SYNTHESIS, MOLECULAR CONFORMATION AND BIOLOGICAL ACTIVITY OF 6-AMINO-5-AZACYTIDINE

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Reaction of N-ethoxycarbonylguanidine *III* with 2,3,5-tri-O-benzoyl-β-D-ribosyl isocyanate (*II*) afforded N-ethoxycarbonylamidinourea *IV*, which was cyclized by bis(trimethylsilyl)acetamide to the 6-ethoxy derivative *VII*. Ammono- and methanolysis of intermediate *VII* yielded 6-amino and 6-methoxy derivatives of 5-azacytidine *I* and *VIII*, respectively. Reaction of amidinourea *IV* with a mixture of chlorotrimethylsilane and triethylamine gave the blocked nucleoside *V* which was also formed by dealkylation of 6-ethoxy derivative *VII* with chlorotrimethylsilane or oxidation of tribenzoyl-5-azacytidine *IX* with hydrogen peroxide in acetic acid. Methanolysis of blocked nucleoside *V* gave ribosylammelide (*VI*). The measurement of ^{1}H NMR spectra of 6-amino-5-azacytidine (*I*) revealed a marked preference of g^+ (80%) rotamer around C(5')–C(4') bond, a predominance of *S* conformation of the ribose ring (K_{eq} 2·12) and a preference of *anti* conformation around the C-N glycosyl bond. These data indicate a conformational resemblance of 6-amino-5-azacytidine (*I*) to purine nucleosides. 6-Amino-5-azacytidine (*I*) inhibits the growth of bacteria *E. coli* while 6-methoxy and 6-oxo derivatives *VIII* and *VI*, respectively, are bacteriostatically inactive. 6-Amino-5-azacytidine (*I*) had an ID_{50} of 33·9 μM against CCRF-CEM cells and inhibited the growth of WI-L2 cells by 39% at 100 μM but did not inhibit L1210 and LoVo/L cells at ≤ 100 μM concentration.

5-Azacytidine[1] is used in clinical treatment of acute leukemia[2] and 2'-deoxy-5-azacytidine[3] has also been shown to be an effective antileukemic agent in children[4]. 1-β-D-Arabinofuranosyl-5-azacytosine was found to display a pronounced antileukemic activitiy[5,6]. Both, 5-azacytidine and 2'-deoxy-5-azacytidine are very useful experimental tools in cell and molecular biology[7]. Wide-spectrum of biological activity of the mentioned 5-azacytosine nucleosides stimulated our interest in substituted congeners of these compounds. Recently, N^4-substituted derivatives of 5-azacytidine and 2'-deoxy-5-azacytidine have been prepared in our laboratory and their antibacterial[8] and antisecretory[9] activity have been described. In continuation of the study of substituted 5-azapyrimidine nucleosides we were also interested in 6-amino-5-azacytidine (*I*). In this paper we wish to present the preparation of this compound, its molecular conformation and biological activity. The preparation of 6-amino-5-azacytidine (*I*) was formerly also included in two symposial presentations[10,11].

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The isocyanate method, which proved to be convenient also for the synthesis of other 6-substituted 5-azacytosine nucleosides[10], has been used for the preparation of 6-amino-5-azacytidine (*I*). Reaction of 2,3,5-tri-O-benzoyl-β-D-ribosyl isocyanate (*II*) with N-ethoxycarbonylguanidine (*III*) in acetone afforded N-ethoxycarbonylamidinourea *IV*. Treatment of this intermediate with excess of equimolar mixture of chlorotrimethylsilane and triethylamine gave blocked ribosylammelide (*V*) which was methanolysed to free nucleoside *VI*. This product was identical with the nucleoside prepared by oxidation of 5-azacytidine with hydrogen peroxide in acetic acid[12]. Cyclization of intermediate *IV* mediated by bis(trimethylsilyl)acetamide led to the 6-ethoxy derivative *VII* which was methanolysed to 6-methoxy-5-azacytidine (*VIII*). In this reaction both deprotection of the glycone and nucleophilic substitution of the ethoxy by the methoxy group took place. Ammonolysis of ethoxy derivative *VII* gave 6-amino-5-azacytidine (4,6-diamino-1-β-D-ribofuranosyl-1,3,5-triazin-2(1*H*)-on, ribosylammeline *I*). This compound was also obtained by ammonolysis of the free 6-methoxy nucleoside *VIII*. Reaction of methoxy nucleoside *VIII* with hydrogen chloride in methanol afforded ribosylammelide (*VI*).

