

Preparative Electrochemical Reduction of 2-Amino-6-chloropurine and Synthesis of 6-Deoxyacyclovir, a Fluorescent Substrate of Xanthine Oxidase and a Prodrug of Acyclovir[§]

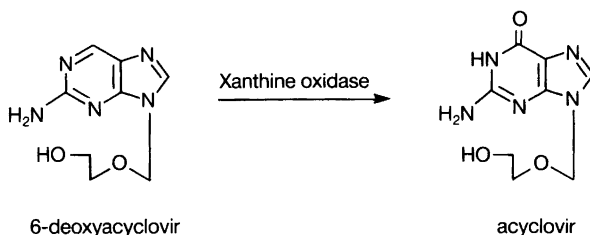
Jaroslaw T. Kusmierek,^a Barbara Czochralska,^b Nils Gunnar Johansson^c and David Shugar^{a,b,*}

^aInstitute of Biochemistry and Biophysics, Academy of Sciences, 02-532 Warszawa, ^bDepartment of Biophysics, Institute of Experimental Physics, University of Warsaw, 02-089 Warszawa, Poland and ^cDepartment of Antiviral Chemotherapy, Research and Development Laboratories, Astra Alab AB, S-151 85 Södertälje, Sweden

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D.c. polarography of 2-amino-6-chloropurine in aqueous medium over a broad pH range revealed two diffusion waves, the first of which corresponds to reduction of the C(6)–Cl bond, leading to formation of 2-aminopurine in high yield. Condensation of the sodium salt of 2-aminopurine with (2-acetoxyethoxy)methyl chloride led to the two isomeric 9- and 7-(2-hydroxyethoxymethyl)-2-aminopurines. The 9- isomer, 6-deoxyacyclovir, a prodrug of acyclovir previously synthesized by another route, was readily converted to the latter by xanthine oxidase; the 7- isomer was not a substrate. The intense fluorescence of 6-deoxyacyclovir makes it a convenient fluorescent substrate for xanthine oxidase, although less sensitive than xanthine; it is shown that 2-aminopurine would be a very sensitive fluorescent substrate. The polarographic behaviour of the riboside of 2-amino-6-chloropurine was virtually identical with that of the parent purine, leading to a simple procedure for conversion of 2-amino-6-chloropurine nucleosides and acyclonucleosides to the corresponding 2-aminopurine congeners.

The demonstration that 6-deoxyacyclovir is a good substrate of xanthine oxidase, which converts it intracellularly to the potent antiherpes agent acyclovir,¹ as shown in Scheme 1, underlines the utility of a convenient procedure for the large-scale preparation of this prodrug. A re-



Scheme 1.

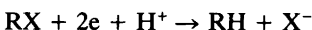
[§]Abbreviations: Acyclovir, 9-(2-hydroxyethoxymethyl) guanine; 6-deoxyacyclovir, 9-(2-hydroxyethoxymethyl)-2-aminopurine.

*To whom correspondence may be addressed.

ported synthesis¹ is based on the classical condensation of 2-amino-6-chloropurine (**1**) with the appropriate protected acyclic fragment, followed by removal of the 6-chloro substituent by catalytic reduction at high pressure.

An alternative pathway would be condensation of 2-aminopurine (**2**) with the protected acyclic fragment, but **2** is relatively expensive (and not readily available commercially), and its synthesis rather arduous. One might also carry out the condensation reaction with the more readily available, less expensive, 2-amino-6-chloropurine (cf. Krenitsky *et al.*)¹ followed by electrolytic, as compared to catalytic, reduction. Another possibility is simply electrochemical reduction of **1**, and subsequent conversion of the resulting **2** to its acyclonucleoside.

