DOI: 10.1002/asia.200700061

Structural Identification of DNA Adducts Derived from 3-Nitrobenzanthrone, a Potent Carcinogen Present in the Atmosphere

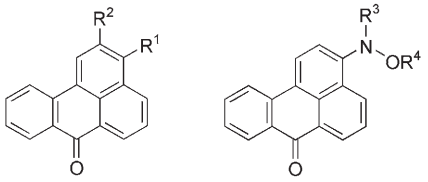
Takeji Takamura-Enya,*[a] Masanobu Kawanishi,[b] Takashi Yagi,[b] and Yoshiharu Hisamatsu[c]

Abstract: 3-Nitrobenzanthrone is a powerful bacterial mutagen and carcinogen to mammals. To obtain precise information on DNA-adduct formation by 3-nitrobenzanthrone, a number of DNA adducts, including *N*-(2′-deoxyguanosin-8-yl)-3-aminobenzanthrone (**13a**), 2-(2′-deoxyguanosin-N^2-yl)-3-aminobenzanthrone (**14a**), *N*-(2′-deoxyadenosin-8-yl)-3-aminobenzanthrone (**15a**), 2-(2′-deoxyadenosin-N^6-yl)-3-aminobenzanthrone (**16a**), and their *N*-acetylated counterparts **13b**, **14b**, **15b**, and **16b** were synthesized by palladium-catalyzed aryl amination of the corresponding nucleoside and bromobenzanthrone derivatives. Among these DNA adducts, DNA adducts **13a**, **13b**, **14a**, **14b**, and **16a** were identified in the reaction mixture of nucleosides (2′-deoxyguanosine, 2′-deoxyadenosine, or DNA) with *N*-acetoxy-3-aminobenzanthrone or *N*-acetyl-*N*-acetoxy-3-aminobenzanthrone, both of which are recognized as activated metabolites of 3-nitrobenzanthrone. The formation of these multiple DNA adducts may help explain the potent mutacarcinogenicity of 3-nitrobenzanthrone.

Keywords: aryl amination · DNA adducts · DNA damage · nitrobenzanthrone · palladium

Introduction

3-Nitrobenzanthrone (**2**) was initially reported by our group to be a powerful bacterial mutagen present in diesel exhaust particles and airborne particulate matter.[1] Owing to its potent mutagenicity, which has been shown to be equal to that of dinitropyrenes, the presence of **2** in the natural environment has raised worldwide concern, and a considerable number of investigations into its environmental concentration, DNA-adduct formation, biological influence, and the mechanisms by which it is formed from the parent compound benzanthrone (**1**), have been reported.[2] In the atmosphere, **2** has been detected at levels of 0–9.8 pg m^{-3}.[1,3] Compound **2** has also been found in soil samples in Japan.[4] Environmental nitration to form **2**, as well as the 2-nitro isomer **3**, from **1** has also been suggested.[5] From biological studies, **2** was found to form DNA adducts and induce DNA damage when applied to certain cell lines.[6] Nitrooxy poly-



1: R^1, R^2 = H
2: R^1 = NO_2, R^2 = H
3: R^1 = H, R^2 = NO_2
4: R^1 = Br, R^2 = H
5: R^1 = NH_2, R^2 = H
6: R^1 = NH_2, R^2= Br
7: R^1 = H, R^2 = Br
8: R^1 = NO_2, R^2 = Br

9: R^3, R^4 = Ac
10: R^3 = H, R^4 = Ac
11: R^3, R^4 = H

[a] Dr. T. Takamura-Enya
Department of Applied Chemistry
Kanagawa Institute of Technology
1030 Shimo-Ogino
Atsugi-shi 243-0292 (Japan)
Fax: (+81) 46-242-8760
E-mail: takamura@chem.kanagawa-it.ac.jp

[b] Dr. M. Kawanishi, Dr. Prof. T. Yagi
Frontier Science Innovation Center
Osaka Prefecture University
1-2 Gakuen-cho, Sakai-city
Osaka 599-8570 (Japan)

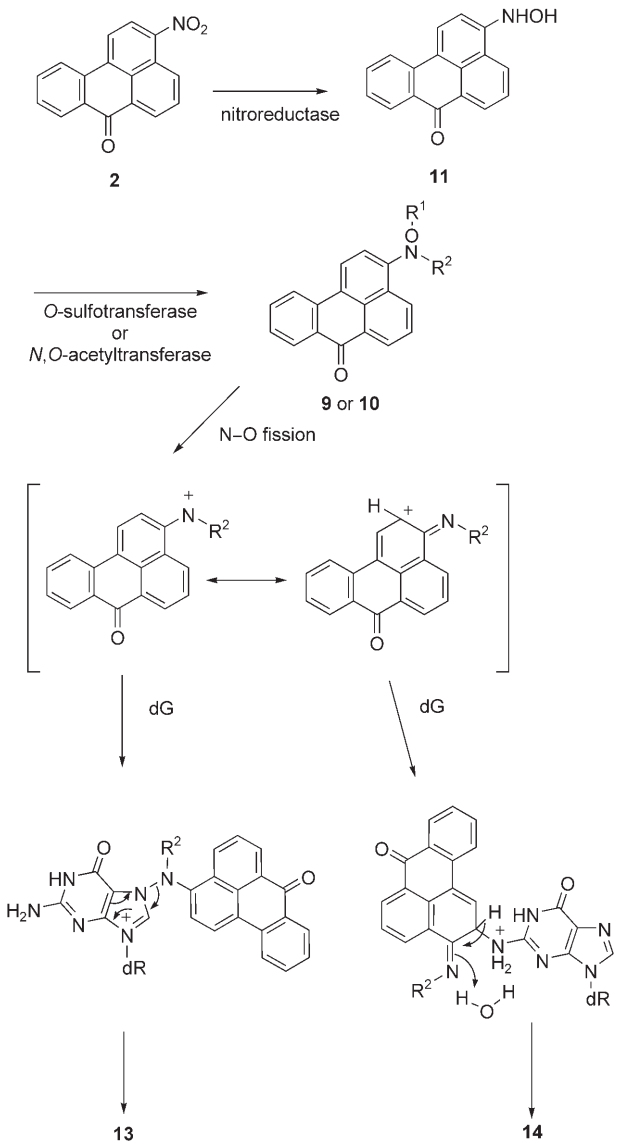
[c] Dr. Y. Hisamatsu
Field Science Center
Tokyo University of Agriculture and Technology
3-5-8 Saiwai-cho
Tokyo 183-8509 (Japan)

Supporting information for this article is available on the WWW under http://www.chemasianj.org or from the author.

cyclic aromatic hydrocarbon (PAH) **2** was reported to induce lung cancer by oral administration.[7]

As with many nitroarenes, **2** requires metabolic activation to induce mutagenicity, in which the initial event is believed to be covalent bond formation with nucleobases in cellular DNA.[8] Generally, nitroarenes are reductively activated to form hydroxyamines, followed by O-esterification or N,O-diesterification by cellular enzymes such as the acetyl- and sulfotransferases (Scheme 1).[9] The resulting *O*-acetyl- or *O*-sulfonylhydroxyamines spontaneously undergo heterolytic N–O fission to form highly reactive nitrenium ions, which attack DNA molecules to form the corresponding DNA adducts.[9] Schmeiser, Phillips, and co-workers reported that **2** is reductively activated by cytochrome P450 systems with NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) as a cofactor.[8b,c] A considerable number of DNA adducts was also found in cells and rat livers treated with **2**.[8b,10]

In our previous work on DNA adducts formed from **2**, we found that *N*-acetyl-*N*-acetoxy-3-aminobenzanthrone (**9**), which is a putative ultimate form of **2**, generated a unique adduct with 2′-deoxyguanosine (dG), that is, *N*-acetyl-2-(2′-deoxyguanosin-8-yl)-3-aminobenzanthrone (**12b**), in which the C2 atom of *N*-acetyl-3-aminobenzanthrone is covalently bound to the C8 atom of dG (Scheme 2).[8a] To the best of our knowledge, this unusual type of DNA adduct, which involves C–C bond formation, has so far been described in only one report, which deals with a DNA adduct of Phe-P-1, one of the heterocyclic amines formed in the course of cooking certain foods.[11] A diphosphate derivative of the adduct **12b** was also detected in a cancer cell line, HepG2, when it was treated with **2**.[6b] The authors also showed the presence of a 2′-deoxyadenosine (dA) adduct from **9** with a calf thymus DNA system.[8a] This dA adduct from **9** was also detected in cellular DNA from HepG2 cells treated with **2**, along with two other unidentified major DNA adducts.[6b] The chemical structure of this dA adduct was recently identified as (9′-(2″-deoxyribofuranosyl))purino[6′,1′:2,3]imidazo-[5,4-*p*]-(1,11b-dihydro-(*N*-acetyl-3-amino))benzanthrone (**17**).[12] The same study also showed that *N*-acetyl-*N*′-(2′-deoxyguanosin-8-yl)-3-aminobenzanthrone (**13b**) was produced from the reaction of dG with **9**.[12]

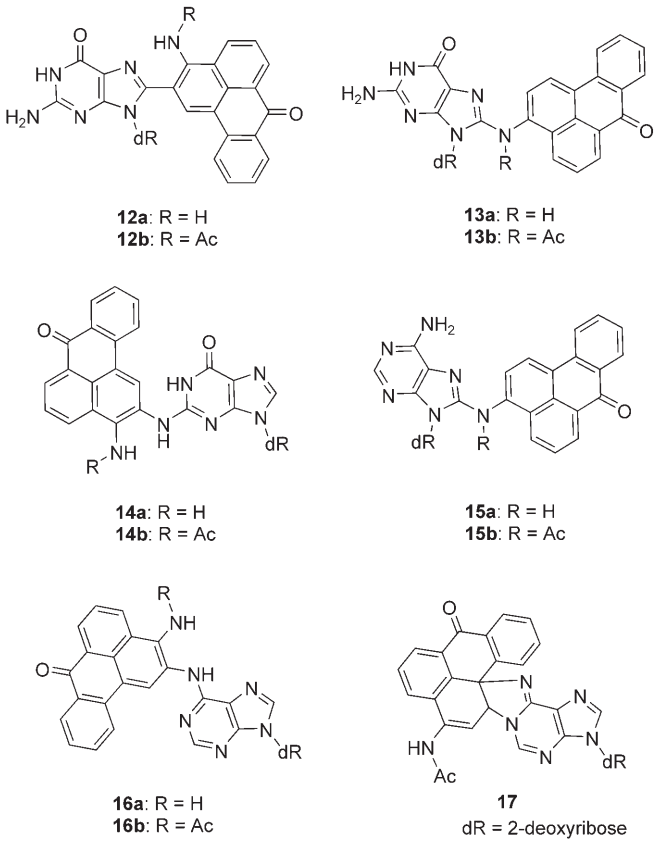


Scheme 1. Pathways of activation and DNA-adduct formation of nitroarenes in vivo. Compound **2** is shown as a representative.

