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Reaction of acrolein with 2′-deoxyadenosine and 9-ethyladenine—Formation of cyclic adducts

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Abstract

Treatment of 2′-deoxyadenosine with acrolein at pH 4.6 in 37 °C affords unstable adducts containing either one or two fused ring systems where the hydroxypropano units are derived from acrolein. Since the use of 2′-deoxyadenosine resulted in the creation of at least four diastereoisomers for the adduct made up of two fused rings, therefore, for identification and assignment of the products, 9-ethyladenine was used instead as the starting material in the reaction. The products, $\mathbf{3}_{\mathbf{E}}$ and $\mathbf{4}_{\mathbf{E}}$, were structurally characterised by UV, mass spectrometry and NMR spectroscopy.

Keywords: Acrolein; 9-Ethyladenine; 2′-Deoxyadenosine adducts; Bicyclic adduct; Structural assignment; Stereochemistry

1. Introduction

α,β-unsaturated aldehydes are a class of compounds of considerable importance in nature and industry. The widespread presence of unsaturated carbonyl compounds in the environment and in vivo has increased concern about their toxicity and their role in carcinogenesis. The simplest α,β-unsaturated aldehyde, acrolein (**1**), is a commonly used

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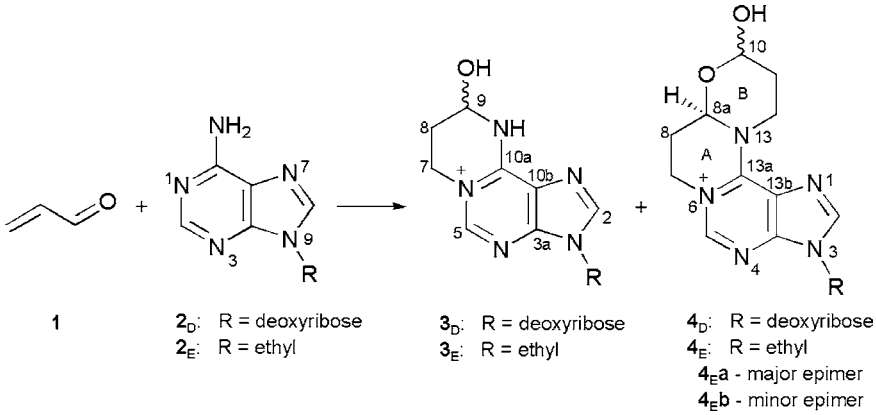


Scheme 1. The structures of adducts formed in the reaction of acrolein and 9-ethyladenine. For **4**, both epimers are racemic but for convenience the discussion centres on the indicated absolute stereochemistry of C-8a where in this depiction H-8a is designated as α.

industrial chemical, environmental pollutant [1–3] and a product of the endogenous oxidative degradation of unsaturated lipids [4–6]. As a result of such concerns, the mutagenic properties of acrolein have been examined in prokaryotic [7] and eukaryotic cells [8,9].

The most likely mode of action for acrolein-induced mutagenicity is by the formation of adducts with DNA. For example, it is known that acrolein (**1**) forms cyclic deoxyguanosine adducts when it reacts with DNA in vitro [10–12]. Of interest also is the formation of adducts in the reaction with 2′-deoxyadenosine ($\mathbf{2_D}$). Previously, Smith et al. [13], isolated and characterised the monoadduct $\mathbf{3_D}$. However, they observed in the chromatogram of their reaction mixture an adduct peak that they proposed to be a unstable diadduct. The diadduct was not further discussed in the paper. We have re-examined the adduct formation between 2′-deoxyadenosine ($\mathbf{2_D}$) and acrolein (**1**), with special emphasis on an adduct where two acrolein units were incorporated into 2′-deoxyadenosine ($\mathbf{4_D}$). However, $\mathbf{4_D}$ was unstable in DMSO solution, and furthermore, the product was found to consist of at least four diastereoisomers, thus rendering the task of identification and assignment prohibitive. This latter complication was simplified by the preferred use of 9-ethyladenine ($\mathbf{2_E}$), resulting in the formation of only two compounds, $\mathbf{4_E}$, as assessed by achiral NMR (Scheme 1). The present paper deals with the structural identification of the adducts, $\mathbf{3_E}$ and $\mathbf{4_E}$, and presents a plausible mechanism for their formation.

