SYNTHESIS OF THE SULFONAMIDO DERIVATIVES OF ARABINONUCLEOSIDES*

Ladislav NOVOTNÝ, Hubert HŘEBABECKÝ and Jiří BERÁNEK

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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Reaction of the sodium salt of 4-aminobenzenesulfonamide with cyclocytidine *I* and cyclouridine *II* led to the 2-sulfonamido derivatives *V* and *VI* while the reaction with the 5'-chloro derivatives of anhydronucleosides *III* and *IV* afforded compounds *VIII* and *IX* containing nitrogen bridge between the carbon atoms in position 2 and 5'. Kinetics of the model cyclization reaction of 5'-chloroarabinosylisocytosine (*XI*) was followed and the structure of prepared compounds was confirmed. Inhibition activity against L 1210 leukemia cells in the experiments *in vitro* was exhibited by compounds *V* ($1{\cdot}4 \cdot 10^{-5}$ mol l^{-1}) and *VIII* ($3{\cdot}3 \cdot 10^{-6}$ mol l^{-1}).

Arabinosylcytosine belongs to the most successful antileukemic agents of the group of nucleosides[1] and it is used in the treatment of acute myeloblastic leukemias. That is why such an attention is devoted to the preparation of its new analogues and derivatives with the aim to find the compounds with enhanced antileukemic activity. This is connected with common effort to prepare and investigate the compounds influencing regulatory processes in living cell. Such an important group of compounds can be seen *e.g.* in the group of sulfonamides which inhibit the biosynthesis of folic acid[2], dihydropteroic acid, and 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine in the presence of Mg^{2+} ions and ATP (ref.[3]) as well as the activity of some enzymes, probably by the coordination bond between metal cation and active site of the enzyme[4-7]. From clinical point of view, one of the most important sulfonamides is sulfodiazine. It inhibits L-dihydroorotase[5] which plays an important role in the biosynthesis of pyrimidine nucleosides. We decided, therefore, to prepare the conjugates of nucleosides with the sulfonamide chain in order to determine in which way the coexistence of these two biologically active groupings in one molecule would influence their biological activity (above all antileukemic but also antibacterial one).

For synthesis of the sulfonamido derivatives of arabinonucleosides we used the opening of the 2,2'-anhydro bond of cyclonucleosides *I*–*IV* with the sodium salt of 4-aminobenzenesulfonamide. Reaction rate and the yields were influenced by the

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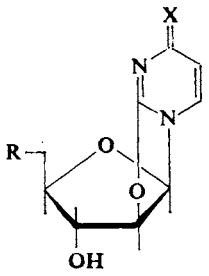
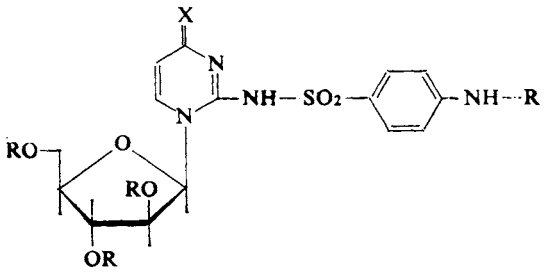
type of nucleobase while the reaction course and the structure of reaction products were also affected by the substitution in the sugar component. More reactive cytosine derivatives *I* and *III* reacted at room temperature already and cyclonucleoside *I* afforded the 2-sulfonamido derivative *V* in a good yield. Less reactive uracil derivatives *II* and *IV* reacted only on heating at 110°C for several hours whereby cyclouridine *II* and 5′-chlorocyclouridine *IV* furnished compounds *VI* and *IX* in a low yield. Lower reactivity of the uracil derivatives *II* and *IV* is in agreement with findings[8] on the conversion of cyclouridine to isocytidine which requires as many as 5 days to termination.

Reaction of the sodium salt of 4-aminobenzenesulfonamide with cyclocytidine *I* or cyclouridine *II* leads to the 2-sulfonamido derivatives *V* and *VI*. In case of the





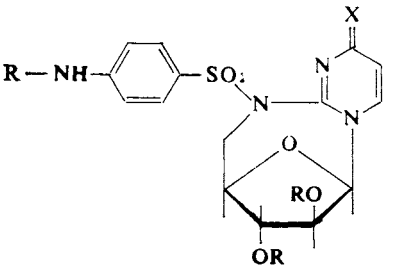
I, R = OH, X = NH
II, R = OH, X = O
III, R = Cl, X = NH
IV, R = Cl, X = O

