REACTION OF 2,2'-ANHYDRO-1-(β-D-ARABINOFURANOSYL)-6-AZAURACIL, 4-CHLOROPYRIMIDINE AND 6-CHLOROPURINE NUCLEOSIDES WITH AMINO ACIDS*

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Reaction of cycloazauridine *I* with glycine and L-lysine in water at pH 10 afforded the glycine derivative *II* and the N^{ε}-lysine derivative *III*, respectively. An identical sample of *III* was prepared by reaction of N^{α}-formyl-L-lysine with *I* followed by deformylation of the formed *IV*. L-Arginine reacts with *I* in water to give the N^{α}-derivative *V*. Under analogous conditions, 6-chloro-9-β-D-ribofuranosylpurine and L-lysine afford the N^{ε}-derivative *X*. Reaction with N^{γ}- and N^{ε}-formyl-L-lysine at pH 10 leads to the N^{ε}- and N^{α}-ribosylpurinyl derivatives *XI* and *XII* which are deformylated with hydrochloric acid to compounds *X* and *XIII*. Benzyl glycinate reacts with 4-chloro-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)- and 4-chloro-1-(2,3,5-tri-O-benzoyl-β-D-arabinofuranosyl)pyrimidin-2(1*H*)-one in chloroform to give benzyl N-(1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrimidin-2(1*H*)-on-4-yl)glycinate (*VI*) and N-(1-(2,3,5-tri-O-benzoyl-β-D-arabinofuranosyl)pyrimidin-2(1*H*)-on-4-yl)glycinate (*VIII*). Their methanolysis with sodium methoxide afforded the free methyl glycinates *VII* and *IX*. The reaction of poly(L-lysine) with *I* and 6-chloro-9-β-D-ribofuranosylpurine was investigated.

Studies of reaction of pyrimidine cyclonucleosides with ammonia[1] have proved high reactivity of O^2,2'-cyclo-6-azauridine (*I*). The marked difference between the reactivities of uracil and 6-azauracil derivatives was also observed in the reaction of cyclonucleosides with primary aliphatic and aromatic amines[2] leading to isocytosine derivatives, again with a much higher reactivity of the 6-aza compound. In the reaction with aqueous ammonia, cycloazauridine *I* is practically solely ammonolyzed to arabinosylisoazacytosine whereas, under the same reaction conditions, cyclouridine is hydrolyzed to arabinosyluracil[1]. According to these results it is assumed that cycloazauridine *I* *in vivo* could interact with proteins of the organism. It was therefore desirable to prove that cycloazauridine *I* can react with the free amino group of amino acids or proteins.

It is known from the literature that the relatively high reactivity of the chlorine atom in 6-chloropurine derivatives enables the preparation of purine analogues

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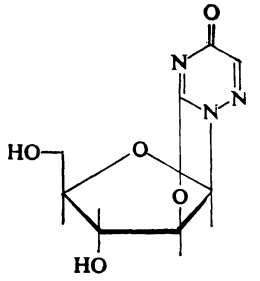
with a bonded amino acid[3-14] such as purinylamino acids[3-7], N-(9-ribofuranosylpurin-6-yl)amino acids[8-11] or N-(2-amino-9-ribofuranosylpurin-6-yl)glycine[12]. Reaction of polylysine with 6-chloropurine[13] and 6-chloro-9-ribosylpurine[14] has been also described as well as reaction of cytosine, cytidine and its 5'-phosphate with amino acids in the presence of bisulfite, affording N^4-substituted cytosine derivatives[15,16].

We made use of these results for the preparation of pyrimidine and purine amino acid derivatives. Glycine reacted with cycloazauridine *I* in water at pH 10 to give the glycine derivative *II* in 71% yield. Reaction of *I* with L-lysine in an aqueous solution afforded 80% of the N^{ε}-derivative *III*. We proved by an unequivocal synthesis that the nucleoside is bonded to the lysine N^{ε}-nitrogen. N^{α}-Formyl-L-lysine with the cyclonucleoside *I* in water at pH 10 afforded the formyl derivative *IV*. The lower yield (55%) was due to a concurrent hydrolysis of *I* to 2-(β-D-arabinofuranosyl)-1,2,4-triazine-3,5(2*H*,4*H*)-dione. The compound *IV* was deformylated with 1M-HCl to give the compound *III*. Arginine reacted with *I* under formation of the N^{α}-derivative *V* (29%), again with simultaneous hydrolysis of *I* to 2-(β-D-arabinofuranosyl)-1,2,4-triazine-3,5(2*H*,4*H*)-dione (69%).