2. Materials and methods

2.1. Chromatographic methods and isolation of products

LC-DAD (liquid chromatography with diode array detection) analyses were performed on an Agilent 1100 Series liquid chromatographic system consisting of a quaternary pump, a vacuum degasser, an autosampler, a thermostated column compartment, a diode-array detector (UV) and an Agilent ChemStation data system using a reversed-phase C-18 analytical column (5 μm, 4 × 125 mm, Hypersil BDS-C18). The column was eluted with a gradient from 1% acetonitrile in 0.01 M ammonium acetate (pH 7.0) to 30% acetonitrile over the

course of 20 min at a flow rate of 1 mL min^{-1}. Preparative isolation of the products was conducted on a semipreparative reversed-phase C-18 column (5 μm, 10 × 250 mm, BDS Hypersil C18) using 0.01 M ammonium bicarbonate solution (pH 6.4) and acetonitrile as the mobile phase. The column was coupled to an Agilent HPLC system equipped with an Agilent 1100 Series G1364C (analytical scale) thermostated fraction collector.

2.2. Spectrometric and spectroscopic methods

LC-ESI-MS/MS (liquid chromatography/electrospray ionisation tandem mass spectrometry) analyses were performed on an Agilent 1100 Series LC/MSD Trap SL instrument equipped with an electrospray source and operated in positive ion mode. Ionisation was carried out using nitrogen as both nebuliser gas (40 psi) and drying gas (12 L min^{-1}) heated to 350 °C. The capillary exit offset had a value of 115.3 and the skim 1 voltage was set at 40 V. The maximum ion accumulation time was 2.00 ms and the target value was 20,000. Collision induced dissociation (CID) experiments coupled with multiple tandem mass spectrometry (MS^n) employed helium as collision gas. The fragmentation amplitude was varied between 0.7 and 1.0 V. The compounds were introduced through the LC system using the same chromatographic conditions as previously described for LC-DAD analyses with the exception of the flow rate which was adjusted to 0.5 mL min^{-1} and the use of an Agilent binary LC pump. Pure compounds were introduced directly into the MS source by a syringe pump at a flow rate of 5 μL min^{-1} using as solvent a 1:1 v/v mixture of 0.01 M aqueous ammonium acetate/acetonitrile. For such analyses, the drying gas temperature was 325 °C set at the rate of 5 L min^{-1} with the nebuliser gas pressure set at 15 psi.

NMR spectra were acquired at 14.1 T using a Bruker Avance NMR spectrometer equipped with a *z*-axis field gradient 5 mm inverse broadband probe operating at 600.13 MHz for ^{1}H, 150.92 MHz for ^{13}C and 60.81 MHz for ^{15}N. Spectra were recorded at 25 °C in D_2O and both ^{1}H and ^{13}C chemical shifts were referenced internally to DSS (0.015 ppm for ^{1}H and 0.0 ppm ^{13}C) whilst ^{15}N spectra were referenced externally to 90% nitromethane in CD_3NO_2 (0.0 ppm). ^{15}N signals were only observed indirectly whereby the f1 dimension taken from a ^{1}H–^{15}N HMBC experiment was forward linear predicted to 16.4 Hz pt^{-1} (acquisition resolution 131 Hz pt^{-1}). Additional details concerning general experimental procedures are available in [14].

