SYNTHESIS AND BIOLOGICAL ACTIVITY OF N^4-METHYL-5-AZACYTIDINES

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Protected N^4-methyl and N^4,N^4-dimethyl derivatives of 5-azacytidine **3** and **4** were prepared by selective aminolysis of benzoylated 4-methoxy-1-(β-D-ribofuranosyl)-1,3,5-triazin-2(1*H*)-one **5**, by glycosylation of silylated N^4-methyl- or N^4,N^4-dimethyl-5-azacytosines **7** and **8** with 2,3,5-tri-*O*-benzoyl-α,β-D-ribofuranosyl chloride (**11**) or by several modifications of the isocyanate method. By the isocyanate approach, also the α-D anomer of protected N^4-methyl-5-azacytidine **17** was obtained as a minor product. The protected dimethyl derivative **4** was also obtained by the reaction of isobiuret **22** with dimethylformamide dimethyl acetal. The free nucleosides **1** and **2** were obtained either by aminolysis of the free methoxy nucleoside **23** with methylamine or dimethylamine, respectively, or by methanolysis or ammonolysis of the corresponding benzoyl derivatives **3** and **4**. The free α-D anomer **24** was obtained by methanolysis of its tribenzoate **17**. Nucleosides **1** and **2** exhibited a lower antibacterial, antitumor and antiviral activity than the unsubstituted 5-azacytidine.

Key words: 5-Azacytidines; 1,3,5-Triazines; 5-Azapyrimidines; Nucleosides; Antitumor activity.

5-Azacytidine[1] (Azacitidine) and 2′-deoxy-5-azacytidine[2] (Decitabine) are used in clinical treatment of acute leukemia[3,4]. Both agents are potent hypomethylating agents and are used as experimental tools in molecular and cell biology for induction of gene expression and cell differentiation[5]. Wide spectrum of biological activity of the mentioned 5-azacytosine nucleosides stimulated our interest in substituted congeners of these compounds. Previously, some 6-substituted 5-azacytidines[6–8] were prepared in our laboratory and their biological activity was described. In continuation of the study of substituted 5-azacytosine nucleosides, we were interested in N^4-methyl-5-azacytidine (**1**) and N^4,N^4-dimethyl-5-azacytidine (**2**). In this paper, we wish to present the preparation and biological activity of these nucleosides.

Benzoylated N^4-methyl- and N^4,N^4-dimethyl-5-azacytidines, **3** and **4**, have been prepared in 44 and 79% yields by short treatment of 4-methoxy-1-(2,3,5-tri-*O*-benzoyl-β-

