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Synthesis of Nucleoside Adducts of Highly Mutagenic Polycyclic Aromatic Imines

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Abstract:

Polycyclic arene imines are a class of highly potent mutagens that exhibit generally higher activity than the corresponding arene oxides. Efficient syntheses of adducts between a model polycyclic aromatic imine derivative of phenanthrene and the nucleosides adenosine, guanosine, cytidine, 2'-deoxyadenosine and 2'-deoxyguanosine are described. These compounds are needed as standards for identification of adducts formed by reaction of polycyclic arene imines with DNA in cells.

Keywords: Polycyclic aromatic compounds; Nucleosides; Biomimetic reactions; Aziridines.

Polycyclic aromatic compounds (PACs) are widespread environmental pollutants that are present in cigarette smoke, automobile exhaust, and many common foods [1]. Some PACs are potent carcinogens [1,2]. The principal mechanism of PAC carcinogenesis is believed to involve activation by microsomal enzymes to reactive PAC arene oxide and diol epoxide metabolites that bind to DNA thereby causing mutations that result in induction of tumors [1,2]. Additional mechanisms of PAC carcinogenesis that may also be operative in certain cases [3] include (i) one electron oxidation to form radical-cations that attack the nucleic acid resulting in depurination [4], (ii) enzymatic dehydrogenation of dihydrodiol metabolites to yield quinones that enter into a redox cycle with O_2 to generate reactive oxygen species prone to attacking DNA [5,6], and (iii) formation of benzylic alcohols that undergo conversion by sulfotransferase enzymes to sulfate esters that react with DNA [7]. Evidence has also been obtained in support of a mechanism that involves transformation of arene oxides to mutagenic arene imines (azirdines) [8]. It was hypothesized that the latter may arise by interaction of arene oxides with amine nucleophiles to form β-amino alcohol derivatives that may be transformed by sulfotransferase enzymes to cancer-producing aziridines [9]. In support of this hypothesis, a number of polycyclic arene imines have been synthesized and shown to be extremely potent mutagens, more potent than the corresponding arene oxides, in *Salmonella typhimurium* cell cultures [10-13]. Moreover, the lifetimes of the arene imines are considerably longer than the arene oxides because of their resistance to the action of detoxifying enzymes. On

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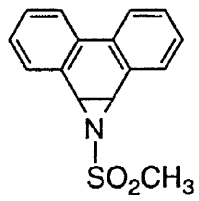
the other hand, however, direct evidence for the formation of adducts of arene imines with DNA in cells is lacking.

We now report the synthesis and structural determination of the first adducts between a model polycyclic arene imine and the nucleosides cytidine, adenosine, guanosine, 2'-deoxyadenosine, and 2'-deoxyguanosine. These adducts are required as reference standards for identification of the transformation products formed by reaction of the polycyclic arene imine with the nucleic acid *in vivo* and *in vitro*. The methodology may, in principle, be extended to the synthesis and characterization of similar nucleoside **adducts** formed by a wide range of other mutagenic polycyclic arene imines in cells.

RESULTS AND DISCUSSION

Although unsubstituted arene imines react only very slowly with purines and pyrimidines their hydrolyzible *N*-sulfonamido derivatives have been found to be sufficiently reactive to undergo such additions under basic conditions [14]. In this study we demonstrate that activated imines react also with nucleosides and ribonucleosides. In fact, the formation of "imine-DNA adducts" does not require application of the multistep procedures by which arene oxide-nucleosides have been synthesized [15-17]. 1a,9b-Dihydro-1-(methanesulfonyl)-1*H*-phenanthro[9,10-*b*]azirine (*N*-methanesulfonylphenanthrene 9,10-imine) (**1**) [18] was selected as a model activated arene imine for studying the syntheses of imine-nucleoside adducts. Reaction of **1** and N^4-acetylcytidine 2',3',5'-triacetate [19,20] in dry DMSO in the presence of K_2CO_3 furnished, after quenching with water and purification on silica gel, a single pair of diastereomers: *trans*-(9*R*,10*R*)- and *trans*-(9*S*,10*S*)-N^4-[9,10-dihydro-10-(methanesulfonamido)phenanthren-9-yl]cytidine 2',3',5'-triacetate (**2a** and **2b**). Thus, the N^4-bound acetyl group underwent hydrolysis during the work up of the reaction mixture with water. Further hydrolysis by methanolic ammonia solution afforded the acetate-free imine-nucleoside adducts which were separated with the aid of an octadecylsilane (RP-18) HPLC column using aqueous MeOH as eluent, into the optically active diastereomers *trans*-(9*R*,10*R*)- and *trans*-(9*S*,10*S*)-N^4-[9,10-dihydro-10-(methanesulfonamido)-phenanthren-9-yl]cytidine (**3a** and **3b**, respectively). The letters **a** and **b** in **3a** and **3b** as well as in the numbers of the following acetate-free adducts indicate the corresponding *early* and *late* eluted isomers. The structures of the pure adducts were deduced from their ^1H, ^{13}C and 2D NMR spectra, from the circular dichroism (CD) measurements and from their mobility on the HPLC column (see Tables 1-3 and Fig. 1). Wherever possible the exact assignment of the peaks to the corresponding hydrogen and carbon atoms was obtained from C-H correlation measurements. When the ^1H NMR was recorded in an acetone-d_6-D_2O mixture, H9 and H10 appeared as doublets with coupling constants of 6.7 and 6.2 Hz for **3a** and **3b**, respectively. These values indicate the *trans* orientation of the protons [16]. The presence of a free carbonyl group in the adducts, has been evidenced by a signal at 164.93 ppm in the ^{13}C NMR spectrum. This observation as well as the fact that the C9 atoms are not bound to ethereal oxygen (they appear at ~54 ppm) exclude the possibility that the addition of **1** to the cytidine has taken place via the O2' atom. Involvement of either N1' or the sterically hindered N3' atoms can also be ruled out, as such a process would have caused rearrangement of the cytosine ring and removal of the ribose moiety. In D_2O-free acetone-d_6 the N4'-bound protons of the diastereomers appear as a doublet at 7.20 ppm ($J_{NH,9}$ = 7.3 Hz). The sulfonamido N*H* peak is centered at 6.62 ppm. These data as well as the absence of a primary amine group suggest that the addition of the cytidine to the dihydrophenanthrene residue has taken place between C9 and N4'. Support for this assumption has been