Independent studies by Arlt et al. showed that other DNA adducts different from **12b**, **13b**, and **17** were mainly produced by DNA from rats treated with **2**, although the chemical structures of these DNA adducts are unknown at present.[10c] Some of these unknown DNA adducts are considered to be from another ultimate form of **2**, such as *N*-acetoxy-3-aminobenzanthrone (**10**).[10b]

From a number of studies of the DNA adducts derived from nitroarenes, certain common structures have been elucidated.[9,13] Nitrenium ions, which result from the cellular activation of nitroarenes, are believed to attack initially the N7 atom of the purine ring of dG or dA, which then rearrange to the C8 adduct of dG (the dG–C8 adduct) or dA (the dA–C8 adduct).[14] Additionally, the most-cationic ring carbon atoms, resonance-stabilized from the initial nitreni-

Abstract in Japanese:
大気環境中の発がん物質である 3–ニトロベンゾアントロン(NBA)の DNA 付加体の別途合成法を確立した。Pd を用いたアミノ化反応を使用することにより、*N*-(2'-deoxyguanosin-8-yl)-3-aminobenzanthrone (**13**)、2-(2'-deoxyguanosin-N^2-yl)-3-aminobenzanthrone (**14a**)、*N*-(2'-deoxyadenosin-8-yl)-3-aminobenzanthrone (**15a**)、2-(2'-deoxyadenosin-N^6-yl)-3-aminobenzanthrone (**16a**) およびそれらの *N*-アセチル化体 **13b**、**14b**、**15b**、**16b** を合成した。得られた DNA 付加体のうち **13a**、**13b**、**14a**、**14b**、**16a**、**16b** は NBA の究極活性体と核酸の反応より得ることが出来る。このことからこれらの付加体が NBA の発がんに寄与していると考えられる。



Scheme 2. Strucuture of DNA adducts derived from **2**.

um ion intermediates, can attack N2 of dG (the dG–N2 adduct) or N6 of dA (the dA–N6 adduct) (Scheme 1). In the case of the DNA adducts of **2** and dG, four general types of DNA adducts can be found. From the reaction of dG with **10**, one of the putative ultimate mutagens of nitrobenzanthrone **2**, the formation of *N*-(2′-deoxyguanosin-8-yl)-3-aminobenzanthrone (**13a**) and 2-(2′-deoxyguanosin-N^2-yl)-3-aminobenzanthrone (**14a**) is deduced. When the ultimate mutagen **9** is used as the putative ultimate form, it gives acetylated dG adducts, that is, **13b** and *N*-acetyl-2-(2′-deoxyguanosin-N^2-yl)-3-aminobenzanthrone (**14b**). In a similar manner, dA with **9** or **10** gives four DNA adducts: *N*-(2′-deoxyadenosin-8-yl)-3-aminobenzanthrone (**15a**), 2-(2′-deoxyadenosin-N^6-yl)-3-aminobenzanthrone (**16a**), and their acetylated forms *N*-acetyl-*N*′-(2′-deoxyadenosin-8-yl)-3-aminobenzanthrone (**15b**) and *N*-acetyl-2-(2′-deoxyadenosin-N^6-yl)-3-aminobenzanthrone (**16b**).

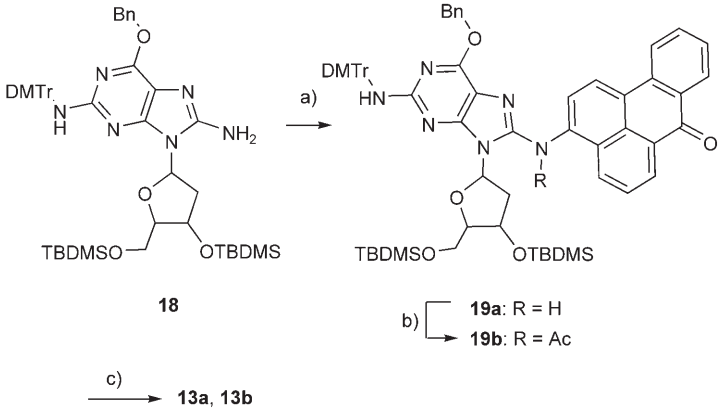
Studies that endeavored to carry out the structural identification of DNA adducts with nitroarenes were performed with reactions between dG/dA/DNA and the putative ultimate forms of the mutagens.[8a,12,15] These reactions are known to be useful as analogues of DNA-adduct formation in vivo to determine the product information of DNA adducts or the reactivity of nitreniun ions.[16] However, in terms of identifying the chemical structures of DNA adducts, approaches with the nitrenium ion are not efficient because many laborious repetitions of HPLC isolation and fractionation are required for spectroscopic analysis.[15,16] Recent progress in aryl amination reactions now makes it possible to prepare general-type DNA adducts by independent chemical synthesis.[17] Herein, we describe a defined synthetic methodology to yield authentic specimens of general-type DNA adducts derived from **2**, as mentioned above. With these authentic samples, some were identifiable from the in vitro reaction of **9** or **10** with dG, dA, and DNA.

Results and Discussion

Synthesis of General-Type DNA Adducts Derived from 3-Nitrobenzanthrone

The synthetic schemes of the DNA adducts derived from **2** are illustrated in Schemes 3–7. In our previous study, for the synthesis of DNA adducts by palladium-catalyzed aryl amination, xantphos (9,9-dimethyl-4,5-bis(diphenylphosphanyl)-xanthene) was found to be an effective phosphine ligand to yield the desired coupling compounds.[17g] In this study, therefore, xantphos was used throughout all the aryl amination reactions.

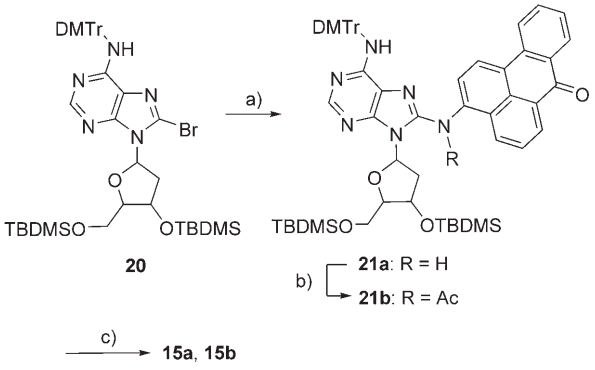
The preparation of compound **19a** and its subsequent deprotection product **13a** has already been reported.[17g] Compound **18** was found to couple efficiently with bromo derivatives **4** under the conditions 10% Pd^0, xantphos, and potassium *tert*-butoxide in toluene at 80 °C (Scheme 3). Acetylation of **19a** was performed with acetic anhydride along with catalytic amounts of 4-dimethylaminopyridine (4-DMAP) to give **19b**, which was subsequently deprotected to give authentic samples of **13b** almost quantitatively. Compound **13b** was recently isolated and identified from the reaction mixture from *N*-acetoxy derivatives **9** with dG.[12] The reported NMR spectrum of the isolated compound was the same as that of our synthetic compound **13b**. In a similar manner, we were also able to prepare authentic samples of the dA–C8 adducts **15a** and **15b** from coupling of 3-amino-



Scheme 3. Preparation of **13**. a) [Pd_2dba_3] (10 mol%), xantphos, Cs_2CO_3, **4**. b) $(CH_3CO)_2O$, 4-DMAP, pyridine. c) 1) $ClCH_2COOH$, CH_3OH/CH_2Cl_2; 2) Pd black, H_2, THF; 3) TEA·3HF, THF. Bn = benzyl, dba = dibenzylideneacetone, DMTr = dimethoxytrityl, TBDMS = *tert*-butyldimethylsilyl.

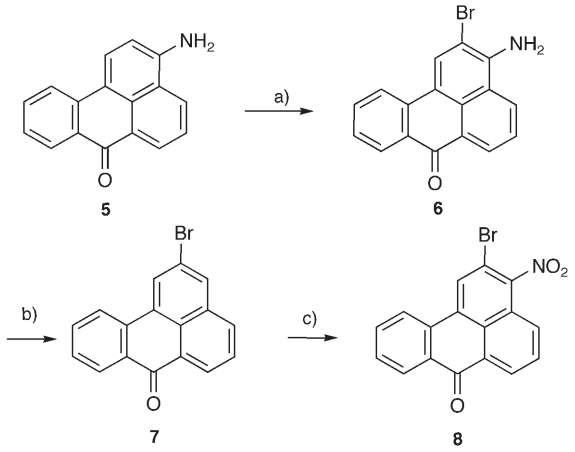
CHEMISTRY
AN ASIAN JOURNAL

benzanthrone (**5**) with the N^6-dimethoxytritylated 8-bromoadenosine derivative **20** (Scheme 4). The coupling reaction was completed within 2 h at 80 °C to yield **21a**, which was converted into **15a** by treatment with triethylamine·trihydrogenfluoride (TEA·3HF). Acetylation of **21a** to **21b** followed by subsequent deprotection gave the desired **15b** in high yields (Scheme 4).



Scheme 4. Preparation of **15**. a) $[Pd_2dba_3]$ (10 mol%), xantphos, Cs_2CO_3, **5**. b) $(CH_3CO)_2O$, 4-DMAP, pyridine. c) 1) CCl_3COOH, CH_3OH/CH_2Cl_2; 2) TEA·3HF, THF.

For the N^2-type dG adduct **14** as well as the N^6-type dA adduct **16**, the bromo derivative **8** was used as the starting material for the coupling partner. Although **8** had not been obtained previously, we prepared it by a series of nitrations of **1**, followed by reduction, bromination, deamination, and further nitration of the resulting 2-bromobenzanthrone (**7**) (Scheme 5). Attempts to oxidize amino derivative **6** to the desired compound **8** with *m*-chloroperbenzoic acid were not successful; they resulted in the formation of complex reaction products as shown by TLC analysis. The positional selectivity of the nitration of **1** is considered to be unchanged by the presence of a bromo group at the 2-position of **1**.



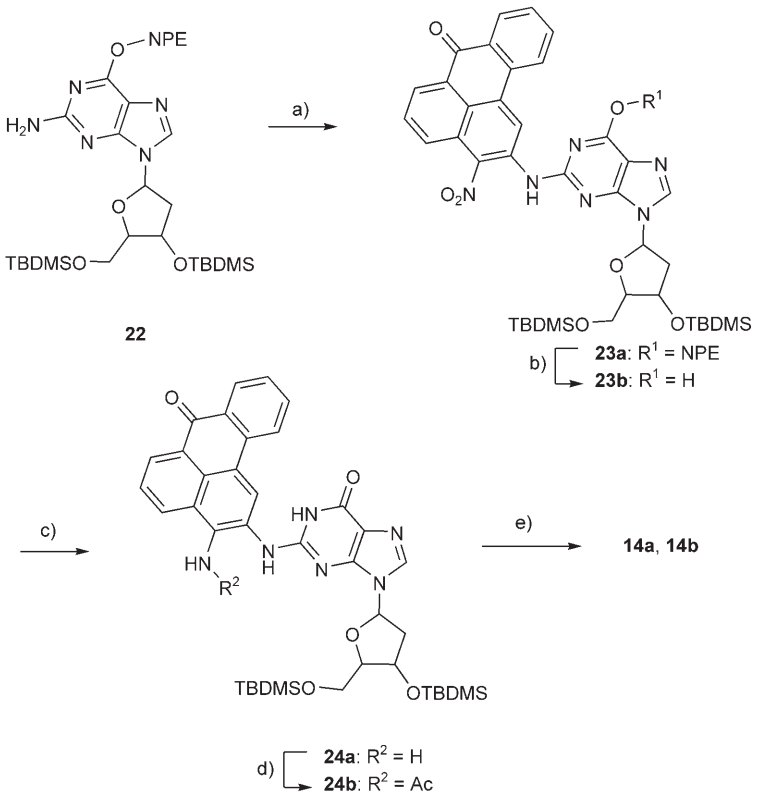
Scheme 5. Preparation of **8**. a) NBS, DMF, −20 °C. b) $NaNO_2$, H_2SO_4 then Cu_2O. c) HNO_3, $C_6H_5NO_2$. DMF = *N*,*N*-dimethyl formamide, NBS = *N*-bromosuccinimide.

After nitration of **7** in nitrobenzene, compound **8** was easily recovered by filtration, and recrystallization in acetic acid gave the pure specimen. The coupling reactions of **8** with the nucleoside derivatives of **22** were complete within 4 h at 100 °C, but the yield varied from 45 to 63%. We initially attempted to use a benzyl group for O^6 protection of dG in place of a nitrophenylethyl (NPE) group as illustrated. Coupling yields (≈80%) were found to be somewhat higher than those with an NPE protective group. Catalytic hydrogenation was expected to deprotect the benzyl group and reduce the nitro group simultaneously to give the desired compound **24a**. However, this expectation was not realized because further reduction of the carbonyl group at O^7 of the benzanthrone moiety was significant. We were not able to control the hydrogenation reaction to remove the O^6 benzyl group selectively. The NPE group, however, was removed by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) without affecting other functional groups. Subsequent reduction of the nitro group to the amine, therefore, required a selective nitro-reduction system. Generally, the reduction of **2** to aminobenzanthrone **5** is performed by Pd/C with hydrazine hydrate at 70 °C in DMF. However, this procedure turned out to produce only decomposed products. Finally, we found $[Fe_3(CO)_{12}]$ to be quite effective for the selective reduction of the nitro group in the presence of the carbonyl group of the benzanthrone moiety.[18] Treatment of **23b** with a stoichiometric amount of $[Fe_3(CO)_{12}]$ in methanol/toluene under reflux conditions gave only one desired product **24a**, which was easily purified by short-column chromatography on silica gel (Scheme 6). DNA adduct **14a** was obtained by removal of the silyl group.