In alkaline solutions, isocytosine and N-alkylisocytosine derivatives are hydrolyzed to arabinosyl-6-azauracil. On the other hand, in acidic media the 2,2'-anhydro bond is again closed, more smoothly in the uracil than in the 6-aza series[15,16]. In 0·1M-NaOH the amino acid derivatives *II*, *III* and *V* are completely hydrolyzed after 20 h at room temperature whereas in acidic media they are stable.

The reaction of poly(L-lysine hydrochloride) with *I* in water was followed after 45% neutralization with sodium hydroxide (pH 10·1) as well as after 68% neutralization (pH 10·7), using an equimolar ratio of the reactants (calculated for monomeric units) in concentration 0·1 mol l^{-1}. In the first case the equilibrium was achie-



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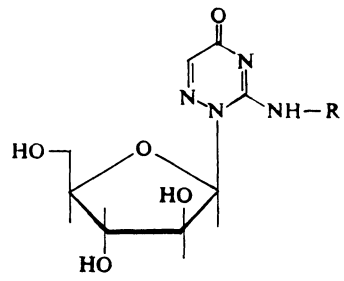
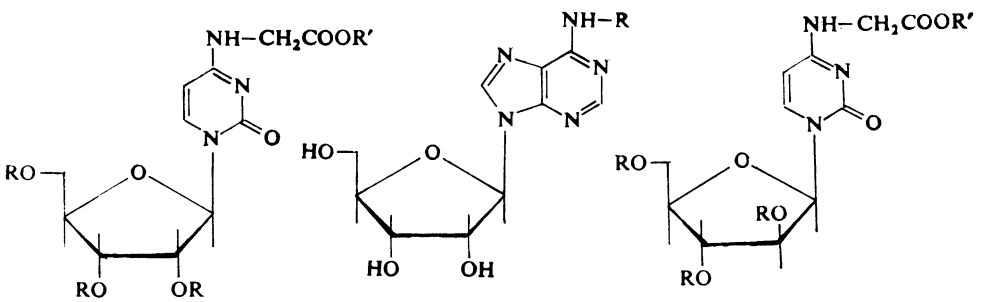


II, R = $-CH_2COOH$
III, R = $-(CH_2)_4CH(NH_2)COOH$
IV, R = $-(CH_2)_4CH(COOH)NHCHO$
V, R = $-CH(COOH)(CH_2)_3NH-C(=NH)-NH_2$

ved at 26% of the reacted compound *I* (13% of *I* reacting in 2 minutes), in the second case (pH 10·7) at 39% of *I* (19·5% in 0·5 min). Attempted isolation of the pure reaction product of poly(L-lysine) and *I* was unsuccessful since already during dialysis the product was hydrolyzed to give *I* and arabinosyl-6-azauracil.

Further nucleoside derivatives, potentially useful for the reaction with amino acids are 1-ribosyl- or 1-arabinosyl-4-chloropyrimidin-2-one and 6-chloro-9-ribosylpurine. Because of high reactivity of 4-chloropyrimidine nucleosides the reaction can be performed only in a non-aqueous medium and with protected reactants. Reaction of 4-chloro-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-pyrimidin-2(1*H*)-one with benzyl glycinate in chloroform afforded in 27% yield the protected derivative *VI* which was methanolyzed to the free compound *VII* in 79% yield. The same conditions were used in the preparation of the protected arabinosyl derivative *VIII* and its methanolysis to the free compound *IX*.