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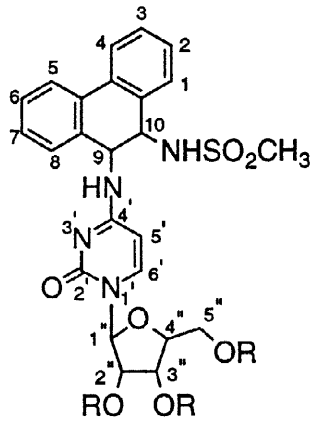
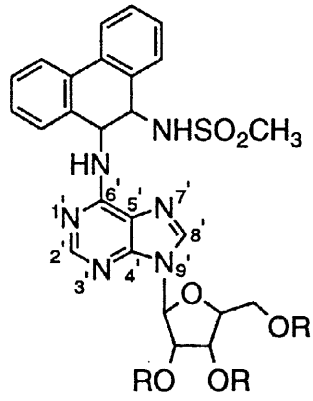
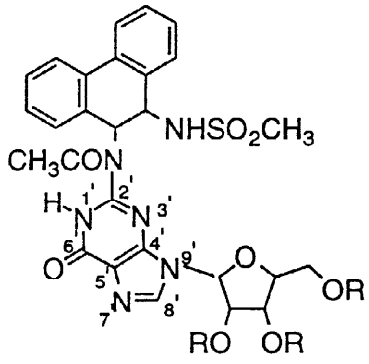


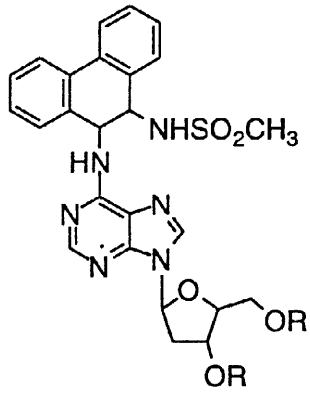
2a, (9*R*,10*R*) - R = Ac
2b, (9*S*,10*S*) - R = Ac
3a, (9*R*,10*R*) - R = H
3b, (9*S*,10*S*) - R = H



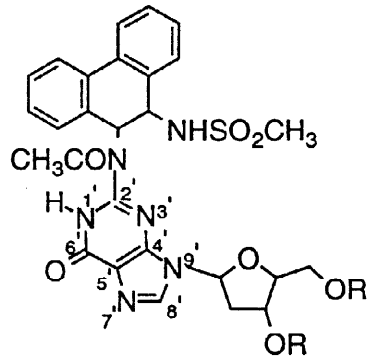
4a, (9*S*,10*S*) - R = Ac
4b, (9*R*,10*R*) - R = Ac
5a, (9*S*,10*S*) - R = H
5b, (9*R*,10*R*) - R = H



6a, (9*R*,10*R*) - R = Ac
6b, (9*S*,10*S*) - R = Ac
7a, (9*R*,10*R*) - R = H
7b, (9*S*,10*S*) - R = H



8a, (9*S*,10*S*) - R = Ac
8b, (9*R*,10*R*) - R = Ac
9a, (9*S*,10*S*) - R = H
9b, (9*R*,10*R*) - R = H



10a, (9*R*,10*R*) - R = Ac
10b, (9*S*,10*S*) - R = Ac
11a, (9*R*,10*R*) - R = H
11b, (9*S*,10*S*) - R = H

provided by 2D NMR COSY measurements [21] that revealed a nuclear Overhauser enhancement (NOE) between *H*N4' of the cytidine and H9. In addition, NOESY measurements [22] showed NOE also between *H*N4' and H10 as well as between H5' and H9. The stereochemistry of **3a** and **3b** at C9 and C10 has been established as 9*R*,10*R* for the former and 9*S*,10*S* for the latter adduct, by comparing their CD spectra and those of the analogous adducts of nucleosides to phenanthrene oxide derivatives (*vide infra*).

By the same procedure described for the reaction of **1** and the *N*,O-protected cytidine derivative, we added the imine to N^6-acetyladenosine 2',3',5'-triacetate [23]. As in the previous case the nitrogen-bound acetyl group underwent hydrolysis during the workup of the reaction mixture. The product consisted of a single pair of the trans-oriented diastereoisomers *trans*-(9*S*,10*S*)- and *trans*-(9*R*,10*R*)-N^6-[9,10-dihydro-10-methanesulfonamido)phenanthren-9-yl]adenosine 2',3',5'-triacetate (**4a** and **4b**). Hydrolysis of the protecting groups with a methanolic ammonia solution followed by HPLC separation on RP-18 of the acetate-free mixture, gave the pure diastereomers **5a** and **5b**. Their structures were established by multinuclear and 2D NMR, as well as by CD measurements. In an acetone-d_6-D_2O solution H9 and H10 of **5a** and **5b** formed doublets with coupling

Table 1. Summary of 400-MHz ^{1}H NMR Spectra of the Pure HPLC Separated Diastereomeric Adducts. The Chemical Shifts, δ, are Given in ppm. The Coupling Constants, *J*, are Presented in Parenthesis in Hz[a]