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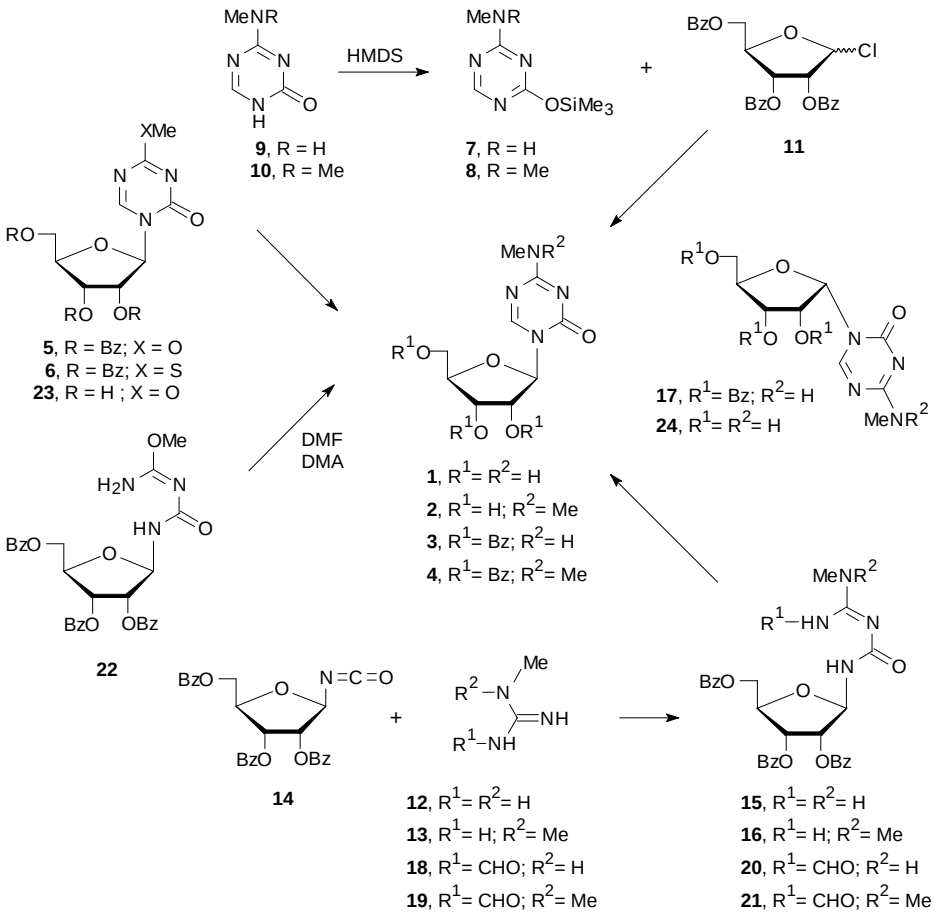
D-ribofuranosyl)-1,3,5-triazin-2(1*H*)-one[9] (**5**) with methylamine and dimethylamine, respectively, in methanol (Scheme 1). The dimethyl derivative **4** was also prepared in 66% yield using 4-methylsulfanyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,3,5-triazin-2(1*H*)-one[10] (**6**) as intermediate. However, the best method for the preparation of nucleosides **3** and **4** consists in ribosylation of silylated N^4-methyl- and N^4,N^4-dimethyl-5-azacytosines **7** and **8** (prepared by the reaction of the respective bases[11] **9** and **10** with hexamethyldisilazane at elevated temperature) with 2,3,5-tri-*O*-benzoyl-α,β-D-ribofuranosyl chloride[6] (**11**) in acetonitrile. Additionally to high yields (almost 90%), this reaction was highly stereoselective (no corresponding α-anomers were detected). Lower yields (12–24%) of methyl derivatives **3** and **4** were obtained by various modifications of the isocyanate method which proved to be convenient for the preparation of 6-substituted 5-azacytosine nucleosides[6–8] or for the preparation of 5-azacytosine and



SCHEME 1

its methyl derivatives[11]. Addition of N-methylguanidine (**12**) or *N*,*N*-dimethylguanidine (**13**) to 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl isocyanate[6] (**14**) afforded carbamoylguanidines **15** and **16**, respectively. Cyclocondensation of these unisolated intermediates with triethyl orthoformate or dimethylformamide dimethyl acetal gave the respective nucleosides **3** and **4**. In the case of the methyl derivative **3**, its α-D anomer **17** was also isolated in a 5% yield. Comparable results were obtained in a modification of this approach. Formyl derivatives of methyl- and dimethylguanidine **18** and **19** (prepared by reaction of the respective guanidines **12** and **13** with ethyl formate) were added to the isocyanate **14** and the unisolated formylcarbamoylguanidines **20** and **21** were cyclized by the treatment of a mixture of chlorotrimethylsilane and triethylamine or *N*,*O*-bis(trimethylsilyl)acetamide to the respective methyl derivatives **3** and **4**. The dimethyl derivative **4** was eventually obtained in a 45% yield by cyclocondensation of 4-methyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)isobiuret[9] (**22**) with dimethylformamide dimethyl acetal in benzene. Free nucleosides **1** and **2** were obtained in good yields by methanolysis or ammonolysis of the respective benzoyl derivatives **3** and **4** or by reaction of 4-methoxy-1-(β-D-ribofuranosyl)-1,3,5-triazin-2(1*H*)-one[12] (**23**) with methylamine and dimethylamine, respectively, in methanol. Methanolysis of benzoylated α-D anomer **17** gave the free nucleoside **24** in a 65% yield.

The assignment of anomeric configuration in compounds **1–4**, **17** and **24** was inferred from the chemical shifts of H-1′ protons on the basis of a well known rule and the sign of Cotton effect in CD spectra of the free nucleosides **1**, **2** and **24**. The β-nucleosides **1** and **2** exhibited positive B_{2u} Cotton effects as 5-azacytidine and the α-anomer **24** showed a negative Cotton effect as the α-anomer of 5-azacytidine. The β-configuration of the nucleosides **1** and **2** follows moreower from their formation by aminolysis of the methoxy nucleoside **23** which is converted by ammonolysis to the known 5-azacytidine[1].