V, R = H, X = NH
VI, R = H, X = O
VII, R = Ac, X = NH

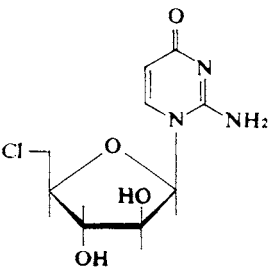
reaction of the 5′-chloro derivatives of cyclonucleosides *III* and *IV*, compounds *VIII* and *IX* containing nitrogen bridge between carbon atoms in position 2 and 5′ are formed. We suppose that compounds *VIII* and *IX* are formed *via* intermediary 2-sulfonamido derivatives, as it can be deduced from the model reaction of 5′-chlorocyclouridine *IV* with methanolic ammonia. During this reaction, compound *IV* affords the 2-amino derivative *XI* first and only then compound *XII* with the 2,5′--nitrogen bridge is formed. The course of the reaction was monitored by HPLC (Fig. 1). Knowledge of the time course of the reaction made it possible to prepare also the isocytosine derivative *XI* on interrupting the reaction after 4 h. Compound *XI* was isolated by crystallization because on chromatography on silica gel it is recyclized under formation of the starting compound *IV* similarly as at 2-amino--1-β-D-arabinofuranosylpyrimidin-4(1*H*)-one[8,11]. The structure of products with the nitrogen bridge *VIII*, *IX*, *XII* was confirmed by NMR and CD spectra.

It was proved that the formation of nitrogen bridge between the base and the sugar component by the described reaction requires anhydrous conditions and the presence of amino group in position 2 of the nucleobase. 5′-Chloro-5′-deoxyarabinosyl-

cytosine in methanolic ammonia was stable for more than 20 h although in aqueous ammonia 2′,5′-anhydroarabinosylcytosine[10] is formed rapidly. 5′-Chloro-5′-deoxyarabinosylisocytosine (*XI*) in 15% aqueous ammonia lost the amino group after 20 h under formation of 5′-chloro-5′-deoxyarabinosyluracil which subsequently cyclized[10] to afford 2′,5′-anhydroarabinosyluracil. The course of the reaction was followed by TLC. Mentioned experiments have evidenced that the isocytosine derivative *XI* in aqueous alkaline medium (in our case, aqueous ammonia) cannot furnish 2′,5′-anhydroarabinosylisocytosine and contrarywise, that anhydrous conditions are necessary for the formation of the 2,5′ nitrogen bridge (at the preparation of compounds *VIII*, *IX*, and *XII*).



VIII, R = H, X = NH
IX, R = H, X = O
X, R = Ac, X = NH



XI

The ^{1}H NMR spectra of compounds *V* and *VI* contain signal of proton of the 5′-hydroxyl at 5·06 ppm. This signal is missing in the NMR spectra of compounds *VIII*, *IX*, *XII* (and also *XI*). ^{1}H NMR spectrum of the cytosine derivative *V* contains two signals of NH protons at 7·62 and 7·64 ppm and spectrum of the uridine derivative *VI* contains signal of the NH group proton at 6·00 ppm. ^{1}H NMR spectrum of the cyclonucleoside *VIII* contains only one signal of the NH group proton at 7·51

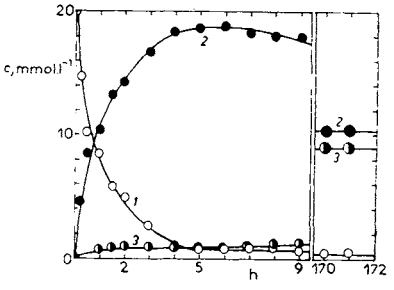
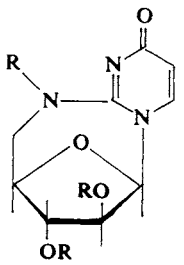


FIG. 1
Time course of the reaction of 5′-chlorocyclouridine (*IV*) with methanolic ammonia; *1* 5′-chlorocyclouridine (*IV*), *2* compound *XI*, *3* compound *XII*

ppm while the spectrum of cyclonucleoside *IX* does not contain any signal of the proton of the NH group. Signal of protons of the NH_2 group of acyclic compound *XI* at 6·86 ppm is replaced by signal of one proton of the NH group at 6·75 ppm in the ^1H NMR spectrum of the cyclic compound *XII*. The ^1H NMR signals of $H_{1'}$ proton at compounds *V*, *VI*, and *XI* which do not contain nitrogen bridge between the carbon atoms in positions 2 and 5′ are doublets at 6·22, 6·03, and 5·88 ppm, while at the cyclic compounds *VIII*, *IX*, and *XII* we can find singlets at 5·95, 5·98, and 6·00 ppm. Infrared spectra in KBr also support possible presence of the isomer with exocyclic double bond in position 2 of the pyrimidine ring in the solid phase, because the band of carbonyl group of compounds *VI* and *XII* can be found 40–50 cm^{-1} higher when compared with the band of carbonyl group of compounds *IX* and *XI*.



