SYNTHESIS OF SOME 6-SUBSTITUTED 5-AZACYTIDINES

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Protected 6-substituted benzyl, phenyl and chloromethyl derivatives of 5-azacytidine **8–10** have been prepared by addition of phenylacetyl- (**2**), benzoyl- (**3**) or (chloroacetyl)guanidine (**4**) to 2,3,5-tri-*O*-benzoyl-β-D-ribosyl isocyanate (**1**) and subsequent silylation-mediated cyclization of the obtained acyl(carbamoyl)guanidines **5–7**. 4-Amino-6-phenyl-1,3,5-triazin-2(1*H*)-one (**12**) was obtained by condensation of carbamoylguanidine (**13**) with methyl benzoate in presence of methanolic sodium methoxide or by condensation of **13** with triethyl orthobenzoate in *N*,*N*-dimethylformamide. Stannic chloride catalyzed condensation of silylated 6-phenyl derivative **11** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose (**14**) in 1,2-dichloroethane afforded a 1.2 : 1 mixture of N^1 and N^3 nucleosides **9** and **15**, respectively. Methanolysis of the protected compounds **8–10** and **15** gave the respective free nucleosides **16–19**. The latter compounds inhibited the growth of bacteria *E. coli* B to a much lower extent than the unsubstituted 5-azacytidine.

Key words: 5-Azapyrimidines; 1,3,5-Triazines; Nucleosides; Circular dichroism; Antibacterial activity.

In continuation of our study of 5-azapyrimidine nucleosides, we were also interested in the synthesis and biological activity of 6-substituted 5-azacytidines. The synthesis of 6-methyl-5-azacytidine was described in a preceding paper[1]. In this communication we wish to give an account of our further studies in this field. The isocyanate method which proved to be convenient for the synthesis of 6-methyl-5-azacytidine[1] has been used for the preparation of 6-substituted benzyl, phenyl and chloromethyl derivatives of 5-azacytidine. Reaction of 2,3,5-tri-*O*-benzoyl-β-D-ribosyl isocyanate (**1**) with phenylacetyl- (**2**), benzoyl- (**3**) and (chloroacetyl)guanidine (**4**) afforded the respective acyl(carbamoyl)guanidines **5–7**. Silylation-mediated cyclization of these intermediates gave the respective protected 6-substituted 5-azacytidines **8–10** (Scheme 1). As silylation agents were used bis(trimethylsilyl)acetamide, bis(trimethylsilyl)trifluoroacetamide or a mixture of chlorotrimethylsilane and triethylamine. This procedure is

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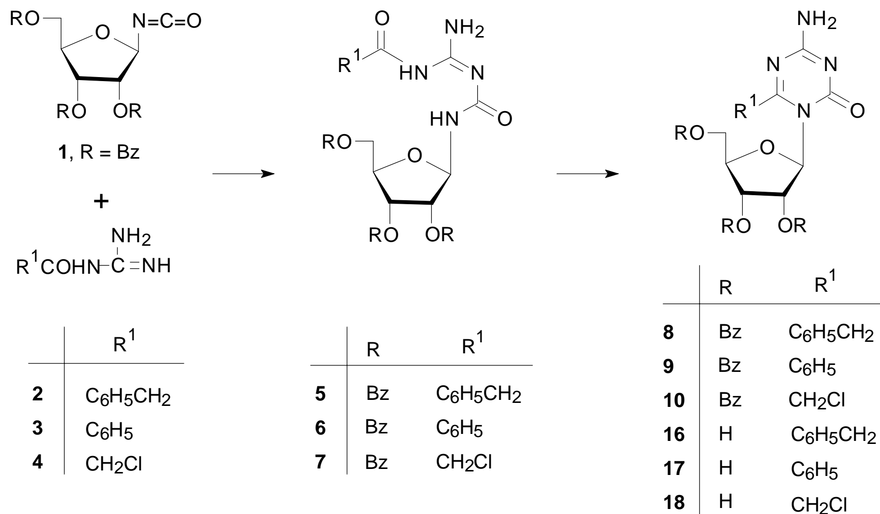
unambiguous in respect to the position of the ribosyl group and also to the anomeric configuration. The structure of the products was confirmed by elemental analysis, mass spectra and spectral data on comparison with the analogous blocked 6-methyl-5-azacytidine[1].