2.3. Reaction of acrolein with 9-ethyladenine to form 3_E and 4_E

9-Ethyladenine ($\mathbf{2_E}$, 216 mg, 1.325 mmol) was allowed to react with acrolein (**1**, 742.1 mg, 13.25 mmol) in 86 mL of 0.1 M phosphate buffer, pH 4.6 at 37 °C for 2 days. The progress of the reaction was monitored by LC-DAD and LC-MS analyses. After 2 days, the reaction was completed and the volume of the aqueous phase was reduced to ca. 10 mL by rotary evaporation. The mixture was then fractionated and purified on a semi-preparative C-18 column. The column was eluted isocratically with 7% acetonitrile in 0.01 M ammonium bicarbonate buffer (pH 6.4) for 13 min at a flow rate of 3 mL min^{-1}. The solutions containing the pure adducts were evaporated to dryness and dried under vacuum. Quantitative ^{1}H NMR analysis, using methanol as an internal standard, was performed on aliquots of the purified adducts. Standard solutions were prepared for LC-DAD analysis by taking an exact volume of the NMR sample and diluting it with an appropriate volume of water. The quantitative determination of adducts in the reaction mixtures was made by comparing the

Table 1
^{1}H and ^{13}C chemical shifts (δ) of protons, carbons, and nitrogens and spin–spin coupling constants ($J_{H,H}$) of protons in $\mathbf{3_E}$

Proton	δ (ppm)	Mult.	$J_{H,H}$ (Hz)	Carbon	δ (ppm)	Nitrogen	δ (ppm)
Purine unit							
H-5	8.55	s		C-5	149.07	N-1	−147.0
H-2	8.38	s		C-2	147.82	N-3	−206.2
				C-10a	149.33	N-4	−155.6
				C-10b	121.04	N-6	−228.1
				C-3a	149.91	N-10	[a]
Ethyl unit							
CH_2	4.36	qt	7.37	CH_2	42.63		
CH_3	1.52	t	7.37	CH_3	17.13		
Acrolein units							
H-7α	4.64	dt	$-13.36_{H7\beta}$; $4.61_{H8\alpha}$; $3.69_{H8\beta}$; -0.82_{H9}	C-7	45.86		
H-7β	4.46	td	$-13.36_{H7\alpha}$; $12.86_{H8\alpha}$; $3.60_{H8\beta}$				
H-8α	2.31	d_{AB}dd	$-14.55_{H8\beta}$; $12.86_{H7\beta}$; $4.61_{H7\alpha}$; 3.24_{H9}	C-8	28.45		
H-8β	2.41	d_{AB}qt	$-14.55_{H8\alpha}$; $3.69_{H7\alpha}$; $3.60_{H7\beta}$; 3.07_{H9}				
H-9	5.51	t	$3.24_{H8\alpha}$; $3.07_{H8\beta}$; $-0.82_{H7\alpha}$	C-9	72.81		
HO-9, HN-10	[a]						

[a] Signal not observed.

peak area of the adducts in the reaction mixture. The adducts were quantified using UV detection at 254 nm. The molar yields (10% for $\mathbf{3_E}$ and 53% for $\mathbf{4_E}$) were calculated from the original amount of 9-ethyladenine in the reaction mixture.

For $\mathbf{3_E}$, λ_{max} (HPLC eluent, approximately 8% acetonitrile in ammonium acetate buffer, pH 7)/nm 264 and 212; λ_{min} 234; *m/z* (ESI) MS: 220 (100%, MH^+), MS^2 202 (100%, MH^+-H_2O), 192 (35%, MH^+−ethyl+H), 164 (15%, $MH^+-C_3H_5O$+H); [HRMS: Calc for ($C_{10}H_{14}N_5O$+H): *m/z*, 220.1198. Found: *m/z*, 220.1204]. ^{1}H and ^{13}C NMR spectroscopic data of compound $\mathbf{3_E}$ are presented in Table 1.

For $\mathbf{4_E}$, λ_{max} (HPLC eluent, approximately 11% acetonitrile in ammonium acetate buffer, pH 7)/nm 274 and 218, λ_{min} 240; *m/z* (ESI) MS: 276 (100%, M^+), MS^2 232 (100%, $M^+-C_2H_4O$), MS^3 176 (100%, $M^+-C_5H_8O_2$); [HRMS: Calc for ($C_{13}H_{18}N_5O_2^+$): *m/z*, 276.1461. Found: *m/z*, 276.1470]. ^{1}H and ^{13}C NMR spectroscopic data of major $\mathbf{4_E a}$ and minor $\mathbf{4_E b}$ epimers are presented in Tables 2 and 3, respectively.

