SYNTHESIS OF N^4-ALKYL-5-AZACYTIDINES AND THEIR BASE-PAIRING WITH CARBAMOYLGUANIDINES – A CONTRIBUTION TO EXPLANATION OF THE MUTAGENICITY OF 2′-DEOXY-5-AZACYTIDINE

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A series of N^4-alkyl-5-azacytidines **3a**–**3h** were prepared by treatment of the 4-methoxy analogue **4** with the respective amines. In the case of propyl-, butyl-, *sec*-butyl-, benzyl- or furfurylamine, aggregates of azacytidines **3a**–**3e** with their hydrolytic products **5a**–**5e** were isolated. Similar aggregates were obtained with 1-methyl-5-azacytosine (**6**) and 2-(methylcarbamoyl)guanidine (**7**). Compound **7** was prepared by the reaction of guanidine with methyl isocyanate; the reaction of 2 or 3 equivalents gave the di- or tricarbamoyl derivatives **11** and **12**, respectively. Cyclization of **7** and **11** with DMF dimethyl acetal afforded azacytosines **6** and **13**, respectively. Aggregates of guanosine with 5-azacytosine nucleosides **1**, **2** and **15** or of 5-aza-5,6-dihydrocytosine nucleosides **16** and **17** with 5-azacytidine (**1**) and its 2′-deoxy congener **2** have been prepared by co-crystallization of the respective pairs of nucleosides. The anomers of (deoxyribosylcarbamoyl)guanidine **20a** and **20b** have been prepared by hydrolysis of the deoxy nucleoside **2**. An aggregate of the picrate (**8a**) of (ribosylcarbamoyl)guanidine **8** with cytidine (**9**) has been obtained by co-crystallization of both components. Reaction of the methoxy nucleoside **4** with *tert*-butylamine gave, by contrast to the above mentioned amines, the α-anomer of *O*-methylribosylisobiuret **22**, which was cyclized by DMF dimethyl acetal to the α-anomer of N^4,N^4-dimethyl-5-azacytidine **24**. The connection of the base-pairing ability of carbamoylguanidines with the mutagenicity of 2′-deoxy-5-azacytidine (**2**) as well as the mechanism of inhibition of DNA methyltransferase by this nucleoside analogue is discussed. In contrast to the unsubstituted 5-azacytidine (**1**) or its N^4-methyl derivatives, none of the N^4-alkyl derivatives exhibited any antibacterial or antitumor activity at 100 μg/ml or 10 μmol/l concentrations, respectively.

Keywords: 1,3,5-Triazines; 5-Azapyrimidines; Nucleosides; 5-Aza-2′-deoxycytidine; Cytostatic activity; Decitabine; Antitumor drugs; Mutagenesis; Self assembly; Nucleobase pairs.

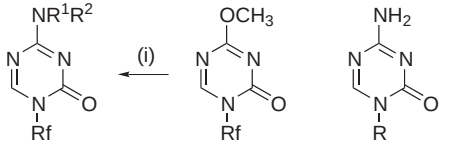
The nucleoside antibiotic 5-azacytidine[1–5] (**1**, azacitidine) and 2′-deoxy-5-azacytidine[6–8] (**2**, decitabine) are active drugs for the therapy of acute leukemia[9–13]. 2′-Deoxy-5-azacytidine (**2**) has also shown clinical activity

against metastatic lung cancer[14] and hormone-independent prostate cancer[15]. 5-Azacytidine (**1**) is phosphorylated and incorporated[16] into RNA and, after deoxygenation, also into DNA. 5-Aza-2′-deoxycytidine (**2**) is incorporated[16] into DNA. 5-Azacytosine-containing DNA [17–20] is a potent inhibitor of DNA methyltransferase due to a covalent interaction[21,22] of the thiol (SH) group in the active site of the enzyme with the labile 5-azacytosine ring[23,24]. Hypermethylation of DNA is a characteristic phenomenon of tumor cells, which allows inhibition of tumor suppressor genes, and hence tumor progression. Association of tumor progression with hypermethylation has been documented in several solid tumors as well as in acute leukemia and chronic myelogenous leukemia[18–20]. It has been shown that **1** and particularly **2**, which is the most potent hypomethylating agent so far available, induce cellular differentiation[25] and enhanced expression of genes involved in tumor suppression, immunogenicity, and programmed cell death[14,15].

In connection with structure–activity relationship investigations in nucleosides related to 5-azacytidine (**1**), we prepared a series of N^4-alkyl-5-azacytidines. Recently, we described N^4-methyl-5-azacytidines possessing an antibacterial activity[26]. In this paper, we are presenting the preparation of other N^4-alkyl-5-azacytidines and their ability to form aggregates with corresponding carbamoylguanidines obtained by their subsequent hydrolysis. Some of these results were formerly included in conference proceedings[27].

RESULTS AND DISCUSSION

N^4-Alkyl-5-azacytidines **3a**–**3h** were prepared by treatment of the 4-methoxy analogue[28] **4** with the respective amines in methanol (Scheme 1). In the case of propyl-, butyl-, *sec*-butyl-, benzyl- or furfurylamine, aggregates of compounds **3a**–**3e** with their hydrolytic products **5a**–**5e** were isolated. The complexes are crystalline with sharp melting points. Their elemental analyses indicate the 1:1 ratio of the components. We suppose a possible analogy with the reversed Watson–Crick base pair guanine–cytosine (G:C) with the expectation that the arrangement of the components in the aggregates could be very close to the binding motif of type **I**. Nevertheless, due to the flexibility and potential existence of several tautomeric forms of carbamoylguanidines **5a**–**5e**, there are more ways how to arrange H-bonds in the aggregates. In aggregates **I**, we assume the ability of carbamoylguanidines to exist not only in the more stable diamino form A, which could comprise an intramolecular hydrogen bond between the free amino group and the carbonyl group, but also in the less stable amino-imino form B,

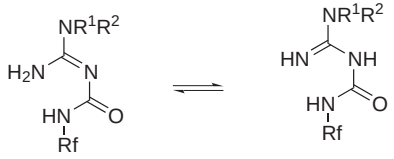


3a-3h **4**

1, R = Rf
2, R = dRf
15, R = Araf
19, R = Bn_3Araf

a, R^1 = Pr, R^2 = H
b, R^1 = Bu, R^2 = H
c, R^1 = i-Bu, R^2 = H
d, R^1 = Bn, R^2 = H
e, R^1 = furfuryl, R^2 = H
f, R^1 = i-Pr, R^2 = H
g, R^1 = R^2 = Bu
h, R^1 = 2-(imidazol-4-yl)ethyl, R^2 = H

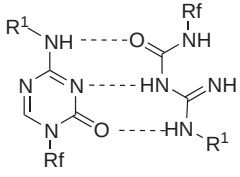
(ii)



A-form B-form

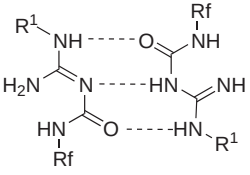
5a-5h

(i) R^1R^2NH, MeOH; (ii) H_2O.



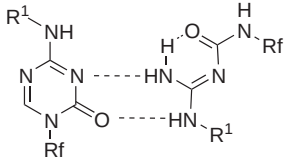
3a-3e **5a-5e** (B-form)

I



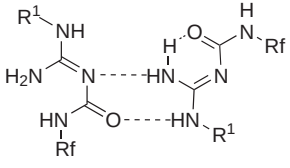
5a-5e (A-form) **5a-5e** (B-form)

V



3a-3e **5a-5e** (A-form)

Ia



5a-5e (A-form) **5a-5e** (A-form)

Va

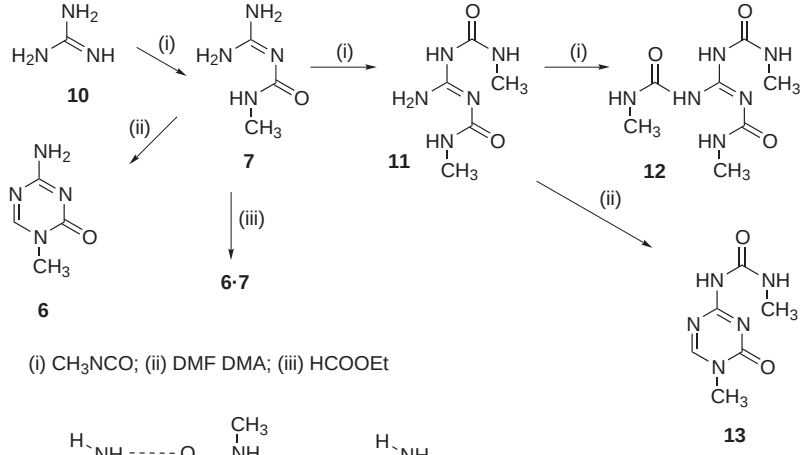
SCHEME 1

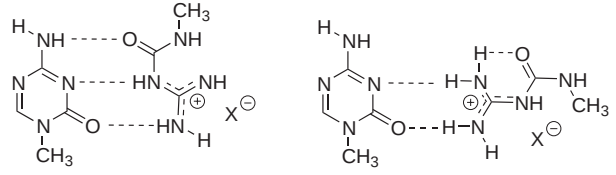
which could associate with 5-azacytosines through a tritopic H-bond interaction, since considerable stabilization is expected through the formation of an aggregate involving three hydrogen bonds. However, due to the proposed intramolecular H-bond between one amino group and the carbonyl group of carbamoylguanidines, we cannot exclude also a ditopic H-bond interaction of type **Ia**. While this base-pair geometry has two primary attractive hydrogen bonds and two attractive secondary interactions, the binding motif **I** has three primary attractive hydrogen bonds, two repulsive and two attractive secondary interactions.

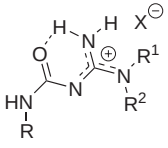
On the contrary, no associates were isolated when isopropylamine, dibutylamine or histamine was used for the aminolysis of **4**. Although, the respective non-associated products **3f**–**3h** were obtained in high yields, the degradation products **5f**–**5h** were also detected in minor amounts in the reaction mixture. Moreover, in the case of the dibutyl derivative **3g**, the formation of an aggregate of type **I** or **Ia** is precluded due to the absence of hydrogen atom on one of the amino groups. The ratio of 5-azacytidines and carbamoylguanidines depends on reaction conditions, especially on the concentration of the amine, the reaction time and also on the relative stability to hydrolysis and/or aminolysis, and on the solubility of the respective 5-azacytidine. By column chromatography on silica gel or Amberlite IRC [H^+], the aggregates are destroyed and the individual components **3a**–**3h** and **5a**–**5h** can be isolated. Both components of the aggregates are also well separable by TLC on silica gel. The carbamoylguanidines **5a**–**5h**, which are not anomerically stable, were obtained after chromatography as a mixture of α- and β-anomers of ribofuranosyl and ribopyranosyl derivatives. In this way, the individual components of the aggregates of **3b** and **3d** with the respective carbamoylguanidines **5b** and **5d** have been separated. Compounds **3b** and **3d** were isolated as individual crystalline compounds. However, the carbamoylguanidine component **5b**, which was after isolation characterized as a crystalline picrate, contains the α-furanosyl as well as α- and β-pyranosyl isomers (β-fur:α-fur:β-pyr:α-pyr = 5:2:1:1.5). The aggregates of 5-azacytidines with carbamoylguanidines are also formed by co-crystallization of 1:1 mixtures of the individual components from methanol or mixtures of methanol and acetonitrile. By this procedure, aggregates of **3b** and **3d** with the respective carbamoylguanidines **5b** and **5d** were prepared. Compounds **5b** and **5d** were obtained by hydrolysis of the respective 5-azacytidines **3b** and **3d** with 1 M ammonia solution in water.

In an attempt to prepare 1-methyl-5-azacytosine (**6**) by condensation of 2-(methylcarbamoyl)guanidine (**7**) with ethyl formate in methanol (Scheme 2), a sharp melting 1:2 aggregate of compound **6** with the starting

compound **7** was obtained. It is of interest to note that 1:1 and 2:1 aggregates of **6** with **7**, prepared on evaporation of water solutions of the respective mixtures of **6** and **7**, exhibited almost the same sharp melting points as the 1:2 aggregate. We suggest for the 1:1 aggregate of **6** with **7** a similar arrangement of hydrogen bonds as shown in binding motifs **I** or **Ia** of the ribosyl derivatives. The structures of the 1:2 or 2:1 associates of **6** with **7** have not been studied. The formation of aggregates between protonated carbamoylguanidines and 5-azacytosines was observed in case of the sharp-melting 1:1 aggregate of 1-methyl-5-azacytosine (**6**) with 2-(methylcarba-



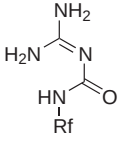




7b, R = R^1= R^2 = H, X = Cl

7c, R = R^1 = R^2 = CH_3, X = Cl

8a, R = Rf, R^1 = R^2 = H, X = picrate



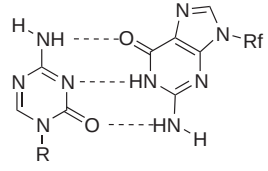
8

SCHEME 2

moyl)guanidinium picrate (**7a**), which was obtained by co-crystallization of both components in methanol. We assume in this case the existence of binding motifs **II** or **IIa** in the crystal lattice. The preparation of an analogous complex of 5-azacytidine (**1**) with picrate **8a** was not successful due to the extremely low solubility of **1** in methanol. However, a similar co-crystallization of cytidine (**9**) with picrate **8a** from ethyl acetate gave the sharp-melting aggregate.

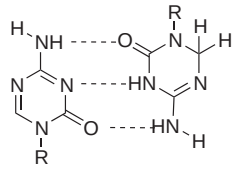
Compound **7** was obtained on reaction of guanidine (**10**) with 1 equivalent of methyl isocyanate in acetone. When 2 or 3 equivalents of methyl isocyanate were used, the respective di- or trisubstituted compounds **11** and **12** were obtained. Condensation[29] of **7** with DMF dimethyl acetal gave non-associated 1-methyl-5-azacytosine (**6**) and the same reaction of the disubstituted derivative **11** afforded 1-methyl-4-(3-methylureido)-1,3,5-triazin-2(1*H*)-one (**13**).

