

SIMULTANEOUS CONVERSION OF *N*(1)-(2-AMINOETHYL)-  
ADENOSINE TO *N*<sup>6</sup>-(2-AMINOETHYL)-ADENOSINE AND  
TRICYCLIC 1,*N*<sup>6</sup>-ETHANOADENOSINE UNDER MILD  
AQUEOUS CONDITIONS

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**Abstract** - Under mild aqueous conditions (50°C, pH range 6 - 7) *N*(1)-(2AE)-adenosine (**2**) can be converted to *N*<sup>6</sup>-(2AE)-adenosine (**7**) by Dimroth rearrangement at unexpectedly high rate. In a parallel reaction tricyclic 1,*N*<sup>6</sup>-ethanoadenosine (**6**) is formed. The latter reaction is new in heterocyclic organic chemistry and is strongly catalysed by the monoanions of phosphoric and arsenic acid and, less strongly, by the acetate anion.

An established strategy for the synthesis of *N*<sup>6</sup>-functionalized adenine containing compounds (AMP, ADP, ATP, NAD, NADP and FAD) follows the typical procedural pattern:<sup>1,2</sup>

1) Alkylation of the *N*(1)-position of the adenine moiety under aqueous conditions in the pH range 3.2 - 5.5 to introduce a reactive group (carboxyl-, epoxy- or primary amino group) leading to *N*(1)-functionalized coenzyme derivatives.

2) Dimroth rearrangement<sup>3-5</sup> of *N*(1)-functionalized coenzyme derivatives<sup>3</sup> to *N*<sup>6</sup>-functionalized coenzyme derivatives under harsh alkaline conditions in aqueous solution (e.g. pH 10.5 - 11, 65 - 70°C, 2 h).

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*Abbreviation:* 2AE: 2-aminoethyl.

It should be emphasized that for the synthesis of  $N^6$ -functionalized NAD or NADP two extra steps are required, namely, specific reduction of the C(4)-position of the nicotinamide of the  $N(1)$ -functionalized derivative with sodium dithionite to  $N(1)$ -functionalized NADH or NADPH to achieve chemical stability of the C-N bond between the ribose and the nicotinamide moiety under the harsh conditions of the Dimroth rearrangement, and final oxidation of  $N^6$ -functionalized NADH or NADPH to  $N^6$ -functionalized NAD or NADP.

For the introduction of a primary amino group by aminoethylation with ethyleneimine of the  $N(1)$ -position of the adenine moiety of NAD or NADP optimized conditions have been elaborated.<sup>6</sup> The purification of the products  $N(1)$ -(2AE)-NAD and  $N(1)$ -(2AE)-NADP by ion exchange chromatography has been described.<sup>7</sup> For the Dimroth rearrangement of these products to  $N^6$ -(2AE)-NAD or  $N^6$ -(2AE)-NADP it has been shown that this step could be carried out with unexpected ease under mild aqueous conditions (e.g. pH 6.0 - 6.5, 50°C, 7 h), omitting the two extra steps mentioned above.<sup>7</sup>

Furthermore, it was observed that after the rearrangement step these  $N^6$ -aminated coenzymes were always accompanied by a byproduct that shows NAD or NADP coenzyme activity and gave a negative reaction with ninhydrin after separation by thin layer chromatography. A striking aspect between the rearrangement behaviour of  $N(1)$ -(2AE)-NAD and  $N(1)$ -(2AE)-NADP is that  $N(1)$ -(2AE)-NAD rearranges to  $N^6$ -(2AE)-NAD with 60 - 65 % transformation and 35 - 40 % to the NAD-byproduct, while for  $N(1)$ -(2AE)-NADP the NADP-byproduct is obtained as main product with 70 - 75 % conversion with 25 - 30 % transformation to  $N^6$ -(2AE)-NADP.<sup>7</sup> This indicated that the phosphate group at the 2-position of the ribose next to the adenine moiety seems to have a catalytic effect on the side reaction. This assessment is supported by the preliminary finding that the formation of the NAD byproduct dominates, if  $N(1)$ -(2AE)-NAD is incubated under mild aqueous conditions in the presence of inorganic phosphate. Recently, it has been proved that the NAD-byproduct is tricyclic 1, $N^6$ -ethanoadenine-NAD.<sup>8</sup> As a consequence of these preliminary observations we decided to investigate both conversions of the  $N(1)$ -(2AE)-adenine moiety in the simpler model compound  $N(1)$ -(2AE)-adenosine (2).

## RESULTS AND DISCUSSION

Initially, our attention was focused on the development of the methodology for the syntheses of *N*(1)-(2AE)-adenosine (**2**), 1,*N*<sup>6</sup>-ethanoadenosine (**6**) and *N*<sup>6</sup>-(2AE)-adenosine (**7**) and their purification. The reaction pathways of the conversions and the supposed transient intermediates are depicted in Figure 1. The procedures developed and given in the experimental section lead conveniently to the products of interest. As is to be expected, the Dimroth rearrangement readily occurred under mild aqueous conditions. The structures of **2**, **6** and **7** have been assigned by uv spectrophotometry, <sup>1</sup>H nmr spectrometry and positive ion FAB-ms. After transformation of **2** and separation by thin layer chromatography compound(**6**) has the lowest *r<sub>f</sub>* value and did not give a positive reaction with ninhydrin reagent indicating the absence of a primary amino group. Uv spectra of the three adenosine analogues in the range 220 - 400 nm at pH 7 and 12 are given in Figure 2. Dimroth rearrangement of **2** to **7** leads to a typical shift of  $\lambda_{\max}$  from 259 nm to 265 nm with an increase in absorption changing the molar absorption coefficient from 15000 M<sup>-1</sup>cm<sup>-1</sup> to 19000 M<sup>-1</sup>cm<sup>-1</sup>. Similar to adenosine (**1**) a very slight shoulder in the uv spectrum in the range 300 -400 nm under strong alkaline conditions is observed (Figure 2B), whereas **2** shows a pronounced shoulder here (Figure 2A), typical for *N*(1)-alkylated adenine containing compounds under strong alkaline conditions due to the presence of a C(6)=NH group in the adenine after deprotonation.<sup>9</sup>

Formation of **6** involves a shift of  $\lambda_{\max}$  to 262 nm and a small increase in absorption, leading to a molar absorption coefficient of 16000 M<sup>-1</sup>cm<sup>-1</sup>. The *N*(1)-position of the adenine of **6** is alkylated since under alkaline conditions a typical shoulder is also observed in the range 300 - 400 nm, but not as pronounced as for **2** (Figure 2C).

<sup>1</sup>H Nmr and positive ion FAB-ms data are given in Table I. From these nmr data it is obvious that the ribose moiety remains intact in all products, while derivatization occurs in the adenine part. The chemical shifts indicate the similarity of the C(1)H<sub>2</sub>-group of **2** and **6** and the C(2)H<sub>2</sub>-group of **2** and **7**.

Characteristic positive ion FAB-ms are obtained for all adenosine derivatives. The equality in mass for **2** and **7** indicates that only isomerization has occurred after the Dimroth rearrangement, while the loss of 17 mass units points to expulsion of NH<sub>3</sub> on formation of the tricyclic product(**6**).

