SYNTHESIS OF N^4-AMINO AND N^4-HYDROXY DERIVATIVES OF 5-AZACYTIDINE. A FACILE REARRANGEMENT OF THE N^4-AMINO DERIVATIVE TO 5-(3-β-D-RIBOFURANOSYLUREIDO)-1*H*-1,2,4-TRIAZOLE

Alois PÍSKALA[1,*], Naeem B. HANNA, Milena MASOJÍDKOVÁ[2], Pavel FIEDLER and Ivan VOTRUBA[3]

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, Czech Republic; e-mail: [1] piskala@uochb.cas.cz, [2] masojidkova@uochb.cas.cz, [3] votruba@uochb.cas.cz

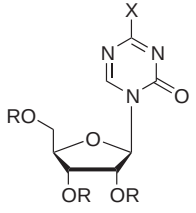
Received November 12, 2003
Accepted February 22, 2004

Treatment of methoxyribosyltriazinone **4** with hydrazine in methanol afforded crude 4-hydrazino-1-β-D-ribofuranosyl-1,3,5-triazin-2(*1H*)-one (N^4-amino-5-azacytidine) (**2**), which rearranged rapidly to isomeric 5-ribosylureidotriazole **6**. The rearrangement proceeds easily also in water solutions of **2**. Alkaline hydrolysis of **6** gave a mixture of 5-ureidotriazole **7** and 5-aminotriazole **8**. Acid hydrolysis of **6** afforded only **7**. This compound was also prepared by thermal rearrangement of 5-amino-1-carbamoyltriazole **9** or on reaction of cyano(formyl)guanidine **10** with hydrazine hydrochloride. Treatment of benzoylated methoxyribosyltriazinone **4a** with hydrazine in methanol gave only the rearranged product **6a**. Reaction of tribenzoylribosyl isocyanate **12** with aminotriazole **8** gave 1-triazolecarboxamidotribenzoylribose **13**, which afforded by methanolysis oxazoloribofuranose **14** and aminotriazole **8**. Compound **14** was also obtained by methanolysis of blocked ribosylcarbamate **16**. Reaction of methoxyribosyltriazinone **4** with hydroxylamine in methanol afforded 4-hydroxylamino-1-β-D-ribofuranosyl-1,3,5-triazin-2(*1H*)-one (N^4-hydroxy-5-azacytidine) (**3**), which on the action of excess hydroxylamine yielded 1-cyano-1-hydroxy-5-β-D-ribofuranosylisobiuret (**19**). Treatment of methoxy-1,3,5-triazinone **18** with a solution of hydroxylamine in methanol gave 4-hydroxylamino-1-methyl-1,3,5-triazin-2(1*H*)-one (N^4-hydroxy-1-methyl-5-azacytosine) (**17**). Heating of cyano(formyl)guanidine **10** with hydroxylamine hydrochloride in water lead to the formation of triuret (**21**). The mechanisms of the reactions of methoxyribosyltriazinone **4** with hydrazine and hydroxylamine are discussed. Compounds **2**, **6** and **19** exhibited no significant antibacterial or cytostatic activity.

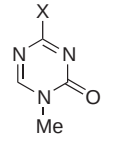
Keywords: 1,3,5-Triazines; 1,2,4-Triazoles; Nucleosides; Ureas; Hydrazones; Cleavage; Antibacterial activity.

In continuation of our study of the nucleoside antibiotic 5-azacytidine[1-4] (**1**) (Chart 1) and its N^4-substituted derivatives[5,6], we were also interested in the synthesis and biological activity of 4-hydrazino-1-β-D-ribofuranosyl-1,3,5-triazin-2(*1H*)-one (N^4-amino-5-azacytidine) (**2**) (Scheme 1) and

4-hydroxylamino-1-β-D-ribofuranosyl-1,3,5-triazin-2(1*H*)-one (N^4-hydroxy-5-azacytidine) (**3**) (Scheme 3). N^4-Methyl derivatives[5] of 5-azacytidine, which exhibited antibacterial activity, have been prepared in good yields by aminolysis of the well available 4-methoxy-1-β-D-ribofuranosyl-1,3,5-triazin-2(1*H*)-one[7] (**4**) (Chart 1). For this reason we have used an analogous approach for the preparation of nucleosides **2** and **3**. In this communication we wish to discuss plausible mechanisms for the formation of nucleosides **2** and **3** from the methoxy nucleoside **4** with hydrazine and hydroxylamine, respectively.



1, R = H, X = NH_2
4, R = H, X = OMe
4a, R = Bz, X = OMe



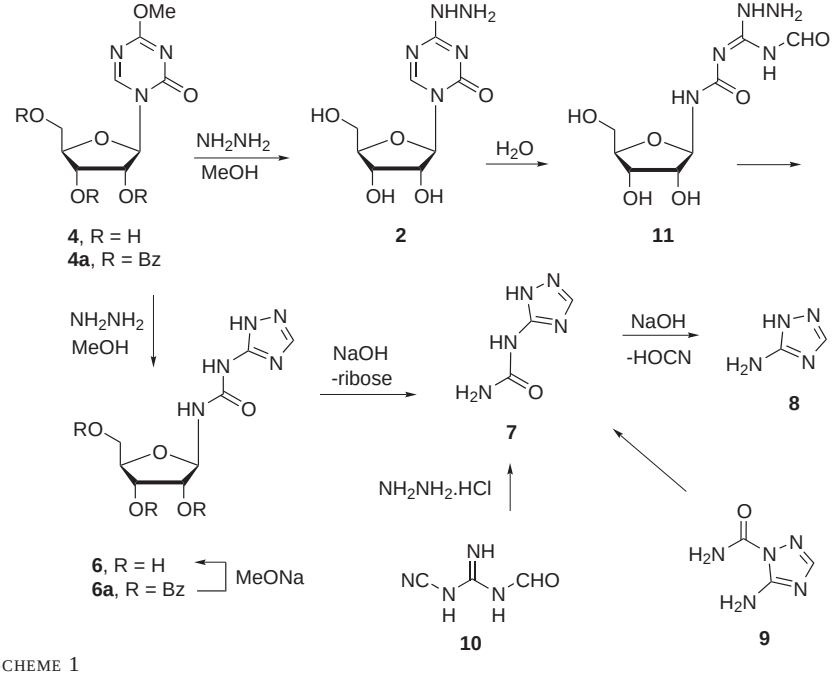
5, X = $NHNH_2$
17, X = NHOH
18, X = OMe

CHART 1

Treatment of methoxy nucleoside **4** with a solution of water-free hydrazine in methanol at room temperature for 4 min gave crude hydrazine derivative **2** (Scheme 1), which precipitated upon addition of ether. The structure of **2** was proved by comparison of its UV spectra with those of the known structurally related 4-hydrazino-1-methyl-1,3,5-triazin-2(1*H*)-one[8] (N^4-amino-1-methyl-5-azacytosine) (**5**) (Chart 1). ^{1}H NMR spectrum of crude **2**, which was not well resolvable, indicated the presence of three species. We assume the presence of **2**, the rearranged product **6** and possibly a ring-opened intermediate of the rearrangement. All attempts to purify crude **2** by recrystallization or chromatography were not successful. Only the rearranged product **6** was isolated. Prolonged treatment of **4** with hydrazine in methanol afforded pure crystalline 5-(3-β-D-ribofuranosyl-ureido)-1*H*-1,2,4-triazole (**6**) in an excellent yield (Scheme 1). The transformation of **2** to **6** proceeded also rapidly in water solutions of crude **2** even at room temperature or very slowly on boiling of crude **2** in methanol.

