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PAPER

Theoretical investigations on the formation of nitrobenzanthrone-DNA Adducts†

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3-Nitrobenzanthrone (3-NBA) is a potent mutagen and suspected human carcinogen identified in diesel exhaust. The thermochemical formation cascades were calculated for six 3-NBA-derived DNA adducts employing its arylnitrenium ion as precursor using density functional theory (DFT). Clear exothermic pathways were found for four adducts, *i.e.*, 2-(2′-deoxyadenosin-N^6-yl)-3-aminobenzanthrone, 2-(2′-deoxyguanosin-N^2-yl)-3-aminobenzanthrone, N-(2′-deoxyguanosin-8-yl)-3-aminobenzanthrone and 2-(2′-deoxyguanosin-8-yl)-3-aminobenzanthrone. All four have been observed to be formed in cell-free experimental systems. The formation of N-(2′-deoxyadenosin-8-yl)-3-aminobenzanthrone is predicted to be not thermochemically viable explaining its absence in either *in vitro* or *in vivo* model systems. However, 2-(2′-deoxyadenosin-8-yl)-3-aminobenzanthrone, can be formed, albeit not as a major product, and is a viable candidate for an unknown adenine adduct observed experimentally. 2-nitrobenzanthrone (2-NBA), an isomer of 3-NBA, was also included in the calculations; it has a higher abundance in ambient air than 3-NBA, but a much lower genotoxic potency. Similar thermochemical profiles were obtained for the calculated 2-NBA-derived DNA adducts. This leads to the conclusion that enzymatic activation as well as the stability of its arylnitrenium ion are important determinants of 2-NBA genotoxicity.

Introduction

Nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) are widely distributed environmental pollutants, *e.g.*, they are found in vehicle exhaust from diesel and gasoline engines.[1] The suspected increased lung cancer risk from exposure to these environmental sources has led to considerable interest in assessing their genotoxic potential.[2–4] Nitro-PAHs generally form their corresponding hydroxylamines after enzymatic reduction, *e.g.*, by nitroreductases including NAD(P)H:quinone oxidoreductase (NQO1),[5] and can undergo heterolytic bond cleavage, in mildly acidic aqueous environment, forming a reactive arylnitrenium ion as shown in Fig. 1.[6,7] This ion can also be formed *via* enzymatic reactions where the acetoxy- or sulphate-esters are formed as intermediates catalysed by N-acetyltransferases or sulfotransferases (see Fig. 1).[5,8–10] Arylnitrenium ions are electrophilic chemical species, and they can form covalent bonds with DNA, resulting in lesions, called DNA adducts, which are pro-mutagenic.[11] It has been shown that the stability of arylnitrenium ions is one of the important factors for them forming DNA adducts.[8,12–17] Also, the interaction of the substrates (nitro-PAHs) and the reductive enzymes (*e.g.* NQO1) plays an important role in the formation of the arylnitrenium ions.[18]

3-Nitrobenzanthrone (3-NBA; Fig. 1), which is found in diesel exhaust and in airborne particles, has received considerable attention due to its unusually high mutagenic potency (see ref. 19–21 and references therein). 3-NBA is a suspected human lung carcinogen and it has been established that it forms DNA adducts in *vivo* (see ref. [22–25] and references therein). In contrast, 2-nitrobenzanthrone (2-NBA), an isomer of 3-NBA, which is detected in ambient air particulate matter, lacks genotoxic activity in rats,[26] despite being genotoxic *in vitro*.[26,27] The structure of several 3-NBA-DNA adducts have been experimentally identified and four of them, namely 2-(2′-deoxyguanosin-N^2-yl)-3-aminobenzanthrone (dG-N^2-3-ABA; adduct G1), N-(2′-deoxyguanosin-8-yl)-3-aminobenzanthrone (dG-C8-*N*-3-ABA; adduct G2), 2-(2′-deoxyguanosin-8-yl)-3-aminobenzanthrone (dG-C8-C2-3-ABA; adduct G3) and 2-(2′-deoxyadenosin-N^6-yl)-3-aminobenzanthrone (dA-N^6-3-ABA; adduct A1), are shown in Fig. 2 (deoxyguanosine adducts) and Fig. 3 (deoxyadenosine adducts).

In Fig. 3 two C8 adducts of deoxyadenosine are also shown, namely *N*-(2′-deoxyadenosin-8-yl)-3-aminobenzanthrone (dA-C8-*N*-3-ABA; adduct A2) and 2-(2′-deoxyadenosin-8-yl)-3-aminobenzanthrone (dA-C2-C8-3-ABA; adduct A3). These are of interest because they have *not* been found experimentally, unlike their deoxyguanosine counterparts.

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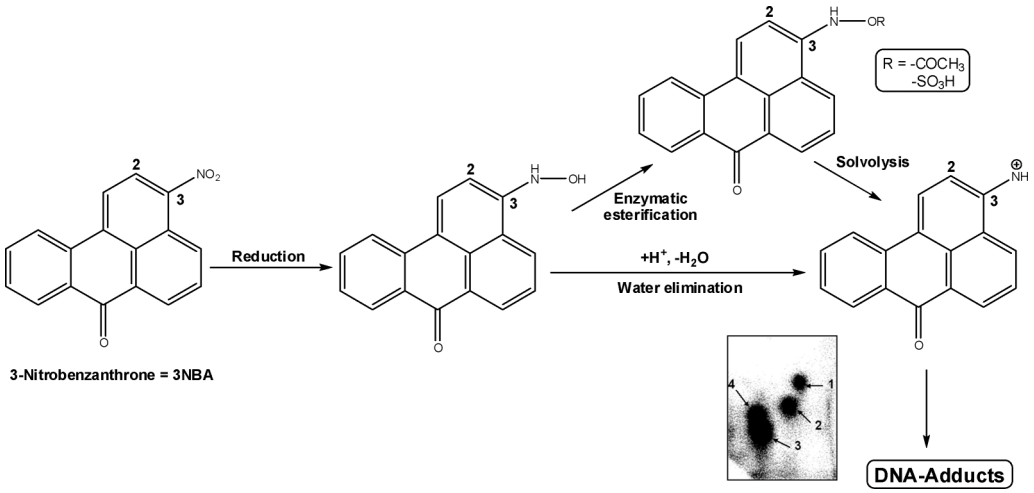


Fig. 1 Metabolic activation of 3-NBA leading to the formation of its arylnitrenium ion and consequent DNA adduct formation. Inset: Autoradiographic profile of 3-NBA-DNA adducts in rat lung after treatment of Wistar rats with a single dose of 3-NBA (2 mg kg^{-1} body weight) by using the butanol enrichment version of the ^{32}P-postlabelling assay [adapted from ref. [19]]. Spot 1, dA-N^6-3-ABA; spot 2, unknown dA adduct; spot 3, dG-N^2-3-ABA; and spot 4, dG-C8-*N*-3-ABA (compare Fig. 2 and 3).