2.4. Reaction of acrolein with 2′-deoxyadenosine to form 3_D and 4_D

2′-Deoxyadenosine ($\mathbf{2_D}$, 250 mg, 0.996 mmol) was dissolved in 100 mL of 0.5 M phosphate buffer at pH 4.6 and acrolein (**1**, 557.77 mg, 9.96 mmol) was added. The reaction mixture was incubated and stirred at 37 °C for 2 days. The isolation of the products were performed on the semipreparative column which was eluted isocratically with 5% acetonitrile in 0.01 M ammonium bicarbonate (pH 6.4) for 15 min and then with a gradient from 5 to 40% acetonitrile over the course of 11 min at a flow rate of 3 mL min^{-1}. *m/z* (ESI) MS of $\mathbf{3_D}$ 308 (100%, MH^+), MS^2 192 (100%, MH^+−deoxyribosyl+H), MS^3 174 (100%, MH^+−deoxyribosyl−H_2O+H), 136 (67%, adenine+H); [HRMS: Calc for ($C_{13}H_{17}N_5O_4$+H): *m/z*, 308.1359. Found: *m/z*, 308.1366]; *m/z* (ESI) MS of $\mathbf{4_D}$ 364 (100%, M^+), MS^2 248 (100%, M^+−deoxyribosyl+H); [HRMS: Calc for ($C_{16}H_{22}N_5O_5^+$): *m/z*, 364.1621. Found: *m/z*, 364.1616].

Table 2
^{1}H and ^{13}C chemical shifts (δ) of protons, carbons, and nitrogens and spin–spin coupling constants ($J_{H,H}$) of protons in $\mathbf{4_E}$**a**

Proton	δ (ppm)	Mult.	$J_{H,H}$ (Hz)	Carbon	δ (ppm)	Nitrogen	δ (ppm)
Purine unit							
H-5	8.55	s		C-5	149.30	N-1	−143.3
H-2	8.43	s		C-2	147.01	N-3	−206.5
				C-13a	148.91	N-4	−155.6
				C-13b	121.78	N-6	−222.3
				C-3a	151.90	N-13	−266.4
Ethyl unit							
CH_2	4.37	q	7.38	CH_2	42.62		
CH_3	1.52	t	7.38	CH_3	17.02		
Acrolein units							
H-7α	4.54	ddd	$-13.27_{H7\beta}$; $4.91_{H8\alpha}$; $3.82_{H8\beta}$	C-7	47.21		
H-7β	4.36	m	$-13.27_{H7\alpha}$; $11.45_{H8\alpha}$; $2.81_{H8\beta}$				
H-8α	2.51	m	$-15.03_{H8\beta}$; $11.45_{H7\beta}$; $4.91_{H7\alpha}$; 3.50_{H8a}	C-8	28.52		
H-8β	2.45	m	$-15.03_{H8\alpha}$; 5.03_{H8a}; $3.82_{H7\alpha}$; $2.81_{H7\beta}$				
H-8a	5.31	t	$5.03_{H8\beta}$; $3.50_{H8\alpha}$	C-8a	83.04		
H-10	5.41	dd	$9.78_{H11\beta}$; $2.43_{H11\alpha}$	C-10	97.93		
H-11α	2.20	ddt	$-13.46_{H11\beta}$; $3.12_{H12\alpha}$; 2.43_{H10}; $2.34_{H12\beta}$	C-11	34.88		
H-11β	1.91	tdd	$-13.46_{H11\alpha}$; $12.85_{H12\alpha}$; 9.78_{H10}; $5.17_{H12\beta}$				
H-12α	3.98	ddd	$-13.93_{H12\beta}$; $12.85_{H11\beta}$; $3.12_{H11\alpha}$	C-12	49.50		
H-12β	5.68	ddd	$-13.93_{H12\alpha}$; $5.17_{H11\beta}$; $2.34_{H11\alpha}$				
HO-10	[a]						

[a] Signal not observed.

