1. Purify the crude oligonucleotides using reversed phase HPLC using a C18 –semi prep column [e.g., Phenomenex Gemini –NX 5μ C18 (250 × 10 mm)] with an eluent of 0.1 M NH₄OAc -1% ACN-H₂O (solvent A) and 80% ACN-20% H₂O (solvent B) with a linear gradient (0 to 40 min, 2% to 30%) at a flow rate of 3.0 mL/ min monitored at both 254 nm and 400 nm. Only the fragments containing

C8-dG-ABA will show absorbance at 400 nm. Additional details for sample preparation and reversed phase HPLC is provided in Sinha and Jung, 2015.

2. Collect the purified oligomers in several 1.5 mL microcentrifuge tubes, filter through Amicon ultracel 3K filters, collect the filtrates and dry in a SpeedVac concentrator. Characterize with HRMS (negative ion mode) and then check the synthetic yield using UV measurements.

Oligonucleotides prepared: **26-mer:** 5'-

CCCGCG*CGAATTCCTGGCCGTCGTT-3' HRMS calcd: 8142.1 Da, found 8142.0 Da, which is 242.9 Da higher than the calculated mass of the unmodified 26-mer (7899.1 Da) indicating the presence of C8-dG-ABA.

12-mer: 5'-CATG*ATGACGCT-3': HRMS calcd: 3888.47 Da, found 3888.00, which is 242.6 Da higher than the calculated mass of the unmodified 26-mer (3645.4 Da).

COMMENTARY

Background Information

Importance of the synthesis of C8-dG-ABA adduct in DNA—Chemical synthesis of 3-nitrobenzanthrone-derived DNA adduct C8-dG-ABA is necessary to study its mutagenic and carcinogenic potential. Once formed, if this bulky DNA-lesion is not repaired, C8-dG-ABA can either stall the replication apparatus, leading to cell death, or undergo error-prone translesion synthesis to produce mutations in the genome. Availability of oligonucleotides containing this synthesized lesion will be helpful to investigate the roles of excision repair proteins that may take a part in its repair. They are also helpful to investigate the mechanisms of C8-dG-ABA bypass by the Y-family DNA polymerases in either error-free or error-prone manner. Indeed, several structural as well as in vitro and cellular replication studies have been performed with the synthesized C8-dG-ABA containing oligonucleotides. In a structural investigation using NMR spectroscopy, it was shown that the C8-dG-ABA adduct in a DNA duplex induces a base-displaced intercalated conformation, in which the adducted guanine is shifted to *syn* conformation about the glycosidic bond (Politica et al., 2015). However, the 5' and 3' base pairs remain intact. Even so, the thermal melting temperature, T_m , is decreased. In comparison to the reported structure of N^2 -dG-ABA in DNA (Lukin et al., 2011), which, in contrast, causes an increased T_m , this structural study rationalizes the more efficient repair of C8-dG-ABA relative to the N^2 -dG-ABA adduct. An in vitro biochemical investigation on a 26-mer oligonucleotide template containing C8-dG-ABA showed that the adduct is a strong replication block of the archaeal DNA polymerase Dpo4 (Godkari et al., 2014). In addition, Dpo4 misincorporates nucleotides at the two pause sites, located at the adducted guanine and its 3' base, on the 26-mer template 5'-CCCGCG*CGAATTCCTGGCCGTCGTT-3' (Godkari et al., 2014). Subsequent studies on the same template using four human translesion synthesis DNA polymerases (hpol η , hpol κ , hpol ι , and hRev1) established that C8-dG-ABA is also a strong replication block of hpol ι and hRev1, but hpol η and hpol κ can efficiently bypass the lesion (Tokarsky et al., 2016). Specifically, dNTP incorporation efficiency and fidelity decreased nearly 10-fold during lesion bypass by hpol η relative to

undamaged DNA. In addition, hpol η exhibited nucleotide concentration-dependent biphasic kinetics at the replication pause sites, implying that several enzyme-DNA complexes form during nucleotide incorporation. In a crystallographic investigation of the C8-dG-ABA bypass by hpol η , a ternary complex of the enzyme with the template:primer duplex and dCTP was captured at the insertion stage (Patra et al., 2016). This structure provided insight as to how the error-free bypass occurs. Specifically, the ABA moiety resides inside a hydrophobic cleft to the side of the hpol η active site, such that an intra-nucleotide hydrogen bond between the phosphate and the ABA amino group is formed, which allows the adducted guanine to create a nearly typical Watson-Crick base-pair with the incoming dCTP. Cellular studies in human embryonic kidney cells confirmed some of the roles of translesion synthesis DNA polymerases, notably, hpol η , hpol κ , hpol ζ , and hRev1 (Pande et al., 2014). Evidently, more research on replication and repair must be carried out, and the convenient synthesis of this DNA adduct, as described in this article, will facilitate these investigations.