	R^1
2	$C_6H_5CH_2$
3	C_6H_5
4	CH_2Cl

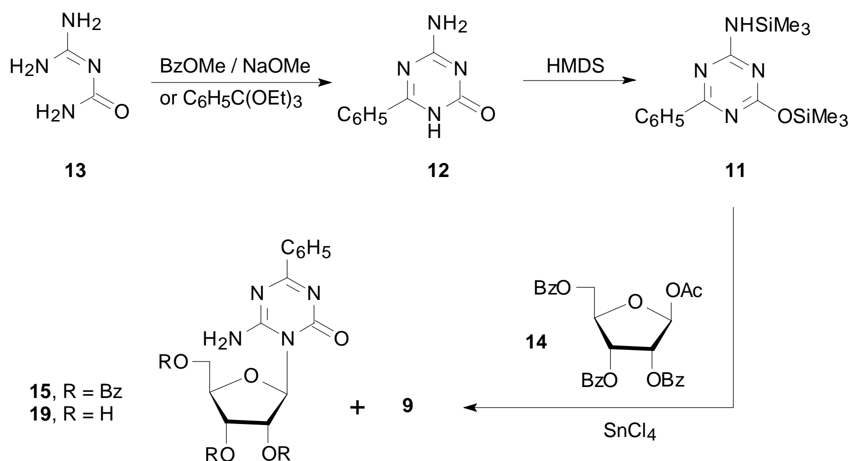
	R	R^1
5	Bz	$C_6H_5CH_2$
6	Bz	C_6H_5
7	Bz	CH_2Cl

	R	R^1
8	Bz	$C_6H_5CH_2$
9	Bz	C_6H_5
10	Bz	CH_2Cl
16	H	$C_6H_5CH_2$
17	H	C_6H_5
18	H	CH_2Cl

SCHEME 1

Protected 6-phenyl-5-azacytidine **9** was also obtained by direct glycosylation of the silylated base **11** (Scheme 2). The starting 4-amino-6-phenyl-1,3,5-triazin-2(1*H*)-one (**12**) was obtained either by condensation of carbamoylguanidine (**13**) with methyl benzoate in presence of sodium methoxide in methanol or by condensation of carbamoylguanidine (**13**) with triethyl orthobenzoate in DMF. Stannic chloride-catalyzed condensation of the silylated 6-phenyl derivative **11** (prepared by treatment of the base **12** with hexamethyldisilazane at elevated temperature) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose (**14**) in 1,2-dichloroethane (reaction time 30 min) gave a 1.2 : 1 mixture of N^1 and N^3 nucleosides **9** and **15**, respectively. The separation of the positional isomers was carried out by column chromatography on silica gel. Crystallization of the N^3 derivative **15** from a mixture of ethanol and ethyl acetate gave a lower-melting 1 : 1 adduct with ethyl acetate. The solvent was removed by heating at 140 °C. The compound changed at its melting point to the higher-melting unsolvated form. In the infrared spectra of the solvate of **15** with ethyl acetate, the typical bands of ethyl acetate were found besides the bands of **15**. This finding indicates that the solvent does not form covalent or hydrogen bonds with **15**. The structure of the N^1 nucleoside **9** was proved on identification with the product obtained by cyclization of benzoyl(carbamoyl)guanidine (**6**) and the structure of the N^3 derivative **15** was inferred from infrared spectra by comparison with the analogous 6-methyl derivative[1].

Methanolysis of the protected nucleosides **8–10** and **15** with sodium methoxide in methanol afforded the respective free nucleosides **16–19**. 6-Chloromethyl-5-azacytidine (**18**) was obtained in the form of a very hygroscopic amorphous dihydrate which hydrolyzed similarly to 5-azacytidine[2], rather rapidly on storage at room temperature.