	Phenanthrene ring protons													Nucleoside and other protons							
Cmpd.	H1 d	H2 m	H7 m	H3/H6 m	H4 d	H5 d	H8 d, m	H9 d	H10 d	H2′ brs	H5′ d	H6′ d	H8′ s	H1″ d, dd, m	H2″ (1) ddd, m	H2″ (2) ddd, m	H3″ m	H4″ dd, m	H5″(1) dd	H5″ (2) dd	NCH$_3$ s	SCH$_3$ s
3a	7.56 (7.5)	7.33		7.42	7.86 (6.9)	7.88 (7.0)	7.47 (7.5)	5.59 (6.7)	4.83 (6.7)		5.90 (7.5)	8.02 (7.5)		5.81 (2.9)	4.16		4.16	4.00	3.72 (2.8) (12.3)	3.83 (2.6) (12.3)		3.16
3b	7.58 (7.5)	7.36		7.42	7.88 (6.3)	7.90 (6.8)	7.49 (7.5)	5.59 (6.2)	4.87 (6.2)		5.86 (7.4)	8.02 (7.4)		5.81 (3.4)	4.19		4.00	3.72 (2.8) (12.3)	3.72 (2.8) (12.3)	3.83 (2.6) (12.3)		3.20
5a	7.62 (7.4)	7.33		7.42	7.88 (6.7)	7.90 (6.8)	7.47 (7.6)	5.82 (7.9)	4.99 (7.9)	8.30			8.22	5.96 (6.6)	4.75		4.34	4.16	3.69 (2.3) (12.6)	3.81 (2.3) (12.6)		3.04
5b	7.63 (7.3)	7.34		7.45	7.89 (7.6)		7.45	5.82 (8.1)	4.99 (8.1)	8.30			8.26	5.96 (6.5)	4.76		4.34	4.16	3.68 (2.4) (12.6)	3.80 (2.4) (12.6)		3.04
7a	7.72 (7.6)	3.45	7.32	7.45	7.92 (7.5)		7.60 (7.7)	6.76 (8.6)	5.05 (8.6)				8.35	6.01 (5.2)	4.67		4.42	4.11	3.72 (3.5) (12.2)	3.81 (3.4) (12.2)	2.22	3.04
7b	7.71 (7.4)	3.44	7.32	7.44	7.92 (7.7)		7.58 (7.6)	6.76 (8.6)	5.04 (8.6)				8.35	6.01 (5.3)	4.68		4.42	4.11	3.72 (3.5) (12.3)	3.81 (3.4) (12.3)	2.22	3.04
9a	7.64 (7.4)	7.36	7.31	7.42	7.88 (7.7)	7.90 (7.5)	7.46 (7.6)	5.82 (8.3)	4.98 (8.3)	8.29			8.20	6.43 (5.9) (8.5)	2.38 (5.9) (13.3) (2.1)	2.80 (8.5) (13.3) (5.4)	4.59	4.08	3.69 (2.9) (12.4)	3.78 (2.9) (12.4)		3.04
9b	7.63 (7.4)	7.35	7.29	7.43	7.87 (7.3)	7.89 (7.5)	7.43	5.79 (8.5)	4.96 (8.5)	8.28			8.25	6.43 (5.9) (8.3)	2.40 (5.9) (13.3) (2.1)	2.79 (8.3) (13.3) (5.5)	4.58	4.07	3.69 (3.0) (12.4)	3.76 (3.0) (12.4)		3.02
11a	7.72 (7.6)	7.45	7.31	7.45	7.91 (7.6)		7.60 (7.6)	6.76 (8.9)	5.10 (8.9)				8.34	6.43	2.42	2.88	4.65	4.02	3.73 (4.0) (12.1)	3.79 (4.0) (12.1)	2.20	3.04
11b	7.71 (7.4)	7.44	7.33	7.44	7.91 (7.7)		7.59 (7.6)	6.74 (8.8)	5.04 (8.8)				8.34	6.44	2.44	2.81	4.64	4.01	3.69 (4.1) (12.0)	3.76 (4.1) (12.0)	2.20	3.02

[a] Recorded in a 9:1 mixture of acetone-d_6 and D_2O.

Table 2. Selected 100-MHz $^{13}C\{^1H\}$ NMR Spectra of the Diastereomeric Mixtures 2-11. The Chemical Shifts, δ, are Given in ppm.[a]

Cmpd.	C4 / C5	C9	C10	C2'/	C4'	C5'	C6'	C8'	C1"	C2" /	C3"	C4"	C5"	$NCOCH_3$	NCO	SCH_3	$COCH_3$	$OCOCH_3$
2	124.85 124.91	54.04	55.59	164.87	156.01	96.16	142.61		91.03	74.03	71.13	80.30	64.01			42.11	20.46	170.28
		54.06	55.69	164.93			142.81		91.22	74.09	71.16		64.07				20.66	170.35
																	20.69	170.89
																		170.94
3	124.85 124.88	53.93	55.86	164.93	157.12	95.47	142.49		92.29	75.61	70.25	85.69	61.44			42.09		
						95.51	142.50		92.36	75.66	70.34	85.71	61.59			42.19		
4	124.90	53.96	56.74	153.74	155.50	120.82	149.96	140.88	87.32	73.50	71.35	80.92	63.87			41.90	20.33	170.20
			56.81								71.38	80.95	63.88				20.48	170.42
																	20.65	171.00
																	20.06	171.01
5	124.99 125.02	54.17	56.74	152.95	155.93	122.04	149.40	141.82	91.32	74.73	72.76	88.39	63.45			41.99		
		54.29								74.84	72.87	88.44	63.59					
6	124.90 125.05	76.02	57.04	161.32	154.12	119.17	153.21	142.52	88.10	73.85	71.74	81.20	63.35	25.00	169.31	42.03	20.39	170.07
					154.15	119.23			88.22	73.91	71.77	81.23					20.50	170.07
																	20.66	170.76
																	20.68	
7	124.89 124.99	75.63	56.92	161.07	154.12	119.06	152.61	142.49	90.35	75.79	71.96	87.21	62.57	24.82	169.87	42.04		
		75.73		161.11		119.15			90.42	75.84	72.01	87.26	62.63	24.84				
8	124.94	54.97	56.86	153.49	155.55	121.22	150.14	140.50	85.23	37.08	75.50	83.18	64.53			41.92	20.67	170.76
									85.28		75.52	83.19	64.56				20.91	170.81
9	124.92 124.96	53.99	56.88	153.03	153.74	121.71	152.31	141.21	87.01	41.14	72.76	89.72	63.35			41.95		
10	124.89 124.98	75.67	57.01	161.19	154.27	119.15	153.01	142.27	85.62	36.72	75.65	83.40	64.65	25.04	169.47	42.04	20.71	170.70
		75.90	57.13			119.21		142.38	85.68	36.88	75.70						20.99	170.86
11	124.89 124.99	75.89	57.19	161.12	154.28	119.05	155.86	142.37	85.53	41.18	73.42	89.21	63.14	25.01	169.86	41.04		
		75.92		161.14	154.30	119.13		142.43	85.58									

