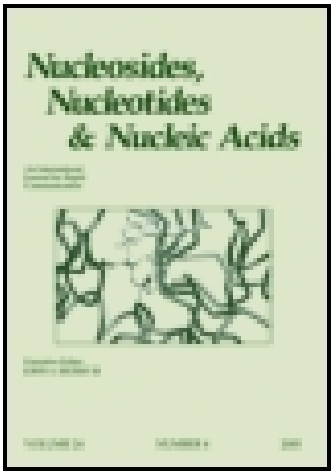

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6-Methyl-5-Azacytidine-Synthesis, Conformational Properties and Biological Activity. A Comparison of Molecular Conformation with 5-Azacytidine

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6-METHYL-5-AZACYTIDINE - SYNTHESIS, CONFORMATIONAL PROPERTIES AND BIOLOGICAL ACTIVITY. A COMPARISON OF MOLECULAR CONFORMATION WITH 5-AZACYTIDINE

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ABSTRACT: The title compound was prepared by the isocyanate procedure and the trimethylsilyl method.The measurement of ^{1}H NMR spectrum of 6-methyl-5-azacytidine (**1**) revealed a preference of γ^t (46%) rotamer around C(5')-C(4') bond, a predominance of N conformation of the ribose ring (K_{eq} O.33) and a preference of syn conformation around the C-N glycosyl bond. An analogous measurement of 5-azacytidine has shown a preference of γ^+ (6O%) rotamer around the C(5')-C(4') bond, a predominance of N conformation of the ribose ring (K_{eq} O.41) and a preference of anti conformation around the C-N glycosyl bond. 6-Methyl-5-azacytidine (**1**) inhibits the growth of bacteria E. coli to the extent of 85% at 4OOO μM concentration and the growth of LoVo/L, a human colon carcinoma cell line, to the extent of 3O% at 1OO μM concentration but did not inhibit L121O cells at ≤1OO μM concentration. 6-Methyl-5-azacytidine (**1**) exhibited no in vitro antiviral activity at ≤1 μM concentration.

INTRODUCTION

5-Azacytidine[1] and 2'-deoxy-5-azacytidine[2] are used in clinical treatment of acute leukemia[3,4] and both agents are also very useful experimental tools in molecular and cell biology[5]. Wide-spectrum of biological activity of the mentioned 5-azacytosine nucleosides stimulated our interest in substituted congeners of these compounds. Formerly, 6-amino-5-azacy-

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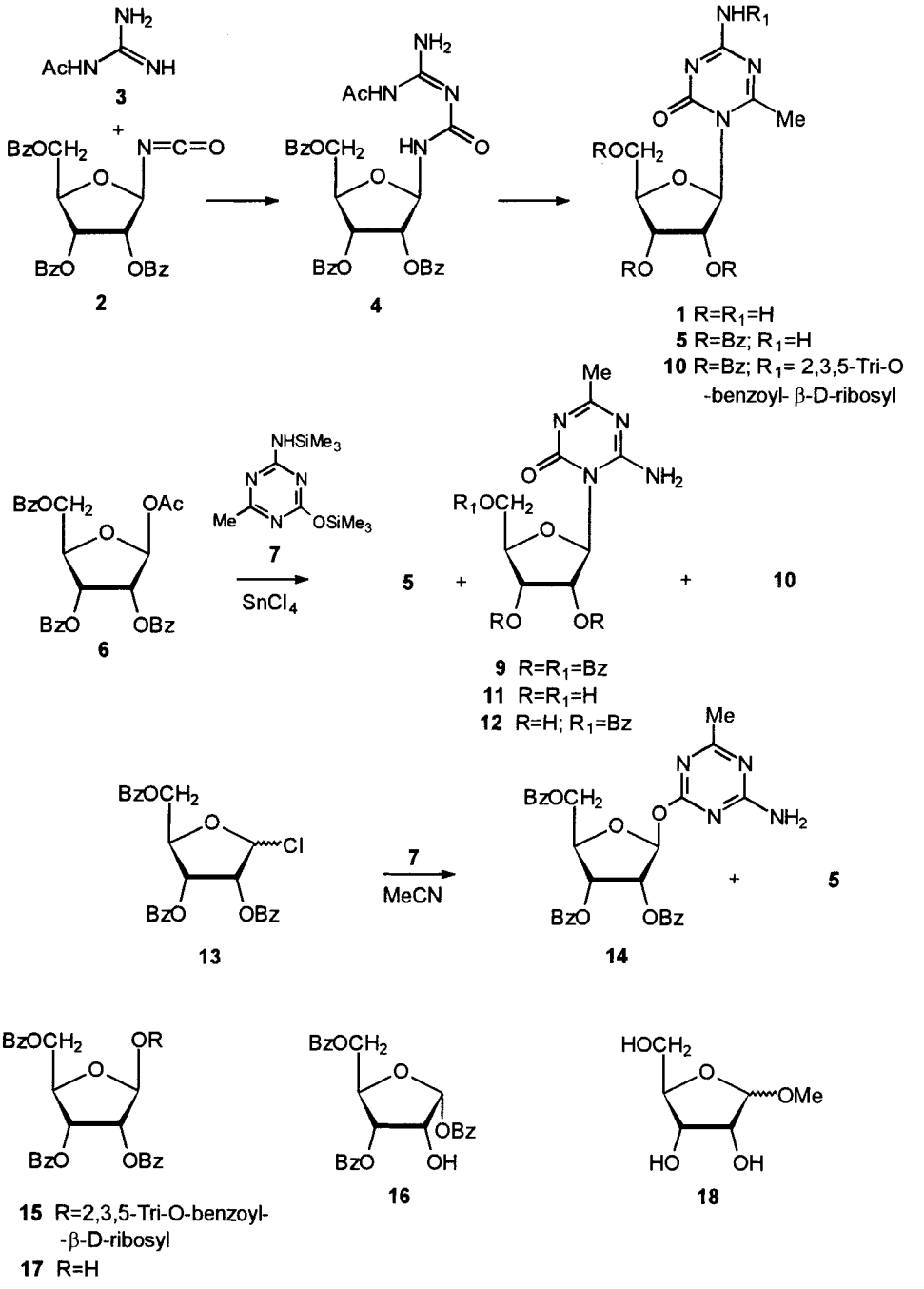
tidine has been prepared in our laboratory and its conformational properties and biological activity have been described[6]. In continuation of the study of substituted 5-azapyrimidine nucleosides we were also interested in 6-methyl-5-azacytidine (**1**). In this paper we wish to present the preparation of this nucleoside, its molecular conformation and biological activity and compare these results with the respective properties of 5-azacytidine. The preparation of 6-methyl-5-azacytidine (**1**) was formerly also included in two symposial presentations[7,8].