The structure of the rearranged product **6** was inferred from spectral data and was confirmed by alkaline hydrolysis, which afforded a mixture of 5-ureido-1*H*-1,2,4-triazole (**7**) and the well known 5-amino-1*H*-1,2,4-triazole[9] (**8**). Compounds **7** and **8** were isolated as picrates. The picrate of

aminotriazole **8** was also previously described[10]. On prolongation of the alkaline hydrolysis only aminotriazole **8** was detected in the reaction mixture. Acid hydrolysis of ribosylureidotriazole **6** gave exclusively ureidotriazole **7**, however in a rather low yield. Both types of hydrolysis were accompanied by cleavage of the C–N glycoside bond so that only the aglycones were isolated. Ureidotriazole **7** was also prepared in low yield by thermal rearrangement of the known 5-amino-1-carbamoyl-1*H*-1,2,4-triazole[11] (**9**) or on heating of hydrazine hydrochloride with 1-cyano-3-formylguanidine[12] (**10**) in water (Scheme 1).

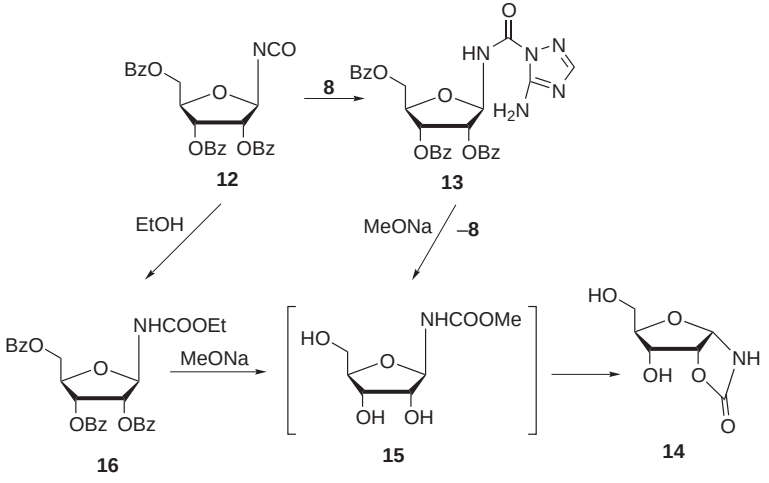


SCHEME 1

The mechanism of the rearrangement of N^4-aminoazacytidine **2** to 5-ribosylureidotriazole **6** in water solutions could be explained as follows. Due to the low electron density in position 6 of the triazine ring, covalent addition of water to the C=N double bond in positions 5 and 6 takes place. The labile covalent hydrate is transformed by a prototropic rearrangement to the ring-opened *N*-formyl derivative **11** (Scheme 1), which cyclizes to ribosylureidotriazole **6**. The reason for this rearrangement is obviously the much lower stability of the 1,3,5-triazine in comparison with the 1,2,4-triazole ring. The reaction of methoxy nucleoside **4** with hydrazine in methanol affords N^4-amino-5-azacytidine (**2**) as the primary product, which

rearranged, either by the action of traces of water in the depicted manner or, more probably, the triazine ring opens by the action of hydrazine and the triazole ring is formed via a formamidrazone intermediate. The rearrangement of the crude aminoazacytidine **2** in methanol proceeds very slowly, because the addition of methanol to the C=N double bond in position 5 and 6 and subsequent opening of the triazine ring proceeds not so easy as with water or hydrazine. Consequently, closing to the triazole is slow. Also, we cannot exclude that the rearrangement is mediated by traces of water present in the solvent.

The reaction of tribenzoyl derivative **4a** (Chart 1) with hydrazine in methanol afforded only the rearranged product **6a** (Scheme 1). From the mother liquor, a second portion of **6a** was obtained, which contained also some α-anomer (α/β = 1:4). This result indicates that the triazine ring of the blocked nucleoside **4a** is more reactive to hydrazine or water than the free nucleoside **4**. The yield of nucleoside **6a** was rather low due to partial debenzoylation of the ribose moiety, which occurs during the reaction. The structure of compound **6a** was inferred from spectral data and was also confirmed by methanolysis, which gave the free rearranged product **6**. An alternative preparation of the blocked ribosylureidotriazole **6a** on condensation of 2,3,5-tri-*O*-benzoyl-β-D-ribosyl isocyanate (**12)** with aminotriazole **8** and subsequent rearrangement of the blocked 5-amino-1-(ribosylcarbamoyl)-1*H*-1,2,4-triazole **13** failed (Scheme 2). The primarily formed intermediate **13** did not rearrange like the unsubstituted carbamoyl derivative **9**. In this connection it should be mentioned that an analogous situation was ob-