[a] Recorded in a 9:1 mixture of acetone-d_6 and D_2O.

constants of 7.9 and 8.1 Hz, respectively (see Table 1). These values indicate that here too, the imine and the ribonucleoside form only *trans* adducts. The fact that during the reaction of **1** and N^6-acetyladenosine triacetate no depurination has taken place, proves that the phenanthrene imine residue is neither bound to N7 nor to N9 of the adenosine moiety [24]. The ^{13}C NMR signal of C8' atoms of both diastereomers (identified by C-H correlation) appeared at 140.88 ppm which proved that they are methine carbon atoms. The peak of atoms C2' at 143.74 ppm reveals that these carbon atoms are both aromatic and methinic in respect to N1' and N3'. Therefore, binding of C9 to either of these nitrogen atoms must be excluded. The ^1H NMR signals of the SN*H* and HN6' protons of **4a** and **4b** in D_2O-free acetone-d_6 appeared as doublets at 6.66 and 7.15 ppm, respectively. Since the HN6' protons were found to split the H9 doublet by $J_{NH,9}$ = 8.1 Hz it is obvious that the C9 is bound to the adenosine *via* N6'. Similar spectral features were observed in the ^1H NMR of the HPLC separated acetate-free adducts **5a** and **5b**. Further evidence for the binding of C9 to N6' in both **5a** and **5b** was provided by 2D COSY NMR that showed an NOE effect between the HN6' and HC9 protons, and by 2D NOESY experiments that indicated NOE between HN6' and H10. The stereochemistry of C9 and C10 in **5a** and **5b** was assigned as 9*S*,10*S* and 9*R*,10*R*, respectively on account of their CD spectra and their relative mobility on an RP-18 HPLC column (*vide infra*).

Imine **1** and N^2-acetylguanosine 2',3',5'-triacetate [25] did not react under the conditions described for the preparation of **2** and **4**. When, however the potassium carbonate was replaced by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) a diastereomeric mixture of *trans*-(9*R*,10*R*)- and *trans*-(9*S*,10*S*)-N^2-acetyl-N^2-[9,10-dihydro-10-(methanesulfonamido)phenanthren-9-yl]guanosine 2',3',5'-triacetate (**6a** and **6b**, respectively) was slowly formed (9% after a reaction period of 20 h). In contrast to **2** and **4** the *N*-acetyl group was not eliminated during the workup of the reaction mixture. Moreover, this group did not undergo hydrolysis even by methanolic ammonia. Thus, under our standard conditions by which the acetate protecting groups are removed the diasteromeric mixture of **7a** and **7b** has been obtained (*cf.* also the very slow hydrolysis of other *N*-acetylguanine derivatives [17,26]). As in the cases of cytidine and adenosine, the addition of guanosine to **1** formed exclusively *trans* adducts ($J_{9,10}$ = 8.6 Hz for both **7a** and **7b**). In addition to the ^1H NMR peaks of **7a** and **7b** listed in Table 1, a singlet corresponding to 1H appeared at 9.55 ppm. This peak was assigned as the amide proton HN1'. This reveals that the binding of the imine to the guanosine residue has not taken place *via* the oxygen atom O6'. Such binding would have resulted in tautomerization, as well as the removal of the proton from N1' and appearance of a signal of *H*N2' at ~ 12 ppm in the NMR spectrum (*cf.* references 17 and 26). 2D NOESY measurements of **7a** as well as of **7b** showed NOE between *H*N1' and both H9 and H10. Thus, it is clear that phenanthrene imine attacked the guanosine at nitrogen N2'.

In analogy to the addition of **1** to the ribonucleosides N^6-acetyladenosine triacetate and N^2-acetylguanosine triacetate, we added the imine derivative also to the corresponding nucleosides, N^6-acetyl-2'-deoxyadenosine 3,5-diacetate [25] and N^2-acetyl-2'-deoxyguanosine-3',5'-diacetate [17]. As for the preparation of **4** and **6** the addition of **1** to the deoxyadenosine derivative could be carried out in the presence of K_2CO_3 while the reaction with deoxyguanosine required the presence of DBU. The ^1H, ^{13}C and 2D NMR spectra of the diastereomeric mixtures of **8a** and **8b** resembled those of **4a** and **4b**, and the spectra of the isomerically pure **9a** and **9b** were practically the same as those of **5a** and **5b** except the features associated with the deficiency of the OH group on the sugar carbon C2'. Likewise, the spectra of the diastereomeric mixture of **10a** and **10b** resembled those of the mixture of **6a** and **6b**, and the NMR data for **11a** and **11b** were similar to

Table 3. Summary of Experimental Conditions for HPLC Separation of the Diastereomeric Mixtures 3, 5, 7, 9 and 11 on Octadecylsilane (RP-18) Columns[a]

compd.	column type[b]	resolution time (min) t_a	t_b	peak width at base (min) W_a	W_b	capacity factor k'_a	k'_b	separation factor, α	resolution factor, R_s
3	A	18.14	23.24	1.50	1.60	10.78	14.09	1.31	3.29
5	A	25.41	34.36	2.14	2.40	15.50	21.38	1.38	3.99
7	A	30.47	39.35	3.93	4.07	18.79	24.55	1.31	2.22
9	A	43.80	49.13	2.23	2.40	27.44	30.90	1.13	2.30
11	B	68.05	73.90	4.00	4.00	34.26	37.29	1.09	1.46

[a] Operation conditions: Samples of 20 µl of 3, 7 and 11 and 50 µl of 5 and 9 of 2 mg/mL solution in aq MeOH were injected; flow rate of eluent was 1 mL/min for 3, 5, 7 and 9 and 0.8 mL/min for 11; a and b refer to the early and late eluted compounds; t_o = 1.54 min for the Lichrospher column, and 1.93 for the Superspher column. $k'_a = t_a - t_o/t_o$ and $k'_b = t_b - t_o/t_o$; $\alpha = k'_b/k'_a$; $R_s = 2\,(t_b-t_a)/W_a + W_b$.
[b] A - 5 µm Merck Lichrospher; B - 4 µm Merck Superspher.