2.5. Formation of product 4_D from 3_D

The monoadduct ($\mathbf{3_D}$, 1.43 mg, 0.0047 mmol) was incubated with acrolein (**1**, 2.63 mg, 0.047 mmol) for several hours in 0.5 M phosphate buffer pH 7.4 at 37 °C. The formation of $\mathbf{4_D}$ was followed by LC-MS.

3. Results and discussion

The reaction of acrolein (**1**) with 9-ethyladenine ($\mathbf{2_E}$), as well as with 2′-deoxyadenosine ($\mathbf{2_D}$), showed the formation of two products by HPLC and UV-detection. The first eluting peaks from both reaction mixtures were isolated and characterised by MS and NMR as monoadduct **3** based on the observation of a molecular ion peak at $m/z = 220$ and 308 a.m.u., respectively. Various chemical shifts (cf. H-7 s, C-7 and N-6 of $\mathbf{3_E}$ and $\mathbf{4_E}$, see Tables 1–3) together with the short retention time indicate that **3** has been isolated as a protonated species. Gross structural characterisation of 9-ethyladenine–acrolein adduct $\mathbf{3_E}$, based on an assumed molecular formula of $C_{10}H_{14}N_5O$ was quite straightforward and realised by the standard application of ^{1}H–^{13}C HSQC, ^{1}H–^{13}C HMBC and COSY and consideration of chemical shifts, e.g., the lack of sp^2-hybridised carbons other than those of the adenine moiety and the singly and twice-bound states of each terminal carbon of the incorporated acrolein segment to electronegative atoms. The correlation spectra readily provided the sites of attachment and orientation to the adenine moiety (e.g., correlations of H-5 to C-7 and of H-9 to

Table 3
^{1}H and ^{13}C chemical shifts (δ) of protons, carbons, and nitrogens and spin–spin coupling constants ($J_{H,H}$) of protons in $\mathbf{4_E b}$

Proton	δ (ppm)	Mult.	$J_{H,H}$ (Hz)	Carbon	δ (ppm)	Nitrogen	δ (ppm)
Purine unit							
H-5	8.52	s		C-5	149.36	N-1	−143.3
H-2	8.41	s		C-2	146.77	N-3	−206.8
				C-13a	148.80	N-4	−156.8
				C-13b	121.64	N-6	−223.4
				C-3a	151.65	N-13	[a]
Ethyl unit							
CH_2	4.36	q	7.38	CH_2	42.59		
CH_3	1.52	t	7.38	CH_3	17.02		
Acrolein unit							
H-7α	4.51	ddd	$-13.39_{H7\beta}$; $6.29_{H8\beta}$; $3.95_{H8\alpha}$	C-7	47.33		
H-7β	4.38	m	$-13.39_{H7\alpha}$; $10.79_{H8\alpha}$; $3.34_{H8\beta}$				
H-8α	2.49	m	$-14.99_{H8\beta}$; $10.79_{H7\beta}$; $3.95_{H7\alpha}$; 4.81_{H8a}	C-8	28.00		
H-8β	2.35	m	$-14.99_{H8\alpha}$; $6.29_{H7\alpha}$; 4.13_{H8a}; $3.34_{H7\beta}$				
H-8a	5.76	t	$4.81_{H8\alpha}$; $4.13_{H8\beta}$	C-8a	77.20		
H-10	5.55	dd	$3.88_{H11\beta}$; $2.50_{H11\alpha}$	C-10	97.93		
H-11α	2.02	dtd	$-14.41_{H11\beta}$; $3.81_{H12\beta}$; $3.43_{H12\alpha}$; 2.50_{H10}	C-11	32.53		
H-11β	2.34	m	$-14.41_{H11\alpha}$; $11.87_{H12\alpha}$; $5.29_{H12\beta}$; 3.88_{H10}				
H-12α	4.25	ddd	$-13.59_{H12\beta}$; $11.87_{H11\beta}$; $3.43_{H11\alpha}$	C-12	46.71		
H-12β	5.34	ddd	$-13.59_{H12\alpha}$; $5.29_{H11\beta}$; $3.81_{H11\alpha}$				
HO-10	[a]						

[a] Signal not observed.