Thanks to the lower reactivity of 6-chloro-9-ribosylpurine the reaction can be performed in an aqueous medium. The compound reacts with 4 equivalents of L-lysine in water at room temperature affording *X* in 58% yield. Japanese authors[11] prepared *X* by reaction of 6-chloro-9-ribosylpurine with N^{α}-acetyllysine in water in the presence of sodium carbonate at 100°C. Reaction of 6-chlororibofuranosylpurine with N^{α}- and N^{ε}-formyl-L-lysine in an aqueous solution at pH 10 afforded the respective N^{ε}- and N^{α}-ribosylpurinyl derivatives *XI* and *XII* which on deformylation with 1M-HCl gave the N^{ε}- and N^{α}-derivatives *X* and *XIII*.



VI, R = benzoyl, R′ = benzyl
VII, R = H, R′ = CH_3

VIII, R = benzoyl, R′ = benzyl
IX, R = H, R′ = CH_3

X, R = $-(CH_2)_4CH(NH_2)COOH$)
XI, R = $-(CH_2)_4CH(COOH)NHCHO$
XII, R = $—CH(COOH)(CH_2)_4NHCHO$
XIII, R = $-CH(COOH)(CH_2)_4NH_2$

The N^{α}- and N^{ε}-derivatives were distinguished on the basis of the fact that the latter form complexes with copper(II) ions whereas the former do not. The cupric

complexes of the N^{ε}-derivatives were detected by their characteristic absorption[17] at 641 nm in the visible spectrum. Also the reaction of the N^{ε}-derivatives with ninhydrin was much faster and more intensive than that of the N^{α}-derivatives.

Reaction of poly(L-lysine hydrochloride) with chlororibosylpurine in water at room temperature in the presence of 2-equivalents of sodium hydroxide afforded a water-insoluble polymer. Comparison of extinction measurements after acid hydrolysis (with 6M-HCl at 110°C) of the polymer and of compound *X* shows that one ribosylpurine unit is bonded to every 8th or 9th unit of lysine. Reaction of poly(L-lysine) with chlororibosylpurine in boiling water was described in the literature[13].

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). The UV spectra were recorded on a Specord apparatus. Optical rotations were measured on an automatic Perkin–Elmer 141 MC polarimeter. The ^{1}H NMR spectra were recorded on Tesla BS 467 60 MHz and Tesla BS 497 100 MHz instruments, using tetramethylsilane as internal standard; chemical shifts (δ values) are expressed in p.p.m. and coupling constants in Hz. Column chromatography was performed on Pitra silica gel (particle size 30–60 μm, produced by Service Laboratories of this Institute) and thin-layer chromatography on ready-for-use Silufol^{R} (Kavalier Glassworks, Votice, Czechoslovakia) silica gel sheets in the solvent systems: S_1, 2-propanol–dioxane–water–26% aqueous ammonia (12 : 5 : 5 : 3) and S_2, 2-propanol–ethyl acetate–water (14 : 5 : 6). Chromatographic mobilities are given in Table I. Poly(L-lysine hydrochloride) (m.w. ~40 000) was prepared by Service Laboratories of this Institute. Dialysis was performed with Dialysierschlauch, Kalle Aktiengesellschaft, Wiesbaden-Biebrich (diameter 17 mm).

N-(2-(β-D-Arabinofuranosyl)-1,2,4-triazine-3,5-(2*H*,4*H*)-dion-3-yl)glycine (*II*)

Cycloazauridine *I* (ref.[18]; 227 mg; 1 mmol) was dissolved in a solution of glycine (316 mg; 4 mmol) in water (2·5 ml) which had been adjusted to pH 10 with conc. sodium hydroxide solution. After standing at room temperature for 6 h, the solution was neutralized with Dowex 50 (H^+-form, 15 ml) which was then filtered and washed with water (50 ml). The combined filtrates were taken down *in vacuo* and the residue was crystallized from aqueous ethanol, affording 180 mg (59·5%) of *II*, m.p. 177–179°C. Mother liquors on crystallization gave 36 mg (12%) of the product, $[\alpha]_D^{25}$ −42° (*c* 0·4; water); UV spectrum (water): λ_{max} 216 nm (log ε 4·36), λ_{sh}