Similarly to unsubstituted 5-azacytidine[13], the CD spectra of methyl derivatives **1** and **2** exhibited intensive positive B_{2u} Cotton effects at about 250 nm. By contrast, the α-D anomer **24** showed a negative Cotton effect nearly at the same wavelength as the respective β-D anomers **1** and **2**. The magnitude of the negative band of **24** is much larger than that of the positive bands of **1** and **2**. The position of the extremes in CD spectra does not correspond to the maxima in UV spectra exactly. 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 effects of nucleosides **1**, **2** and **24** has the same direction as the sign of B_{2u} Cotton effects of the analogous pyrimidine nucleosides[14–16]. This indicates an *anti* conformation around the C–N glycosyl bond for N^4-substituted methyl derivatives of 5-azacytidine **1**, **2** and **24**.

Nucleosides **1** and **2** were tested for their antibacterial activity using a culture of *E. coli* B growing on a mineral medium with glucose[17]. N^4-Methyl-5-azacytidine (**1**) inhibited

the growth of bacteria to the extent of 17% at 4 μM concentration while N^4,N^4-dimethyl-5-azacytidine (**2**) was much less active (15% growth inhibition at 400 μM concentration). By contrast, the unsubstituted 5-azacytidine inhibited the growth of *E. coli* B to the extent of 50% even at 1 μM concentration[18]. N^4-Methyl-5-azacytidine (**1**) and N^4,N^4-dimethyl-5-azacytidine (**2**) were also tested for their ability to inhibit the growth of four tumor cell lines *in vitro*: L1210 murine lymphocytic leukemia, WI-L2 human B-lymphoblastic leukemia, CCRF-CEM human T-lymphoblastic leukemia, and LoVo/L, a human colon carcinoma. N^4-Methyl-5-azacytidine (**1**) produced 29, 34 and 39% inhibition of growth of WI-L2, CCRF-CEM and LoVo/L, respectively, at 100 μM concentrations but was inactive in inhibiting L1210 at the same concentration. N^4,N^4-Dimethyl-5-azacytidine (**2**) inhibited to the extent of 29% the growth of LoVo/L at 100 μM concentration but did not inhibit growth of other tumor cell lines at the same concentration. These results indicate a lower antitumor activity of nucleosides **1** and **2** in comparison with unsubstituted 5-azacytidine[3]. N^4-Methyl-5-azacytidine (**1**) and N^4,N^4-dimethyl-5-azacytidine (**2**) exhibited no *in vitro* antiviral activity against HSV-1, HSV-2, adenoviruses, rhinoviruses, influenza and parainfluenza viruses at ≤1 μM concentration. N^4-Methyl-5-azacytidine (**1**) reduced rhinovirus 1-A cytopathology in HeLa cells by 50% at 100 μM concentration but was inactive against other viruses. In this connection, it is worth mentioning that unsubstituted 5-azacytidine and 2′-deoxy-5-azacytidine were active against HIV-1 even at 1 μM concentrations[19]. Eventually, it is of interest to note that nucleosides **1** and **2** exhibited a lower potential carcinogenity than 5-azacytidine[20] or 6-methyl-5-azacytidine[21] when estimated by a polarographic method[22–24]. Nucleosides **1** and **2** have also shown interesting antisecretory effects[25].

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 the 5,6 position of the 1,3,5-triazine ring[26]. The CD spectra of methyl derivatives **1** and **2** indicate an *anti* conformation around the C–N glycosyl bond of these nucleosides similarly to unsubstituted 5-azacytidine. However, substitution of hydrogen atoms on the amino group of 5-azacytidine by methyl groups prevents, predominantly for sterical reasons, the incorporation into nucleic acids and this fact may explain the lower activity of these nucleosides in comparison with the unsubstituted 5-azacytidine.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. 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, Czech Republic) in solvent systems chloroform–methanol (98 : 2, I) and butan-1-ol–acetic acid–water (5 : 2 : 3, II). The spots were detected visually in UV light (254 nm). Column chromatography was performed with silica gel according to Pitra (Service Laboratories of this Institute). UV spectra were measured on a Unicam SP 8000 spectrophotometer (Pye Unicam, Cambridge, U.K.) in

buffer solutions of ionic strength 0.01 prepared according to Perrin[27], λ are given in nm and ε in $m^2 mol^{-1}$. CD spectra were recorded on a Roussel–Jouan/II dichrographe. Optical rotations were registered on a Perkin–Elmer polarimeter, type 141 MCA at 22 °C. ^{1}H NMR spectra of the nucleosides **1** and **2** were measured on a Varian XL-200 instrument (200 MHz) in D_2O with sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) as internal standard. ^{1}H NMR spectra of the nucleosides **3**, **4**, **17** and **24** were measured on a Varian UNITY 500 instrument at 500 MHz in hexadeuteriodimethyl sulfoxide with the solvent signal as the internal reference [δ(^{1}H) = 2.50 ppm]. The chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hz. The mass spectra (*m/z*) were measured on a ZAB-EQ (VG Analytical Ltd, Manchester, U.K.) spectrometer using the FAB technique (ionization by Xe, accelerating voltage 8 kV), matrices glycerol and thioglycerol. Stationary cultivation of *E. coli* B was performed at 37 °C in mineral medium with glucose[17]. The tested compounds were added before inoculation and the growth of bacteria was measured 16 h later.