C-10a) and thus the provision of a newly formed ring fused to the purine base unit. In addition, ^{15}N NMR has been demonstrated to be of great utility in determining the structures of purine base adducts [14–17] and consequently ^{1}H–^{15}N HMBC spectra were also recorded to obtain four of the five nitrogen chemical shifts indirectly. The chemical shifts for the N-1, N-3, and N-4 nitrogens of $\mathbf{3_E}$ (and also $\mathbf{4_E}$) indicated that they were all quite similar to adenine. Notable was the chemical shift for N-6 in $\mathbf{3_E}$ (and similarly in $\mathbf{4_E}$), which, if depicted as in Scheme 1 with a positive charge, is well within expectations [18] for such a structure. Furthermore, protonation or platination of N-1 in adenines (i.e., N-6 in $\mathbf{3_E}$/$\mathbf{4_E}$) is known [19,20] to deshield H-2 significantly (H-5 in $\mathbf{3_E}$/$\mathbf{4_E}$), thus the placement of charge on N-6 in $\mathbf{3_E}$ (and hence also $\mathbf{4_E}$) is consistent with the deshielding of H-5 by ca. 0.5 ppm.

The close similarity of the pertinent chemical shifts and coupling constants between $\mathbf{3_E}$ and $\mathbf{4_E}$ suggest not only the same ring A conformation for $\mathbf{3_E}$ and $\mathbf{4_E}$, but more specifically the same N-6 geometry and charge distribution between N-6 and N-10/N-13. That is to say, surreptitiously, placement of either the proton or the charge elsewhere in $\mathbf{3_E}$ (e.g., N-6, N-3 in the former case, N-10, N-3 in the latter) would prove difficult to rationalise the convergent NMR data of $\mathbf{3_E}$ and $\mathbf{4_E}$. Thus, despite the lack of its direct observation, the acidic proton is clearly positioned on N-10 which is represented as a secondary amine. Based on vicinal couplings of H-8a (vide infra), the newly formed ring adopts an $^{8a}H_8$ half-chair conformation which places the OH group in an axial position. This conformation also provides a viable w-coupling pathway between H-9 and H-7α, hence the observation of such.

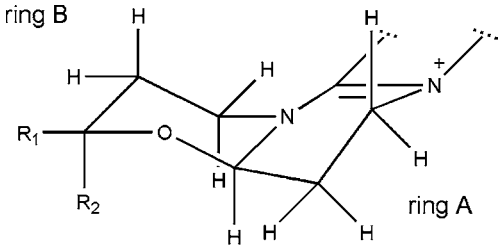
The peaks with longer retention times were isolated and upon examination by NMR, $\mathbf{4_D}$ was found to consist of four diastereomers while $\mathbf{4_E}$ consisted of two diastereomers, $\mathbf{4_E a}$

and **4$_E$b**. The diastereomers were formed in unequal amounts, but they could not be separated by HPLC analyses on a reversed-phase C-18 column. Furthermore, the compounds were found to be unstable in DMSO-d_6 solution, yielding **3** and acrolein (**1**). The problem of stability was serendipitously surmounted as the products were found to be stable as their immonium ions in water solution, in which state they were subjected to full characterisation and identification by NMR.

In the ESI-MS of **4$_E$a** and **4$_E$b**, the molecular ion peak was observed at $m/z = 276$ a.m.u., while **4$_D$** provided a molecular ion at $m/z = 364$ a.m.u. Gross structural characterisation of **4$_E$**, based on an assumed molecular formula of $C_{13}H_{18}N_5O_2$, was essentially uncomplicated based on the aforementioned experiments and consideration of chemical shifts, e.g., the lack of sp^2-hybridised carbons other than those of the adenine moiety and the singly and twice-bound states of each terminal carbon of each acrolein segment to electronegative atoms. The correlation spectra readily provided the distinction of two $-CH_2CH_2CH-$ segments originating from two acrolein units and their attachment not only to the adenine moiety (e.g., correlations of H-5 to C-7 and of H-8a to C-13a), but also their attachment to each other through an oxygen link (e.g., correlation of H-8a to C-10) and thus the provision of two newly formed rings fused to each other and with one of the rings fused to the purine base unit. Exocyclic N-13 was observed only in the major product **4$_E$a** and it resonated at −266.4 ppm, within expectations for a secondary amine in the proximity of electronegative groups and an aromatic moiety, thus lending further support to the placement of the charge on N-6.