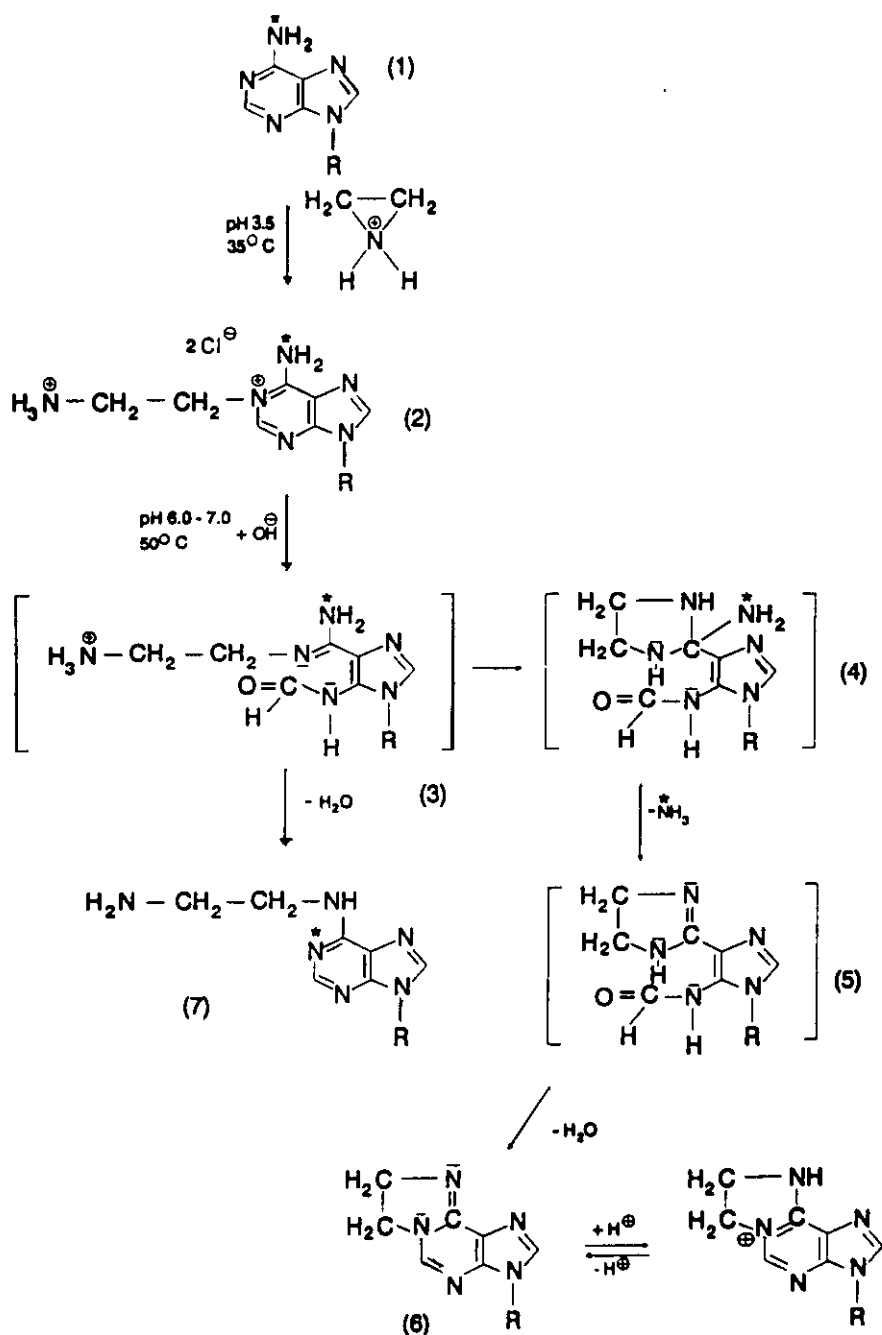


Figure 1: Reaction pathway for the synthesis of  $N^6$ -(2AE)-adenosine (7) and tricyclic  $1,N^6$ -ethanoadenosine (6) from  $N(1)$ -(2AE)-adenosine (2) obtained by aminoethylation with ethyleneimine of the  $N(1)$ -position of the adenine moiety of adenosine (1) including transient intermediates reflecting the reaction mechanism proposed. The symbol (\*) indicates  $^{15}N$  labeling for the  $^{15}N$  labeled adenosine derivatives.

R: D-Ribose

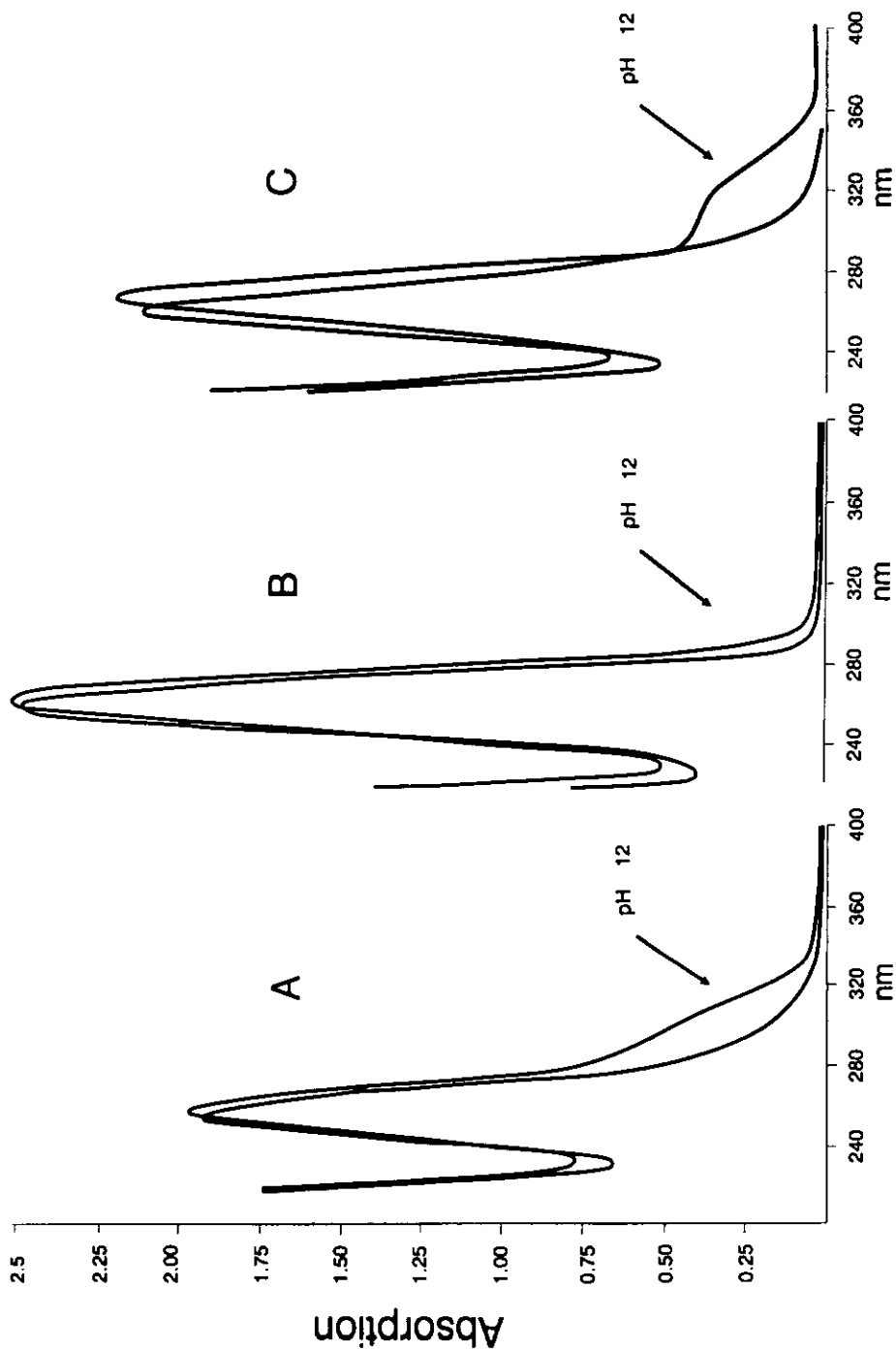


Figure 2: Uv-spectra of (A) *N*(1)-(2AE)-adenosine (2), (B) *N*<sup>6</sup>-(2AE)-adenosine (7) and (C) tricyclic 1,*N*<sup>6</sup>-ethanoadenosine (6).