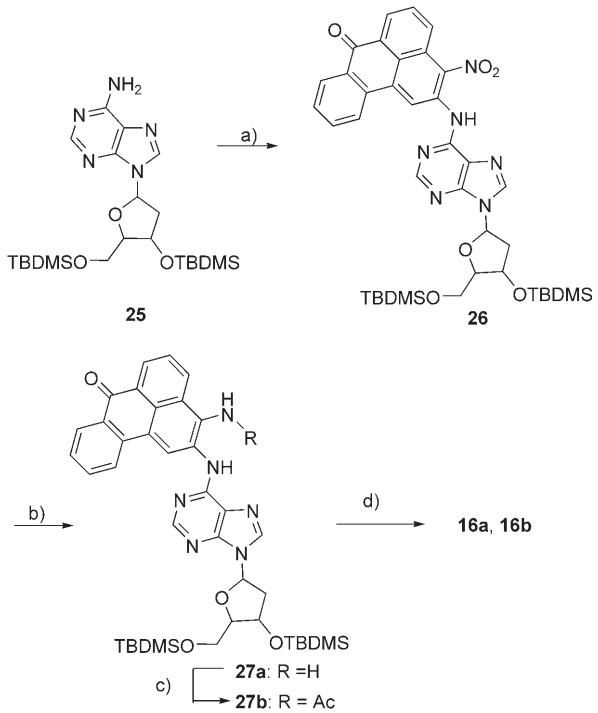
This procedure was applied to the synthesis of **16a**. The coupling reaction of 2′-deoxyadenosine derivative **25** and bromoarene **8** yielded **26**, and subsequent selective nitro reduction with $[Fe_3(CO)_{12}]$ gave **27a** (Scheme 7). The silyl protective groups of **27a** were removed by TEA·3HF to give **16a**. The acetylated derivatives **14b** and **16b** were obtained by general acetylation and removal of the silyl protective groups of **24a** and **27a**, respectively.

Analysis of the Reaction Products of Ultimate Mutagen 10 with dG, dA, and DNA

In this study, two types of metabolic ultimate forms of nitrobenzanthrone (NBA) were prepared to elucidate both the reactivity to nucleobases and the structures of the DNA adducts produced. The synthesis of **9** has already been reported. The other ultimate mutagen, **10**, was synthesized from **11** by the reported method with a slight modification.[19] Compound **10** was too unstable to be isolated from the reaction mixture; therefore, a polymer-bound base (TBD–methylpolystyrene; TBD = 1,5,7-triazabicyclo[4.4.0]dec-5-ene) was used, which could be easily removed from the reaction. With this method, 2 mg of **11** in THF was converted into the *N*-acetoxy form **10** within just a few minutes at room temperature. After addition of methanol to destroy excess pyruvonitrile, the polymer base was filtered off. TLC analysis



Scheme 6. Preparation of **14**. a) $[Pd_2dba_3]$ (10 mol%), xantphos, Cs_2CO_3, **8**. b) DBU, CH_3CN. c) $[Fe_3(CO)_{12}]$, CH_3OH/toluene. d) $(CH_3CO)_2O$, 4-DMAP, pyridine. e) TEA·3HF, THF.



Scheme 7. Preparation of **16**. a) $[Pd_2dba_3]$ (10 mol%), xantphos, Cs_2CO_3, **8**. b) $[Fe_3(CO)_{12}]$, CH_3OH/toluene. c) $(CH_3CO)_2O$, TEA, CH_2Cl_2. d) TEA·3HF, THF.

showed the formation of only one product, which was immediately used for the reaction with dG and DNA.

From LC–MS analysis of the reaction mixture of dG with **10** at 37 °C in THF/water (1:1) buffered at pH 7.5, we found the presence of three peaks of parent ions of *m*/*z* 511 ($[M+1]^+$) corresponding to the dG adducts derived from **10**. From the retention volume of HPLC and the UV/Vis spectrum of the corresponding peaks with *m*/*z* 511, **12a, 13a**, and **14a** were found to be formed in 9, 10, and 2% yields, respectively (Figure 1). The preparation of **12a** has previously been reported, but the formation of this compound in the reaction of dG and the ultimate form of **2** is noted for the first time in this report.

In the reaction of dA with **10**, LC–MS analysis showed that only one dA adduct, **16a**, formed in the reaction mixture (Figure 2). Possible dA adduct **15a** was not detected by the present methodology. Furthermore, we could not find corresponding peaks of **15a** with the LC–MS technique; however, it would be possible to

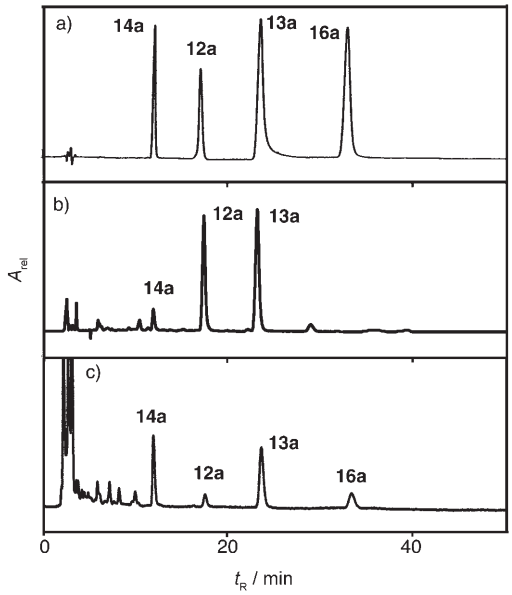


Figure 1. HPLC profiles of the reaction mixture of **10** with dG and DNA. a) Authentic samples, b) reaction mixture of dG with **10**, c) enzymatic hydrolysates from the reaction mixture of DNA with **10**.

CHEMISTRY
AN ASIAN JOURNAL

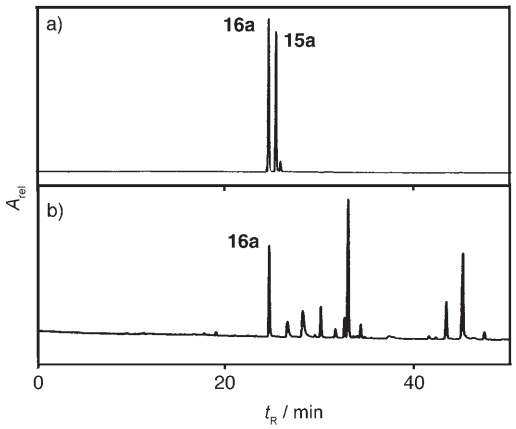


Figure 2. HPLC profiles of the reaction mixture of **10** with dA. a) Authentic samples, b) reaction mixture of dA with **10**.

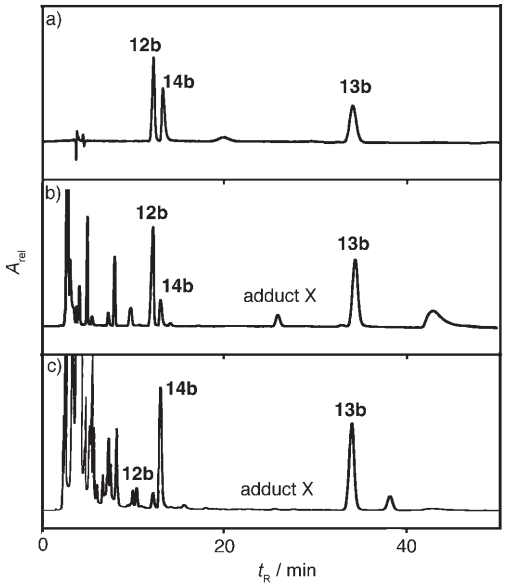


Figure 3. HPLC profile of the reaction mixture of **9** with dG and DNA. a) Authentic samples, b) reaction mixture of dG with **9**, c) enzymatic hydrolysates from the reaction mixture of DNA with **9**. An unidentified DNA adduct is marked "adduct X".

detect **15a** by using a much more sensitive detection method such as ^{32}P postlabeling. Other nucleobases such as dC and T did not give any new peaks corresponding to adducts when incubated with **10**.

Calf thymus DNA was also treated with **10**, and enzymatic hydrolysates were then further applied to HPLC. These four DNA adducts, **12a**, **13a**, **14a**, and **16a**, were also confirmed to be included in the reaction mixture by HPLC (Figure 1c). Interestingly, the product ratio for the reaction mixture of dG alone and that of DNA was different. The ratio **12a**/**13a**/**14a** for dG was approximately 1:1:0.2, but became 0.1:1:1 in the reaction with DNA.

Analysis of the Reaction Products of Ultimate Mutagen 9 with dG, dA, and DNA

We previously reported that compound **9** reacts with dG to form the unusual adduct **12b**. In a subsequent study by Osborne and co-workers, compound **13b** from dG and **17** from dA were found to be produced from **9** with dG/dA.[20] With authentic specimens in hand, we reinvestigated the reaction mixture of dG and **9**. The freshly prepared ultimate mutagen **9** was treated with dG at 37 °C overnight. On an HPLC chromatogram, we found the known peak of **12b** and the peak of **13b**, the retention times and UV patterns of which were identical to those of authentic samples (Figure 3). DNA adduct **14b** was further identified in the same reaction mixture. LC–MS analysis showed that these three peaks correspond to a parent ion of *m*/*z* 553 ($[M+1]^+$). Notably, the glycosidic bond of **13b** is very labile to acid treatment or protonation under ESI, and MS analysis revealed a very small peak of the parent ion $[M+H]^+$, which makes the identification of the dG adduct by a LC–MS difficult. The sensitivity of **13b** to H^+ probably comes from enhancement of the nucleophilicity of the N7 atom of dG by the addition of an electron-donating amine group to C8. Furthermore, the introduction of an acetyl group to the amine group may result in the loss of one resonance-stabilization pathway by which the amine group at the 8-position is converted into an imino group after protonation of N7. Compound **13b** was also isolated from the same reaction system by another research group, and its NMR spectrum is the same as that of our synthetic compound. Moreover, an additional unknown peak of the parent ion of *m*/*z* 553 was found (adduct X in Figure 3), although its UV absorption peak on HPLC was the smallest among the peaks of the adducts identified. This compound is probably an adduct at the O^6 positon of dG or a hydrazine-type adduct at the N^2 position of dG, recently identified as a novel dG adduct from 4-aminobiphenyl (ABP).[21]

In the hydrolysates of calf thymus DNA treated with **9**, dG adducts of **12b**, **13b**, and **14b** were also detected. Again, the product ratio of these three adducts was different between the reaction mixture of dG alone and that of DNA; the formation ratio of the N^2 adduct increased, whereas the ratio of the C–C adduct of **12b** decreased. (Figure 3c)

In the reaction mixture of dA and **9**, a compound with a parent ion of *m*/*z* 537 was confirmed by LC–MS analysis, but the HPLC retention time and UV spectrum of this adduct were different from those of the putative dA adducts of **15b** and **16b** (Figure 4). Instead of general-type adducts, HPLC showed one UV peak corresponding to the new type of dA adduct **17**. This adduct **17** was also present in enzymatic hydrolysates of calf thymus DNA with **9**.[8a] However, in the same hydrolysates, **15b** and **16b** were not detected, although dA–N^6 adduct formation was confirmed in the reaction of **10** with dA or calf thymus DNA. In the case of the dA adduct from **9**, the *N*-acetyl group turned out to be essential for forming this unique adduct **17**.

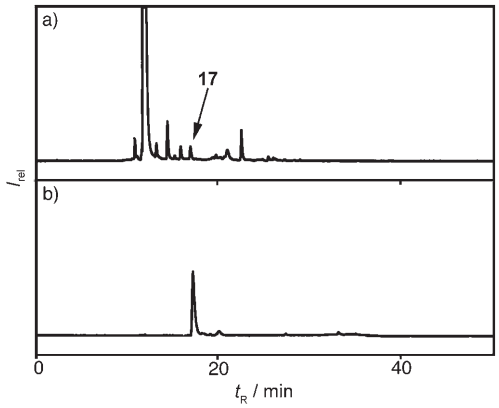
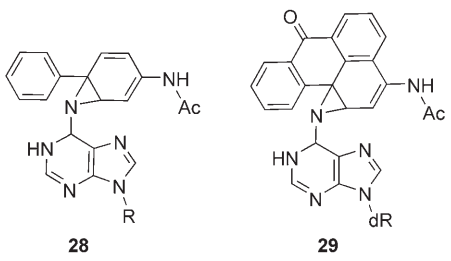


Figure 4. HPLC profile of the reaction mixture of **9** with dA. a) Reaction mixture of dA with **9** (UV monitoring at 260 nm), b) LC–MS analysis (selected-ion monitoring) of *m*/*z* 537, corresponding to a molecular ion of a putative dA–NBA adduct.

