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Stable ion study of benzo[*a*]pyrene (B*a*P) derivatives: 7,8-dihydro-B*a*P, 9,10-dihydro-B*a*P and its 6-halo derivatives, 1- and 3-methoxy-9,10-dihydro- B*a*P-7(8*H*)-one, as well as the proximate carcinogen B*a*P 7,8-dihydrodiol and its dibenzoate, combined with a comparative DNA binding study of regioisomeric (1-, 4-, 2-) pyrenylcarbinols †

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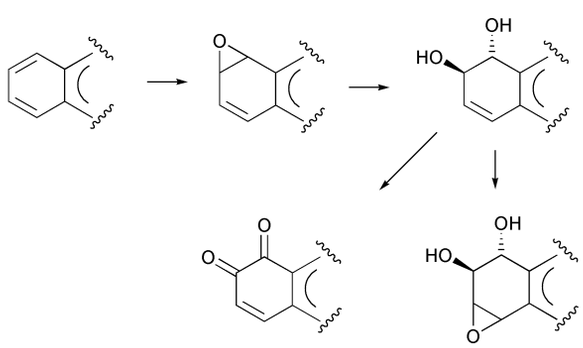
A stable ion study of a series of B*a*P derivatives is reported. 7,8-Dihydro-B*a*P **1** gives a persistent bay-region benzylic-like carbocation which shows extensive charge delocalization into the pyrene moiety. In contrast, a "benzylic" carbocation can not be generated from 9,10-dihydro-B*a*P **2**. Introduction of bulky substituents at *peri* C-6 of 9,10-dihydro-B*a*P (as in **4** and **5**) prevents side reactions (dimerization) to the extent that the initially formed carbocation undergoes rearrangement to generate the corresponding bay-region "benzylic" carbocation as a persistent species. Introduction of methoxy substituents into the 1- or 3- positions of 9,10-dihydro-B*a*P-7(8*H*)-one (**6**,**7**) increases its electrophilic reactivity to the extent that stable carboxonium–arenium dications are produced in FSO_3H–SO_2ClF. A detailed NMR study (at 500 MHz) of the resulting mono- and dications is reported, and charge delocalization mode (as well as conformational aspects) are addressed. Other oxidized derivatives of B*a*P such as the 7,8-dihydrodiol **9** and the 7,8-dihydrodibenzoate **8** are not suitable models for stable ion study because of competing *O*-protonation (and elimination). Energies for various possible arenium ions and regioisomeric "benzylic" cations were computed by the DFT method at the B3LYP/6-31G(d)//B3LYP/6-31G(d) level or by AM1 for comparison with the experimental results. These findings provide further evidence in support of the stability sequence: 1-pyrenyl > 4-pyrenyl > 2-pyrenyl in α-pyrene-substituted carbocations as models for the intermediates arising from B*a*P-epoxide ring opening. In an effort to provide a parallel, a series of α-pyrenylcarbinols were subjected to a DNA binding study using human MCF-7 cells. The results/trends are discussed and compared with the stable ion data.

Introduction

Alteration of genetic material by metabolic activation of polycyclic aromatic hydrocarbons (PAHs) requires the formation of electrophiles which are then capable of forming covalent adducts with the nucleophilic sites within DNA, such as the nucleobases.[1–3] Both the diol epoxides (formed *via* the sequence PAH-epoxide ⟶ PAH-dihydrodiol ⟶ PAH-diol epoxide) and the radical cations (formed *via* single electron oxidation) have been implicated in carcinogenesis by PAHs.[4] The dihydrodiols (proximate carcinogens produced by the combined action of cytochrome-P450 and epoxide hydrolase) can also form conjugates. In addition, dihydrodiols could be transformed to *o*-quinones which could act as Michael acceptors towards nucleophilic sites in DNA (Scheme 1).[4] There is much evidence that depending on the PAH structure, the epoxide ring opening mechanism could become substantially S_N1-like or proceed through proton stabilized transition states that attain significant carbocationic character.[5–8]



Scheme 1 PAH metabolic activation.

Benzo[*a*]pyrene B*a*P is a potent carcinogen which has played a paramount role in the development of the bay-region theory and structure–activity correlations in PAH-induced carcinogenesis. Its oxidized metabolites (among which (+)-*anti*-BPDE is most potent) and their adducts have been extensively studied from numerous angles including synthetic, stereochemical, kinetics, NMR, substituent effects, and structural/theoretical.[9–15] Binding of *anti*-BPDE to calf thymus DNA is suggested to proceed *via* initial rapid intercalation followed by rate determin-

† Electronic supplementary information (ESI) available: Selected NMR spectra (Fig. S1 and Charts S1-S10) and DFT computed energies for carbocations (Table S1). See http://www.rsc.org/suppdata/ob/b2/b212412b/

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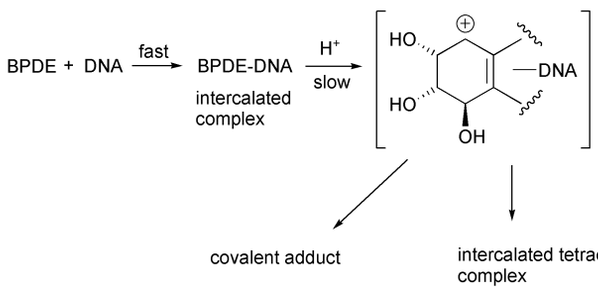
ing protonation to give an intercalated triolcarbenium ion which undergoes (a) hydrolysis (major pathway) to give tetraol which is physically associated with DNA, and (b) covalent attachment to DNA (minor pathway)[2] (Scheme 2). An additional activation pathway for B*a*P involves enzymatic methylation at C-6 and subsequent formation of a "benzylic" ester which could generate an α-B*a*P-substituted carbocation.[16,17]



Scheme 2 Formation of intercalated triolcarbenium ion and subsequent events.