SCHEME 2

The free 6-substituted 5-azacytidines **16–18** and the N^3 isomer **19** were tested for their antibacterial activity using a culture *E. coli* B growing on a mineral medium with glucose. Similarly to 6-methyl-5-azacytidine[1] (85% inhibition of growth at 1 000 μg/ml concentration), all of these nucleosides exhibited a much lower degree of growth inhibition in comparison with the parent nucleoside 5-azacytidine[3] (50% inhibition of growth at 0.25 μg/ml concentration) and also in comparison with 6-amino-5-azacytidine[4] (46% inhibition of growth at 100 μg/ml concentration). The highest degree of growth inhibition exhibited 6-chloromethyl-5-azacytidine (**18**) (39% at 100 μg/ml concentration), 6-benzyl-5-azacytidine (**16**) was less active (18% at 1 000 μg/ml concentration) and 6-phenyl-5-azacytidine (**17**) was not active at all even at 1 000 μg/ml concentration. However, the N^3 isomer of the latter nucleoside **19** inhibited the growth of bacteria *E. coli* B to the extent of 32% at 100 μg/ml concentration. By contrast, the N^3 isomer of 6-methyl-5-azacytidine[1] was inactive even at 1 000 μg/ml concentration. Eventually it is of interest to note that 6-methyl-5-azacytidine[1] exhibited a higher potential carcinogenity[5] than 5-azacytidine[6,7] when estimated by a polarographic method[8–10]. The 6-substituted 5-azacytidines described in this paper are also tested for their potential carcinogenity by the same method. The results of this study will be published later.

CD spectra of nucleosides **16** and **17** are very similar to those of 6-methyl-5-azacytidine[1] and indicate a *syn* conformation aroung the C–N glycosyl bond of 6-substituted

5-azacytidines. By contrast, unsubstituted 5-azacytidine[1] is a typical *anti* nucleoside. The high biological activity of 5-azacytidine is based on its structural and conformational resemblance to 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[11]. Due to remarkable changes in molecular conformation, 6-subtituted 5-azacytidines **16–18** are obviously not able to be incorporated into nucleic acids. Moreover, the substituents in the position 6 prevent covalent interactions of the double bond in the position 5,6 of the triazine ring with enzymes. These facts explain the dramatic differences in biological activity of 6-substituted 5-azacytidines **16–18** and the unsubstituted 5-azacytidine.

EXPERIMENTAL

The 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 A: chloroform–methanol (98 : 2) and B: butan-1-ol–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).

^{1}H NMR spectra were measured on a Varian XL-200 instrument (200 MHz) in hexadeuteriodimethyl sulfoxide and referenced to the signal of solvent (δ(^{1}H) 2.50), chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. 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[12], 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 polarimeter Perkin–Elmer, type 141 MCA at 22 °C. 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 technique (ionization by Xe, accelerating voltage 8 kV), matrices glycerol or thioglycerol. Stationary cultivation of *Escherichia coli* B was performed at 37 °C in mineral medium with glucose[13]. The tested compounds were added before inoculation and the growth of bacteria was measured 16 h later.

4-Amino-6-phenyl-1,3,5-triazin-2(1*H*)-one (**12**)

Method A. A solution of carbamoylguanidine[14] (**13**) (10.2 g, 0.10 mol) in methanolic 1 M NaOMe (100 ml) was treated with methyl benzoate (15 ml, 0.12 mol) and the mixture refluxed for 7 h at 100 °C (bath temperature). The mixture was evaporated, the residue dissolved in water (100 ml), the solution acidified with acetic acid (7 ml) and the precipitate filtered off with suction to give 9.5 g (50%) of the compound **12**, m.p. 330–333 °C (dec.); the sample for analysis was recrystallized from ethanol-water (1 : 1) and dried at 120 °C/30 Pa for 3 h, m.p. 333–335 °C (dec.), ref.[15] m.p. 334–335 °C (dec.), R_F 0.73 (B). UV spectrum, λ_{max} (log ε): (MeOH), 252 (4.34), 210 (4.78); (pH 2.26), 276 (4,34), 226 (4.18); (pH 6.94), 260 (4.23), 203 (4.75). Mass spectrum: 189 (MH^+). ^{1}H NMR spectrum: 11.60 br, 1 H (NH); 7.50 br, 2 H (NH_2); 8.17 m, 2 H (arom.); 7.60–7.42 m, 3 H (arom.). For $C_9H_8N_4O$ (188.2) calculated: 57.44% C, 4.28% H, 29.77% N; found: 57.47% C, 4.19% H, 29.57% N.