2′-Deoxyadenosine adduct **17** was newly identified in recent years.[12] dA adducts of similar structural type from nitroarenes have not been reported before. Novak and co-workers reported the formation of the unique benzene–imine-type adduct from the reaction of the ultimate form of ABP and adenosine (A).[14b] They used *N*-*tert*-butyloxy-4-aminobiphenyl as an ultimate form of ABP and found that benzene–imine adduct **28** formed from A even though an *N*-acetyl group was not attached to the ultimate mutagen. It is possible that this benzene–imine-type dA adduct **29** is the real structure of the dA adduct from **9** instead of the five-membered-ring adduct **17**, although the five-membered-ring structure is more likely than the benzene–imine adduct to form on the basis of structural stability. However, Osborne and co-workers deduced the five-membered-ring structure based on NOESY correlation and computational modeling. Moreover, based on our 2D NMR studies (NOESY, HMQC, HMBC) of **17** in dimethyl sulfoxide (DMSO), we cannot at present exclude the possibility of the formation of this benzene–imine structure **29** (Scheme 8) as the dA adduct from **9**. We did not observe cross-correlation peaks between the carbon atom at the 1-position of benzanthrone and the proton at the 2-position of dA by HMBC studies, which are expected to show a long-range correlation peak of these carbon and proton atoms in the five-membered-ring structure.



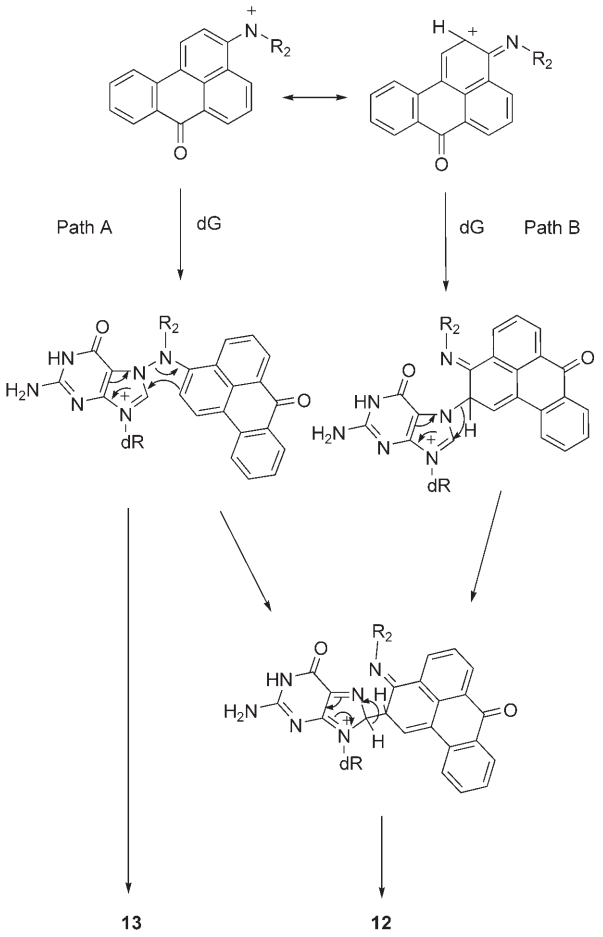
Scheme 8. Benzene–imine-type DNA adduct reported by Novak and co-workers[14b] (left) and possible alternative structure of **17** (right).

The formation ratios of the three dG adducts **12a/b**, **13a/b**, and **14a/b** in the reaction mixture from dG are clearly different from those from DNA, and a strong preference for dG–N^2 adduct formation in the reaction of calf thymus DNA with ultimate mutagens was revealed. This phenomenon is also reported in the case of DNA adducts from *N*-acetyl-3-aminofluorene.[22] At first glance, this is curious because the C8 atom of dG is located in the major groove of double-stranded DNA and is thus accessible by ultimate mutagens; the N^2 position is in the minor groove and is thus not likely to be easily accessed.

From extensive studies carried out by Novak and Kadlubar and their co-workers,[14] the mechanism for the formation of dG–C8 adducts is now shown to be initiated by the attack of the N7 atom of the purine ring on the N atom of nitrenium ions, followed by 1,2-migration and deprotonation. They showed the high selectivity of N7 in basic purine for the N atom of the nitrenium ions, and that the initial product of N7 attack on the *ortho* or *para* carbon atom of the aromatic ring proximal to the nitrenium N atom may be prohibited by steric congestion. In our case, the general-type dG–C8 adducts **13a** and **13b**, as well as the C–C-bond-type dG–C8 adducts **12a** and **12b**, are formed with nearly equal efficiency from a dG monomer and the corresponding nitrenium ions. These adduct formations are explained by the same mechanism of initial attack on N7, whether on the N atom of nitrenium ions or the C atom *ortho* to the nitrenium group. The steric effects with respect to the N7 atom, therefore, would not significantly contribute to the high selectivity of N7 on the N atom of the nitrenium ions. The possibility of initial attack of N7 on the N atom of nitrenium ions to form C–C-bond-type dG–C8 adducts by path A in Scheme 9 may be excluded, because this mechanism is not able to account for the drastic changes of the formation ratio **12**/**13** that appeared in the reaction of DNA and dG from **9** or **10**. Indeed, in the case of DNA, the C–C-bond-type dG–C8 adduct **12** was formed at only about one-tenth the efficiency of general-type dG–C8 adduct **13**. It is likely that with DNA, the basicity of N7 of the purine nucleoside as well as N^2 is different from that of the original monomer nucleoside due to the effect of the stacking of other bases, which could lead to a drastic change in the product formation ratio. If the basicity of N7 decreases, the rate constant of the trapping of N7 decreases, while other types of DNA adducts (e.g., dG–N^2, dA–N^6, or dA–imino adducts) predominantly form. Certain steric effects including intercalation may also be interpreted to be related to this preference of N^2-adduct formation, although more precisely detailed mechanistic studies will be needed to clarify this point.

The DNA adduct formation of **2** in vivo has been extensively studied, not only by our laboratory, but also by Arlt and co-workers.[2] In a study of the treatment of **2** on HepG2 cells in vivo, we found **12b** and **17** by a ^{32}P postlabeling method followed by HPLC fractionation, as well as two unidentified major adduct spots.[6b] Arlt et al. also found the formation of several DNA adducts in cell or rats treated with **2** and concluded that the formation of **12b** is not very

CHEMISTRY
AN ASIAN JOURNAL



Scheme 9. Mechanism of DNA-adduct formation of nitrobenzanthrone **2**.

significant, and that other DNA adducts contributed to the formation of DNA adducts derived from **2**.[10c] From a number of studies of the metabolites of nitroarenes in cells, nitro reduction followed by O-acetylation is the main pathway, and N,O-diacetylation may be a minor one. It is probable that it is the DNA adducts from **10** that mainly contribute to the formation of DNA adducts in vivo. The quantification of DNA-adduct formation in cells treated with **2** has been undertaken and is now a subject of active investigation.

Conclusions

We described here multiple-DNA-adduct formation by the reaction of DNA and ultimate metabolites of **2**. The structures of these adducts were confirmed definitively by independent chemical synthesis through a palladium-catalyzed aryl amination reaction. After this study was completed, collaborative studies with Phillips and co-workers showed that diphosphate derivatives of DNA adducts **13a**, **14a**, and **16a** were really produced in the DNA of rats treated with 3-nitrobenzanthrone.[22] This finding indicates that formation of compound **10** is predominant in vivo, and subsequent adduct formation by 3-nitrobenzanthrone mainly contributes to carcinogenesis.[20]

Experimental Section

Materials and Methods

All solvents were of organic-synthesis grade and used without further purification. HPLC was performed with a Shimadzu LC10Avp system equipped with a Shimazdu PDA10Avp photodiode array detector. All HPLC eluents used were of HPLC grade. NMR spectroscopy was performed on a JEOL JNM-400 spectrometer and *J* values are given in Hz. EI MS, FAB MS, and HRMS were performed on a JEOL JMS-α700 mass spectrometer. ESI MS was performed on a Waters ZQ2000 mass spectrometer. 3-Hydroxyaminobenzanthrone (**11**), **9**, **12b**, and its deacetylation product, 2-(2′-deoxyguanosin-8-yl)-3-aminobenzanthrone (**12a**), were synthesized by the method previously reported.[8a]

Synthetic Procedure

Coupling procedure: Amine (0.1 mmol), bromoarene (0.1 mmol), [Pd_2dba_3] (0.05 mmol), xantphos (0.02 mmol), and Cs_2CO_3(0.2 mmol) were dissolved in toluene (1 mL) and stirred for 2 h at the temperature indicated. After confirmation of the consumption of starting material by TLC, the solvent was evaporated and the desired coupling compounds were purified by column chromatography.

Detritylation procedure 1: Trichloroacetic acid (3%, 5 mL) in CH_2Cl_2 was added to a solution of the *N*-4,4′-dimethoxytritylated nucleoside (0.1 mmol) in MeOH/CH_2Cl_2. After confirmation of the disappearance of the starting material, the reaction mixture was added to a buffer solution of ammonium formate (pH 6.8). The organic layer was extracted with brine, dried over sodium sulfate, and evaporated. The residues were subjected to chromatography on silica gel.

Detritylation procedure 2: Same as detritylation procedure 1 except for the use of monochloroacetic acid (5%) instead of trichloroacetic acid for cleaving the 4,4′-dimethoxytrityl group.

Deprotection procedure 1: The benzyl group at the O^6 position of the dG adduct was removed by catalytic hydrogenation with Pd black/H_2. Typically, the O^6-benzylated dG derivative (0.1 mmol) was dissolved in THF. Pd black (20–30 mg) was added to the solution, which was stirred until the initial spot on TLC under a balloon filled with H_2 gas disappeared. After the reaction was complete, the catalyst was removed by filtration and washed with THF. The filtrate was used for a further deprotection procedure without any purification.

Deprotection procedure 2: The nitrophenylethyl group of **23** was removed by treatment with DBU. Typically, the protected adducts (0.5 mmol) were dissolved in acetonitrile/DMF (9:1), DBU (100 μL) was added, and the mixture was stirred overnight. The reaction mixture was then extracted with chloroform three times. The organic layer was then extracted with brine, dried over sodium sulfate, and evaporated. The residue was subjected to silica-gel column chromatography.

Deprotection procedure 3: The silyl protective groups of the ribose moiety of the DNA adducts were removed by a TEA·3HF. The silylated DNA adducts (≈0.1 mmol) were dissolved in THF, and TEA·3HF (100 μL) was added. After reaction overnight, the reaction mixture was evaporated and the resulting residue was subjected to octadecyl silica gel (ODS) column chromatography.

Acetylation procedure 1: Acetylation of the amine moiety of C8 of **19a**, **21a**, and **24a** was performed by the method of Gillet and Schärer.[17e] Acetic anhydride (5 equiv), triethylamine (5 equiv), and 4′-dimethylaminopyridine (0.5 equiv) was added to a solution of pyridine (1 mL). After confirmation of disappearance of the initial spot on TLC, methanol was added to the reaction mixture, which was then evaporated and subjected to chromatography on silica gel.

Acetylation procedure 2: Acetylation of dA–N^6 adduct derivative **27a** was performed with acetic anhydride (5 equiv) and triethylamine (5 equiv) in dichloromethane. The reaction mixture was then extracted

with aqueous Na_2CO_3 and then brine. The organic layer was dried over Na_2SO_4 and then evaporated. The residues were subjected to chromatography on silica gel.