N^4-Methyl-2′,3′,5′-tri-*O*-benzoyl-5-azacytidine (**3**) and Its α-D Anomer **17**

Method A. A mixture of the blocked methoxy nucleoside[9] **5** (0.572 g, 1 mmol), methanol (7 ml) and a 13% solution of dry methylamine in methanol (1.5 ml) was stirred at room temperature for 5 min and crystallization of the product induced by scratching. The mixture was chilled to –15 °C (30 min), the product filtered off with suction and recrystallized from methanol to yield 0.250 g (44%) of **3**, m.p. 245 °C (dec.), R_F 0.60 (I), $[\alpha]_D$ –60.2 (*c* 0.20, DMF). UV spectrum (MeOH), λ_{max} (log ε): 260, inflexion (4.22), 229 (4.63), 203 (4.38). ^{1}H NMR spectrum: 8.42 s, 1 H (H-6); 8.25 q, 1 H, *J*(NH,CH_3) = 4.9 (NH); 8.00 d, 2 H (arom.); 7.89 t, 2 H (arom.); 7.64 m, 3 H (arom.); 7.50–7.40 m, 6 H (arom.); 6.06–6.00 m, 3 H (H-1′, H-2′, H-3′); 4.74 m, 1 H (H-4′); 4.69 dd, 1 H, *J*(5′a,4′) = 3.9, *J*(gem.) =12.0 (H-5′a); 4.62 dd, 1 H, *J*(5′b,4′) = 5.9, *J*(gem.) = 12.0 (H-5′b); 2.77 d, 3 H, *J*(CH_3,NH) = 4.9 (N-CH_3). Mass spectrum: 571 (MH^+). For $C_{30}H_{26}N_4O_8$ (570.3) calculated: 63.15% C, 4.59% H, 9.82% N; found: 63.45% C, 4.73% H, 9.90% N.

Method B. A mixture of N^4-methyl-5-azacytosine[11] **9** (1.68 g, 13 mmol), hexamethyldisilazane (5 ml) and ammonium sulfate (0.01 g) was refluxed on an oil bath (160–170 °C) for 5 h. The mixture was then evaporated at 50–60 °C (bath temperature), the semicrystalline residue coevaporated with toluene (20 ml) and dried *in vacuo* at 50 °C for 4 h to give 2.7 g of crude silylated N^4-methyl-5-azacytosine **7**. A mixture of crude **7** (2.7 g), acetonitrile (15 ml) and tri-*O*-benzoyl-α,β-D-ribofuranosyl chloride **11** (prepared from 5.30 g, 10.4 mmol of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose[28] by the known procedure[6]) was kept overnight at room temperature. The crystalline precipitate was filtered off by suction and washed with acetonitrile to yield 3.0 g (portion A) of crude **3**, m.p. 240–242 °C (dec.). The mother liquor was evaporated, the residue dissolved in chloroform (50 ml), the solution washed with ice-cold 5% solution of sodium hydrogencarbonate (100 ml), dried (anhydrous sodium sulfate) and evaporated. The residue was dissolved in 1,2-dichloroethane (10 ml) and petroleum ether (10 ml) was added. The mixture was allowed to stand overnight to yield 2.6 g (portion B) of **3**, m.p. 243–244 °C (dec.). Both portions (5.6 g) were combined and recrystallized from a mixture of 1,2-dichloroethane–petroleum ether to afford 5.20 g (88%, based on starting 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose) of **3**, m.p. 243–245 °C (dec.) without depression on admixture with a sample of **3** prepared by method *A*.