The determination of the relative stereochemistries relied on an assessment of the available conformational states derived from an inspection of models and consideration of the extracted coupling constants available by spin simulation [21]. With an endocyclic double bond, ring A should only be able to access half-chair conformations, viz. $^{8}H_{8a}$ and $^{8a}H_{8}$. However, the conformational mobility and geometrical flexibility of nitrogen can permit the permissive participation of a number of ring conformations in which the nitrogens are situated, or it can lead simply to the confluence of a single structure [22–25]. Irrespective of the geometry and configuration of N-13, the disposition of the H-8s to H-9 must be such to give rise to two small couplings as observed. A conformation pertaining to $^{8a}H_{8}$ should possess dihedral angles of ca. 60° between H-8a and both H-8α and H-8β, whilst conformer $^{8}H_{8a}$ should possess dihedral angles of ca. 60° and 180° between H-8a and H-8α and H-8β, respectively. Thus, with H-8a in both **4$_E$a** and **4$_E$b** bearing two small couplings (5.03, 3.50 and 4.81, 4.13 Hz, respectively), ring A adopts an $^{8a}H_{8}$ conformation in both compounds. Moreover, the ring fusion is best considered as *cis* (the $^{8a}H_{8}$ conformation is inaccessible for *trans* fusion) and thus anancomeric (Fig. 1). With only one chair conformation accessible for ring B, H-10 can thus either be axially, or for the epimer, equatorially orientated. In the major product **4$_E$a**, H-10 possesses a large (9.78 Hz) and a small (2.43 Hz) coupling and therefore H-10 is axial and the relative configuration is 8a*R**,10*R** (and the product is racemic). Conversely, in the minor product **4$_E$b**, H-10 possesses two small couplings (3.88 and 2.50 Hz) and is thus equatorial in disposition and the relative configuration is 8a*R**,10*S**. The assertion of a chair conformation for ring B and a half-chair conformation for ring A for both **4$_E$a** and **4$_E$b** was clearly supported by all extracted coupling constants. The observed homonuclear NOEs were also consistent with these conclusions.

Comparisons of the three sets of NMR data provided some interesting revelations. For example, the γ-effect of the axial hydroxyl group in **4$_E$b** was clearly evident on several relevant nuclei in **4$_E$b** in comparison to **4$_E$a**; C-8a and C-12 were both shielded (by 5.8 and 2.8 ppm, respectively), H-8a and H-12α were both deshielded (by 0.45 and 0.28 ppm,