Dealkylation of 6-ethoxy derivative *VII*, performed by short heating with chlorotrimethylsilane in acetonitrile, afforded a high yield of blocked ribosylammelide (*V*). This product was also prepared in a good yield by oxidation of 2',3',5'-tri-O-benzoyl--5-azacytidine[13,14] (*IX*) with hydrogen peroxide in acetic acid. The structures of the new nucleosides *I*, *VII* and *VIII* were inferred from analytical and spectral data, as well as from the way of their synthesis and chemical correlation with the known ribosylammelide *VI*.

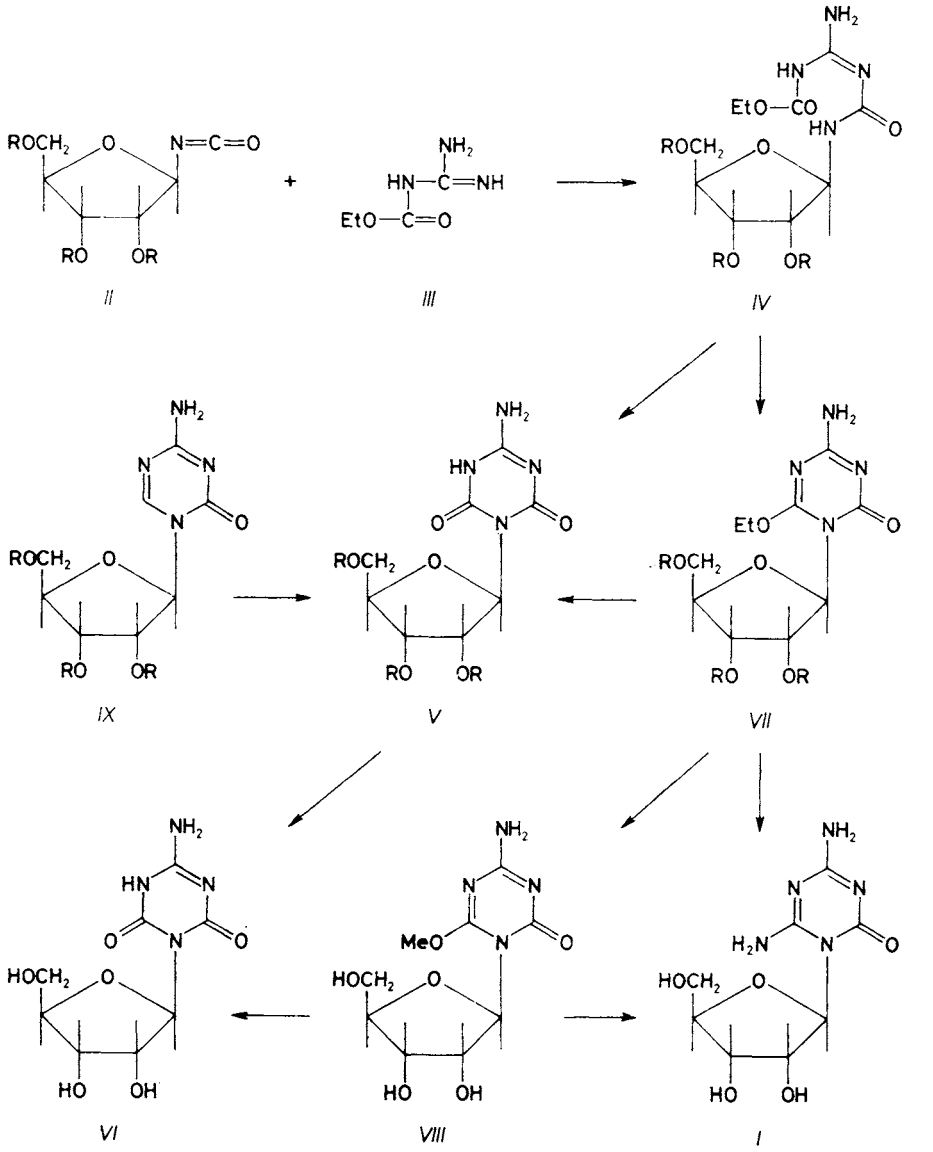
For determination of the molecular conformation of 6-amino-5-azacytidine (*I*) ^{1}H NMR spectrum has been used (Table I). Vicinal coupling constants of the ribose