Method C. To a solution of *N*-methylguanidine hydrochloride (0.220 g, 2 mmol) in methanol (10 ml), methanolic 1 M NaOMe (2 ml) was added and the mixture diluted with benzene (10 ml). Sodium chloride was filtered off with suction, the filtrate evaporated and dried *in vacuo* to give *N*-methylguanidine (**12**). To a stirred mixture of **12** (2 mmol), a solution of crude isocyanate **14** (prepared from 1.008 g, 2 mmol of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose[28] by a known procedure[6]) was added dropwise at room temperature. The mixture was stirred for 1 h, the small insoluble portion removed

by filtration through a layer of celite and evaporated. A solution of the residue in chloroform (10 ml) was washed with water (10 ml), dried (anhydrous sodium sulfate) and evaporated to give the crude carbamoylguanidine **15**. A solution of crude **15** (2 mmol) in triethyl orthoformate (10 ml) was heated (bath temperature 115 °C) in a distillation apparatus in a stream of nitrogen for 2.5 h. The mixture was evaporated and chromatographed on a column of silica gel (50 g). Elution was performed with toluene–ethyl acetate (100 : 0 – 0 : 100, v/v). The more mobile portion was crystallized from methanol to yield 0.140 g (12%) of **3**, m.p. 243–245 °C (dec.) without depression on admixture with the sample prepared by method *A*. The less mobile portion gave, on crystallization from methanol and acetonitrile, 0.060 g (5%) of the α-D anomer **17**, m.p. 238–239 °C (dec.), R_F 0.50 (I), $[\alpha]_D$ –58.4 (*c* 0.12, $CHCl_3$). ^{1}H NMR spectrum: 8.63 s, 1 H (H-6); 8.20 d, 2 H (arom.); 8.12 q, 1 H, *J*(NH,CH_3) = 4.9 (NH); 7.83 d, 2 H (arom.); 7.78 d, 2 H (arom.); 7.70–7.59 m, 3 H (arom.); 7.53 t, 2 H (arom.); 7.45 t, 2 H (arom.); 7.39 t, 2 H (arom.); 6.59 d, 1 H, *J*(1′,2′) = 4.9 (H-1′); 6.05 t, 1 H, *J*(2′,1′) = *J*(2′,3′) = 5.0 (H-2′); 5.98 dd, 1 H, *J*(3′,2′) = 5.2, *J*(3′4′) = 6.1 (H-3′); 5.22 m, 1 H (H-4′); 4.66 dd, 1 H, *J*(5′a,4′) = 3.9, *J*(gem.) = 12.0 (H-5′a); 4.59 dd, 1 H, *J*(5′b,4′) = 4.9, *J*(gem.) = 12.0 (H-5′b); 2.68 d, 3 H, *J*(CH_3,NH) = 4.9 (N-CH_3). Mass spectrum: 571 (MH^+). For $C_{30}H_{26}N_4O_8$ (570.3) calculated: 63.15% C, 4.59% H, 9.82% N; found: 63.36% C, 4.66% H, 10.10% N.

Method D. To a solution of crude amidinourea **15** prepared by method *C* from crude isocyanate **14** (2 mmol) in benzene (5 ml), dimethylformamide dimethyl acetal (0.5 ml) was added. The solution was kept overnight at room temperature and the precipitate filtered off with suction to give 0.180 g (portion A) of **3**, m.p. 241–244 °C (dec.). The mother liquor was evaporated and the residue chromatographed in the way indicated under method *C* to yield 0.120 g (portion B) of **3**, m.p. 241–244 °C (dec.) (methanol) and 0.030 g (2.6%) of the α-D anomer **17**, m.p. 238–239 °C (dec.) (acetonitrile). Both portions of the β-D anomer **3** were combined and recrystallized from methanol to give 0.250 g (22%) of pure **3**, m.p. 243–245 °C (dec.). Both products were identical with the respective samples of **3** and **17** prepared by method *C* (mixed m.p., TLC).

Method *E*. A solution of *N*-methylguanidine (**12**) (prepared from 0.220 g, 2 mmol of its hydrochloride as given under method *C*) in a mixture of ethanol (1 ml) and ethyl formate (0.17 ml, 2.2 mmol) was kept at room temperature for 1 h, evaporated and the residue dried *in vacuo* for 2 h to give crude 1-formyl-3-methylguanidine (**18**). To a solution of crude **18** (2 mmol) in acetonitrile (5 ml), a solution of crude isocyanate **14** (prepared from 1.008 g, 2 mmol of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose[28] by a known procedure[6]) in acetonitrile (10 ml) was added dropwise at room temperature with stirring. The solution was treated with chlorotrimethylsilane (1.5 ml) and triethylamine (1.5 ml) and allowed to stand at room temperature for 30 min. The mixture was diluted with benzene (20 ml), the crystals of triethylamine hydrochloride were filtered off with suction, the filtrate was evaporated and the residue chromatographed on a column of silica gel (25 g). Elution was performed as given under method *C*. The major portion was crystallized from a mixture of 1,2-dichloroethane–petroleum ether to yield 0.180 g (16%) of **3**, m.p. 242–244 °C (dec.) without depression on admixture with the sample prepared by method *A*.