In order to extend our knowledge about the complementary base-pairing of 5-azacytosine nucleosides, we prepared aggregates by crystallization of 1:1 mixtures of guanosine with 5-azacytosine nucleosides **1**, **2** and 1-β-D-arabinofuranosyl-5-azacytosine[30] (**15**), which could comprise the H-bond motifs **III** (Scheme 3), analogous to the normal Watson–Crick base pair



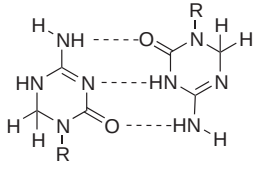
1, R = Rf **14**
2, R = dRf
15, R = β-D-arabinofuranosyl

III



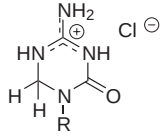
1, R = Rf **16**, R = Rf (B-form)
2, R = dRf **17**, R = dRf (B-form)

IV



16, R = Rf (A-form) **16**, (B-form)
17, R =dRf (A-form) **17**, (B-form)
18, R = 2,3,5-tri-*O*--benzyl-β-D-arabino-furanosyl (A-form) **18**, (B-form)

VI

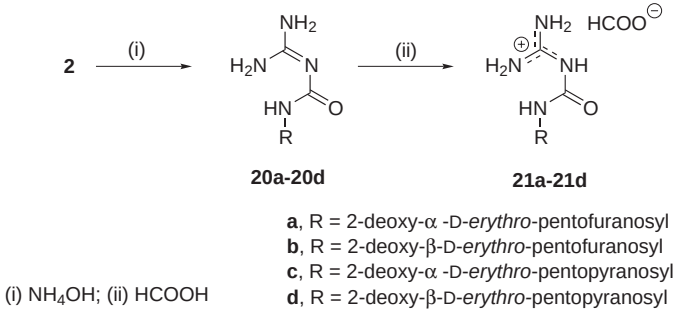


18a, R = 2,3,5-tri-*O*-benzyl--β-D-arabinofuranosyl

SCHEME 3

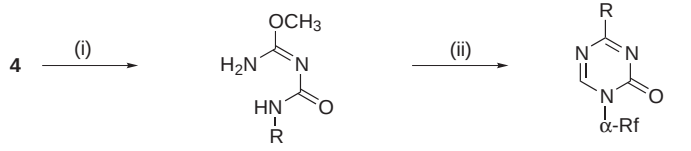
C:G. Also aggregates of **1** and **2** with the respective dihydro derivatives[31] **16** and **17**, which could involve a tritopic H-bond motif **IV** with the tautomeric B-form of the dihydro derivatives, have been prepared. For a spectral study of the base-pairing ability of 5-aza-5,6-dihydrocytosine nucleosides, the tribenzyl derivative **18**, which was isolated as its hydrochloride **18a**, was prepared by reduction of the known 1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-5-azacytosine[30] (**19**).

Although the kinetics of hydrolysis of 2′-deoxy-5-azacytidine (**2**) to 2-[(2-deoxy-β-D-*erythro*-pentofuranosyl)carbamoyl]guanidine (**20b**) was studied by analytical methods[24] and the hydrolytic product **20b** was considered in many papers, its preparation has not been described as yet. We prepared a mixture of anomers of carbamoylguanidines **20a** and **20b** in analogy to the ribosyl[23] derivative by hydrolysis of the deoxy nucleoside **2** with 1 M ammonia in water (Scheme 4). The basic hydrolytic products **20a** and **20b** were isolated in high yields as carbamoylguanidinium formates **21a** and **21b** (contaminated with about 10% of the pyranosyl isomers **21c** and **21d**). A mixture of free bases **20a**–**20d** was liberated from their salts using a basic ion exchange resin (Dowex 2X8) and anomeric carbamoylguanidines **20a** and **20b** (α:β = 1:1) were obtained on crystallization from methanol. Isolation of any isomer of **20** in pure state was impossible due to spontaneous anomerization and ring rearragement during the procedure. An attempt to prepare an aggregate of 2′-deoxy-5-azacytidine (**2**) with a mixture of anomers of carbamoylguanidines **20a** and **20b** (α:β = 1:1) by co-crystallization of both components from methanol was not successful due to a very low solubility of **2** in this solvent. However, FAB-MS (*vide infra*) of a mixture of **2** with **20a** and **20b**, indicates the ability of these components to form a 1:1 aggregate.



SCHEME 4

When *tert*-butylamine was used for aminolysis of the methoxy nucleoside **4**, the displacement proceeded much more slowly because of steric hindrance; even in boiling methanol, cleavage of the triazine ring followed by subsequent anomerization occurred mainly under the formation of 1-α-D-ribofuranosyl-4-*O*-methylisobiuret (**22**) (Scheme 5). The α-D configuration of **22** was inferred from the opposite sign of the Cotton effect in CD spectra in comparison with the known β-D-ribofuranosylisobiuret[1] **23**, which was used as an intermediate for the first synthesis of 5-azacytidine (**1**); an improved preparation of **23** is described in the Experimental. The monohydrate of compound **23** crystallizes well, which facilitates its isolation even from a complex reaction mixture. However, it is not convenient, in contrast to its syrupy anhydrous form, as an intermediate for the synthesis of 5-azacytidine because of the occurrence of hydrolytic side reactions in the condensation step with orthoformate[1].



22, R = α–Rf
23, R = 2,3,5-tri-*O*-acetyl-β-Rf

24, R = NMe_2
25, R = OMe

(i) *t*-$BuNH_2$; (ii) DMF dimethyl acetal

SCHEME 5

The anomeric configuration of isobiuret **22** was also confirmed by its condensation with DMF dimethyl acetal in methanol to 4-(dimethylamino)-1-α-D-ribofuranosyl-1,3,5-triazin-2(1*H*)-one (**24**). The α-D configuration of compounds **22** and **24** follows also from comparison of ^{1}H NMR spectra of these compounds and the β-D anomer **23**. The formation of **24** can be explained by subsequent reaction of the primarily formed 4-methoxy-1-α-D-ribofuranosyl-1,3,5-triazin-2(1*H*)-one (**25**) with dimethylamine released during condensation of ribosylisobiuret **22** with DMF dimethyl acetal.

Mass Spectrometry

The first evidence on the nature of the complexes of carbamoylguanidines with 5-azacytosines came from fast-atom-bombardment mass spectrometric (FAB-MS) experiments, whose outstanding suitability for detection of non-

covalent H-bond-mediated complexes was described in the literature[32–38]. The composition of these associates was proved by MS in the cases of the non-hygroscopic aggregates of 5-azacytidines **3d** and **3e** with the respective carbamoylguanidines **5d** and **5e**. In the spectrum, both types of molecular peaks – of the individual components and of their associates – were present. Even the peaks of the dimers of individual components **3d** and **3e** or **5d** and **5e** as well as the peaks attributable to the associates of benzyl- or furfurylguanidine (formed by fragmentation of carbamoylguanidines **5d** and **5e**) with the respective 5-azacytidines **3d** and **3e**, respectively, and also associates of benzyl- or furfurylguanidine with the respective carbamoylguanidines **5d** and **5e** were detected. The formation of non-covalent dimers of carbamoylguanidines could be explained by association of their tautomeric A- and B-forms through a tritopic H-bond motif **V** (Scheme 1). A similar situation was formerly observed in the case of 2-aminopyrimidin-4-one (isocytosine)[39,40], cyclic benzylidene derivatives of carbamoylguanidine (6-benzyl-5,6-dihydro-5-azacytosine)[41] and some other potentially tautomeric compounds (for recent reviews on H-bonding in non-covalent synthesis, see refs[42,43]). However, also in these cases, the self-association of carbamoylguanidines **5d** and **5e** by complementary hydrogen bonds as shown in the motif **Va** (Scheme 1) or by an analogous donor-donor–acceptor-acceptor (DD–AA) dimerization of carbamoylguanidines in the A-form without an intramolecular hydrogen bond could be considered. In the cases of dimers of 5-azacytidines and of their associates with guanidines, we assume the involvement of protonated species of these compounds, which could serve as proton donors and form complementary associates with the respective neutral molecules of 5-azacytidines or carbamoylguanidines as acceptors. Due to the isolation of unprotonated aggregates, we assume that the associates of 5-azacytidines with carbamoylguanidines or the dimers of carbamoylguanidines are also formed between the neutral molecules.

FAB-MS of the 1:2 aggregate of **6** with **7** has shown, besides peaks of the individual components, also peaks attributable to their 1:1, 1:2 and 2:1 associates. Moreover, peaks of dimers of **6** and **7** and the trimer of **7** as well as peaks of guanidine and methyl isocyanate formed by fragmentation of **7** and also peaks of the aggregates of guanidine with **6** and **7** were observed. The intensities of the peaks of the aggregates are dependent on concentration of the sample in the matrix. When a forty-times-diluted sample of the 1:1 aggregate of **6** with **7** was applied, no associates were detected at all. This result strongly indicates that the formation of these aggregates is based on hydrogen bonding and not on covalent interactions, which could be

also taken into consideration. For the 1:1 aggregate of **6** with **7** we assume assembly modes analogous to **I** or **Ia** and for the dimer of **7** assembly modes analogous to **V** or **Va**. The structure of the heterotrimers of **6** with **7** or the homotrimer of **7** was not clarified. Because the peaks of these aggregates were much smaller than the peaks of the dimeric structures, we assume that the third component of the hetero- or homotrimers is bound to the dimers by a weaker H-bond interaction. In the case of the dimer of **6** and the aggregates with guanidine, we assume the involvement of protonated species as in the case of the above mentioned ribosyl derivatives. In FAB-MS of the (methylcarbamoyl)guanidinium picrate (**7a**), only a negligible peak of a dimer of **7** was observed. However, a 1:1 mixture of 1-methyl-5-azacytosine (**6**) and picrate **7a** or the aggregate of both components exhibited, in addition to the molecular peaks of the individual components, also peaks of the associate of **6** with **7** and the dimer of **6**. Analogous results were obtained in the case of the aggregate of cytidine (**9**) with picrate **8a**. These facts confirm that non-covalent dimeric structures could be formed also by aggregation of protonated with unprotonated molecules. We suggest for the aggregate **6·7a** the binding modes **II** or **IIa**. An analogous base-pairing could be formulated also for the aggregate **9·8a**. The methyl derivative **6** is at lower pH protonated at position 3 of the triazine ring, similarly to cytosine, and could be able to form complementary H-bonds with the neutral molecule of **6**. In this connection, it is of interest to note that protonated cytosine is known to be involved in the Hoogsteen mode of base pairing[44] or in some C^+:C mismatch base pairs involving protonated cytosine[45]. FAB-MS of compounds **11**–**13** indicated also the presence of dimeric structures. Despite many possibilities of the location of hydrogen bonds, we assume a similar arrangement to that in 2-ureidopyrimidin-4(1*H*)-one derivatives[46].

In the cases of aggregates **III** or **IV**, in addition to the molecular peaks of the individual components, also the peaks of their associates were observed by FAB-MS. Additionally, the peaks corresponding to the dimers of dihydro derivatives **16** or **17** and dimers of **1**, **2** or **15** were observed. Similarly, dihydro derivative **18** exhibited, in addition to the molecular peak, also a peak of the dimer. For the dimers of dihydro derivatives **16**–**18** we assume the existence of a tritopic H-bond interaction between the tautomeric forms A and B as shown in the binding motif **VI**. As mentioned above, aggregates analogous to **VI** were considered to exist in the crystalline state of some 6-benzyl-5,6-dihydro-5-azacytosines[41].

Although the preparation of an aggregate of 2′-deoxy-5-azacytidine (**2**) with a mixture of **20a** and **20b** was not successful, FAB-MS of a 1:1 mixture

of **2** with **20a** and **20b** has shown the associate of **2** with **20a** or **20b**, the dimers of **20a** or **20b** and the dimer of **2**.

^{1}H NMR Spectroscopy

Insolubility of 5-azacytidines and their noncovalent heterodimers in $CDCl_3$ and the competitive hydrogen bonding with the solvent oxygen in DMSO-d_6 are responsible for almost no evidence of the nature of these aggregates from ^{1}H NMR spectra. As the only indication of hydrogen bonding in these aggregates can be considered a downfield shifted signal of one NH proton in the picrates of **5a**, **7a** and **8a**, which can be assigned to intramolecular hydrogen bonds persisting even in this strong hydrogen bond acceptor solvent. Similar results were obtained with unsubstituted carbamoylguanidinium chloride (**7b**) and 1,1-dimethyl-2-(methylcarbamoyl)guanidinium chloride (**7c**), which were prepared earlier in connection with another project[29]. Also the downfield shifts of NH protons in compound **11** and **12** indicate the existence of intramolecular hydrogen bonds.

^{13}C NMR Spectroscopy

For the aforementioned reason, also ^{13}C NMR spectra, measured in DMSO-d_6, have not shown any hydrogen bonding in the aggregates of **6** with **7** or **7a**. However, the broad signals of two carbonyl carbons in the spectra of compounds **11** and **12** could be considered as an evidence for the existence of two intramolecular hydrogen bonds.

IR Spectroscopy

IR spectra of the aggregates of **3d** and **3e** with **5d** and **5e**, respectively, in Nujol suspensions or KBr pellets showed the existence of strong hydrogen bonds in their crystal lattice. In this connection it should be mentioned that a specific hydrogen bonding between 1,6-dimethyl-5-azacytosine and 2′,3′,5′-tri-*O*-acetylguanosine was described in the literature[47]. The authors assumed this complex would have a similar structure as the G:C base pair in DNA. We suggest the existence of H-bond-mediated aggregates of type **I** or **Ia** in the solid state which could moreover associate by hydrogen bonds into extended supramolecular arrays. The mode of association of type **I** is obviously very stable and has been shown to exist in crystalline complexes modeled on the G:C base pair[48].

However, IR spectra of the 1:2 aggregate of **6** with **7** in KBr pellets indicate the existence of more than three different H-bonds. We assume a similar arrangement of the molecules in the solid state to that in the aforementioned aggregates of the ribosyl derivatives **3** with **5**. IR spectra of **7** in KBr pellets indicate the presence of the diamino form but not the coexistence of the amino with the imino form, and a simpler arrangement of the molecules in the crystal lattice in comparison with its aggregate with **6**. IR spectra of compounds **11**–**13** in KBr pellets exhibited also the presence of intermolecular hydrogen bonds; however, there are no indications to assume their arrangement.

IR spectra of the dihydro derivative **18** in chloroform exhibited $\nu_{as}(NH_2)$ bands at 3484 and 3392 cm^{-1}, indicating the presence of 1:1 aggregates (one hydrogen atom of an amino group involved in a hydrogen bond, presumably H–N–H···O=C), as well as a band at 3324 cm^{-1}, attributable to higher associates (a fully bound amino group). The ν(C=O) band at 1724 cm^{-1} suggests the presence of a partial positive charge on the unsaturated nitrogen atom in position 3 of the 5,6-dihydro-5-azacytosine ring, which could be expected because of the existence of an N–H···N=C hydrogen bond in the aggregate **VI**. The formation of the aggregate **VI** was moreover supported by deuteration experiments (see Experimental). Similar results were also obtained from IR spectra of **18** measured in tetrachloromethane and acetonitrile.

CD Spectroscopy

CD spectra of N^4-substituted 5-azacytidines **3b** and **3f**–**3h** exhibited intensive B_{2u} Cotton effects at 250 nm similarly to the N^4-substituted methyl derivatives of 5-azacytidine[26] and also to the unsubstituted 5-azacytidine[5]. This indicates an anti conformation around the C–N glycosyl bond of these nucleosides.