TABLE I

^{1}H NMR spectral and conformational parameters of 6-amino-5-azacytidine (*I*)

Proton	Chemical shifts δ, ppm	Vicinal interaction constants, Hz	Pseudorotational and equilibrium parameters	Population of C(5')–C(4') rotamers
H-1'	6·299	$J(1',2')$ = 7·0	τ_m = 38°	g^+ = 0·80
H-2'	4·618	$J(2',3')$ = 6·4	^NP = 36°	$t + g^-$ = 0·20
H-3'	4·306	$J(3',4')$ = 4·0	^SP = 144°	
H-4'	4·125	$J(4',5') + J(4',5'')$ = 5·0	^NX = 0·32	
H-5'	3·855		^SX = 0·68	
H-5''	3·855		K_{eq} = 2·12	
			$^3_4T \leftrightarrows {}^2_1T$	

protons have been used for determination of the conformation of the furanose ring and fractional population of rotamers around the C(5′)—C(4′) bond. For the calculation of the conformational parameters τ_m ("puckering amplitude"), angles of pucker ${}^{N}P$, ${}^{S}P$ (${}^{S}P = 180 - {}^{N}P$) and the fractional population of the furanose ring conformers



R = C_6H_5CO

^{N}X, ^{S}X a program worked out by Guschlbauer[15] has been used. The values 10·2, −0·8 and 0 have been used for the constants A, B and C, respectively, in the Karplus equation[16]

$$J(\mathrm{HiHj}) = A\cos^2\phi(\mathrm{HiHj}) + B\cos\phi(\mathrm{HiHj}) + C\,. \qquad (1)$$

These values of the constants A, B and C were formerly used for the calculation of conformational parameters of many other nucleosides[15,17]. The fractionational population of rotamers g_+, t and g_- (Fig. 1) can be calculated using the known[17] Equations (2)–(4).

$$g_+ = (13{\cdot}0 - (J(4',5') + J(4',5'')))/10 \qquad (2)$$

$$t = (J(4',5'') - 1{\cdot}5)/10 \qquad (3)$$

$$g_- = (J(4',5') - 1{\cdot}5)/10 \qquad (4)$$

The calculated value g_+ (Table I) indicates a pronounced predominance of this rotamer with a gauche arrangement of the protons H-4', H-5', H-5'' and with the oxygen atom O-5' projected over the furanose ring. On the basis of the calculated values of τ_m, ^{N}P, ^{S}P, ^{N}X and ^{S}X (Table I) it is possible to characterize the conformational properties of the furanose ring of 6-amino-5-azacytidine as an interconversion between conformations $^{3}_{4}T$ and $^{2}_{1}T$. The population of $^{2}_{1}T$ conformation is about two times higher. The pronounced predominance of g_+ rotamer allows to assume the preference of anti conformation around the C—N glycosyl bond. A similar conclusion can be drawn by comparison of the chemical shifts of protons H-2', H-3' and H-4' of 6-amino-5-azacytidine (*I*) and 5-azacytidine[18] which also adopts preferentially anti conformation. Generally, a change from anti to syn conformation, caused by the influence of substituents in position 6 of pyrimidine nucleosides, is connected with greater downfield shifts of H-2' (~0·5 ppm) than for H-3' (~0·15 ppm),