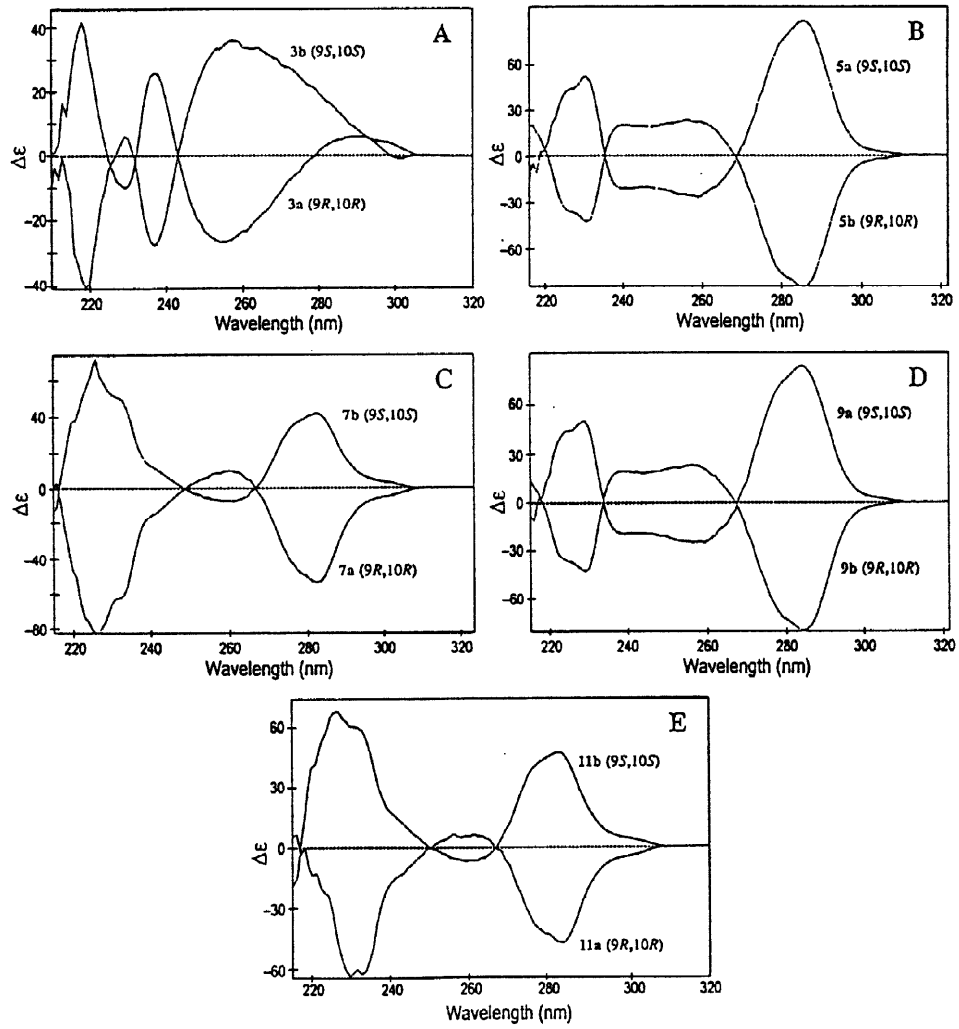


Fig. 1. CD spectra of separated 3a and 3b (A); of 5a and 5b (B); of 7a and 7b (C); of 9a and 9b (D); and of 11a and 11b (E).

those of **7a** and **7b**. Therefore, the structure determination of **9a, 9b, 11a** and **11b** involved the same considerations discussed above for the adducts of **1** and the ribonucleosides.

Various studies have revealed a correlation between the absolute configuration of the adducts of nucleophiles (including nucleosides) to arene oxides, and their CD spectra [27-32]. Such a correlation has also been found for adducts of 1a,9b-dihydrophenanthro[9,10-*b*]oxirene (phenanthrene 9,10-oxide) [16,33]. According to these studies the ellipticity in the 250-300 nm region reflects on the configuration at the benzylic positions (C9 and C10 in adducts of phenanthrene 9,10-oxide). When the sign of the largest optical rotation is negative the binding carbon atom has the *R* configuration. A positive ellipticity indicates an *S* configuration. In addition it was found that upon separation of the diastereomeric adduct mixtures of adenosine (or 2'-deoxyadenosine) to arene oxides on a RP-18 HPLC column (using aqueous MeOH as eluent) the isomer with the *S* configuration at the linking carbon atom is the first of the two diastereomers to be eluted. On the other hand, upon separation of guanosine (or 2'-deoxyguanosine) adducts of arene oxides on this column, the early eluted diastereomer has the *R* configuration at the binding carbon atom [16,27,29,33]. Because the structural similarity between the arene imine-nucleoside adducts and the corresponding adducts of arene oxides, and because the features of the CD spectra depend in the first place on the interactions and orientations of the main chromophores, we postulate that these empirical findings about the arene oxide-nucleoside adducts may be extended also to the corresponding arene imine derivatives. The adenosine and 2'-deoxyadenosine adducts, **5a** and **9a**, respectively, that are the early eluted products (see Table 3), are characterized by strong positive ellipticities at 284 nm (see Fig. 1). Thus, the C9 and consequently also the *trans*-oriented C10 positions are tentatively assigned as 9*S* and 10*S*. The late eluted compounds **5b** and **9b** that show strong negative ellipticities have then the 9*R*,10*R* configuration. The CD spectra of **7a** and **11a** shown in Figure 1 indicate strong negative optical rotation at 284 nm. Thus, **7a** and **11a** are formulated as 9*R*,10*R* and the late eluting compounds as 9*S*,10*S*. In analogy to the analyses of the previous adenine and guanine derivatives we assessed the configuration of **3a** that shows negative ellipticity at 255 nm as 9*R*,10*R* and the late eluted **3b** as 9*S*,10*S* [34].

In conclusion, we wish to point out (a) that the strategy used for binding of **1** to nucleosides can be applied also to other *N*-methanesulfonylarene imines, and (b) that the structures of the nucleoside-arene imine adducts closely resemble those of the adducts of the corresponding arene oxides. In both cases, the principal sites of covalent binding to cytidine, adenosine and guanosine bases are the amino groups, and addition occurs *trans*-stereospecifically. It should also be noted that although hydrolysis of the methanesulfonamido group of the products takes place in dilute acid [35], the resulting free amines are air sensitive and therefore, this operation should be carried out only shortly prior to the biological tests.