Biological Activity

N^4-Substituted 5-azacytidines **3b**, **3d** and **3f-3h** as well as the aggregates of **3a**–**3e** with **5a**–**5e** were tested for their antibacterial activity using a culture of *E. coli B* growing on a mineral medium with glucose[49]. None of these compounds exhibited any antibacterial activity at ≤100 μg/ml concentrations. Also *in vitro* inhibition of cell growth with compounds **3d**, **3f**–**3h**, the aggregates **3d·5d**, **3e·5e** and the mixture of hydrolytic products **21a**–**21d** was evaluated in the following cell cultures: human T-lymphoblastoid

CCRF-CEM cell line (ATCC CCL 119), human promyelocytic 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.

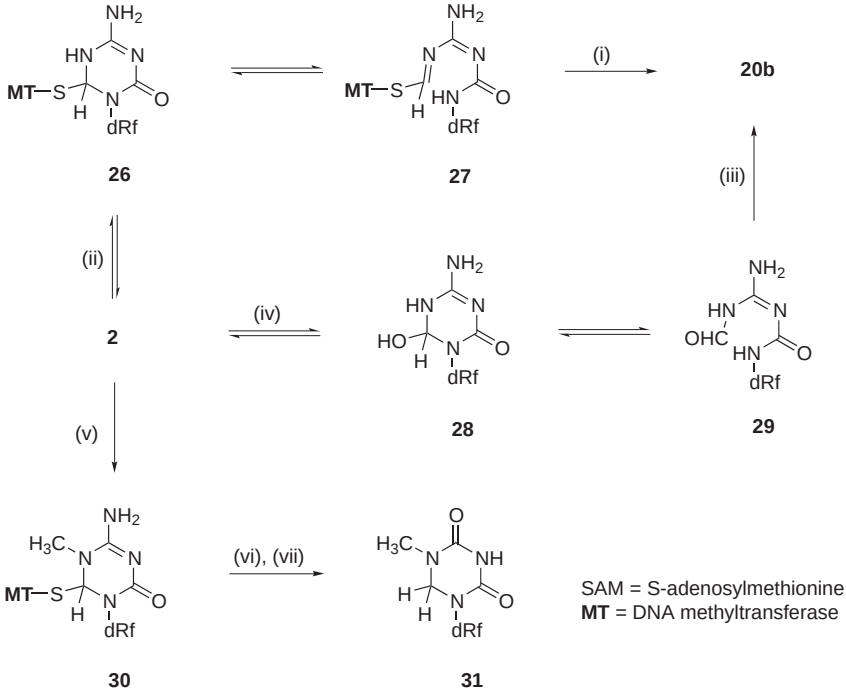
Potential Carcinogenicity

Eventually it is of interest to note that N^4-substituted 5-azacytidines **3d**, **3f** and **3g** exhibited a much lower potential carcinogenicity than unsubstituted 5-azacytidine[50,51] or 6-methyl-5-azacytidine[52], when estimated by a polarographic method[53–55].

*A Remark to the Mutagenicity of 2′-Deoxy-5-azacytidine (**2**) and Its Mechanism of Inhibition of DNA Methyltransferase*

In absence of X-ray crystallographic data, we were not able to clarify the exact location of the hydrogen bonds in the aforementioned associates; however, the formation of aggregates of type **I** or **II** suggests that, in respect of hydrogen-bonding ability, carbamoylguanidines or carbamoylguanidinium salts could mimic guanine and bind cytosine. Unprotonated carbamoylguanidine in the A-form with an intramolecular hydrogen bond could in our opinion, also form DA–AD associates similar to adenine; in the A-form without an intramolecular hydrogen bond it could mimic cytosine similarly to 5-azacytosine. We assume carbamoylguanidine could behave as a universal base with a high affinity to cytosine. The above mentioned aggregates represent in fact models for a possible pairing of carbamoylguanidine incorporated into DNA with cytosine. As we found formerly, 5-azacytosine is able to form a pair with guanine, although less stable than cytosine[56], and incorporation of 5-azacytosine nucleosides into nucleic acids is followed by rapid hydrolytic cleavage of the base, which can change genetic information[23]. Recently, it was published by Jackson-Grusby *et al.*[57] that the hydrolysis of 5-azacytosine to carbamoylguanidine in 5-azacytosine-containing DNA may really be a reason for mutagenicity of 2′-deoxy-5-azacytidine (**2**). Its hydrolysis to the carbamoylguanidine **20b** could proceed *via* the covalent adduct **26** of the SH group of methyltransferase to the highly reactive double bond in positions 5 and 6 of the triazine ring in **2** and the ring-opened intermediate **27** (Scheme 6). An enzyme-independent way *via* the covalent hydrate **28** and the formyl derivative **29** is also possible. In both cases, the 5-azacytosine ring functions as a

formylating agent affording the *S*-formyl derivative of methyltransferase in the first case and in the second case formic acid, which forms salt **21b** with the strongly basic product **20b**.

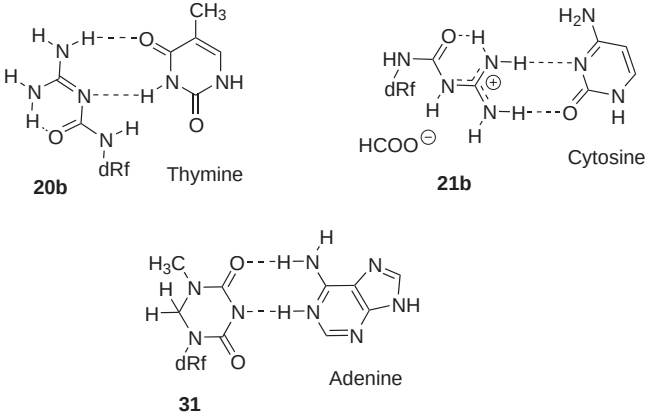


(i) H_2O, - OHCS-**MT**; (ii) HS-**MT**; (iii) H_2O, - HCOOH; (iv) H_2O; (v) $^-$S-**MT**.SAM$^+$; (vi) reduction, - HS-**MT**; (vii) deamination

SCHEME 6

Mutations described in the paper[57] consist predominantly in C:G → G:C transversions; nevertheless, C:G → T:A and C:G → A:T transitions were also observed. The C:G → G:C transversion is explained by the ability of the aglycone of carbamoylguanidine **20b** formed by the aforementioned enzyme-assisted or spontaneous hydrolysis of the 1,3,5-triazine ring of 5-azacytosine-containing DNA, to mimic guanine. The authors assume the existence of an intramolecular H-bond in **20b** analogous to the A-form of the substituted derivatives **5a**–**5h**, which would be able to bind cytosine through two amino groups, similarly to the binding motif **Ia**. This pair would be isosteric with the natural G:C pair in the minor groove. The C:G → T:A transition is explained by the formation of abasic sites on the basis of

the known A-rule and the C:G → A:T transition has not been clarified at all by this authors. In contrast to this paper, we assume the involvement of carbamoylguanidinium ion in the formation of a pair with cytosine. The C:G → A:T transition could be explained, in our opinion, by the aforementioned ability of neutral carbamoylguanidine in the tautomeric A-form with an intramolecular H-bond to mimic adenine. Also the C:G → T:A transition need not be explained only by the formation of abasic sites, as proposed in the mentioned paper[57], but also by a partial methylation at N-5 of the covalent adduct **26** with formation of the N^5-methyl derivative **30**, followed by reductive cleavage of the C–S bond and deamination of this intermediate affording 5,6-dihydro-5-azathymidine (**31**), which could mimic thymine. This idea is also supported by the fact that the known antibiotic 5,6-dihydro-5-azathymidine (**31**) was isolated from the culture filtrates of *Streptomyces platensis* var. *clarensis*[58,59] and that 5-azacytidine (**1**) is produced by *Streptoverticillium ladakanus* var. *ladakanus*[3,4]. Moreover, methylation at N-5 in presence of *S*-adenosylmethionine was more recently documented by Gabbara and Bhagwat[60]. The putative binding modes of the aglycones of carbamoylguanidine **20b**, carbamoylguanidinium formate **21b** and dihydro-5-azathymidine **31** with thymine, cytosine and adenine, respectively, are shown in Scheme 7.



SCHEME 7

The earlier studies[21,22] of the inhibition of DNA methyltransferase by 5-azacytosine-containing DNA proposed two mechanisms for covalent bond formation with methylase. The first consists in the reaction of a nucleophilic catalyst, presumably a thiol (SH) group of the enzyme, with the highly reactive carbon-6 under formation of the 5,6-dihydrotriazine **26**,

which could be slowly reversible. The second mechanism is based on the ability of 5-azacytosine nucleosides to function as formylating agents. The initially formed covalent adduct **26** could undergo a reversible prototropic rearrangement to form the ring-opened intermediate **27**, which could hydrolyze irreversibly to yield an inactive formyl derivative of the methylase and the hydrolytic product **20b**. Because it was known[61] that 5-azacytosine-containing DNA does not require *S*-adenosylmethionine to inhibit the methylase, Santi *et al.*[21,22] concluded that methylation at N-5 of the adduct **26** need not be involved. In our opinion, the initially formed adduct **26** is not stable enough to explain the inhibition of DNA methylase by 2′-deoxy-5-azacytidine (**2**). It is well known[62,63] that covalent adducts of 5-azacytosine nucleosides are detectable only in strongly acidic media, in which the 5-azacytosine ring is protonated in position 3. Benjamin[62] has shown that covalent adducts of 5-azacytosine nucleosides, in neutral media present in negligible, not detectable amounts, are extremely unstable being rapidly transformed either by elimination back to the respective 5-azacytosine nucleoside and the free nucleophile or by a prototropic rearrengement to a ring-opened product, which could be hydrolyzed to the respective formylated nucleophile and the corresponding glycosyl derivative of carbamoylguanidine. In our opinion, DNA methylase is inactivated in absence of *S*-adenosylmethionine by formylation of the catalytic thiol group of the enzyme *via* **26** and **27** as proposed earlier by Santi *et al.*[21,22] and Jackson-Grusby *et al.*[57] or in presence of *S*-adenosylmethionine also by methylation of the labile intermediate **26** at N-5 to form a tightly bound covalent adduct **30** as found by Gabbara and Bhagwat[60]. The free active DNA methylase could be released from its formyl derivative by hydrolysis with the formation of formic acid and from the relatively stable adduct **30** on the action of reductive enzymes with the formation of 2′-deoxy-5,6-dihydro-5-methyl-5-azacytidine, which could be eventually deaminated to 5,6-dihydro-5-azathymidine (**31**) as mentioned above. The formation of the stable dihydro derivative **31** was not taken into consideration in any of the previous studies on the mechanism of inhibition of DNA methylase by 2′-deoxy-5-azacytidine (**2**). Gabbara and Bhagwat[60] have speculated that the methylated covalent adduct **30** could eliminate on heating the methylase with restoration of the double bond between N-5 and C-6 to form a 5-methyltriazinium cation. However, they have not ruled out the possibility of other rearrangements of the triazine ring[60]. In our experience, the putative triazinium cation would be very unstable under neutral conditions and would react with water to a 6-hydroxy derivative, which could undergo a Dimroth-type rearrangement to give N^4-methyl-5-azacytosine. However,

there has been no evidence so far, which would substantiate the formation of a 5-methyltriazinium cation or any subsequent products.

CONCLUSION

The high biological activity of 5-azacytidine (**1**) and 2′-deoxy-5-azacytidine (**2**) is based on their structural and conformational resemblance with cytidine and 2′-deoxycytidine, which enables their incorporation into nucleic acids and subsequent covalent interaction of the reactive double bond in the 5,6 position of the 1,3,5-triazine ring with regulatory proteins. The CD spectra of N^4-substituted 5-azacytidines 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 the bulky alkyl groups prevents (predominantly because of steric hindrance) their incorporation into nucleic acids. This is probably the main reason for the low biological activity in comparison with the N^4-methyl-5-azacytidines and especially with the unsubstituted 5-azacytidine (**1**). The formation of aggregates of carbamoylguanidines or their protonated forms with 5-azacytosines or cytosine, which represent in fact models for the base-pairing ability of carbamoylguanidine incorporated into DNA, is in agreement with the observed[57] C:G → G:C transversion caused by 2′-deoxy-5-azacytidine (**2**). The C:G → T:A transition, which was also observed[57] in the mutational spectrum of **2**, could be explained by methylation at N-5 of 5-azacytosine-containing DNA and subsequent transformation to 5,6-dihydro-5-azathymine-containing DNA. This idea is supported by the microbial production[58] of 5,6-dihydro-5-azathymidine (**31**) and by a more recent investigation of Gabbara and Bhagwat[60], who have documented methylation at N-5 of 5-azacytosine-containing DNA. The formation of the stable dihydro derivative **31** has not been taken into consideration in any of the earlier studies on the mechanism of inhibition of DNA methylase by 2′-deoxy-5-azacytidine (**2**).

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block) and are not corrected. 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 the following solvent systems: A chloroform–methanol (98:2), B butan-1-ol–acetic acid–water (5:2:3), C ethyl acetate–acetone–ethanol–10 mM phosphate buffer pH 7.0 (4:1:1:1) and D acetic acid–water–methanol–propan-1-ol–chloroform (1:2:3:4:5). The spots of 5-azacytidines **3a**–**3h**

were detected visually in UV light (254 nm) (D_1,) the spots of carbamoylguanidines **5a**–**5h** which are not visible in UV light were detected with the sodium nitroprusside–potassium ferricyanide reagent (orange spots) (D_2) or with ninhydrin (D_3) and the spot of isobiuret **22** was detected by heating (D_4). Column chromatography was performed with silica gel according to Pitra (Service Laboratories of this Institute). IR spectra (ν in cm^{-1}) were recorded on a FTIR spectrometer Bruker IFS 88 in the region 3800–400 cm^{-1} in KBr pellets or in the indicated solvent. UV spectra were measured on a Unicam SP 8000 spectrophotometer (Pye–Unicam, Cambridge, England) in buffer solutions of ionic strength 0.01 prepared according to Perrin[64], 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 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 ($\delta(^1H)$ 2.50 and $\delta(^{13}C)$ 39.70 ppm, respectively). For the measurement of ^1H NMR in D_2O, DSS as internal standard was used. The chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hz. Mass spectra (*m/z* (% rel. int.)) were measured on a ZAB-EQ mass spectrometer (Micromass, Manchester, U.K.) using a FAB ionization (bombarding gas Xe at 8 kV). A mixture of glycerol and thioglycerol (1:3, v/v) was used as a matrix and samples were dissolved in methanol or water. Elemental analysis was provided on a Perkin–Elmer CHN Analyser 2400, Series II Sys (Perkin–Elmer, Norwolk (CT), U.S.A.). Stationary cultivation of *Escherichia coli B* was performed at 37 °C in a mineral medium with glucose[49]. The tested compounds were added before inoculation and the growth of bacteria was measured 16 h later. The ratios of the individual components in aggregates of type **III**, **V** and **VI** as well as the ratios of isomers in compounds **20a**–**20d** and **21a**–**21d** were estimated by ^1H NMR spectra measured in D_2O.