RESULTS AND DISCUSSION

The isocyanate method which proved to be convenient also for the synthesis of other 6-substituted 5-azacytosine nucleosides[6,8] has been used for the preparation of 6-methyl-5-azacytidine (**1**). Reaction of 2,3,5-tri-O-benzoyl-ß-D-ribosyl isocyanate[6] (**2**) with N-acetylguanidine (**3**) in acetone afforded N-acetylamidinourea **4**. Treatment of this intermediate with excess of an equimolar mixture of chlorotrimethylsilane and triethylamine or bis(trimethylsilyl)acetamide gave a high yield of 2',3',5'-tri-O-benzoyl-6-methyl-5-azacytidine (**5**). Similar results were obtained using bis(trimethylsilyl)trifluoroacetamide as condensating agent. Methanolysis of the blocked derivative **5** afforded the corresponding free nucleoside **1**. The isocyanate procedure is unambiguous in respect to the position of the ribosyl group and also the anomeric configuration. The structure of the products was confirmed by elemental analysis and spectral data.

The second method used for the preparation of **1** was direct glycosylation. Stannic chloride catalyzed condensation of 1-O-acetyl-2,3,5-tri-O-benzoyl-ß-D-ribose (**6**) with silylated 6-methyl-5-azacytosine 7 (prepared on treatment of the base[9] **8** with hexamethyldisilazane at elevated temperature) in 1,2-dichloroethane (reaction time 3O min) yielded a 3:1 mixture of N^1 and N^3 nucleosides **5** and **9.** On prolongation of the reaction time to 4 h a 7:1 mixture of N^1 and N^3 nucleosides was formed. When acetonitrile was used as solvent (reaction time 3 h) a 2.3:1 mixture of N^1 and N^3 nucleosides resulted, however, in a lower overall yield. On reaction of the silylated base 7 with excess of blocked ribose **6** in presence of stannic chloride in 1,2-dichloroethane and prolongation of reaction time to 18 h, in addition to a 8.2:1 mixture of N^1 and N^3 derivatives, the N^1,N^4-disubstituted ribonucleoside **1O** was formed in a low yield. The structure of the N^1 nucleoside **5** was proved by identification with the product obtained by cyclization of amidinourea **4** and the structures of the N^3 derivative **9** and the N^1,N^4-disubstituted derivative **1O** were inferred from IR and ^{1}H NMR spec-



tra by comparison with the respective methyl derivatives of 5-azacytosine[9,10]. Methanolysis of protected N^3 nucleoside **9** afforded the free nucleoside **11**. Short treatment of **9** with sodium methoxide gave the mono-benzoate **12** in addition to the free N^3 nucleoside **11**.

The N^1 nucleoside **5** was also obtained in a 4% yield on direct glycosylation of the silylated 6-methyl-5-azacytosine **7** with halogenose **13** in acetonitrile at room temperature. In addition to **5** also the O^2-ribosyl derivative **14** was isolated in a 17% yield by column chromatography on silica gel.

The mixture of N^1 and N^3 nucleosides **5** and **9** was also formed by stannic chloride catalyzed transribosylation of any of the tribenzoates **5**, **9**, or **14.** In addition the diriboside[11] **15**, the isomeric tribenzoylriboses[12-14] **16** and **17** and the base[9] **8** were isolated as side products. The ratio of N^1 and N^3 nucleosides depends on the reaction time and the concentration of stannic chloride used. The lowest overall yield of this nucleosides was obtained in the reaction of N^3 nucleoside **9** with stannic chloride. In this case the formation of diriboside **15** predominated. Transribosylation of O^2-ribosyl derivative **14** by action of stannic chloride in presence of chlorotrimethylsilane gave higher overall yields of N^1 and N^3 nucleosides.