EXPERIMENTAL

Infrared spectra were taken on a Perkin Elmer spectrophotometer model 457. ^{1}H and ^{13}C NMR measurements were carried out on a Bruker AMX 400 instrument. EIMS were recorded on a Hewlett Packard mass spectrometer model 4989A equipped with both an HP gas chromatograph model 5890 series II and a particle beam interface system with an HPLC model HP 1050. CD spectra were obtained with the aid of a Jobin Yvon CD6 dichromograph and HPLC separations were performed on a Jasco TRI-ROTAR IV machine equipped with a DG-3510 degasser and a UVIDEC 100-VI UV spectrophotometer.

Diastereomeric Mixture of *trans*-(9*R*,10*R*)-, and *trans*-(9*S*,10*S*)-N^4-9,10-Dihydro-10-(methanesulfanamido)phenanthren-9-yl]cytidine 2',3',5'-Triacetate (2a and 2b). To a solution of 380 mg (0.90 mmol) of N^4-acetylcytidine 2',3',5'-triacetate [19,20] in 10 ml of dry DMSO was added under

exclusion of air, 130 mg (0.90 mmol) of K_2CO_3 and 500 mg (1.80 mmol) of 1a,9b-dihydro-1-(methanesulfonyl)-1*H*-phenanthro[9,10-*b*]azirine (**1**) [18] and the mixture was stirred at 35-40 °C for 7 d. Cold water (50 ml) was added and the organic material was extracted with ethyl acetate. The solution was concentrated and chromatographed on silica gel, using a 4:1 mixture of ethyl acetate-hexane as eluent to give 76 mg (12%) of diastereomers **2a** and **2b** as colorless crystals; mp 144-148 °C; 400-MHz ^{1}H NMR (9:1 mixture of acetone-d_6-D_2O) δ 2.06 (s, 3H), 2.065 (s, 3H), 2.07 (s, 3H), 3.21 (s, 1.5H), 3.22 (s, 1.5H), 4.29-4.37 (m, 3H), 4.89 (d, 0.5H, $J_{9,10}$ = 5.8 Hz), 4.90 (d, 0.5H, $J_{9,10}$ = 5.6 Hz), 5.44 (m, 1H), 5.53 (m, 1H), 5.57 (d, 0.5H, $J_{9,10}$ = 5.6 Hz), 5.58 (d, 0.5H, $J_{9,10}$ = 5.8 Hz), 5.90 (d, 1H, $J_{5',6'}$ = 7.5 Hz), 5.95 (d, 0.5H, $J_{1'',2''}$ = 4.3 Hz), 5.97 (d, 0.5H, $J_{1'',2''}$ = 4.4 Hz), 7.33-7.37 (m, 2H), 7.41-7.46 (m, 2H), 7.50 (d, 0.5H, $J_{7,8}$ = 7.5 Hz), 7.51 (d, 0.5H, $J_{7,8}$ = 7.5 Hz), 7.57 (d, 0.5H, $J_{1,2}$ = 6.9 Hz), 7.58 (d, 0.5H, $J_{1,2}$ = 7.4 Hz), 7.65 (d, 0.5H, $J_{5',6'}$ = 7.5 Hz), 7.67 (d, 0.5H, $J_{5',6'}$ = 7.5 Hz), 7.89 (d, 1H, $J_{4,5}$ = 7.3 Hz), 7.90 (d, 1H, $J_{4,5}$ = 7.4 Hz); the 100-MHz ^{13}C{^{1}H} NMR spectrum is listed in Table 2. Anal. Calcd for $C_{30}H_{32}N_4O_{10}S$: C, 56.24; H, 5.03; N, 8.75; S, 5.0. Found: C, 55.95, H, 5.05; N, 8.42; S, 5.01.

***trans*-(9*R*,10*R*)- and *trans*-(9*S*,10*S*)-*N*^{4}-[9,10-Dihydro-10-(methanesulfonamido)-phenanthrene-9-yl]cytidine (3a and 3b, Respectively).** A solution of 100 mg (0.16 mmol) of the mixture of diastereomers **2a** and **2b** in 15 ml of MeOH and 15 ml of concentrated ammonia solution was stirred at room temperature until the starting acetates were fully hydrolyzed (*ca.* 60 min, as evidenced by TLC monitoring on silica gel with a 1:9 mixture of MeOH-CH_2Cl_2 as eluent). Extraction of the hydrolyzed material into ethyl acetate, concentration of the extract and chromatography of the residue on silica gel (using a 1:10 mixture of MeOH and CH_2Cl_2 as eluent) afforded 75 mg (94%) of the mixture of **3a** and **3b** as colorless crystals; mp 198 - 201 °C. The 100-MHz ^{13}C{^{1}H} NMR of the isomeric mixture of **3a** and **3b** is given in Table 2. Anal. Calcd for $C_{24}H_{26}N_4O_7S$: C, 56.02; H, 5.09; N, 10.89; S, 6.23. Found: C, 55.93; H, 5.04; N, 10.64; S, 6.20.

The two diastereomers were separated, using a 5 μm, 250 x 4 mm Merck Lichrospher RP-18 HPLC column under the conditions listed in Table 3. Selected 400-MHz ^{1}H NMR peaks of pure **3a** and **3b** are given in Table 1. Their CD spectra are shown in Fig. 1.