N^4-Substituted 5-Azacytidines **3b**, **3d**, **3f**–**3h** and Aggregates of 5-Azacytidines **3a**–**3e** with Carbamoylguanidines **5a**–**5e**. General Procedure

A mixture of methoxytriazinone[28] **4** (0.259 g, 1 mmol), methanol and the respective amine was stirred at room temperature. The solution was evaporated, the residue co-evaporated with methanol (4 × 10 ml) and crystallized from an appropriate solvent or worked up by column chromatography (*vide infra*).

N^4-Isopropyl-5-azacytidine (**3f**)

Methanol (6 ml), isopropylamine (1 ml), reaction time 1 h. Yield: 0.223 g (78%) of the nucleoside **3f**, m.p. 172–173 °C (dec.) (i-PrOH), R_F (B) 0.82 (D_1), $[\alpha]_D$ +27.5 (*c* 0.5, water). UV, λ_{max} (log ε): (MeOH), 255 (3.97), 213 (4.38); (pH 2.46), 258 (3.79); (pH 6.93) 250, inflexion (3.84), 213 (4.23); (pH 11.1), 227 (4.40). CD (pH 6.93), λ_{max} ($[\Theta]_{max}$): 250 (+9450). ^1H NMR: 8.52 s, 1 H (H-6); 7.99 d, 1 H, *J*(NH,CH) = 8.1 (NH); 5.66 d, 1 H, *J*(1′,2′) = 3.9 (H-1′); 5.41 d, 1 H, *J*(OH,2′) = 5.4 (OH); 5.10 t, 1 H, *J*(OH,5′) = 5.1 (OH); 5.01 d, 1 H, *J*(OH,3′) = 5.8 (OH); 4.08 ddd, 1 H, *J*(2′,1′) = 3.9, *J*(2′,3′) = 5.0 *J*(2′,OH) = 5.4 (H-2′); 4.00 td, 1 H, *J*(3′,2′) = 5.0, *J*(3′,4′) = *J*(3′,OH) = 5.7 (H-3′); 3.97 dsept, 1 H, *J*(CH,CH_3) = 6.6, *J*(CH,NH) = 8.1 (N-CH); 3.84 dt, 1 H, *J*(4′,5′) = 3.0, *J*(4′,3′) = 5.6 (H-4′); 3.68 ddd, 1 H, *J*(5′a,4′) = 2.9, *J*(5′a,OH) = 4.9, *J*(gem) = 12.0 (H-5′a); 3.55 ddd, 1 H, *J*(5′b,4′) = 3.2, *J*(5′b,OH) = 5.2, *J*(gem) = 12.0 (H-5′b); 1.115 d, 3 H and 1.11 d, 3 H, *J*(CH_3,CH) = 6.6 (CH_3). ^{13}C NMR: 162.86 (C-4), 155.57 (C-6), 153.41 (C-2), 89.34 (C-1′), 84.445 (C-4′), 74.01 (C-2′), 69.17 (C-3′), 60.32 (C-5′), 42.20 (N-CH), 22.00 (2 × CH_3). FAB-MS: 595 (1) [2 $M_{\mathbf{3f}}$ + Na]$^+$, 573 (4) [2 $M_{\mathbf{3f}}$ + H]$^+$,

309 (5) [$M_{\mathbf{3f}}$ + Na]$^+$, 287 (18) [$M_{\mathbf{3f}}$ + H]$^+$, 155 (100) [aglycone$_{3f}$ + H]$^+$. For $C_{11}H_{18}N_4O_5$ (286.3) calculated: 46.15% C, 6.34% H, 19.57% N; found: 46.34% C, 6.55% H, 19.30% N.

N^4-Butyl-5-azacytidine (**3b**)

Methanol (5 ml), butylamine (0.2 ml, 2 mmol), reaction time 4 h. A solution of the syrupy crude product in methanol (5 ml) was applied onto a column of Amberlite IRC-50 [H$^+$] ion exchange resin (10 ml), which was prepared in methanol. The column was eluted with methanol (100 ml) and the effluent evaporated. A solution of the residue in acetonitrile (3 ml) was kept at room temperature for 3 days to yield 0.141 g (47%) of the nucleoside **3b**, m.p. 86–88 °C, R_F (B) 0.72 (D_1), $[\alpha]_D$ +25.8 (*c* 0.1, water). UV, λ_{max} (log ε): (MeOH), 252 (3.88), 213 (4.26); (pH 2.30), 258 (3.87), 220 (3.92); (pH 6.95), 252 (3.90), 213 (4.27); (pH 10.92), 250, inflexion (3.76), 226 (4.17). CD (pH 7.00), λ_{max} ($[\Theta]_{max}$): 250 (+8970). For $C_{12}H_{20}N_4O_5$ (300.3) calculated: 47.99% C, 6.71% H, 18.66% N; found: 47.69% C, 6.65% H, 18.63% N. Nucleoside **3b** was hygroscopic and decomposed on storage to hydrolytic products.

In a simultaneous experiment, to a solution of the crude product in methanol (5 ml) silica gel (1 g) was added and the mixture evaporated to a powder, which was applied onto a column of silica gel (10 g) prepared in chloroform. The column was eluted with chloroform–methanol (100:0–85:15, v/v). Fractions containing the product were collected, evaporated and the residue crystallized from acetonitrile to give 0.150 g (50%) of nucleoside **3b**, m.p. 86–88 °C without depression on admixture with a sample prepared by the isolation procedure mentioned above.

N^4,*N*^{4}-Dibutyl-5-azacytidine (**3g**)

Methanol (2.5 ml), dibutylamine (0.34 ml, 2 mmol), reaction time 5 days. Yield: 0.182 g (51%) of nucleoside **3g**, m.p. 125–127 °C (acetonitrile), R_F (B) 0.47 (D_1), $[\alpha]_D$ +8.4 (*c* 0.2, water). UV, λ_{max} (log ε): (MeOH), 261 (3.96), 219 (4.30); (pH 2.30), 268 (3.92), 225 (4.10); (pH 7.03), 263 (3.90); (pH 11.0), 263 (3.96), 221 (4.28). CD (pH 7.00), λ_{max} ($[\Theta]_{max}$): 250 (+6990). ^{1}H NMR: 8.64 s, 1 H (H-6); 5.68 d, 1 H, *J*(1′,2′) = 3.9 (H-1′); 5.43 d, 1 H, *J*(OH,2′) = 5.1 (OH); 5.12 dd, 1 H, *J*(OH,5′) = 4.9 and 5.1 (OH); 5.03 d, 1 H, *J*(OH,3′) = 5.8 (OH); 4.10 td, 1 H, *J*(2′,1′) = 3.9, *J*(2′3′) = *J*(2′,OH) = 5.0 (H-2′); 4.02 td, 1 H, *J*(3′,2′) = 5.0, *J*(3′,4′) = *J*(3′,OH) = 5.6 (H-3′); 3.85 dt, 1 H, *J*(4′,5′) = 3.0, *J*(4′,3′) = 5.6 (H-4′); 3.69 ddd, 1 H, *J*(5′a,4′) = 2.9, *J*(5′a,OH) = 4.9, *J*(gem) = 12.2 (H-5′a); 3.56 ddd, 1 H, *J*(5′b,4′) = 3.2, *J*(5′b,OH) = 5.1, *J*(gem) = 12.2 (H-5′b); 3.52 m, 2 H and 3.43 m, 2 H (N-CH_2); 1.52 m, 4 H and 1.27 m, 4 H (C-CH_2); 0.90 t, 3 H and 0.885 t, 3 H, *J*(CH_3,CH_2) = 7.3 (CH_3). ^{13}C NMR: 162.76 (C-4), 155.79 (C-6), 153.02 (C-2), 89.37 (C-1′), 84.48 (C-4′), 74.09 (C-2′), 69.17 (C-3′), 60.30 (C-5′), 47.15 and 46.83 (N-CH_2), 30.225, 29.095, 19.84 and 19.69 (C-CH_2), 14.01 and 13.96 (CH_3). FAB-MS: 379 (2) [$M_{\mathbf{3g}}$ + Na]$^+$, 357 (15) [$M_{\mathbf{3g}}$ + H]$^+$, 225 (100) [aglycone$_{\mathbf{3g}}$ + H]$^+$. For $C_{16}H_{28}N_4O_5$ (356.4) calculated: 53.92% C, 7.92% H, 15.72% N; found: 53.73% C, 7.85% H, 16.02% N.

N^4-Benzyl-5-azacytidine (**3d**)

Methanol (2 ml), benzylamine (0.22 ml, 2 mmol), reaction time 3 h. The crude product was triturated with ether (5 ml) and crystallized from methanol (1 ml). Recrystallization from methanol gave 0.224 g (67%) of pure nucleoside **3d**, m.p. 168–170 °C (dec.), R_F (B) 0.71 (D_1), $[\alpha]_D$ +23.9 (*c* 0.1, water). UV, λ_{max} (log ε): (MeOH), 252 (3.99), 213 (4.37); (pH 2.30), 252 (3.89), 222 (4.01); (pH 7.03), 250 (3.97), 212 (4.37); (pH 10.97), 229 (4.34), 217 (4.24). ^{1}H NMR: 8.60 t, 1 H, *J*(NH, CH_2) = 6.3 (NH); 8.59 s, 1 H (H-6); 7.35–7.20 m, 5 H (arom.);

5.66 d, 1 H, *J*(1′,2′) = 3.7 (H-1′); 5.42 d, 1 H, *J*(OH,2′) = 5.4 (OH); 5.12 t, 1 H, *J*(OH,5′) = 5.1 (OH); 5.01 d, 1 H, *J*(OH,3′) = 5.8 (OH); 4.44 d, 2 H, *J*(CH_2,NH) = 6.3 (arom.-CH_2); 4.09 ddd, 1 H, *J*(2′,1′) = 3.7, *J*(2′,3′) = 5.0, *J*(2′,OH) = 5.4 (H-2′); 4.01 td, 1 H, *J*(3′,2′) = 5.0, *J*(3′,4′) = *J*(3′,OH) = 5.6 (H-3′); 3.85 dt, 1 H, *J*(4′,5′) = 3.1, *J*(4′,3′) = 5.6 (H-4′); 3.69 ddd, 1 H, *J*(5′a,4′) = 2.9, *J*(5′a,OH) = 5.1, *J*(gem) = 12.2 (H-5′a); 3.50 ddd, 1 H, *J*(5′b,4′) = 3.2, *J*(5′b,OH) = 5.1, *J*(gem) = 12.2 (H-5′b). ^{13}C NMR: 164.13 (C-4), 155.95 (C-6), 153.37 (C-2), 138.98, 128.505, 127.38 and 127.07 (C-arom), 89.46 (C-1′), 84.43 (C-4′), 74.04 (C-2′), 69.10 (C-3′), 60.25 (C-5′), 43.71 (N-CH_2). FAB-MS: 691 (<1) [2 $M_{\mathbf{3d}}$ + Na]$^+$, 669 (2) [2 $M_{\mathbf{3d}}$ + H]$^+$, 357 (2) [$M_{\mathbf{3d}}$ + Na]$^+$, 335 (42) [$M_{\mathbf{3d}}$ + H]$^+$, 203 (100) [aglycone$_{\mathbf{3d}}$ + H]$^+$. For $C_{15}H_{18}N_4O_5$ (334.3) calculated: 53.89% C, 5.43% H, 16.76% N; found: 53.82% C, 5.42% H, 16.90% N.

N^4-[2-(Imidazol-4-yl)ethyl]-5-azacytidine (**3h**)

Methanol (2 ml), histamine (4 mmol, prepared from 0.736 g of its dihydrochloride by the action of 8 ml of 1 M NaOMe, dilution with 6 ml benzene, removal of sodium chloride by filtration and evaporation of the filtrate), reaction time 2.5 h. The deposited nucleoside **3h** was filtered off with suction. A solution of the crude product (0.220 g, m.p. 175–182 °C (dec.)) in methanol was evaporated. During evaporation the less soluble and higher melting modification of the product was formed. Recrystallization from methanol gave 0.142 g (42%) of pure nucleoside **3h**, m.p. 230–232 °C (dec.), R_F (B) 0.51 (D_1), $[\alpha]_D$ +23.8 (*c* 0.1, water). UV, λ_{max} (log ε): (MeOH), 252 (3.91), 213 (4.33); (pH 2.30), 250 (3.92), 222 (4.09); (pH 7.03), 250 (3.91), 212 (4.35); (pH 10.97), 226 (4.37). CD (pH 6.86): λ_{max} ($[\Theta]_{max}$): 250 (+10 650). ^{1}H NMR: 11.80 brs, 1 H (NH); 8.54 s, 1 H (H-6); 8.14 t, 1 H, *J*(NH,CH_2) = 5.6 (NH); 7.52 s, 1 H and 6.81 brs, 1 H (CH-imidazole); 5.67 d, 1 H, *J*(1′,2′) = 3.9 (H-1′); 5.44 d, 1 H, *J*(OH,2′) = 5.1 (OH); 5.12 t, 1 H, *J*(OH,5′) = 5.0 (OH); 5.03 d, 1 H, *J*(OH,3′) = 5.6 (OH); 4.08 td, 1 H, *J*(2′,1′) = 3.9, *J*(2′,3′) = *J*(2′,OH) = 5.0 (H-2′); 4.00 td, 1 H, *J*(3′,2′) = 4.9, *J*(3′,4′) = *J*(3′,OH) = 5.6 (H-3′); 3.85 dt, 1 H, *J*(4′,5′) = 3.0, *J*(4′,3′) = 5.6 (H-4′); 3.69 ddd, 1 H, *J*(5′a,4′) = 2.9, *J*(5′a,OH) = 5.0, *J*(gem) = 12.2 (H-5′a); 3.55 ddd, 1 H, *J*(5′b,4′) = 3.2, *J*(5′b, OH) = 5.0, *J*(gem) = 12.2 (H-5′b); 3.43 m, 2 H (N-CH_2); 2.73 brt, 2 H, *J*(CH_2,CH_2) = 7.5 (CH_2-imidazole). ^{13}C NMR: 163.795 (C-4), 155.64 (C-6), 153.395 (C-2), 134.89 (CH-imidazole), 132.79 (C-imidazole), 89.39 (C-1′), 84.43 (C-4′), 74.05 (C-2′), 69.115 (C-3′), 60.27 (C-5′), 40.63 (N-CH_2), 29.74 (CH_2). FAB-MS: 699 (<1) [2 $M_{\mathbf{3h}}$ + Na]$^+$, 677 (2) [2 $M_{\mathbf{3h}}$ + H]$^+$, 361 (3) [$M_{\mathbf{3h}}$ + Na]$^+$, 339 (40) [$M_{\mathbf{3h}}$ + H]$^+$, 207 (100) [aglycone$_{\mathbf{3h}}$ + H]$^+$. For $C_{13}H_{18}N_6O_5$ (338.3) calculated: 46.15% C, 5.36% H, 24.85% N; found: 46.13% C, 5.44% H, 25.12% N.

In a simultaneous experiment, in which only 2 mmol of histamine were used, a lower melting modification of nucleoside **3h** was obtained. Recrystallization of the crude product from methanol gave 0.092 g (27%) of pure nucleoside **3h**, m.p. 193–197 °C (dec.). According to TLC and UV spectra, the product was identical with the higher-melting modification.