Nitro-reduction procedure: Reduction of the nitro group of **23b** and **26** to the amine group was performed with $[Fe_3(CO)_{12}]$ in methanol/toluene under reflux conditions as previously indicated.[18]

8: *N*-bromosuccinimide (1 mmol) was added with stirring to a solution of 3-aminobenzanthrone (**5**; 1 mmol) in DMF at −20 °C. After confirmation of the disappearance of the initial TLC spot, water was added, and the resulting reddish-orange precipitate was collected and lyophilized to give 2-bromo-3-aminobenzanthrone (**6**; 320 mg) almost quantitatively. ^{1}H NMR ([D$_6$]DMSO): δ = 8.84 (d, J = 8.8 Hz, 1H), 8.70 (s, 1H), 8.64 (dd, J = 7.6, 0.8 Hz, 1H), 8.45 (d, J = 8.0 Hz, 1H), 8.26 (dd, J = 8.0, 0.8 Hz, 1H), 7.82 (t, J = 8.0 Hz, 1H), 7.74 (dt, J = 8.0, 0.1 Hz, 1H), 7.47 (t, J = 7.9 Hz, 1H), 6.88 ppm (s, 2H); ^{13}C NMR ([D$_6$]DMSO): δ = 181.6, 147.9, 134.3, 133.1, 130.5, 130.4, 130.3, 130.2, 130.2, 127.7, 127.6, 127.3, 126.0, 125.2, 125.2, 124.0, 112.2 ppm; HRMS (EI): *m/z* calcd for $C_{17}H_{10}BrNO$: 322.9945 $[M]^+$; found: 322.9969, 324.9928 (1:1). The resulting **6** was dissolved in sulphuric acid, and nitrosyl sulphate (10 equiv), which was freshly prepared from sodium nitrite in sulphuric acid, was added at 0 °C. After 1 h, this solution was added dropwise to acetic acid at a temperature not in excess of 20 °C. Copper(I) oxide (10 equiv with respect to starting material) was then added, and the mixture was stirred at room temperature for 3 h. The reaction mixture was poured into iced water and extracted with ethyl acetate three times. The combined organic layer was then treated with aqueous sodium bicarbonate until evolution of CO_2 gas ceased, then further extracted with brine, dried over sodium sulfate, and evaporated. The resulting residue was subjected to chromatography on silica gel with hexane/ethyl acetate (5:1) as an eluent to give 2-bromobenzanthrone (**7**; 150 mg, 50%). ^{1}H NMR ([D$_6$]DMSO): δ = 8.54 (dd, J = 7.6, 1.6 Hz, 1H), 8.33 (dd, J = 7.6, 1.6 Hz, 1H), 8.23 (d, J = 1.6 Hz, 1H) 8.05 (dd, J = 8.0, 0.4 Hz, 1H), 7.93 (d, J = 1.6 Hz, 1H), 7.92 (dd, J = 6.4, 0.8 Hz, 1H), 7.65–7.60 (m, 2H), 7.46 ppm (dt, J = 8.0 1.2 Hz, 1H); ^{13}C NMR ([D$_6$]DMSO): δ = 182.9, 134.6, 133.8, 133.4, 131.4, 131.0, 129.7, 128.9, 128.7, 128.3, 127.5, 127.0, 126.2, 123.0, 120.7 ppm; HRMS (EI): *m/z* calcd for $C_{17}H_9BrO$: 307.9836 $[M]^+$; found: 307.9840, 309.9857 (1:1). Bromobenzanthrone 7 thus obtained was dissolved in nitrobenzene. Concentrated nitric acid (d = 1.5, 5 equiv) was added to this solution, and a brownish-yellow solid separated from the solution. The solid materials were collected, washed with methanol, and recrystallized from acetic acid to give 2-bromo-3-nitrobenzanthrone (**8**; 110 mg, 65%). ^{1}H NMR ([D$_6$]DMSO): δ = 9.02 (s, 1H), 8.69 (d, J = 8.0 Hz, 1H), 8.66 (d, J = 7.2 Hz, 1H), 8.29 (d, J = 7.6 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 8.04 (t, J = 7.2 Hz, 1H), 7.88 (dt, J = 7.6 Hz, 1H), 7.72 ppm (t, J = 7.6 Hz, 1H); ^{13}C NMR ([D$_6$]DMSO): δ = 181.6, 147.9, 134.3, 133.1, 130.5, 130.4, 130.3, 130.2, 130.2, 127.7, 127.6, 127.3, 126.0, 125.2, 125.2 124.0, 112.2 ppm; HRMS (EI): *m/z* calcd for $C_{17}H_8BrNO_3$: 352.9688 $[M]^+$; found: 352.9796.

23a: 2-(3′,5′-Bis-*O*-*tert*-butyldimethylsilyl-O^6-nitrophenylethyl-2′-deoxyguanosin-N^2-yl)-3-nitrobenzanthrone (**23a**; 57 mg, 63%) was obtained from 3′,5′-bis-*O*-*tert*-butyldimethylsilyl-O^6-nitrophenylethyl-2′-deoxyguanosine (**22**) and **8** by the coupling procedure at a reaction temperature of 100 °C. ^{1}H NMR ($CDCl_3$): δ = 9.79 (s, 1H), 9.12 (s, 1H), 8.68 (d, J = 7.6 Hz, 1H), 8.51 (d, J = 8.8 Hz, 1H), 8.39 (d, J = 8.7, 1.1 Hz, 1H), 8.28 (d, J = 8.1 Hz, 1H), 8.13 (s, 1H), 8.10 (dt, J = 9.0, 2.0 Hz, 2H), 7.86 (t, J = 7.2 Hz, 1H), 7.72–7.60 (m, 1H), 7.59 (t, J = 7.6 Hz, 1H), 7.43 (d, J = 8.8 Hz, 1H), 6.47 (t, J = 6.5 Hz, 1H), 4.82 (t, J = 6.8 Hz, 2H), 4.56 (s, 1H), 4.03 (s, 1H), 3.82 (ddd, J = 19.0, 11.3, 3.4 Hz, 2H), 3.22 (t, J = 6.4 Hz, 1H), 2.62–2.50 (m, 1H), 2.44–2.42 (m, 1H), 0.92 (s, 9H), 0.81 (s, 9H), 0.08 (s, 3H) 0.07 (s, 3H), 0.04 (s, 3H), −0.01 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): δ = 183.0, 160.5, 153.9, 152.6, 146.7, 145.4, 139.1, 134.8, 134.5, 133.9, 131.3, 131.2, 129.8, 129.7, 129.3, 128.9, 128.4, 128.3, 128.1, 126.1, 124.0, 123.7, 123.6, 119.0, 118.9, 117.7, 88.1, 83.8, 72.0, 66.8, 62.9, 41.5, 35.1, 26.0, 25.7, 18.5, 18.0, −4.5, −4.7, −5.2, −5.4 ppm; HRMS (FAB; NBA): *m/z* calcd for $C_{46}H_{56}N_7O_9Si_2$: 918.3678 $[M+H]^+$; found: 918.3585.

2-(3′,5′-Bis-*O*-*tert*-butyldimethylsilyl-O^6-benzyl-2′-deoxyguanosin-N^2-yl)-3-nitrobenzanthrone: This compound (73 mg, 85%) was obtained from 3′,5′-bis-*O*-*tert*-butyldimethylsilyl-O^6-benzyl-2′-deoxyguanosine and **8** by the coupling procedure at a reaction temperature of 100 °C. ^{1}H NMR ($CDCl_3$): δ = 9.75 (s, 1H), 9.11 (s, 1H), 8.56 (d, J = 7.2 Hz, 1H), 8.41 (d, J = 8.0 Hz, 1H), 8.38 (d, J = 7.2 Hz, 1H), 8.24 (d, J = 8.0 Hz, 1H), 8.06 (s, 1H), 7.77 (t, J = 8.0 Hz, 1H), 7.63 (t, J = 7.2 Hz, 1H), 7.52 (t, J = 7.6 Hz, 1H), 7.42 (d, J = 7.2 Hz, 2H), 7.29–7.22 (m, 3H), 6.41 (t, J = 6.4 Hz, 1H), 5.57 (s, 2H), 4.50 (t, J = 2.8 Hz, 1H), 3.96 (s, 1H), 3.72 (m, 2H), 2.56–2.52 (m, 1H), 2.45–2.40 (m, 1H), 0.82 (s, 9H), 0.76 (s, 9H), 0.10 (s, 6H) 0.09 (s, 3H), 0.07 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): δ = 183.0, 160.8, 153.9, 152.6, 135.7, 134.6, 134.1, 133.8, 131.3, 131.2, 129.9, 129.3, 128.9, 128.4, 128.3, 128.2, 128.1, 126.1, 123.9, 123.8, 118.9, 117.9, 88.0, 83.8, 71.9, 68.9, 62.9, 41.5, 26.0, 25.7, 18.5, 18.0, −4.5, −4.7, −5.2, −5.4 ppm; HRMS (FAB; NBA): *m/z* calcd for $C_{46}H_{55}N_6O_7Si_2$: 859.3671 $[M+H]^+$; found: 859.4843.

26: 2-(3′,5′-Bis-*O*-*tert*-butyldimethylsilyl-2′-deoxyadenosin-N^6-yl)-3-nitrobenzanthrone (**26**; 54 mg, 72%) was obtained from 3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxyadenosine (**25**) and **8** by the coupling procedure at a reaction temperature of 100 °C. ^{1}H NMR ($CDCl_3$): δ = 9.96 (s, 1H), 8.65 (s, 1H), 8.59 (d, J = 8.0 Hz, 1H), 8.42 (dd, J = 8.5, 0.8 Hz, 2H), 8.30 (s, 1H), 8.27 (d, J = 3.2 Hz, 1H), 7.84–7.70 (m, 2H), 7.61 (t, J = 7.5 Hz, 1H), 6.51 (t, J = 6.3 Hz, 1H), 4.67–4.65 (m, 1H), 4.05 (dd, J = 7.0, 3.2 Hz, 1H), 3.89 (dd, J = 11.3, 4.2 Hz, 1H) 3.79 (dd, J = 11.3, 3.1 Hz, 1H), 2.73–2.60 (m, 1H), 2.52–2.40 (m, 1H), 0.92 (s, 18H), 0.11 ppm (s, 12H); ^{13}C NMR ([D$_6$]DMSO): δ = 182.9 157.2, 152.0, 150.8, 150.0, 140.8, 140.7, 136.2, 135.3, 134.3, 133.8, 132.9, 131.6, 131.1, 130.0, 129.9, 129.4, 129.3, 128.9, 128.7, 128.1, 128.0, 127.9, 126.1, 125.9, 124.3, 123.8, 121.9, 119.2, 88.0, 84.6, 71.9, 62.7, 60.1, 41.3, 26.0, 25.8, 18.5, 18.0, −4.6, −4.7, −5.2 −5.3 ppm; HRMS (FAB; NBA): *m/z* calcd for $C_{39}H_{49}N_6O_6Si_2$: 753.3252 $[M+H]^+$; found: 753.3317.

21a: *N*-[3′,5′-Bis-*O*-*tert*-butyldimethylsilyl-N^{6-}-4,4′-dimethoxytrityl-2′-deoxyadenosin-8-yl]-3-aminobenzanthrone (**21a**; 87 mg, 85%) was obtained from 3′,5′-bis-*O*-tert-butyldimethylsilyl-N^{6-}-4,4′-dimethoxytrityl-8-bromo-2′-deoxyadenosine (**20**) and **5** by the coupling procedure at a reaction temperature of 80 °C. ^{1}H NMR ($CDCl_3$): δ = 8.67 (d, J = 7.2 Hz, 1H), 8.6 (br s, 1H), 8.36–8.34 (m, 2H), 8.23 (d, J = 8.2 Hz, 1H), 8.17 (d, J = 8.2 Hz, 1H), 7.82 (s, 1H), 7.67 (t, J = 7.8 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.38 (t, J = 7.7 Hz, 1H), 7.22–7.06 (m, 14H), 6.63 (d, J = 8.7 Hz, 4H), 6.42 (s, 1H), 6.34 (dd, J = 7.6, 5.5 Hz, 1H), 4.49–4.45 (m, 1H), 4.08 (q, J = 2.7 Hz, 1H), 3.81 (dd, J = 11.5, 2.9 Hz, 1H) 3.70 (dd, J = 11.5, 3.0 Hz, 1H), 3.63 (s, 6H), 3.00–2.92 (m, 1H), 2.35–2.31 (m, 1H), 0.81 (s, 12H), 0.45 (s, 12H), 0.01 (s, 3H), 0.00 (s, 3H), −0.33 (s, 3H), −0.47 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): δ = 183.8, 157.9, 151.5, 149.6, 148.3, 148.0, 145.7, 137.8, 137.2, 136.3, 133.3, 130,2, 130.1, 129.8, 128.9, 128.8, 128.5, 127.9, 127.7, 127.6, 127.4, 126.5, 126.3, 126.1, 125.4, 122.7, 121.9, 118.4, 118.3, 112.9, 88.4, 85.7, 72.3, 70.3, 62.9, 55.1, 39.9, 29.7, 25.8, 25.6, 18.2, 18.0, 14.2, −4.44, −4.66, −5.62, −5.64 ppm; HRMS (FAB): *m/z* calcd for $C_{60}H_{69}N_6O_6Si_2$: 1025.4817 $[M+H]^+$; found: 1025.7704.