Method B. A mixture of carbamoylguanidine[14] (**13**) (0.408 g, 4 mmol), DMF (3 ml) and triethyl orthobenzoate (3 ml) was heated for 1.5 h at 145–150 °C (bath temperature). The mixture was evapo-

rated *in vacuo* at 55–60 °C (bath temperature), the residue triturated with ether (10 ml) and crystallized from water to give 0.35 g (46%) of **12**, m.p. 328–331 °C (dec.). The recrystallized product melted at 333–335 °C (dec.) undepressed with the sample prepared by method *A*.

2-Trimethylsilylamino-4-trimethylsilyloxy-6-phenyl-1,3,5-triazine (**11**)

A mixture of triazine **12** (3.44 g, 18 mmol), hexamethyldisilazane (8 ml) and ammonium sulfate (0.05 g) was refluxed at 160–165 °C (bath temperature) for 4 h. The excess of hexamethyldisilazane was evaporated *in vacuo* at 55–60 °C (bath temperature). The residue was coevaporated with toluene (10 ml), dried *in vacuo* and minced to a powder which was dried again at 50 °C *in vacuo* for 30 min. The crude product was used in glycosylation without any further purification. Yield, 5.9 g (98%) of compound **11**, m.p. 110–115 °C (dec.). IR spectrum (Nujol): ν(ring)(triazine) 1 559 s, 1 540 s, sh, 1 515 m; ν(ring)(phenyl) 1 594 s, 1 491 m; δ rock (CH_3) asym. 847 s, 873 m, sh, sym. 750 w, sh, 760 w; ν(Si–C) asym. 682 w, sym. 623 w, 650 w. For $C_{18}H_{24}N_4OSi_2$ (332.6) calculated: 54.17% C, 7.27% H, 16.84% N; found: 53.81% C, 7.10% H, 17.05% N.

N-(Benzoylcarbamimidoyl)-*N*′-(2,3,5-tri-*O*-benzoyl-β-D-ribosyl)urea (**6**)

A solution of crude isocyanate **1** (prepared by a known procedure[4] from 1.008 g, 2 mmol of blocked ribose[16] **14**) in dry acetone (10 ml) was added dropwise at room temperature to a magnetically stirred mixture of benzoylguanidine[17] (**3**) (0.326 g, 2 mmol) and dry 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 to give crude acyl(carbamoyl)guanidine **6** which was used in the next step without purification. An analytical sample was obtaine 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 1.0 g (77%) of the pure **6** as a chromatographically homogeneous solid foam; R_F 0.61 (A), $[\alpha]_D$ –50 ° (*c* 0.1, $CHCl_3$). IR spectrum ($CHCl_3$): ν(NH) 3 384 m; ν(C=O) 1 727 *vs* (benzoate), 1 690 m (amide), 1 657 s (urea); ν(C=N) 1 620 m; ν(ring) arom. 1 603 m, 1 584 m (benzoyl), amide II bound 1 563 m, amide II free 1 525 m, sh. UV spectrum (MeOH), λ_{max} (log ε): 260, inflexion (4.42), 230 (4.80), 210 (4.66). Mass spectrum: 651 (MH^+). ^{1}H NMR spectrum: 10.60 br, 1 H (NH); 9.20 br, 1 H (NH); 8.70 br, 1 H (NH_2); 8.55 br, 1 H (NH_2); 8.10–7.80 m, 8 H (arom.); 7.70–7.35 m, 12 H (arom.); 5.81–5.60 m, 3 H (H-1′ + H-2′ + H-3′); 4.63–4.46 m, 3 H (H-4′ + 2 × H-5′). For $C_{35}H_{30}N_4O_9$ (650.7) calculated: 64.61% C, 4.65% H, 8.61% N; found: 64.63% C, 4.60% H, 8.43% N.