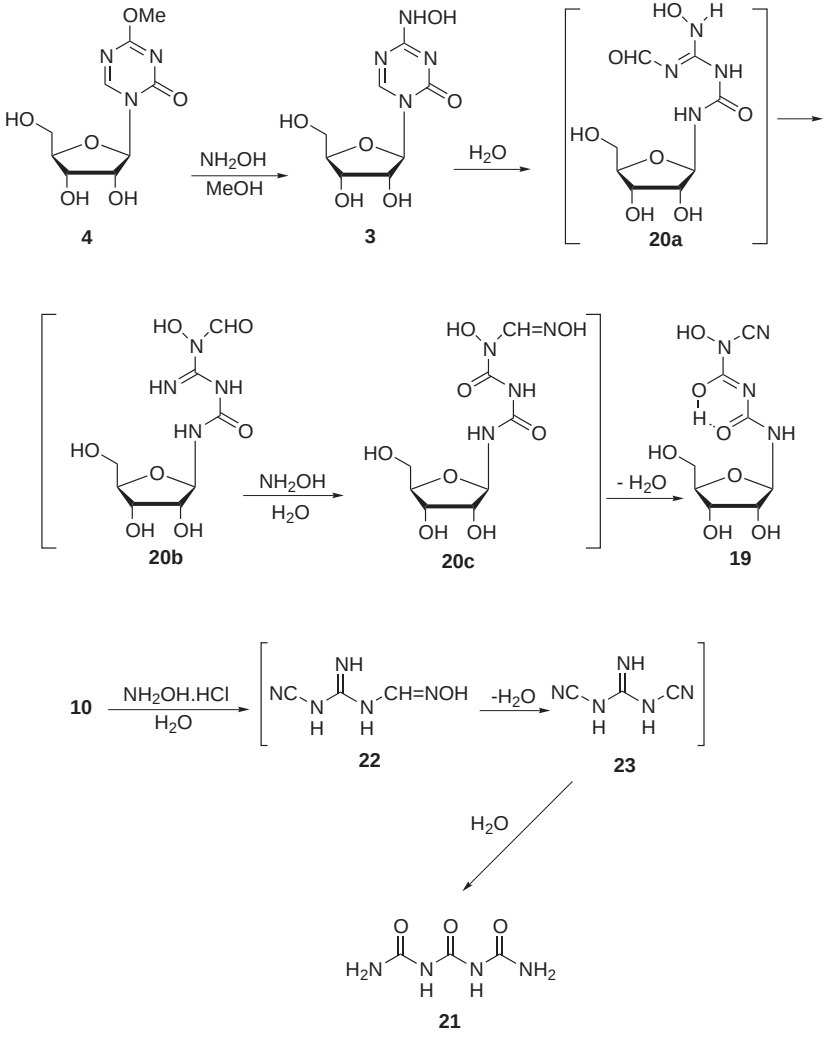
SCHEME 2

served in the case of 1-(methylcarbamoyl)-5-amino-1*H*-1,2,4-triazole[13]. Methanolysis of the blocked nucleoside **13**, instead of giving the respective free nucleoside, afforded aminotriazole[9] **8**, isolated as picrate[10], and oxazoloribofuranose[12] **14**, which were both identified by comparison with authentic samples. The formation of oxazoloribofuranose **14** proceeds obviously via methyl *N*-β-D-ribofuranosylcarbamate (**15**), which undergoes anomerization and cyclization to yield the final product **14**. To prove this assumption we prepared ribofuranosooxazole **14** by methanolysis of the blocked ribosylurethane **16**, which was prepared by a known procedure from isocyanate **12** and ethanol[14].

4-Hydroxylamino-1-β-D-ribofuranosyl-1,3,5-triazin-2(1*H*)-one (N^4-hydroxy-5-azacytidine) (**3**) was obtained by reaction of methoxy nucleoside **4** with a small excess of hydroxylamine in methanol (Scheme 3). The product was isolated as amorphous monohydrate by column chromatography on silica gel. The highly hygroscopic product was characterized by comparison of its UV spectra with those of the structurally related 4-hydroxylamino-1-methyl-1,3,5-triazin-2(1*H*)-one (N^4-hydroxy-1-methyl-5-azacytosine) (**17**), which was prepared by reaction of 4-methoxy-1-methyl-1,3,5-triazin-2(1*H*)-one[15] (**18**) with a solution of hydroxylamine in methanol (Chart 1). NMR spectra of **17** indicate the prevalence of the hydroxyimino over the hydroxyamino tautomeric form in solution. Prolonged action of a large excess of hydroxylamine on methoxy nucleoside **4** afforded 1-cyano-1-hydroxy-5-β-D-ribofuranosylisobiuret (**19**) (Scheme 3). The structure of this ring-opened product was inferred from elemental analysis, mass spectrum, IR spectrum (exhibiting characteristic bands of the cyano group, C=N bond and also of the N–O bond), and eventually from ^{1}H and ^{13}C NMR spectra. The formation of **19** can be explained by hydrolytic opening of the triazine ring, in analogy to the above mentioned N^4-aminoazacytidine **2**, yielding the formyl derivative **20a**, followed by transformylation to **20b,** hydrolysis of the imino group and formation of oxime **20c,** which eliminates water to yield the final product **19**. This course of the reaction was also supported by the formation of triuret (**21**) by the action of hydroxylamine hydrochloride on 1-cyano-3-formylguanidine (**10**) (Scheme 3). The final product **21** was obviously formed via oxime **22** and 1,3-dicyanoguanidine (**23**).

Compounds **2**, the rearranged product **6** and the decomposition product **19** were tested for antibacterial activity against *Escherichia coli*. N^4-Amino-5-azacytidine (**2**) exhibited only an 87% inhibition of growth at 1 mg/ml concentration and the product **19** has shown a 100% growth inhibition under the same conditions. Both compounds were inactive at lower concen-

trations. The rearranged product **6** was completely inactive even at ≤1 mg/ml concentrations. Also in vitro inhibition of cell growth with compounds **2**, **6** and **19** was evaluated in the following cell cultures: human T-lymphoblastoid CCRF-CEM cell line (ATCC CCL 119), human promyeolocytic leukemia HL-60 (ATCC CCL 240) cells, human cervix carcinoma HeLa S3 cells (ATCC CCL 2.2) and mouse leukemia L1210 cells (ATCC CCL 219). None of the mentioned compounds exhibited any considerable activity at 10 μM concentrations.



SCHEME 3

EXPERIMENTAL

Melting points were taken on a heated microscope stage (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), or B, butan-1-ol–acetic acid–water (5:2:3). The spots of the studied compounds were detected either in UV light (254 nm) (D_1) or with ninhydrin (D_2). Column chromatography was performed with silica gel according to Pitra (Service Laboratories of this Institute). IR spectra were recorded on a IR spectrometer Zeiss UR-20 and on a FTIR spectrometer Bruker IFS 55 in KBr pellets, Nujol or DMSO solutions. UV spectra were measured on a Unicam SP 8000 spectrometer (Pye–Unicam, Cambridge, U.K.) in buffer solutions of ionic strength 0.01 prepared according to Perrin[16], λ are given in nm and ε in m^2 mol^{-1}. Optical rotations were recorded on a polarimeter Perkin–Elmer, type 141 at 22 °C and are given in deg cm^3 g^{-1} dm^{-1}. ^1H and ^{13}C NMR spectra were measured at 500 and 125.7 MHz on a Varian Unity 500 instrument in DMSO-d_6 with the solvent signal as the internal reference ($\delta(^1H)$ 2.5 and $\delta(^{13}C)$ 39.70 ppm). The chemical shifts (δ-scale) are given in ppm and coupling constants (*J*) in Hz. The mass spectra (*m*/*z* (% rel. int.)) were measured on a ZAB-EQ (Micromass, Manchester, U.K.) spectrometer using a FAB ionization (bombarding gas Xe at 8 kV). A mixture of glycerol and thioglycerol (1:3, v/v) was used as matrix; samples were dissolved in methanol or water. Stationary cultivation of *Escherichia coli* was performed at 37 °C in a mineral medium with glucose[17]. The tested compounds were added before inoculation and bacterial growth was measured 16 h later.