TABLE I

Chromatographic mobilities of amino acid derivatives of nucleosides (R_F in the systems S_1 and S_2)

Compound	*II*	*III*	*IV*	*V*	*VII*	*IX*	*X*	*XI*	*XII*	*XIII*
S_1	0·36	0·26	0·44	0·13	0·55	0·61	0·34	0·48	0·50	0·17
S_2	0·47	0·26	0·51	0·24	0·59	0·65	0·28	0·54	0·56	0·15

250 nm (log ε 3·88). ^{1}H NMR spectrum (60 MHz, hexadeuteriodimethyl sulfoxide-deuteriochloroform): 3·63 (s, 2 H, $H_{5'}$), 3·87 (d, 2 H, —CH_2—, $J_{CH_2,NH}$ = 5), 5·93 (d, 1 H, $H_{1'}$, $J_{1',2'}$ = = 6), 7·35 (s, 1 H, H_5), 8·03 (broad t, 1 H, —NH—, J_{NH,CH_2} = 5); after exchange with ^2H_2O: 3·63 (s, 2 H, $H_{5'}$), 3·87 (s, 2 H, —CH_2—), 5·93 (d, 1 H, $H_{1'}$, $J_{1',2'}$ = 6), 7·35 (s, 1 H, H_5). For C_{10}·.$H_{14}N_4O_7$ (302·2) calculated 39·74% C, 4·67% H, 18·53% N; found: 40·04% C, 4·67% H, 18·69% N.

According to TLC, the mother liquors contained only 2-(β-D-arabinofuranosyl)-1,2,4-triazine-·3,4(2*H*,4*H*)-dione in addition to small amount of *II*.

N$^\varepsilon$-(2-(β-D-Arabinofuranosyl)-1,2,4-triazine-3,5(2*H*,4*H*)-dion-3-yl)-L-lysine (*III*)

A) The cycloazauridine *I* (227 mg; 1 mmol) was dissolved with stirring in a solution of L-lysine (585 mg; 4 mmol) in water (3 ml). After 1 h the solution was neutralized with IRC-50 (H^+-form), the resin was filtered through a thin layer of Celite and washed with water (50 ml). The combined filtrates were taken down *in vacuo*, the residue was dissolved in water (3 ml) and 2-propanol was gradually added to the hot solution. The separated product *III* (314 mg; 80%) melted at 176 to 177°C (dec.); $[\alpha]_D^{25}$ −31° (*c* 0·4; water). UV spectrum (water): λ_{max} 215 nm (log ε 4·36), λ_{sh} 248 nm (log ε 3·90); copper complex: λ_{max} 641 nm (log ε 1·78). ^{1}H NMR spectrum (60 MHz, hexadeuteriodimethyl sulfoxide): 1·50 (m, 6 H, —$(CH_2)_3$—), 3·23 (m, 2 H, $NHCH_2$), 3·60 (m, 3 H, 2 $H_{5'}$, CH), 5·92 (d, 1 H, $H_{1'}$, $J_{1',2'}$ = 6), 7·32 (s, 1 H, H_5). For $C_{14}H_{23}N_5O_7.H_2O$ (391·4) calculated: 42·96% C, 6·44% H, 17·90% N; found: 42·91% C, 6·45% H, 17·77% N. Chromatography of the concentrated mother liquors on a column of silica gel (30 g) in ethyl acetate–-acetone–ethanol–water (18 : 3 : 2 : 2) afforded 9 mg (4%) of 2-(β-D-arabinofuranosyl)-1,2,4-·-triazine-3,5(2*H*,4*H*)-dione. Further elution with the system S_2 gave 20 mg (5%) of *III*.

B) A solution of the formyl derivative *IV* (100 mg; 0·24 mmol) in 1M-HCl (2 ml) was allowed to stand at room temperature for 3 days, applied on a column of Dowex 3 (CH_3COO^--form 15 ml) and eluted with water. The UV-absorbing fraction was taken down *in vacuo* and the residue was chromatographed on a column of silica gel (12 g) in 2-propanol–ethyl acetate–water (14 : 4 : 7). Crystallization of the UV-absorbing fraction from water–2-propanol gave 74 mg (79%) of *III*, m.p. 175—177°C.