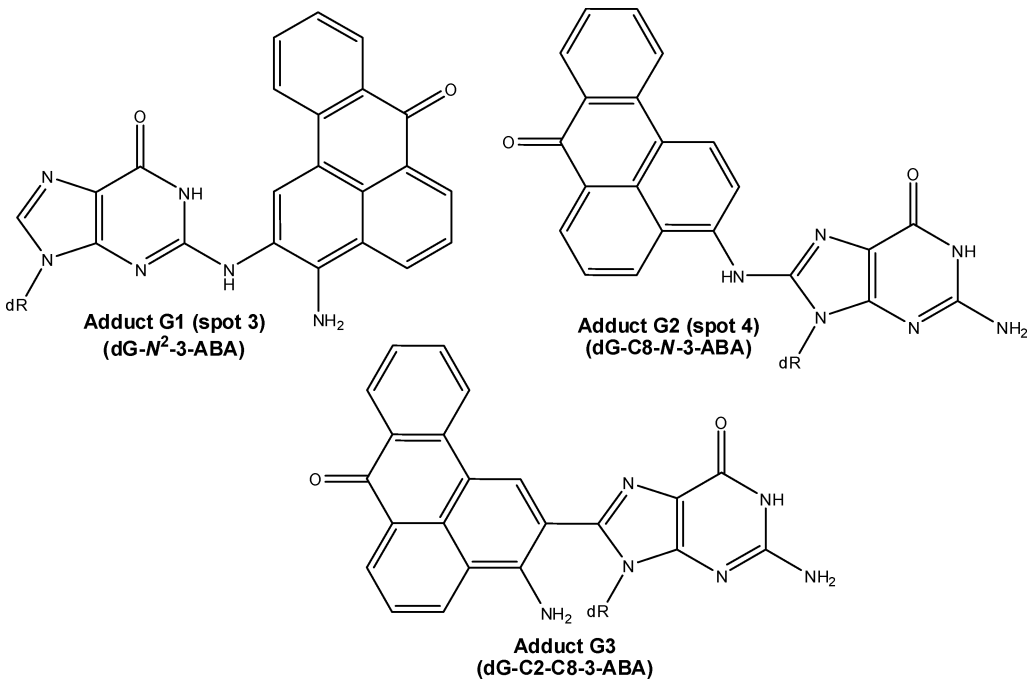


Fig. 2 3-NBA-DNA adducts with deoxyguanosine, of which adduct G1 and G2 have been detected in biological systems. dR = 2′-deoxyribose moiety.

In order to gain further understanding of the mechanism of formation of these DNA adducts we performed density functional theory (DFT) calculations on their thermochemical formation cascades. Six DNA adducts of 3-NBA were investigated, three for deoxyguanosine and three for deoxyadenosine. Furthermore, the formation by 2-NBA of adducts with deoxyguanosine were calculated for comparison.

Theoretical methods

The energy calculations and geometry optimisations were performed with the GAUSSIAN 09 program suite[28] utilizing restricted DFT. The non-local B3LYP functional hybrid method was employed.[29–31] The standard 6-31G(d,p) basis set[32] was used for the geometry optimization and frequency analysis. The zero-point vibrational energies (ZPE) were scaled according to Wong (0.9804).[33] Subsequent single-point electronic energy calculations were performed with the larger 6-311G(2df,p) basis set. Proton affinities (PA) were calculated as described by Foresman and Frisch[34] and adduct formation energies, H-shifts, rearrangements, tautomerisations as in ref.[35] The results of the single-point electronic energy calculations and the ZPE of the calculated structures are listed in Tables S1–S6 in the ESI.†

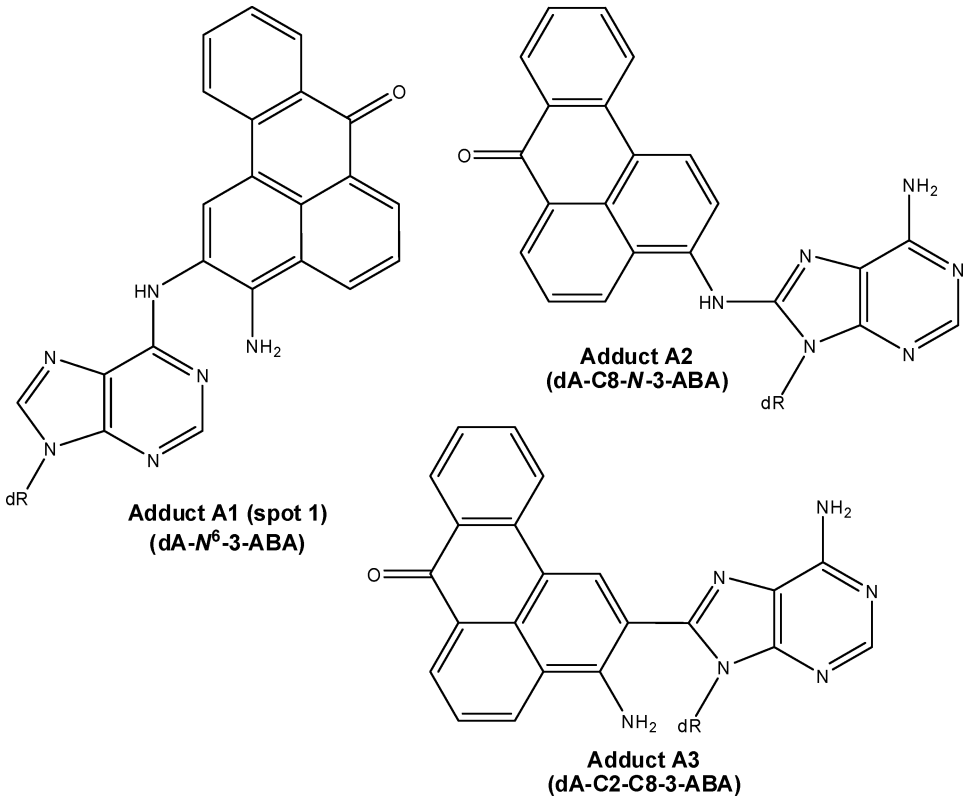


Fig. 3 3-NBA-DNA adducts with deoxyadenosine, of which only adduct A1 has been detected in biological systems. dR = 2′-deoxyribose moiety.