Methanolysis as well as ammonolysis of O^2-ribosyl derivative **14** afforded 6-methyl-5--azacytosine **8** and in the mother liquor anomeric methyl ribofuranosides[15] **18a,b** were detected by TLC.

The molecular conformation of 6-methyl-5-azacytidine (**1**) has been deduced from the ^{1}H NMR spectral parameters (Table 1). Conformations of the furanose ring have been determined by analyzing the vicinal coupling constants of ribose protons $J_{i,j}$ in the terms of pseudorotation parameters[16] of two conformations undergoing interconversion and their relative populations in equilibrium. One of the conformers belongs to the N region and the other is from the S region of the pseudorotation pathway. For the calculation of conformational parameters (Table 2), the puckering amplitudes $\tau_m N$, $\tau_m S$, the phase angles P^N, P^S, and the relative populations x_N, x_S the PSEUROT 6.2 program worked out by Altona[17] has been used. The fractional populations of rotamers about the C4'-C5' bond γ^+, γ^t and γ^- (Table 2) were calculated using the equations[18] with *trans* and *gauche* H-H coupling constants J_t of 11.5 Hz and J_g of 1.5 Hz.

For comparison analogous conformational studies of 5-azacytidine (Table 1 and 2) were performed. The sugar-base conformation of this nucleoside was previously studied by Hruska and co-workers[19]. These authors assumed on the basis of comparison of the ^{1}H

Table 1. ^{1}H NMR parameters of 6-methyl-5-azacytidine (**1**) and 5-azacytidine.

Compound	Chemical shifts, ppm							
	1'-H	2'-H	3'-H	4'-H	5'-H	5"-H	6-CH_3	6-H
6-methyl-5-azacytidine	5.76	4.78	4.39	4.00	3.88	3.74	2.57	
5-azacytidine	5.78	4.41	4.26	4.15	3.95	3.82		8.52
	Coupling constants, Hz							
	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{4',5''}$	$J_{5',5''}$		
6-methyl-5-azacytidine	3.5	6.4	7.0	3.0	6.1	-12.3		
5-azacytidine	3.2	5.0	6.9	2.8	4.2	-12.8		

Table 2. Conformational properties of 6-methyl-5-azacytidine (**1**) and 5-azacytidine.

Compound	Pseudorotation parameters and rotamer populations about the C4'-C5' bond								
	$\tau_m N$	P^N	$\tau_m S$	P^S	x_N	K_{eq}	γ^+	γ^t	γ^-
6-methyl-5-azacytidine	32	39	32	146	0.75	0.33	0.39	0.46	0.15
5-azacytidine	39	20	38	152	0.71	0.41	0.60	0.27	0.13

NMR spectra of series of pyrimidine nucleosides with 5-azacytidine a preference of anti conformation about the C-N glycosyl bond of the latter nucleoside. However, the conformational properties of the ribofuranose ring of 5-azacytidine as well as the conformation about the C(4')-C(5') bond of this nucleoside have not been studied.

The x_N values (Table 2) show that both 6-methyl-5-azacytidine and 5-azacytidine exist predominantly in the N-type conformation. However, 5-azacytidine exihibits a preference for the γ^+ conformation about the C(4')-C(5') bond with a gauche arrangement of the protons 4'-H, 5'-H and 5"-H and with the oxygen atom 5'-O projected over the furanose ring. This is in agreement with previous finding by Hruska and co-workers[19] who preferred anti conformation about the C-N glycosyl bond. On the other side, 6-methyl-5-azacytidine shows a preference for the γ^t conformation. In this case, the oxygen atom 5'-O is projected outside of the furanose ring. The predominance of the γ^t rotamer allows to assume the preference

of syn conformation about the C-N glycosyl bond. A similar conclusion can be drawn by comparison of the chemical shifts of the protons 2'-H, 3'-H and 4'-H of 6-methyl-5-azacytidine (**1**) and 5-azacytidine (Table 1) which adopts preferentially anti conformation.

Generally, a change from anti to syn conformation caused by the influence of substituents in the position 6 of pyrimidine nucleosides is connected with downfield shifts for the 2'-H (~ +0.5 ppm) and for the 3'-H (~ +0.15 ppm) whereas upfield shifts are observed for the 4'-H (~ -0.15 ppm) as compared to the unsubstituted nucleosides[20]. As evident from Table 1 chemical shift differences of the respective protons between 5-azacytidine and 6--methyl-5-azacytidne (**1**) are in agreement with this general presumption (+0.37, +0.13 and -0.15 ppm for the protons 2'-H, 3'-H and 4'-H, respectively). Obviously, the methyl group in 6-methyl-5-azacytidine (**1**) changes markedly the conformations about the C-N glycosyl and the exocyclic C(5')-C(4') bonds due to repulsive interactions between the methyl group and the glycone, but the conformation of the ribose ring is less influenced.