These data confirm the structures of **7** and tricyclic **6** as products of the transformation of **2**.

The reaction pathway for the transformations to 6-<sup>15</sup>NH<sub>2</sub>-*N*(1)-(2-aminoethylaminoethyl)-adenosine (**8**) and <sup>15</sup>*N*(1)-*N*<sup>6</sup>-(2-aminoethylaminoethyl)-adenosine (**9**) starting from 6-<sup>15</sup>NH<sub>2</sub>-*N*(1)-(2AE)-adenosine (<sup>15</sup>*N*-**2**) is given in Figure 6. The uv spectral characteristics of **8** and **9** conform to that of **2** and **7** respectively. <sup>1</sup>H Nmr, <sup>15</sup>N nmr and positive FAB ms data for these <sup>15</sup>*N* labeled adenosine derivatives are given in Table I and confirm their structures. The slightly higher mass (357) for **9** compared to that for **8** (355) is presumably related to the protonated form of **9**. The conversion of **2** was compared with that of *N*(1)-methyladenosine under mild aqueous conditions, using thin layer chromatography and uv scanning to follow their disappearance and the formation of products. Figures 3A and 3B summarize the results of these conversions. It is obvious that *N*(1)-methyladenosine (*r<sub>f</sub>* 0.40) is very slowly converted to *N*<sup>6</sup>-methyladenosine (*r<sub>f</sub>* 0.56) at pH 6.0 and 7.0, which points to normal Dimroth rearrangement behaviour (requirement of harsh alkaline conditions to achieve an acceptable rearrangement rate).<sup>10</sup> On the contrary, **2** rearranges to **7** in an unexpected fast reaction, while byproduct (**6**) is formed to a lesser extent. At pH 7.0 the rearrangement proceeds to give a final conversion of approximately 65 % **7** and 32 % **6**. It should be emphasized that these final percentages do not change even after prolonged incubation, indicating that **2** is converted by two parallel reactions which rules out a consecutive reaction with **7** as intermediate product. Even at pH 6.0 there is 35 % conversion to **7** with formation of 12 % **6** within 200 min.

As preliminary observations have shown that the presence of sodium phosphate causes the predominant formation of 1,*N*<sup>6</sup>-(ethanoadenosine)-NAD from *N*(1)-(2AE)-NAD, the influence of sodium phosphate on the conversion of **2** was studied. The condition pH 6.0 and 50°C was chosen, as at pH 7.0 an instantaneous conversion to **7** (10%) and **6** (90%) was observed. Comparison of Figures 3A and 4A indicates that at pH 6.0 sodium phosphate causes approximately a 2-fold increase in the conversion rate similar to that at pH 7.0 without phosphate. From the same comparison it is clear that the rate of conversion to **7** is little affected by the introduction of phosphate, while that to **6** is greatly enhanced. As a consequence, the formation of **6** becomes the dominating reaction.

This phenomenon must arise from the presence of a phosphoric acid anion (see later) in the solution, as in the presence of NaCl a conversion pattern is observed similar to Figure 3A.

It is generally accepted that in the mechanism of the Dimroth rearrangement of *N*(1)-alkylated adenine derivatives there is a preliminary base catalysed ring opening between the *N*(1)- and the *C*(2)-position leading to a transient intermediate with a formylamino group at the *C*(5)-position of the imidazole ring as in 3 (Figure 1).<sup>3-5</sup> Figures 3A and 3B indicate that the presence of a primary amino group at the *C*(2)-position of the CH<sub>2</sub>CH<sub>2</sub> moiety attached to the *N*(1)-position of the adenine ring of 2 appears to cause a strong intramolecular H-bond formation between the NH<sub>2</sub> group and the *C*(2)-bound OH group of an intermediate still with a closed ring preceding 3 after attack of the *C*(2)-position by an OH<sup>-</sup> ion as proposed by Wilson and McCloskey.<sup>3</sup>

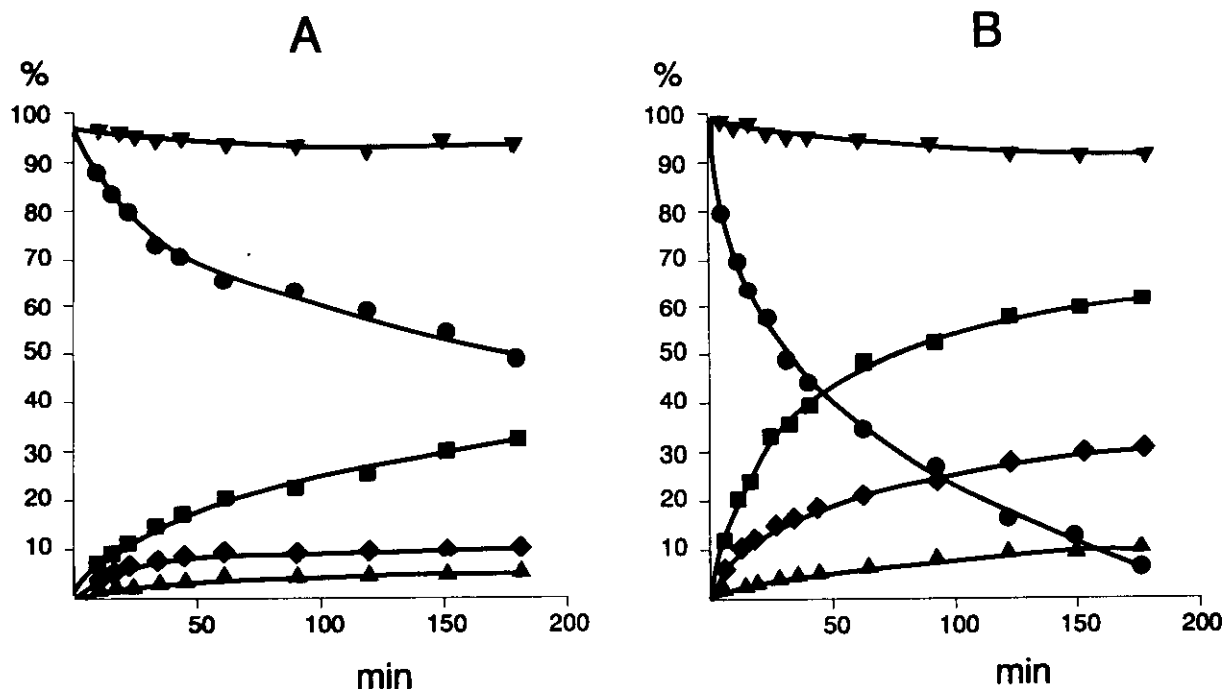


Figure 3: Comparison of the transformation (%) of *N*(1)-(2AE)-adenosine (2) (●) to *N*<sup>6</sup>-(2AE)-adenosine (7) (■) and tricyclic 1,*N*<sup>6</sup>-ethanoadenosine (6) (◆) and of *N*(1)-methylenadenosine (2) (▼) to *N*<sup>6</sup>-methylenadenosine (7) (▲).

Conditions: initial concentration 15 mM *N*(1)-(2AE)-adenosine in aqueous solution at pH 6.0 (A) and pH 7.0 (B) at 50 °C.

Presumably, this H-bond formation leads to a very facile deprotonation of the C(2)-bound OH group resulting in a fast formation of intermediate (3).

Given the FAB-ms result of a loss of 17 mass units corresponding to expulsion of  $\text{NH}_3$  for the tricyclization reaction we postulate that transient intermediate (4), derived from 3 is responsible and is in turn converted to transient intermediate (5) and finally to 6 (Figure 1). The expulsion of the  $\text{NH}_2$  group from the C(6)-position of the adenine of intermediate (4) and the subsequent cyclization to intermediate (5) is plausible taking into account the results of  $^{15}\text{N}$  tracer studies by Chheda *et al.*<sup>11</sup> for the conversion of  $N^6$ -glycyladenine to tricyclic 3H-imidazo-(2,1-*i*)-purine-8-(7H)one under mild aqueous conditions, that point to participation of transient intermediates similar to 4 and 5 in this tricyclization reaction.