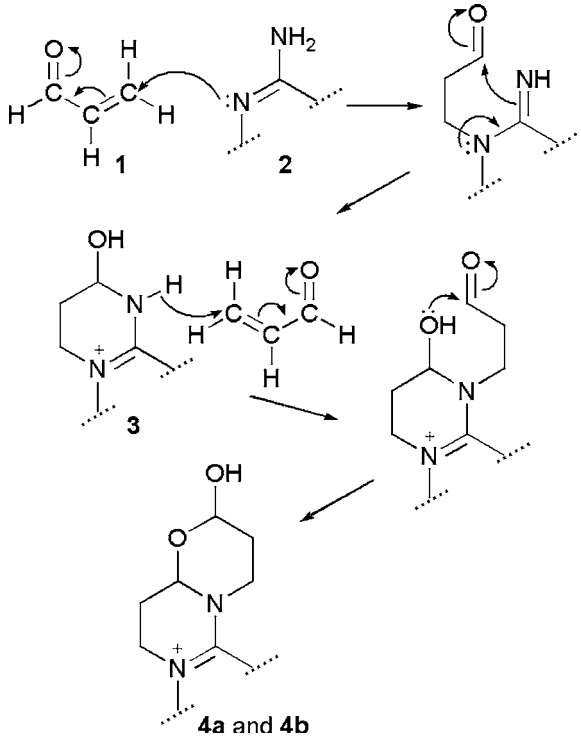
4Ea: R_1 = OH, R_2 = H

4Eb: R_1 = H, R_2 = OH

Fig. 1. The alicyclic portions of $\mathbf{4_E a}$ and $\mathbf{4_E b}$ indicating the preferred conformations and relative stereochemistry. Both compounds adopt $^{8a}H_8$ half-chair conformations for ring A whilst ring B is in an $^{11}C_{8a}$ chair conformation; the relative stereochemistry for the major product (OH equatorial) was 8a*R**,10*R** and that for the minor product 8a*R**,10*S**.

respectively), whilst H-12 β was shielded by 0.34 ppm. Furthermore, slight differences were apparent for some of the *J* values of the acrolein segments for the two structures, therefore either there is some slight distortion in the geometry of the minor product $\mathbf{4_E b}$ or there is a small contribution of a skew form to a fast conformational equilibrium for this compound. The former scenario would provide direct relief for the steric hindrance imposed by an axial hydroxyl, whilst the latter would implicate the diminished energetic favourability of the chair conformation arising from the same interaction. Of the seven possible 2- or 3-bond ^{1}H couplings to N-13 for each of the diastereomers, only one correlation was observed by ^{1}H–^{15}N HMBC, that for H-11α in $\mathbf{4_E a}$. The dihedral angle between these two nuclei is essentially 180°, an optimum for such coupling, though it is noted that H-8β should also be similarly disposed. The sign of this large coupling was also discerned to be negative, in accordance with the expectation for a large coupling extending over three bonds and involving one $-\gamma$ nucleus [15]. Interestingly, H-2 is also deshielded in both $\mathbf{3_E}$ and $\mathbf{4_E}$ relative to adenines [19,20], however, for differing reasons. In $\mathbf{3_E}$, the deshielding is attributed to intramolecular H-bonding to the amine proton; in $\mathbf{4_E}$, from steric compression by the C-12 methylene group. Also concomitant with the deshielding of H-2 in $\mathbf{4_E}$ is the shielding of N-1 in these compounds relative to N-1 in $\mathbf{3_E}$.

Regarding the course of the reaction, several observations and the results of additional experiments are worth noting. Only two peaks were ever observed for the treatment of $\mathbf{2_E}$ with excess acrolein (**1**) in phosphate buffer at pH 4.6. The treatment of pure monoadduct $\mathbf{3_D}$ with acrolein (**1**) provided $\mathbf{4_D}$ as the main products of the reaction. The compounds **4** were found to degrade back to the monoadduct **3** and free acrolein (**1**) based on the ^{1}H NMR spectrum in DMSO-d_6 which displayed signals characteristic of free acrolein (**1**). From these observations, the conclusion can be drawn that the reaction proceeds via adduct formation first at N-1 and subsequently a ring closure takes place at the carbonyl carbon by the exocyclic NH_2 to form the ring-fused monoadduct **3** (Scheme 2). N-1 adducts are known to undergo the Dimroth rearrangement to provide the corresponding N-6 adducts [26,27]. In this case, however, we did not observe the N-6 adduct and we were not able to substantiate the case for Dimroth rearrangement by performing the reaction at higher pH. The first Michael addition at N-1 of **2** is followed by a second Michael reaction of free acrolein (**1**) at the formerly exocyclic nitrogen of 9-ethyladenine (**2**). Compound **4** is



Scheme 2. Proposed mechanism for the formation of **4**.

finally obtained by a second cyclisation, but this time by the hydroxyl of the initially formed ring onto the carbonyl of this second acrolein unit to complete the reaction sequence. The structure **3** actually accounts for an increase in reactivity towards acrolein as we found that **4** was the major adduct formed with time.

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