4-Hydrazino-1-β-D-ribofuranosyl-1,3,5-triazin-2(1*H*)one (**2**)

A solution of methoxytriazinone[7] **4** (0.259 g, 1 mmol) in methanol (20 ml) was treated with a 0.5 M solution of water-free hydrazine in methanol (3.0 ml, 1.5 mmol) and the mixture kept at room temperature for 4 min. The solution was precipitated with ether (20 ml), the product filtered off by suction and washed with ether to yield 0.20 g (72%) of the crude monohydrate of **2**, m.p. 105–115 °C (dec.), R_F 0.47 (B, D_1, D_2), $[\alpha]_D$ –22.7 (*c* 0.20, water). All attempts to purify the sample by recrystallization or column chromatography were unsuccessful, only the rearranged product **6** was isolated. UV, λ_{max} (log ε), pH 2.33: 258 (3.53), 223 (3.76); pH 7.05: 211 (4.16) (rearrangement to **6**); pH 10.99: 216 (4.01) (rearrangement to **6**). MS: 260 (10) $[M + H]^+$, 128 (7) $[M + H]^+$ – aglycone. For $C_8H_{13}N_5O_5 \cdot H_2O$ (277.2) calculated: 34.66% C, 5.45% H, 25.56% N; found: 34.38% C, 5.15% H, 25.16% N.

4-Hydrazino-1-methyl-1,3,5-triazin-2(1*H*)-one (**5**)

This compound was prepared by a known[8] procedure. UV, λ_{max} (log ε), pH 2.30: 258 (3.76), 223 (3.65); pH 7.03: 250 (3.75), 209 (4.11); pH 10.97: 252 (3.84), 216 (4.03). ^1H NMR: 9.05 br s, 1 H (NH); 8.175 s, 1 H (H-6); 3.20 s, 3 H (CH_3); 4.41 br s, 2 H (NH_2). ^{13}C NMR: 154.595 (C-2), 165.21 (C-4), 158.535 (C-6), 34.005 (CH_3). MS: 142 (4) $[M + H]^+$.

5-(3-β-D-Ribofuranosylureido)-1*H*-1,2,4-triazole (**6**)

Method A. A mixture of methoxytriazinone[7] **4** (0.259 g, 1 mmol) and a 0.5 M solution of water-free hydrazine in methanol (3.0 ml, 1.5 mmol) was stirred at room temperature for

20 min. The crystals were filtered off by suction to give 0.23 g (89%) of triazole **6**, m.p. 202–205 °C (dec.), recrystallization from water raised the melting point to 206–209 °C (dec.), R_F 0.48 (B, D_2), $[\alpha]_D$ –62.2 (*c* 0.50, water). UV, λ_{max} (log ε), MeOH: 220 (3.99); pH 0: 213 (4.04); pH 7.0: 213 (4.09). IR (Nujol): 3389 m, 3370 m, 3294 s, 3207 s, 3110 m, 3045 m [ν(OH, NH)]; 1701 [ν(C=O)]. ^{1}H NMR: 13.10 br, 1 H, 9.80 br s, 1 H and 7.90 br, 1 H (3 × NH); 7.90 br, 1 H (H-3); 5.08 br s, 1 H, 4.93 br s, 1 H and 4.78 br s, 1 H (3 × OH); 5.23 dd, 1 H, *J*(1′,2′) = 5.6, *J*(1′,NH) = 9.4 (H-1′); 3.72 t, 1 H, *J*(2′,1′) = *J*(2′,3′) = 5.5 (H-2′); 3.85 dd, 1 H, *J*(3′,4′) = 3.9, *J*(3′,2′) = 5.4 (H-3′); 3.68 br q, 1 H, *J* = 3.9 (2 ×) and 4.4 (H-4′); 3.45 dd, 1 H, *J*(5′a,4′) = 3.9, *J*(gem) = 11.7 (H-5′a); 3.39 dd, 1 H, *J*(5′b,4′) = 4.4, *J*(gem) = 11.7 (H-5′b). ^{13}C NMR: 147.0 br (2 × C) (C-3 and C-5), 153.72 (C=O), 84.515 (C-1′), 74.55 (C-2′), 70.56 (C-3′), 83.45 (C-4′), 62.06 (C-5′). MS: 260 (1) $[M + H]^+$, 128 (3) $[M + H]^+$ – aglycone. For $C_8H_{13}N_5O_5$ (259.2) calculated: 37.07% C, 5.05% H, 27.02% N; found: 37.15% C, 5.12% H, 26.75% N.

Method B. A solution of N^4-amino derivative **2** (29 mg, 0.1 mmol) in hot water (1.5 ml) was kept after cooling at room temperature for 3 h and evaporated. The residue was triturated with ethanol and the solid recrystallized from a mixture of methanol and water to yield 15 mg (52%) of **6**, m.p. 204–205 °C (dec.) without depression on admixture of a sample prepared by method *A*.

Method C. A mixture of **2** (29 mg, 0.1 mmol) and methanol (5 ml) was refluxed for 24 h. The crystals were filtered off by suction and recrystallized from a mixture of methanol and water to afford 17 mg (57%) of **6**, m.p. 203–205 °C (dec.) without depression on admixture of a sample prepared by method *A*.

Method D. A solution of blocked nucleoside **6a** (28.5 mg, 0.05 mmol) in methanolic 0.2 M NaOMe (0.5 ml) was kept at room temperature for 90 min. The solution was treated with acetic acid (0.01 ml) and applied onto a column of Amberlite IRC $[H^+]$ ion exchange resin (10 ml, packed in methanol). The column was eluted with methanol (100 ml), the eluate evaporated and the residue co-evaporated with water (3 × 5 ml). Crystallization of the residue from ethanol afforded 5 mg (38%) of compound **6**, m.p. 202–205 °C without depression on admixture of a sample prepared by method *A*.

Alkaline Hydrolysis of **6**

A solution of ribosylureidotriazole **6** (0.259 g, 1 mmol) in 2 M KOH (2 ml) was refluxed for 90 min. The solution was applied onto a column of Amberlite IRC-50 $[H^+]$ ion exchange resin (30 ml, packed in methanol) and the column eluted with methanol (150 ml). The eluate was evaporated, the residue boiled with 96% ethanol (3 ml), the solution filtered and the clear filtrate precipitated with a solution of picric acid (0.120 g) in ethanol (2 ml). The mixture was kept at room temperature for 1 h, the yellow crystals filtered off with suction and washed with ethanol to yield 0.125 g (35%) of the picrate of ureidotriazole **7**, m.p. 218–220 °C (dec.), R_F 0.51 (B, D_2, **7**) and 0.90 (B, D_1, picric acid). The analytical sample was recrystallized from methanol, m.p. 220–222 °C (dec.). UV, λ_{max} (log ε), MeOH: 356 (4.16), 240 (infl.) (4.11), 213 (4.45). ^{1}H NMR: 10.59 br s, 1 H, 9.0 br, 2 H, 6.83 br s, 2 H (3 × NH + NH_2); 8.70 s, 1 H (triazole H-3); 8.59 s, 2 H (picrate H-3 + H-5). ^{13}C NMR: 160.95 (triazole C-5), 141.37 (triazole C-3), 153.67 (ureido C=O), 148.43 (picrate C-1), 142.04 (picrate C-2), 125.44 (picrate C-3), 124.67 (picrate C-4). MS: 128 (1) $[M_7 + H]^+$, 85 (12) $[M_8 + H]^+$. For $C_3H_5N_5O \cdot C_6H_3N_3O_7$ (356.2) calculated: 30.34% C, 2.26% H, 31.46% N; found: 30.62% C, 2.34% H, 31.78% N.