The interpretation of NMR chemical shifts in terms of syn-anti conformation about the C-N glycosyl bond is also supported by CD spectral data. 5-Azacytidine[21] shows an intensive positive B_{2u} Cotton effect ([Θ] +11600) at 249 nm. The position of the extreme in CD spectra does not corresponds exactly with the maximum of UV spectra. This fact can be explained by superposition of the B_{2u} with the B_{1u} band. The latter band is obviously of a smaller magnitude and the same sign as the more intensive B_{2u} band. The sign of the B_{2u} Cotton effect of 5-azacytidine has the same direction as the B_{2u} Cotton effects of the analogous pyrimidine nucleosides[22-24]. This indicates an anti conformation about the C-N glycosyl bond for 5-azacytidine. In contrast, 6-methyl-5-azacytidine (**1**) exhibits a positive B_{2u} Cotton effect of small magnitude ([Θ] +880) at 262 nm and a more intensive negative B_{1u} band ([Θ] -4410) at 231 nm. The extrema of the bands are shifted in comparison with UV spectra in opposite direction due to superposition of a negative and a positive curve. This pronounced change of the CD spectrum can be explained by a change of the sugar-base conformation from anti to syn.

Nucleosides **1** and **11** were tested for their antibacterial activity using a culture of E. coli B growing on a mineral medium with glucose[25]. 6-Methyl-5-azacytidine (**1**) inhibited the growth of bacteria to the extent of 85% at 4000 μM concentration while the isomeric N^3 nucleoside **11** was completely inactive at the same concentration. The unsubstituted 5-azacytidine inhibited by contrast the growth of E. coli B to the extent of 50% even at 1 μM

concentration[26]. It is of interest to note that the isomeric 6-azacytidine and also 6-azauridine exhibit similarly to 6-methyl-5-azacytidine (**1**) only a weak bacteriostatic effect (5O% growth inhibition at 2OOO μM and 5OOO μM concentrations, respectively)[26].

6-Methyl-5-azacytidine (**1**) was also tested for its ability to inhibit the growth of four tumor cell lines in vitro: L121O murine lymphocytic leukemia, WI-L2 human B-lymphoblastic leukemia, CCRF-CEM T-lymphoblastic leukemia, and LoVo/L, a human colon carcinoma. Nucleoside **1** inhibited to the extent of 3O% the growth of LoVo/L at 1OO μM concentration but did not inhibit growth of other tumor cell lines at ≤1OO μM concentration. These results indicate a much lower antitumor activity of nucleoside **1** in comparison with the parent 5-azacytidine[3].

6-Methyl-5-azacytidine (**1**) exhibited no in vitro antiviral activity against HSV-1, HSV-2, adeno, rhino, influenza and parainfluenza viruses at ≤1 μM concentration. Nucleoside **1** was also inactive against HIV-1. In contrast, 5-azacytidine and 2'-deoxy-5-azacytidine were highly active against HIV-1 at 1 μM concentrations[27].

Eventually it is of interest to note that 6-methyl-5-azacytidine (**1**) exhibited a higher potential carcinogenity[28] than 5-azacytidine[29] when estimated by a polarographic method[30-32].

CONCLUSION

The data presented in this paper allow to conclude that 6-methyl-5-azacytidine (**1**) represents a syn-type nucleoside in contrast to unsubstituted 5-azacytidine which is a typical anti nucleoside similarly to cytidine. The high biological activity of 5-azacytidine is based on its structural and conformational resemblance with cytidine which enables its incorporation into nucleic acids and subsequent covalent addition of mercapto groups of enzymes to the reactive double bond in 5,6 position of the 1,3,5-triazine ring[33]. Due to remarkable changes of conformation about both the C-N glycosyl and C(5')-C(4') bonds, 6-methyl-5-azacytidine (**1**) is obviously not able to be incorporated into nucleic acids. Moreover, the methyl group in position 6 prevents covalent interactions of the double bond in position 5,6 of the triazine ring with enzymes. These facts explain the dramatic differences in biological activity of 6--methyl-5-azacytidine (**1**) and the unsubstituted 5-azacytidine.

In this connection it is of interest to note that also 6-azauridine, a specific inhibitor of orotidylate decarboxylase, exhibits similar conformational properties of the ribose ring as 6--methyl-5-azacytidine (**1**). 6-Azauridine is not incorporated[34] into nucleic acids and the

double bond in position 5,6 of the 6-azauracil ring is much more resistent towards nucleophilic agents than the respective double bond of the 1,3,5-triazine ring of 5-azacytidine. We do believe that the differences in molecular conformation and reactivity of the double bond of the triazine ring in 5-azacytidine and 6-azauridine are responsible for the remarkable differences in the mechanism of action of these nucleosides.

EXPERIMENTAL

General Remarks. 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) was performed on Silufol UV 254 plates (Kavalier, Votice, The Czech Republic) in solvent systems A chloroform-methanol (98:2) and B 1-butanol-acetic acid--water (5:2:3). The spots were detected visually in UV light (254 nm). Column chromatography was performed with silica gel according to Pitra (Service Laboratories of the Institute).