XII, R = H
XIII, R = Ac

The comparison of CD spectra of the pairs of compounds *V* and *VIII*, *VI* and *IX*, *XI* and *XII* shows that the intensity of positive band at 233 nm at compound *V* ([θ] + 16 000) is only half this magnitude at compound *VIII* (237 nm, [θ] + 8 200) and on the contrary, the weak positive band at 255 nm ([θ] + 6 000) at compound *V* is very intensive (264 nm, [θ] + 16 000) at compound *VIII*. In the CD spectrum of compound *VI* there is a weak negative band ([θ] − 3 000) at 236 nm while compound *IX* exhibits a positive band at 238 nm ([θ] + 9 600). A very intensive positive band of compound *VI* at 267 nm ([θ] + 54 400) is replaced by weak negative band at 264 nm ([θ] − 3 200) at compound *IX*. In the CD spectrum of compound *XI* there is an intensive positive band ([θ] + 15 100) at 244 nm which is much weaker at compound *XII* (230 nm, [θ] + 5 600). The CD spectrum of compound *XI* further contains the negative band of low intensity at 275 nm ([θ] −2 100). In contrast to that, the CD spectrum of compound *XII* contain a strong positive band at 264 nm ([θ] + 12 400). The values of wavelengths of the CD-bands as well as the molar elipticities [θ] are summarized in Table I. Because the CD spectra have already been utilized[9] for the determination of conformations of pyrimidine nucleosides substituted in position 2 or 6, we consider the change of intensities and/or signs

of the bands in the CD spectra of pairs of compounds *V* and *VIII*, *VI* and *IX*, *XI* and *XII* as a proof for the *syn*-conformation of compounds *VIII*, *IX*, and *XII* where this conformation is fixed by the formation of the nitrogen bridge between carbon atoms in position 2 and 5′.

Treatment of the 2-sulfonamido derivatives *V* and *VI* with 50% acetic acid resulted in a quantitative formation of cyclocytidine and cyclouridine which is in agreement with the earlier described reactions[11]. The cyclic nucleoside *XII*, however, was stable after standing in 60% acetic acid for 2 days.

In biological tests, compounds *V* and *VIII* inhibited the growth of L1210 leukemia cells in the concentrations of $1{\cdot}4 \cdot 10^{-5}$ mol l^{-1} and $3{\cdot}3 \cdot 10^{-6}$ mol l^{-1}, resp. Compounds *V*, *VIII*, and *IX* as well as the starting 4-aminobenzenesulfonamide did not inhibit (in concentration 1 mg/ml) the growth of *Escherichia coli* 326/71, *Staphylococcus* M78/71, *Klepsiela* Klp NCTC 11228, and *Streptococcus* of the group A.

EXPERIMENTAL

Melting points were determined on a heated microscope stage (Kofler block). Samples for analyses were dried under reduced pressure (65 Pa) at 35°C for 12 h. Thin-layer chromatography was carried out on ready-for-use Silufol UV_{254} silica gel sheets (Kavalier Glassworks, Votice, Czechoslovakia) in the following systems: S_1, ethyl acetate–acetone–ethanol–water 3 : 1 : 1 : 1; S_2, ethyl acetate–acetone–ethanol–water 4 : 1 : 1 : 1; S_3, ethyl acetate–acetone–ethanol–water

TABLE I
Wavelengths (λ) of CD bands and molar elipticities ([θ]) of some compounds studied

Compound	λ, nm; ([θ] . 10^{-3}), (10^{-5} deg m^2 mol^{-1})					
V	208 (−7·6)	219 (−2·4)	233 (+16·0)	255 (+6·0)	275 (+1·8)	302 (−0·8)
VIII	—	—	237 (+8·2)	264 (+16·0)	—	—
VI	—	223 (+4·0)	236 (−3·0)	267 (+54·4)	—	298 (−28·0)
IX	—	—	238 (+9·6)	264 (−3·2)	—	292 (+20·0)
XI	205 (−8·5)	217 (−8·2)	244 (+15·1)	—	275 (−2·1)	—
XII	—	—	230 (+5·6)	264 (+12·4)	—	—

18 : 1 : 1 : 1. Detection was performed by UV light. The R_F values are summarized in Table II. Column chromatography was performed on the Pitra silica gel (30—60 μ). High-performance liquid chromatography was carried out on an instrument set including the pump (miniPump, Milton Roy Co.), the differential UV analyzer 254 nm (Construction Department of the Czechoslovak Academy of Sciences), and the TZ 4100 line recorder (Laboratory Instruments, Prague). The column was packed with Separon SI C_{18} (8 μ) reversed phase. Dimensions of the analytical column, 4 × 250 mm. Solvent system of 1% tetrahydrofuran in water was used, 10 μl samples were injected. Column flow, 0·5 ml min^{-1}; pressure, 6·9 MPa. Capacity factors of the studied compounds are given in Table II. ^{1}H NMR spectra were recorded on Tesla BS 467 instrument at 60 MHz and on Varian XL-200 instrument at 200 MHz. Tetramethylsilane was used as internal standard. Chemical shifts are given in ppm (δ values) and coupling constants in Hz. Ultraviolet spectra were recorded on Unicam SP 8000 spectrophotometer and infrared spectra were measured on a UR-20 (Carl Zeiss, Jena) apparatus. Optical rotations were taken on an automatic Perkin–Elmer 141 MC polarimeter and CD spectra were measured on a Roussel–Jouan II CD 185 dichrograph. The solvents were distilled off on a rotatory evaporator at the temperature of 20 to 60°C, depending on the solvent used. Purification of the solvents was performed according to standard procedures. 4-Aminobenzenesulfonamide for syntheses was a commercial product. Cyclouridine[12], cyclocytidine[13], 5′-chlorocyclouridine[14,15], and 5′-chlorocyclocytidine[16] were prepared according to the literature.