The use of electrochemical techniques in organic syntheses is a subject of growing interest, in part because of high yields and purity of the products.^{2,3} In the case of halogenated saturated and unsaturated compounds, removal of the halogen by controlled potential electrolysis usually proceeds smoothly, with few or no side reactions,⁴ via cleavage of the C–X (X = halogen) bond, with simultaneous transfer of 2 electrons as follows



and formation of a single, or single type of, product(s). Kinetic data for alkyl halide reduction by electrons transfer reagents have been interpreted in terms of the Marcus theory, postulating a transition state structure with an almost completely cleaved C-halogen bond.⁵

Electroreduction of halopyrimidines, including analogues of natural pyrimidines and their nucleosides, has been extensively investigated.⁶ However, relatively little attention has been devoted to the corresponding halopurines. Bearing in mind that reduction of purines usually occurs in the pyrimidine moiety, imidazoles being polarographically inert,⁷ it might be anticipated that reduction of 2-amino-6-chloropurine would proceed as for 2-amino-4-chloropyrimidine.⁸ A previous report described the zinc reduction of the riboside of 2-amino-6-chloropurine, followed by paper chromatographic isolation of the product, viz. the riboside of 2-aminopurine, in 70% yield.⁹ During the preparation of this manuscript, we were informed of a procedure based on the

photoinduced reductive dehalogenation of 2-amino-6-chloro-9-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)purine, also applicable on a preparative scale, to obtain 2-aminopurine riboside in about 70% yield.¹⁰

Results

D.c. polarography of **1** in aqueous medium revealed two diffusion waves, with a pH-dependence of $E_{1/2}$ and I_d as shown in Fig. 1. It should be noted that the pH-dependence of $E_{1/2}$ for wave II is virtually identical with that for **2**. Hence, as in the case of 2-amino-4-chloropyrimidine,⁸ it is wave I which controls reduction of the C–Cl bond.

Electrolysis of a 2×10^{-3} M solution of **1** at pH 2 or at pH 5, at potentials of -0.75 V or -1.0 V, respectively, led to disappearance of wave I, whereas the $E_{1/2}$ of wave II corresponded to that of **2**. The UV absorption spectrum of the electrolyzed solution corresponded to that for **2** at various pH values, and the isolated product was chromatographically identical with authentic **2** on TLC with several solvent systems. The product of electrolysis also exhibited the fluorescence typical for **2** (see below).

Electrolysis at the more acidic pH is advantageous because of the more positive reduction potential and elimination of buffer ions. In effect, replacement of buffer ions by 0.25 M HCl in preparative electrolysis facilitates isolation of the product simply by evaporation of solvent and crystallization of the residue. Preparative electroreduction of **1** under such conditions proved reproducible and, because of its simplicity and high yield (85%), should be of wider applicability. The scale on which such electroreduction may be carried out is limited largely by the volume of the electrolytic cell and the power supply current. Because of the interest attaching to **5** as a pro-drug of acyclovir, it would obviously be desirable to carry out this reaction on an even larger scale. Preliminary trials with aqueous DMF did not improve solubility, and further attempts in this direction are under way.

The range of applicability of this procedure is even broader than initially envisaged, since we have found that the polarographic behaviour of 9- β -D-ribofuranosyl-2-amino-6-chloropurine is almost identical to that of the parent aglycone. It