UV spectra were measured on a Unicam SP 8000 spectrophotometer (Pye Unicam, Cambridge, England) in buffer solutions of ionic strength 0.01 prepared according to Perrin[35], λ are given in nm and ε in $m^2\ mol^{-1}$. Optical rotations were registered on a polarimeter Perkin-Elmer, type 141 MCA at 22 °C. ^1H NMR spectra of **1** and 5-azacytidine were 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. The signals of the geminal 5'-H and 5"-H protons were assigned according to Remin and Shugar[36] such that $\delta_{5'\text{-H}} > \delta_{5''\text{-H}}$. The 5'-H refers to the proton which is gauche to the 4'-H and the 4'-O atoms in the staggered γ^+ conformation. The chemical shifts (δ) and coupling constants (J) for the sugar protons were determined with an accuracy of ±0.01 ppm and ±0.1 Hz, respectively, by spectral simulation-iteration procedures using the SPIN program which is a part of the spectrometer program equipment. The IR spectra were recorded on a Zeiss UR-20 instrument, the wavenumbers are given in cm^{-1}. The mass spectra (m/z) were measured on a ZAB-EQ (VG Analytical Ltd, Manchester, U.K.) spectrometer using the FAB (ionization by Xe, accelerating voltage 8 kV) technique, matrices glycerol and thioglycerol or 2-hydroxyethyl disulfide. Stationary cultivation of Escherichia coli B was performed at 37 °C in mineral medium with glucose[25]. The tested

compounds were added before inoculation and the growth of bacteria was measured 16 h later.

2-Methyl-4-(trimethylsilylamino)-6-(trimethylsilyloxy)-1,3,5-triazine (7). A mixture of triazine[9] **8** (12.6 g, 100 mmol), hexamethyldisilazane (40 mL) and ammonium sulfate (200 mg) was refluxed at 165-170 °C (metal bath temperature) for 16 h. The excess of hexamethyldisilazane was evaporated at 55-60 °C (bath temperature). The residue was coevaporated with toluene (50 mL), minced to a powder and dried for 30 min at 50 °C in vacuo. The crude product was used in glycosylations without further purification. Yield, 25 g (92%) of compound 7, m.p. 82-85 °C (dec.) (seald capillary tube). The sample for the elemental analysis was sublimed at 90 °C / 30 Pa. IR (nujol): ν (ring) 1569 s, 1548 m, 1504 m, δ s (CH_3) 1253 s, 1262 m, δ rock (CH_3) as.: 853 s, 871 s, sym.: 762 w. Anal. $C_{10}H_{21}N_4OSi_2$: C, 44.56; H, 7.85; N, 20.79, Found: C, 44.28; H, 7.62; N, 21.03.

N-(Acetylamidino)-N'-(2,3,5-tri-O-benzoyl-ß-D-ribosyl)urea (4). A solution of crude isocyanate[6] **2** (prepared from 2 mmol of blocked ribose[15] **6**) in acetone (10 mL) was added dropwise at room temperature to a magnetically stirred mixture of acetylguanidine **3** (202 mg, 2 mmol) and acetone (5 mL). The mixture was stirred for 30 min, evaporated and the residue treated with toluene (20 mL). The small insoluble portion was filtered off, the clear filtrate evaporated and the crude amidinourea **4** used without purification in the next step (purification is connected with high lost of product due to hydrolytical cleavage of the acetyl group during chromatography on silica gel). An analytical sample was obtained by chromatography of the product from a parallel experiment on a column of silica gel (30 g). Elution was performed with toluene-ethyl acetate (100:0 - 80:20, v/v). The major portion was dried at 60 °C/40 Pa for 2 h to give 510 mg (43%) of pure **4** as a solid foam; R_F 0.64 (A), $[\alpha]_D$ -26° (c 0.21, $CHCl_3$). IR($CHCl_3$): ν(NH) 3384 m, ν(C=O) 1727 vs (benzoate), 1710 s (amide), 1668 s (urea), ν(C=N) 1622 m. Mass spectrum: 589 (MH^+). Anal. $C_{30}H_{28}N_4O_9$: C, 61.22; H, 4.80; N, 9.52. Found: C, 60.98; H, 4.73; N, 9.25.

2',3',5'-Tri-O-benzoyl-6-methyl-5-azacytidine (5). A solution of crude amidinourea **4** (prepared from 2 mmol of isocyanate **2**) in acetonitrile (10 mL) was treated with chlorotrimethylsilane (1.5 mL, 12 mmol) and triethylamine (1,5 mL, 11 mmol). The mixture was kept at room temperature for 20 min, benzene (20 mL) added, the insoluble triethylammonium chloride 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 to give 880

mg of crude **5**, m.p. 230-233 °C (dec.). Recrystallization from acetonitrile gave 796 mg (70%) of pure **5**, m.p. 239-241 °C (dec.), R_F 0.59 (A), $[\alpha]_D$ -21.7° (c 0.51, DMF). IR ($CHCl_3$): $\nu(NH_2)$ 3548 m, 3427 m, ν(C=O) 1729 m (benzoate), 1714 m, ν(C=N) 1623 m. ^{1}H NMR (DMSO-d_6) δ 2.47 (s, 3H, 6-CH_3), 4.67 (cm, 3H, 4'-H, 5'-H, 5"-H), 6.10 (cm, 3H, 1'-H, 2'-H, 3'-H). Mass spectrum: 571 (MH^+). Anal. $C_{30}H_{26}N_4O_8$: C, 63.15; H, 4.59; N, 9.82. Found: C, 62.93; H, 4.40; N, 9.59.

When purified amidinourea **4** was used as starting compound a 84% yield of **5** was obtained. In a parallel experiment crude amidinourea **4** (2 mmol) in acetonitrile (10 mL) was treated with bis(trimethylsilyl)acetamide (2 mL, 8 mmol) and the mixture evaporated after 30 min standing. The residue was dried, dissolved in ethanol (4 mL) and allowed to stand overnight. The crystalline precipitate was filtered off with suction and recrystallized to give 910 mg (80%) of pure **5**. The same result was obtained when bis(trimethylsilyl)trifluoroacetamide was used for mediating the cyclization.