N-[3′,5′-Bis-*O*-*tert*-butyldimethylsilyl-2′-deoxyadenosin-8-yl]-3-aminobenzanthrone: This compound (50 mg, 80%) was obtained from **21a** with detritylation procedure 1. ^{1}H NMR ($CDCl_3$): δ = 8.61 (d, J = 7.2, 1H), 8.6 (br s, 1H), 8.36–8.34 (m, 2H), 8.20–8.14 (m, 2H), 8.1–8.0 (m, 3H), 7.6–7.49 (m, 1H), 7.35 (t, J = 7.6 Hz, 1H), 7.14–7.12 (m, 3H), 7.01 (d, J = 6.8 Hz, 1H), 6.67 (dd, J = 8.0, 2.0 Hz, 1H), 6.34 (s, 1H), 5.46 (s, 2H), 4.45 (br s, 1H), 4.01 (s, 1H), 3.81 (d, J = 2.0 Hz, 1H), 3.64 (d, J = 2.0 Hz, 1H), 3.00–2.85 (m, 1H), 2.39–2.31 (m, 1H), 0.79 (s, 9H), 0.42 (s, 9H), 0.02 (s, 3H), 0.01 (s, 3H), −0.38 (s, 3H), −0.49 ppm (s, 3H); ^{13}C NMR ($CDCl_3$): δ = 183.8, 158.4, 152.4, 150.0, 149.2, 148.0, 147.2, 139.4, 136.9, 136.0, 133.3, 130.1, 129.8, 129.0, 128.8, 128.5, 128.0, 127.8, 127.7, 127.6, 127.5, 126.4, 126.2, 126.1, 125.4, 124.9, 122.7, 121.9, 118.4, 112.9, 88.3, 85.5, 81.3, 72.3, 62.9, 55.1, 39.9, 31.9, 29.7, 25.8, 25.6, 18.2, 18.0, 14.2, −4.44, −4.66, −5.62, −5.64 ppm; HRMS (FAB; NBA): *m/z* calcd for $C_{39}H_{51}N_6O_4Si_2$: 723.3511 $[M+H]^+$; found: 723.3436.

15a: *N*-(2′-Deoxyadenosin-8-yl)-3-aminobenzanthrone (**15a**) was obtained from *N*-[3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxyadenosin-8-yl]-3-aminobenzanthrone by deprotection procedure 3 in almost quantitative yield (33 mg). ^{1}H NMR ([D$_6$]DMSO): δ = 9.49 (s, 1H, NH of BA), 8.73–8.63 (m, 4H, 4-H, 6-H, 11-H, 8-H of BA), 8.56 (d, J = 7.8 Hz, 1H, 1-H of BA), 8.33 (d, J = 8.0 Hz, 1H, 2-H of BA), 8.03 (s, 1H, 2-H of A), 7.94–7.81 (m, 3H, 8-H, 5-H, 10-H of BA), 7.58 (t, J = 7.2 Hz, 1H, 9-H of BA),

6.84 (s, 2H, NH_2 of A), 6.56 (br s, 1H, 1′-H of dR), 5.73 (s, 1H, 5′-OH of dR), 5.32 (s, 1H, 3′-OH of dR), 4.46 (s, 1H, 3′-H of dR), 3.97 (s, 1H, 4′-H of dR), 3.77–3.72 (m, 2H, 5′-H of dR), 3.62 (br s, 1H, 5′-H of dR), 3.05–3.00 (m, 1H, 2″-H of dR), 2.27–2.20 ppm (m, 1H, 2′-H of dR); ^{13}C NMR ($CDCl_3$): δ = 182.5, 153.5, 150.1, 148,5, 147.4, 139.9, 135.8, 133.8, 130.5, 129.6, 129.3, 127.7, 127.1, 126.3, 126.1, 126.0, 123.4, 120.8, 118.7, 116.5, 87.7, 84.0, 71.3, 61.7 ppm; HRMS (FAB): *m/z* calcd for $C_{27}H_{23}N_6O_4$: 495.1780 $[M+H]^+$; found: 495.1686.

15b: Compound **21a** was acetylated by acetylation procedure 1. The resulting compound **21b** was purified by column chromatography and further treated with detritylation procedure 2 followed by deprotection procedure 3 to give *N*-(2′-deoxyadenosin-8-yl)-3-acetylaminobenzanthrone (**15b**; 30 mg, 35% from **21a**). ^{1}H NMR (CD_3OD): δ = 8.75–8.50 (m, 3H), 8.28–8.23 (m, 2H), 8.14 (s, 1H), 8.01–7.60 (m, 3H), 7.47 (t, *J* = 7.3 Hz, 1H), 6.57 (br s, 1H), 4.69 (br s, 1H), 4.24 (br s, 1H), 3.94 (br s, 1H), 3.81 (br s, 1H), 3.25–3.00(m, 1H), 2.52–2.30 (m, 1H), 2.20 ppm (s, 3H); ^{13}C NMR (CD_3OD): δ = 184.6, 157.3, 153.3, 149.7, 145.7, 139.3, 136.5, 135.0, 132.2, 131.7, 131.2, 130.0, 129.8, 129.4, 128.6, 125.7, 124.9, 90.7, 88.1, 73.8, 64.4, 39.8 ppm; HRMS (FAB): *m/z* calcd for $C_{29}H_{25}N_6O_5$: 537.1886 $[M+H]^+$; found 537.3231.

16a: Compound **26** was treated with the nitro-reduction procedure, and the product was purified by column chromatography to give 2-(3′,5′-bis-*O*-tert-butyldimethylsilyl-2′-deoxyadenosin-N^6-yl)-3-aminobenzanthrone (**27a**; 51 mg, 76%). ^{1}H NMR ($CDCl_3$): δ = 8.72 (d, *J* = 7.2 Hz, 1H), 8.45–8.36 (m, 3H), 8.28–8.26 (m, 1H), 8.19 (d, *J* = 8.8 Hz, 1H), 8.10–8.02 (m, 2H), 7.93 (s, 1H), 7.69–7.38 (m, 2H), 6.47 (dt, *J* = 6.4, 2.7 Hz, 1H), 4.63–4.60 (m, 1H), 4.03 (q, *J* = 3.3 Hz, 1H), 3.89 (dt, *J* = 11.2, 4.1 Hz, 1H), 3.78 (dt, *J* = 11.4, 2.7 Hz, 1H), 2.72–2.60 (m, 1H), 2.50–2.43 (m, 1H), 0.91 (s, 9H), 0.90 (s, 9H), 0.10 (s, 6H), 0.09 ppm (s, 1H); ^{13}C NMR ($CDCl_3$): δ = 183.6, 141.8, 140.9, 139.5, 139.4, 136.4, 135.2, 133.0, 131.7, 130.8, 129.7, 129.6, 128.7, 128.5, 128.2, 127.9, 127.8, 127.7, 127.4, 127.3, 127.2, 126.5, 126.0, 125.3, 125.2, 123.5, 123.2, 122.7, 122.1, 120.3, 118.5, 117.1, 116.6, 116.2, 87.9, 84.5, 71.9, 71.7, 62.8, 41.5, 41.3, 26.1, 25.8, 18.5, 18.1, −4.4, −4.5, −5.1, −5.2 ppm; HRMS (FAB; NBA): *m/z* calcd for $C_{39}H_{51}N_6O_4Si_2$: 723.3511 $[M+H]^+$; found: 723.2997. Compound **27a** was further treated with deprotection procedure 3, and after being left overnight the reaction mixture was then purified by ODS column chromatography to give *N*-(2′-deoxyadenosin-N^6-yl)-3-aminobenzanthrone (**16a**; 30 mg, 82%). ^{1}H NMR ([D_6]DMSO): δ = 9.38 (s, 1H, NH of A6), 8.80 (d, *J* = 8.3 Hz, 1H, 4-H of BA), 8.65 (d, *J* = 7.3 Hz, 1H, 6-H of BA), 8.48 (s, 1H, 1-H of BA), 8.42 (s, 1H, 8-H of BA), 8.26 (d, *J* = 8.5 Hz, 1H, 11-H of BA), 8.24 (d, *J* = 8.3 Hz, 1H, 8-H of BA), 8.19 (s, 1H, 2-H of BA), 7.79 (t, *J* = 8.0 Hz, 1H, 5-H of BA), 7.67 (t, *J* = 6.4 Hz, 1H, 10-H of BA), 7.42 (t, *J* = 8.0 Hz, 1H, 9-H of BA), 6.56 (s, 2H, NH_2 of BA3), 6.39 (t, *J* = 6.0 Hz, 1H, 1′-H of dR), 5.36 (s, 1H, 5′-OH of dR), 5.23 (t, *J* = 6.4 Hz, 1H, 3′-OH of dR), 4.41–4.38 (m, 1H, 3′-H of dR), 3.86 (dd, *J* = 6.6, 3.9 Hz, 4′-H of dR), 3.63–3.57 (m, 1H, 5′-H of dR), 3.52–3.46 (m, 1H, 5′-H of dR), 2.76–2.69 (m, 1H, 2″-H of dR), 2.26 ppm (ddd, *J* = 13.1, 5.9, 2.6 Hz, 1H, 2′-H of dR); ^{13}C NMR ($CDCl_3$): δ = 182.5 (C=O of BA), 154.2 (A5), 152.1 (A2), 149.2 (A4), 144.5 (BA3), 140.0 (A8), 136.8 (BA11a), 133.4 (BA10), 130.3 (BA4), 129.2 (BA6), 128.4 (BA7a), 128.3 (BA1), 127.6 (BA6a), 126.8 (BA8) 126.5 (BA11c), 125.8 (BA9), 124.4 (BA5), 122.9 (BA3a), 122.5 (BA11), 120.4 (A6), 118.3 (BA2), 112.3 (BA11b), 88.0 (dR4′), 84.1 (dR1′), 71.0 (dR3′), 61.9 (dR5′), ≈40.0 ppm (dR2′; overlapping with DMSO); HRMS (FAB; NBA): *m/z* calcd for $C_{27}H_{23}N_6O_4$: 495.1780 $[M+H]^+$; found 495.1758.

16b: Compound **27a** was treated with acetylation procedure 2 followed by column chromatography to give *N*-[3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxyadenosin-N^6-yl]-3-acetylaminobenzanthrone (**27b**; 33 mg, 61%). HRMS (FAB): *m/z* calcd for $C_{41}H_{53}N_6O_5Si_2$: 765.3616 $[M+H]^+$; found: 765.3782. Compound **27b** was further treated with deprotection procedure 3. The reaction mixture was evaporated and subjected to chromatography with ODS to give *N*-(2′-deoxyadenosin-N^6-yl)-3-acetylaminobenzanthrone (**16b**; 23 mg) quantitatively. ^{1}H NMR ([D_6]DMSO): δ = 9.94 (s, 1H), 9.09 (s, 1H), 8.55 (dd, *J* = 7.2, 1.1 Hz, 1H), 8.50 (s, 1H), 8.43 (d, *J* = 8.5 Hz, 1H), 8.36 (dd, *J* = 7.8, 1.5 Hz, 1H), 8.20 (d, *J* = 8.3 Hz, 2H), 8.09 (s, 1H), 7.90 (t, *J* = 7.2 Hz, 1H), 7.77 (t, *J* = 7.9 Hz, 1H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.32–7.25 (m, 1H), 6.41 (t, *J* = 7.0 Hz, 1H), 5.32 (s, 1H), 5.13 (s, 1H), 4.43 (s, 1H), 3.89 (s, 1H), 3.65–3.50 (m, 2H), 2.81–2.74 (m, 1H), 2.50 (s, 3H), 2.30 ppm (ddd, *J* = 13.0, 6.5, 3.0 Hz, 1H); ^{13}C NMR ([D_6]DMSO): δ = 182.4, 169.7, 153.9, 151.8, 149.3, 140.6, 135.1, 134.0, 133.5, 131.5, 130.8, 130.0, 128.6, 128.1, 127.7, 127.3, 127.0, 126.8, 125.1, 124.4, 123.4, 122.1, 120.2, 87.9, 83.9, 70.9, 61.8, 13.5 ppm; HRMS (FBA; NBA): *m/z* calcd for $C_{29}H_{25}N_6O_5$: 537.1886 $[M+H]^+$; found: 537.1910.