The closely related benzo[*e*]pyrene B*e*P with two identical bay-regions is not a carcinogen. Its non-K-region (9,10)- and K-region (4,5)-*trans*-dihydrodiols as well as *syn*- and *anti*-diol epoxides have been synthesized (Fig. 1).[18]

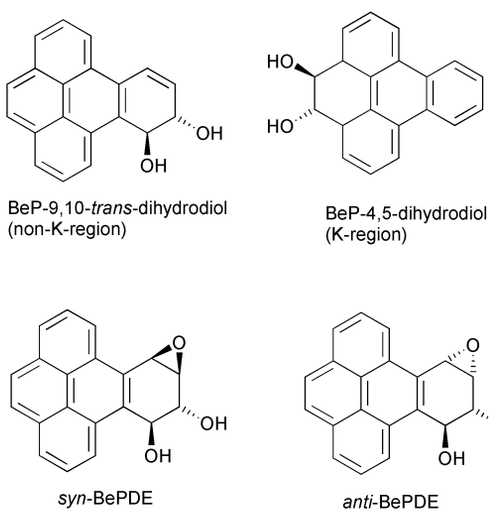


Fig. 1 B*e*P dihydrodiol and diol epoxide.

The dihydrodiols were shown not to be carcinogenic and were not further transformed to diol epoxides enzymatically.[18,19] Computational studies (molecular modeling and *ab initio* calculations) on the B-DNA intercalated physical complexes of bay-region PAH-triol epoxides (as in Scheme 2) have suggested that "correct " preorganization is important for subsequent covalent bonding to the nucleotide and this could explain the shape and stereochemical dependence of carcinogenesis.[20]

We are engaged in *direct* studies of carbocations generated from various classes of PAHs and their derivatives and in probing charge delocalization mode(s), substituent effects on charge delocalization and relative stability, as well as their structural/conformational aspects.[21–23] In our studies, protonated arenium ions and α-PAH-substituted "benzylic" carbocations serve as models for the generation of biological electrophiles. In continuation, we report here a low temperature protonation study on a series of B*a*P derivatives namely: 7,8-dihydro-**1**, 9,10-dihydro-**2** and its 6-halo-derivatives **3**–**5**, the methoxy-substituted ketones **6** and **7**, as well as the *trans*-dihydrodiol dibenzoate, dihydrodiol derivatives **8** and **9** and the 7,8,9,10-tetrahydro-7-ol derivative (**10**) (Fig. 2). DFT and AM1 calculations were performed to compute and compare the relative energies for various carbocations.

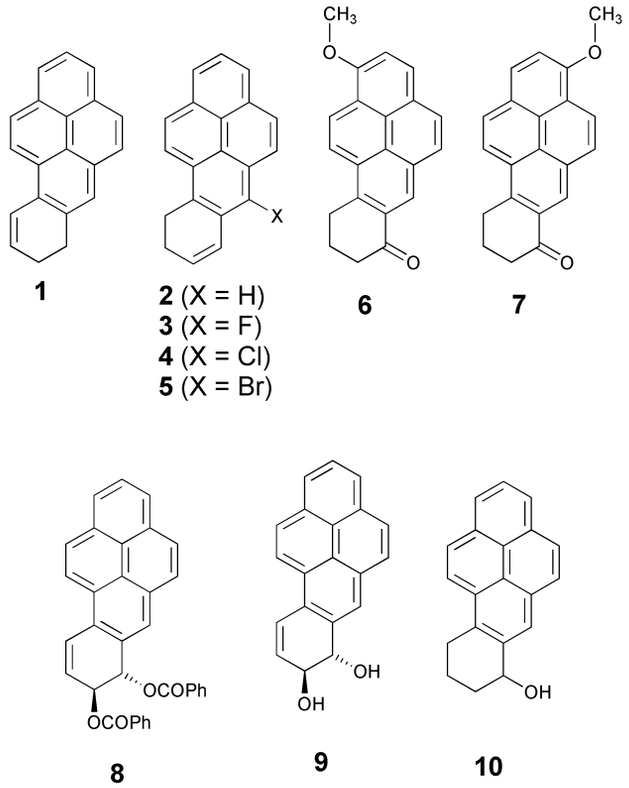


Fig. 2 List of substrates.

For comparative purposes, the primary, secondary and tertiary alcohols: 1-pyrenyl, 4-pyrenyl- and 2-pyrenylcarbinols (regioisomeric pyrenyl-CR_2OH) (Fig. 3) were subjected to a DNA binding study using human MCF-7 cells.

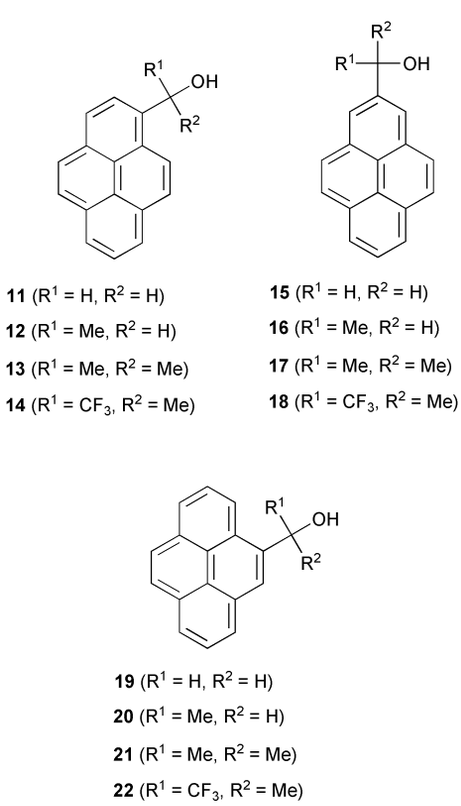
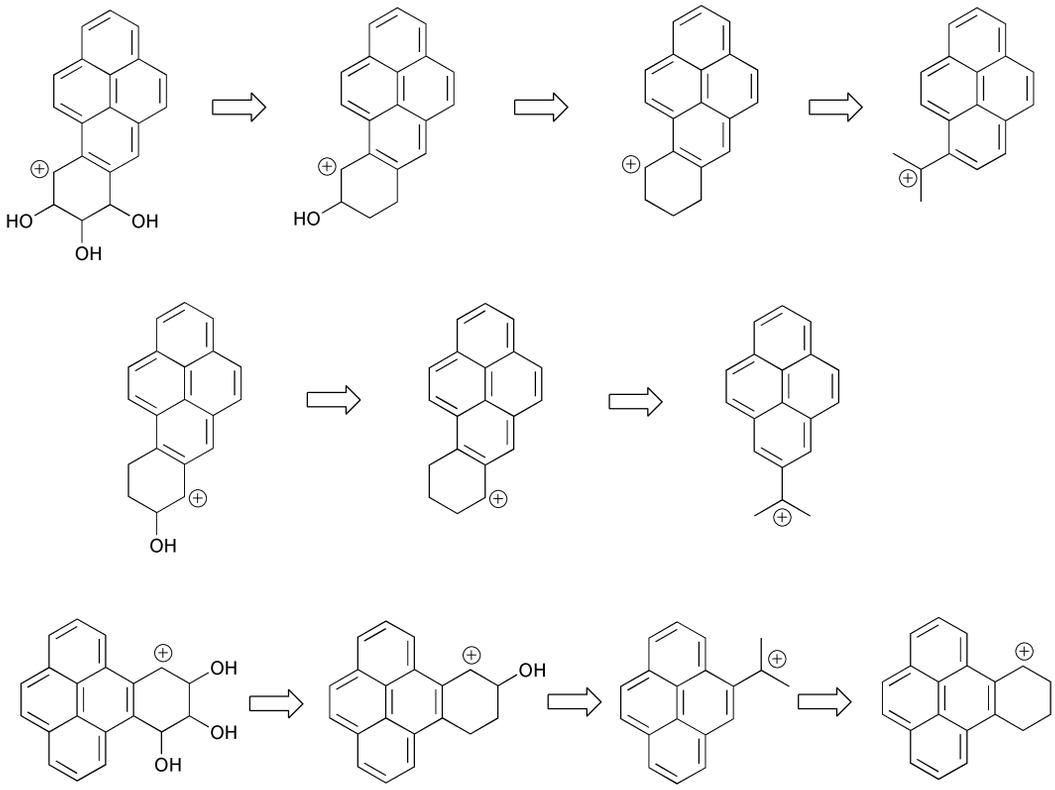