2-(4-Aminobenzenesulfonylamino)-1-(1-β-D-arabinofuranosyl)-4(1*H*)-iminopyrimidine (*V*)

To a mixture of 4-aminobenzenesulfonamide (516 mg; 3 mmol) and dimethylformamide (7 ml), sodium hydride (72 mg; 3 mmol) and cyclocytidine hydrochloride (392 mg; 1·5 mmol) were added under stirring. The mixture was stirred at room temperature for 60 min, evaporated,

TABLE II

Chromatographic mobilities and retention factor k for HPLC (reversed phase) of the compounds studied

Compound	R_F in solvent system			k
	S_1	S_2	S_3	
V	0·65	0·52	0·10	25·2
VI	0·86	0·77	0·45	10·8
VII	0·85	0·80	0·20	—
VIII	0·90	0·85	0·10	28·2
IX	0·81	0·71	0·28	9·0
X	0·90	0·87	0·27	—
XI	0·70	0·56	0·23	14·0
XII	0·32	0·20	0	3·5
Cyclouridine	—	—	—	7·2
Cyclocytidine	—	—	—	6·5
5′-Chlorocyclouridine				8·5

and the residue was chromatographed on a column of silica gel (100 g) in the system ethyl acetate–acetone–ethanol–water 3 : 1 : 1 : 1 (250 ml). Fractions of 10 ml were collected. Evaporation of the fraction 17–21 afforded compound *V* (475 mg; 70%) in the form of a solid foam. Analytical sample was obtained on crystallization from ethanol, m.p. 305–306°C (decomp.). $[\alpha]_D^{25}$ +45° (*c* 0·4, ethanol). IR spectrum (KBr): 3 228 cm^{-1} (NH), 1 646 cm^{-1} (NH), 1 646 cm^{-1} (C=N exocyclic), 1 548 cm^{-1} (C=N), 1 597 and 1 500 cm^{-1} (ring), 1 300 and 1 127 cm^{-1} (SO_2). UV spectrum, pH 2·32: λ_{max} 235, sh 272 nm (log ε 4·37, 4·11); pH 6·94: λ_{max} 230 and 260 nm (log ε 4·44, 4·38), λ_{min} 255 nm (log ε 4·36); pH 10·93: λ_{max} 229 and 260 nm (log ε 4·41, 4·36), λ_{min} 251 nm (log ε 4·35). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide, 200 MHz): 3·58 (broad t, 2 H, 2 $H_{5'}$, $J_{5',4'}$ = 4·0, $J_{5',OH}$ = 5·0), 3·79 (broad t, 1 H, $J_{4',5'}$ = 4·0), 3·90 (broad d, 1 H, $H_{3'}$, $J_{3',2'}$ = 1·0), 4·02 (broad t, 1 H, $H_{2'}$, $J_{2',1'}$ = 3·0, $J_{2',OH}$ = 5·0), 5·06 (t, 1 H, 5'-OH), 5·52 (broad s, 2 H, NH_2), 5·53 (broad d, 2 H, 2'-OH, 3'-OH), 5·87 (d, 1 H, H_5, $J_{5,6}$ = 7·6), 6·22 (d, 1 H, $H_{1'}$, $J_{1',2'}$ = 3·2), 6·48 (d, 2 H_m, $J_{m,o}$ = 8·4), 7·51 (broad s, 1 H, NH), 7·62 (d, 2 H, 2 H_o, $J_{o,m}$ = 8·4), 7·64 (broad s, 1 H, NH), 7·70 (d, 1 H, H_6, $J_{6,5}$ = 7·6). For $C_{15}H_{19}N_5O_6S.C_2H_5OH$ (443·5) calculated: 46·04% C, 5·68% H, 15·79% N, 7·23% S; found: 45·81% C, 5·50% H, 16·00% N, 7·24% S.

2-(4-Aminobenzenesulfonylamino)-1-(1-β-D-arabinofuranosyl)pyrimidin-4(1*H*)-one (*VI*)