Aggregate of *N*^{4}-Propyl-5-azacytidine (**3a**) with *N*^{1}-Propyl-*N*^{2}-(β-D-ribofuranosylcarbamoyl)-guanidine (**5a**)

Methanol (2 ml), propylamine (0.16 ml, 2 mmol), reaction time 2 h. Yield: 0.126 g (45%) of the aggregate, m.p. 134–136 °C (dec.) (acetonitrile), R_F (B) 0.62 (D_1, **3a**) and 0.43 (D_2, **5a**), $[\alpha]_D$ –25.6 (*c* 0.1, water). UV, λ_{max} (log ε): (MeOH), 258, inflexion (3.90), 226 (4.51); (pH 2.30), 257 (3.83), 223 (3.86); (pH 7.03), 250 (3.88); (pH 10.97), 227 (4.68). For $C_{21}H_{38}N_8O_{10}$ (562.6) calculated: 44.83% C, 6.81% H, 19.92% N; found: 44.85% C, 6.83% H, 20.14% N. The aggregate of **3a** with **5a** was hygroscopic and decomposed on storage to products of hydrolysis.

Aggregate of N^4-Butyl-5-azacytidine (**3b**) with N^1-Butyl-N^2-(β-D-ribofuranosylcarbamoyl)-guanidine (**5b**)

Methanol (5 ml), butylamine (0.7 ml, 7 mmol), reaction time 40 min. Yield: 0.148 g (50%) of the aggregate, m.p. 130–132 °C (dec.) (acetonitrile), R_F (B) 0.72 (D_1, **3b**) and 0.55 (D_2, **5b**), $[\alpha]_D$ –22.1 (*c* 0.5, water). UV, λ_{max} (log ε): (MeOH), 255 (3.68), 227 (4.25); (pH 2.46), 258 (3.85), 219 (4.56); (pH 6.93), 255, inflexion (3.98); (pH 11.1), 228 (4.72). For $C_{23}H_{42}N_8O_{10}\cdot 1/2\ H_2O$ (590.7) calculated: 46.07% C, 7.23% H, 18.69% N; found: 46.05% C, 7.25% H, 18.63% N. The aggregate **3b** with **5b** was hygroscopic and decomposed on storage to products of hydrolysis.

In a simultaneous experiment, a solution of crude **3b** (prepared from 1 mmol of **4** as described above) in1 M ammonia (5 ml) was kept at room temperature for 1 h, evaporated and co-evaporated with ethanol (3 × 5 ml) to give crude carbamoylguanidine **5b**. A solution of this product in methanol (5 ml) was treated with a solution of crude **3b** (prepared from 1 mmol of **4** by the above mentioned procedure) in methanol (5 ml). Evaporation of this mixture and crystallization of the residue from acetonitrile gave 0.408 g (69%) of the aggregate of **3b** with **5b**, m.p. 130–132 °C (dec.) without depression on admixture with the sample described above.

Separation of the Aggregate of **3b** with **5b**

Method A. A solution of the aggregate of **3b** with **5b** (0.118 g, 0.2 mmol) in methanol (2 ml) was applied onto a column of Amberlite IRC 50 [H^+] ion exchange resin (5 ml), which was prepared in methanol. The column was eluted with methanol (50 ml), the effluent evaporated and the residue crystallized from acetonitrile to afford 0.024 g (40%) of **3b**, m.p. 86–88 °C (dec.), without depression on admixture with a sample of the product described above. The column was further eluted with a 9:1 mixture of methanol–acetic acid (50 ml) and the effluent evaporated. A solution of the residue in ethanol (0.5 ml) was treated with a solution of picric acid (0.025 g) in ethanol (0.5 ml) to yield 0.025 g (24%) of the picrate of N^1-butyl-N^2-(ribosylcarbamoyl)guanidine (**5b** and isomers), m.p. 142–147 °C (dec.), R_F (B) 0.58 (D_2, **5b** and isomers) and 0.90 (D_1, picric acid). ^{1}H NMR: 9.40 s, 1 H, 8.88 br s, 1 H, 8.47 br s, 1 H and 8.38 br, 1 H (guanidinium NH); 7.94 br s, 1 H (carbamoyl NH); 8.58 s, 2 H (picrate CH); 5.13 br t, $J \approx 6.0$ [+ DOAc: 5.13 d, $J(1',2') = 4.8$] (H-1′ of **5b**); 5.42 dd, $J(1',2') = 4.9$, $J(1',NH) = 9.0$ [+ DOAc: 5.42 d, $J(1',2') = 4.9$] (H-1′ of the α-furanosyl isomer); 5.01 dd, $J(1',2') = 3.2$, $J(1',NH) = 8.5$ [+ DOAc: 5.00 d, $J(1',2') = 3.4$] (H-1′ of the α-pyranosyl isomer); 4.84 br t, $J \approx 8.0$ [+ DOAc: 4.84 d, $J(1',2') = 8.8$] (H-1′ of the β-pyranosyl isomer) (**5b** (β-fur):α-fur:α-pyr:β-pyr ≈ 5:2:1.5:1). The other signals were not resolvable. FAB-MS: 291 (42) [$M_{\mathbf{5b}}$ and isomers + H]$^+$, 116 (20) [M + H]$^+$ of butylguanidine. For $C_{11}H_{22}N_4O_5\cdot C_6H_3N_3O_7$ (519.4) calculated: 39.29% C, 4.84% H, 18.87% N; found: 39.00% C, 4.55% H, 18.78% N.

Method B. A solution of the aggregate of **3b** with **5b** (0.118 g, 0.2 mmol) in methanol (5 ml) was worked up by column chromatography on silica gel (10 g) in analogy to the preparation of **3b**. The fractions containing **3b** were collected, evaporated and the residue crystallized from acetonitrile to yield 0.024 g (40%) of **3b**, m.p. 86–88 °C (dec.) without depression on admixture with a sample of the product prepared by method *A*. The very polar carbamoylguanidine **5b** was not eluted from the column.

Aggregate of N^4-*sec*-Butyl-5-azacytidine (**3c**) with N^1-*sec*-Butyl-N^2-(β-D-ribofuranosylcarbamoyl)guanidine (**5c**)

Methanol (2 ml), *sec*-butylamine (0.2 ml, 2 mmol), reaction time 4 h. The crude product was applied onto a column of Amberlite IRC-50 [H^+] ion exchange resin (10 ml), which was prepared in methanol. The column was eluted with methanol (100 ml), the effluent evaporated and the residue crystallized from acetonitrile (3 weeks, room temperature) to yield 0.074 g (25%) of the aggregate of **3c** with **5c**, m.p. 130–132 °C (dec.), R_F (B) 0.72 (D_1, **3c**) and 0.52 (D_2, **5c**), $[\alpha]_D$ –1.5 (*c* 0.1, water). UV, λ_{max} (log ε): (MeOH), 255, inflexion (3.98), 226 (4.53); (pH 2.30), 258 (3.85), 219 (4.02); (pH 7.03), 250, inflexion (3.90); (pH 10.07), 227 (4.68). For $C_{23}H_{42}N_8O_{10}\cdot 1/2H_2O$ (590.7) calculated: 46.07% C, 7.23% H, 18.69% N; found: 46.06% C, 7.14% H, 18.95% N. The aggregate of **3c** with **5c** was hygroscopic and decomposed on storage to products of hydrolysis.

Aggregate of N^4-Benzyl-5-azacytidine (**3d**) with N^1-Benzyl-N^2-(β-D-ribofuranosylcarbamoyl)-guanidine (**5d**)

Methanol (2 ml), benzylamine 0.22 ml, 2 mmol), reaction time 5 h. Yield: 0.059 g (18%) of the aggregate, m.p. 136–139 °C (dec.) (acetonitrile), R_F (B) 0.71 (D_1, **3d**) and 0.57 (D_2, **5d**), $[\alpha]_D$ –27.4 (*c* 0.1, water). UV, λ_{max} (log ε): (MeOH), 255, inflexion (3.95), 228 (4.57); (pH 2.30), 255 (3.95), 219 (4.25); (pH 7.03), 255, inflexion (3.96), 225, inflexion (4.44); (pH 10.97), 228 (4.69). IR (KBr): 3512 m [ν(OH)]; 3408 s [ν(NH) free, urea]; 3363 s [ν(NH), imino group]; 3275 s, br, 3240 s, br, sh [ν(NH) bonded]; 1757 m, 1678 s, 1655 s [ν(C=O) + β(NH), triazine]; 1621 vs [amide I, urea, ν(C=N), triazine]; 1598 s [ν(ring), phenyl]; 1576 s, 1560 s, sh [amide II, urea + ν(C=N)]; 1515 s, sh, 1497 s [ν(C=N), ν(C–N), triazine + ν(ring), phenyl]; 1288 s (amide III, urea); 796 s (ring breathing, triazine); 600 m [γ(NH)]. FAB-MS: 669 (<1) [2 $M_{\mathbf{3d}}$ + H]$^+$, 659 (1) [$M_{\mathbf{3d}}$ + $M_{\mathbf{5d}}$ + H]$^+$, 649 (<1) [2 $M_{\mathbf{5d}}$ + H]$^+$, 484 (2) [$M_{\mathbf{3d}}$ + benzylguanidine + H]$^+$, 474 (2) [$M_{\mathbf{5d}}$ + benzylguanidine + H]$^+$, 357 (2) [$M_{\mathbf{3d}}$ + Na]$^+$, 347 (3) [$M_{\mathbf{5d}}$ + Na]$^+$, 335 (4) [$M_{\mathbf{3d}}$ + H]$^+$, 325 (16) [$M_{\mathbf{5d}}$ + H]$^+$, 203 (12) [aglycone$_{\mathbf{3d}}$ + H]$^+$, 150 (100) [M + H]$^+$ of benzylguanidine. For $C_{29}H_{38}N_8O_{10}$ (658.7) calculated: 52.87% C, 5.81% H, 17.02% N; found: 52.69% C, 5.75% H, 17.30% N.

In a simultaneous experiment, a solution of crude **3d** (prepared from 1 mmol of **4** by the aforementioned procedure) in 1 M ammonia (6 ml) was kept at room temperature for 1 h, evaporated and the residue co-evaporated with ethanol (3 × 5 ml). The crude carbamoylguanidine **5d** thus obtained was treated with a solution of crude **3d** (prepared by the above mentioned procedure from 1 mmol of **4**) in methanol (5 ml). The mixture was evaporated and the residue crystallized from acetonitrile–methanol (95:5, v/v) to give 0.382 g (58%) of the aggregate of **3d** with **5d**, m.p. 136–139 °C (dec.) without depression on admixture with a sample prepared by method *A*.

Aggregate of N^4-Furfuryl-5-azacytidine (**3e**) with N^1-Furfuryl-N^2-(β-D-ribofuranosylcarbamoyl)-guanidine (**5e**)

Methanol (2 ml), furfurylamine (0.18 ml, 2 mmol), reaction time 7 h. Yield: 0.150 g (47%) of the aggregate, m.p. 141–143 °C (dec.) (acetonitrile–methanol 9:1, v/v), R_F (B) 0.68 (D_1, **3e**) and 0.61 (D_2, **5e**), $[\alpha]_D$ –19.7 (*c* 0.5, water). UV, λ_{max} (log ε): (MeOH), 260 (3.63), 225 (4.36); (pH 2.30), 254 (3.64), 222 (4.06); (pH 7.03), 253 (3.70), 215 (4.31); (pH 10.97), 228 (4.54), 215 (4.38). IR (KBr): 3526 m, sh [ν(OH)]; 3410 s, br, sh [ν(NH) free, urea]; 3368 s [ν(NH), imino group]; 3275 s, br, sh [ν(NH) bonded]; 1749 w, sh, 1730 w, sh, 1678 s, 1655 s [ν(C=O) + β(NH), triazine]; 1623 vs [amide I, urea, ν(C=N), triazine]; 1571 s, 1563 s [amide II +

ν(C=N)]; 1512 m, sh, 1502 s [ν(C=N), ν(C–N) + ν(ring), furan]; 1302 m, 1294 m (amide III, urea); 1011 m (ring breathing, furan); 798 w (ring breathing, triazine); 599 w [γ(NH)]. FAB-MS: 649 (1) [2 M_{3e} + H]$^+$, 639 (4) [M_{3e} + M_{5e} + H]$^+$, 629 (2) [2 M_{5e} + H]$^+$, 464 (<1) [M_{3e} + furfurylguanidine + H]$^+$, 454 (<1) [M_{5e} + furfurylguanidine + H]$^+$, 325 (17) [M_{3e} + H]$^+$, 315 (47) [M_{5e} + H]$^+$, 193 (35) [aglycone$_{3e}$ + H]$^+$, 140 (15) [M + H]$^+$ of furfurylguanidine. For $C_{25}H_{34}N_8O_{12}$ (638.6) calculated: 47.02% C, 5.36% H, 17.55% N; found: 47.09% C, 5.38% H, 17.51% N.