Results

Thermochemical calculations were performed on six 3-NBA-DNA adduct structures, *i.e.*, three deoxyguanosine and three deoxyadenosine adducts. Furthermore, the formation cascades were also calculated for the 2-NBA- deoxyguanosine adducts. In all the calculations 9-methylguanine and 9-methyladenine are used as models for the deoxyguanosine and deoxyadenosine moieties.

1) Formation of 2-(2′-deoxyguanosin-N²-yl)-3-aminobenzanthrone (adduct G1)

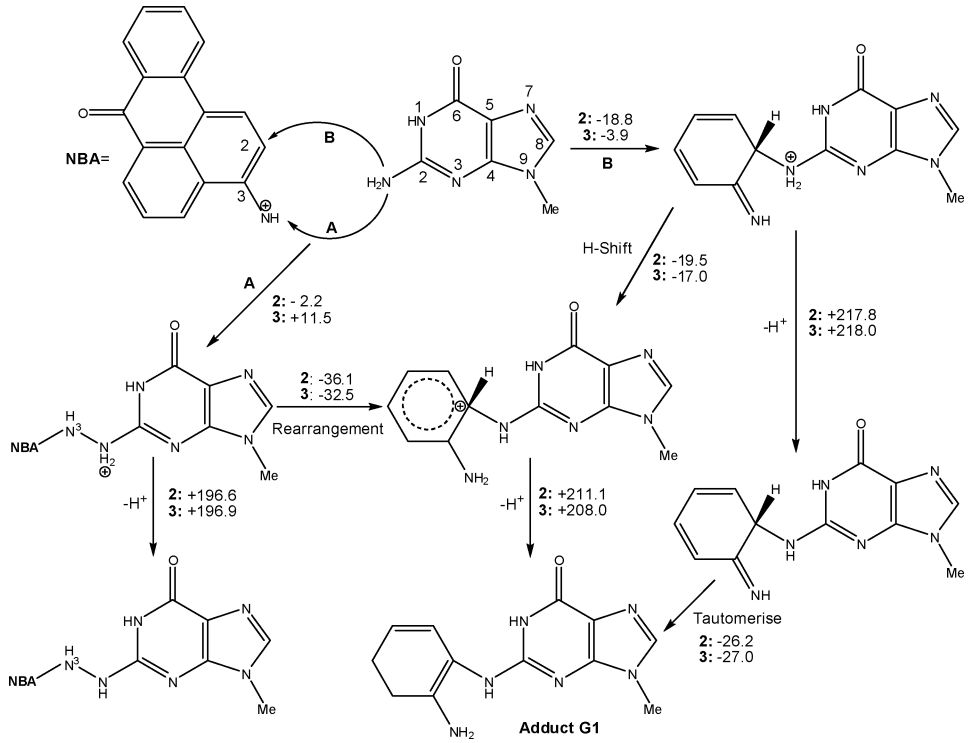
For the electrophilic attack on the exocyclic nitrogen of the guanine moiety for the 3-NBA arylnitrenium ion two reaction pathways were investigated and the results are shown in Scheme 1. First, a nitrogen - nitrogen bond formation (pathway **A**) was calculated, which can subsequently rearrange to form the C2 (on the arylnitrenium ion) bond with the exocyclic nitrogen of the guanine moiety. The first step in this pathway is endothermic by +11.5 kcal mol^{-1} and is therefore not plausible. Pathway **B**, is initiated by a direct electrophilic attack on the exocyclic guanine moiety's nitrogen from the C2 on the arylnitrenium ion and is exothermic by –3.9 kcal mol^{-1}. It can either shift its proton from the exocyclic guanine moiety's nitrogen to its counterpart on the benzanthrone scaffold forming an amine (H-shift) or directly deprotonate to the aqueous phase ($-H^+$). The former pathway is exothermic by –17.0 kcal mol^{-1} and the direct deprotonation endothermic by +218.0 kcal mol^{-1} which means it can be deprotonated easily due to the high solvation energy of the proton (–263.9 kcal mol^{-1}).[36] It is therefore a question whether deprotonation or tautomerisation (H-shift) is the most prominent pathway, but according to the results presented in Scheme 1 both processes are active.

The 2-NBA ion is more reactive than its 3-NBA counterpart, which is reflected in that pathway **A** is slightly exothermic for 2-NBA but endothermic for 3-NBA and for pathway **B** 2-NBA is more exothermic than 3-NBA. In general, a clear thermochemical path is present for adduct formation by the arylnitrenium ions of both 2-NBA and 3-NBA.

2) Formation of N-(2′-deoxyguanosin-8-yl)-3-aminobenzanthrone (adduct G2)

The formation of this adduct involves an electrophilic attack on the C8 on the guanine moiety. The possibility is explored as to whether this reaction takes place *via* an electrophilic attack on the N7 and a consequent rearrangement or *via* direct attack on the C8. The reaction cascades were calculated for the arylnitrenium ions of both the 2- and 3-isomers of NBA for comparison. The results are shown in Scheme 2.

The thermochemical profile of the reaction cascades for the arylnitrenium cations of the 2- and 3-isomers of NBA are in general similar, *i.e.*, the electrophilic attack on the guanine moiety is exothermic for both the pathways investigated (**A** and **B** in Scheme 2). The rearrangement step to the C8 adduct from the N7 structure is in both cases endothermic and the deprotonation steps are all endothermic by ~230 kcal mol^{-1}. The solvation energy of the proton is measured as –263.9 kcal mol^{-1} [36] facilitating these reactions in the water phase. Finally, the tautomerisation and H-shift steps also have similar exothermic profiles. What differs between the two reaction cascades presented in Scheme 2