N$^\varepsilon$- 2-(β-D-Arabinofuranosyl)-1,2,4-triazine-3,5(2*H*,4*H*)-dion-3-yl)-N$^\alpha$-formyl-L-lysine (*IV*)

A solution of N$^\alpha$-formyl-L-lysine[22] (700 mg; 4 mmol) in water (3 ml) was adjusted to pH 10 with concentrated sodium hydroxide. The cycloazauridine *I* (227 mg; 1 mmol) was added, the solution being kept at pH 10 in the course of 6 h. The solution was applied on a column of Dowex 50 (H^+-form; 5 ml) and eluted with water until the UV absorption of the eluate disappeared. The eluate was taken down and the residue chromatographed on a column of silica gel (30 g) in the system S_2. The first UV-absorbing fraction gave 90 mg (37%) of 2-(β-D-arabinofuranosyl)-·-1,2,4-triazine-3,5(2*H*,4*H*)-dione, the second fraction furnished the monohydrate of *IV* (230 mg; 55%) as a foam. UV spectrum (water): λ_{max} 212 nm (log ε 4·39), λ_{sh} 245 nm (log ε 3·88). IR spectrum (KBr): 1 728 cm^{-1} (COOH), 1 650 cm^{-1} (amide I), 1 560 cm^{-1} (amide II), 1 569 and 1 505 cm^{-1} (C=N). ^{1}H NMR spectrum (100 MHz, hexadeuteriodimethyl sulfoxide): 1·10—1·86 (m, 6 H, —$(CH_2)_3$—), 3·19 (m, 2 H, $NHCH_2$), 3·65 (s, 3 H, 2 $H_{5'}$, CH), 5·92 (d, 1 H, $H_{1'}$, $J_{1',2'}$ = 6·5), 7·29 (s, 1 H, H_5), 7·61 (broad s, 1 H, N$^\varepsilon$H), 8·05 (s, 1 H, CHO), 8·26 (d, 1 H, N$^\alpha$H, $J_{NH,CH}$ = 8). For $C_{15}H_{23}N_5O_8.H_2O$ (419·4) calculated: 42·95% C, 6·01% H, 16·70% N; found: 42·76% C, 6·09% H, 16·56% N.

N^{α}-(2-(β-D-Arabinofuranosyl)-1,2,4-triazine-3,5(2*H*,4*H*)-dion-3-yl)-L-arginine (*V*)

A solution of L-arginine (871 mg; 5 mmol) and the cycloazauridine I (227 mg; 1 mmol) in water (3 ml) was set aside at room temperature for 1 h and neutralized with Amberlite IRC-50 (H^+-form; 10 ml). The ion-exchange resin was filtered, washed with water (100 ml) and the combined filtrates were taken down *in vacuo*. The residue was chromatographed on a column of silica gel (25 g). Elution with ethyl acetate–acetone–ethanol–water (15 : 3 : 4 : 3) afforded 150 mg (61%) of 2-(β-D-arabinofuranosyl)-1,2,4-triazine-3,5(2*H*,4*H*)-dione. Further elution with 70% aqueous methanol, followed by crystallization from water–methanol–2-propanol, gave the monohydrate of *V* (122 mg; 29%), decomposing at 235°C; $[\alpha]_D^{25}$ 2·9° (*c* 0·3; water). UV spectrum (water): λ_{max} 216 nm (log ε 4·40), λ_{sh} 250 nm (log ε 3·95). For $C_{14}H_{23}N_7O_7.H_2O$ (419·4) calculated: 40·09% C, 6·01% H, 23·38% N; found: 40·39% C, 5·82% H, 23·13% N.