Ribosylation of silylated 6-methyl-5-azacytosine 7.

Method A. To a solution of silylated 6-methyl-5-azacytosine **7** (2.80 g, 10.4 mmol) in 1,2-dichloroethane (50 mL) and stannic chloride (1,7 mL, 14.6 mmol) the blocked ribose[15] **6** (5.04 g, 10 mmol) was added with magnetical stirring. The solution was kept for 30 min at room temperature and cautiously shaken with ice-cold 5% aqueous solution of sodium hydrogen carbonate (200 mL). The mixture was extracted with chloroform (500 mL), filtered through a layer of celite, the organic layer dried (anhydrous sodium sulfate) and evaporated. The residue was crystallized from 1,2-dichloroethane and the crude product (2.5 g) recrystallized from acetonitrile to give 2.281 g (40%) of pure **5**, m.p. 239-241 °C (dec.). The product was identical with the sample prepared via amidinourea **4** (TLC, mixed m.p., $[\alpha]_D$, IR).

The mother liquor after crystallization of crude **5** from 1,2-dichloroethane was precipitated with ether to give crude **9** (1.0 g). Recrystallization from acetonitrile afforded 741 mg (13%) of pure **9**, mp 204-206 °C (dec.) (N^1:N^3 = 3:1), R_F 0.65 (A), $[\alpha]_D$ +42.6° (c 0.48, DMF). ^{1}H NMR($CDCl_3$) δ 2.12 (s, 3H, 6-CH_3), 4.50-4.85 (m, 3H, 4'-H, 5'-H, 5"-H), 5.90 (m, 2H, 2'-H, 3'-H), 6.93 (d, 1H, $J_{1',2'}$ = 3.6, 1'-H). Anal. $C_{30}H_{26}N_4O_8$: C, 63.15; H, 4.59; N, 9.82. Found: C, 63.12; H, 4.52; N, 10.02.

Prolongation of reaction time to 4 h gave a 49% yield of N^1 derivative **5** and a 7% yield of N^3 derivative **9** (N^1:N^3 = 7:1). When the reaction was carried out in acetonitrile (re-

action time 3 h) a 25% yield of N^1 derivative **5** and a 11% yield of N^3 derivative **9** was obtained (N^1:N^3 = 2.3:1).

In a further experiment the silylated triazine 7 (700 mg, 2.5 mmol) was reacted with blocked ribose **6** (2.52 g, 5 mmol) and stannic chloride (0.8 mL, 7 mmol) in 1,2-dichloroethane (15 mL) at room temperature for 18 h and the mixture worked up in analogy to the above procedure to yield 470 mg (33%) of N^1 derivative **5** and 60 mg (4%) of N^3 derivative **9** (N^1:N^3 = 8.2:1). The last mother liquor was chromatographed on silica gel (50 g) with toluene - ethyl acetate (100:0 - 60:40, v/v) and chloroform - methanol (95:5, v/v). Fractions containing compound **10** were collected, evaporated and the residue crystallized from ethanol to give 75 mg (3%) of N^1,N^4 - disubstituted derivative **10**, mp 115-120 °C (dec.), R_F 0.71 (A). IR($CHCl_3$): ν(N-H) 3414 w, ν(C=O) 1721 vs, ν(C=N) 1607 s, 1537 s, ν(ring) 1602 s, 1584 m, 1491 w, 1450 s. Mass spectrum: 1015 (MH^+). Anal. $C_{56}H_{46}N_4O_{15}$: C, 66.28; H, 4.57; N, 5.52. Found: C, 66.52; H, 4.54; N, 5.50.

Method B. A mixture of protected ribosyl chloride[6] **13** (prepared from 40 mmol of **6**), silylated triazine **7** (11.0 g, 40 mmol) and acetonitrile (40 mL) was magnetically stirred at room temperature for 3 days. The mixture was evaporated, dissolved in chloroform (200 mL), the solution washed with ice-cold 5% solution of sodium hydrogen carbonate and the mixture filtered with suction through a layer of celite and the material on the filter washed with chloroform - methanol (95:5, v/v) . The organic layer of the filtrate was separated, dried (anhydrous sodium sulfate), evaporated and chromatographed on silica gel (300 g) with benzene - ethyl acetate (80:20 - 0:100, v/v). The more mobile portion (5.5 g) was crystallized from benzene (20 mL) and recrystallized from acetonitrile to give 3.9 g (17%) of O^2 derivative **14** , mp 185-186 °C (dec.), R_F 0.85 (A), $[\alpha]_D$ -9.1° (c 0.51, DMF). IR ($CHCl_3$): ν(NH_2) 3541 m, 3426 m, ν(C=O)(benzoate) 1731 m, ν(ring) 1615 m, 1583 m, 1553 m. ^{1}H NMR(DMSO-d_6) δ 2.15 (s, 3H, 6-CH_3), 4.52 (m, 2H, 5'- H, 5"- H), 4.78 (m, 1H, 4'- H), 5.68-6.00 (cm, 2H, 2'- H, 3'- H), 6.62 (bs, 1H, $J_{1',2'}$ < 1.0, 1'- H). Anal. $C_{30}H_{26}N_4O_8$: C, 63.15; H, 4.59; N, 9.82. Found: C, 63.45; H, 4.58; N, 10.09. The less mobile portion (1.5 g) was crystallized from benzene and recrystallized from acetonitrile to give 900 mg (4%) of N^1 derivative **5**, mp 238-240 °C (dec.). The product was identical (TLC, $[\alpha]_D$ and IR) with the sample prepared via amidinourea **4**.