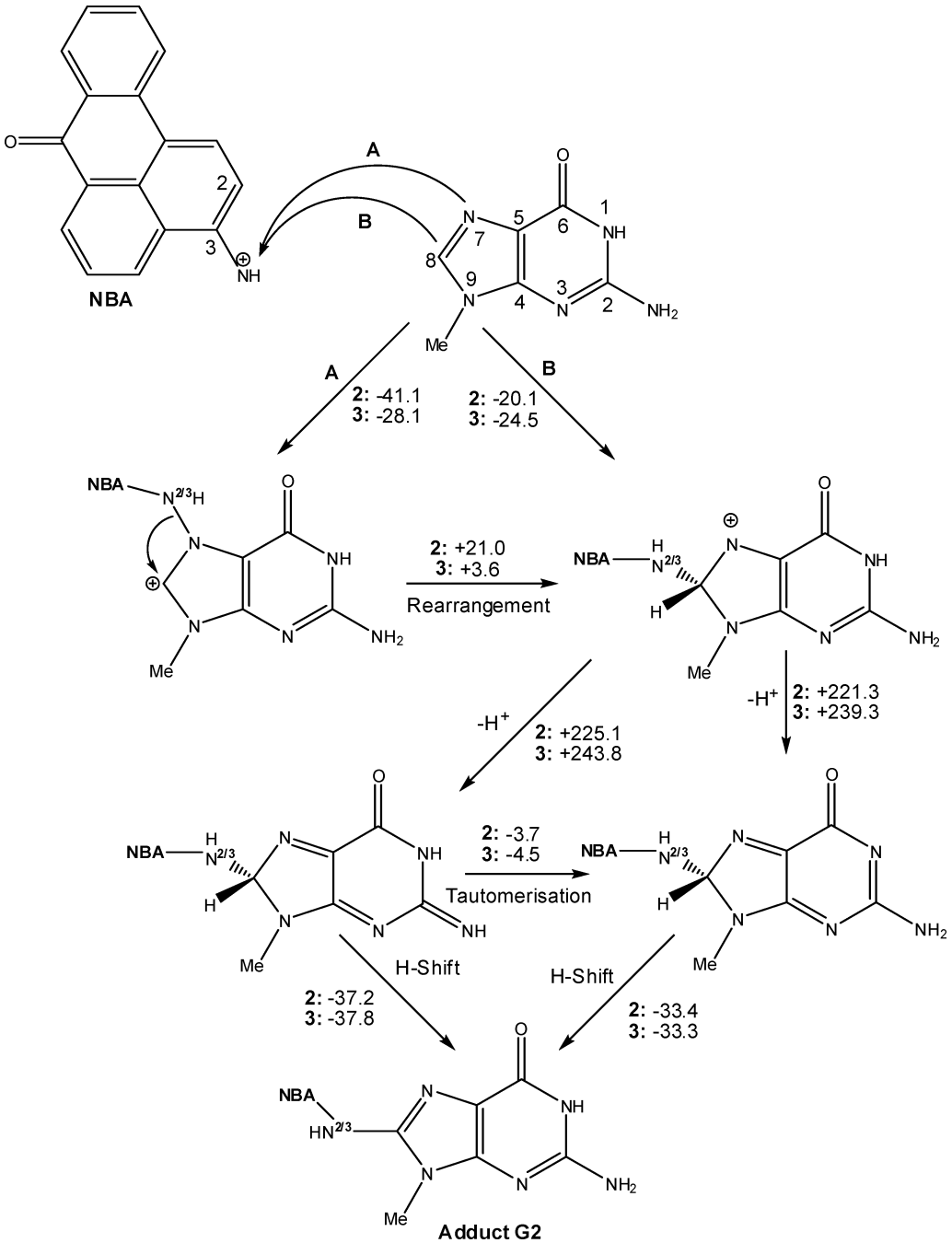
Scheme 1 Electrophilic attack of the arylnitrenium ions of 2-NBA and 3-NBA on the exocyclic amine on the guanine moiety. All the values are in kcal mol^{-1}. 2′-deoxyribose is substituted with a methyl moiety to ease the computational load.

is the higher exothermic addition energy of the arylnitrenium ion of 2-NBA to the N7 position of the guanine moiety and substantial rearrangement energy to form the C8 transient. The latter step is much lower for the 3-NBA adduct at +3.6 kcal mol^{-1} and is therefore plausible with the thermal energy available at physiological temperature. This means that a rearrangement is unlikely for the N7 adduct of 2-NBA and the formation of a stable DNA adduct is *not* viable *via* this mechanism. When the tertiary structure of DNA is considered the N7 position of the guanine moiety is more exposed to the aqueous environment than the C8 position in the major groove of the DNA helix. Hence, the probability of an addition on N7 is higher but this reaction pathway does not lead to stable adduct formation due to the low bond dissociation energy of nitrogen-nitrogen single bonds.[37–39] The deprotonation from the C8 adduct is formulated from the exocyclic amine group and the endocyclic N1 rather than a straight deprotonation from C8. This is based on the analogy of the large decrease in the pK_a values of one-electron oxidised organic ions compared to their parent compounds predominantly occurring at a hydrogen bond donor moieties interacting with the water phase followed by H-shift (*e.g.* Steenken[40–46]). This deprotonation mechanism is pH dependent favouring C–H bond cleavage at acidic conditions.[47–51] The calculations here are performed *in vacuum* and are therefore not directly affected by the solvent. However, the solvent effects need to be considered. At physiological pH there are plenty of hydrogen bond acceptors inherently available in water, which can accept protons from N–H or O–H moieties in organic cations. Upon increased acidity less lone pairs become available to interact with these moieties and direct deprotonation from C–H moieties becomes dominant. Since the adduct formation under consideration occurs at physiological pH the deprotonation reaction are formulated from the amine groups in the purines followed by H-shifts.

3) Formation of 2-(2′-deoxyguanosin-8-yl)-3-aminobenzanthrone (adduct G3)

The electrophilic attack was investigated on the N7 and C8 in the guanine moiety from C2 in the 3-NBA arylnitrenium ion. The former is shown as path **A** in Scheme 3 and is exothermic by −34.6 kcal mol^{-1}. However, the adduct formed cannot rearrange further and pathway **A** does not lead to stable adduct formation. Pathway **B** describes the C2 and C8 bond formation and it is predicted to be exothermic by −11.0 kcal mol^{-1}. Rapid deprotonation from the guanine moiety can occur either at the endocyclic N1 or the exocyclic N^2 nitrogen atoms and both are thermochemically plausible. Hydrogen shift of these deprotonated species from the C8 atom to the endocyclic N1 or the exocyclic N^2 atoms is exothermic (~ −30 kcal mol^{-1}). Also, a hydrogen shift reinstating the aromaticity of the aniline ring moiety is exothermic in both scenarios by more than −20 kcal mol^{-1} and a consequent hydrogen shift from the C8 in the guanine moiety to either N1 or N^2 is also considerably exothermic.

The 2-NBA isomer has a very similar reaction profile as the 3-NBA ion with the exception that it is more reactive in the initial electrophilic attacks. Both isomers have a clear thermochemical path to the DNA adducts.