Benzyl N-(1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)pyrimidin-2(1*H*)-on-4-yl)glycinate (*VI*)

1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-4-chloropyrimidin-2(1*H*)-one[20] (1·72 g; 3 mmol) was added to a solution of benzyl glycinate[19] (prepared from 2·53 g (7·5 mmol) of its *p*-toluenesulfonate) in chloroform (20 ml) and the mixture was stirred until the mixture was homogeneous. After standing for 2 h at room temperature, the solution was diluted with chloroform (40 ml), washed with water (3 × 10 ml), saturated sodium chloride solution (10 ml), dried over magnesium sulfate and taken down *in vacuo*. The residue was chromatographed on a column of silica gel (150 g) in ethyl acetate–toluene (4 : 1), affording 1·52 g (72%) of *VI*. For $C_{39}H_{33}N_3O_{10}$ (703·7) calculated: 66·56% C, 4·72% H, 5·97% N; found: 66·80% C, 4·85% H, 5·72% N.

Methyl N-(1-β-D-Ribofuranosylpyrimidin-2(1*H*)-on-4-yl)glycinate (*VII*)

A solution of the benzoate *VI* (704 mg; 1 mmol) in 0·1 mol l^{-1} methanolic sodium methoxide (15 ml) was set aside for 2 h at room temperature and neutralized with Dowex 50 (H^+-form pre-washed with methanol). The resin was filtered, washed with methanol (50 ml) and the combined filtrates were concentrated *in vacuo*. Crystallization of the residue from methanol afforded 263 mg (79%) of *IX* as the monohydrate, m.p. 106–109°C; $[\alpha]_D^{25}$ 31·4° (*c* 0·45; water). UV spectrum (water): λ_{max} 236 and 272 nm (log ε 3·95 and 4·04), λ_{min} 226 and 251 nm (log ε 3·93 and 3·90). ^{1}H NMR spectrum (60 MHz, hexadeuteriodimethyl sulfoxide): 3·63 (s, 5 H, CH_3, $H_{5'}$), 3·77–4·13 (m, 5 H, $H_{2'}$, $H_{3'}$, $H_{4'}$, $—CH_2—$), 4·95–5·37 (m, 3 H, OH), 5·77 (d, 1 H, $H_{1'}$, $J_{1',2'} = 4$), 5·87 (d, 1 H, H_5, $J_{5,6} = 7$), 6·22 (d, 1 H, H_6, $J_{6,5} = 7$), 8·13 (broad t, 1 H, NH, $J_{NH,CH_2} = 5·5$). For $C_{12}H_{17}N_3O_7.H_2O$ (333·3) calculated: 43·24% C, 5·75% H, 12·61% N; found: 43·20% C, 5·71% H, 12·55% N.

Benzyl N-(1-(2,3,5-Tri-O-benzoyl-β-D-arabinofuranosyl)pyrimidin-2(1*H*)-on-4-yl)glycinate (*VIII*)

The title compound was prepared from 4-chloro-1-(2,3,5-tri-O-benzoyl-β-D-arabinofuranosyl)-pyrimidin-2(1*H*)-one (prepared according to ref.[20]; 1·15 g; 2 mmol) and benzyl glycinate (5 mmol) by the same procedure as described for compound *VIII*; yield 806 mg (57%) after crystallization from toluene; m.p. 187–189°C. For $C_{39}H_{33}N_3O_{10}$ (703·7) calculated: 66·56% C, 4·72% H, 5·97% N; found: 66·47% C, 4·80% H, 5·88% N.

Methyl N-(1-β-D-Arabinofuranosylpyrimidin-2(1*H*)-on-4-yl)glycinate (*IX*)

The compound *IX* was prepared by methanolysis of the benzoate *VIII* (704 mg; 1 mmol) as described for *VII*; yield 304 mg (91%); m.p. 210·5–211·5°C (methanol). UV spectrum (water):

λ_{max} 235 and 274 nm (log ε 3·96 and 4·06), λ_{min} 227 and 250 nm (log ε 3·94 and 3·87). ^{1}H NMR spectrum (60 MHz, hexadeuteriodimethyl sulfoxide): 3·65 (s, 5 H, CH_3, $H_{5'}$), 3 77—4·17 (m, 5 H, $H_{2'}$, $H_{3'}$, $H_{4'}$, —CH_2—), 4·90—5·53 (m, 3 H, OH), 5·82 (d, 1 H, H_5, $J_{5,6}$ = 7), 6·00 (d, 1 H, $H_{1'}$, $J_{1',2'}$ = 3), 7·60 (d, 1 H, H_6, $J_{6,5}$ = 7), 8·02 (broad t, 1 H, J_{NH,CH_2} = 6). For $C_{12}H_{17}N_3O_7 . H_2O$ (333·3) calculated: 43·24% C, 5·75% H, 12·61% N; found: 43·36% C, 5·69% H, 12·44% N.