2′,3′,5′-Tri-*O*-benzoyl-6-phenyl-5-azacytidine (**9**)

A solution of crude acyl(carbamoyl)guanidine **6** (prepared from 1 mmol of **14** *via* crude 1) in acetonitrile (5 ml) was treated with chlorotrimethylsilane (1 ml, 7.9 mmol) and triethylamine (1 ml, 7.3 mmol). The mixture was kept for 30 min at room temperature, diluted with benzene (20 ml), 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 at room temperature. The precipitate was filtered off with suction and applied on a column of silica gel (30 g). Elution was performed with toluene–ethyl acetate (100 : 0 – 0 : 100, v/v). The major portion was crystallized from ethanol–petroleum ether and recrystallized from ethanol to afford 0.253 g (43%) of compound **9**, m.p. 118–120 °C (dec.), R_F 0.31 (A), $[\alpha]_D$ +61° (*c* 0.21, $CHCl_3$). IR spectrum ($CHCl_3$): ν(NH_2) 3 543 w, 3 423 m; ν(C=O) 1 729 m, 1 715 s, sh, 1 695 m, sh; ν(C=N) 1 621 s; δ(NH_2) 1 649 s, sh. UV spectrum (MeOH), λ_{max} (log ε): 265, inflexion (4.41), 229 (4.78), 210 (4.73). Mass spectrum: 633 (MH^+). ^{1}H NMR spectrum: 7.88 br, 1 H (NH_2); 7.81 br, 1 H (NH_2); 7.98 m, 2 H (arom.); 7.78–6.90 m, 4 H (arom.);

7.66–7.50 m, 6 H (arom.); 7.50–7.27 m, 8 H (arom.); 6.15 m, 2 H (H-2′ + H-3′); 5.66 d, 1 H, $J(1',2')$ = 1.2 (H-1′); 4.64–4.50 m, 3 H (H-4′ + 2 × H-5′). For $C_{35}H_{28}N_4O_8$ (632.6) calculated: 66.45% C, 4.46% H, 8.86% N; found: 66.39% C, 4.67% H, 8.67% N.

In a parallel experiment a solution of purified acyl(carbamoyl)guanidine **6** (0.325 g, 0.5 mmol) in acetonitrile (4 ml) was treated with bis(trimethylsilyl)trifluoroacetamide (1.2 ml, 4.5 mmol), kept overnight at room temperature, evaporated and the residue crystallized from ethanol. Recrystallization of the product from the same solvent afforded 0.237 g (75%) of **9**, m.p. 118–120 °C (dec.), that was in all respects identical with the above mentioned product.