Sodium hydride (36 mg; 1·5 mmol) and cyclouridine (363 mg; 1·5 mmol) were added to a stirred mixture of 4-aminobenzenesulfonamide (516 mg; 3 mmol) and dimethylformamide (7 ml). The mixture was stirred at 110°C for 3 h, evaporated to dryness and chromatographed on a silica gel column (150 g) in the system ethyl acetate–acetone–ethanol–water 15 : 3 : 2 : 2 (450 ml); 10 ml fractions were collected. Crystallization of the residue of fractions 37–46 from aqueous ethanol afforded compound *VI* (102 mg; 16%), m.p. 149–153°C. $[\alpha]_D^{25}$ −51° (*c* 0·5; methanol). IR spectrum (KBr): 3 505, 3 450, 3 369 and 3 281 cm^{-1} (OH, NH, NH_2), 1 703 and 1 683 cm^{-1} (C=O), 1 634 cm^{-1} (C=C), 1 593 and 1 508 cm^{-1} (ring), 1 342 and 1 331 cm^{-1} (SO_2 asym.), 1 136 cm^{-1} (SO_2 sym.); in dimethyl sulfoxide: 1 713 and 1 699 cm^{-1} (C=O), 1 367 cm^{-1} (SO_2 asym), 1 139 cm^{-1} (SO_2 sym.). UV spectrum, pH 2·41: λ_{max} 265, sh 240, sh 225 nm (log ε 4·27, 4·15, 3·93); pH 7·10: λ_{max} 201, sh 223, 259 nm (log ε 4·53, 4·37, 4·39), λ_{min} 241 nm (log ε 4·30); pH 11·15: λ_{min} 225 and 258 nm (log ε 4·38, 4·36), λ_{min} 243 nm (log ε 4·30). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide, 200 MHz): 3·58 (broad t, 2 H, 2 $H_{5'}$, $J_{5',4'}$ = 5·3), 3·81 (t d, 1 H, $H_{4'}$, $J_{4',3'}$ = 2·9, $J_{4',5'}$ = 5·4), 3·89 (broad t, 1 H, $H_{3'}$), 3·99 (broad t, 1 H, $H_{2'}$), 5·06 (t, 1 H, 5'-OH, $J_{OH,H5'}$ = 5·5), 5·53 (d, 1 H, 3'-OH, $J_{OH,H3'}$ = 4·2), 5·61 (d, 1 H, 2'-OH, $J_{OH,H2'}$ = 4·9), 5·85 (d, 1 H, H_5, $J_{5,6}$ = 8·3), 6·00 (broad s, 1 H, NH), 6·03 (d, 1 H, $H_{1'}$, $J_{1',2'}$ = 3·6), 6·61 (d, 2 H, 2H_m, $J_{m,o}$ = 8·9), 7·50 (d, 2 H, 2 H_o, $J_{o,m}$ = 8·9), 7·78 (d, 1 H, H_6, $J_{6,5}$ = 8·3). For $C_{15}H_{18}N_4O_7S.1/2\ H_2O$ (407·4) calculated: 44·22% C, 4·70% H, 13·75% N, 7·87% S; found: 43·99% C, 4·72% H, 13·53% N, 7·68% S.

2-(4-Acetylaminobenzenesulfonylamino)-1-(2',3',5'-tri-O-acetyl-1-β-D-arabinofuranosyl)-4(1*H*)-iminopyrimidine (*VII*)

Compound *VII* was prepared on acetylation of compound *V* with the mixture pyridine–acetic anhydride. Crystallization from 2-propanol yielded the product of m.p. 144–147°C. $[\alpha]_D^{25}$ +115° (*c* 0·1; ethanol). IR spectrum (chloroform): 3 530 and 3 439 cm^{-1} (free NH_2), 3 510 and 3 340 cm^{-1} (NH_2, bonded), 3 416, 3 345 and 3 245 cm^{-1} (NH), 1 752 cm^{-1} (C=O acetate), 1 594 cm^{-1} (ring), 1 371 cm^{-1} (CH_3), 1 311 and 1 144 cm^{-1} (SO_2), 1 247 cm^{-1} (C—O acetate). ^{1}H NMR spectrum (pentadeuteriopyridine, 60 MHz): 1·79–2·17 (m, 18 H, 6 CH_3CO). For $C_{23}H_{27}N_5O_{10}S.2\ CH_3COOH$ (685·7) calculated: 47·30% C, 5·15% H, 10·21% N, 4·68% S; found: 47·27% C, 5·02% H, 10·20% N, 4·68% S.

(6*R*,7*S*,8*S*,9*R*)-4-(4-Aminobenzenesulfonyl)-4,5,6,7,8,9-hexahydro-7,8-dihydroxy-2(10*H*)--imino-furo[1,2,5:1,2,7]oxadiazepino[3,4-*a*]pyrimidine (*VIII*)