N$^{\varepsilon}$-(9-β-D-Ribofuranosylpurin-6-yl)-L-lysine (*X*)

A) 6-Chloro-9-ribofuranosylpurine[21] (143 mg; 0·5 mmol) was added to a solution of L-lysine (292 mg; 2 mmol) in water (3 ml). The mixture was stirred at room temperature for 6 h, diluted with water (15 ml), neutralized with Amberlite IRC-50 (H^+-form; 2·5 ml) and filtered through Celite which was then washed with water (100 ml). The combined filtrates were taken down *in vacuo* and the residue was mixed with methanol (8 ml). The separated solid was filtered and crystallized from water to give 120 mg (58%) of *X*, m.p. 197—200°C (dec.) (reported[11] m.p. 197—203°C).

B) A solution of the formyl derivative *XI* (100 mg; 0·23 mmol) in 1M-HCl (2 ml) was left to stand for 6 days at room temperature, applied on a column of Dowex 3 (CH_3COO^--form; 15 ml) and eluted with water. The UV-absorbing fraction was taken down *in vacuo* and the crystalline residue was boiled with methanol (1 ml), cooled and filtered. Crystallization from water afforded 74 mg (79%) of the compound *X*, m.p. 198—200° (dec.).

N$^{\alpha}$-Formyl-N$^{\varepsilon}$-(9-β-D-ribofuranosylpurin-6-yl)-L-lysine (*XI*)

6-Chloro-9-ribosylpurine (143 mg; 0·5 mmol) was added to a stirred solution of N$^{\alpha}$-formyl-L-lysine (350 mg; 2 mmol) in water (2 ml) which had been adjusted to pH 10 with concentrated sodium hydroxide solution. The mixture was stirred at room temperature for 24 h, the pH value being kept at 10. The formed solution was applied on a column of Dowex 50 (H^+-form; 15 ml) which was eluted with water (50 ml) and then with 2·5% aqueous ammonia. The UV-absorbing fraction was taken down and the residue chromatographed on a column of silica gel (30 g) in ethyl acetate–acetone–ethanol–water (15 : 3 : 4 : 3). The main, UV-absorbing, fraction was concentrated and the residue crystallized from methanol, giving monohydrate of the formyl derivative *XII* (151 mg; 68%), m.p. 140—143°C. UV spectrum (water): λ_{max} 266 nm (log ε 4·24), λ_{min} 230 nm (log ε 3·39). IR spectrum (KBr): 1 729 cm^{-1} (COOH), 1 671 cm^{-1} (amide I), 1 535 cm^{-1} (amide II). ^{1}H NMR spectrum (60 MHz, hexadeuteriodimethyl sulfoxide): 1·10 to 1·90 (m, 6 H, —$(CH_2)_3$—), 3·19—3·75 (m, 5 H, $NHCH_2$, CH, 2 $H_{5'}$), 5·88 (d, 1 H, $H_{1'}$, $J_{1',2'}$ = 6), 7·83 (broad s, 1 H, N$^{\varepsilon}$H), 8·03 (s, 1 H, CHO), 8·18 and 8·33 (2 s, 3 H, H_8, N$^{\alpha}$H, H_2); after exchange with ^2H_2O: 8·18 (s, 1 H, H_8), 8·33 (s, 1 H, H_2). For $C_{17}H_{24}N_6O_7 . H_2O$ (442·4) calculated: 46·15% C, 5·92% H, 19·00% N; found: 46·32% C, 5·93% H, 18·72% N.