Support for the occurrence of the successive transient intermediates (4) and (5) after the generally accepted intermediate (3) is demonstrated by the  $^{15}\text{N}$ -nmr result, that the 6- $^{15}\text{NH}_2$  group of the adenine of ( $^{15}\text{N}$ -2) is expelled in the form of  $^{15}\text{NH}_4^+$  under conversion conditions where the tricyclization to 6 dominates (Table I). For the tricyclization of  $N^6$ -glycyladenine Chheda *et al.*<sup>11</sup> propose a mechanism where the primary amino group of the aminoacyl moiety adds across the C(6)-N(1) bond of the adenine to form an intermediate with a heterocyclic 5-ring attached to the adenine *via* the C(6)-position, that is converted to an intermediate similar to 4 after ring opening between the C(2)- and the N(1)-position. It should be stressed that a similar tricyclization reaction for 7, which should lead to 6, could not be observed either under the conditions of Chheda *et al.* (in water, pH 5.5, 100°C) or in water in the absence or presence of 0.05 M sodium phosphate, pH 7.5 and 10.7 at 50°C and 100°C. This emphasizes again that the conversion of 2 occurs by two parallel reactions.

Given the strong catalysis of the tricyclization in the presence of phosphate, the final tricyclization by ring closure between the N(1)- and C(6)-position of transient intermediate (5) may proceed similarly to the acid catalysed semicarbazone formation studied by Cordes and Jencks<sup>12</sup> where the mono anions of phosphoric and arsenic acid are extremely effective catalysts. Consequently, we studied the influence of sodium arsenate on the conversion of 2 under similar conditions to those using sodium phosphate. The transformation curves of Figure 4B show that sodium arsenate catalyses the tricyclization of 2 to 6 at least as strongly as sodium phosphate.



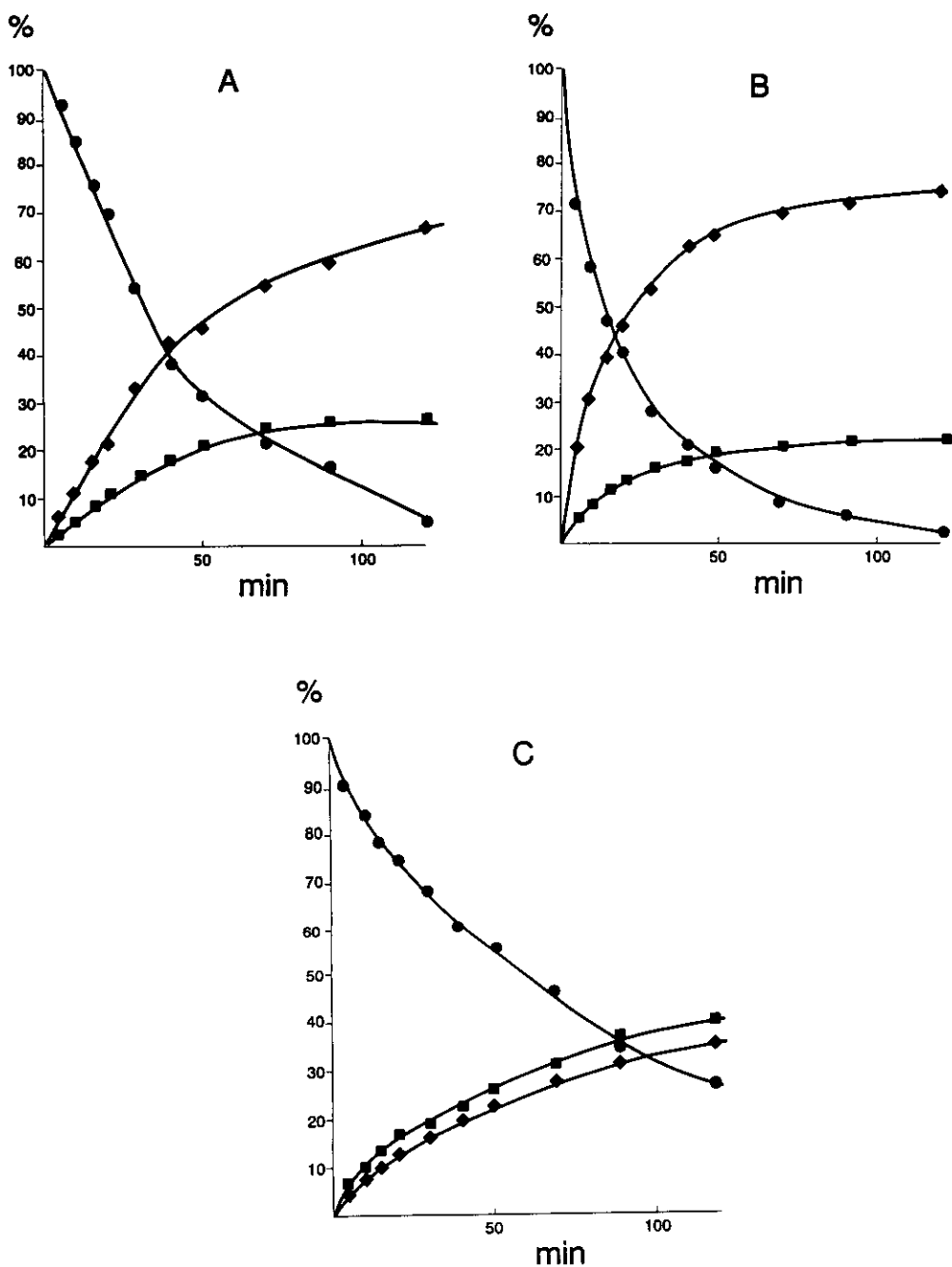


Figure 4: Transformation (%) of *N*(1)-(2AE)-adenosine (2) (●) to *N*<sup>6</sup>-(2AE)-adenosine (7) (■) and tricyclic 1,*N*<sup>6</sup>-ethanoadenosine (6) (◆) in the presence of (A) sodium phosphate (0.025 mM), (B) sodium arsenate (0.025 mM) and (C) sodium acetate (0.025 mM)  
 Conditions: initial concentration 15 mM *N*(1)-(2AE)-adenosine at pH 6.0 and 50°C.

Table I:  $^1\text{H}$ Nmr data (from 1D and 2D COSY spectra),  $^{15}\text{N}$  nmr data, and positive ion FAB ms data of adenosine (1),  $N(1)$ -(2AE)-adenosine without and with  $^{15}\text{N}$  labeled 6-NH<sub>2</sub> (2),  $N^6$ -(2AE)-adenosine without and with  $^{15}\text{N}$  labeled  $N(1)$  (7), 1, $N^6$ -ethanoadenosine (6), 6- $^{15}\text{NH}_2$ - $N(1)$ -(2-aminoethylaminoethyl)adenosine (8) and  $^{15}\text{N}(1)$ - $N^6$ -(2-aminoethylaminoethyl)adenosine (9).