Sodium hydride (72 mg; 3 mmol) and 5'-chlorocyclocytidine hydrochloride (420 mg; 1·5 mmol) were added to a stirred mixture of 4-aminobenzenesulfonamide (516 mg; 3 mmol) and dimethylformamide (7 ml). The mixture was stirred at room temperature for 60 min, evaporated to dryness and chromatographed on a silica gel column (120 g) in the system ethyl acetate–acetone–ethanol–water 4 : 1 : 1 : 1 (200 ml). Fractions of 10 ml were collected. Evaporation of the residue of fractions 14—17 afforded compound *VIII* (97 mg; 14%) in the form of a solid foam. Analytical sample was obtained on crystallization from aqueous ethanol, m.p. 198—201°C (decomp.). $[\alpha]_D^{25}$ +114° (*c* 0·1; water). IR spectrum (KBr): 3 400 and 3 244 cm^{-1} (OH, NH), 1 646 cm^{-1} (NH_2), 1 555 cm^{-1} (pyrimidine ring), 1 599 and 1 507 cm^{-1} (ring), 1 298 and 1 131 cm^{-1} (SO_2), 1 079 cm^{-1} (C—O). UV spectrum, pH 2·32: λ_{max} 237, sh 275 (log ε 4·26, 3·96); pH 6·94: λ_{max} 230 and 258 nm (log ε 4·10, 4·08), λ_{min} 249 nm (log ε 4·07); pH 10·93: λ_{max} 230 and 258 nm (log ε 4·24, 4·22), λ_{min} 250 nm (log ε 4·21). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide, 60 MHz): 3·91 (s, 2 H, 2 $H_{5'}$), 4·21—4·54 (m, 3 H, $H_{2'}$, $H_{3'}$, $H_{4'}$), 5·52 (broad s, 2 H, NH_2), 5·82—6·20 (m, 4 H, $H_{1'}$, H_5, 2 OH), 6·49 (d, 2 H, 2 H_m, $J_{m,o}$ = 8·5), 7·51) (broad s, 1 H, NH), 7·60 (d, 2 H, 2 H_o, $J_{o,m}$ = 8·5), 7·98 (d, 1H, H_6, $J_{6,5}$ = 8·0); after ^2H_2O exchange: 4·31—4·54 (m, 3 H, $H_{2'}$, $H_{3'}$, $H_{4'}$), 5·80 (d, 1 H, H_5, $J_{5,6}$ = 8·0), 5·95 (s, 1 H, $H_{1'}$), 6·50 (d, 2 H, 2 H_m, $J_{o,m}$ = 8·5), 7·60 (d, 2 H, 2 H_o, $J_{o,m}$ = 8·5), 7·98 (d, 1 H, H_6, $J_{6,5}$ = 8·0). For $C_{15}H_{17}N_5O_5S.H_2O$ (397·4) calculated: 45·34% C, 4·81% H, 17·62% N, 8·07% S; found: 45·26% C, 4·74% H, 17·53% N, 8·06% S.

(6*R*,7*S*,8*S*,9*R*)-4-(4-Aminobenzenesulfonyl)-4,5,6,7,8,9-hexahydro-7,8-dihydroxy-furo-[1,2,5:1,2,7]oxadiazepino[3,4-*a*]pyrimidin-2(10*H*)-one (*IX*)

Sodium hydride (36 mg; 1·5 mmol) and 5'-chlorocyclouridine (368 mg; 1·5 mmol) were added under stirring to a mixture of 4-aminobenzenesulfonamide (516 mg; 3 mmol) and dimethylformamide (6 ml). The mixture was stirred at 110°C for 4 h, evaporated, and chromatographed on a column of silica gel (120 g) in the system ethyl acetate–acetone–ethanol–water 15 : 3 : 2 : 1 (360 ml). Fractions of 10 ml were collected. Crystallization of the residue of fractions 28—34 from ethanol furnished 74 mg (13%) of compound *IX*, m.p. 219—222°C. $[\alpha]_D^{25}$ +138° (*c* 0·25; ethanol). IR spectrum (KBr): 3 460, 3 366 cm^{-1} (NH_2), 3 255 and 3 432 cm^{-1} (OH), 1 709 and 1 637 cm^{-1} (C=O), 1 693 cm^{-1} (NH_2), 1 525 cm^{-1} (C=N), 1 506 cm^{-1} (ring), 1 367, 1 358, 1 138, 558 cm^{-1} (SO_2). UV spectrum, pH 2·41: λ_{max} 273, sh 243 nm (log ε 4·32, 4·20); pH 7·10: λ_{max} 201, 224 and 259 nm (log ε 4·46, 4·39, 4·34), λ_{min} 217 and 245 nm (log ε 4·34, 4·27); pH 11·15: λ_{max} 224 and 259 nm (log ε 4·39, 4·36), λ_{min} 245 nm (log ε 4·29). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide, 60 MHz): 3·89 (s, 2 H, 2 $H_{5'}$), 4·04 (d, 1 H, $H_{4'}$, $J_{4',3'}$ = 2·5), 4·39 (d, 1 H, $H_{3'}$, $J_{3',4'}$ = 2·5), 4·46 (s, 1 H, $H_{2'}$), 5·81 (d, 1 H, H_5, $J_{5,6}$ = 8·5), 5·88—6·08 (583·6) (m, 4 H, $H_{1'}$, NH_2, OH), 6·58 (d, 2 H, 2 H_m, $J_{m,o}$ = 9·0), 7·48 (d, 2 H, 2 H_o, $J_{o,m}$ = 9·0), 8·04 (d, 1 H, H_6, $J_{6,5}$ = 8·5); after ^2H_2O exchange: 4·49 (t, 2 H, $H_{2'}$, $H_{3'}$), 5·88 (d, 1 H, H_5, $J_{5,6}$ = = 8·5), 5·98 (s, 1 H, $H_{1'}$), 6·64 (d, 2 H, 2 H_m, $J_{m,o}$ = 9·0), 7·52 (d, 2 H, 2 H_o, $J_{o,m}$ = 9·0), 8·04 (d, 1 H, H_6, $J_{6,5}$ = 8·5). For $C_{15}H_{16}N_4O_6S$ (380·4) calculated: 47·36% C, 4·23% H, 14·72% N, 8·43% S; found: 47·17% C, 4·07% H, 14·51% N, 8·29% S.