The mother liquor after isolation of the picrate of **7** was evaporated and the residue crystallized from ethanol (2 ml) to afford 0.045 g (14%) of the picrate of aminotriazole **8**, m.p. 228–232 °C (dec.). An analytical sample was recrystallized from water, m.p. 233–236 °C (dec.) without depression on admixture of an authentic[10] sample, R_F 0.48 (B, D_2, **8**) and 0.90 (B, D_1, picric acid). UV, λ_{max} (log ε), MeOH: 355 (4.15), 240 (4.13), 211 (4.37). ^{1}H NMR: 8.31 s, 1 H (H-3); 8.00 br, 4 H (N_1-H + N_2-H + NH_2); 8.59 s, 2 H (picrate H-3 + H-5). ^{13}C NMR: 160.99 (C-5), 139.54 (C-3), 151.02 (picrate C-1), 142.04 (picrate C-2), 125.38 (picrate C-3), 124.37 (picrate C-4). MS: 85 (12) $[M_{\mathbf{8}} + H]^+$. For $C_2H_4N_4O \cdot C_6H_3N_3O_7$ (313.2) calculated: 30.68% C, 2.26% H, 31.31% N; found: 30.97% C, 2.15% H, 31.51% N.

On prolongation of the time of hydrolysis to 2 h, 22% of the picrate of **7** and 41% of the picrate of **8** were obtained by the same working-up procedure. Prolongation of the time of hydrolysis to 8 h led to complete hydrolysis of the intermediate **7**. TLC indicated only the presence of the final product **8**.

Acid Hydrolysis of **6**

A solution of ribosylureidotriazole **6** (0.259 g, 1 mmol) in 10% hydrochloric acid (6 ml) was heated at 100 °C (bath temperature) for 1 h and evaporated. The residue was triturated with methanol (10 ml), a small insoluble portion filtered off and the filtrate applied onto a column of Amberlite IR-45 [OH^-] (40 ml, packed in methanol). The column was eluted with methanol (200 ml), the eluate filtered with charcoal and evaporated. The residue was dissolved in ethanol (6 ml) and, after addition of two drops of water and filtration of the solution (to remove slight turbidity), a solution of picric acid (0.240 g) in ethanol (6 ml) was added. Concentration of the solution gave 0.14 g of crude picrate of **7**, m.p. 195–210 °C (dec.). Recrystallization of the crude material from methanol afforded in two portions 0.10 g (28%) of the picrate of **7**, m.p. 217–219 °C (dec.) without depression on admixture with the sample prepared by alkaline hydrolysis.

5-Ureido-1*H*-1,2,4-triazole (**7**)

Method A. A solution of the picrate of **7** (0.178 g, 0.5 mmol) in methanol (60 ml) was applied onto a column of Amberlite IR-45 [OH^-] (60 ml, packed in methanol). The column was washed with methanol (600 ml) and the eluate evaporated. The residue was crystallized from 9:1 ethanol–water to yield 0.050 g (78%) of ureidotriazole **7**; the compound decomposed at 245–250 °C without melting up to 350 °C, R_F 0.51 (B, D_2). UV, λ_{max} (log ε), pH 2.30: 222 (3.81); pH 6.90: 210 (4.07); pH 10.8: 218 (3.92). MS: 128 (100) $[M + H]^+$. For $C_3H_5N_5O$ (127.1) calculated: 28.35% C, 3.97% H, 55.10% N; found: 28.56% C, 3.90% H, 55.12% N.

Method B. 5-Amino-1-carbamoyltriazole[11] **9** (0.381 g, 3 mmol) was heated at 180 °C for 10 min. The starting compound melted and then resolidified. The crude product was powdered and extracted with boiling 9:1 ethanol–water (50 ml). The insoluble portion was filtered off after cooling and the filtrate evaporated. A solution of the residue in 9:1 ethanol–water (5 ml) was treated with a solution of picric acid (0.12 g) in ethanol (2 ml), the yellow precipitate filtered off with suction and washed with ethanol to afford 0.07 g (7%) of the picrate of **7**, m.p. 218–220 °C (dec.) without depression on admixture of a sample obtained from hydrolysis of ribosylureidotriazole **6**. The free ureidotriazole **7** was obtained from the picrate using method *A*. Yield 0.018 g (5%, based on **9**), the product decomposed at 245–250 °C without depression on admixture of a sample prepared by method *A*.

Method C. A mixture of cyano(formyl)guanidine **10** (0.448 g, 4 mmol), hydrazine hydrochloride (0.274 g, 4 mmol) and water (2 ml) was refluxed for 20 min. The mixture was cooled to 0 °C, the crystals were filtered off with suction and washed with ice water and methanol to give 0.11 g of the crude hydrochloride of ureidotriazole **7** contaminated with the free base. ^{1}H NMR: 10.46 br s, 1 H, 9.15 br, 2 H and 6.92 br s, 2 H (3 × NH + NH_2); 8.30 s, 1 H (H-3). ^{13}C NMR: 150.16 (C-5), 143.47 (C-3), 154.36 (C=O). MS: 128 (100) $[M + H]^+$. The free base **7** was obtained by method *A*. Yield 0.055 g (11%), the product decomposed at 245–250 °C without depression on admixture of a sample prepared by method *A*.