2-(Methylcarbamoyl)guanidine (**7**)

A mixture of guanidine hydrochloride (3.84 g, 40 mmol) and 1 M sodium ethoxide in ethanol (40 ml) was stirred at room temperature for 15 min and the insoluble sodium chloride filtered off with suction. The filtrate was evaporated at 35 °C (bath temperature) and the residue dried for 1 h in vacuum to give crude syrupy guanidine. To a stirred mixture of this product with dry acetone (40 ml) a solution of methyl isocyanate (2.4 ml, 40 mmol) in dry acetone (20 ml) was added dropwise at 0 °C. The mixture was then stirred at room temperature for 2 h and kept overnight in a refrigerator. The crystalline precipitate was filtered off with suction to give 3.50 g (75%) of **7**, m.p. 132–135 °C, R_F (B) 0.28 (D_2). Recrystallization of the unique crude product from ethanol raised the melting point to 137–139 °C. UV, λ_{max} (log ε): (EtOH), 225 (4.21); (pH 11.15), 225 (4.38). IR (KBr): 3403 s [ν(NH) free trans amide]; 3365 s [$\nu_{as}(NH_2)$]; 3266 s, 3123 m, sh [$\nu_s(NH_2)$ bonded]; 1730 m, sh [ν(C=0)]; 1668 s (amide I); 1611 s, 1597 s [$\beta_s(NH_2)$]; 1552 s [ν(C=N)]; 1496 vs, br (amide II); 1463 s, br [δ^+_{as}(Me)]; 1408 s [δ_s(Me)]; 1302 s (amide III); 1153 m [δ^-_{as}(Me) + ν(C=O)]; 1092 m [$\beta_{as}(NH_2)$]; 1062 w, br, sh [ν(N–Me)]; 665 m, vbr, sh (amide V); 588 m, br, 565 m, br [$\gamma_s(NH_2)$]; 527 m [δ(N–C–N), urea]. ^{1}H NMR: 6.56 br s, 4 H (guanidine NH); 6.04 br q, 1 H, J(NH,CH_3) = 4.4 (carbamoyl NH); 2.49 d, 3 H, J(CH_3,NH) = 4.4 (CH_3). ^{13}C NMR: 166.19 (C=O), 160.92 (C=N), 26.53 (CH_3). FAB-MS: 349 (6) [3 M_7 + H]$^+$, 255 (8) [2 M_7 + Na]$^+$, 233 (100) [2 M_7 + H]$^+$, 176 (7) [M_7 + M_{10} + H]$^+$, 139 (14) [M_7 + Na]$^+$, 117 (100) [M_7 + H]$^+$, 60 (14) [M_{10} + H]$^+$, 57 (5) $M^{\bullet+}$ of methyl isocyanate. For $C_3H_8N_4O$ (116.1) calculated: 31.03% C, 6.94% H, 48.26% N; found: 31.20% C, 7.02% H, 48.54% N. Crystallization of a mixture of **7** (0.058 g, 0.5 mmol) and picric acid (0.115 g, 0.5 mmol) from ethanol–water gave 0.156 g (90%) of 2-(methylcarbamoyl)guanidinium picrate (**7a**), m.p. 235–236 °C (dec.). IR (Nujol): 3449 m, 3439 s, 3404 m [$\nu_{as}(NH_2)$, ν(NH) free]; 3375 m, sh, 3323 m, 3294 m [ν(NH) bonded]; 3245 m, sh, 3215 m, 3183 m [$\nu_s(NH_2)$]; 1744 s [ν(C=O), CO–NH$^+$=]; 1687 s, 1653 s [$\beta_s(NH_2)$]; 1629 s, sh, 1610 s, br [ν(C=N), ν(ring) of the picrate]; 1567 s, 1559 s [$\nu_{as}(NO_2)$]; 1505 m, sh, 1497 s (amide II); 1430 m [ν(ring) of the picrate]; 1416 m [δ_s(Me)]; 1364 s [ν(C–N)]; 1339 s, 1320 s [$\nu_s(NO_2)$]; 1057 w, br [ν(N–Me)]; 743 m, 726 m [$\gamma_s(NO_2)$]; 568 m, 559 m [$\gamma_s(NH_2)$]; 526 m [δ(N–C–N), urea]. ^{1}H NMR: 9.65 br s, 1 H and 8.01 br s, 4 H (guanidinium NH); 7.36 br q, 1 H, J(NH,CH_3) = 4.5 (carbamoyl NH); 2.645 d, 3 H, J(CH_3,NH) = 4.5 (CH_3); 8.60 s, 2 H (picrate CH). ^{13}C NMR: 161.01 (C=O), 155.345 (N-C), 153.88, 142.03, 125.41 and 124.41 (picrate C), 26.27 (CH_3). FAB-MS: 117 (35) [M_7 + H]$^+$, 60 (12) [M_{10} + H]$^+$, 57 (26) $M^{\bullet+}$ of methyl isocyanate. For $C_3H_8N_4O{\cdot}C_6H_3N_3O_7$ (345.2) calculated: 31.31% C, 3.21% H, 28.40% N; found: 31.60% C, 3.00% H, 28.16% N.

1-Methyl-5-azacytosine (**6**)

A solution of 2-(methylcarbamoyl)guanidine (**7**) (0.116 g, 1 mmol) in a mixture of methanol (1.5 ml) and dimethylformamide dimethyl acetal (0.13 ml, 1 mmol) was kept at room tem-

perature for 3 days and the precipitate filtered off with suction to give 0.083 g (66%) of **6**, m.p. 258–260 °C (dec.) without depression on admixture with an authentic sample[65], R_F (B) 0.33 (D_1). UV, λ_{max} (log ε): (MeOH), 209 (4.02), 243 (3.72); (pH 6.94), 201 (4.36), 245 (3.68); (pH 10.93), 214 (3.89), 245 (3.70). IR (KBr): 3500 m, br, sh, 3408 m, br [$\nu_{as}(NH_2)$]; 3340 s, br, sh, 3303 s, br, 3249 s, br, 3183 s, br, 3045 m, br, sh [$\nu_s(NH_2)$]; 1757 m, sh, 1734 s, sh, 1707 s, br, sh, 1681 s, br, 1668 s, vbr, sh [ν(C=O) + $\beta_s(NH_2)$]; 1616 s, 1513 s, 1487 s, br, 1354 s [ν(C=N), ν(C–N)]; 1123 s [$\beta_s(NH_2)$]; 795 s (ring breathing); 711 w, vbr [$\gamma_s(NH_2)$]. ^{1}H NMR: 8.24 s, 1 H (H-6); 7.33 brs, 1 H and 7.31 brs, 1 H (NH_2); 3.19 s, 3 H (CH_3). ^{13}C NMR: 166.87 (C-4), 159.65 (C-6), 154.755 (C-2), 34.20 (CH_3). FAB-MS: 275 (17) [2 M_6 + Na]$^+$, 253 (28) [2 M_6 + H]$^+$, 149 (27) [M_6 + Na]$^+$, 127 (100) [M_6 + H]$^+$.

Aggregates of 1-Methyl-5-azacytosine (**6**) with 2-(Methylcarbamoyl)guanidine (**7**)

Method A. A solution of 2-(methylcarbamoyl)guanidine (**7**) (0.464 g, 4 mmol) in a mixture of methanol (4 ml) and ethyl formate (0.5 ml) was kept at room temperature for 5 days and crystallization of the product induced by scratching. The precipitate was filtered off with suction to give 0.165 g (32%) of the monohydrate of a 1:2 aggregate of **6** and **7**, m.p. 166–167 °C (dec.), R_F (D) 0.48 (D_3, **7**) and 0.55 (D_1, **6**). UV, λ_{max} (log ε): (MeOH), 211 (4.33), 225 (4.40); (pH 2.50), 254 (3.64); (pH 7.06), 243, inflexion (3.56), 225 (3.80); (pH 11.15), 225 (4.57). IR (KBr): 3505 m [$\nu_s(NH_2)$ free]; 3381 s, 3330 s, sh, 3290 s, br, sh [$\nu_{as}(NH_2)$, ν(NH) bonded]; 3219 s, 3080 m, br, sh [$\nu_s(NH_2)$]; 1706 s [ν(C=O) triazine]; 1673 s, 1663 s, sh [$\beta_s(NH_2)$, triazine + amide I free, urea]; 1628 s [amide I bonded, urea + ν(C=N), triazine]; 1604 s, 1595 s, sh [$\beta_s(NH_2)$, ν(C=N), guanidine]; 1552 s, br, sh (amide II bonded, urea); 1500 vs, br [amide II free, urea, ν(C=N), triazine]; 1462 s, sh, 1445 s, sh [δ^+_{as}(Me)]; 1410 s [δ_s(Me), urea]; 1365 m [δ_s(Me), triazine]; 1310 s, br (amide III, urea); 1121 m [$\beta_s(NH_2)$, triazine]; 1086 m [δ^-_{as}(Me)]; 791 s (ring breathing, triazine); 714 m, vbr, [$\gamma_s(NH_2)$, triazine]; 650 m, vbr, sh (amide V, urea); 607 m [δ(C=O), triazine]; 544 m, br [δ(N–C–N), urea]. ^{1}H NMR: 8.24 s, 1 H (H-6 of **6**); 7.31 br s, 2 H (NH_2 of **6**); 6.52 br, 8 H (4 × NH_2 of **7**); 5.98 br s, 2 H (2 × NH of **7**); 3.20 s, 3 H (CH_3 of **6**); 2.49 d, 6 H, J(CH_3,NH) = 4.6 (2 × CH_3 of **7**) (**6**:**7** = 1:2). ^{13}C NMR: 166.84 (C-4 of **6**), 166.22 (C=O of **7**), 160.91 (C=N of **7**), 159.63 (C-6 of **6**), 154.73 (C-2 of **6**), 34.18 (CH_3 of **6**), 26.52 (CH_3 of **7**). FAB-MS: 369 (7) [2 M_6 + M_7 + H]$^+$, 359 (5) [M_6 + 2 M_7 + H]$^+$, 349 (3) [3 M_7 + H]$^+$, 275 (2) [2 M_6 + Na]$^+$, 265 (3) [M_6 + M_7 + Na]$^+$, 255 (4) [2 M_7 + Na]$^+$, 253 (5) [2 M_6 + H]$^+$, 243 (100) [M_6 + M_7 + H]$^+$, 233 (57) [2 M_7 + H]$^+$, 186 (10) [M_6 + M_{10} + H]$^+$, 176 (7) [M_7 + M_{10} + H]$^+$, 149 (8) [M_6 + Na]$^+$, 139 (7) [M_7 + Na]$^+$, 127 (79) [M_6 + H]$^+$, 117 (100) [M_7 + H]$^+$, 60 (20) [M_{10} + H]$^+$, 57 (7) $M^{\bullet+}$ of methyl isocyanate. For $C_4H_6N_4O\cdot(C_3H_8N_4O)_2\cdot H_2O$ (376.4) calculated: 31.91% C, 6.42% H, 44.66% N; found: 31.95% C, 6.56% H, 44.32% N.

Method B. A solution of a mixture of **6** (21.5 mg) and **7** (20 mg) in water was evaporated and the residue dried to constant weight to give the monohydrate of a 1:1 aggregate of **6** with **7**, m.p. 163–165 °C (dec.). FAB-MS: 253 (1) [2 M_6 + H]$^+$, 243 (8) [M_6 + M_7 + H]$^+$, 233 (4) [2 M_7 + H]$^+$, 127 (29) [M_6 + H]$^+$, 117 (100) [M_7 +H]$^+$, 60 (22) [M_{10} + H]$^+$, 57 (12) $M^{\bullet+}$ of methyl isocyanate. When the same solution of a sample of the 1:1 aggregate of **6** with **7** was diluted with a forty times larger volume of water, no aggregates have been detected at all. For $C_4H_6N_4O\cdot C_3H_8N_4O\cdot H_2O$ (260.2) calculated: 32.31% C, 6.20% H, 43.07% N; found: 32.02% C, 6.38% H, 43.00% N.

Method C. A solution of a mixture of **6** (21.5 mg) and **7** (10 mg) in water was evaporated and the residue dried to constant weight to give the dihydrate of a 2:1 aggregate of **6** with

7, m.p. 162–163 °C (dec.). For $(C_4H_6N_4O)_2 \cdot C_3H_8N_4O \cdot 2H_2O$ (404.2) calculated: 32.68% C, 5.98% H, 41.58% N; found: 32.40% C, 6.12% H, 41.73% N.

Aggregate of 1-Methyl-5-azacytosine (**6**) with 2-(Methylcarbamoyl)guanidinium Picrate (**7a**)

A mixture of **6** (25.2 mg, 0.2 mmol) and the picrate **7a** (69 mg, 0.2 mmol) was dissolved in boiling methanol and the solution evaporated. The residue (m.p. 205–208 °C (dec.)) was recrystallized from methanol to give 60 mg (64%) of the aggregate, m.p. 211–213 °C (dec.), R_F (D) 0.48 (D_3, **7**); 0.55 (D_1, **6**) and 0.78 (D_1, picric acid). ^{1}H NMR: 8.24 s, 1 H (H-6 of **6**); 7.34 br s, 2 H (NH_2 of **6**); 3.20 s, 3 H (CH_3 of **6**); 8.59 s, 2 H (picrate CH); 9.67 br s, 1 H and 8.03 br s, 4 H (guanidinium NH of **7a**); 7.36 br t, 1 H, J(NH,CH_3) = 4.5 (carbamoyl NH of **7a**); 2.65 d, 3 H, J(CH_3,NH) = 4.5 (methylcarbamoyl CH_3 of **7a**) (**6**:**7a** ≈ 1:1, based on the signals of the methyl groups). ^{13}C NMR: 166.83 (C-4 of **6**), 161.025 (C=O of **7a**), 159.63 (C-6 of **6**), 155.36 (C-N of **7a**), 154.80 (C-2 of **6**), 153.92, 142.04, 125.43 and 124.41 (picrate C), 34.17 (CH_3 of **6**), 26.29 (CH_3 of **7a**). FAB-MS: 253 (<1) [2 M_6 + H]$^+$, 243 (5) [M_6 + M_7 + H]$^+$, 233 (1) [2 M_7 + H]$^+$, 127 (27) [M_6 + H]$^+$, 117 (100) [M_7 + H]$^+$, 60 (67) [M_{10} + H]$^+$, 57 (7) $M^{\bullet+}$ of methyl isocyanate. For $C_4H_6N_4O \cdot C_3H_8N_4O \cdot C_6H_3N_3O_7$ (471.3) calculated: 33.13% C, 3.64% H, 32.69% N; found: 32.99% C, 3.66% H, 32.39% N.

2-(β-D-Ribofuranosylcarbamoyl)guanidinium Picrate (**8a**) and Its α-D Anomer

The product was prepared by a known procedure[23]. ^{1}H NMR: 8.59 s, 2 H (benzene CH); 9.58 br s, 1 H and 8.10 br s, 4 H (guanidinium NH); β-D anomer: 7.96 d, 1 H, J(NH,1′) = 8.1 (carbamoyl NH); 5.13 br dd, 1 H, J(1′,2′) = 4.7 (H-1′); 3.87 br t, 1 H, J(2′,3′) = 4.4 (H-2′); 3.77 br t, 1 H, J(3′,4′) = 4.0 (H-3′); 3.71 br q, 1 H (H-4′); 3.46 dd, 1 H, J(5′a,4′) = 4.1, J(gem) = 11.8 (H-5′a); 3.40 dd, 1 H, J(5′b,4′) = 4.3, J(gem) = 11.8 (H-5′b); 5.13 br s, 1 H, 4.99 br s, 1 H and 4.78 br s, 1 H (OH); α-D anomer: 7.69 d, 1 H, J(NH,1′) = 9.1 (carbamoyl NH); 5.42 dd, 1 H, J(1′,2′) = 4.6, J(1′,NH) = 9.1 (H-1′); 3.98 br q, 1 H, J(2′,3′) = 4.6 (H-2′); 3.89 br t, 1 H, J(3′,4′) ≈ 4.5 (H-3′); 3.74 (covered), 1 H (H-4′); 3.47 dd, 1 H, J(5′a,4′) = 4.0 , J(gem) = 12.0 (H-5′a); 3.36 dd, 1 H, J(5′b,4′) = 4.6, J(gem) = 12.0 (H-5′b); 5.47 br s, 1 H, J ≈ 4.0, 4.80 br s, 1 H and 4.70 br s, 1 H (OH) (β:α ≈ 6.5:1, based on the signals of H-1′). FAB-MS: 235 (12) [M_8 + H]$^+$, 60 (100) [M_{10} + H]$^+$.