14a: Compound **23a** was treated according to deprotection procedure 2. The reaction mixture was purified by column chromatography to give (2-(3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxyguanosin-N^2-yl)-3-nitrobenzanthrone (**23b**; 54 mg, 75%). ^{1}H NMR ($CDCl_3$): δ = 13.41 (s, 1H), 12.46 (s, 1H), 8.17 (d, *J* = 4.0 Hz, 1H), 8.09 (s, 1H), 7.91 (d, *J* = 4.8 Hz, 1H), 7.72 (s, 1H), 7.44–6.94 (m, 5H), 6.65 (s, 2H), 6.57 (br s, 1H), 5.02 (s, 1H), 3.98 (s, 1H), 3.27 (br s, 2H), 2.89–2.85 (m, 1H), 2.37–2.31 (m, 1H), 0.64 (s, 9H), 0.12 (s, 9H), −0.04 (s, 3H), −0.16 (s, 3H), −0.97 (s, 3H), −1.26 ppm (s, 3H). The resulting **23b** was further treated according to the nitro-reduction procedure to give 2-(3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxyguanosin-N^2-yl)-3-aminobenzanthrone (**24a**; 33 mg, 65%). ^{1}H NMR ($CDCl_3$): δ = 8.76–8.37 (m, 3H), 8.00–7.77 (m, 4H), 6.13 (s, 1H), 4.44 (s, 1H), 3.95 (s, 2H), 3.71 (br s, 1H), 2.58–2.27 (m, 2H), 0.91 (s, 18H), 0.80 (s, 12H), −0.24 (s, 9H), −033 ppm (s, 3H). Compound **24a** was immediately treated with deprotection procedure 3 to give 2-(2′-deoxyguanosin-N^2-yl)-3-aminobenzanthrone (**14a**; 23 mg, 54%). ^{1}H NMR ([D_6]DMSO): δ = 8.81 (d, *J* = 7.7 Hz, 1H, 4-H of BA), 8.72 (s, 1H, 8-H of G), 8.64 (dd, *J* = 7.3, 1.0 Hz, 1H, 6-H of BA), 8.36 (d, *J* = 7.9 Hz, 1H, 8-H of BA), 8.27 (dd, *J* = 7.9, 1.3 Hz, 1H, 11-H of BA), 7.97 (s, 1H, 1-H of BA), 7.79 (t, *J* = 7.8 Hz, 1H, 5-H of BA), 7.75–7.70 (m, 1H, 9-H of BA), 7.44 (t, *J* = 7.4 Hz, 1H, 10-H of BA), 6.71 (s, 2H, NH_2 of BA), 6.00 (t, *J* = 6.1 Hz, 1H, 1′-H of dR), 5.05 (s, 1H, 3′-OH of dR), 4.69 (t, *J* = 4.9 Hz, 1H, 5′-OH of dR), 4.09 (s, 1H, 3′-H of dR), 3.65 (td, *J* = 4.7, 2.8 Hz, 1H, 4′-H of dR), 3.36–3.30 (m, 1H, 5′-H of dR), 2.58–2.51 (m, 1H, 2″-H of dR), 2.09 ppm (dq, *J* = 13.2, 3.1 Hz, 1H, 2′-H of dR); ^{13}C NMR ([D_6]DMSO): δ = 182.4 (C=O of BA), 156.6 (G6), 151.4 (G2), 150.1 (G4), 143.3 (G8), 136.8 (BA3), 135.7 (BA11a), 133.3 (BA10), 130.1 (BA4), 129.0 (BA6), 128.5 (BA7a), 127.6 (BA6a), 126.9 (BA1), 126.8 (BA8), 126.2 (BA11c), 125.7 (BA9) 124.3 (BA5), 122.9 (BA11), 122.4 (BA3a), 117.7 (BA2), 117.1 (G5), 112.6 (BA11b), 87.6 (dR1′), 82.6 (dR4′), 70.7 (dR3′), 61.6, (dR5′) 55.9 ppm (dR2′); HRMS (FAB): *m/z* calcd for $C_{27}H_{23}N_6O_5$: 511.1729 $[M+H]^+$; found: 511.1767.

14b: Compound **24a** was acetylated with acetylation procedure 1 to give **24b**, followed by deprotection procedure 3 to give 2-(2′-deoxyguanosin-N^2-yl)-3-acetylaminobenzanthrone (**14b**; 18 mg, 52%). ^{1}H NMR ([D_6]DMSO): δ = 10.1 (s, 1H), 9.84 (s, 1H), 8.54 (dd, *J* = 7.4, 1.1 Hz, 1H), 8.49 (d, *J* = 7.9 Hz, 1H), 8.44 (s, 1H), 8.35–8.33 (m, 2H), 8.13 (s, 1H), 7.96 (t, *J* = 7.0 Hz, 1H), 7.90 (t, *J* = 7.8 Hz, 1H), 7.66 (d, *J* = 7.3 Hz, 1H), 6.32 (t, *J* = 6.8 Hz, 1H), 5.27 (d, *J* = 3.6 Hz, 1H), 4.86 (s, 1H), 4.28 (s, 1H) 3.86 (dt, *J* = 7.2, 2.4 Hz, 1H), 2.72–2.4 (m, 1H), 2.31 (s, 3H), 2.28–2.26 ppm (m, 1H); ^{13}C NMR ([D_6]DMSO): δ = 182.4, 170.1, 156.6, 149.4, 149.3, 136.1, 135.3, 134.4, 134.3, 133.9, 130.4, 130.2, 130.1, 128.6, 127.7, 127.6, 127.3, 124.0, 123.9, 123.5, 120.7, 87.9, 82.8, 70.7, 61.6, 22.5 ppm; HRMS (FAB): *m/z* calcd for $C_{29}H_{25}N_6O_6$: 553.1836 $[M+H]^+$; found: 553.2184.

13b:[12] Compound **19a** was prepared by the reported procedure.[17g] Compound **19a** was treated according to acetylation procedure 1, detritylation procedure 2, then deprotection procedure 1. The resulting filtrate was further treated with deprotection procedure 3. After the solvent was evaporated, the resulting residue was subjected to ODS column chromatography to give *N*-(2′-deoxyguanosin-8-yl)-3-acetylaminobenzanthrone (**13b**; 25 mg, 45%). ^{1}H NMR ([D_6]DMSO): δ = 10.77 (s, 1H), 8.80–8.50 (m, 4H), 8.31 (t, *J* = 7.4 Hz, 1H), 8.20–7.60 (m, 4H), 6.60–6.25 (m, 3H), 5.30 (s, 1H), 4.69 (br s, 1H), 4.49 (br s, 1H), 3.98 (br s, 1H), 3.75–3.58 (m, 2H), 3.15–3.00 (m, 1H), 2.25 (s, 3H), 2.0–1.95 ppm (m, 1H); ^{13}C NMR ([D_6]DMSO): δ = 182.8, 171.9, 171.2, 156.3, 155.8, 153.4, 153.1, 150.3, 150.1, 140.2, 138.6, 138.4, 134.9, 134.0, 131.8, 131.4, 129.9, 129.6, 129.0, 128.1, 127.9, 88.2, 73.8, 64.4, 39.8 ppm; HRMS (FAB): *m/z* calcd for $C_{29}H_{25}N_6O_6$: 553.1835 $[M+H]^+$; found: 553.1959.

13a:[17g] *N*-(2′-Deoxyguanosin-8-yl)-3-aminobenzanthrone (**13a**) was obtained by the same procedure previously reported.[17g] ^{1}H NMR ([D_6]DMSO, 600 MHz): δ = 10.61 (s, 1H, N1H of dG), 9.11 (s, 1H, NH of

BA), 8.69 (d, $J=7.6$ Hz, 2H, 1-H, 4-H of BA), 8.66 (d, $J=8.3$ Hz, 1H, 4-H of BA), 8.53 (d, $J=8.3$ Hz, 1H, 11-H of BA), 8.32 (d, $J=8.8$ Hz, 1H, 8-H of BA), 7.90 (t, $J=7.7$ Hz, 1H, 5-H of BA), 7.83 (t, $J=7.7$ Hz, 1H, 10-H of BA), 7.73 (d, $J=7.7$ Hz, 1H, 2-H of BA), 7.57 (t, $J=7.7$ Hz, 1H, 9-H of BA), 6.37 (s, 2H, NH_2 of G), 6.32 (t, $J=6.6$ Hz, 1H, 1′-H of dR), 5.33 (s, 1H, 3′-H of dR), 5.25 (d, $J=3.8$ Hz, 1H, 5′-H of dR), 4.38 (br s, 1H, 3′-H of dR), 3.89 (br s, 1H, 4′-H of dR), 3.79–3.69 (m, 1H, 5′-H of dR), 3.62–3.58 (m, 1H, 5′-H of dR), 2.88–2.80 (m, 1H, 2′-H of dR), 2.19–2.10 ppm (m, 1H, 2′-H of dR); MS (FAB): $m/z=511.3$ $[M+H]^+$.

Reaction of **10** with 2′-deoxyribonucleosides: TBD-methyl-polystyrene (Novabiochem; 10 mg) was added to a solution of hydroxyamine derivative **10** (2 mg) in THF (200 μL), followed by pyruvonitrile (2.4 μL). After 10 min reaction at room temperature, MeOH (10 μL) was added, and the mixture was filtered. The filtrate was added to a solution of dA, dG, or DNA (1%, 200 μL) and incubated at 37°C for 4 h. The reaction mixture was extracted with ethyl acetate. In the case of dA or dG, the aqueous layer was subjected to HPLC. (HPLC condition A for dG: a column of Cosmosil C-18 ARII (Nacalai tesque, 4.6×250 mm^2) was used at a flow rate of 1.0 mL min^{-1} with 24.6% acetonitrile in 20 mM triethylamine acetate buffer at pH 7. HPLC condition B for dA: a column of Cosmosil C-18 ARII (Nacalai tesque, 4.6×250 mm) was used at a flow rate of 1.0 mL min^{-1} with a linear gradient of 15→40% acetonitrile in 20 mM triethylamine acetate buffer at pH 7 for 45 min. Monitoring was done under UV light of wavelength 320 nm. In the case of DNA, the DNA was recovered by ethanol precipitation and digested with nuclease mix and bacterial alkaline phosphatase as indicated in the literature.[8a]

Reaction of **9** with 2′-deoxyribonucleotides: Compound **9** was added to a solution of 2′-deoxynucleotide (1%, 0.5 mL) and incubated for 12 h at 37°C, followed by extraction and HPLC as with the reaction of **10** with nucleobases. The following compounds were isolated by the reported procedure.[8a,12]

14b:[8a] 2-(2′-Deoxyguanosin-8-yl)-3-acetylaminobenzanthrone (≈6%); ^{1}H NMR ([D$_6$]DMSO): $\delta=10.82$ (br s, N1H of G), 8.89 (d, $J=8.6$ Hz, 1H, 6-H of BA), 8.71 (d, $J=7.4$ Hz, 1H, 4-H of BA), 8.57 (s, 1H, 1-H of BA), 8.37 (d, $J=8.1$ Hz, 1H, 11-H of BA), 8.30 (d, $J=8.1$ Hz, 1H, 8-H of BA), 7.86 (t, $J=8.1$ Hz, 1H, 5-H of BA), 7.74 (dt, $J=8.1$, 7.4 Hz, 1H, 10-H of BA), 7.47 (t, $J=7.4$ Hz, 1H, 9-H of BA), 7.31 (s, 2H, NH_2 of BA), 6.45 (s, 2H, NH_2 of G), 6.01 (t, $J=6.8$ Hz, 1H, 1′-H of dR), 5.04 (d, $J=4.4$ Hz, 1H, 5′-OH of dR), 5.09–4.98 (m, 1H, 3′-OH of dR), 4.30 (ddd, $J=8.2$, 3.6, 2.7 Hz, 1H, 3′-H of dR), 3.74 (dd, $J=4.9$, 3.6 Hz, 1H, 4′-H of dR), 3.59 (ddd, $J=11.5$, 5.1, 4.4 Hz, 1H, 5′-H of dR), 3.56 (ddd, $J=11.5$, 4.9, 4.4 Hz, 1H, 5″-H of dR), 3.11–2.80 (br m, 1H, 2′-H of dR), 2.07 ppm (dddd, $J=19.1$, 6.8, 6.5, 2.7 Hz, 1H, H-2″ (dR); MS (ESI): $m/z=510.9$ $[M+1]^+$.