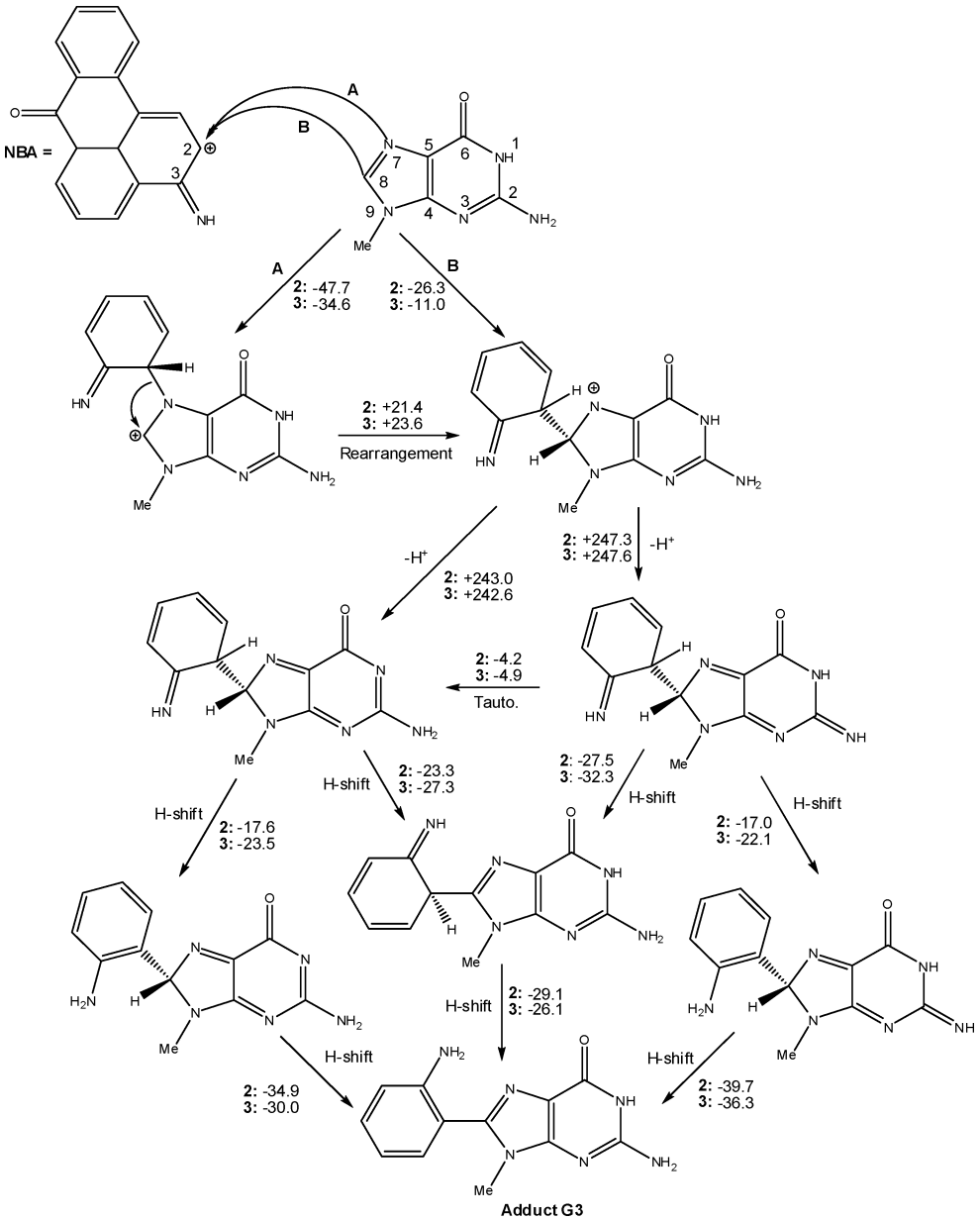


Scheme 2 Electrophilic attack on the guanine moiety by the arylnitrenium ions of 2-NBA and 3-NBA and the resulting reaction cascade. All the values are in kcal mol^{-1}. 2′-deoxyribose is substituted with a methyl moiety.

4) Formation of 2-(2′-deoxyadenosin-N^6-yl)-3-aminobenzanthrone (adduct A1)

The next molecular system calculated was the addition of the arylnitrenium ion of 3-NBA to the exocyclic nitrogen on the adenine moiety and the results are shown in Scheme 4. This reaction cascade is similar to the one depicted in Scheme 1 as both involve the addition to an exocyclic amine moiety. Unlike the addition to deoxyguanosine (Scheme 1) pathways **A** and **B** are both exothermic. However, pathway **A** is only slightly exothermic compared to the direct electrophilic addition to the C2 on the arylnitrenium ion (pathway **B**). Furthermore, if the transient species in pathway **A** is formed it can easily rearrange (exothermic by –28.5 kcal mol^{-1}) to form a carbon–nitrogen bond between the 3-NBA molecular scaffold and the adenine moiety.

In the case of pathway **B** it is exothermic by –11.1 kcal mol^{-1}. The resulting cation can either deprotonate directly or shift its proton to the imine group on the NBA moiety forming an amine. Both of these steps are thermochemically plausible. Furthermore, the direct deprotonation path does lead to the final product.



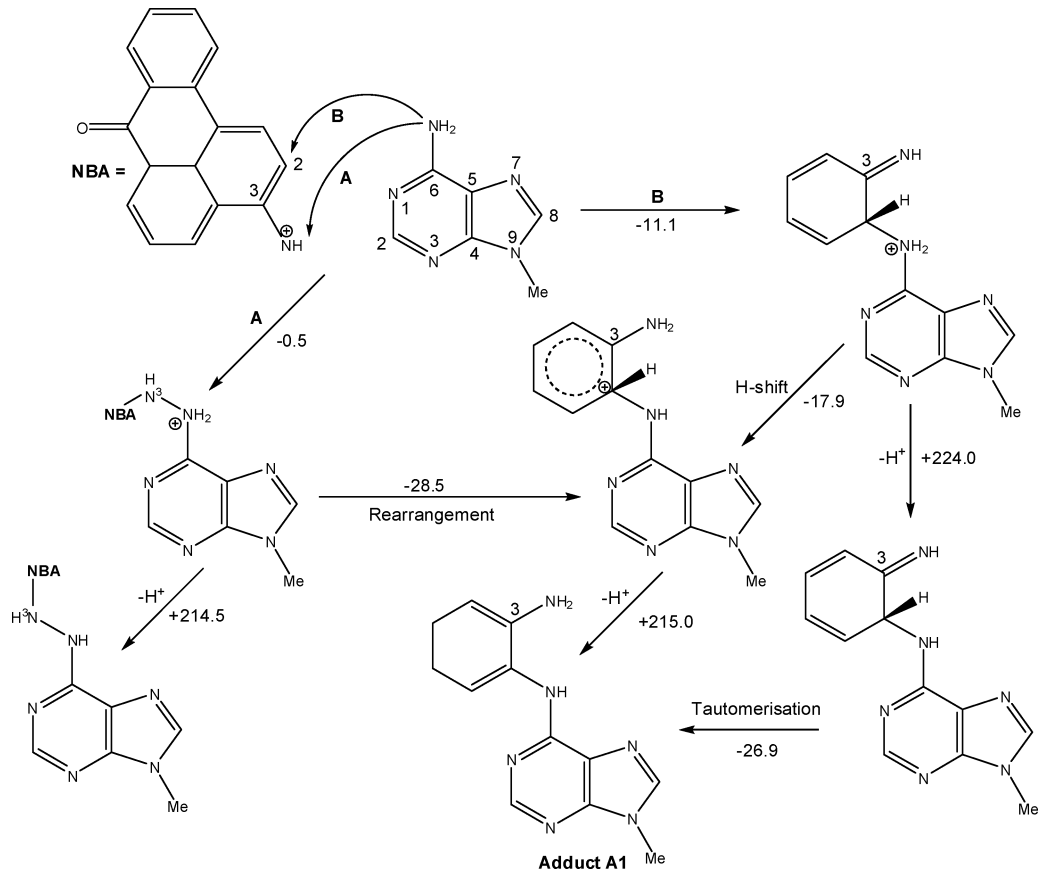
Scheme 3 Electrophilic attack of the arylnitrenium ion of 3-NBA from C2 on C8 of the guanine moiety. The 2-NBA isomer calculated for comparison. All the values are in kcal mol^{-1}. 2′-deoxyribose is substituted with a methyl moiety.