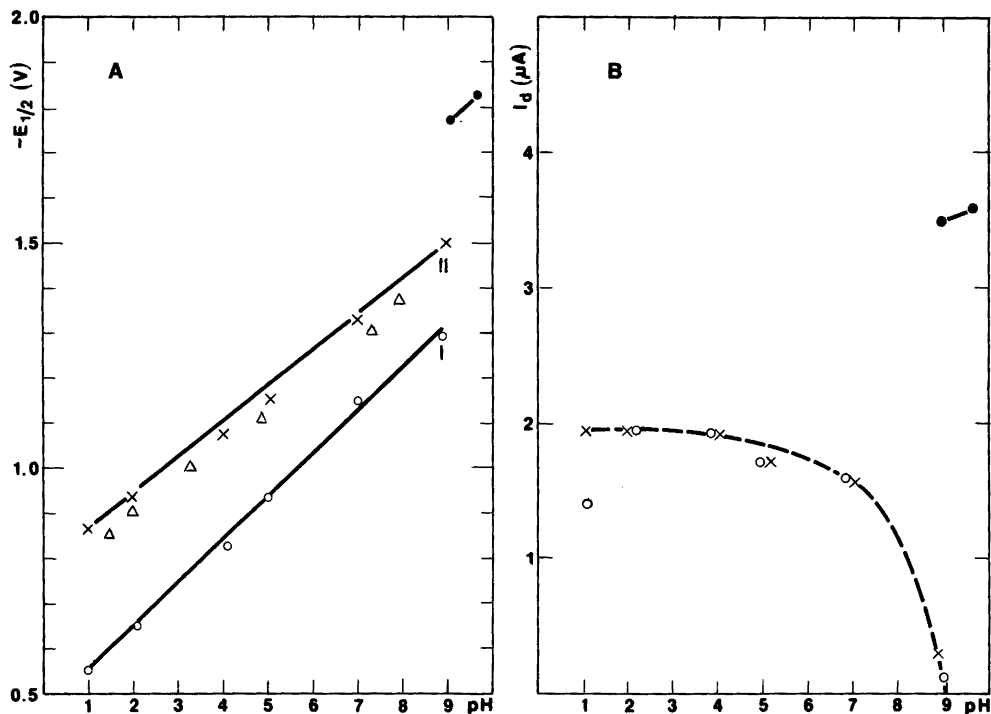


Fig. 1. pH-dependence of: (A) half-wave potential, $E_{1/2}$, and (B) limiting current, I_d , for 2-amino-6-chloropurine (4×10^{-4} M): $\circ\circ\circ\circ$, wave I; $\times\times\times\times$, wave II; and for 2-aminopurine (4×10^{-4} M): $\triangle\triangle\triangle\triangle$, wave 1; $\bullet\text{---}\bullet$, wave 2.

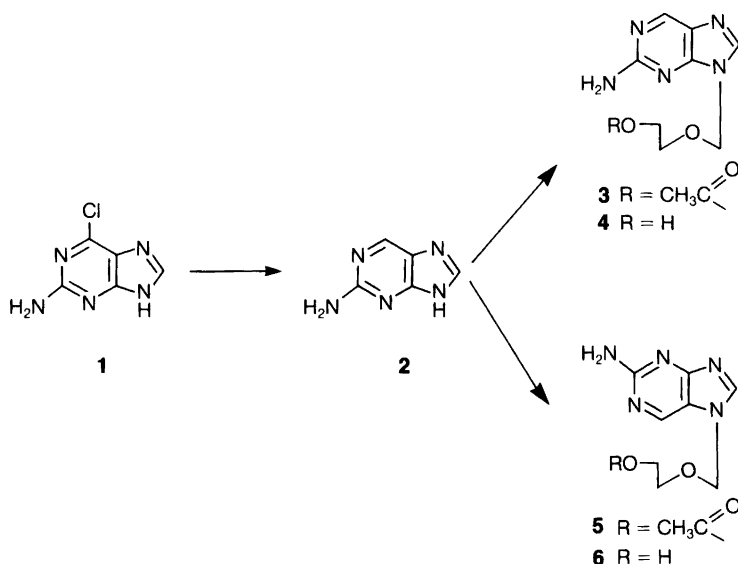
may therefore be applied to reduction of chloropurine nucleosides and analogues of these.

Condensation of the sodium salt of 2-aminopurine (2, Scheme 2) with (2-acetoxyethoxy)methyl chloride in dimethylformamide at room temperature gave, as the major product, the 9- isomer (3), and smaller proportions of several side-products, one of which was isolated and characterized as the 7- isomer (4). The isomers 3 and 4 were isolated by column chromatography on silica gel and obtained in crystalline form in yields of 25% and 5%, respectively. These products were deacetylated with Dowex (OH^-) at room temperature to yield the 9- and 7-(2-hydroxyethoxymethyl) derivatives 5 and 6, the structures of which were confirmed by elementary analysis, UV and NMR spectroscopy, and fluorescence spectroscopy.

The ^1H NMR spectra of 3 and 5 exhibit shifts of 8.2 ppm and 6.6 ppm, assigned to the C(8) and NH_2 protons, respectively. The corresponding shifts for 4 and 6 are 8.4 and 6.3, respectively. The relative positions of the regioisomeric signals

are in agreement with the spectra of a variety of alkylated 2-aminopurine and guanine derivatives,¹¹ for which the positions of alkylation at N(9) and N(7) were correlated with unequivocally prepared regioisomers.¹² It was then found that the H(8) signals for the N(9) isomers are shifted upfield relative to those for the corresponding N(7) isomers, while the NH_2 signals are shifted downfield for the N(9) isomer relative to that for the corresponding N(7) isomer.