N^4,N^4-Dimethyl-2′,3′,5′-tri-*O*-benzoyl-5-azacytidine (**4**)

Method A. A mixture of blocked methoxy nucleoside[9] **5** (0.572 g, 1 mmol) and a 7% solution of dry dimethylamine in methanol (2.5 ml) was stirred at room temperature for 5 min. The mixture was kept at 0 °C for 30 min and the product filtered off by suction to afford 0.460 g (79%) of **4**, m.p. 216–219 °C. The sample for analysis was recrystallized from acetonitrile [m.p. 220 °C (dec.)], R_F 0.73 (I), $[\alpha]_D$ –61.9 (*c* 0.45, DMF). UV spectrum (MeOH), λ_{max} (log ε): 265, inflexion (4.05), 226 (4.69), 204 (4.39). ^{1}H NMR spectrum: 8.52 s, 1 H (H-6); 7.99 d, 2 H (arom.); 7.88 t, 4 H (arom.); 7.64 m, 3 H (arom.); 7.50–7.41 m, 6 H (arom.); 6.04–6.00 m, 3 H (H-1′, H-2′, H-3′); 4.75 td, 1 H,

J(4′,5′a) = 3.9, J(4′,3′) ≈ J(4′,5′a) ≈ 6.1 (H-4′); 4.69 dd, 1 H, J(5′a,4′) = 3.9, J(gem.) = 12.0 (H-5′a); 4.62 dd, 1 H, J(5′b,4′) = 5.9, J(gem.) = 12.0 (H-5′b); 3.17 s, 3 H (N-CH_3); 3.08 s, 3 H (N-CH_3). Mass spectrum: 585 (MH^+). For $C_{31}H_{28}N_4O_8$ (584.3) calculated: 63.69% C, 4.83% H, 9.58% N; found: 63.93% C, 4.92% H, 9.81% N.

Method B. The blocked methylsulfanyl nucleoside[10] **6** (0.588 g, 1 mmol) was treated in analogy to method *A* with a 7% solution of dry dimethylamine in methanol (2.5 ml) to afford 0.386 g (66%) of **4**, m.p. 216–218 °C (dec.) without depression on admixture with the sample prepared by method *A*.

Method C. N^4,N^4-Dimethyl-5-azacytosine[11] **10** (1.812 g, 13 mmol) was silylated and the syrupy silylated base **8** (2.65 g) ribosylated in analogy to the preparation of **3** (method *B*). The product deposited from the reaction mixture was filtered off by suction to give 5.02 g (portion A) of the nucleoside **4** m.p. 211–214 °C (dec.). The mother liquor was worked up in analogy to the preparation of **3** (method *B*) and the syrupy product crystallized from a mixture of 1,2-dichloroethane–petroleum ether (1 : 1) to afford 0.35 g (portion B) of **4**, m.p. 211–214 °C (dec.). Both portions (5.37 g) were combined and recrystallized from a mixture of 1,2-dichloroethane–petroleum ether (1 : 1) to yield 5.0 g (86%, based on starting 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose) of **4**, m.p. 216–219 °C (dec.) without depression on admixture with the sample prepared by method *A*.

Method D. A solution of crude isocyanate **14** (prepared from 1.008 g, 2 mmol of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose[28] by a known procedure[6]) in acetone (5 ml) was added dropwise at room temperature to a magnetically stirred mixture of 1,1-dimethylguanidine (**13**) [prepared from 0.248 g, 2 mmol of its hydrochloride as described for **12** (see preparation of **3**, method *C*)] and acetone (5 ml). The mixture was allowed to stand for 1 h and evaporated to yield the crude carbamoylguanidine **16** (2 mmol) which was further worked up and reacted with orthoformate in analogy to the preparation of **3** (method *C*) to yield 0.140 g (12%) of **4**, m.p. 216–219 °C (dec.) without depression on admixture with the sample prepared by method *A*.

Method E. To a solution of crude carbamoylguanidine **16** (2 mmol) prepared by method *D* in benzene (5 ml), dimethylformamide dimethyl acetal (0.5 ml, 3.3 mmol) was added. The solution was kept overnight at room temperature and worked up in analogy to the preparation of **3** (method *D*) to give 0.280 g (24%) of **4**, m.p. 216–219 °C (dec.) without depression on admixture with the sample prepared by method *A*.