Critical Parameters and Troubleshooting

To obtain the products in moderate to high yields, it is critical to maintain dry and inert atmosphere, as indicated in the protocols. Throughout the protocols critical parameters are stated as “NOTE” which can be helpful to the investigators. A well-ventilated fume hood is obligatory for this protocol. The anhydrous solvents used in the reactions should be freshly distilled. Because of extreme acid sensitivity of the dimethoxytrityl group, prior to silica gel column chromatography it is recommended that the column be neutralized with solvent containing 1% triethylamine.

For the solid phase DNA synthesis, standard protocol is used, except the time for the coupling step of C8-dG-ABA monomer is increased to 20 min. After releasing from the solid support, the oligonucleotide is deprotected with ammonium hydroxide under standard conditions (55° C for 16 h) for DNA synthesis, except 0.25 M 2-mercaptoethanol is added to avoid oxidative degradation of C8-dG-ABA.

Anticipated Results

The protocols described in this unit allow one to synthesize C8-dG-ABA-adducted oligonucleotides in any desired DNA sequence using a commercial DNA synthesizer at any scale (0.2–10 micromole). Usually synthesis at 0.2–1 micromole scale provides sufficient material for in vitro repair and replication studies or for preparing C8-dG-ABA containing plasmids for in vivo work, whereas NMR or crystallographic investigations require 5–10 micromole scale synthesis. The yield is dependent on the purity of the phosphoramidite monomer and the length of the oligonucleotide. But for most oligonucleotides containing less than 30-mers, HPLC and polyacrylamide gel electrophoresis allows separation of highly purified (>99% purity) adducted oligonucleotides.

Time Considerations

If all the reagents and analyses methods are readily available, a skilled synthetic chemist can accomplish the 15-step synthesis of the protected C8-dG-ABA phosphoramidite in approximately three weeks. The synthesis and purification of the adducted oligonucleotide would take another week.

Acknowledgments

This research was supported by grant ES09127 from NIEHS, NIH.

Literature Cited

- Arlt VM. 3-Nitrobenzanthrone, a potential human cancer hazard in diesel exhaust and urban air pollution: a review of the evidence. *Mutagenesis*. 2005; 20:399–410. [PubMed: 16199526]
- Arlt VM, Bieler CA, Mier W, Wiessler M, Schmeiser HH. DNA adduct formation by the ubiquitous environmental contaminant 3-nitrobenzanthrone in rats determined by (32)P-postlabeling. *Int J Cancer*. 2001; 93:450–454. [PubMed: 11433414]
- Arlt VM, Glatt H, Muckel E, Pabel U, Sorg BL, Seidel A, Frank H, Schmeiser HH, Phillips DH. Activation of 3-nitrobenzanthrone and its metabolites by human acetyltransferases, sulfotransferases and cytochrome P450 expressed in Chinese hamster V79 cells. *Int J Cancer*. 2003; 105:583–592. [PubMed: 12740904]
- Arlt VM, Hewer A, Sorg BL, Schmeiser HH, Phillips DH, Stiborova M. 3-Aminobenzanthrone, a human metabolite of the environmental pollutant 3-nitrobenzanthrone, forms DNA adducts after metabolic activation by human and rat liver microsomes: evidence for activation by cytochrome P450 1A1 and P450 1A2. *Chem Res Toxicol*. 2004; 17:1092–1101. [PubMed: 15310241]
- Arlt VM, Stiborova M, Henderson CJ, Osborne MR, Bieler CA, Frei E, Martinek V, Sopko B, Wolf CR, Schmeiser HH, Phillips DH. The environmental pollutant and potent mutagen 3-nitrobenzanthrone forms DNA adducts after reduction by NAD(P)H:quinone oxidoreductase and conjugation by acetyltransferases and sulfotransferases in human hepatic cytosols. *Cancer Res*. 2005; 65:2644–2652. [PubMed: 15805261]
- Basu AK, Essigmann JM. Site-specifically modified oligodeoxynucleotides as probes for the structural and biological effects of DNA-damaging agents. *Chem Res Toxicol*. 1988; 1:1–18. [PubMed: 2979705]
- Bieler CA, Cornelius MG, Klein R, Arlt VM, Wiessler M, Phillips DH, Schmeiser HH. DNA adduct formation by the environmental contaminant 3-nitrobenzanthrone after intratracheal instillation in rats. *Int J Cancer*. 2005; 116:833–838. [PubMed: 15856450]
- Bieler CA, Wiessler M, Erdinger L, Suzuki H, Enya T, Schmeiser HH. DNA adduct formation from the mutagenic air pollutant 3-nitrobenzanthrone. *Mutat Res*. 1999; 439:307–311. [PubMed: 10023091]
- Chakraborti D, Colis L, Schneider R, Basu AK. Synthesis of N^2 2'-deoxyguanosine adducts formed by 1-nitropyrene. *Org Lett*. 2003; 5:2861–2864. [PubMed: 12889893]
- Delaney JC, Essigmann JM. Biological properties of single chemical-DNA adducts: a twenty year perspective. *Chem Res Toxicol*. 2008; 21:232–252. [PubMed: 18072751]
- Gillet LC, Alzeer J, Scharer OD. Site-specific incorporation of *N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene (dG-AAF) into oligonucleotides using modified 'ultra-mild' DNA synthesis. *Nucleic Acids Res*. 2005; 33:1961–1969. [PubMed: 15814813]
- Gillet LC, Scharer OD. Preparation of C8-amine and acetylamine adducts of 2'-deoxyguanosine suitably protected for DNA synthesis. *Org Lett*. 2002; 4:4205–4208. [PubMed: 12443059]
- Gadkari VV, Tokarsky EJ, Malik CK, Basu AK, Suo ZC. Mechanistic investigation of the bypass of a bulky aromatic DNA adduct catalyzed by a Y-family DNA polymerase. *DNA Repair*. 2014; 21:65–77. [PubMed: 25048879]
- Hartwig JF. Carbon-heteroatom bond-forming reductive eliminations of amines, ethers, and sulfides. *Acc Chem Res*. 1998; 31:852–860.
- Huang H, Das RS, Basu AK, Stone MP. Structure of (5'S)-8,5'-cyclo-2'-deoxyguanosine in DNA. *J Am Chem Soc*. 2011; 133:20357–20368. [PubMed: 22103478]
- Lukin M, Zaliznyak T, Johnson F, de Los Santos CR. Incorporation of 3-nitrobenzanthrone into 2'-deoxyoligonucleotides and its impact on duplex stability. *J Nucleic Acids*. 2011 521035.
- Murata M, Tezuka T, Ohnishi S, Takamura-Enya T, Hisamatsu Y, Kawanishi S. Carcinogenic 3-nitrobenzanthrone induces oxidative damage to isolated and cellular DNA. *Free Radic Biol Med*. 2006; 40:1242–1249. [PubMed: 16545693]