Fig. 3 List of regioisomeric carbinols used in DNA binding study.

These studies underscore the importance of bay-region α-PAH-substituted carbocations in chemical carcinogenesis and provide an experimental, carbocation-based rationale for the



Scheme 3 Formation of carbocation *via* diol epoxide ring opening and its "simplified" models.

relative reactivity and possibly the DNA binding of metabolically-formed electrophiles.

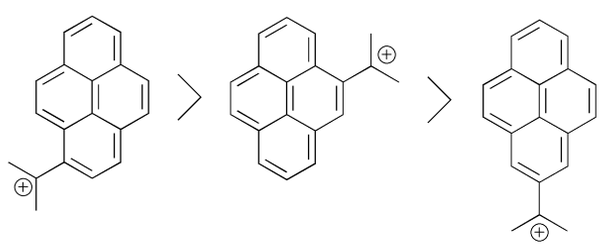
Results and discussion

In a previous stable ion study,[21c] the regioisomeric 1-pyrenyl-, 2-pyrenyl- and 4-pyrenyl methylcarbenium ions were generated from precursor alcohols by protonation–ionization in superacids. Charge delocalization mode and conformational aspects were probed by NMR and their relative stabilities were computed by AM1. The carbocations served as simplified models for the carbocations produced *via* diol epoxide ring opening (see Scheme 3). It was shown that 1-pyrenyl-substituted carbocations are most effectively delocalized and the stability order: 1-pyrenyl-C^+(R)R′ > 4-pyrenyl-C^+(R)R′ > 2-pyrenyl-C^+(R)R′ was established (Scheme 4). Introduction of α-CF_3 groups increased π-participation and arenium ion character (more extensive positive charge delocalization into the pyrene moiety), and increased Py–C^+ bond order.[21d] Because of inherent destabilization of the 2-pyrenylmethyl carbocation, introduction of an α- CF_3 group prevented carbinol ionization and ring protonation occurred instead.[21] The present study seeks to provide a carbocation-based structure–activity relationship utilizing precursors which are more closely related to the metabolic products.

NMR assignments

Detailed NMR assignments for the precursors and carbocations were based on ^{1}H, ^{13}C, H/H COSY, C/H HETCOR (or HMQC), COLOC (or HMBC), and NOED spectra.

NMR features in the neutrals. For most of the substrates employed in this study, ^{1}H NMR, and in some cases ^{13}C NMR data, had been reported in the literature.[24–26] However, except for the 6-halo derivatives,[25] no specific assignments of the carbon resonances were previously made. Complete NMR assignments for compounds **1**, **4**, **5**, **6** and **7** are included in Fig. S1 (supplementary material †). For the methoxy derivatives