5) Formation of N-(2′-deoxyadenosin-8-yl)-3-aminobenzanthrone (adduct A2)

Scheme 5 depicts the thermochemical formation cascade of the C8 adducts to the adenine moiety. The addition to N7 is shown in pathway **A** and it is exothermic by –18.2 kcal mol^{-1}. The transient chemical species formed cannot rearrange to form the carbon-nitrogen bond because that step is endothermic by +25.9 kcal mol^{-1}. Therefore, pathway **A** does not lead to a stable DNA adduct. Pathway **B** is endothermic by +7.7 kcal mol^{-1}, which makes it unlikely to occur but not impossible. Hence, the results presented in Scheme 5 show that there is *not* a clear thermochemical route to C8 adduct formation for deoxyadenosine, which is in line with experimental evidence.

6) Formation of 2-(2′-deoxyadenosine-8-yl)-3-aminobenzanthrone (adduct A3)

Scheme 6 shows the thermochemical formation cascade of the C8 (adenine) C2 (3-NBA) carbon bond adduct. Pathway **A** is exothermic by –20.8 kcal mol^{-1} but the transient chemical species formed cannot rearrange to form the carbon–carbon bond due to its endothermicity of +23.6 kcal mol^{-1}. Pathway **B** is endothermic by +2.7 kcal mol^{-1}, which is not insurmountable obstacle for this reaction to take place. Nevertheless, the reaction is endothermic and is not expected to lead to a major product. The results presented in Scheme 6 show that the thermochemically route to C8–C2 adduct for deoxyadenosine is less likely than for deoxyguanosine (see Scheme 3). However, adduct A3 is a plausible



Scheme 4 Electrophilic attack of the arylnitrenium ion of 3-NBA on the exocyclic amine on the adenine moiety. All the values are in kcal mol^{-1}. 2′-deoxyribose is substituted with a methyl moiety.

candidate for spot 2 shown in Fig. 1 emphasising the necessity to synthesise this molecule to test this hypothesis.

7) The overall energy balance for each pathway

The total, or overall energy balance, was calculated for all of the cascaded investigated. The energy of the adducts (products) were subtracted from the combined total energy of the nucleotides and the nitrenium ions with the added solvation energy of the proton (–263.9 kcal mol^{-1}).[36] It can be stated that this solvation energy is the driving force for these reactions. The results are shown in Table 1.

It is clear that the overall energy balance is larger for the 2-NBA nitrenium ion adducts by ~14 kcal mol^{-1}. This means that the 2-NBA ion reacts quite readily with DNA but the resulting adducts are not observed experimentally leading to the conclusion that the 2-NBA nitrenium ion is not formed *in vivo* or does not have a sufficiently long lifetime in the cytosol to reach the DNA helix. Adducts A1 and A3 have similar overall energy balance as its guanine counterparts but A2 has a much lower total energy.

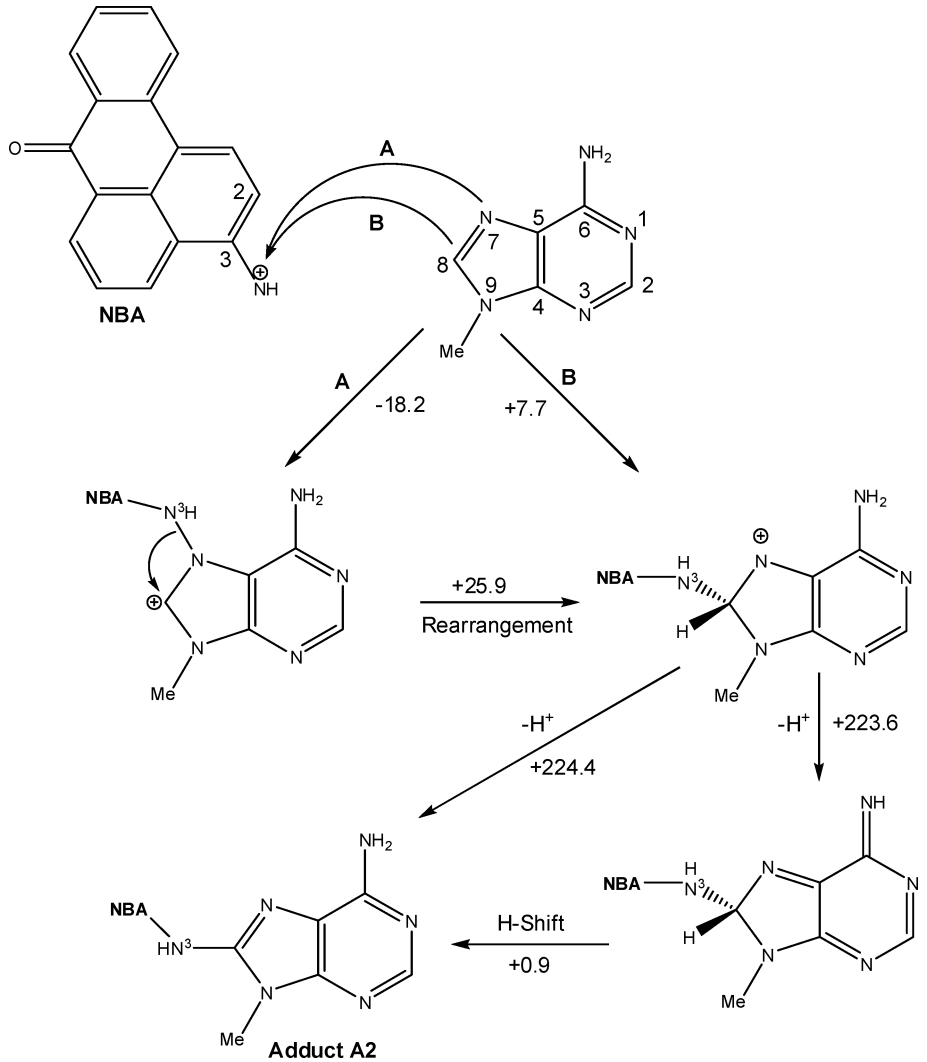
Table 1 The total energy balance of the cascades investigated. The difference (Δ) is given between the 2-NBA and 3-NBA nitrenium ions. All the values are in kcal mol^{-1}