Fluorescence emission properties. 2-Aminopurine and its N(9)-substituted analogues are known to be strongly fluorescent; 5, with a UV absorption maximum at 305 nm at pH 7, exhibited a strong emission band at 366 nm on excitation in the range 290–320 nm. With $\lambda_{\text{exc}} = 306$ nm, the fluorescence quantum yield was 0.6. For 4, with a UV absorption maximum at 316 nm, excitation in the range 300–330 nm gave a strong emission band centered at 380 nm, with a quantum yield (on excitation at 317 nm) essentially the same, i.e. 0.6. By comparison, both 9-ethyl-2-aminopurine



Scheme 2.

and the N(9)-ribose of 2-aminopurine, each with a UV absorption maximum at 303 nm, exhibit fluorescence maxima at 370 nm with quantum yields of 0.68.¹³

Since 9-substituted guanines do not exhibit fluorescence at neutral pH at room temperature, the xanthine oxidase oxidation of 6-deoxyacyclovir may be followed by monitoring the decrease in fluorescence emission at 366 nm. This was, in fact, observed. However, in accordance with the higher K_m (0.9 mM) for 6-deoxyacyclovir than that for xanthine¹ (0.008 mM), a 30-fold higher concentration of enzyme was required to monitor the reaction. Under the same conditions the N(7) isomer of 6-deoxyacyclovir exhibited no decrease in fluorescence intensity, even on incubation for up to 2 h, and is therefore not a substrate for xanthine oxidase.

In retrospect, bearing in mind that the K_m value for 2-aminopurine is identical with that for xanthine while its V_{\max} value is 20% of that for xanthine, it is this compound which, with its equally high fluorescence quantum yield, should be an ideal fluorescent substrate for xanthine oxidase.

Biological activity. With plaque assays in a system of HSV-1 infected Vero cells, all four 2-aminopurine derivatives at up to 100 μM concentrations showed no inhibition of HSV-1 plaque formation.

Under the same conditions, acyclovir at a concentration of 0.5 μM inhibited plaque formation by 50%.¹⁴

Topical treatment of the infected backs of guinea pigs with **5** and **3** led to a reduction in the cumulative scores from 23 and 22 to 15 and 18, respectively. This is identical to the results reported with acyclovir in this model, and indicates that **5** and **3** are both metabolically transformed to acyclovir.¹⁴ It follows that **3** also undergoes deacetylation under these *in vivo* conditions.

In contrast, the N(7) isomer (**6**) was totally inactive in both systems. It had, in fact, been shown by Krenitsky *et al.*¹ that 7- and 9-methyl purines are, in general, poor substrates of milk xanthine oxidase. It is, in fact, of interest that the N(9) isomer is a reasonable substrate, albeit not nearly as good as xanthine (see above).

It should, on the other hand, be noted that there are a number of reports on biological activities of 7-glycosylpurines; e.g., the nucleoside moiety of pseudovitamin B₁₂ is 7- α -D-ribofuranosyladenine,¹⁵ and it has been shown that 7- β -D-ribofuranosyl-3-deazaguanine is a potent antibacterial agent vs. several gram-negative strains, due to its cleavage to 3-deazaguanine in *E. coli* by a presumed phosphorylase.¹⁶ It has also been reported that N-deoxyribosyl transferase from both bacterial and mammalian cells can convert 3-deazaguanine to the 7- β -D-deoxyribofuranoside.¹⁶

Finally, a recent communication describes the intracellular cleavage of 9-alkylpurines to liberate free purine;¹⁷ although the nature of the enzyme system involved was not established, it clearly is not a conventional purine nucleoside phosphorylase, and it will be of interest to determine whether the N(7) isomer (**6**) is a substrate or an inhibitor of this enzyme system.

Experimental

2-Amino-6-chloropurine and its riboside were purchased from Sigma (St. Louis, MO, USA).