5-[3-(2,3,5-Tri-*O*-benzoyl-β-D-ribosyl)ureido]-1*H*-1,2,4-triazole (**6a**)

A mixture of the blocked methoxy nucleoside[7] **4a** (1.143 g, 2 mmol) and methanol (50 ml) was shortly heated to dissolve the starting compound. The solution, after cooling to room temperature, was treated with 0.5 M water-free hydrazine in methanol (6 ml). The product started to deposit from solution almost immediately. The mixture was kept overnight at room temperature and the precipitate filtered off with suction to give 0.220 g of crude **6a**, m.p. 182–190 °C (dec.). Recrystallization of the crude material from methanol–chloroform afforded 0.170 g (15%) of pure **6a**, m.p. 195–200 °C (dec.), R_F 0.51 (A, D_1), $[\alpha]_D$ –52.6 (*c* 0.20, DMF). ^{1}H NMR: 13.30 br, 1 H, 10.05 br, 1 H and 8.00 br, 1 H (3 × NH); 7.87 br s, 1 H (H-3); 8.03 d, 2 H, 7.88 d, 4 H, 7.67 t, 1 H, 7.64 t, 2 H, 7.51 t, 2 H and 7.45 t, 4 H (arom.); 5.82 dd, 1 H, *J*(1′,2′) = 5.7, *J*(1′,NH) = 9.0 (H-1′); 5.79 dd, 1 H, *J*(2′,3′) = 4.9, *J*(2′,1′) = 5.7 (H-2′); 5.68 br t, 1 H, *J*(3′,2′) = *J*(3′,4′) = 4.8 (H-3′); 4.60 m, 2 H (H-4′ + H-5′a); 4.55 dd, 1 H, *J*(5′b,4′) = 5.6, *J*(gem.) = 12.6 (H-5′b). ^{13}C NMR: 151.80 br (C-5); 146.50 br (C-3); 153.58 (ureido C=O); 165.68, 164.89 and 164.87 (3 × benzoyl C=O); 134.08, 133.97, 133.71, 129.47 (2 × C); 129.45 (4 × C); 128.98 (2 × C); 128.94 (2 × C); 128.91 (2 × C); 128.84 and 128.654 (2 × C) (arom.); 83.44 (C-1′); 73.835 (C-2′); 71.33 (C-3′); 77.86 (C-4′); 64.41 (C-5′). MS: 572 (8) $[M + H]^+$, 445 (5) $[M]^+$ of the tribenzoylribosyl ion. For $C_{29}H_{25}N_5O_8$ (571.6) calculated: 60.94% C, 4.41% H, 12.25% N; found: 60.95% C, 4.35% H, 12.04% N.

The mother liquor of the crude product was evaporated and applied onto a column of silica gel (50 g), prepared in chloroform. The column was eluted with 250-ml portions of chloroform containing 5, 10, 20 and 40% of methanol. The fractions containing the major portion of the product were evaporated and the residue (0.4 g) was crystallized from a mixture of chloroform–methanol to yield another portion of **6a** (0.040 g, 3.5%). Evaporation of the mother liquor and crystallization of the residue from methanol gave 0.050 g (4.3%) of a mixture of **6a** and its α-anomer, m.p. 162–166 °C (dec.), $[\alpha]_D$ –33.6 (*c* 0.21, DMF). ^{1}H NMR: 10.10 br and 8.0 br (NH); 8.05–7.40 m, 15 H (arom.); β-anomer: 5.82 dd, 1 H, *J* = 5.4 and 9.6 (H-1′); 5.79 dd, 1 H, *J* = 5.4 and 5.6 (H-2′); 5.68 br t, 1 H, *J* = 5.5 (H-3′); 4.60 m, 2 H and 4.54 m, 1 H (H-4′ + 2 × H-5′); α-anomer: 6.21 dd, 1 H, *J* = 4.5 and 9.2 (H-1′); 6.02 m, 2 H (H-2′ + H-3′) (α/β = 1:4). ^{13}C NMR, β-anomer: 83.45 (C-1′), 73.85 (C-2′), 71.345 (C-3′), 77.87 (C-4′), 64.41 (C-5′); α-anomer: 80.19 (C-1′), 73.96 (C-2′), 72.31 (C-3′), 78.03 (C-4′), 64.45 (C-5′); the other signals were not distinguished. MS: 572 (3) $[M + H]^+$, 445 (7) $[M]^+$ of the tribenzoylribosyl ion. For $C_{29}H_{25}N_5O_8$ (571.6) calculated: 60.94% C, 4.41% H, 12.25% N; found: 60.62% C, 4.39% H, 11.90% N.

5-Amino-1-[(2,3,5-tri-*O*-benzoyl-β-D-ribosyl)carbamoyl]-1*H*-1,2,4-triazole (**13**)

A solution of crude isocyanate **12** (prepared from 5 mmol of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose[18] by a known procedure[19]) in toluene (25 ml) was added dropwise at room tem-

perature to a stirred mixture of aminotriazole **8** (0.42 g, 5 mmol) in dry acetone (10 ml). The mixture was stirred at room temperature for 1 h and then evaporated. A solution of the residue in chloroform (50 ml) was washed with water (3 × 10 ml), dried (anhydrous $MgSO_4$), evaporated and the syrupy residue applied onto a column of silica gel (50 g). The column was eluted successively with 250 ml portions of toluene, mixtures of toluene with 10, 30 and 50% of ethyl acetate and ethyl acetate. The elution was followed by TLC in solvent system A. Fractions containing the major product were collected, evaporated and the residue dried at 80 °C/40 Pa to yield 1.5 g (52%) of the blocked nucleoside **13** as a foam, R_F 0.24 (A, D_1), $[\alpha]_D$ –32.1 (*c* 0.20, chloroform). UV, λ_{max} (log ε), MeOH: 229 (4.73), 208 (4.57). ^{1}H NMR: 9.60 d, 1 H, *J*(NH,1′) = 9.0 (NH); 7.32 br s, 2 H (NH_2); 7.64 s, 1 H (H-3); 8.03 d, 2 H, 7.91 d, 2 H, 7.84 d, 2 H, 7.65 m, 3 H, 7.50 t, 2 H, 7.47 t, 2 H and 7.42 t, 2 H (arom.); 5.83 dd, 1 H, *J*(1′,2′) = 4.0, *J*(1′,NH) = 9.0 (H-1′); 5.92 m, 2 H (H-2′ + H-3′); 4.62 m, 2 H (H-4′ + H-5′a); 4.53 dd, 1 H, *J*(5′b,4′) = 6.0, *J*(gem.) = 12.9 (H-5′b). ^{13}C NMR: 165.70, 164.86 and 164.80 (benzoyl C=O); 134.05, 133.94, 133.66, 129.57 (2 × C); 129.48 (2 × C); 129.42 (2 × C); 128.99 (2 × C); 128.86 (4 × C); 128.80 and 128.72 (2 × C) (arom.); 157.15 (C-5); 150.52 (C-3); 151.14 (carbamoyl C=O); 83.815 (C-1′); 73.77 (C-2′); 70,90 (C-3′); 78.02 (C-4′); 63.96 (C-5′). MS: 572 (75) $[M + H]^+$, 445 (100) $[M]^+$ of the tribenzoylribosyl ion. For $C_{29}H_{25}N_5O_8$ (571.6) calculated: 60.94% C, 4.41% H, 12.25% N; found: 61.15% C, 4.25% H, 11.98% N.