Adduct	2-NBA	3-NBA	Δ
G1	–91.1	–76.8	14.3
G2	–96.2	–82.5	13.7
G3	–99.7	–85.8	13.9
A1	X	–77.9	X
A2	X	–31.8	X
A3	X	–85.5	X

X: no data

Discussion

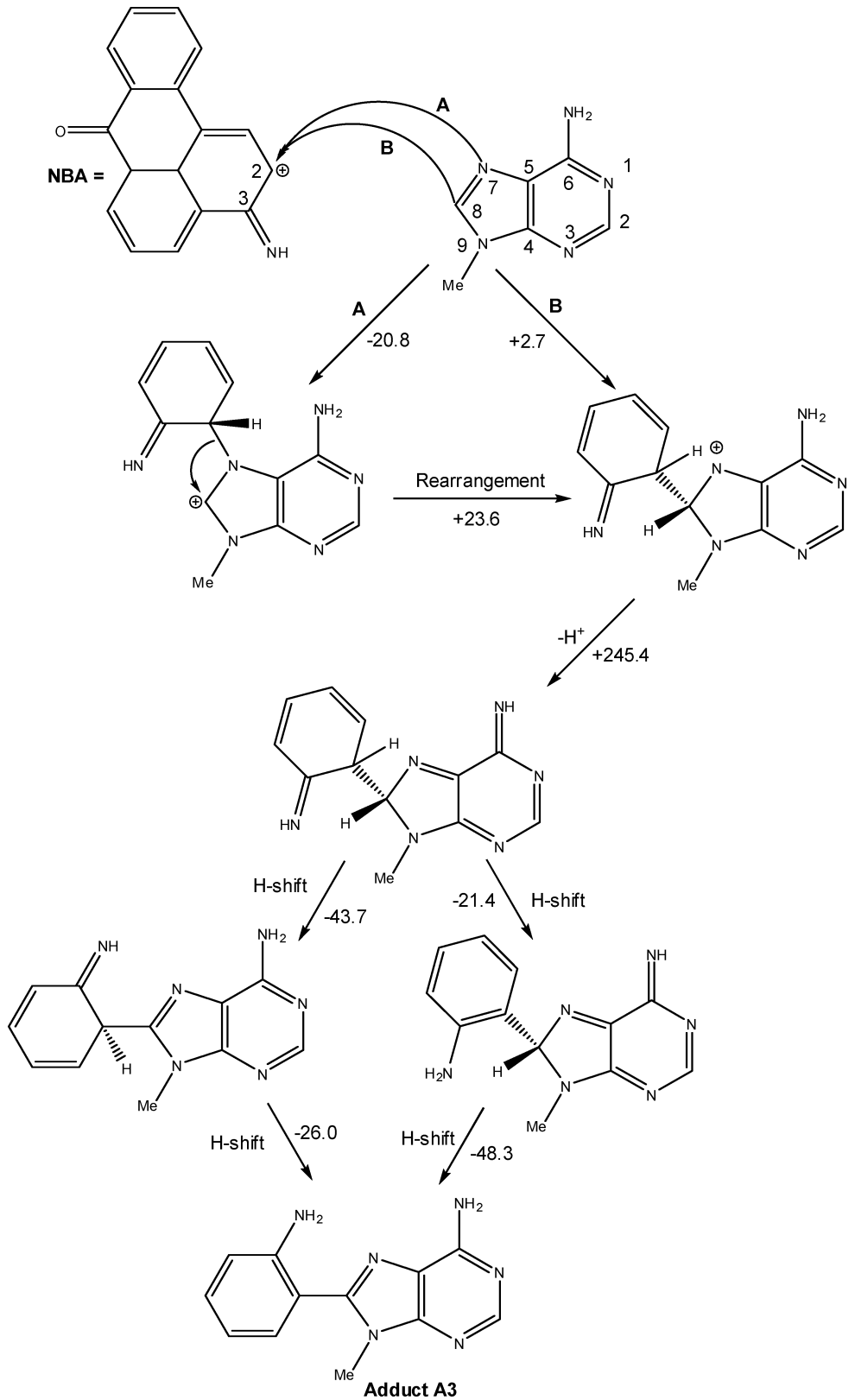
An ultrasensitive method that has been widely applied in the detection of DNA adducts is ^{32}P-postlabelling analysis.[52] One advantage of the method is that prior structural characterisation of adducts is not required although some assumptions about their likely chromatographic properties may be necessary. We found that 3-NBA forms multiple characteristic DNA adducts *in vivo* in rodents (see Fig. 1, inset).[5,23] In previous studies we have shown that all the major 3-NBA-derived DNA adducts detected by ^{32}P-postlabelling are derived from reductive metabolites bound to deoxyadenosine (dA, adduct spots 1 and 2) or deoxyguanosine (dG, adduct spots 3 and 4). Three of these DNA adducts were structurally identified as dA-N^6-3-ABA (spot 1; adduct A1), dG-N^2-3-ABA (spot 3; adduct G1) and dG-C8-N-3-ABA (spot 4; adduct G2), whereas the other dA adduct (spot 2) remains to be identified.[23,53] In order to gain further knowledge of the mechanism(s) of 3-NBA-derived DNA adduct formation we performed electronic



Scheme 5 Electrophilic attack of arylnitrenium ion of 3-NBA on C8 of the adenine moiety. All the values are in kcal mol^{-1}. 2′-deoxyribose is substituted with a methyl moiety.

energy calculations using DFT. For 3-NBA the calculations presented revealed that all experimentally observed dG adducts have clear exothermic formation pathways (see Schemes 1–3). This includes an unusual C2–C8 adduct, *e.g.*, dG-C2-C8-3-ABA (adduct G3), which is formed *in vitro* after reacting *N*-hydroxy-3-aminobenzanthrone with DNA but is not detectable *in vivo* in 3-NBA-treated experimental animals.[24,25,54] The formation of dA-N^6-3-ABA (spot 1; adduct A1) is thermochemically possible whereas dA-C8-*N*-3-ABA (adduct A2) is *not* (Scheme 5), which offers an explanation for its absence in biological systems. Indeed, using an authentic standard preliminary data indicate that the structurally unidentified dA adduct detected by ^{32}P-postlabelling (*e.g.* adduct spot 2) is not dA-C8-*N*-3-ABA (Arlt & Takamura-Enya, unpublished data). Also, our calculations show that dA-C2-C8-3-ABA (adduct A3, Scheme 6) can be formed, albeit not as a major product, which makes it a candidate for spot 2. In order to check this prediction an effort should be made to synthesise an authentic standard of dA-C2-C8-3-ABA which can be used for ^{32}P-postlabelling analysis.