Ribosylation of the Silylated Base **11**

A stirred mixture of silylated base **11** (0.997 g, 3 mmol) and blocked ribose[16] **14** (1.51 g, 3 mmol) in 1,2-dichloroethane (15 ml) was treated dropwise at 0 °C with a solution of stannic chloride (0.51 ml, 4 mmol) in 1,2-dichloroethane (5 ml). The solution was kept for 30 min at room temperature and cautiously shaken with an icecold 5% solution of sodium hydrogencarbonate (100 ml). The mixture was extracted with chloroform (100 ml), filtered through a layer of cellite, the organic layer dried (anhydrous sodium sulfate) and evaporated. The residue was applied on a column of silica gel (50 g) packed in toluene. Elution was performed with toluene–ethyl acetate (75 : 25 – 0 : 100, v/v). Fractions containing the more mobile portion were collected, evaporated and the residue triturated with ethanol to give 0.64 g (34%) of the N^3 nucleoside **15** m.p. 225–228 °C (dec.), R_F 0.60 (A), $[\alpha]_D$ –57.2° (*c* 0.20, $CHCl_3$), IR spectrum ($CHCl_3$): $\nu(NH_2)$ 3 494 m, 3 373 s; ν(C=O) 1 729 vs, 1 714 s, sh, 1 695 s, sh; ν(C=N) 1 560 s; $\delta(NH_2)$ 1 613 s. UV spectrum (MeOH), λ_{max} (log ε): (MeOH), 261 (4.53), 229 (4.72), 212 (4.71). Mass spectrum: 633 (MH^+). ^{1}H NMR spectrum: 8.46 br, 2 H (NH_2); 8.26 m, 2 H (arom.); 8.02–7.82 m, 6 H (arom.); 7.70–7.36 m, 12 H (arom.); 6.22 br s, 1 H (H-1′); 6.10 m, 2 H (H-2′ + H-3′); 4.75–4.55 m, 3 H (H-4′ + 2 × H-5′). For $C_{35}H_{28}N_4O_8$ (632.6) calculated: 66.45% C, 4.46% H, 8.86% N; found: 66.16% C, 4.35% H, 8.82% N. Recrystallization of the product from ethanol–ethyl acetate afforded the solvate of **15** with ethyl acetate, m.p. 198–200 °C (resolidification) and 220–224 °C (dec.); at 130 °C the sample changed (release of ethyl acetate). For $C_{35}H_{28}N_4O_8 \cdot C_4H_8O_2$ (720.7) calculated: 64.99% C, 5.03% H, 7.77% N; found 64.83% C, 4.98% H, 7.54% N. The IR spectra of the solvated sample exhibited besides of the bands of nucleoside **15** also typical bands of ethyl acetate at 1 045 (m) and 1 376 (m) cm^{-1}. Drying of the solvate *in vacuo* at 140 °C for 5 h afforded the lower melting modification of nucleoside **15**, m.p. 198–200 °C (resolidification) and 220–224 °C (dec.), without any changes at 130 °C. For $C_{35}H_{28}N_4O$ (632.6) calculated: 66.45% C, 4.46% H, 8.86% N; found 66.16% C, 4.35% H, 8.82% N.

Fractions containing the less mobile isomer were collected, evaporated and the residue crystallized from ethanol to give 0.759 g (40%) of nucleoside **9**, m.p. 118–120 °C (dec.) underpressed with a sample of **9** prepared by cyclization of **6**.

6-Phenyl-5-azacytidine (**17**)

A mixture of blocked nucleoside **9** (0.316 g, 0.5 mmol), methanol (1.5 ml) and methanolic 1 M NaOMe (0.15 ml) was stirred at room temperature for 2.5 h and kept overnight. The solution was acidified with acetic acid (0.02 ml) and decationized on a column of Amberlite IRC-50[H^+] ion exchange resin (10 ml) which was prepared in methanol. The column was washed with methanol (100 ml), the effluent evaporated, the residue dissolved in isopropyl alcohol (1 ml), the solution precipitated with ether, the amorphous solid filtered off and crystallized from isopropyl alcohol to yield 0.102 g (64%) of nucleoside **17**, m.p. 200–201 °C (dec.), R_F 0.54 (B), $[\alpha]_D$ –1.0° (*c* 0.1, H_2O). The sample for analysis was dried at 80 °C/40 Pa for 2 h. UV spectrum, λ_{max} (log ε): (MeOH), 260 (4.21), 210 (4.69); (1 M HCl), 277 (4.22), 208 (4.40); (pH 2.26), 277, inflexion (4.10), 248 (4.35), 227, inflexion

(4.25); (pH 6.94), 248 (4.28); (pH 10.84), 254 (4.42), 218 (4.34). CD spectrum (pH 6.86), λ_{max} ($[\Theta]_{max}$): 268 (+720), 234 (–360). Mass spectrum: 321 (MH^+). For $C_{14}H_{16}N_4O_5$ (320.3) calculated: 52.50% C, 5.04% H, 17.49% N; found: 52.21% C, 4.92% H, 17.77% N.