Transribosylation of 14. A mixture of **14** (571 mg, 1 mmol), 1,2-dichloroethane (5 mL) and stannic chloride (0.2 mL, 1.7 mmol) was magnetically stirred for 3 h at room tem-

perature. The solution was worked up in analogy to ribosylation of **7** (method A) to yield 120 mg (21%) of N^1 nucleoside **5**, mp 239-241 °C (dec.) and 45 mg (8%) of N^3 nucleoside **9**, mp 203-205 °C (dec.) (N^1:N^3 = 2.6:1). The last mother liquor containing according to TLC ($CHCl_3$) mainly diriboside **15** and the isomeric tribenzoylriboses **16** and **17** was chromatographed on silica gel (20 g) with toluene - ethyl acetate (100:0 - 50:50, v/v). The more mobile portion was crystallized twice from ethanol to yield 60 mg (13%) of diriboside **15**, mp 138-141 °C undepressed with an authentic specimen[11], R_F 0.71 ($CHCl_3$). UV (MeOH), λ_{max} (logε): 273 (3.94), 229 (4.83). Mass spectrum: 907 (MH^+). Anal. $C_{52}H_{42}O_{15}$: C, 68.85; H, 4.67. Found: C, 69.09; H, 4.51. The less mobile portion was crystallized from ethanol to give 45 mg (9.7%) of 1,3,5-tri-O-benzoyl-α-D-ribose (**16**), mp 142-143 °C undepressed with an authentic specimen[12-14], $[\alpha]_D$ +85.3° (c 1.03, $CHCl_3$). UV(MeOH), λ_{max} (logε): 273 (3.47), 228 (4.57). Anal. $C_{26}H_{22}O_8$: C, 67.53; H, 4.80. Found: C, 67.25; H, 4.81. Crystallization of the mother liquor from ether-petroleum ether afforded 25 mg (5.4%) of the isomeric 2,3,5-tri-O-benzoyl-ß-D-ribose (**17**), mp 104-106 °C undepressed with an authentic sample[12-14]; $[\alpha]_D$ +64.8° (c 1.0, $CHCl_3$). UV(MeOH), λ_{max} (logε): 274 (3.66), 229 (4.59). Anal. $C_{26}H_{22}O_8$: C, 67.53; H, 4.80. Found: C, 67.58; H, 4.82. The layer of celite through which the crude mixture after washing with sodium hydrogen carbonate was filtered gave by washing with chloroform-methanol (95:5, v/v) and evaporation of the filtrate 45 mg (36%) of 6-methyl-5-azacytosine (**8**), mp > 350 °C (dec.), identical with an authentic sample[9] (TLC, UV, IR).

In a further experiment (1 mmol) in addition of stannic chloride (0.1 mL, 0.87 mmol) also chlorotrimethylsilane (0.1 mL, 0.79 mmol) was added to the mixture. After 1 h standing at room temperature the mixture was worked up in analogy to the above procedure to afford 240 mg (42%) of N^1 nucleoside **5**, mp 238-240 °C (dec.) and 100 mg (18%) of N^3 nucleoside **9**, mp 202-204 °C (dec.) (N^1:N^3 = 2.4:1). In the last mother liquor the presence of the sugar derivatives **15, 16** and **17** was detected by TLC ($CHCl_3$). From the layer of celite 20 mg (16%) of the base **8** was extracted.

Transribosylation of 5. A mixture of nucleoside **5** (571 mg, 1 mmol), 1,2-dichloroethane (5 mL) and stannic chloride (0.2 mL, 1.7 mmol) was magnetically stirred for 3 h at room temperature and worked up in analogy to the ribosylation of 7 (method A) to give 200 mg (35%) of starting N^1 nucleoside **5**, mp 238-240 °C (dec.) and 35 mg (6%) of N^3

nucleoside **9**, mp 202-204 °C (dec.) (N^1:N^3 = 6:1). In the last mother liquor the presence of sugar derivatives **15, 16** and **17** was detected by TLC ($CHCl_3$). In the water phase the presence of the base **8** was proved by TLC in solvent system B.

Transribosylation of 9. A mixture of nucleoside **9** (571 mg, 1 mmol), 1,2-dichloroethane (5 mL) and stannic chloride (0.1 mL, 0.86 mmol) was magnetically stirred at room temperature for 1 h and the mixture worked up in analogy to the ribosylation of **7** (method A) to yield 18 mg (3%) of N^1 nucleoside **5**, mp 238-240 °C (dec.) and 100 mg (18%) of starting compound **9**, mp 202-204 °C (dec.) (N^1:N^3 = 1:6). The last mother liquor was chromatographed on silica gel (20 g) with toluene - ethyl acetate (100:0 - 50:50, v/v). The more mobile portion was crystallized twice from ethanol to give 70 mg (15%) of diriboside **15**, mp 136-140 °C, undepressed with an authentic sample. The less mobile portion (60 mg) which contained sugar derivatives **16** and **17** was not worked up in this experiment. In the water phase the base **8** was detected by TLC in solvent system B.