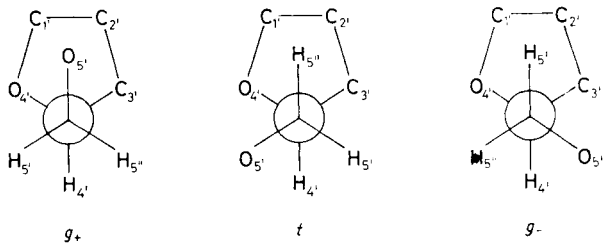


FIG. 1
Classical staggered rotamers for C(5')–C(4') bond

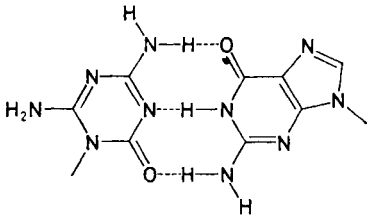
whereas upfield shifts are observed for H-4' ($\sim -0{\cdot}15$ ppm) compared to the unsubstituted nucleosides[17]. Chemical shift differences of the respective protons between 5-azacytidine and 6-amino-5-azacytidine are, however, much smaller (0·21, 0·05 and 0·01 ppm for the protons H-2', H-3' and H-4', respectively). Also these results indicate that the amino group in position 6 of the triazine ring do not change considerably the conformation of the C—N glycosyl bond. However, striking differences have been observed between the ribose ring conformations of 5-azacytidine and 6-amino-5-azacytidine. A comparison of the equilibrium constants ($K_{eq} = {}^{S}X/{}^{N}X$) of both nucleosides has shown that 5-azacytidine (K_{eq} 0·45) resembles cytidine (K_{eq} 0·69) whereas 6-amino-5-azacytidine (K_{eq} 2·12) is similar to adenosine (K_{eq} 1·78). The same conclusion can be drawn on the basis of vicinal coupling constants $J(1',2')$ and $J(3',4')$ the ratio of which corresponds[17] approximately to the equilibrium constant ($K_{eq} \approx J(1',2')/J(3',4')$). Also these data indicate that 5-azacytidine ($J(1',2')/J(3',4') = 0{\cdot}46$) resembles cytidine ($J(1',2')/J(3',4') = 0{\cdot}67$) while 6-amino-5-azacytidine ($J(1',2')/J(3',4') = 1{\cdot}75$) is similar to adenosine ($J(1',2')/J(3',4') = 1{\cdot}90$). All these results indicate a conformational resemblance of 6-amino-5-azacytidine to purine nucleosides.

Molecular conformation of pyrimidine nucleosides with the amino group in ortho position to C—N glycosyl bond has not been studied as yet. In the case of 8-aminopurine nucleosides no intramolecular hydrogen bond is considered between the amino group in position 8 of the purine ring and the glycone, but the influence of the amino group on the conformation of the ribose ring is not excluded[19]. In contrast to these results, Neidle and co-workers have established the formation of an intramolecular hydrogen bond between N—H substituted on C-8 and the O-5' atom in 9-β-D-arabinofuranosyl-8-butylaminoadenine[20].

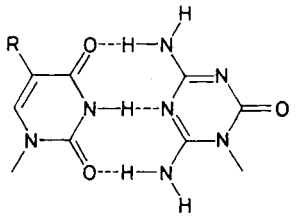
The high portion of g_+ rotamer in 6-amino-5-azacytidine indicates the existence of slight attractive forces between the amino group in position 6 of the triazine ring and the glycone which may be based on hydrogen bonds or dipolar interactions. These interactions are obviously also responsible for the change of the ribose ring conformation. In this connection it is worth mentioning that the methyl group in 6-methyl-5-azacytidine changes markedly due to repulsive interactions between the methyl group and the glycone, the conformations around the C—N glycosyl and the exocyclic C(5')—C(4') bonds but not the conformation of the ribose ring (K_{eq} 0·41)[21].

Very important, from the biochemical point of view, is also the nature of the heterocyclic moiety of the nucleoside *I*. The amino group in position 6 of the triazine ring can change chemical properties of the base and its selectivity of binding to enzymes or complementary nucleobases. 5-Azacytosine binds presumably guanine by three hydrogen bonds, similarly to cytosine[22]. 6-Amino-5-azacytosine could bind guanine (*X*) in analogy to cytosine and uracil (*XIa*) or thymine (*XIb*) in analogy to adenine. In contrast to adenine which binds the complementary pyrimidines only by two hydrogen bonds, in the respective pairs with 6-amino-5-azacytosine (*XIa*, *XIb*)

three hydrogen bonds can be considered. For these reasons, 6-amino-5-azacytosine could serve after incorporation into oligonucleotides as an "universal" base[23].