(6*R*,7*S*,8*S*,9*R*)-4-(4-Acetylaminobenzenesulfonyl)-7,8-diacetoxy-4,5,6,7,8,9-hexahydro-2(10*H*)--imino-furo[1,2,5:1,2,7]oxadiazepino[3,4-*a*]pyrimidine (*X*)

Compound *X* was prepared on acetylation of compound *VIII* with the mixture pyridine–acetic anhydride. It was purified by column chromatography on silica gel in the system ethyl acetate--acetone–ethanol–water (4 : 1 : 1 : 1) and by the crystallization from 2-propanol. M.p., 213 to

216°C (decomp.). IR spectrum (KBr): 3 422 cm^{-1} (NH sec.), 3 329 cm^{-1} (NH imine), 3 235, 3 195 and 3 118 cm^{-1} (NH bonded), 1 750 cm^{-1} (C=O), 1 647 cm^{-1} (C=N) and C=C, (*para*-quinoid), 1 594 and 1 466 cm^{-1} (ring), 1 547 cm^{-1} (amide), 1 371 cm^{-1} (CH_3), 1 308 cm^{-1} (SO_2 asym.), 1 143 cm^{-1} (SO_2 sym.), 1 231 cm^{-1} (C—O) acetate. ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide, 60 MHz): 1·98 (s, 3 H, CH_3COOH), 2·03 (s, 3 H, CH_3CO), 2·10 (s, 3 H, CH_3CO), 2·13 (s, 3 H, CH_3CON). For $C_{21}H_{23}N_5O_8S.CH_3COOH.H_2O$ (583·6) calculated: 47·34% C, 5·01% H, 12·00% N, 5·49% S; found: 47·11% C, 5·01% H, 12·10% N, 5·36% S.

2-Amino-1-(5-chloro-5-deoxy-1-β-D-arabinofuranosyl)pyrimidin-4(1*H*)-one (*XI*)

A solution of 5'-chlorocyclouridine (226 mg; 1 mmol) in 17% methanolic ammonia (40 ml) was left to stand at room temperature for 5 h. Evaporation and crystallization of the residue from aqueous ethanol afforded 195 mg (80%) of compound *XI*, m.p. 181—185°C. $[\alpha]_D^{25}$ +97° (*c* 0·1; water). IR spectrum (KBr): 3 395, 3 350, 3 308, 3 263 and 3 215 cm^{-1} (OH, NH_2), 1 667 and 1 641 cm^{-1} (CO, NH_2), 1 588 cm^{-1} (C=C). UV spectrum, pH 2·41: λ_{max} 251, sh 225 nm (log ε 3·76, 3·37); pH 7·10: λ_{max} 199 and 248 nm (log ε 4·07, 3·77), λ_{min} 233 nm (log ε 3·72); pH 11·15: λ_{max} 216 and 257 nm (log ε 4·00, 3·74), λ_{min} 240 (log ε 3·69). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide, 60 MHz): 3·80—4·33 (m, 5 H, $H_{2'}$, $H_{3'}$, $H_{4'}$, 2 $H_{5'}$), 5·52 (d, 1 H, H_5, $J_{5,6}$ = 8·0), 5·75 (m, 2 H, 2 OH), 5·88 (d, 1 H, $H_{1'}$, $J_{1',2'}$ = 4·5), 6·86 (broad s, 2 H, NH_2), 7·36 (d, 1 H, H_6, $J_{6,5}$ = 8·0); after ^2H_2O exchange: 3·80—4·42 (m, 5 H, $H_{2'}$, $H_{3'}$, $H_{4'}$, 2 $H_{5'}$), 5·79 (d, 1 H, $H_{1'}$, $J_{1',2'}$ = 4·5), 5·72 (d, 1 H, H_5, $J_{5,6}$ = 8·0), 7·52 (d, 1 H, H_6, $J_{6,5}$ = 8·0). For $C_9H_{12}ClN_3O_4$ (262·5) calculated: 41·18% C, 4·92% H, 13·51% Cl, 16·01% N; found: 41·32% C, 4·72% H, 13·35% Cl, 16·28% N.