 **$^1\text{H}$  Nmr data:**

Chemical shifts:	(1)	(2)	(7)	(6)	(8)	(9)
<b>Ribose moiety</b>						
H-1'	5.95	6.16	6.04	6.13	6.12	6.06
H-2'	4.79	4.78	4.78	4.75	4.80	4.80
H-3'	4.33	4.45	4.42	4.44	4.44	4.44
H-4'	4.21	4.27	4.27	4.26	4.28	4.28
H-5'A	3.83	3.91	3.91	3.91	3.92	3.92
H-5'B	3.74	3.84	3.82	3.82	3.84	3.84
<b>Adenosine moiety</b>						
H-2	8.21	8.59	8.31	8.57	8.46	8.27
H-8	8.08	8.58	8.27	8.54	8.48	8.31
<b>2-(AE)-moiety</b>						
CH <sub>2</sub> (1)		4.75	3.91	4.79	4.44	4.44
CH <sub>2</sub> (2)		3.60	3.33	4.23	3.12	3.12
CH <sub>2</sub> (3)					3.09	3.09
CH <sub>2</sub> (4)					2.95	2.91
<b>Coupling constants ribose moiety</b>						
J(1'-2')	5.9	5.2	6.2	5.2	5.5	6.2
<b>Coupling constants 2-(AE)-moiety</b>						
J(1-2)		6.6	5.4	9.1	6.5(1-2)	6.3(1-2)
2)					6.5(3-4)	6.3(3-4)

 **$^{15}\text{N}$  Nmr data:****Chemical shifts:**

$\text{N}^6$		94.7			93.7	
$\text{N}(1)$			229.7			227.3

**Coupling constants**

J(N-2)		15.2			15.3	
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**FAB-ms data:**

(M + H) <sup>+</sup>	268	311	311	294	355	357
(M + H) <sup>+</sup>			317			

Additionally, it was shown that acetate has a catalytic effect on the formation of **6**, but lower compared to the other catalysts (Figure 4C).

The influence of the pH on the final conversion percentage for **6** and **7** was studied in the pH range 3 - 11 for the conversion of **2** in the absence and presence of sodium phosphate, arsenate and acetate at lower and higher concentration than that of the starting compound. The curves of Figures 5A, 5B, 5C and 5D present the results of this study. The reaction pattern of Figure 5A reflects that the intermediates (**4**) and (**5**) of the tricyclization reaction seem to be optimally formed from **3** in the pH range 8 - 9.

From a synthetic point of view a pH range 6 - 7 is optimal for the preparation of *N*<sup>6</sup>-(2AE)-adenine containing compounds, while high yields can be expected in combination with acceptable conversion times (7 - 20 h at 50°C). Given the pK<sub>2</sub> values of the order of 7 for phosphoric and arsenic acid (see below), the pattern of the conversion curves, particularly for the high concentrations of the catalysts, depicted in Figures 5A and 5B, point to their monoanions as catalytically active species. Arsenate is a stronger catalyst than phosphate, since even at the lower arsenate concentration the tricyclization reaction still dominates the Dimroth rearrangement in the pH range 6.5 - 9.5. Some influence of differences in the concentration of the monoanions in this pH range may be assumed to be due to a significant difference in pK<sub>2</sub> (6.8 for arsenic acid versus 7.2 for phosphoric acid).<sup>13</sup> The catalytic activity of acetate is the weakest as at the higher concentration of acetate the catalytic activity approximates that of arsenate at low concentration. In Figure 5D the conversion curve for the highest acetate concentration indicates that the acetate anion is the catalytically active species.

The parallel conversions of **2** to **6** and **7** (100 % conversion) were studied at 40, 45, 50, 55, 60, 65 and 70°C in the absence and presence of sodium phosphate, arsenate and acetate. Straight plots of log C(*N*(1)-(2AE)-adenosine) against reaction time were always obtained, indicating a (pseudo)first order reaction for the transformation of **2**.

From these plots the rate constants  $k(N(1)-(2AE)\text{-adenosine})$  for the decrease of the concentration of **2** at the different temperatures were calculated. On the assumption that the formation of **6** and **7** proceed by two parallel (pseudo)first order reactions the following equations hold according to Jencks:<sup>14</sup>

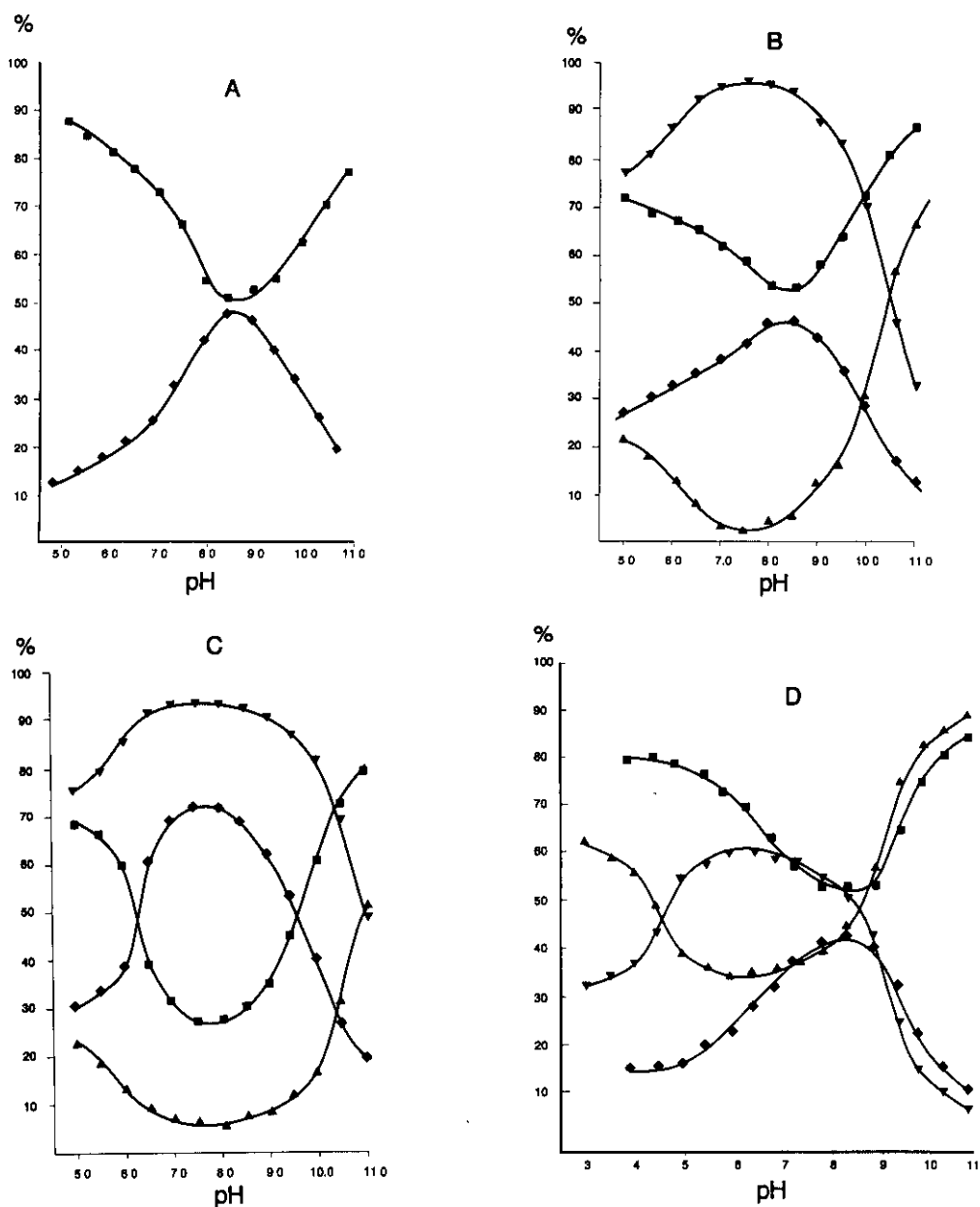


Figure 5: Terminal transformation percentages for the conversion of *N*(1)-(2AE)-adenosine (2) to *N*<sup>6</sup>-(2AE)-adenosine (7) and 1,*N*<sup>6</sup>-ethanoadenosine (6) at different pH in the range 3 - 11; Conditions: initial concentration 15 mM *N*(1)-(2AE)-adenosine (2) in aqueous solution at 50 °C.  
 (A) *N*<sup>6</sup>-(2AE)-adenosine (7) (■) and 1,*N*<sup>6</sup>-ethanoadenosine (6) (◆);  
 (B) *N*<sup>6</sup>-(2AE)-adenosine (7) (2.5 mM (■) and 25 mM (▲) sodium phosphate) and 1,*N*<sup>6</sup>-ethanoadenosine (6) (2.5 mM (◆) and 25 mM (▼) sodium phosphate);  
 (C) *N*<sup>6</sup>-(2AE)-adenosine (7) (2.5 mM (■) and 25 mM (▲) sodium arsenate) and 1,*N*<sup>6</sup>-ethanoadenosine (6) (2.5 mM (◆) and 25 mM (▼) sodium arsenate);  
 (D) *N*<sup>6</sup>-(2AE)-adenosine (7) (2.5 mM (■) and 25 mM (▲) sodium acetate) and 1,*N*<sup>6</sup>-ethanoadenosine (6) (2.5 mM (◆) and 25 mM (▼) sodium acetate)