X



XI a, R = H
XI b, R = CH_3

Nucleosides *I*, *VI* and *VIII* were tested for their antibacterial activity using a culture of *E. coli* B growing on mineral medium with glucose. 6-Amino-5-azacytidine (*I*) inhibited the growth of bacteria (46% inhibition at 100 μg/ml concentration) while 6-oxo derivative *VI* and 6-methoxy-5-azacytidine (*VIII*) were completely inactive at the same concentration. The inactivity of nucleosides *VI* and *VIII* can be explained by the change of *anti* to *syn* conformation caused by repulsive interactions of substituents in position 6 of the triazine ring with the glycone.

A more detailed biochemical investigation[24] has shown that the antibacterial activity of 6-amino-5-azacytidine (*I*) can be reversed even by low levels of natural purine bases and/or nucleosides, while natural pyrimidine bases and thymidine do not reverse the inhibition at all; only a partial reversion of antibacterial activity of 6-amino-5-azacytidine (*I*) has been achieved by high doses of uridine and cytidine.

6-Amino-5-azacytidine (*I*) was also tested for its ability to inhibit the growth of four tumor cell lines in vitro: L1210 murine lymphocytic leukemia, WI-L2 human B-lymphoblastic leukemia, CCRF-CEM T-lymphoblastic leukemia, and LoVo/L, a human colon carcinoma. Nucleoside *I* had an ID_{50} of 33·9 μM against CCRF-CEM and inhibited WI-L2 by 39% at 100 μM concentration but did not inhibit growth of L1210 and LoVo/L tumor lines at $\leq$100 μM concentration.

6-Amino-5-azacytidine (*I*) exhibited no in vitro antiviral activity against herpes simplex, adeno, rhino, influenza and parainfluenza viruses at $\leqq 1$ μM concentration.

The data presented in this paper allow to conclude that 6-amino-5-azacytidine (*I*) represents an *anti*-type nucleoside. The conformation of the ribose ring of this nucleoside resembles the conformation of the ribose ring in purine nucleosides. The amino group in position 6 of nucleoside *I* changes the binding properties of the base which could in principle bind complementary pyrimidine bases in analogy to adenine. These conclusions correlate well with the results of biochemical investigations[24] which have shown that 6-amino-5-azacytidine (*I*) interferes with the metabolism of purines rather than pyrimidines and that it can be therefore considered a purine antimetabolite.

EXPERIMENTAL

Unless stated otherwise, the solutions were evaporated at 35°C/2·5 kPa and analytical samples were dried at 40 Pa (room temperature). Thin-layer chromatography (TLC) on silica gel was performed on Silufol UV 254 plates (Kavalier, Votice, Czechoslovakia) in solvent systems S1 chloroform–methanol (98 : 2), S2 1-butanol–acetic acid–water (5 : 2 : 3) and S3 chloroform–methanol (7 : 3). The spots of blocked nucleosides were detected visually in UV light (254 nm) and the spots of free nucleosides were detected chemically by a modification of the method of Reindel and Hoppe[25]: the plates were sprayed with a 1 : 1 mixture of acetone and ethanol, chlorinated for 5 min, the excess of chlorine was removed by hot air and the compounds were revealed by spraying the plates with a 1 : 1 mixture of saturated solution of *o*-tolidine in 2% acetic acid and 0·05M solution of potassium iodide (blue spots). HPLC analyses were performed on a 4 × 250 mm column of Separon SGX C18 (10 μ), flow rate 1 ml min^{-1}, in 0·02M triethylammonium acetate, pH 6·2, containing 2% methanol; detection at 254 nm. Column chromatography was performed with silica gel according to Pitra (30–60 μ, Service Laboratories of this Institute).