4,5-Dihydro-(1,2-dideoxy-α-D-ribofuranoso[1,2-*d*]-1,3-oxazol-2-one (**14**)

Method A. A solution of the blocked nucleoside **13** (0.285 g, 0.5 mmol) in 1 M NaOMe (2.5 ml) was kept at room temperature for 3 h. The solution was acidified with acetic acid (0.2 ml) and applied onto a column of Amberlite IRC-50 $[H^+]$ ion exchange resin (30 ml), which was prepared in methanol. The column was washed with methanol (200 ml) and the eluent evaporated. The residue was crystallized from ethanol (3 ml) to afford 0.045 g (51%) of the oxazolone **14**, m.p. 165–167 °C without depression on admixture of an authentic sample[12], R_F 0.82 (B, D_2), $[\alpha]_D$ +111.3 (*c* 0.108, water). UV, λ_{max} (log ε), MeOH: 209 (3.77). ^{1}H NMR: 8.60 br s, 1 H (NH); 5.52 d, 1 H, *J*(1,2) = 5.3 (H-1); 4.78 t, 1 H, *J*(2,1) ≈ *J*(2,3) = 5.4 (H-2); 3.78 br dt, 1 H, *J*(3,2) ≈ *J*(3,OH) = 6.0, *J*(3,4) = 9.3 (H-3); 5.38 d, 1 H, *J*(OH,3) = 6.5 (3-OH); 3.59 ddd, 1 H, *J*(4,5a) = 2.0, *J*(4,5b) = 5.3, *J*(4,3) = 9.3 (H-4); 3.66 ddd, 1 H, *J*(5a,4) = 2.0, *J*(5a,OH) = 5.0, *J*(gem) = 12.2 (H-5a); 3.42 dt, 1 H, *J*(5b,4) ≈ *J*(5b,OH) = 5.0, *J*(gem) = 12.2 (H-5b); 4.73 br t, 1 H, *J*(OH,5) = 5.2 (5-OH). ^{13}C NMR spectra: 158.28 (C=O), 84.73 (C-1), 78.73 (C-2 or C-4), 70.54 (C-3), 78.94 (C-4 or C-2), 60.15 (C-5). MS: 176 (100) $[M + H]^+$. For $C_6H_9NO_5$ (175.1) calculated: 41.14% C, 5.18% H, 8.00% N; found: 41.35% C, 5.25% H, 8.23% N.

The mother liquor was evaporated and a solution of the residue in ethanol (2 ml) was treated with a solution of picric acid (0.12 g) in ethanol (2 ml) to deposit immediately the picrate of aminotriazole **8**. Yield 0.125 g (80%), m.p. 233–236 °C (dec.) without depression on admixture of an authentic sample[10], R_F 0.48 (B, D_2, **8**) and 0.90 (B, D_1, picric acid). For $C_2H_4N_4 \cdot C_6H_3N_3O_7$ (313.2) calculated: 30.68% C, 2.26% H, 31.31% N; found: 30.97% C, 2.31% H, 31.60% N.

Method B. A solution of (tribenzoylribosyl)carbamate[14] **16** (0.266 g, 0.5 mmol) in 1 M NaOMe (2.5 ml) was allowed to stand at room temperature for 1.5 h. The solution was decationized with Amberlite IRC-50 $[H^+]$, as described by method *A*. The eluent was evaporated, the residue co-evaporated with water (3 × 5 ml) and crystallized from ethanol (1 ml)

with seeding to yield 0.038 g (43%) of compound **14**, m.p. 165–167 °C without depression on admixture of an authentic sample[12].

4-Hydroxylamino-1-methyl-1,3,5-triazin-2(1*H*)-one (**17**)

A solution of 4-methoxy-1-methyl-1,3,5-triazin-2-one[15] (**18**; 0.070 g, 0.5 mmol) in 1 M hydroxylamine in methanol (6 ml) was allowed to stand at room temperature for 1.5 h. The deposited crystals were filtered off with suction to give 0.05 g (70%) of compound **17**, m.p. 224–226 °C (dec.), R_F 0.54 (B, D_1). UV, λ_{max} (log ε), MeOH: 275 (4.43), 216 (4.66); pH 2.30: 265 (3.91), 227 (3.93); pH 6.90: 275 (3.45), 217 (4.11); pH 10.80: 254 (3.89), 225 (3.91). IR (KBr): 3307 m, 3095 m, br [ν(OH,NH)]; 1724 s, 1714 s, sh [ν(C=O)]; 1660 s, 1645 s, sh [ν(C=N)]. IR (DMSO): 1726 s, 1717 s, sh [ν(C=O)]; 1659 s (asymmetric) [ν(C=N)]. ^{1}H NMR: 9.90 br, 1 H and 9.82 br, 1 H (NH, OH); 7.72 s, 1 H (H-6); 3.105 s, 3 H (CH_3). ^{13}C NMR: 151.58 br, 2 C (C-2 and C-4); 149.24 br (C-6); 32.30 (CH_3). MS: 143 (6) $[M + H]^+$. For $C_4H_6N_4O_2$ (142.1) calculated: 33.81% C, 4.26% H, 39.42% N; found: 33.79% C, 4.20% H, 39.69% N.

4-Hydroxylamino-1-β-D-ribofuranosyl-1,3,5-triazin-2(1*H*)-one (**3**)

A solution of methoxytriazinone[7] **4** (0.259 g, 1 mmol) in 1 M hydroxylamine in methanol (3 ml) was kept at room temperature overnight. The deposited crystals were filtered off with suction to yield 0.04 g (14%) of the decomposition product **19**, m.p. 215–219 °C (dec.). The mother liquor was evaporated and the residue applied onto a column of silica gel (10 g), which was prepared in chloroform. The column was eluted with 50 ml portions of chloroform containing 10, 20, 30 and 40% of methanol. The fractions containing major portions of the product were collected, evaporated and the residue dissolved in a 2:8 mixture of methanol–isopropyl alcohol (3 ml). The deposited amorphous hygroscopic solid was filtered off with suction to afford 0.10 g (36%) of the monohydrate of **3**, m.p. 110–120 °C (dec.), R_F 0.37 (B, D_1). UV, λ_{max} (log ε), MeOH: 280 (3.34), 215 (3.96); pH 2.30: 268 (3.34), 225 (3.89); pH 7.03: 250 (infl.) (3.81), 217 (4.00) (partly decomposes). For $C_8H_{12}N_4O_6 \cdot H_2O$ (278.2) calculated: 34.54% C, 5.07% H, 20.14% N; found: 34.84% C, 4.78% H, 19.89% N. The product was very hygroscopic and decomposed rapidly on standing at room temperature.