Diastereomeric Mixture of *trans*-(9*S*,10*S*)-, and *trans*-(9*R*,10*R*)-*N*^{6}-[9,10-Dihydro-10-(methanesulfonamido)phenanthren-9-yl]adenosine 2',3',5'-Triacetate (4a and 4b). In a similar manner to the preparation of the mixture of **2a** and **2b**, 250 mg (0.96 mmol) of **1** in 10 ml of dry DMSO was reacted for 30 h with 200 mg (0.46 mmol) of *N*^{6}-acetyladenosine 2',3',5'-triacetate [23] in the presence of 95 mg (0.69 mmol) of K_2CO_3. After the usual workup 165 mg (51%) of the diasteromers **4a** and **4b** were isolated. Colorless crystals; mp 135 - 138 °C; 400 MHz ^{1}H NMR (9:1 mixture of acetone-d_6-D_2O) δ 2.06 (s, 3H), 2.07 (s, 3H), 2.11 (s, 3H), 3.06 (s, 1.5H), 3.065 (s, 1.5H), 4.33-4.67 (m, 3H), 5.03 (d, 1H, $J_{9,10}$ = 7.6 Hz), 5.74 (m, 1H), 5.83 (d, 1H, $J_{9,10}$ = 7.6 Hz), 6.07 (m, 1H), 6.27 (d, 1H, $J_{1'',2''}$ = 5.2 Hz), 7.30-7.39 (m, 2H), 7.41-7.46 (m, 2H), 7.49 (d, 1H, $J_{7,8}$ = 7.6 Hz), 7.65 (d, 1H, $J_{1,2}$ = 7.4 Hz), 7.82-7.92 (m, 2H), 8.19 (s, 0.5H), 8.21 (s, 0.5H), 8.35 (br s, 1H); the 100-MHz ^{13}C{^{1}H} NMR spectrum is given in Table 2. Anal. Calcd for $C_{31}H_{32}N_6O_9S$: C, 56.02; H, 4.85; N, 12.64; S, 4.82. Found: C, 55.78; H, 4.84; N, 12.38; S, 4.61.

***trans*-(9*S*,10*S*)- and *trans*-(9*R*,10*R*)-N^6-[9,10-Dihydro-10-(methanesulfonamido)-phenanthren-9-yl]adenosine (5a and 5b, Respectively).** Hydrolysis of 100 mg (0.15 mmol) of the foregoing mixture of **4a** and **4b** by the method described for the preparation of **3a** and **3b** gave 80 mg (96%) of the mixture of **5a** and **5b** as colorless crystals of mp 157 - 162 °C. The 100-MHz $^{13}C\{^1H\}$ NMR of the mixture is given in Table 2. Anal. Calcd for $C_{25}H_{26}N_6O_6S$: C, 55.75; H, 4.87; N, 15.60; S, 5.95. Found: C, 55.58; H, 4.71; N, 15.26; S, 5.64.

The mixture of the diastereomers were separated by HPLC on a 5 μm, 250 x 4 mm, Merck Lichrospher RP-18 column under the conditions of Table 3. Selected ^1H NMR peaks of **5a** and of **5b** are given in Table 1. The CD spectra of the pure diastereomers are shown in Fig. 1.

Diastereomeric Mixture of *trans*-(9*R*,10*R*)- and *trans*-(9*S*,10*S*)-N^2-Acetyl-N^2-[9,10-dihydro-10-(methanesulfonamido)phenanthren-9-yl]guanosine 2',3',5'-Triacetate (6a and 6b). In analogy to the preparation of **2a** and **2b**, 1.20 g (4.4 mmol) of **1** was reacted for 20 h with 1.00 g (2.2 mmol) of N^2-acetylguanosine 2',3',5'-triacetate [25] in 80 ml of DMSO. However, instead of the K_2CO_3, 3.4 ml (2.3 mmol) of 1.8-diazabicyclo[5.4.0]-undec-7-ene were used. Yield 140 mg (9%) of colorless crystals; mp 133-134 °C; 400-MHz ^1H NMR (9:1 mixture of acetone-d_6-D_2O) δ 2.01 (s, 1.5H), 2.02 (s, 1.5H), 2.06 (s, 3H), 2.12 (s, 3H), 2.25 (s, 1.5H), 2.26 (s, 1.5H), 3.07 (s, 1.5H), 3.08 (s, 1.5H), 4.41-4.51 (m, 3H), 5.10 (m, 1H), 5.88 (m, 1H), 6.02 (m, 1H), 6.23 (d, 1H, $J_{1'',2''}$ = 4.6 Hz), 6.72 (d, 0.5H, $J_{9,10}$ = 8.9 Hz), 6.73 (d, 0.5H, $J_{9,10}$ = 8.7 Hz), 7.32 (m, 1H), 7.44-7.51 (m, 3H), 7.63 (d, 0.5H, $J_{7,8}$ = 7.5 Hz), 7.65 (d, 0.5H, $J_{7,8}$ = 7.4 Hz), 7.76 (d, 1H, $J_{1,2}$ = 7.5 Hz), 7.95 (d, 2H, $J_{4,5}$ = 7.7 Hz), 8.22 (s, 1H); the 100-MHz $^{13}C\{^1H\}$ NMR spectrum is shown in Table 2. Anal. Calcd for $C_{33}H_{34}N_6O_{11}S$: C, 54.84; H. 4.74; N, 11.63; S, 4.44. Found: C, 54.55; H, 4.75; N, 11.29; S, 4.51.

***trans*-(9*R*,10*R*)-, and *trans*-(9*S*,10*S*)-N^2-Acetyl-N^2-[9,10-dihydro-10-(methanesulfonamido)phenanthren-9-yl]guanosine (7a and 7b, Respectively).** Hydrolysis of 180 mg (0.30 mmol) of the foregoing mixture of **6a** and **6b** yielded 140 mg (96%) of the diastereomeric mixture of **7a** and **7b** as colorless crystals of mp 198-203 °C. The ^{13}C NMR of the isomeric mixture is summarized in Table 2. Anal. Calcd for $C_{27}H_{28}N_6O_8S$: C, 54.36; H, 4.73; N, 14.09; S, 5.37. Found: C, 54.26; H, 4.69; N, 13.95; S, 5.52.

The diastereomeric mixture was separated on a 5μm, 250 x 4 mm Merck, Lichrospher RP-18 column under the conditions of Table 3. The ^1H NMR spectra of the separated isomers are given in Table 1. Their CD spectra are shown in Fig. 1.