Ultraviolet absorption spectra were measured on a Unicam SP 8000 UV recording spectrophotometer (Pye Unicam, Cambridge, England) in buffer solutions of ionic strength 0·01 prepared according to Perrin[26]: $CH_2ClCOOH$–KOH (pH ~ 2·3); KH_2PO_4–Na_2HPO_4 (pH ~ 7·0); $C_4H_9NH_2$–HCl (pH ~ 11·0), λ are given in nm and ε in mol m^{-2}. Optical rotations were registered on a polarimeter Perkin–Elmer, type 141 MCA. ^1H NMR spectrum of *I* was measured on a Varian XL-200 instrument (200 MHz) in D_2O (c. 10 mg of sample/0·5 ml) with sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) as internal standard at 22°C. The signals were assigned to individual protons on the basis of chemical shifts, the observed multiplicities and decoupling experiments. Chemical shifts (δ) and coupling constants (J) were determined with an accuracy of ±0·003 ppm and ±0·1 Hz, respectively, by comparison of experimental and simulated spectra. Due to the character of the respective part of the experimental spectrum, protons H-5′ and H-5″ were treated in the calculation as magnetically equivalent and protons of the ribose ring were considered an $ABCDE_2$ spin system. In consequence of this presumption it was possible to determine unambiguously only the sum of $J(4', 5')$ and $J(4', 5'')$. For simulation the program SPIN which is a part of the program equipment of the spectrometer, has been used. Stationary cultivation of *Escherichia coli* B was performed at 37°C in mineral medium with glucose[27]. The tested compounds were added before inoculation and the growth of bacteria was measured 16 h later.

2,3,5-Tri-O-benzoyl-β-D-ribosyl Isocyanate (*II*)

A solution of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribose (1·01 g; 2 mmol) in 1,2-dichloroethane (10 ml) was saturated for 2 h at 0°C with dry hydrogen chloride under exclusion of atmospheric moisture. The solution was kept at room temperature overnight and the solvents were evaporated at 25°C/2 KPa. The syrupy residue was coevaporated under the same conditions with toluene (3 × 5 ml) to remove the remaining acetic acid. A mixture of thus obtained crude 2,3,5-tri-O--benzoyl-D-ribosyl chloride, silver isocyanate (0·9 g; 6 mmol) and toluene (10 ml) was vigorously stirred at room temperature for 24 h. The insoluble silver salts were rapidly filtered off with suction through a Celite layer and the material on the filter was washed with toluene (3 × 5 ml). Evaporation of the filtrate gave crude syrupy isocyanate *II* which was used directly in the subsequent step with better results in comparison to the product prepared by a formerly described procedure[28].

2′,3′,5′-Tri-O-benzoyl-6-ethoxy-5-azacytidine (*VII*)

A solution of crude isocyanate *II*, prepared from 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribose (1.01 g; 2 mmol), in acetone (10 ml) was added dropwise to a magnetically stirred mixture of guanidine *III* (0·262 g; 2 mmol) and acetone (5 ml) at room temperature. The mixture was stirred for 30 min, evaporated and the residue treated with toluene (20 ml). The small insoluble portion was filtered off and the clear filtrate evaporated to give crude amidinourea *IV* which was without purification dissolved in acetonitrile (10 ml) and the solution treated with bis(trimethylsilyl)acetamide (2 ml; 8 mmol). The mixture was allowed to stand for 1 h at room temperature, evaporated and coevaporated with toluene (2 × 10 ml). The residue was chromatographed on a column of silica gel (50 g) which was prepared in chloroform. The column was rapidly eluted with 300 ml portions of chloroform and chloroform–methanol (99 : 1, 97 : 3, 95 : 5). Fractions containing the major portion were collected, evaporated, the syrup dissolved in methanol (10 ml), the solution filtered to remove small amount of insoluble nucleoside *V*, evaporated and the residue dried at 80°C/40 Pa for 2 h to yield 0·804 g (67%) of nucleoside *VII* as a solid foam; R_F 0·24 (S1), $[\alpha]_D^{22}$ −21·5° (*c* 0·21, chloroform). For $C_{31}H_{28}N_4O_9$ (600·6) calculated: 62·00% C, 4·70% H, 9·33% N, 7·49% OC_2H_5; found: 61·85% C, 4·64% H, 9·39% N, 7·20% OC_2H_5.