Method F. A mixture of blocked isobiuret[9] **22** (0.561 g, 1 mmol), benzene (5 ml) and dimethylformamide dimethyl acetal (0.13 ml) was stirred at room temperature for 10 days. The precipitate was filtered off by suction to yield 0.264 g (45%) of **4**, m.p. 216–219 °C (dec.) without depression on admixture with the sample prepared by method *A*.

4-Methoxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,3,5-triazin-2(1*H*)-one (**5**)

A mixture of blocked methylsulfanyl nucleoside **6** (0.588 g, 1 mmol) and a 3.7% solution of dry methylamine in methanol (5 ml) was stirred at room temperature for **5** min. The mixture was kept in the refrigerator (–20 °C) for 30 min and the precipitate filtered off by suction to yield crude **5**, m.p. 150–155 °C (dec.). Recrystallization of the crude product (0.240 g) from ethanol afforded 0.200 g (35%) of the pure methoxy derivative **5**, m.p. 161–163 °C (dec.) without depression on admixture with an authentic sample[9], $[\alpha]_D$ –23.3 (*c* 0.6, DMF). Mass spectrum: 572 (MH^+). For $C_{30}H_{25}N_3O_9$ (571.3) calculated: 63.05% C, 4.41% H, 7.35% N; found: 63.27% C, 4.32% H, 7.61% N.

N^4-Methyl-5-azacytidine (**1**)

Method A. A mixture of the free methoxy nucleoside[12] **23** (0.259 g, 1 mmol) and a 7% solution of dry methylamine in methanol (2 ml) was stirred at room temperature for 1.5 h and the crystals filtered off by suction to yield 0.20 g of crude **1**, m.p. 149–151 °C (dec.). The crude product was re-

crystallized from methanol and dried at 110 °C/40 Pa for 8 h to give 0.164 g (64%) of pure **1**, m.p. 155–157 °C (dec.), R_F 0.24 (II), $[\alpha]_D$ +34.5 (*c* 0.5, H_2O). UV spectrum (MeOH), λ_{max} (log ε): 250 (3.87), 213 (4.22). CD spectrum (H_2O), λ_{max} ($[\Theta]_{max}$): 249 (+7 800). ^{1}H NMR spectrum: 2.89 s, 3 H (N^4-CH_3); 8.45 s, 1 H (H-6); 3.88 m, 2 H (2 × H-5′); 4.00–4.50 m, 3 H (H-2′ + H-3′ + H-4′); 5.78 d, 1 H, *J*(1′,2′) = 3.0 (H-1′). Mass spectrum: 258 (M^+). For $C_9H_{14}N_4O_5$ (258.2) calculated: 41.86% C, 5.46% H, 21.70% N; found: 41.89% C, 5.76% H, 21.83% N.

Method B. A mixture of blocked nucleoside **3** (0.571 g, 1 mmol), methanol (4 ml) and methanolic 1 M NaOMe (0.2 ml) was stirred at room temperature for 30 min. The mixture was kept overnight in the refrigerator (–20 °C) and then acidified with acetic acid (0.05 ml) to yield 0.226 g of crude **1**, m.p. 144–147 °C (dec.). The crude product was recrystallized from methanol and dried at 110 °C/40 Pa for 8 h to give 0.191 g (74%) of pure **1**, m.p. 155–157 °C (dec.) without depression on admixture with the sample prepared by method *A*.

N^4,N^4-Dimethyl-5-azacytidine (**2**)

Method A. A mixture of the free methoxy nucleoside[12] **23** (0.259 g, 1 mmol) and a 7% solution of dry dimethylamine in methanol (2 ml) was stirred at room temperature for 3 min. The mixture was kept for 45 min at room temperature, evaporated and coevaporated with methanol (5 ml). The crystalline residue was triturated with ethanol (1 ml) and the slurry kept overnight in the refrigerator (–20 °C) to afford 0.220 g of crude **2**, m.p. 127–129 °C (dec.). Recrystallization of the crude product from ethanol gave 0.193 g (71%) of pure **2**, m.p. 128–130 °C (dec.), R_F 0.25 (II), $[\alpha]_D$ +24.1 (*c* 0.5, H_2O). UV spectrum, λ_{max} (log ε): (MeOH), 258 (3.89), 219 (4.24); (pH 2.32), 262 (3.88), 211 (4.11); (pH 6.94), 258 (3.89), 217 (4.31); (pH 10.93), 257 (3.85), 218 (4.25). CD spectrum (H_2O), λ_{max} ($[\Theta]_{max}$): 248 (+6 450). ^{1}H NMR spectrum: 3.12 s, 3 H ($N^{4'}$-CH_3); 3.21 s, 3 H ($N^{4''}$-CH_3); 3.88 m, 2 H (2 × H-5′); 4.09 m, 1 H (H-4′); 4.24 m, 1 H (H-3′); 4.49 dd, 1 H, *J*(2′,1′) = 3.0, *J*(2′,3′) = 5.0 (H-2′); 5.78 d, 1 H, *J*(1′,2′) = 3.0 (1′-H). Mass spectrum: 273 (MH^+). For $C_{10}H_{16}N_4O_5$ (272.2) calculated: 44.12% C, 5.92% H, 20.58% N; found: 44.31% C, 5.96% H, 20.48% N.