Mixture of the Diastereomers *trans*-(9*S*,10*S*)-, and *trans*-(9*R*,10*R*)-N^6-[9,10-Dihydro-10-(methanesulfonamido)phenanthren-9-yl]-2'-deoxyadenosine 3',5'-Diacetate (8a and 8b). Reaction of 290 mg (1.06 mmol) of **1** and 200 mg (0.53 mmol) of N^6-acetyl-2'-deoxyadenosine 3',5'-diacetate [26] and 110 mg (0.80 mmol) K_2CO_3 in 10 ml DMSO afforded after 60 h, 103 mg (32%) of a colorless mixture of **8a** and **8b**; mp 134-139 °C; 400-MHz ^1H NMR (9:1 mixture of acetone-d_6-D_2O) δ 2.00 (s, 1.5H), 3.06 (s, 1.5H), 2.09 (s, 3H), 2.63 (ddd, 1H, $J_{1'',2''(1)}$ = 6.2 Hz, $J_{2''(1),2''(2)}$ = 14.2 Hz, $J_{2''(1),3''}$ = 2.6 Hz), 3.05 (s, 1.5H), 3.06 (s, 1.5H), 3.18 (m, 1H), 4.28-4.38 (m,3H), 5.02 (d, 1H, $J_{9,10}$ = 8.1 Hz), 5.48 (m, 1H), 5.84 (br s, 1H), 6.47 (m, 1H), 7.29-7.38 (m, 2H), 7.41-7.46 (m, 2H), 7.48 (d, 1H, $J_{7,8}$ = 7.5 Hz), 7.65 (d, 1H, $J_{1,2}$ 7.4 Hz), 7.89 (d,1H, $J_{4,5}$ = 7.4 Hz), 7.91 (d, 1H, J = 7.4 Hz), 8.185 (s, 0.5H), 8.19 (s, 0.5H), 8.33 (br s, 1H); the

^{13}C NMR spectrum is given in Table 2. Anal. Calcd for $C_{29}H_{30}N_6O_7S$: C, 57.42; H, 4.98; N, 13.85; S, 5.29. Found: C, 57.15; H, 5.02; N, 13.58; S, 5.02.

***trans*-(9*S*,10*S*)-, and *trans*-(9*R*,10*R*)-N^6-[9,10-Dihydro-10-(methanesulfonamido)-phenanthren-9-yl]-2'-deoxyadenosine (9a and 9b, Respectively).** In the manner described for the hydrolysis of **2a** and **2b**, 154 mg (0.25 mmol) of the diastereomeric mixture of **8a** and **8b** was hydrolyzed to give 126 mg (95%) of the diasteromeric mixture of **9a** and **9b**; mp 149-150 °C. The ^{13}C NMR spectrum of the mixture is given in Table 2. Anal. Calcd for $C_{25}H_{26}N_6O_5S$: C, 57.46; H, 5.01; N, 16.08; S, 6.13. Found: C, 57.46; H, 5.07; N, 15.80; S, 5.78.

Separation of the diastereoisomers was accomplished by using a 5 μm 250 x 4 mm Merck Lichrospher RP-18 HPLC column under the conditions of Table 3. The ^{1}H NMR and CD spectra of **9a** and **9b** are shown in Table 1 and Fig. 1, respectively.

Diastereomeric Mixture of *trans*-(9*R*,10*R*)- and *trans*-(9*S*,10*S*)-N^6-Acetyl-N^2-[9,10-dihydro-10-(methanesulfonamido)phenanthren-9-yl]-2'-deoxyguanosine 3',5'-Diacetate (10a and 10b). In analogy to the preparation of the ribonucleoside adducts **8a** and **8b** the stirring of the mixture of 300 mg (0.76 mmol) of N^2-acetyl-2'-deoxyguanosine 3',5'-diacetate [17], 0.114 ml (0.76 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene, 420 mg (1.52 mmol) of **1** and 12 ml DMSO for 48 h, afforded 140 mg (28%) of a diastereomeric mixture of **10a** and **10b**. Colorless crystals; mp 140-145 °C; 400-MHz ^{1}H NMR (mixture of 90% acetone-d_6-and 10% D_2O) δ 2.00 (s, 1.5H), 2.01 (s, 1.5H), 2.09 (s, 3H), 2.26 (s, 1.5H), 2.27 (s, 1.5H), 2.62 (ddd, 1H, $J_{1'',2''(1)}$ = 6.3 Hz, $J_{2''(1),2''(2)}$ = 14.3 Hz, $J_{2''(1),3''}$ = 2.6 Hz), 3.05 (s, 1.5H), 3.06 (s, 1.5H), 3.31 (m, 1H), 4.31-4.43 (m, 3H), 5.09 (d, 1H, $J_{9,10}$ = 8.7 Hz), 5.53 (m, 1H), 6.45 (m, 1H), 6.73 (d, 0.5H, $J_{9,10}$ = 8.7 Hz), 6.74 (d, 0.5H, $J_{9,10}$ = 8.7 Hz), 7.35 (m, 1H), 7.44-7.51 (m, 3H), 7.65 (m, 1H), 7.77 (d, 1H, $J_{1,2}$ = 7.6 Hz), 7.95 (d, 2H, $J_{4,5}$ = 7.7 Hz), 8.24 (s, 1H); Selected 100-MHz ^{13}C{^{1}H} NMR peaks are listed in Table 2. Anal. Calcd for $C_{31}H_{32}N_6O_9S$: C, 56.02; H, 4.85; N, 12.64; S, 4.82. Found: C, 56.04; H, 4.81; N, 12.45; S, 4.79.

***trans*-(9*R*,10*R*)-, and *trans*-(9*S*,10*S*)-N^2-Acetyl-N^2-[9,10-dihydro-10-(methanesulfonamido)phenanthren-9-yl]-2'-deoxyguanosine (11a and 11b, Respectively).** Hydrolysis of the foregoing diastereomeric mixture formed a mixture of **11a** and **11b** as colorless crystals in a 94% yield, mp 152-156 °C. The 100-MHz ^{13}C{^{1}H} NMR peaks are listed in Table 2. Anal. Calcd for $C_{27}H_{28}N_6O_7S$: C, 55.85; H, 4.86; N, 14.47; S, 5.52. Found: C, 55.63; H, 4.85; N, 14.13; S. 5.4.

Separation of the diastereoisomers was accomplished by using a 4 μm 250 x 4 mm Merck Superspher RP-18 HPLC column under the conditions of Table 3. Selected 400-MHz ^{1}H NMR data for **11a** and **11b** and their CD spectra are shown in Table 1 and Fig. 1, respectively.

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