4-Amino-6-phenyl-3-β-D-ribofuranosyl-1,3,5-triazin-2(1*H*)-one (**19**)

A mixture of blocked nucleoside **15** (0.070 g, 0.11 mmol), methanol (1 ml) and methanolic 1 M NaOMe (0.1 ml) was magnetically stirred at room temperature for 3 h. The solution was acidified with acetic acid (0.01 ml) and decationized in analogy to the above preparation of **17**. The residue after evaporation of the methanolic effluent from the column was crystallized from isopropyl alcohol (1 ml) to give 0.030 g (86%) of the free nucleoside **19**, m.p. 200–202 °C (dec.), R_F 0.54 (B), $[\alpha]_D$ +38.7° (*c* 0.07, H_2O). UV spectrum, λ_{max} (log ε): (MeOH), 263 (4.33), 212 (4.39); (pH 2.36), 279 (4.29); (pH 6.86), 263 (4.38), 210 (4.35); (pH 10.96), 259 (4.27), 216 (4.14). The sample for analysis was dried at 80 °C/40 Pa for 2 h. For $C_{14}H_{16}N_4O_5$ (320.3) calculated: 52.50% C, 5.04% H, 17.49% N; found: 52.64% C, 5.07% H, 17.75% N.

2′,3′,5′-Tri-*O*-benzoyl-6-benzyl-5-azacytidine (**8**)

A solution of crude isocyanate **1** prepared by a known procedure[4] from 1.008 g, 2 mmol of blocked ribose[16] **14**) in dry acetone (10 ml) was added dropwise at room temperature to stirred mixture of (phenylacetyl)guanidine[17] (**2**) (0.334 g, 2 mmol) and dry acetone (5 ml). The mixture was stirred for 30 min and worked up in analogy to the preparation of **6** to yield the crude syrupy acyl(carbamoyl)guanidine **5**. A solution of crude **5** (2 mmol) in acetonitrile (10 ml) was treated with chlorotrimethylsilane (1.7 ml, 13 mmol) and triethylamine (1.7 ml, 12 mmol), the mixture kept for 30 min at room temperature and the mixture worked up in analogy to the preparation of **9**. The crude product was crystallized from ethanol (4 ml) and recrystallized from methanol–1,2-dichloroethane (2 : 1) to give 0.970 g (70%) of nucleoside 8, m.p. 223–226 °C (dec.), R_F 0.35 (A), $[\alpha]_D$ +10.1° (*c* 0.51, $CHCl_3$). IR spectrum ($CHCl_3$); $\nu(NH_2)$ 3 545 m, 3 427 m; ν(C=O) 1 728 s, 1 714 s, sh, 1 694 m, sh; ν(C=N) 1 621 s; $\delta(NH_2)$ 1 651 m, sh. UV spectrum (MeOH), λ_{max} (log ε): 265, inflexion (4.41), 229 (4.73), 210 (4.69). Mass spectrum: 647 (MH^+). ^1H NMR spectrum: 7.95 m, 2 H (arom. + NH_2); 7.80 m, 17 H (arom. + NH_2); 7.00 m, 3 H (arom. + NH_2); 6.23 d, 1 H, *J*(1′,2′) = 1.8 (H-1′); 6.06 br t, 1 H, *J* = 7.0 (H-3′); 5.98 dd, 1 H, *J*(2′,1′) = 1.8, *J*(2′,3′) = 6.4 (H-2′); 4.65–4.45 m, 3 H (H-4′ + 2 × H-5′); 4.25 d, 1 H (C**H**$_2$-Ph); 4.03 d, 1 H, *J*(gem) = 15.6 (C**H**$_2$-Ph). For $C_{36}H_{30}N_4O_8$ (646.7) calculated: 66.87% C, 4.56% H, 8.66% N; found: 66.79% C, 4.57% H, 8.36% N.