Direct current polarography was carried out with a Radiometer Polariter PO₄ instrument, the characteristics of the mercury electrode being $n = 2.54 \text{ mg s}^{-1}$, $t = 3.7 \text{ s}$ at 60 cm Hg. Curves were recorded at room temperature, and all potentials are relative to the saturated calomel electrode. Solutions were flushed with argon to remove oxygen.

Preparative electrolytic reduction was performed with a mercury electrode (surface area $\sim 50 \text{ cm}^2$) in a three-compartment cell at a constant potential controlled by a type OH-404/A Potentiostat (Radelkis, Hungary). The counter compartment contained a platinum electrode immersed in saturated KCl. Agar salt bridges were inserted on the reference and counter electrode sides of the medium, the compartments being separated by fine porosity glass frits. The cell was flushed with argon, and magnetic stirring was employed.

Fluorescence emission and excitation spectra (uncorrected) were recorded on an Aminco-Bowman spectrofluorimeter fitted with an Hanovia 901 xenon lamp as light source, and a Hamamatsu 1P28 photomultiplier as detector. Fluorescence quantum yields were measured relative to quinine sulphate ($\Phi = 0.55$).

Melting points (uncorr.) were measured on a Boetius hot-stage microscope.

Elementary analyses were performed by Mikrokemi AB (Uppsala, Sweden).

¹H NMR spectra of solutions in (CD₃)₂SO were recorded on a Jeol JNM-FX 200 instrument, and

chemical shifts are in ppm relative to internal TMS.

Herpes simplex virus (HSV-1) tests. Plaque assays were performed as described previously,¹⁸ using the HSV-1 strain C-42 in African green monkey kidney (Vero) cells.

In vivo tests. For cutaneous infections, guinea pigs were inoculated as described elsewhere¹⁹ with ca. 10⁵ PFU of HSV-1, strain C-42, applied to each of four sites on the back. The animals were treated three times daily with 25 μ l of a 5% (w/v) solution in dimethyl sulfoxide, starting 24 h after infection.

2-Aminopurine (2). A suspension of 1.5–5 g of 2-amino-6-chloropurine (**1**) in 500 ml of 0.25 M HCl was heated to 50°C, with stirring, in an electrochemical cell. Electrolysis was conducted at a potential of -0.75 V relative to the calomel electrode, the initial current being 50–60 mA. Following overnight electrolysis, a clear solution resulted, accompanied by a decrease in current. Electrolysis was continued until the current fell below 5 mA (additional 12 h), after which TLC demonstrated the presence of **2** as the major product ($R_f = 0.30$), with traces of **1** ($R_f = 0.45$) and of two side-products ($R_f = 0.0$ and 0.1).

The pooled solutions from several runs (total 14.5 g of **1**) were evaporated to dryness under reduced pressure and the residue taken up in 80 ml of boiling water with addition of 20 ml of methanol. To the dark solution was added 1 g of charcoal; after stirring, the latter was removed by filtration and washed with $3 \times 20 \text{ ml}$ of hot methanol. The combined filtrates were reduced in volume to 50 ml, giving a clear yellow solution. Addition of 20 ml of conc. NH₄OH, and storage overnight in an open vessel at room temperature led to the formation of a pale yellow precipitate. This was collected by filtration, washed with $2 \times 10 \text{ ml}$ of water and dried at 100°C. The product (9.85 g, 85%) was chromatographically homogeneous 2-aminopurine, in the form of an amorphous powder which was slowly converted to thick needles on heating to above 250°C; m.p. 281–283°C, as compared to 277–278°C reported by Lister.²⁰

9- and 7-(2-Acetoxyethoxymethyl)-2-aminopurine (3 and 4). To a suspension of 9.85 g (73 mmol) of