N$^{\varepsilon}$-Formyl-N$^{\alpha}$-(9-β-D-ribofuranosylpurin-6-yl)-L-lysine (*XII*)

6-Chloro-9-β-D-ribofuranosylpurine (143 mg; 0·5 mmol) was converted into the monohydrate *XII* (foam; 190 mg, 86%) as described for the derivative *XI*. UV spectrum (water): λ_{max} 268 nm (log ε 4·26), λ_{min} 230 nm (log ε 3·44). IR spectrum (KBr): 1 724 cm^{-1} (COOH), 1 662 cm^{-1} (amide I), 1 544 cm^{-1} (amide II). ^{1}H NMR spectrum (60 MHz, hexadeuteriodimethyl sulfoxide): 1·20—1·63 (m, 4 H, —$(CH_2)_2$—), 1·63—2·17 (m, 2 H, $CHCH_2$), 2·83—3·25 (m, 2 H, $NHCH_2$), 3·63 (s, 3 H, 2 $H_{5'}$, CH), 5·92 (d, 1 H, $H_{1'}$, $J_{1',2'}$ = 6), 7·57—8·33 (m, 4 H, N$^{\varepsilon}$H, CHO, N$^{\alpha}$H, H_8), 8·37 (s, 1 H, H_2); after exchange with ^2H_2O: 7·97 (s, 1 H, CHO), 8·20 (s, 1 H, H_8), 8·37 (s, 1 H, H_2). For $C_{17}H_{24}N_6O_7 . H_2O$ (442·4) calculated: 46·15% C, 5·92% H, 19·00% N; found: 46·24% C, 5·81% H, 19·26% N.

N^{α}-(9-β-D-Ribofuranosylpurin-6-yl)-L-lysine (*XIII*)

A solution of the formyl derivative *XII* (100 mg; 0·23 mmol) in 1M-HCl (2 ml) was set aside at room temperature for 13 days and then applied on a column of Dowex 3 (CH_3COO^- form; 15 ml). After elution with water, the UV-absorbing fraction was concentrated and chromatographed on a column of silica gel (10 g) in 2-propanol–ethyl acetate–water (12 : 5 : 8). The UV-absorbing fraction upon evaporation of the solvents gave 51 mg (56%) of *XIII*. UV spectrum (water): λ_{max} 269 nm (log ε 4·22), λ_{min} 230 nm (log ε 3·38). For $C_{16}H_{24}N_6O_6$ (396·4) calculated: 48·47% C, 6·10% H, 21·20% N; found: 48·18% C, 6·34% H, 21·05% N.

Reaction of Poly(L-lysine) with *I*

To a stirred solution of poly(L-lysine hydrochloride) (165 mg) in water (0·8 ml) was added 1M-NaOH (45 μl), followed by a solution of *I* (22·7 mg; 0·1 mmol) in water (0·2 ml). Aliquotes (10 μl), taken from the reaction mixture, were neutralized with 5 μl of 1M-HCl and applied on a column (12 mm × 300 mm) of Sephadex G 25 (medium). The absorbing fractions were collected in volumetric flasks, made up to 25 ml, and the extinction at 250 nm or 254 nm was measured. The reaction after neutralization of the solution of poly(L-lysine hydrochloride) to pH 10·7 (68 μl of 1M-NaOH) was followed in the same manner.

Reaction of Poly(L-lysine) with 6-Chloro-9-β-D-ribofuranosylpurine

To a solution of poly(L-lysine) (82 mg) in water (5 ml) was added 1M-NaOH (1 ml) followed by 6-chloro-9-ribofuranosylpurine (143 mg; 0·5 mmol). After stirring at room temperature for 20 h, the mixture was dialyzed for 3 days against water and freeze-dried, affording 115 mg of lyophylizate. A sample (5·421 mg) was hydrolyzed with 6M-HCl (2 ml) at 110°C for 8 h, the mixture was made up to 100 ml and its extinction at 274 nm was measured. A sample of *X* was hydrolyzed in the same manner, its extinction was measured and the molar extinction coefficient calculated ($1{\cdot}095 \cdot 10^{-4}$). Using this value, the sample of the lyophilizate was found to contain 29% of *X* (calculated for mol.wt. 396·4), *i.e.* ribosylpurine is bonded approximately to every 8th—9th lysine unit.

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