6-Methyl-5-azacytidine (1). A mixture of blocked nucleoside **5** (571 mg, 1 mmol), methanol (5 mL) and methanolic 1M-$NaOCH_3$ (0.2 mL) was magnetically stirred for 24 h at room temperature. The mixture was acidified with acetic acid, the crystals filtered off with suction and the crude product [250 mg, mp 140-147 °C (dec.)] recrystallized from ethanol to afford 209 mg (81%) of **1**, mp 142-147 °C (dec.), $[\alpha]_D$ -41.1° (c 0.10, H_2O), R_F 0.42 (B). CD(pH 6.9), λ_{max} ($[\Theta]_{max}$): 262 (+880), 231 (-4 410). UV(MeOH), λ_{max} (logε): 228 (4.02), 209 (4.14); (pH 2.4): 245 (inflexion) (3.51), 226 (3.72); (pH 6.9): 230 (inflexion) (3.99), 206 (4.24); (pH 11.0): 243 (4.04), 227 (4.24). For ^{1}H NMR see Table 1. Mass spectrum: 258 (M^+). Anal. $C_9H_{14}N_4O_5$: C, 41.86; H, 5.46; N, 21.70. Found: C, 42.06; H, 5.66; N, 21.38.

4-Amino-6-methyl-3-ß-D-ribofuranosyl-1,3,5-triazin-2(3H)-one (11). A mixture of the blocked nucleoside **9** (571 mg, 1 mmol), methanol (5 mL) and methanolic 1M-NaOMe (0.4 mL) was magnetically stirred for 8 h at room temperature and the solution kept overnight. The solution was acidified with acetic acid and decationized on a column of Amberlite IRC-50[H^+] ion exchange resin (10 mL) which was prepared in methanol. The product was eluted with methanol and the eluate evaporated. The residue was crystallized from ethanol and the crude product [230 mg, mp 193-195 °C (dec.) (resolidification)] recrystallized to give 181 mg (70%) of the free nucleoside **11**, mp 198-201 °C (dec.) (resolidification), $[\alpha]_D$ +27.4° (c 0.12, H_2O), R_F 0.41 (B). CD(pH 6.9), λ_{max} ($[\Theta]_{max}$): 257

(+1O 76O), 242 sh (+8 23O). UV(pH 6.9), λ_{max} (logε): 263 (3.93). Mass spectrum: 259 (MH^+). Anal. $C_9H_{14}N_4O_5$: C, 41.86; H, 5.46; N, 21.7O. Found: C, 41.69; H, 5.44; N, 21.45.

4-Amino-3-(5-O-benzoyl-ß-D-ribofuranosyl)-6-methyl-1,3,5-triazin-2(3H)-one (12). A mixture of the blocked nucleoside **9** (571 mg, 1 mmol), methanol (5 mL) and methanolic 1M-NaOMe (O.2 mL) was magnetically stirred for 1 h at room temperature. The crystalline precipitate was filtered off by suction and the crude product [14O mg, mp 215-218 °C (dec.)] recrystallized from methanol-water to give 76 mg (21%) of the monobenzoate **12**, mp 22O-222 °C (dec.), R_F O.63 (B), $[\alpha]_D$ -34.2° (O.1O, DMF). UV(pH 2.3), λ_{max} (logε): 233 (4.2O); (pH 7.O): 263 (3.85), 231 (4.OO); (pH 11.O): 266 (3.7O), 22O (3.98). Mass spectrum: 362 (M^+). Anal. $C_{16}H_{18}N_4O_6$: C, 53.O4; H, 5.O1; N, 15.46. Found: C, 52.84; H, 5.O7; N, 15.35. The original mother liquor was acidified with acetic acid and worked up in analogy to the preparation of **11** to give 65 mg (25%) of free nucleoside **11**, mp 195-198 °C (dec.).

Cleavage of O^2-ribosyl triazine 14. Method A. A mixture of O^2-ribosyltriazine **14** (285 mg, O.5 mmol), methanol (5 mL) and methanolic 1M-NaOMe (O.5 mL) was magnetically stirred at room temperature for 45 min. The solution was acidified with acetic acid and the precipitate filtered off with suction to yield 5O mg (79%) of the base **8**, mp > 35O °C (dec.). The product was identified (UV, IR) with an authentic sample[9]. Anal. $C_4H_6N_4O$: C, 38.O9; H, 4.8O; N, 44.43. Found: C, 38.O7; H, 4.74; N, 44.15. In the mother liquor the presence of anomeric ribofuranosides **18a,b** was detected by TLC in solvent system B. For comparison an authentic sample[15] of **18a,b** was used (detected as black spots by heating with a flame).

Method B. A mixture of O^2-ribosyl derivative **14** (285 mg, O.5 mmol) and 7M ammonia in methanol (1O mL) was magnetically stirred at room temperature for 7 h and kept overnight. The mixture was evaporated, the residue treated with methanol (5 mL), acidified with acetic acid and the precipitate filtered off to give 5O mg (79%) of the base **8**, mp > 35O °C (dec.). In the mother liquor the presence of **18a,b** was proved by TLC.

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