2 in 200 ml of anhydrous DMF was added, portionwise, 3.5 g (~85 mmol) of NaH (as a 50–60% suspension in oil). The mixture was stirred at room temperature; after liberation of hydrogen had ceased (~30 min), 10.3 ml (80 mmol) of (2-acetoxyethoxy)methyl chloride was added. After 5 min, TLC demonstrated the disappearance of **2** ($R_f = 0.30$). After 15 min, acetic acid (~1 ml) was added to bring the pH to about 5. The mixture was then filtered and the precipitate washed with 2×10 ml of dimethylformamide. TLC of the combined filtrates indicated two major products ($R_f = 0.70$ and 0.40) corresponding to **3** and **4**, respectively, and several minor products ($R_f < 0.3$) more polar than **2**. The mixture was evaporated under reduced pressure to an oil (~30 ml) and loaded on a 3.5×27 cm column of MN-Kieselgel 60 (0.05–0.2 mm). The column was washed with 500 ml of methylene chloride and eluted successively with 2-propanol in methylene chloride as follows: 2.5% (500 ml), 10% (1000 ml), 15% (1000 ml) and 50% (1000 ml). Fractions of 250 ml were collected and examined by TLC.

Fractions 2–10 were brought to dryness and the residue crystallized from 2-propanol to yield 4.06 g (22%) of virtually pure **3**, which was subjected to deacetylation without further purification. An analytical sample of **3**, in the form of colourless needles, m.p. 135–136°C, was obtained by recrystallization from 2-propanol with addition of charcoal.

Fractions 11–16 were brought to dryness and the residue crystallized twice from 2-propanol:methanol (2:1, v/v) with addition of charcoal to give 0.57 g (3%) of chromatographically homogeneous **4**, m.p. 180–182°C.

The mother liquors from the foregoing were combined with fractions 17–22, brought to dryness, and chromatographed on silica gel, as above, to yield an additional 0.6 g (3%) of **3** and 0.38 g (2%) of **4**. Overall yields were 25% (**3**) and 5% (**4**). Anal. $C_{10}H_{12}N_5O_3$ (**3** and **4**): C, H, N. 1H NMR (**3**), δ (ppm): 1.94 (s, CH_3), 3.69 (b, CH_2O), 4.08 (b, CH_2OCO), 5.48 (s, NCH_2), 6.62 (s, NH_2), 8.20 (s, H-8), 8.61 (s, H-6); (**4**): 1.92 (s, CH_3), 3.62 (b, CH_2O), 4.05 (b, CH_2OCO), 5.63 (s, NCH_2), 6.30 (s, NH_2), 8.43 (s, H-8), 8.68 (s, H-6).

9-(2-Hydroxyethoxymethyl)-2-aminopurine (6-deoxyacyclovir, **5**). To a solution of 4.5 g (18

mmol) of **3** in 150 ml of 50% aqueous methanol was added 50 ml of 200/400 mesh Dowex 1×4 (OH^-); the slurry was stirred overnight at room temperature, then reduced to half-volume and loaded on a 2.6×60 cm column of Dowex 1×4 (OH^-). Elution with water, and collection of 25 ml fractions every 6 min, gave the desired product in fractions 13–14 (~90% UV-absorbing material). These were combined, brought to dryness, and the residue crystallized from 2-propanol:methanol (1:1) to yield 2.86 g (76%) of **5** as colourless needles, m.p. 193.5–194.5°C (lit.¹ 187–189°C). Anal. $C_8H_{11}N_5O_2$: C, H, N. 1H NMR, δ (ppm): 3.49 (s, CH_2CH_2), 4.68 (b, OH), 5.47 (s, NCH_2), 6.60 (s, NH_2), 8.19 (s, H-8), 8.61 (s, H-6).

7-(2-Hydroxyethoxymethyl)-2-aminopurine (**6**). To 0.36 g (1.4 mmol) of **4** in 50% ethanol was added 5 ml of Dowex 1×4 (OH^-); the slurry was stirred for 30–60 min (prolonged deacetylation led to appearance of side products) and loaded on a 2×25 cm column of Dowex 1×4 (OH^-). The column was eluted with water, with collection of 25 ml fractions every 6 min. Fractions 3–10, containing the major component, were brought to dryness and the residue crystallized from 10 ml of 95% ethanol to give 0.14 g (48%) of **6** in the form of needles, m.p. 183–185°C. Anal. $C_8H_{11}N_5O_2$: C, H, N. 1H NMR, δ (ppm): 3.44 (s, CH_2CH_2), 4.69 (b, OH), 5.62 (s, NCH_2), 6.29 (s, NH_2), 8.42 (s, H-8), 8.69 (s, H-6).

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