2-Carbamoylguanidinium Chloride (**7b**) and
1,1-Dimethyl-2-(methylcarbamoyl)guanidinium Chloride (**7c**)

For the preparation of guanidinium chlorides **7b** and **7c**, see ref.[29]. ^{1}H NMR of **7b**: 10.42 br s, 1 H and 8.21 br s, 4 H (guanidinium NH); 7.20 br s, 2 H (carbamoyl NH); on dilution (1:4) the following signals of the NH protons were observed: 10.45 br s, 1 H and 8.23 br s, 4 H (guanidinium NH); 7.22 br s, 2 H (carbamoyl NH). ^{1}H NMR of **7c**: 10.36 s, 1 H, 9.16 br, 1 H and 8.47 br, 1 H (guanidinium NH); 8.46 br q, 1 H, J(NH,CH_3) = 4.6 (methylcarbamoyl NH); 3.10 br s, 6 H (2 × guanidinium CH_3); 2.68 d, 3 H, J(CH_3,NH) = 4.6 (methylcarbamoyl CH_3); on dilution the following signals of the NH protons were observed: 10.30 s, 1 H, 9.16 br, 1 H and 8.45 br, 1 H (guanidinium NH); 8.38 br q, 1 H (methylcarbamoyl NH).

Aggregate of Cytidine (**9**) with 2-(β-D-Ribofuranosylcarbamoyl)guanidinium Picrate (**8a**) and Its α-D Anomer

A mixture of cytidine (**9**) (48.6 mg, 0.2 mmol) and 2-(β-D-ribofuranosylcarbamoyl)-guanidinium picrate (**8a**) (92.6 mg, 0.2 mmol) was dissolved in boiling methanol and the solution evaporated. Crystallization of the residue (m.p. 96–98 °C) from ethyl acetate gave 85 mg (60%) of the aggregate, m.p. 94–96 °C, R_F (D) 0.29 (D_3, **8**); 0.36 (D_1, **9**) and 0.78 (D_1, picric acid). ^{1}H NMR: 7.42 br s, 1 H and 7.28 br s, 1 H (NH_2 of **9**); 10.00 br, 1 H and 8.26 br s, 4 H (guanidinium NH of **8a**); 7.92 d, 1 H (carbamoyl NH of β-**8a**); 7.65 d, 1 H (carbamoyl NH of α-**8a**) (**9**:**8a** = 1.2:1 and β-**8a**:α-**8a** ≈ 3.2:1, based on the signals of H-1′ of the ribosyl group); on dilution (1:4) the following signals of the NH protons were observed: 7.29 br s, 1 H and 7.18 br s, 1 H (NH_2 of **9**); 10.00 br, 1 H and 8.15 br s, 4 H (guanidinium NH of **8a**); 7.93 d, 1 H, J = 7.8 (carbamoyl NH of β-**8a**); 7.66 d, 1 H, J = 9.0 (carbamoyl NH of α-**8a**); the signals of the other protons were not evaluated in this measurement. FAB-MS: 487 (5) [2 M_9 + H]$^+$, 478 (15) [M_8 + M_9 + H]$^+$, 244 (100) [M_9 + H]$^+$, 235 (95) [M_8 + H]$^+$. For $C_9H_{13}N_3O_7 \cdot C_7H_{14}N_4O_5 \cdot C_6H_3N_3O_7$ (706.5) calculated: 37.40% C, 4.28% H, 19.82% N; found: 37.58% C, 4.51% H, 18.70% N.

1,2-Bis(methylcarbamoyl)guanidine (**11**)

To a stirred mixture of guanidine, (prepared from guanidine hydrochloride (1.92 g, 20 mmol) in analogy to the preparation of **7**) in dry acetone (20 ml), a solution of methyl isocyanate (2.4 ml, 40 mmol) in dry acetone (10 ml) was added dropwise at 0 °C. The mixture was then stirred at room temperature for 2 h and kept overnight in a refrigerator. The crystals were filtered off with suction to give 2.80 g (81%) of **11**, m.p. 174–176 °C (dec.). Recrystallization from ethanol raised the melting point to 179–181 °C (dec.), R_F 0.36 (B, D_2). UV, λ_{max} (log ε): (EtOH), 233 (4.27); (0.1 M HCl), 214 (4.24); (pH 9.53), 209 (4.09), 216 (4.06), 234 (4.33). IR (KBr): 3405 m, sh, 3388 m, 3349 s, 3333 s, sh, 3291 m, 3113 w, br, sh [ν(NH), $\nu_{as}(NH_2)$, $\nu_s(NH_2)$]; 1700 m, 1680 s, 1638 vs (amide I); 1601 m, sh [$\beta_s(NH_2)$]; 1565 s, br, 1537 s, br (amide II); 1472 m, sh, 1451 m [δ^+_{as}(Me) + ν(C=O)]; 1403 m [δ_s(Me)]; 1303 s, 1268 s (amide III); 1171 w, 1159 m [δ^-_{as}(Me) + ν(C=O)]; 526 m, br [δ(N–C–N)]. ^{1}H NMR: 8.90 br s, 1 H, 8.60 br s, 1 H, 7.70 br s, 1 H, 7.40 br s, 1 H and 7.00 br s, 1 H (5 × NH); 2.60 br d, 6 H, J(CH_3,NH) = 4.4 (2 × CH_3). ^{13}C NMR: 157.42 br and 156.38 br (2 × C=O), 156.21 (C=N), 26.70 br and 26.17 (2 × CH_3). FAB-MS: 347 (4) [2 M_{11} + H]$^+$, 174 (100) [M_{11} + H]$^+$, 117 (25) [M_7 + H] $^+$, 60 (36) [M_{10} + H]$^+$, 57 (14) M$^{\bullet+}$ of methyl isocyanate. For $C_5H_{11}N_5O_2$ (173.2) calculated: 34.68% C, 6.40% H, 40.44% N; found: 34.93% C, 6.58% H, 40.67% N.

1,2,3-Tris(methylcarbamoyl)guanidine (**12**)

To a stirred mixture of guanidine (prepared from guanidine hydrochloride (0.96, 10 mmol) in analogy to the preparation of **7**) in dry acetone, a solution of methyl isocyanate (1.8 ml, 30 mmol) in dry acetone (5 ml) was added dropwise at 0 °C. The mixture was then stirred at room temperature for 3 h. During this time, the voluminous crystalline precipitate dissolved. The clear solution was filtered off with suction to remove a small insoluble portion, kept overnight in a refrigerator and the crystals were filtered off with suction to give 1.40 g of **12**, m.p. 185–190 °C (resolidification). Evaporation of the mother liquor and crystallization of the residue from acetone (3 ml) gave a second crop of **12**. Overall yield: 1.90 g (82%). Recrystallization of the unique crude product from ethanol raised the melting point to

191–194 °C (resolidification), R_F 0.50 (B, D_1). UV, λ_{max} (log ε): (EtOH), 224 (4.30), 233 (4.27), 239 (4.29); (0.1 M HCl), 228 (4.41); (pH 2.40), 231 (4.27); (pH 6.88), 234 (4.27); (pH 11.15), 231 (4.10). IR (KBr): 3398 m, br, 3327 s, br, sh, 3286 s, 3223 s, br, 3102 m, br [ν(NH)]; 1718 s, 1707 s, 1695 s, 1670 s, 1623 s, br [amide I, ν(C=N)]; 1568–1520 s, br (amide II); 1470 m, 1447 m, br [δ^{+}_{as}(Me)]; 1410 s, 1401 s [δ_s(Me)]; 1320 s, br, 1250 s, br (amide III); 1174 m, 1158 m [ν(C=O) + δ^{-}_{as}(Me)]; 690 m, 678 m (amide V). ^{1}H NMR: 11.98 br s, 1 H, 10.78 br s, 1 H, 9.14 br s, 1 H, 8.18 br s, 1 H and 7.76 br q, 1 H, J(NH,CH_3) = 4.5 (5 × NH); 2.72 br s, 3 H, 2.60 br s, 3 H and 2.64 d, 3 H, J(CH_3,NH) = 4.5 (3 × CH_3). ^{13}C NMR: 162.93 (C=N), 154.685 br, 153.03 br and 151.41 (3 × C=O), 26.535 br, 26.15 br and 26.01 (3 × CH_3). FAB-MS: 461 (3) [2 M_{12} + H]$^+$, 231 (100) [M_{12} + H]$^+$, 174 (22) [M_{11} + H]$^+$, 117 (24) [M_7 + H]$^+$, 60 (46) [M_{10} + H]$^+$, 57 (26) $M^{\bullet+}$ of methyl isocyanate. For $C_7H_{14}N_6O_3$ (230.2) calculated: 36.52% C, 6.13% H, 36.51% N; found: 36.80% C, 6.19% H, 36.26% N.

1-Methyl-4-(3-methylureido)-1,3,5-triazin-2(1*H*)-one (**13**)

A mixture of 1,2-bis(methylcarbamoyl)guanidine (**11**) (0.173 g, 1 mmol), methanol (2 ml) and dimethylformamide dimethyl acetal (0.13 ml, 1 mmol) was stirred at room temperature for 24 h. The precipitate was filtered off with suction to give 0.152 g (83%) of **13**, m.p. 241–243 °C (dec.), R_F 0.45 (B, D_1). UV, λ_{max} (log ε): (MeOH), 211 (4.03), 239 (4.03); (pH 2.50), 242 (4.04); (pH 7.06), 238 (4.06); (pH 11.15), 228 (4.07). IR (KBr): 3372 m, sh, 3305 m, 3244 m, 3213 m, sh, 3143 m, br, 3100 m, sh [ν(NH) bonded]; 3065 m, 3045 m, sh [ν(C–H)]; 3010 m, 2988 m [ν_{as}(Me)]; 2890 w, sh, 2858 w, sh [ν_s(Me)]; 1713 s, 1685 vs, br [ν(C=O), triazine + amide I, free urea]; 1630 vs, 1614 s, sh [amide I, bonded urea + ν(C=N), triazine]; 1569 m, sh, 1545 s (amide II, bonded urea); 1524 vs, 1498 vs [ν(C=N), triazine]; 1477 s, 1453 m, sh [δ^{+}_{as}(Me)]; 1426 s [ν(C–N)]; 1410 m, 1403 s, 1373 m, 1364 w, sh [δ_s(Me), urea + triazine]; 1298 vs, 1251 m (amide III, urea); 1077 m [δ^{-}_{as}(Me), triazine]; 796 m (ring breathing, triazine); 651 w, br (amide V, urea); 537 w [δ(N–C–N), urea). ^{1}H NMR: 10.09 br s, 1 H (ureido NH in position 1); 8.90 br q, 1 H, J(NH,CH_3) = 4.6 (ureido NH in position 3); 8.51 s, 1 H (H-6); 3.30 s, 3 H (triazine CH_3); 2.77 d, 3 H, J(CH_3,NH) = 4.6 (ureido CH_3). FAB-MS: 367 (4) [2 M_{13} + H]$^+$, 184 (45) [M_{13} + H]$^+$, 127 (74) [M_6 + H]$^+$, 57 (100) $M^{\bullet+}$ of methyl isocyanate. For $C_6H_9N_5O_2$ (183.2) calculated: 39.34% C, 4.95% H, 38.23% N; found: 39.63% C, 5.11% H, 38.09% N.

Aggregates of Guanosine (**14**) with 5-Azacytidine (**1**), 2′-Deoxy-5-azacytidine (**2**) and 1-β-D-Arabinofuranosyl-5-azacytosine (**15**)

Method A. A mixture of **1** (0.120 g) and **14** (0.140 g) was crystallized from water–acetone (1:1, vv) (3 ml) to give 0.147 g of a 1:2 aggregate of **1** with **14**, m.p. 214–218 °C (dec.). FAB-MS: 528 (6) [M_1 + M_{14} + H]$^+$, 489 (5) [2 M_1 + H]$^+$, 284 (100) [M_{14} + H]$^+$, 245 (84) [M_1 + H]$^+$, 152 (100) [aglycone$_{14}$ + H]$^+$, 113 (100) [aglycone$_1$ + H]$^+$.

Method B. A mixture of **2** (0.110 g) and **14** (0.140 g) was crystallized from methanol–water (98:2, v/v) (10 ml) to give 0.143 g of a 1:1 aggregate of **2** with **14**, m.p. 141–143 °C (dec.). FAB-MS: 512 (4) [M_2 + M_{14} + H]$^+$, 457 (2) [2 M_2 + H]$^+$, 284 (34) [M_{14} + H]$^+$, 229 (12) [M_2 + H]$^+$, 152 (52) [aglycone$_{14}$ + H]$^+$, 113 (45) [aglycone$_2$ + H]$^+$.

Method C. A mixture of **15** (0.050 g) and **14** (0.060 g) was crystallized from methanol to give 0.044 g of a 1:1 aggregate of **15** with **14**, m.p. 219–223 °C (dec.). FAB-MS: 528 (1) [M_{14} + M_{15} + H]$^+$, 489 (<1) [2 M_{15} + H]$^+$, 284 (13) [M_{14} + H]$^+$, 245 (5) [M_{15} + H]$^+$, 152 (35) [aglycone$_{14}$ + H]$^+$, 113 (9) [aglycone$_{15}$ + H]$^+$.

Aggregates of 5-Azacytidine (**1**) and 2′-Deoxy-5-azacytidine (**2**) with the Respective Dihydro Derivatives **16** and **17**

Method A. A mixture of **1** (0.120 g) and **16** (0.120) was crystallized from water–acetone (1:1, v/v) (4 ml) to give 0.145 g of a 1:1 aggregate of **1** with **16**, m.p. 205–207 °C (dec.). FAB-MS: 493 (4) [2 M_{16} + H]$^+$, 491 (7) [M_1 + M_{16} + H]$^+$, 489 (5) [2 M_1 + H]$^+$, 247 (100) [M_{16} + H]$^+$, 245 (53) [M_1 + H]$^+$, 115 (45) [aglycone$_{16}$ + H]$^+$, 113 (50) [aglycone$_1$ + H]$^+$.

Method B. A mixture of **2** (0.115 g) and **17** (0.115 g) was crystallized from methanol (10 ml) to give a 1:1 aggregate of **2** with **17**, m.p. 182–190 °C (dec.). FAB-MS: 461 (6) [2 M_{17} + H]$^+$, 459 (5) [M_2 + M_{17} + H]$^+$, 457 (1) [2 M_2 + H]$^+$, 231 (58) [M_{17} + H]$^+$, 229 (8) [M_2 + H]$^+$, 115 (25) [aglycone$_{17}$ +H]$^+$, 113 (28) [aglycone$_2$ + H]$^+$.