Method B. A mixture of the tribenzoate **4** (0.585 g, 1 mmol), methanol (4 ml) and methanolic 1 M NaOMe (0.2 ml) was stirred at room temperature for 1 h. The solution was kept overnight, acidified with acetic acid (0.05 ml) and applied onto a column of Amberlite IRC-50 [H^+] ion exchange resin (10 ml) prepared in methanol. The column was washed with methanol (100 ml) and the effluent evaporated. The residue was crystallized from ethanol to yield 0.196 g (72%) of **2**, m.p. 128–130 °C (dec.) without depression on admixture with the sample prepared by method *A*.

Method C. A mixture of the tribenzoate **4** (0.585 g, 1 mmol) and a 7 M solution of dry ammonia in methanol (20 ml) was stirred at room temperature for 3 days. The solution was evaporated, the residue triturated with ether (20 ml) and crystallized from ethanol to yield 0.152 g (56%) of **2**, m.p. 128–130 °C (dec.) without depression on admixture with the sample prepared by method *A*.

Method D. A mixture of the tribenzoate **4** (0.585 g, 1 mmol) and a 7% solution of dry dimethylamine in methanol (15 ml) was stirred at room temperature for 3 days. The solution was evaporated, the residue triturated with ether (10 ml) and crystallized from ethanol to give 0.190 g (70%) of **2**, m.p. 128–130 °C (dec.) without depression on admixture with the sample prepared by method *A*.

4-Methylamino-1-(α-D-ribofuranosyl)-1,3,5-triazin-2(1*H*)-one (**24**)

A mixture of the blocked α-D anomer **17** (0.035 g, 0.06 mmol), methanol (0.5 ml) and methanolic 1 M NaOMe (0.02 ml) was magnetically stirred for 20 min and kept overnight at room temperature. The crystalline product was filtered off with suction to yield 0.010 g (65%) of the free nucleoside **24**, m.p. 241–243 °C (dec.), R_F 0.24 (II). UV spectrum, λ_{max} (log ε): (MeOH), 252 (3.94), 213 (4.25); (pH 2.36), 257 (3.48), 226 (3.50); (pH 6.86), 252 (3.84), 213 (4.22); (pH 10.96), 227 (4.32). CD

spectrum (pH 6.86), λ_{max} ($[\Theta]_{max}$): 248 (–16 570), 226 sh (–10 650). ^{1}H NMR spectrum: 8.46 s, 1 H (H-6); 7.97 q, 1 H, J(NH,CH_3) = 4.9 (NH); 6.03 d, 1 H, J(1′,2′) = 4.2 (H-1′); 5.41 br, 1 H (OH); 5.22 br, 1 H (OH); 4.82 br t, 1 H (OH); 4.10 t, 1 H, J(2′,1′) = J(2′,3′) = 4.4 (H-2′); 4.06 dd, 1 H, J(3′,2′) = 4.6, J(3′,4′) = 6.8 (H-4′); 4.01 ddd, 1 H, J(4′,5′a) =2.7, J(4′,5′b) = 4.2, J(4′,3′) = 6.8 (H-4′); 3.60 dd, 1 H, J(5′a,4′) = 2.7, J(gem.) = 12.2 (H-5′a); 3.42 dd, 1 H, J(5′b,4′) = 4.2, J(gem.) = 12.2 (H-5′b); 2.73 d, 3 H, J(CH_3,NH) = 4.9 (N-CH_3). Mass spectrum: 259 (MH^+). For $C_9H_{16}N_4O_5$ (258.2) calculated: 41.86% C, 5.46% H, 21.70% N; found: 41.63% C, 5.34% H, 21.42% N.

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