6-Methoxy-5-azacytidine (*VIII*)

A solution of the purified ethoxy derivative *VII* (0·10 g; 0·166 mmol) in methanol (1 ml) and methanolic 1M-$NaOCH_3$ (0·05 ml) was allowed to stand 24 h at room temperature. The solution was acidified with acetic acid (0·01 ml) and decationized on a column of Amberlite IRC-50[H^+] ion exchange resin (5 ml) which was prepared in methanol. The product was eluted with methanol and the eluate evaporated. Crystallization of the residue from ethanol afforded 0·031 g (72%) of the nucleoside *VIII*, m.p. 165—168°C (resolidification) and 222—225°C (dec.). R_F 0·42 (S2), $[\alpha]_D^{22}$ −24·9° (*c* 0·21, water). UV (CH_3OH), λ_{max} (log ε): 220 (4·31); (pH 2·4): 230 (4·16); (pH 6·9): 219 (4·27); (pH 11·0): 217 (4·27). For $C_9H_{14}N_4O_6$ (274·2) calculated: 39·42% C, 5·15% H, 20·43% N, 11·32% OCH_3; found: 39·70% C, 5·05% H, 20·17% N, 11·55% OCH_3. When crude *VII* was used as starting material, the yield of nucleoside *VIII* decreased to 44%.

6-Amino-5-azacytidine (*I*)

A) A solution of crude 6-ethoxy derivative *VII*, prepared by the above procedure from 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribose (2·52 g; 5 mmol), in methanolic 7M ammonia (30 ml) was allowed to stand for 24 h at room temperature. The solution was filtered to remove a small

insoluble impurity, evaporated, the residue triturated with ether (50 ml), the syrup dissolved in methanol (20 ml) and the crystalline slurry allowed to stand for 30 min at room temperature to give 0·810 g (62%) of the nucleoside *I* which decomposed at 240—242°C without melting. The sample for analysis was purified by dissolution in water, filtration of the solution to remove a slight turbidity, concentration of the filtrate in vacuo to a syrup and addition of methanol to cause crystallization. The purified sample decomposed at the same temperature as the crude product. R_F 0·41 (S2), $[\alpha]_D^{22}$ +15·3° (*c* 0·1, water). HPLC: $k = 2{\cdot}4$. UV (CH_3OH), λ_{max} (log ε): 213 (4·37), 240 (4·05); (pH 2·3): 235 (4·58); (pH 6·9): 205 (4·91), 235 (4·46); (pH 10·8): 218 (4·39), 235 (4·24). For ^{1}H NMR spectra see Table I. For $C_8H_{13}N_5O_5$ (259·2) calculated: 37·07% C, 5·05% H, 27·02% N; found: 37·35% C, 4·91% H, 26·74% N.

In a parallel experiment a solution of the chromatographically purified ethoxy derivative *VII* (0·1 g; 0·166 mmol) in methanolic 7M ammonia was kept overnight at room temperature to deposit 0·030 g of the free nucleoside *I* which decomposed at 240—242°C without melting. Concentration of the mother liquor afforded 0·005 g of the same product. Overall yield, 0·035 g (81%) of the free nucleoside *I*.

B) A mixture of the free nucleoside *VIII* (0·060 g; 0·22 mmol) and methanolic 7M ammonia (1 ml) was magnetically stirred at room temperature for 2 h and kept overnight to deposit 0·05 g (88%) of the nucleoside *I* which decomposed at 240—242°C without melting. The product was identical (HPLC, $[\alpha]_D$, IR) with the sample prepared by method *A*).