$$k(N(1)-(2AE)\text{-adenosine}) = k(N^6-(2AE)\text{-adenosine}) + k(1,N^6\text{-ethanoadenosine}) \quad (\text{I})$$

$$\frac{\text{Final } C(N^6-(2AE)\text{-adenosine})}{\text{Final } C(1,N^6\text{-ethanoadenosine})} = \frac{k(N^6-(2AE)\text{-adenosine})}{k(1,N^6\text{-ethanoadenosine})} = (\text{constant}) \quad (\text{II})$$

$$\text{Final } C(1,N^6\text{-ethanoadenosine}) \quad k(1,N^6\text{-ethanoadenosine})$$

(C - concentration)

Using equations I and II  $k(N^6-(2AE)\text{-adenosine})$  and  $k(1,N^6\text{-ethanoadenosine})$  at different temperatures were calculated from experimentally obtained  $k(N(1)-(2AE)\text{-adenosine})$  and  $c$ . From Arrhenius plots of  $\log k(N^6-(2AE)\text{-adenosine})$  and  $\log k(1,N^6\text{-ethanoadenosine})$  against  $1/T$  the activation energies for both parallel reactions were calculated in the absence and presence of the catalysts (Table II).

The activation energy of the Dimroth rearrangement to 7 is lower than that of the tricyclization reaction to 6 when the strong catalysts are absent. The lowest activation energy was calculated for the presence of the monoanion of arsenic acid which underlines that this compound is the most effective catalyst.

The course of the conversion curves of Figures 3A and 3B and Figures 4A, 4B and 4C are in agreement with the activation energies listed in Table II.

The rearrangement of 8 to 9 in distilled water and 0.05 M sodium phosphate at pH 7.0 and 50°C is summarized in Figures 7A and 7B respectively. Compared to  $N(1)$ -methyladenosine (see Figure 3B) 8 can also be relatively quickly rearranged at a similar rate under mild aqueous conditions both in the absence or presence of phosphate (approximately 50 % slower than the rearrangement of 2 to 7 under the conditions related to Figure 3B). No parallel formation of a second tricyclic compound could be detected by thin layer chromatography. This was further demonstrated by  $^1\text{H}$  and  $^{15}\text{N}$  nmr (Table I) and the absence of  $^{15}\text{NH}_4^+$  after 100 % conversion in the  $^{15}\text{N}$  nmr spectrum. Obviously, the secondary amine of the  $N(1)$ -attached 2-aminoethylaminoethyl group cannot expel the  $C(6)\text{-NH}_2$  group of the adenine.

Table II: Activation energies ( $E_a$ ) for the two parallel conversions of *N*(1)-(2AE)-adenosine (2) to *N*<sup>6</sup>-(2AE)-adenosine (7) and 1,*N*<sup>6</sup>-ethanoadenosine (6) in the absence or presence of sodium phosphate, arsenate and acetate (0.025 M).  
 Conditions: initial concentration of 2 15 mM in aqueous solution at pH 6.0 at different temperatures in the range 40-70°C (see text).

	$E_a$ (7) (kJ/Mol)	$E_a$ (6) (kJ/Mol)
In H <sub>2</sub> O	90.960	108.365
In sodium phosphate	90.793	78.659
In sodium acetate	88.868	98.115
In sodium arsenate	87.738	61.923

kJ: kilo Joules

The finding that under mild aqueous conditions (pH 6 - 7, 50°C) the Dimroth rearrangement of the *N*(1)-(2AE)-adenine moiety to the *N*<sup>6</sup>-(2AE)-adenine moiety still proceeds relatively fast has been used to develop a convenient simple synthesis of *N*<sup>6</sup>-(2AE)-NAD, -NADP and -FAD.<sup>15</sup>

The tricyclization of the *N*(1)-(2AE)-adenine moiety to the 1,*N*<sup>6</sup>-ethanoadenine moiety under mild aqueous conditions, effectively catalysed in the presence of phosphate or arsenate, can be considered as a new reaction in heterocyclic chemistry in addition to the method described by Ganapati and Townsend<sup>16</sup> using organic solvents for a similar tricyclization of adenine containing compounds by reacting 6-Cl adenine derivatives with ethyleneimine to effect nucleophilic displacement of the chlorine followed by a final rearrangement.

Both 6 and 7 may be starting compounds of interest for further derivatization in the development of new adenosine receptor ligands for medical purposes.<sup>17</sup>

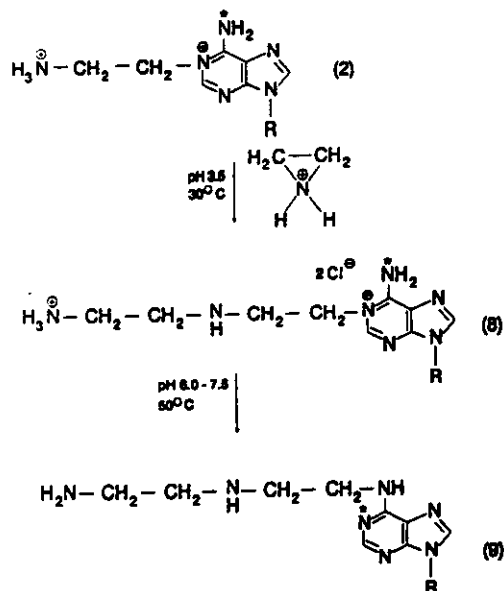


Figure 6: Reaction pathway for the conversion of (<sup>15</sup>N-2) to 6-<sup>15</sup>NH<sub>2</sub>-N(1)-(2-aminoethylaminoethyl)-adenosine (8) and rearrangement of 8 to <sup>15</sup>N(1)-N<sup>6</sup>-(2-aminoethylaminoethyl)adenosine (9). The symbol (\*) indicates <sup>15</sup>N labeling  
R: D-Ribose