6-Benzyl-5-azacytidine (**16**)

A mixture of blocked nucleoside **8** (0.20 g, 0.31 mmol), methanol (2 ml) and methanolic 1 M NaOMe (0.1 ml) was stirred at room temperature for 4 h and the solution worked up in analogy to the preparation of **17**. A solution of the crude product in methanol (2 ml) was precipitated with ether and the amorphous solid crystallized from ethanol to give 0.080 g (77%) of free nucleoside **16**, m.p. 206–208 °C (dec.), R_F 0.71 (B), $[\alpha]_D$ –33.8° (*c* 0.1, DMF). UV spectrum, λ_{max} (log ε): (MeOH), 240, inflexion (3.95), 209 (4.32); (0.1 M HCl), 245, inflexion (3.44), 209 (4.46); (pH 2.32), 222 (4.05); (pH 6.94), 239, inflexion (3.94); (pH 10.93), 221 (4.46). CD spectrum (pH 6.93), λ_{max} ($[\Theta]_{max}$): 271 (+50), 237 (–210). For $C_{15}H_{18}N_4O_5$ (334.3) calculated: 53.89% C, 5.43% H, 16.76% N; found: 53.77% C, 5.36% H, 17.00% N.

2′,3′,5′-Tri-*O*-benzoyl-6-chloromethyl-5-azacytidine (**10**)

A solution of crude isocyanate **1** (prepared by a known procedure[4] from 1.008 g, 2 mmol of blocked ribose[16] **14**) in dry acetone (10 ml) was added dropwise at room temperature to a stirred mixture of (chloroacetyl)guanidine[17] (**4**) (0.271 g, 2 mmol) and dry acetone (5 ml). The mixture was stirred for 30 min and worked up in analogy to the preparation of **6** to yield the crude syrupy acyl(carbamoyl)guanidine. A solution of crude **7** (2 mmol) in acetonitrile (10 ml) was treated with chlorotrimethylsilane (1.7 ml, 13 mmol) and triethylamine (1.7 ml, 12 mmol), the mixture kept for 30 min at room temperature and worked up in analogy to the preparation of **9**. The crude product was crystallized from ethanol (2 ml) and recrystallized from 1,2-dichloroethane–methanol (2 : 1) to afford 0.726 g (60%) of nucleoside **10**, m.p. 217–218 °C (dec.), R_F 0.39 (A), $[\alpha]_D$ +10.5° (*c* 0.21, $CHCl_3$). IR spectrum ($CHCl_3$): $\nu(NH_2)$ 3 539 m, 3 422 m; ν(C=O) 1 724 s; ν(C=N) 1 630 vs; $\delta(NH_2)$ 1 656 m, sh. UV spectrum (MeOH), λ_{max} (log ε): 266, inflexion (4.43), 230 (4.76), 210 (4.64). ^{1}H NMR spectrum: 8.05–7.80 m, 8 H (arom. + NH_2); 7.60 m, 3 H (arom. + NH_2); 7.50–7.30 m, 6 H (arom. + NH_2); 6.13 d, 1 H, *J*(1′,2′) = 1.5 (H-1′); 6.07 m, 2 H (H-2′ + H-3′); 4.79 d, 1 H (CH_2-Cl); 4.67 d, 1 H, *J*(gem) = 13.8 (CH_2-Cl); 4.70–4.50 m, 3 H (H-4′ + 2 × H-5′). For $C_{30}H_{26}ClN_4O_8$ (605.1) calculated: 59.86% C, 4.10% H, 9.32% N, 5.98% Cl; found: 59.55% C, 4.17% H, 9.26% N, 5.86% Cl.

6-Chloromethyl-5-azacytidine (**18**)

A mixture of protected nucleoside **10** (0.20 g, 3 mmol), methanol (2 ml) and methanolic 1 M NaOMe (0.1 ml) was stirred at room temperature for 3 h and worked up in analogy to the preparation of **16**. A solution of the crude product in methanol (2 ml) was precipitated with ether and the amorphous powder dried for 3 h at 80 °C/40 Pa to give 0.064 g (65%) of the dihydrate of **18**, m.p. 77–80 °C, R_F 0.41 (B), $[\alpha]_D$ +24.4° (*c* 0.1, DMF). For $C_9H_{13}ClN_4O_5 \cdot 2\,H_2O$ (328.7) calculated: 33.89% C, 5.21% H, 17.05% N, 10.76% Cl; found: 33.62% C, 5.00% H, 16.86% N, 10.43% Cl. The very hygroscopic product hydrolyzed rapidly on storage at room temperature for several days.

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