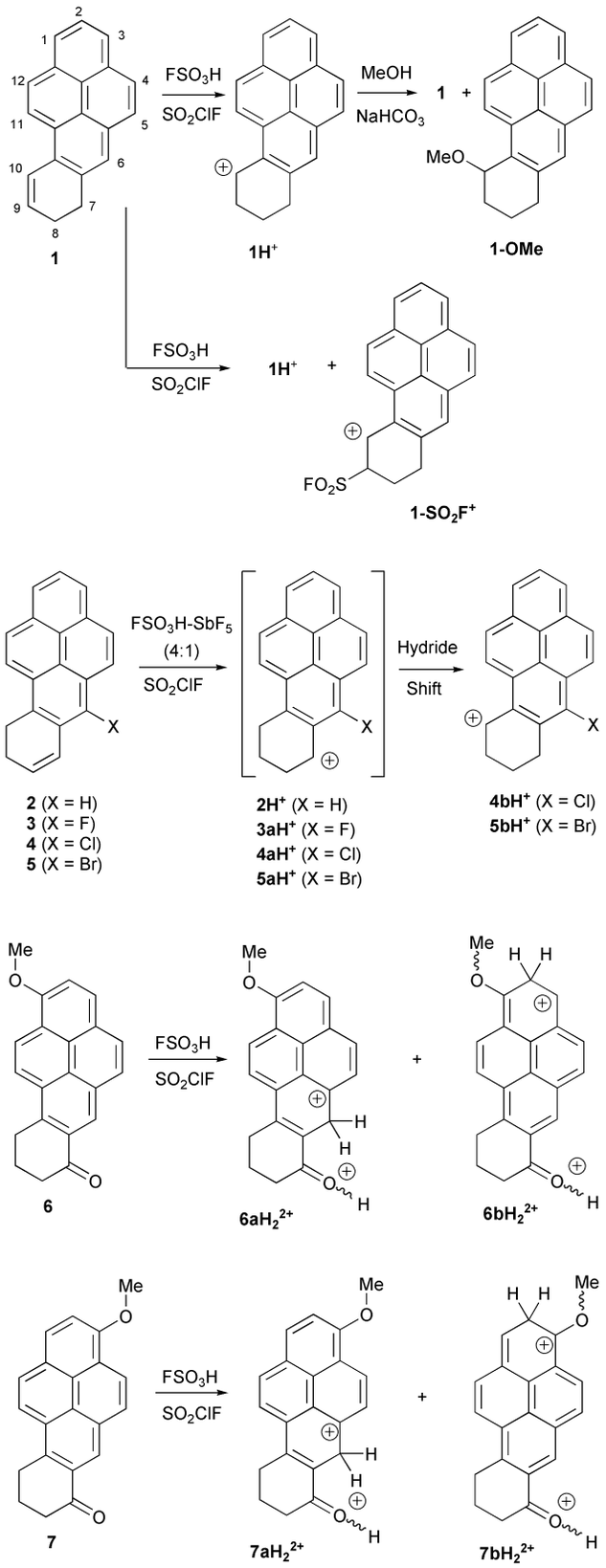


Scheme 4 Relative stability order for the regioisomeric carbocations.

(**6** and **7**) irradiation of OMe protons gave concurrent NOE enhancements for H-2/H-12 in **6** and H-2/H-4 in **7**. However, these NOE effects were larger for the H-2 protons (*ortho*), suggesting that the conformation in which OMe points toward H-2 (the *out-form*) predominates. A notable feature for **6** and **7** is the observation of *peri*-NOE effects for H-5/H-6 and H-11/CH_2.

Stable ion study (Scheme 5, Fig. S1 and selected NMR spectra in supplementary material †)

Protonation of 7,8-dihydro-B*a*P (1). Low temperature reaction of **1** with FSO_3H–SO_2ClF cleanly produced **1H$^+$** (in *ca.* 95% yield) with protonation taking place at C-9 (a dark-red solution). In the ^{1}H NMR spectrum (at −60 °C), the most deshielded proton is H-10 at δ 9.66 (supplementary material †). The aromatic protons are all deshielded except for H-6 (singlet at δ 7.73). The latter exhibits NOE with H-5 (at δ 8.0) and with the methylene protons at C-7. The upfield shift of H-6 is possibly due to a conformational change when the cyclohexadiene ring is transformed into the more flexible cyclohexenyl cation. Additional NOE effects were detected between H-3 and H-4. There are seventeen aromatic resonances in the ^{13}C NMR with the most deshielded at δ180.2 for the "carbocation center". Positive charge is extensively delocalized into the pyrene moiety and establishes a clear charge alternation path. The NMR features for **1H$^+$** are very similar to those of 1-(1-pyrenyl)ethyl cation 1-PyC^+(Me)H[21c](Fig. S1 †), confirming the previous



Scheme 5 Stable ion study.

prediction that the ring opened "simplified" version is a reasonable model for α-PAH carbocations derived *via* B*a*P epoxide ring opening.

Quenching with methanol gave a mixture of **1**-OMe and **1** (in 3 : 1 ratio). When this mixture was reacted with FSO_3H–SO_2ClF, $\mathbf{1H^+}$ was regenerated quantitatively. In a separate experiment, a higher concentration of **1** was used in reaction with FSO_3H–SO_2ClF. This produced a *ca.*1 : 1 mixture of $\mathbf{1H^+}$ and the arenium ion of fluorosulfonation $[\mathbf{1}\text{-}SO_2F]^+$. In the fluorosulfonation cation, positive charge is more extensively delocalized into the pyrene moiety and the carbocation center is comparatively more shielded (δ147.0 *versus* δ180.2). The aromatic protons are generally more deshielded in $[\mathbf{1}\text{-}SO_2F]^+$ whereas H-10 is relatively shielded. NOE effects were observed between H-6 and H-5/H-7. Overall, charge alternation modes in $\mathbf{1H^+}$ and $[\mathbf{1}\text{-}SO_2F]^+$are analogous. Local overheating in the more concentrated sample is probably responsible for the competing formation of the arenium ion of fluorosulfonation.

Monoprotonation of 9,10-dihydro-B*a*P (2). Contrary to the behavior of the 7,8-dihydro compound **1**, low temperature reaction of the 9,10-dihydro-B*a*P **2** with FSO_3H–SO_2ClF did not produce a persistent carbocation. Instead, complex proton and carbon spectra were obtained with far too many resonances (quenching of the superacid solution produced unknown compounds). Based on DFT calculations, $\mathbf{2H^+}$ is 9.8 kcal mol^{-1} higher in energy than $\mathbf{1H^+}$. In line with previous stable ion studies on regioisomeric 1-pyrenyl-substituted carbocations[21c] and with DFT, it is plausible that $\mathbf{2H^+}$ is the initially formed carbocation which immediately undergoes dimerization to produce dimeric carbocations with complex spectra. Dimer formation as a logical reaction route for the less favored carbocation derived from **2** is borne out by the results obtained with the 6-halo derivatives discussed below.

Monoprotonation of 6-halo-9,10-dihydro-B*a*P (3–5). *a) 6-Fluoro derivative* **3**: low temperature reaction of **3** with FSO_3H or with FSO_3H–SbF_5 (4 : 1)–SO_2ClF at dry-ice–acetone temperature gave dark-red solutions (with formation of black precipitates), whose NMR spectra exhibited a large number of resonances with broad features, compatible with rapid initial formation of the carbocation and subsequent dimerization–oligomerization. Carbocation $\mathbf{3aH^+}$ is computed to be 10.7 kcal mol^{-1} less stable than its bay-region benzylic carbocation ($\mathbf{3bH^+}$) (as in $\mathbf{4bH^+}$ but with X = F).

b) 6-Chloro derivative **4**: Low temperature reaction of **4** with FSO_3H–SbF_5 (4 : 1)–SO_2ClF gave the rearranged "benzylic" carbocation $\mathbf{4bH^+}$ as a persistent species (a dark-red solution). Cation $\mathbf{4bH^+}$ would be formed *via* a 1,4-hydride shift in the initially generated cation $\mathbf{4aH^+}$. On the basis of DFT calculations, the bay-region carbocation $\mathbf{4bH^+}$ is preferred over $\mathbf{4aH^+}$ by 10.5 kcal mol^{-1}. For this substrate, steric hindrance is likely preventing the formation of a dimer carbocation from the initially formed $\mathbf{4aH^+}$, allowing it to undergo rearrangement to the more stable "benzylic" carbocation. The cation center in $\mathbf{4bH^+}$ is at δ181.9 with the H-10 at δ 9.71. The latter gives NOE enhancement with H-11. A similar shielding of H-5 is observed for $\mathbf{4bH^+}$, all other aromatic protons are deshielded. Similar to $\mathbf{1H^+}$, positive charge is delocalized extensively into the pyrene moiety resulting in significant pyrenium ion character, exhibiting a clear charge alternation path. Quenching of the superacid solution gave 6-chloro-B*a*P[22b,27] as a major product.