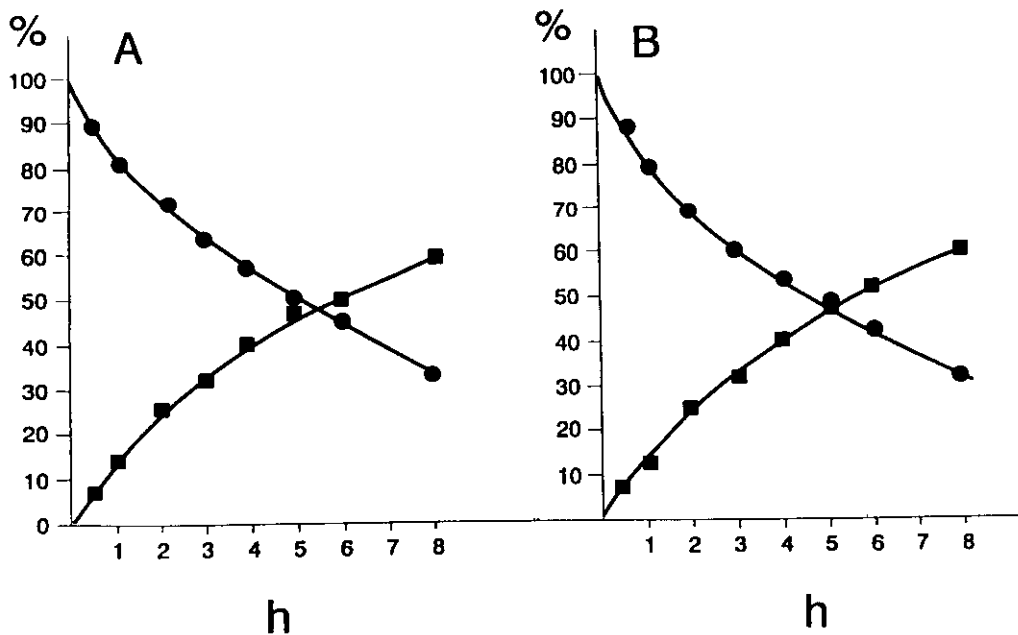


Figure 7: Rearrangement of 6-<sup>15</sup>NH<sub>2</sub>-N(1)-(2-aminoethylaminoethyl)adenosine (8) (●) to <sup>15</sup>N(1)-N<sup>6</sup>-(2-aminoethylaminoethyl)adenosine (9) (■) under mild aqueous conditions in the absence (A) and the presence of sodium phosphate (50 mM) (B). Conditions: initial concentration 15 mM 8, pH 7.0 and 50 °C.

## EXPERIMENTAL SECTION

Ethyleneimine was purchased from Serva (Heidelberg, Germany); 98 % enriched  $^{15}\text{N}$ -ammonium chloride from Isotec (Dayton, Ohio, USA); adenosine from Boehringer (Mannheim, Germany); *N*(1)-methyladenosine, *N*<sup>6</sup>-methyladenosine, inosine and 6-chloropurine riboside from Sigma (Deisenhofen, Germany); Biorex-70 (50 - 100 mesh) and Chelex-100 (minimal 400 mesh) from Biorad (Munich, Germany); Sephadex G-10 from Pharmacia (Uppsala, Sweden); silicagel-60 (0.063 - 0.2 mm particle size, 70 - 230 mesh), silica gel 60-F<sub>254</sub> (0.2 mm) aluminum thin layer chromatography plates and all other chemicals of reagent grade were from Merck (Darmstadt, Germany).

$^1\text{H}$  Nmr spectra were obtained in  $\text{D}_2\text{O}$  (pD 7.0) on a Bruker WM-400 nmr spectrometer (400.1 MHz), using 3-(trimethylsilyl)propanesulphonic acid -  $\text{d}_4$  sodium salt - as reference.  $^{15}\text{N}$  Nmr spectra were obtained in  $\text{D}_2\text{O}$  (pD 7.0 - 7.5 range) on a Bruker AM-600 nmr spectrometer. Positive ion FAB-ms spectra were recorded on a Kratos M 5-50 mass spectrometer equipped with a Kratos FAB source and using glycerol as matrix. In Table I the  $^1\text{H}$  nmr,  $^{15}\text{N}$  nmr and positive ion FAB-ms data are summarized for **1**, **2**, **6**, **7**, **8** and **9**. To follow the conversion of 6-chloropurine riboside to 6- $^{15}\text{NH}_2$  labeled **1** by thin layer chromatography the solvent system methylene chloride / methanol, 8/2 (v/v) was used. For all other transformations isobutyric acid / 25 % aqueous  $\text{NH}_3$  /  $\text{H}_2\text{O}$ , 66/1/33 (v/v/v) was used as thin layer chromatography solvent system. Spots were qualitatively visualized under uv light at 254 nm and by spraying the plates with ninhydrin. Quantitative determinations were performed at the applicable  $\lambda_{\text{max}}$  by scanning using a Shimadzu CS-920 high speed scanner. Uv spectra were obtained in the standard medium 0.2 M potassium phosphate, pH 7.0, using a Perkin-Elmer 554 spectrophotometer. By adding 0.2 ml NaOH (10 M) the pH could be changed to 12. The concentrations of **1** and the other adenosine derivatives were determined spectrophotometrically at  $\lambda_{\text{max}}$  using the following molar absorption coefficient: **1**, **2** and **8**,  $15000 \text{ M}^{-1}\text{cm}^{-1}$  at 259 nm, **6** and **9**,  $19000 \text{ M}^{-1}\text{cm}^{-1}$  at 265 nm and **7**,  $16000 \text{ M}^{-1}\text{cm}^{-1}$  at 262 nm.



***N*(1)-(2AE)-Adenosine (2).** Ethyleneimine (1.1 ml, 21.45 mmol) was added slowly to a solution of unlabeled **(1)** (5.34 g, 20 mmol) in distilled water (21 ml), maintaining the pH at 3.5 with 70 % HClO<sub>4</sub> (total volume 22 ml). The reaction mixture was gently stirred in the dark for 8 h at 35 °C. After dilution to 50 ml with distilled water, the product was precipitated with cold technical grade ethanol at 4 °C (5 x 1 l). The precipitate was recovered by subsequent centrifugation. In this way unreacted ethyleneimine could be removed. The final precipitate was dried under vacuum at 25 °C and stored in a desiccator at 4 °C over NaOH. After thin layer chromatography and high speed scanning, it was determined that the precipitate contained **1** (3.1 mmol, 14.5 %, *r<sub>f</sub>* 0.66), **2** (10.6 mmol, 49.5 %, *r<sub>f</sub>* 0.46) and unidentified byproducts with lower *r<sub>f</sub>* values than that of **2**, that are probably further aminoethylated derivatives of the main product (totally 7.75 mmol, 36 %). Dry reaction mixture containing **2** (4.04 mmol) was dissolved in 80 ml water. After adjusting the pH to 3.5 with LiOH (5N), this solution was applied to a Chelex-100 cation exchange column (1.5 x 130 cm), preequilibrated against HCl (0.316 mM, pH 3.5) at 4°C. After elution with 1 l HCl (0.316 mM, pH 3.5), the column was eluted with a gradient 0-0.2 M CaCl<sub>2</sub> in HCl (0.316 mM, pH 3.5) (2.5 l). Fractions with pure **2** were collected (totally 850 ml) and concentrated by rotation evaporation to a final volume of 25 ml. CaCl<sub>2</sub> was removed by gel filtration by applying this concentrated fraction on a Sephadex G-10 column (5 x 100 cm), preequilibrated against HCl (0.316 mM, pH 3.5). After lyophilization, pure hydrochloride salt of **2** (0.55 g, overall yield 21.6 %) was obtained.

***N*<sup>6</sup>-(2AE)-Adenosine (7) and 1,*N*<sup>6</sup>-Ethanoadenosine (6).** **(2)** (191 mg, 0.61 mmol) was dissolved in 40 ml distilled water. After adjusting the pH to 8.0 with LiOH (1N), the solution was incubated at 50 °C for 8 h to convert **(2)** to *N*<sup>6</sup>-(2AE)-adenosine and 1,*N*<sup>6</sup>-ethanoadenosine. After thin layer chromatography and high speed scanning it was determined that the reaction mixture contained **7** (0.346 mmol, 56.8 %, *r<sub>f</sub>* 0.55) and **6** (0.264 mmol, 43.2 %, *r<sub>f</sub>* 0.40). The reaction mixture was lyophilized and stored over NaOH at 4 °C in a desiccator. Lyophilized reaction mixture containing **7** (0.181 mmol) and **6** (0.138 mmol) was dissolved in 3 ml distilled water. After adjusting the pH to 10.5 with LiOH (1N) the solution was applied to a Biorex-70 cation exchange column (1.5 x 60 cm), preequilibrated against H<sub>2</sub>O, pH 10.5 at 4 °C. After elution under

equilibrium conditions, fractions containing pure 6 were collected (totally 700 ml) and concentrated by rotation evaporation to 50 ml. After lyophilization pure 6 (40 mg, yield 9.1 %) was obtained. By applying a 0 - 0.5 M LiCl gradient (11 / 11) at pH 10.5 7 was eluted. Fractions with pure 7 were collected (totally 1 l) and concentrated by rotation evaporation to 25 ml. LiCl was removed by two precipitations with 600 ml cold technical grade ethanol and subsequent centrifugation. After dissolving the precipitate in 5 ml and lyophilization, pure 7 (54 mg, yield 11.7 %) was obtained.