6-Amino-3-(2,3,5-tri-O-benzoyl-β-D-ribosyl)-1,3,5-triazine-2,4(1*H*, 3*H*)-dione (*V*)

A) A solution of crude amidinourea *IV* (1·24 g; 2 mmol) in acetonitrile (10 ml) was treated with chlorotrimethylsilane (1·7 ml; 13 mmol) and triethylamine (1.7 ml; 12 mmol). The mixture was kept 30 min at room temperature, diluted with benzene (20 ml), the insoluble triethylamine hydrochloride filtered off with suction and the filtrate evaporated. The residue was dried in vacuo for 30 min, dissolved in ethanol (4 ml) and allowed to stand overnight at room temperature to deposit 0·82 g of crude nucleoside *V*; m.p. 295—298°C (dec.). Recrystallization of the crude product from dimethylformamide and drying at 120°C/40 Pa for 4 h afforded 0·504 g (44%) of pure nucleoside *V*; m.p. 306—308°C (dec.), R_F 0·52 (S3), $[\alpha]_D^{22}$ −27·3° (*c* 0·19, dimethylformamide). For $C_{29}H_{24}N_4O_9$ (572·5) calculated: 60·84% C, 4·23% H, 9·79% N; found: 60·58% C, 4·20% H, 10·05% N.

B) A mixture of the purified ethoxy derivative *VII* (0·10 g; 0·166 mmol), acetonitrile (1 ml) and chlorotrimethylsilane (0·05 ml; 0·39 mmol) was heated at 100°C (bath temperature) for 15 min. The mixture was cooled, the product filtered off with suction and dried at 120°C/40 Pa to give 0·9 g (94%) of the nucleoside *V*; m.p. 303—305°C (dec.) without depression on admixture with the product prepared by method *A*). The identification was also carried out by IR spectra. Using a mixture of chlorotrimethylsilane (0·05 ml) and triethylamine (0·05 ml) in acetonitrile (1 ml) for the dealkylation of *VII* (0·10 *g*; 0·166 mmol) a 63% yield of *V* was obtained on heating at 100°C (bath temperature) for 6 h.

C) A solution of blocked 5-azacytidine *IX* (1·113 g; 2 mmol) in a mixture of acetic acid (8 ml) and 30% hydrogen peroxide (8 ml) was allowed to stand for 48 h at room temperature. The precipitated product was filtered off with suction and washed with water to yield 0·7 g of crude nucleoside *V*; m.p. 298—301°C (dec.). Recrystallization of the crude product from dimethylformamide and drying at 120°C/40 Pa gave 0·469 g (41%) of nucleoside *V*; m.p. 305—308°C (dec.) without depression on admixture with the product prepared by method *A*). The identification was also carried out by IR spectra.

6-Amino-3-β-D-ribofuranosyl-1,3,5-triazine-2,4(1*H*, 3*H*)-dione (*VI*)

A) A mixture of blocked nucleoside *V* (0·15 g; 0·262 mmol), methanol (2 ml) and methanolic 1M-$NaOCH_3$ (0·45 ml) was stirred at room temperature for 8 h. The mixture was allowed to stand overnight, acidified with acetic acid (0·2 ml) and the precipitate collected by suction to yield 0·055 g of crude nucleoside *VI* which decomposed over 250°C without melting. Recrystallization of the crude product from water and drying at 120°C/40 Pa afforded 0·04 g (59%) of nucleoside *VI* which decomposed at the same temperature as the crude product; R_F 0·43 (S2): $[\alpha]_D^{22}$ −22·9° (*c* 0·1, water). The nucleoside *VI* was identified with an authentic sample[12] by IR spectra. UV (pH 2·3), λ_{max} (log ε): 227 (4·09); (pH 6·9): 211 (4·21, inflection), 226 (4·14); (pH 10·8): 217 (4·06), 228 (3·99). For $C_8H_{12}N_4O_6$ (260·2) calculated: 36·93% C, 4·65% H, 21·53% N; found: 36·64% C, 4·67% H, 21·25% N.

B) A mixture of the methoxy nucleoside *VIII* (0·04 g; 0·146 mmol) and a 2% solution of dry hydrogen chloride in ethanol (0·4 ml) was stirred at room temperature for 8 h, kept overnight and the product filtered off with suction. Yield, 0·024 g (63·2%) of the nucleoside *VI* which decomposed above 250°C without melting and was identical (TLC, IR) with the product prepared by method *A*).

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