6-Bromo derivative **5**: low temperature reaction of **5** with FSO_3H–SbF_5 (4 : 1)–SO_2ClF gave $\mathbf{5bH^+}$(as a dark-red solution) whose carbocation center is at δ 181.6 and H-10 at δ 9.67. The latter exhibits NOE with H-11 (at δ 8.80). In concert with the experiment, DFT preferred $\mathbf{5bH^+}$ over $\mathbf{5aH^+}$ by as much as 10.5 kcal mol^{-1}. Charge delocalization mode in $\mathbf{5bH^+}$ is analogous to those of $\mathbf{4bH^+}$ and $\mathbf{1H^+}$. Cation $\mathbf{5bH^+}$ could be formed *via* a 1,4-hydride shift in the initially generated cation $\mathbf{5aH^+}$. Quenching of the superacid solution gave 6-bromo-B*a*P[27] as a major product.

It may be concluded that, under stable ion conditions, steric hindrance at C-6 is the key to preventing dimerization–oligomerization, providing the opportunity for the initial carbocation to rearrange to the more stable bay-region benzylic-like cation.

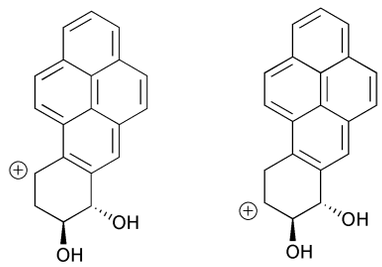
Monoprotonation of 1-methoxy-9,10-dihydrobenzo[*a*]pyren-7-(8*H*)-one (6). Low temperature reaction of **6** with FSO_3H–SO_2ClF gave a mixture of two carboxonium–arenium dications $\mathbf{6aH_2}^{2+}$ and $\mathbf{6bH_2}^{2+}$ (dark-purple with some black precipitate) with ring protonation taking place at C-6 and C-2 (*ortho* to methoxy) in 8 : 1 ratio respectively (at −70 °C). After about 1 hour, the ratio increased to 19 : 1 in favor of $\mathbf{6aH_2}^{2+}$. Upon warming the superacid solution (to −50 °C), $\mathbf{6bH_2}^{2+}$ completely isomerized to $\mathbf{6aH_2}^{2+}$ and the solution became dark-red and homogeneous (no precipitate).

According to AM1 calculations, initial CO-protonation (-> carboxonium ion) is slightly favored over ring protonation at C-6 (by about 0.7 kcal mol^{-1}). For the carboxonium ion, the conformation in which COH^+ points towards *peri*-H-6 is somewhat better than a "down" COH^+. Starting with the carboxonium ion, relative energies were computed for all possible ring protonated dications. Protonation at C-6 (observed) and C-11 (not observed) were equally favored. Protonation at C-5 (not observed) and at C-2 (initially observed) were the next best possibilities. The difference in energy between $\mathbf{6aH_2}^{2+}$ and $\mathbf{6bH_2}^{2+}$ (both in the "*E*-out" conformation; OMe pointing towards H-2 with "down" COH^+) was computed by DFT to be 3.9 kcal mol^{-1} in favor of $\mathbf{6aH_2}^{2+}$. It is conceivable that $\mathbf{6bH_2}^{2+}$ appeared because of local overheating, then slowly isomerized to $\mathbf{6aH_2}^{2+}$(alternatively, $\mathbf{6bH_2}^{2+}$ may be the kinetically controlled carbocation and $\mathbf{6aH_2}^{2+}$ the thermodynamic one). NMR data clearly rule out the formation of any other potential dications.

In $\mathbf{6aH_2}^{2+}$ the protons are all deshielded, with H-12 (at δ 9.63) and H-3 (at δ 9.23) being most downfield. The COH^+ is seen at 15.1 ppm. NOE effects were observed between H-5/H-6s and between the H-10 and H-11. When the methoxy protons of $\mathbf{6aH_2}^{2+}$ were irradiated, NOE enhancement in H-2 was observed, suggesting that the "out-form" is more favored (this agrees with AM1). The most deshielded carbons are COH^+ (δ 216.5), C-1 (δ 177.6) and C-10a (δ 171.4). Positive charge is delocalized throughout the system. The most deshielded proton in $\mathbf{6bH_2}^{2+}$ is H-3 (δ 9.33). Quenching of the superacid solution cleanly returned the skeletally intact material.

Monoprotonation of 3-methoxy-9,10-dihydrobenzo[*a*]pyren-7(8H)-one (7). Stable ion chemistry of compound **7** is very much analogous to **6**. Its low temperature reaction with FSO_3H–SO_2ClF gave a mixture of carboxonium–arenium dications $\mathbf{7aH_2}^{2+}$ and $\mathbf{7bH_2}^{2+}$ (a dark-red solution), with protonation taking place at C-6 and C-2 (in 8 : 2 ratio at −70 °C). Upon warming the superacid solution (to −50 °C), $\mathbf{7bH_2}^{2+}$ completely isomerized to $\mathbf{7aH_2}^{2+}$. In concert with the experiment, DFT calculations predict that $\mathbf{7bH_2}^{2+}$ is the lowest energy dication, with $\mathbf{7aH_2}^{2+}$ computed to be only 0.5 kcal mol^{-1} higher. For $\mathbf{7aH_2}^{2+}$ the protons are all deshielded, with H-4 (*peri* to OMe) appearing at δ 9.45 (the COH^+ is at δ 14.50). NOE effects were observed between H-5/H-6s, between the H-10 and H-11, and between the methoxy protons and H-2/H-4. The most deshielded carbons are COH^+ (δ 215.8), C-3 (δ 180.4) and C-10a (δ 172.8). The charge delocalization pattern in $\mathbf{7aH_2}^{2+}$ and $\mathbf{6aH_2}^{2+}$ is very similar. To explore the possibility of further protonation (a trication), protonation of **7** with FSO_3H–SbF_5 (4 : 1) was also examined, however, the same two dications were formed. A quenching experiment gave the intact substrate as the only product.