(6*R*,7*S*,8*S*,9*R*)-4,5,6,7,8,9-Hexahydro-7,8-dihydroxy-furo[1,2,5:1,2,7]oxadiazepino[3,4-*a*]-pyrimidin-2(10*H*)-one (*XII*)

A solution of 5'-chlorocyclouridine (204 mg; 0·9 mmol) in 17% methanolic ammonia (40 ml) was set aside at room temperature for 10 days and then evaporated under diminished pressure. Crystallization of the residue from methanol afforded 45 mg (24%) of compound *XII*. The residue of mother liquors was chromatographed on a column of silica gel (50 g) in the system ethyl acetate–acetone–ethanol–water 3 : 1 : 1 : 1 (240 ml). Fractions of 8 ml volume were collected. Fractions 9—12 contained 76 mg (36%) of 5'-chlorocyclouridine, the structure of which was confirmed by comparison of its IR and ^{1}H NMR spectra with those of an authentic sample[15]. Fractions 20—26 contained compound *XII* (63 mg; 33·5%), m.p. 224·5—226·5°C. $[\alpha]_D^{25}$ +12° (*c* 0·4, methanol). IR spectrum (KBr): 3 110 cm^{-1} (OH, NH), 1 690 cm^{-1} (C=O), 1 639 and 1 593 cm^{-1} (C=N). UV spectrum, pH 2·41: λ_{max} 227 and 256 nm (log ε 3·76, 3·90), λ_{min} 237 nm (log ε 3·68); pH 7·10: λ_{max} 204, sh 226 and 254 nm (log ε 4·44, 4·18, 3·92), λ_{min} 245 nm (log ε 3·91); pH 11·15: λ_{max} 217, sh 225 and 256 nm (log ε 4·12, 4·08, 3·81), λ_{min} 245 nm (log ε 3·79). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide, 60 MHz): 3·93 (s, 2 H, 2 $H_{5'}$), 4·39 (t, 3 H, $H_{2'}$, $H_{3'}$, $H_{4'}$), 5·56 (d, 1 H, H_5, $J_{5,6}$ = 8·0), 5·93 (broad s, 1 H, OH), 6·00 (s, 1 H, $H_{1'}$), 6·75 (broad s, 1 H, NH), 7·69 (d, 1 H, $J_{6,5}$ = 8·0); after ^2H_2O exchange: 3·98 (s, 2 H, 2 $H_{5'}$), 4·47 (t, 3 H, $H_{2'}$, $H_{3'}$, $H_{4'}$), 5·68 (d, 1 H, H_5, $J_{5,6}$ = 8·0), 6·00 (s, 1 H, $H_{1'}$), 7·78 (d, 1 H, H_6, $J_{6,5}$ = 8·0). For $C_9H_{11}N_3O_4$ (225·2) calculated: 48·00% C, 4·92% H, 18·65% N; found: 47·74% C, 4·83% H, 18·51% N.

(6*R*,7*S*,8*S*,9*R*)-4,7,8-Triacetoxy-4,5,6,7,8,9-hexahydro-furo[1,2,5:1,2,7]oxadiazepino[3,4-*a*]-pyrimidin-2(10*H*)-one (*XIII*)

Compound *XIII* was prepared by the acetylation of compound *XII* with the mixture of pyridine and acetic anhydride. IR spectrum (KBr): enhanced absorption in the region 2 900—3 500 cm^{-1}

(NH), 1 748 cm^{-1} (C=O acetate), 1 713, 1 689, 1 637 and 1 591 cm^{-1} (C=O, C=N, C=C), 1 372 cm^{-1} (CH_3), 1 242 cm^{-1} (C—O); chloroform: 3 456 cm^{-1} (NH), 1 748 cm^{-1} (C=O acetate), 1 700 cm^{-1} (C=O isocytosine), 1 642 cm^{-1} (C=C), 1 582 cm^{-1} (C=N), 1 373 cm^{-1} (CH_3). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide, 60 MHz): 2·06 (s, 6 H, 2 CH_3CO), 2·15 (s, 3 H, CH_3CON), 4·08 (s, 2 H, 2 $H_{5'}$), 4·73 (dd, 1 H, $H_{3'}$, $J_{3',2'} = 2·0$, $J_{3',4'} = 1·0$), 4·85 (d, 1 H, $H_{4'}$, $J_{4',3'} = 1·0$), 5·27 (d, 1 H, $H_{2'}$, $J_{2',3'} = 2·0$), 6·09 (d, 1 H, H_5, $J_{5,6} = 8·0$), 6·14 (s, 1 H, $H_{1'}$), 8·11 (d, 1 H, H_6, $J_{6,5} = 8·0$). For $C_{15}H_{17}N_3O_7$ (351·3) calculated: 51·28% C, 4·87% H, 11·96% N; found: 51·32% C, 4·82% H, 11·88% N.

Reaction of Compounds *V* and *VI* with Acetic Acid

10 mg Amounts of compounds *V* and *VI* were dissolved in 50% acetic acid (2 ml) and left to stand at room temperature. The conversion of compound *V* to compound *I* and that of *VI* to *II* were followed by means of HPLC in the course of 24 h (see Table III).

Kinetics of the Formation of Compounds *VII* and *VIII*

5'-Chlorocyclouridine (10 mg) was dissolved in 17% methanolic ammonia (2 ml) and the time changes of the proportion of compounds *XI* and *XII* in the reaction mixture were followed by HPLC (Fig. 1).

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