17:[12] (9′-(2″-Deoxyribofuranosyl))purino[6′,1′:2,3]imidazo[5,4-*p*]-(1,11b-dihydro-(*N*-acetyl-3-amino))benzanthrone (**17**; ≈5%) was obtained as a mixture of enantiomers by the same procedure previously reported.[12] ^{1}H NMR ([D$_6$]DMSO): $\delta=9.54$ (s, NH(amide)), 8.68 (s, 1H, A2), 8.20 (s, 1H, A8), 8.14 (d, $J=6.8$ Hz, 1H, 8-H of BA), 8.12 (d, $J=7.1$ Hz, 1H, 6-H of BA), 7.79 (d, $J=7.3$ Hz, 1H, 4-H of BA) 7.71 (d, $J=7.1$ Hz, 1H, 11-H of BA), 7.70 (t, $J=7.6$ Hz, 1H, 9-H of BA), 7.64 (t, $J=7.7$ Hz, 1H, 5-H of BA), 7.58 (t, $J=7.0$ Hz, 1H, BA10), 6.56 (br s, 1H, 1-H of BA), 6.35 (br s, 1H, 2-H of BA), 6.29 (br s, 1H, 1′-H of dR), 4.42 (s, 3′-H of dR), 3.91 (s, 4′-H of dR), 3.59 (dd, $J=26.5$, 9.1 Hz, 1H, 5′-H of dR), 2.63–2.60 (m, 1H, 2″-H of dR), 2.35–2.28 (m, 1H, 2′-H of dR), 2.13 ppm (s, 3H, CH_3); MS (ESI): $m/z=537$ $[M+1]^+$.

Acknowledgements

We are grateful to Dr Yosihiro Matano of Kyoto University for many helpful discussions and suggestions.

[1] T. Enya, H. Suzuki, T. Watanabe, T. Hirayama, Y. Hisamatsu, *Environ. Sci. Technol.* **1997**, *31*, 2772–2776.

[2] See the review and references cited therein: a) V. M. Arlt, *Mutagenesis*, **2005**, *20*, 399–410; recent publications of 3-nitrobenzanthrone after the review: b) C. A. Bieler, M. G. Cornelius, M. Stiborova, V. M. Arlt, M. Wiessler, D. H. Phillips, H. H. Schmeiser, *Carcinogenesis* **2007**, *28*, 1117–1121; c) T. Takamura-Enya, H. Suzuki, Y. Hisamatsu, *Mutagenesis* **2006**, *21*, 399–404; d) E. Nagy, S. Adachi, T. Takamura-Enya, M. Zeisig, L. Moller, *Environ. Mol. Mutagen.* **2006**, *47*, 541–552; e) M. Stiborova, H. Dracinska, J. Hajkova, P. Kaderabkova, E. Frei, H. H. Schmeiser, P. Soucek, D. H. Phillips, V. M. Arlt, *Drug. Metab. Dispos.* **2006**, *34*, 1398–1405; f) M. Stiborova, V. M. Arlt, C. J. Henderson, C. R. Wolf, E. Frei, H. H. Schmeiser, D. H. Phillips, *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc. Czech. Repub.* **2005**, *149*, 191–197; g) M. Murata, T. Tezuka, S. Ohnishi, T. Takamura-Enya, Y. Hisamatsu, S. Kawanishi, *Free Radical Biol. Med.* **2006**, *40*, 1242–1249; h) E. Nagy, C. Johansson, M. Zeisig, L. Moller, *J. Chromatogr. B* **2005**, *827*, 94–103.

[3] a) A. Feilberg, T. Ohura, T. Nielsen, M. W. B. Poulsen, T. Amagai, *Atmos. Environ.* **2002**, *36*, 3591–3600; b) N. Tang, R. Taga, T. Hattori, K. Tamura, A. Toroba, R. Kizu, K. Hayakawa, *Anal. Sci.* **2004**, *20*, 119–123.

[4] a) T. Murahashi, T. Watanabe, S. Otake, Y. Hattori, T. Takamura, K. Wakabayashi, T. Hirayama, *J. Chromatogr. A* **2003**, *992*, 101–107; b) T. Watanabe, Y. Hasei, S. Takahashi, T. Otake, T. Murahashi, T. Takamura, T. Hirayama, K. Wakabayashi, *Mutat. Res.* **2003**, *538*, 121–131.

[5] a) T. Enya, H. Suzuki, Y. Hisamatsu, *Bull. Chem. Soc. Jpn.* **1998**, *71*, 2221–2228; b) P. T. Phousongphouanga, J. Arey, *Atmos. Environ.* **2003**, *37*, 3189–3199.

[6] a) C. A. Bieler, M. Wiessler, L. Erdinger, H. Suzuki, T. Enya, H. H. Schmeiser, *Mutat. Res.* **1999**, *439*, 307–311; b) M. Kawanishi, T. Enya, H. Suzuki, H. Takebe, S. Matsui, T. Yagi, *Mutat. Res.* **2000**, *470*, 133–139; c) P. T. Phousongphouang, A, J. Grosovsky, D. A. Eastmond, M. Covarrubias, J. Arey, *Mutat. Res.* **2000**, *472*, 93–103; d) C. A. Bieler, V. M. Arlt, M. Wiessler, H. H. Schmeiser, *Cancer Lett.* **2003**, *200*, 9–18; e) E. Lamy, F. Kassie, R. Gminski, H. H. Schmeiser, V. Mersch-Sundermann, *Toxicol. Lett.* **2004**, *146*, 103–109.

[7] a) S. Adachi, K. Kawanura, K. Takemoto, H. Suzuki, Y. Hisamatsu in *Relationship between Respiratory Disease and Exposure to Air Pollution* (Eds.: U. Heinrich, U. Mohr), ILSI Press, Washington, D.C., **2000**, pp. 315–319; b) E. Nagy, M. Zeisig, K. Kawamura, Y. Hisamatsu, A Sugeta, S. Adachi, S. L. Möller, *Carcinogenesis* **2005**, *26*, 1821–1828.

[8] a) T. Enya, M. Kawanishi, H. Suzuki, S. Matsui, Y. Hisamatsu, *Chem. Res. Toxicol.* **1998**, *11*, 1460–1467; b) V. M. Arlt, H. Glatt, E. Muckel, U. Pabel, B. L. Sorg, A. Seidel, H. Frank, H. H. Schmeiser, D. H. Phillips, *Int. J. Cancer* **2003**, 105, 583–592; c) V. M. Arlt, M. Stiborova, A. Hewer, H. H. Schmeiser, D. H. Phillips, *Cancer Res.* **2003**, *63*, 2752–2761.

[9] a) F. A. Beland, M. M. Marques, *DNA Adducts: Identification and Biological Significance*, *Vol 125*, IARC, Lyon, **1984**, pp. 229–244; b) F. A. Beland, F.F Kadlubar, *Handb. Exp. Pharmacol.* **1990**, *94*/I, 267–325.

[10] a) V. M. Arlt, C. A. Bieler, W. Mier, M. Wiessler, H. H. Schmeiser, *Int. J. Cancer* **2001**, *93*, 450–454; b) V. M. Arlt, H. Glatt, E. Muckel, U. Pabel, B. L. Sorg, H. H. Schmeiser, D. H. Phillips, *Carcinogenesis* **2002**, *23*, 1937–1945; c) V. M. Arlt, B. L. Sorg, M. Osborne, A. Hewer, A. Seidel, H. H. Schmeiser, D. H. Phillips, *Biochem. Biophys. Res. Commun.* **2003**, *300*, 107–114.

[11] M. Novak, L. Xu, R. A. Wolf, *J. Am. Chem. Soc.* **1998**, *120*, 1643–1644.

[12] M. R. Osborne, V. M. Arlt, C. Kliem, W. E. Hull, A. Mirza, C. A. Bieler, H. H. Schmeiser, D. H. Phillips, *Chem. Res. Toxicol.* **2005**, *18*, 1056–1070.

[13] P. P. Fu, *Drug Metab. Rev.* **1990**, *22*, 217–267.

[14] a) W. G. Humphrey, F. F. Kadlubar, F. P. Guengerich, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 8278–8282; b) S. A. Kennedy, M. Novak, B. A. Kolb, *J. Am. Chem. Soc.* **1997**, *119*, 7654–7664.

[15] a) Y. Hashimoto, K. Shudo, T. Okamoto, *J. Am. Chem. Soc.* **1982**, *104*, 7636–7640; b) H. Frederiksen, H. Frandsen, W. Pfau, *Carcinogenesis* **2004**, *25*, 1525–1533; c) E. G. Snydewine, P. P. Roller, R. H. Adamson, S. Sato, S. S. Thorgeirssom, *Carcinogenesis* **1988**, *9*, 411–418.

[16] a) M. Novak, K. Toth, S. Rajagopal, M. Brooks, L. L. Hott, M. Moslener, *J. Am. Chem. Soc.* **2002**, *124*, 7972–7981; b) M. Novak, T.-M. Nguyen, *J. Org. Chem.* **2003**, *68*, 9875–9881.

[17] See reviews and references cited therein: a) M. K. Lakshman, *J. Organomet. Chem.* **2002**, *653*, 234; b) M. K. Lakshman, *Curr. Org. Synth.* **2005**, *2*, 83; typical references: c) F. De Riccardis, R. R. Bonola, F. Johnson, *J. Am Chem. Soc.* **1999**, *121*, 10453–10460; d) Z. Wang, C. J. Rizzo, *Org Lett.* **2001**, *3*, 565–568; e) L. C. J. Gillet, O. D. Schärer, *Org. Lett.* **2002**, *4*, 4205–4208; f) C. Meier, S. Gräsel, *Synlett* **2002**, 802–804; g) T. Takamura-Enya, S. Ishikawa, M. Mochizuki, K. Wakabayashi, *Tetrahedron Lett.* **2003**, *44*, 5969–5973; h) D. Chakraborti, L. Colis, R. Schneider, A. K. Basu, *Org Lett.* **2003**, *5*, 2861–2864.

[18] J. M. Landesberg, L. Katz, C. Olsen, *J. Org. Chem.* **1972**, *37*, 930–936.

[19] a) A. M. Lobo, M. M. Marques, S. Prabhakar, H. S. Rzepa, *J. Org. Chem.* **1987**, *52*, 2925–2927; b) A. M. Lobo, M. M. Marques, S. Prabhakar, H. S. Rzepa, *J. Chem. Soc. Chem. Commun.* **1985**, *16*, 1113–1115; c) M. Famulok, G. Boche, *Angew. Chem.* **1989**, *101*, 470–471; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 468–469.

[20] V. M. Arlt, H. H. Schmeiser, M.R Osborne MR, M. Kawanishi M, T. Kanno T. Yagi, D. H. Phillips, T. Takamura-Enya, *Int. J. Cancer* **2006**, 118, 2139–2146.

[21] a) J. F. Hatcher, S. Swaminathan, *Environ. Mol. Mutagen.* **2002**, *39*, 314–322; b) T. Haack, G. Boche, C. Kliem, M. Wiessler, D. Albert, H. H. Schmeiser, *Chem. Res. Toxicol.* **2004**, *17*, 776–784.

[22] M. Novak, S. A. Kennedy, *J. Phys. Org. Chem.* **1998**, *11*, 71–76.

Received: February 20, 2007
Revised: April 30, 2007
Published online: August 21, 2007