Protonation of *trans*-7,8-dibenzoyloxy-7,8-dihydrobenzo[*a*]-pyrene (8) and *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene 9. Despite the presence of a reactive double bond in a strategically favored position, protonation of **8** and **9** did not proceed according to our expectations. Low temperature reaction of **8** with FSO_3H–SO_2ClF gave a dark-red solution, whose NMR spectral data were consistent with generation of a CO-protonated benzoic acid (δ ^{13}C: 181.1, 141.5, 133.6, 131.1, 122.1)[28] as the predominant species whose resonances were surrounded by a group of (small) broader unresolved peaks (the data are not consistent with the alternative formation of a benzoyl cation[29]). Quenching with MeOH did not produce a dihydrodiol derivative or a methoxy addition compound. Despite mild conditions, protonation of the *trans*-dihydrodiol **9** with FSO_3H–SO_2ClF was not selective, resulting in a mixture of ions. Clear preference for the formation of the bay-region ($\mathbf{9aH^+}$) relative to non-bay-region carbocation ($\mathbf{9bH^+}$) (Fig. 4) may be inferred from DFT calculations which compute the former to be 35.1 kcal mol^{-1} lower in energy. Quenching of the superacid solution with MeOH produced B*a*P as a major product (comparison with NMR of authentic sample).



$\mathbf{9aH^+}$ $\mathbf{9bH^+}$

Fig. 4 Bay-region *versus* non bay-region carbocation *via* **9**.

Reaction of 7,8,9,10-tetrahydrobenzo[*a*]pyren-7-ol (10). The secondary alcohol **10** is the precursor to the least stable α-pyrenyl-substituted carbocation (see Scheme 1). Its low temperature reaction with FSO_3H–SO_2ClF gave a dark-red solution, exhibiting broad and deshielded (unresolved) proton spectra, indicative of rapid dimerization–oligomerization. This infers that the initially formed carbocation undergoes alkylation probably with **2** formed *in situ* by competing dehydration. In an attempt to make an adduct between a "solvolytic" carbenium ion and a suitable base, compound **10** was refluxed in the presence of imidazole in CH_2ClCH_2Cl solvent without any acid. This returned only the intact material.

Comparative discussion of the stable ion data

Compound **1** with a reactive double bond at the bay-region cleanly produced the biologically important carbocation $\mathbf{1H^+}$ as a persistent species, exhibiting significant pyrenium ion character. No stable benzylic-like carbocation could be generated from **2**, **3** (or **10**). By increasing steric hindrance at C-6 (**4** and **5**), dimerization of the initially formed high energy carbocation could be prevented and this led to facile rearrangement to the corresponding bay-region carbocations.

Methoxy substitution in the remote α-positions of pyrene (compounds **6** and **7**) increases the electrophilic reactivity of the parent ketone. In our previous study,[23c] the unsubstituted ketone was shown to produce a carboxonium ion in FSO_3H–SO_2ClF. Protonation with the higher acidity superacid FSO_3H–SbF_5 (4 : 1)–SO_2ClF led to dication formation (ring protonation occurring at C-1 and C-3 in 2 : 1 ratio). The present study shows that with **6** and **7**, dications are produced in FSO_3H–SO_2ClF. Furthermore, methoxy-substitution directs the attack to C-6 whereas in the parent ketone attack is directed to C-1/C-3. Compounds **8** and **9** are not suitable models for stable ion study in superacid solvents because *O*-protonation becomes competitive and side reactions dominate.

DNA binding experiments for substituted pyrene system

In an effort to bridge the reactivity trend that emerges from stable ion studies of α-pyrene-substituted carbocations with DNA binding ability, a series of regioisomeric (1-pyrenyl-, 4-pyrenyl- and 2-pyrenyl-) primary, secondary and tertiary

Table 1 Results of DNA binding experiments (pmol adducts mg^{-1} DNA)[a]

Carbinols	1 μM	Average	10 μM	Average
11	1.45, 0.6, 0.51, 2, 0	**0.91**	2.41, 2.1, 1.9, 2.5	**2.22**
12	0.72, 0.29, 0, 1.3	**0.58**	0.29, 0, 0, 0	**0.07**
13	1.25, 1.6, 2, 0	**1.21**	0.34, 0, 0	**0.11**
14	0.32, 2, 1.42, 0	**0.94**	0.16, 0, 0	**0.05**
15	0.24, 1.36, 0.5, 2.68	**1.2**	0.27, 0, 1.76, 0.63	**0.67**
16	0.08, 0, 0.45, 0	**0.13**	0, 0, 0	**0**
17	0.56, 6.33, 7.05, 0.69	**3.66**	0.37, 0.77, 0, 0.158	**0.325**
18	0.31, 6.75, 0, 0, 0	**1.41**	0.24, 0.96, 2.64, 0, 0	**0.77**
19	0.09, 1.26, 0.67	**0.673**	0.12, 1.05, 2	**1.06**
20	1.48, 6.83, 2.8, 1.08	**3.05**	0.27, 1.07, 0	**0.45**
21	0.98, 3.83, 2.14	**2.32**	0.27, 0.185	**0.23**
22	0.29, 2.5, 2.63	**1.81**	0.21, 2, 0.26	**0.82**
B*a*P	21.7, 10.38, 9.08, 13, 3.9	**11.61**		
DMSO	0, 0, 0, 1.26, 1.38, 0	**0.44**		

[a] MCF-7 cell cultures were exposed to the substrate for 24 hours. DNA was isolated and DNA adducts were analyzed by HPLC.