The relative yields of adducts dG-N^2-3-ABA (adduct G1), dG-C8-*N*-3-ABA (adduct G2) and dG-C2-C8-3-ABA (adduct G3) in reactions involving dG with *N*-acetoxy-3-ABA are reported to be 2%, 10% and 9%, respectively.[25] The low yield of dG-N^2-3-ABA (adduct G1) can be attributed to the low exothermicity (–3.9 kcal mol^{-1}) of the electrophilic attack of the 3-NBA arylnitrenium ion on N^2 (pathway **B**, Scheme 1). Furthermore, pathway **A** is endothermic. However, for dG-C8-*N*-3-ABA (adduct G2) and dG-C2-C8-3-ABA (adduct G3) clear exothermic electrophilic reactions are predicted (pathway **B**, Schemes 2 and 3) facilitating higher yields than for dG-N^2-3-ABA (adduct G1). Only dA-N^6-3-ABA (adduct A1) has been observed for the adenine adducts and the yield was not given.[25] Takamura-Enya *et al.* [25] also reported that the yield ratio was changed considerably when calf thymus DNA was used instead of dG. When double stranded DNA is used the three dimensional structure plays an important role. In this study simple bases of guanine and adenine were used as models for DNA and therefore the results presented here relate to the experiments performed with dG and dA.



Scheme 6 Electrophilic attack of the arylnitrenium ion of 3-NBA from C2 on C8 of the adenine moiety. All the values are in kcal mol^{-1}. 2′-deoxyribose is substituted with a methyl moiety.

Previously we found that a combination of physicochemical and enzymatic properties related to 2-NBA and 3-NBA activation may account for their large differences in mutagenicity and DNA adduct formation.[17,26] For comparison the formation cascades of 2-NBA and 3-NBA were calculated for the guanine DNA adducts (see Schemes 1, 2 and 3) as they are the predominant DNA adducts formed by 3-NBA *in vivo* and are most probably responsible for the GC to TA transversion mutations observed *in vivo*.[55] We observed relatively small thermodynamic differences in the formation cascades, *i.e.*, both can be readily formed according to these results. However, it has been established that 2-NBA has a much lower mutagenic potency than 3-NBA[26,56] leading to the conclusion that the arylnitrenium ion of 2-NBA hardly reaches DNA to react.[17] We proposed that the stability of these ions play a pivotal role, *i.e.*, more stable ions have longer lifetimes and can therefore diffuse to react with electron rich macromolecules (*e.g.*, DNA) whereas unstable arylnitrenium ions are more promiscuous.[17,26] An alternative explanation is that the 2-NBA arylnitrenium ion is not formed because 2-NBA is *not* metabolised by the nitroreductase NQO1.[18] Molecular docking of 2-NBA and 3-NBA to the active site of human NQO1 showed similar binding affinities; however, the binding orientation of 2-NBA does not favour the reduction of the nitro group. This was confirmed by inhibition experiments which showed that 2-NBA can compete with 3-NBA for binding to human NQO1, resulting in inhibition of 3-NBA-DNA adduct formation by 2-NBA.[18] However, although 2-NBA is not well activated by NQO1, these results alone do not necessarily explain the results of a previous study[5] in which we found that 2-NBA forms DNA adducts in human lung A549 and liver HepG2 cells, whereas in breast MCF-7 and colorectal HCT116 cells it did not. As the metabolite 2-hydroxy-2-aminobenzanthrone was able to form DNA adducts in all four cell lines, these data indicate that nitroreduction catalysed by enzymes like NQO1 seems to be the critical step in the metabolic activation of 2-NBA.[26] However, preliminary data show that NQO1 protein is expressed in all cells but no direct correlation between 2-NBA-DNA adduct levels and NQO1 expression was found (Arlt *et al.*, unpublished data). This raises the possibility that other nitroreductases (*e.g.* NADPH:cytochrome P450 oxidoreductase)[57] or other enzymes that can reduce nitro-PAHs (*e.g.* cytochrome P450s), may reduce 2-NBA in these cells leading to the formation of its arylnitrenium ion. Our calculations show that 2-NBA can, in principle, form guanine adducts analogous to those formed by 3-NBA and that they are all thermodynamically stable. Therefore, adducts such as dG-N^2-2-ABA or dG-C8-*N*-2-ABA should be synthesised in the future to use them as standards for ^{32}P-postlabelling analysis in order to help to structurally identify 2-NBA-derived DNA adducts formed in A549 and HepG2 cells.

Summary and conclusions

The thermochemical reaction cascades were calculated for four experimentally established DNA adducts of 3-NBA (*i.e.* dG-N^2-3-ABA (adduct G1), dG-C8-*N*-3-ABA (adduct G2), dG-C2-C8-3-ABA (adduct G3) and dA-N^6-3-ABA (adduct A1)). In addition, for comparison the reaction pathways of two hypothetical 3-NBA-DNA adducts were performed (*i.e.* dA-C8-*N*-3-ABA (adduct A2) and dA-C2-C8-3-ABA (adduct A3)). A clear exothermic path was observed for the known adducts whereas the hypothetical ones are less thermochemically feasible offering an explanation for their absence. These results excludes the A2 hypothetical candidate for further investigation into the unknown adduct (*i.e.* adduct spot 2, Fig. 1) that has been observed using the ^{32}P-postlabelling method but A3 adduct remains an interesting candidate.

For guanine adducts their formation by 2-NBA was also calculated. In all cases a similar thermochemical profile to 3-NBA is observed leading to the conclusion that the 2-NBA arylnitrenium ion is capable of forming DNA adducts provided it reaches DNA. However, it has been shown that both the reductive enzyme systems and the physicochemical properties of 2-NBA play a crucial part in determining its genotoxic potency.[17,18,26]

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