- Pande P, Malik CK, Bose A, Jasti VP, Basu AK. Mutational analysis of the C8-guanine adduct of the environmental carcinogen 3-nitrobenzanthrone in human cells: The critical roles of DNA polymerase η , κ and Rev1 in error-prone translesion synthesis. *Biochemistry*. 2014; 53:5323. [PubMed: 25080294]
- Patra A, Politica DA, Chatterjee A, Tokarsky EJ, Suo Z, Basu AK, Stone MP, Egli M. Mechanism of error-free bypass of the environmental carcinogen *N*-(2'-deoxyguanosin-8-yl)-3-aminobenzanthrone adduct by human DNA polymerase η . *ChemBioChem*. 2016; 17:2033–2037. [PubMed: 27556902]
- Pearce F. Devil in the diesel: lorries belch out what may be the most carcinogenic chemical ever discovered. *New Scientist*. 1997:5.
- Phousongphouang PT, Grososky AJ, Eastmond DA, Covarrubias M, Arey J. The genotoxicity of 3-nitrobenzanthrone and the nitropyrene lactones in human lymphoblasts. *Mutat Res*. 2000; 472:93–103. [PubMed: 11113702]
- Politica DA, Malik CK, Basu AK, Stone MP. Base-Displaced Intercalated Structure of the *N*-(2'-deoxyguanosin-8-yl)-3-aminobenzanthrone DNA Adduct. *Chem Res Toxicol*. 2015; 28:2253–2266. [PubMed: 26641105]
- Sinha ND, Jung KE. Analysis and purification of synthetic nucleic acids using HPLC. *Curr Protoc Nucleic Acid Chem*. 2015; 61:10.5.1–10.5.39. [PubMed: 26344225]
- Purohit V, Basu AK. Synthesis and characterization of oligodeoxynucleotides containing the major DNA adducts formed by 1,6- and 1,8-dinitropyrene. *Org Lett*. 2000; 2:1871–1874. [PubMed: 10891179]
- Takamura-Enya T, Kawanishi M, Yagi T, Hisamatsu Y. Structural identification of DNA adducts derived from 3-nitrobenzanthrone, a potent carcinogen present in the atmosphere. *Chem Asian J*. 2007; 2:1174–1185. [PubMed: 17712830]
- Tokarsky EJ, Gadkari VV, Zahurancika WJ, Malik CK, Basu AK, Suo Z. Pre-Steady-State Kinetic Investigation of Bypass of a Bulky Guanine Lesion by Human Y-family DNA Polymerases. *DNA Repair*. 2016; 46:20–28. [PubMed: 27612622]
- Wang Z, Rizzo CJ. Synthesis of the C8-deoxyguanosine adduct of the food mutagen IQ. *Org Lett*. 2001; 3:565–568. [PubMed: 11178826]
- Wolfe JP, Wagaw S, Marcoux JF, Buchwald SL. Rational development of practical catalysts for aromatic carbon-nitrogen bond formation. *Acc Chem Res*. 1998; 31:805–818.

Figure 1.
Chemical structures of the major DNA adducts formed by 3-NBA.

Figure 2.
Synthetic route of the key intermediate **5**.

Figure 3.
Synthetic route of the 3-aminobenzanthrone.

Figure 4.

Formation of protected 3-aminobenzanthrone-2'-deoxyguanosine adduct by Buchwald-Hartwig coupling.

Figure 5.

Conversion of the protected 3-aminobenzanthrone-2'-deoxyguanosine adduct **9** to a fully protected phosphoramidate monomer **15** for solid phase DNA synthesis.