carbinols (see Fig. 3) were subjected to a DNA binding study using human MCF-7 cells at various doses. For comparison, MCF-7 cell binding data for B*a*P itself were also measured under the same set of conditions. Although the pyrenyl-substituted benzylic alcohols exhibit potencies substantially lower than B*a*P, in several cases measurable DNA binding occurred. The results are summarized in Table 1. Among the primary "benzylic" alcohols, only the 1-pyrenyl isomer (**11**) shows DNA binding ability that gives a detectable adduct peak (see Fig. 5). Among the secondary alcohols, the 4-pyrenylcarbinol (**20**) binds to DNA. The tertiary alcohols **13**, **17**, and **21** also exhibit some DNA binding ability to MCF-7 cells, whereas α-CF_3 substituted alcohols exhibit little or no DNA binding ability. The observed variations in binding among primary "benzyl" alcohols (**11**, **15**, and **19**) infer that some degree of carbocationic character is developed in the transition state for DNA binding, otherwise they should have all exhibited nearly equal binding ability (this is assuming no significant steric bias among regioisomers). On a purely steric argument, the 2-pyrenyl alcohol is least crowded (no *peri* interactions) and may be better accessible to a bulky nucleophile. However, the observed trend for regioisomeric $PyCH_2OH$ appears to be more in line with charge stabilization than a purely steric phenomenon. DNA binding data for regioisomeric PyCH(Me)OH (**12**, **16**, and **20**) reflect lack of reactivity for the 2-pyrenyl system. For this group, the 4-pyrenylcarbinol is most reactive.

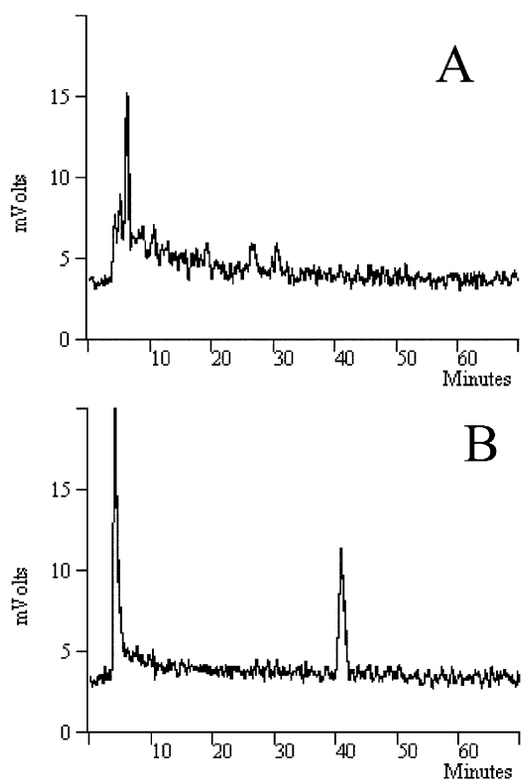


Fig. 5 HPLC elution profiles of ^{33}P-postlabeled DNA adducts formed in MCF-7 cells treated with 1 μM (A) or 10 μM (B) of compound **11**.

We interpret these trends as a balance between relative carbocation stability and steric factors (charge is better delocalized in 1-pyrenyl but steric crowding is relatively less in 4-pyrenyl). Among regioisomeric $PyC(Me)_2OH$ (**13**, **17**, and **21**), the 2-pyrenyl and 4-pyrenyl derivatives exhibit activity. The higher than expected average binding value for the 2-pyrenyl isomer could imply a contribution from a more favorable/less sterically crowded nucleophile approach. Poor DNA binding activity for the α-CF_3-substituted derivatives ties in with a carbocation mechanism which becomes severely retarded by an α-CF_3 group. As part of this study, in selected cases, the alcohols were also reacted with calf-thymus (CT)-DNA and the adducts were analyzed. For the primary "benzylic" carbinols the level of binding between MCF-7 cells and CT-DNA was similar but higher levels were measured for CT-DNA in the case of 1-pyrenylpropanol (**13**) and most notably for the secondary 4-pyrenylethanol (**20**). It is conceivable that this is a juxtaposition of carbocation stability and steric effects, with the latter obviously playing an important role in DNA binding (as compared to conventional solvolysis with trapping by "conventional" nucleophiles). Significance of steric effects and the geometrical compatibility of the bay-region carbocation with DNA (pre-organization in the physical complex) to form the PAH–DNA covalent adduct were underscored in previous computational studies.[20]

The goal of the present investigation was to search for possible trends between stability patterns emerging *via* direct studies of the carbocation intermediates and the much more complex phenomena of DNA intercalation and covalent binding. We surmise that DNA binding data are compatible overall with the relative stability orders deduced from direct studies of regioisomeric α-pyrene-substituted carbocations; they also emphasize the importance of steric contribution to DNA binding.

Experimental

FSO_3H (Allied and Aldrich) and SbF_5 (Aldrich and Fluorochem) were freshly distilled in an all-glass distillation unit under a dry nitrogen atmosphere. SO_2ClF was synthesized from SO_2Cl_2, ammonium fluoride, and trifluoroacetic acid according to a modified procedure of Reddy *et al.*[30] Several distillations provided pure SO_2ClF. Calf-thymus DNA (CT-DNA) was purchased from Sigma Chemical Co. Other commercially available reagents were used as received.

NMR spectra were recorded on a 500 MHz spectrometer. Those of neutral PAHs were recorded in $CDCl_3$ at room temperature. Carbocations were studied between −80 °C and −30 °C. NMR analyses included ^1H, ^{13}C, H/H COSY, C/H HETCOR (or HMQC), COLOC (or HMBC), and NOED experiments.

DFT calculations

Geometry optimizations were performed at the B3LYP/6-31G(d) level using the Gaussian 98 package.[31] Computed geometries were verified by frequency calculations at the B3LYP/6-31G(d)//B3LYP/6-31G(d) level (calculated energies are listed in Table S1 in the supplementary information †).

AM1 calculations

These were carried out using standard methods as implemented in the Hyperchem package 5.11 (Hypercube Inc, 1999) or Insight II Release 97.0 (MSI, 1999).