**6-<sup>15</sup>NH<sub>2</sub>-Adenosine (<sup>15</sup>N-1).** <sup>15</sup>NH<sub>4</sub>Cl (3 g, 55 mmol) dissolved in 5 ml distilled water was added dropwise to a refluxing aqueous solution of NaOH (10.6 N, 14 ml with 148.4 mmol NaOH). The <sup>15</sup>NH<sub>3</sub> generated was swept for 1.5 h with nitrogen into 30 ml methanol incubated at -20 °C through the reflux condenser and two KOH drying tubes. The methanolic <sup>15</sup>NH<sub>3</sub> solution was added to a pressure proof glass tube containing 6-chloropurine riboside (1 g, 3.48 mmol). The reaction was incubated at 90 °C for 6 h with stirring (clear solution after 10 min). The reaction solution contained as main compounds 6-chloropurine riboside (0.786 mmol, 22.6 %, *r<sub>f</sub>* 0.48), (<sup>15</sup>N-1) (2.33 mmol, 67 %, *r<sub>f</sub>* 0.66 - same as unlabeled 1 -) and inosine (0.3 mmol, 7.3 %, *r<sub>f</sub>* 0.19). The composition did not change significantly after further incubation at 90 °C. The reaction solution was kept at -20 °C for 16 h to precipitate the nucleoside mixture. As described above <sup>15</sup>NH<sub>3</sub> was dissolved in the separated methanolic fraction. After adding this methanolic <sup>15</sup>NH<sub>3</sub> solution to the precipitated nucleosides this reaction solution was incubated at 90 °C for 8 h. The final reaction mixture contained 6-chloropurine riboside (0.6 mmol, 17.3 %) (<sup>15</sup>N-1) (2.62 mmol, 75.4 %) and inosine (0.3 mmol, 7.3 %). Any residual <sup>15</sup>NH<sub>3</sub> was removed by rotation evaporation together with methanol. The residue was dissolved in methanol by stirring at 100 °C (total volume 80 ml). No precipitation occurred after cooling to room temperature. 40 ml of the nucleoside solution was loaded on a silica gel-60 column (3.5 x 250 cm) equilibrated against methylene chloride / methanol, 8/2 (v/v), at room temperature. By elution with this solvent two separate peaks were obtained (respectively in 300 and 1000 ml). The first contained 6-chloropurine riboside. The first part of the second peak (600 ml) contained pure (<sup>15</sup>N-1), while the tail contained the latter compound contaminated with inosine. The fractions with pure (<sup>15</sup>N-1) were collected and the solvent removed by rotation

evaporation. This purification procedure was repeated for the other 40 ml nucleoside solution using a new silica gel column. Finally pure ( $^{15}\text{N-1}$ ) (571 mg) was obtained with an overall yield of 61.2 %.

**( $^{15}\text{N-2}$ ) and 6- $^{15}\text{NH}_2\text{-N(1)-(2-Aminoethylaminoethyl)-adenosine (8)}$ .** Ethyleneimine (0.5 ml, 10 mmol) was added slowly to a solution of ( $^{15}\text{N-1}$ ) (571 mg, 2.13 mmol) in distilled water (5 ml), maintaining the pH at 3.2 - 3.3 with 70 %  $\text{HClO}_4$  (total volume 6 ml). The reaction solution was gently stirred in the dark for 26.5 h at 30 °C, maintaining the pH in the range 3.2 - 3.3 with 70 %  $\text{HClO}_4$ . The reaction solution contained ( $^{15}\text{N-1}$ ) (0.128 mmol, 6 %,  $r_f$  0.66), ( $^{15}\text{N-2}$ ) (1.14 mmol, 53.5 %,  $r_f$  0.46) and byproducts with different  $r_f$  values lower than that of the main product (0.85 mmol, 40.3 %). The reaction mixture adjusted to pH 3.0 with HCl (5N) was loaded on a Chelex-100 column (3.5 x 200 cm) equilibrated against HCl (1mM, pH 3.0) at 4 °C. The column was eluted with a gradient 0 - 0.15 M  $\text{CaCl}_2$  in HCl (1 mM) (3.5 l). After 1 l a first main peak (in 1.5 l) could be eluted containing ( $^{15}\text{N-1}$ ), ( $^{15}\text{N-2}$ ) and minor amounts of ( $^{15}\text{N(1)-N}^6\text{-(2AE)-adenosine (}^{15}\text{N-7)}$ ) and tricyclic **6** together with some unreacted ethyleneimine ( $r_f$  0.85). The second peak (400 ml) appearing after elution of 3 l contained the main byproduct(**8**) with  $r_f$  0.22.

After concentration to 10 ml by rotation evaporation, the contents of both peaks were gelfiltrated on a Sephadex G-10 column (3.5 x 210 cm) equilibrated against HCl (1 mmol, pH 3.0) at 4 °C to remove salt. Gel filtration of the concentrated first peak lead to some fractionation. Successive fractions containing ( $^{15}\text{N-2}$ ) slightly contaminated with ( $^{15}\text{N-7}$ ) and **6** without and with unreacted ethyleneimine were separately pooled and lyophilized leading to respectively ethyleneimine free ( $^{15}\text{N-2}$ ) (106 mg with 3 % contamination) and ethyleneimine containing ( $^{15}\text{N-2}$ ) (260 mg with 4 % contamination). ( $^{15}\text{N-1}$ ) in the tailing fraction of the first peak points to a retardation presumably due to interaction with the Sephadex material. Ethyleneimine free ( $^{15}\text{N-2}$ ) without further purification was chosen for conversion to demonstrate the expulsion of the 6- $^{15}\text{NH}_2$ -group after tricyclization to **6** by  $^{15}\text{N}$  nmr. Gel filtration of the concentrated second peak led similarly to pure **8**. Totally, 105 mg **8** was obtained as hydrochloride salt.

**1,*N*<sup>6</sup>-Ethanoadenosine (6) from (<sup>15</sup>*N*-2).** Ethyleneimine free (<sup>15</sup>*N*-2) (10 mg, 0.032 mmol) dissolved in 2 ml 0.05 M sodium phosphate buffer, pH 7.5, was incubated for 2 h at 50 °C. Under these conditions a reaction mixture was obtained containing **6** (0.03 mmol, 94 %) and (<sup>15</sup>*N*-7) (0.002 mmol, 6 %). After lyophilization, the solid material was dissolved in 1 ml D<sub>2</sub>O for nmr.

**<sup>15</sup>*N*(1)-*N*<sup>5</sup>-(2-Aminoethylaminoethyl)-adenosine (9) from 8.** **8** (5 mg, 0.0143 mmol) was dissolved in 1 ml distilled water and in 1 ml 0.05 M sodium phosphate buffer, pH 7.0. In the first solution the pH was adjusted to 7.0 with NaOH (0.1 N). Both solutions were incubated at 50 °C for 8 h maintaining the pH at 7.0 with NaOH (0.01 N) in the case of the aqueous solution. For nmr experiments 10 mg (**8**) was dissolved in D<sub>2</sub>O and the pH adjusted to 7.5. For 35 h the reaction solution was incubated at 30 °C to attain 50 % conversion. For 100 % transformation incubation was continued for 22 h at 50 °C.

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