1-Cyano-1-hydroxy-5-β-D-ribofuranosylisobiuret (**19**)

A solution of methoxytriazinone[7] **4** (0.259 g, 1 mmol) in 1 M hydroxylamine in methanol (12 ml) was kept at room temperature overnight to deposit 0.170 g (61.5%) of compound **19**, m.p. 213–218 °C (dec.), R_F 0.34 (B, D_1), $[\alpha]_D$ –31.4 (*c* 0.15, water). UV, λ_{max} (log ε), MeOH: 220 (4.22); pH 2.3: 226 (4.02); pH 7.0: 221 (4.28); pH 11.0: 221 (4.30). IR (KBr): 3348 s, 3315 s, 3235 s [ν(OH,NH)]; 2195 s, ≈2170 m, sh [ν(C≡N)]; 1673 vs (amide I); 1632 vs [ν(C=N)]; 1547 vs (trans-amide II); 1479 s [β(NOH)]; 1336 s, 1322 s [amide III, ν(C–N)]; 1089 vs, 1040 s [ν(C–O)]; 920 s [ν(N–O)]; 774 m, 766 m [γ(NOH)]; 592 m, br (amide VI). IR (DMSO): ≈3490 vs, sh, 3435 vs, ≈3285 s, sh [ν(NH,OH)]; 2166 s [ν(C≡N)]; 1690 vs (amide I); 1627 vs [ν(C=N)]; 1538 vs (trans-amide II); 1323 s [amide III, ν(C≡N)]; 900 w [ν(N–O)]; 772 m [γ(NOH)]. ^{1}H NMR: 9.27 d, 1 H, *J*(NH,1′) = 9.5 (5-NH); 8.33 s, 1 H (3-NH); 5.08 br s, 1 H, 4.93 br s, 1 H and 4.76 br t, 1 H, *J* ≈ 5.0 (3 × ribosyl OH); 5.17 dd, 1 H, *J*(1′,2′) = 5.6, *J*(1′,NH) = 9.5 (H-1′); 3.65 t, 1 H, *J*(2′,1′) = *J*(2′,3′) = 5.5 (H-2′); 3.79 dd, 1 H, *J*(3′,4′) = 4.0, *J*(3′,2′) = 5.4 (H-3′); 3.62 br q, *J* = 4.0 (2 ×), *J* = 5.0 (H-4′); 3.40 m, 2 H (2 × H-5′); + AcOD:

3.42 dd, 1 H, *J*(5′a,4′) = 4.1, *J*(gem) = 11.6 (H-5′a); 3.36 dd, 1 H, *J*(5′b,4′) = 4.9, *J*(gem) = 11.6 (H-5′b). The resonance signal of the N-OH proton, which appeared as a very broad singlet, was not evaluated. ^{13}C NMR: 163.08 (C=N), 155.33 (C=O), 121.245 (C≡N), 83.955 (C-1′), 74.48 (C-2′), 70.60 (C-3′), 83.56 (C-4′), 62.36 (C-5′). MS: 279 (18) $[M + 3 H]^+$, 277 (3) $[M + H]^+$, 147 (10) $[M + 3 H]^+$ – aglycone of the dihydro derivative, 145 (6) $[M + H]^+$ – aglycone. For $C_8H_{12}N_4O_6$ (276.2) calculated: 34.79% C, 4.38% H, 20.28% N; found: 34.50% C, 4.09% H, 19.99% N.

Triuret (**21**)

A mixture of cyano(formyl)guanidine[12] **10** (0.112 g, 1 mmol), hydroxylamine hydrochloride (0.070 g, 1 mmol) and water (2 ml) was refluxed for 30 min. The mixture was cooled and the deposited crystals filtered off with suction to yield 0.050 g (34%) of triuret (**21**), m.p. > 350 °C (dec.), R_F 0.76 (B, D_2). UV, λ_{max} (log ε), MeOH: 208 (4.01); pH 2.36: 220 (2.98); pH 6.86: 206 (4.12); pH 10.96: 227 (4.22). ^{1}H NMR: 9.67 br s, 2 H (2 × NH); 7.23 br s, 2 H and 6.95 br s, 2 H (2 × NH_2). ^{13}C NMR: 154.23 (2 × C), 153.01 (3 × C=O). MS: 147 (2) $[M + H]^+$. For $C_3H_6N_4O_3$ (146.1) calculated: 24.66% C, 4.14% H, 38.35% N; found: 24.84% C, 4.12% H, 38.61% N.

The authors are indebted to Ms I. Křížková for measurement of UV spectra, Ms Z. Ledvinová for determination of optical rotations, to Ms Y. Černá for technical assistance, to Dr A. Čihák for estimation of antibacterial effects, to the staff of the Analytical Laboratory (Dr V. Pechanec, Head) for elemental analyses and the staff of the Central Laboratory of Mass Spectrometry (Dr K. Ubik, Head) for mass spectral data.

REFERENCES

1. Pískala A., Šorm F.: *Collect. Czech. Chem. Commun.* **1964**, *29*, 2060.
2. Šorm F., Pískala A., Čihák A., Veselý J.: *Experientia* **1964**, *20*, 202.
3. Hanka L. J., Evans J. S., Mason D. J., Dietz A.: *Antimicrob. Agents Chemother.* **1966**, 619.
4. Bergy M. E., Herr R. R.: *Antimicrob. Agents Chemother.* **1966**, 625.
5. Hanna N. B., Masojídková M., Pískala A.: *Collect. Czech. Chem. Commun.* **1998**, *63*, 713.
6. Pískala A., Hanna N. B., Masojídková M., Otmar M., Fiedler P., Ubik K.: *Collect. Czech. Chem. Commun.* **2003**, *68*, 711.
7. Šorm F., Pískala A.: *Ger. Offen.* 1 922 702; *Chem. Abstr.* **1970**, *72*, 79427.
8. Kobe J., Stanovnik B., Tišler M.: *Tetrahedron* **1970**, *26*, 3357.
9. Allen C. F. H., Bell A.: *Org. Synth.* **1946**, *26*, 11.
10. Levin Ya. A., Kukhtin V. A.: *Zh. Obshch. Khim.* **1963**, *33*, 2673.
11. Taylor E. C., Hendess R. W.: *J. Am. Chem. Soc.* **1965**, *87*, 1980.
12. Piťhová P., Pískala A., Piťha J., Šorm F.: *Collect. Czech. Chem. Commun.* **1965**, *30*, 2801.
13. Hirata T., Wood H. B., Driscoll J. S.: *J. Chem. Soc., Perkin Trans. 1* **1973**, 1209.
14. Ukita T., Hamada A., Yoshida M.: *Chem. Pharm. Bull.* **1964**, *12*, 454.
15. Pískala A., Gut J.: *Collect. Czech. Chem. Commun.* **1963**, *28*, 1681.
16. Perrin D. D.: *Aust. J. Chem.* **1963**, *16*, 572.
17. Čihák A., Šorm F.: *Collect. Czech. Chem. Commun.* **1965**, *30*, 2091.
18. Recondo E. F., Rinderknecht H.: *Helv. Chim. Acta* **1959**, *42*, 1171.
19. Pískala A., Hanna N. B., Zajíček J., Čihák A.: *Collect. Czech. Chem. Commun.* **1989**, *54*, 2502.