Neutral substrates

The B*a*P derivatives **1**–**3** and **5**–**9** are all known compounds whose syntheses have previously been reported in the literature.[24–26] Synthesis of the 6-chloro derivative **4** (not previously reported) is detailed below. Complete NMR assignments for **1**, **4**, **5**, **6** and **7** are included in Fig. S1 †. Compound **10** was commercially available (Aldrich). The regioisomeric carbinols (**11**–**22**) were prepared according to previously reported procedures.[21c,21d]

On the synthesis of 4

Compound **4** was prepared in 2 steps by chlorination of 7,8,9,10-tetrahydrobenzo[*a*]pyren-7-ol using *N*-chlorosaccharin (NCSac) followed by dehydration as described below:

(a) *6-Chloro-7,8,9,10-tetrahydrobenzo[a]pyren-7-ol*: to a stirred solution of 7,8,9,10-tetrahydrobenzo[*a*]pyren-7-ol (1.360 g, 5 mmol) in benzene (218 cm^3) at 5 °C was added a solution of NCSac[32] (1.196 g, 5.5 mmol) in benzene (125 cm^3) (cooled to just above the freezing point) over 30 min. The reaction mixture was stirred for another hour at 5 °C and then for 24 h at rt. The mixture was diluted with ethyl acetate and enough THF was added for the suspension to dissolve. The mixture was washed with aqueous $Na_2S_2O_5$, aqueous $NaHCO_3$ and water, the organic layer was dried over anhydrous sodium sulfate and evaporated. The crude product was washed first with boiling MeOH and subsequently purified by dry column chromatography (SiO_2, eluted with CH_2Cl_2). The 6-chloro derivative was isolated as a pale yellow powder (1.180 g, 77%); ν_{max} (5 mM solution in CCl_4)/cm^{-1} 3610 (OH); ^1H NMR ($CDCl_3$) δ 1.98 (1 H, tt, *J* 13.4 and 3.4, H-8_{ax}), 2.13 (1 H, m, H-9_{eq}), 2.30 (1 H, qdd, *J* 13.4, 5.3 and 2.7, H-9_{ax}), 2.42 (1 H, dm, *J* 13.5, H-8_{eq}), 2.68 (1 H, broadened d, *J* 2.7, OH), 3.25 (1 H, ddd, *J* 17.9, 11.8 and 6.0, H-10_{ax}), 3.71 (1 H, broad dd, *J* 17.3 and 4.9, H-10_{eq}), 5.61 (1 H, broad q, J_{app} 3.2, H-7), 8.02 (1 H, t, *J* 7.6, H-2), 8.11 (1 H, d, *J* 9.3, H-4), 8.13 (1 H, d, *J* 9.3, H-12), 8.19 (1 H, d, *J* 7.6, H-1), 8.20 (1 H, d, *J* 7.6, H-3), 8.27 (1 H, d, *J* 9.3, H-11), 8.51 (1 H, d, *J* 9.3, H-5); ^{13}C NMR ($CDCl_3$) δ 16.99 (C-9), 27.00 (C-10), 30.46 (C-8), 65.29 (C-7), 122.78 (C-11), 123.55 (C-5), 123.98 (C-12b), 125.15 (C-12c), 125.54 (C-3), 125.68 (C-1), 126.48 (C-2), 127.01 (C-5a), 127.68 (C-12), 128.05 (C-10b), 128.10 (C-4), 130.20 (C-6), 131.08 (C-12a), 131.13 (C-3a), 132.85 (C-10a), 133.57 (C-6a); HRMS *m/z* 306.0820 (M^+. $C_{20}H_{15}ClO$ requires 306.0811).

(b) Synthesis of **4**: to a stirred solution of 6-chloro-7,8,9,10-tetrahydrobenzo[*a*]pyren-7-ol (122 mg, 0.398 mmol) in benzene (10 cm^3) was added *p*-toluenesulfonic acid monohydrate (15 mg, 0.079 mmol) and the suspension was heated (70 °C, 30 min). After cooling it was diluted with ethyl acetate and the mixture was washed with aqueous $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4, the solvent was evaporated and the crude product was purified by dry column chromatography (SiO_2, eluted with PhH). Compound **4** was obtained as a yellow powder (105 mg, 91%); ^1H and ^{13}C NMR (see Fig. S1 †); HRMS *m/z* 288.0714 (M^+. $C_{20}H_{13}Cl$ requires 288.0706).

General procedure for stable ion generation

SO_2ClF (*ca.* 0.4 cm^3) was distilled into a 5 mm NMR tube containing the PAH (10–20 mg) cooled to dry ice–acetone temperature. To the resulting suspension cold FSO_3H (2 drops) or cold FSO_3H–SbF_5 (4 : 1) (2 drops) was carefully added followed by efficient mixing (vortex) until homogeneous. Then two drops of cold CD_2Cl_2 were added on the top of the solution and the combined solution was thoroughly mixed (vortex).

Quenching experiments with water

The superacid solution was carefully poured into ice–$NaHCO_3$ and the mixture was extracted with CH_2Cl_2. The organic extract was washed (10% NaCl) and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was analyzed by NMR.

Quenching with methanol

The superacid solution was carefully poured into cold MeOH–$NaHCO_3$. Most of the solvent was evaporated and the residue was dissolved in CH_2Cl_2. The solution was washed (sat. NaCl) and dried ($MgSO_4$) and the solvent was removed under reduced pressure and examined directly by NMR.

DNA binding experiments with human MCF-7 cells

Stable DNA adducts were analyzed *via* ^{33}P-Postlabeling technique using previously established procedures.[33] Briefly, DNA samples were isolated from the cells and 10 μg of each sample was degraded with nuclease P1 and prostatic acid phosphatase. This treatment cleaves DNA to nucleosides. However, it blocks one nucleotide 3′ of any nucleotide containing a PAH adduct. The dinucleotides containing the PAH adducts were then 5′ labeled using ^{33}P-ATP. The nucleosides without an adduct cannot be labeled, since the phosphorolation of the 5′ requires the presence of a 3′ phosphate. This only occurs in the PAH-adducted nucleotides. They were then cleaved with snake venom phosphodiesterase to give 5′ ^{33}P-labeled PAH-mononucleotides that were analyzed by reverse phase HPLC as previously described.[33]

DNA binding experiments with CT-DNA

1-Pyrenylcarbinol (**11**, **13**, or **16**; 0.5 mg) dissolved in DMSO (0.5 cm^3) was added to a solution of calf-thymus DNA (1.5 mg) in water (0.5 cm^3). After the mixture was stirred under nitrogen for 24 h, 5 M NaCl (0.1 cm^3) and cold 95% EtOH (2 cm^3) were added. The precipitated DNA was washed with cold 70% EtOH and water and the solution was analyzed by HPLC.

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