1-(2,3,5-Tri-*O*-benzyl-β-D-arabinofuranosyl)-5,6-dihydro-5-azacytosine (**18**)

A mixture of **19** (0.514 g, 1 mmol), acetic acid (5 ml), 2,2-dimethoxypropane (2.5 ml) and zinc powder (0.65 g) was stirred at room temperature for 5 h and the insoluble material filtered off with suction. The filtrate was diluted with water and extracted with chloroform (3 × 20 ml). The organic phase was shaken with a 5% solution of $NaHCO_3$, dried (anhydrous $MgSO_4$) and evaporated. The residue was dissolved in methanolic hydrogen chloride (38 mg HCl/5 ml), concentrated to a smaller volume and precipitated with ether (15 ml). The precipitate crystallized slowly to give the crude product, which was recrystallized three times from benzene (4 ml) to yield 0.400 g (72%) of the pure hydrochloride of **18a**, m.p. 154–156 °C (dec.), $[\alpha]_D$ –22.8 (*c* 0.1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): 10.30 br, 1 H and 9.25 br, 1 H (2 × NH); 8.20 br, 2 H (NH_2); 7.36–7.15 m, 15 H (arom.); 6.01 d, 1 H, *J*(1′,2′) = 5.2 (H-1′); 4.82 d, 1 H and 4.59 d, 1 H, *J*(gem) = 10.36 (2 × H-6); 4.53 d, 1 H and 4.44 d, 1 H, *J*(gem) = 12.0 (*O*-benzyl); 4.50 d, 1 H and 4.48 d, 1 H, *J*(gem) = 12.0 (*O*-benzyl); 4.42 d, 1 H and 4.38 d, 1 H, *J*(gem) = 11.5 (*O*-benzyl); 4.10 dd, 1 H, *J*(2′,3′) = 3.7, *J*(2′,1′) = 5.2 (H-2′); 3.96 q, 1 H, *J*(4′,3′) = *J*(4′,5) = 4.9 (H-4′); 3.93 dd, 1 H, *J*(3′,2′) = 3.7, *J*(3′,4′) = 4.9 (H-3′); 3.56 d, 2 H, *J*(H5′,4′) = 4.9 (2 × H-5′). For $C_{29}H_{32}N_4O_5$·HCl (553.0) calculated: 62.98% C, 5.83% H, 6.41% Cl, 10.13% N; found: 62.80% C, 5.92% H, 6.43% Cl, 10.18% N. A mixture of hydrochloride **18a** (0.055 g, 0.1 mmol), methanol (1 ml) and 1 M NaOMe (0.1 ml) was diluted with ethyl acetate (5 ml) and the mixture washed with a saturated solution of sodium chloride (3 × 5 ml). The organic phase was dried (anhydrous $MgSO_4$) and evaporated to give 0.045 g of the unique free base **18** as a syrup, R_F (A) 0.58 (D_1). ^{1}H NMR ($CDCl_3$): the signals of NH and NH_2 groups were not detectable; 7.34–7.20 m, 15 H (arom.); 6.14 d, 1 H, *J*(1′,2′) = 5.6 (H-1′); 4.72 d, 1 H and 4.60 d, 1 H, *J*(gem) = 9.8 (2 × H-6); 4.53 d, 1 H and 4.46 d, 1 H, *J*(gem) = 12.0 (*O*-benzyl); 4.51 d and 4.48 d, *J*(gem) = 11.5 (*O*-benzyl); 4.50 d, 1 H and 4.38 d, 1 H, *J*(gem) = 11.5 (*O*-benzyl); 4.12 dd, 1 H, *J*(3′,2′) = 4.3, *J*(3′,4′) = 5.4 (H-3′); 3.95 dd, 1 H, *J*(2′,3′) = 4.3, *J*(2′,1′) = 5.6 (H-2′); 3.89 br q, 1 H (H-4′); 3.59 dd, 1 H, *J*(5′a,4′) = 4.4, *J*(gem) = 10.7 (H-5′a); 3.55 dd, 1 H, *J*(5′b,4′) = 4.8, *J*(gem) = 10.7 (H-5′b). IR ($CHCl_3$): 3484 w, 3392 w, br, sh, 3324 m, br [$\nu_{as}(NH_2)$]; 3174 m, br [$\nu_s(NH_2)$]; 1724 s [ν(C=O)] (on deuteration shifted down by 1–2 cm^{-1}); 1702 s, 1669 s, sh [ν(C=O) + $\beta_s(NH_2)$] (on deuteration in CCl_4 a pronounced shift down and the lower band disappears); 1653 s [$\beta_s(NH_2)$] (on deuteration in CCl_4 the band disappears); 1613 s [ν(C=N) + β(NH)] (on deuteration in CCl_4 and in acetonitrile a pronounced shift up); 1548 s [ν(C=N) + β(NH) + ν(C–N)] (on deuteration a shoulder appeared on the down side of the band); 1281 s (ν(C–N)]. A very similar IR has been obtained also in CCl_4. IR (MeCN): 3459 m, 3356 m, vbr [$\nu_{as}(NH_2)$]; 3191 m, br [$\nu_s(NH_2)$]; 1724 s [ν(C=O)]; 1702 vs, 1680 s

[ν(C=O) + $\beta_s(NH_2)$]; 1656 s, 1651 s, 1638 s [$\beta_s(NH_2)$]; 1620 vs, 1551 s [ν(C=N) + β(NH)]; 1729 s [ν(C–N)]. FAB-MS: 1033 (3) [2 M_{18} + H]$^+$, 517 (100) [M_{18} + H]$^+$.

Anomeric 2-(D-*erythro*-2-Deoxypentosylcarbamoyl)guanidinium Formates (**21a**–**21d**)

A mixture of 2′-deoxy-5-azacytidine (**2**) (2.28 g, 10 mmol) and 1 M amonia (30 ml) was stirred at room temperature for 2 h, the solution evaporated and the residue co-evaporated with ethanol (1 × 50 ml). The residue was dissolved in ethanol (20 ml), acidified with formic acid (1 ml) and kept overnight at room temperature. The crystalline product was filtered off by suction to give 2.41 g (91%) of a mixture of guanidinium formates **21a**–**21d** (**21a:21b:21c:21d** = 9.9:7.1:2.7:1), m.p. 149–154 °C (dec.), $[\alpha]_D$ +13.9 (*c* 0.1, 1 M ammonia). UV, λ_{max} (log ε): (pH 2.30), 217 (3.44); (pH 6.95), 221 (3.85); (pH 10.92), 223 (4.38). ^{1}H NMR (D_2O): 8.46 s 1 H (formate CH); α-D-furanosyl derivative **21a**: 5.77 dd, 1 H, *J* = 3.5 and 7.0 (H-1′); 2.49 dt, 1 H, *J* = 6.3, 7.1 and 14.2 (H-2′a); 1.96 dt, 1 H, *J* = 3.0 (2×) and 14.2 (H-2′b); 4.37 dt, 1 H, *J* = 2.9 (2×) and 6.3 (H-3′); 4.10 br q, 1 H, *J*(average) = 4.5 (H-4′); 3.65 dd, 1 H, *J* = 3.8 and 12.4 (H-5′a); 3.59 dd, 1 H, *J* = 5.2 and 12.4 (H-5b); β-D-furanosyl derivative **21b**: 5.78 t, *J* = 7.0 (H-1′); 2.26 ddd, 1 H, *J* = 2.9, 6.2 and 14.0 (H-2′a); 2.14 dt, 1 H, *J* = 6.7, 7.2 and 14.0 (H-2′b); 4.39 dt, 1 H, *J* = 2.7 (2×) and 6.5 (H-3′); 3.96 td, 1 H, *J* = 2.8 and 4.5 (2×) (H-4′); 3.67 dd, 1 H, *J* = 4.5 and 12.0 ((H-5′a); 3.62 dd, 1 H, *J* = 4.2 and 12.0 (H-5′b); α-D-pyranosyl derivative **21c**: 5.04 dd, 1 H, *J* = 2.0 and 10.0 (H-1′); 1.96, 1 H (H-2′a, covered by the H-2′b signal of **21a**); 1.87 br q, 1 H, *J* = 11.5 (2×) and 12.0 (H-2′b); 4.00 ddd, *J* = 3.4, 4.3 and 11.0 (H-3′); 3.91 dd, 1 H, *J* = 3.2 and 12.6 (H-5′a); 3.68 dd, 1 H, *J* = 1.0 and 12.6 (H-5′b); β-D-pyranosyl derivative **21d**: 5.34 dd, 1 H, *J* = 2.8 and 8.1 (H-1′); 2.09 ddd, 1 H, *J* = 3.0, 6.4 and 13.9 (H-2′a); 1.89 ddd, 1 H, *J* = 3.2, 8.4 and 13.9 (H-2′b); 4.16 dt, 1 H, *J* = 3.2 (2×) and 6.4 (H-3′); 3.84 m, 3 H (H-4′, H-5′a and H-5′b). ^{1}H NMR (DMSO-d_6): 8.35 s, 1 H (formate CH); 8.25 br, 5 H (guanidinium NH); 7.63 d, 1 H (carbamoyl NH); the signals of the other protons were not evaluated. For $C_8H_{16}N_4O_6$ (264.2) calculated: 36.36% C, 6.10% H, 21.20% N; found: 36.30% C, 6.26% H, 21.30% N.

Anomeric 2-(D-*erythro*-2-Deoxypentofuranosylcarbamoyl)guanidines (**20a** and **20b**)

A solution of a mixture of **21a**–**21d** (2.642 g, 10 mmol) in water (50 ml) was applied onto a column of Dowex 2X8 (50 ml) and the column eluted with water (1200 ml). Evaporation of the eluate, co-evaporation of the residue with ethanol and crystallization from ethanol gave a mixture of **20a**–**20d** (2.16 g, m.p. 124–133 °C). Recrystallization of the crude product from methanol (10 ml) gave 1.545 g (70.8%) of a mixture of **20a** and **20b** (**20a:20b** = 1:1), m.p. 155–162 °C. ^{1}H NMR (D_2O): α-D anomer **20a**: 5.70 brm, 1 H (H-1′); 2.48 dt, 1 H, *J* = 6.6, 6.9 and 13.9 (H-2′a); 1.90 dt, 1 H, *J* = 3.5 (2×) and 13.9 (H-2′b); 4.31 dt, 1 H, *J* = 4.1 (2×) and 6.4 (H-3′); 4.01 dt, 1 H, *J* = 4.0 (2×) and 4.7 (H-4′); 3.68–3.58 m, 2 H (H-5′); β-D anomer **20b**: 5.75 brm, 1 H (H-1′); 2.18 ddd, 1 H, *J* = 2.3, 6.0 and 13.9 (H-2′a); 2.07 ddd, 1 H, *J* = 6.7, 7.2 and 13.9 (H-2′b); 4.33 dt, 1 H, *J* = 2.9 (2×) and 6.5 (H-3′); 3.89 td, 1 H, *J* = 2.7 and 4.8 (2×) (H-4′); 3.68–3.58 m, 2 H (H-5′). FAB-MS: 437 (9) [2 $M_{\mathbf{20a}\text{ and }\mathbf{20b}}$ + H]$^+$, 219 (87) [$M_{\mathbf{20a}\text{ and }\mathbf{20b}}$ + H]$^+$, 60 (25) [M_{10} + H]$^+$. For $C_7H_{14}N_4O_4$ (218.2) calculated: 38.53% C, 6.47% H, 25.68% N; found: 38.49% C, 6.63% H, 25.46% N.

Mass spectrum of a 1:1 Mixture of 2′-Deoxy-5-azacytidine (**2**) and the Anomeric 2-(D-*erythro*-2-Deoxypentofuranosylcarbamoyl)guanidines (**20a** and **20b**)

A mixture of **2** (5 mg) and and a 1:1 mixture of **20a** and **20b** (5 mg) was powdered in an agate mortar and the sample used for measurement. FAB-MS: 479 (5) [2 M_2 + Na]$^+$, 469 (4) [M_2 + $M_{\mathbf{20a}\text{ or }\mathbf{20b}}$ + Na]$^+$, 459 (2) [2 $M_{\mathbf{20a}\text{ or }\mathbf{20b}}$ + Na]$^+$, 457 (7) [2 M_2 + H]$^+$, 447 (14) [M_2 + $M_{\mathbf{20a}\text{ or }\mathbf{20b}}$ + H]$^+$, 437 (12) [2 $M_{\mathbf{20a}\text{ or }\mathbf{20b}}$ + H]$^+$, 251 (11) [M_2 + Na]$^+$, 241 (13) [$M_{\mathbf{20a}\text{ or }\mathbf{20b}}$ + Na]$^+$, 229 (14) [M_2 + H]$^+$, 219 (100) [$M_{\mathbf{20a}\text{ or }\mathbf{20b}}$ + H]$^+$, 113 (79) [aglycone$_2$ + H]$^+$, 103 (10) [aglycone$_{\mathbf{20a}\text{ or }\mathbf{20b}}$ +H]$^+$, 60 (30) [M_{10} + H]$^+$.

4-*O*-Methyl-1-(α-D-ribofuranosyl)isobiuret (**22**)

A mixture of methoxytriazinone[28] **4** (0.259 g, 1 mmol), methanol (7 ml) and *tert*-butylamine (0.7 ml) was stirred at room temperature for 26 h. The solution was refluxed for 2 h, kept at room temperature overnight and evaporated. The residue was co-evaporated with ethanol (3 × 5 ml) and crystallized from the same solvent to yield 0.105 g of isobiuret **22**, m.p. 158–160 °C (dec.). Recrystallization of the crude product from ethanol afforded 0.080 g (32%) of pure isobiuret **22**, m.p. 170–172 °C (dec.), R_F 0.58 (C, D_4), $[\alpha]_D$ +123.8 (*c* 0.3, water). UV (MeOH), λ_{max} (log ε): 220 (4.27). CD (pH 6.93), λ_{max} ($[\Theta]_{max}$): 223 (+10 170). IR (Nujol): 3390 s, 3296 s, 3234 m, sh [ν(OH, NH_2, NH)]; 1665 s (amide I); 1631 m, sh [ν(C=N)]. ^{1}H NMR: 8.10 br s, 1 H and 7.70 br s, 1 H (NH_2); 6.40 d, 1 H, *J*(NH,1′) = 9.8 (NH); 5.40 dd, 1 H, *J*(1′,2′) = 4.9, *J*(1′,NH) = 9.8 (H-1′); 5.25 d, 1 H, *J*(OH,2′) = 5.0 (OH); 5.08 d, 1 H, *J*(OH,3′) = 5.5 (OH); 4.66 t, 1 H, *J*(OH,5′) = 5.6 (OH); 3.91 q, *J*(2′,1′) = *J*(2′,OH) = *J*(2′,3′) = 5.0 (H-2′); 3.85 dt, 1 H, *J*(3′,2′) = *J*(3′,4′) = 5.0, *J*(3′,OH) = 5.5 (H-3′); 3.70 td, 1 H, *J*(4′,5′a) = 3.7, *J*(4′,5′b) = *J*(4′,3′) = 4.9 (H-4′); 3.65 s, 3 H (OCH_3); 3.42 ddd, 1 H, *J*(5′a,4′) = 3.7, *J*(5′a,OH) = 5.2, *J*(gem) = 12.0 (H-5′a); 3.34 ddd, 1 H, *J*(5′b,4′) = 4.9, *J*(5′b,OH) = 5.8, *J*(gem) = 12.0 (H-5′b). ^{13}C NMR: 163.20 (C=N), 163.04 (C=O), 82.79 (C-1′), 80.64 (C-4′), 71.44 (C-2′), 70.05 (C-3′), 61.93 (C-5′). FAB-MS: 250 (45) [M_{22} + H]$^+$. For $C_8H_{15}N_3O_6$ (249.2) calculated: 38.56% C, 6.07% H, 16.86% N, 12.45% OCH_3; found: 38.67% C, 6.12% H, 16.85% N, 12.37% OCH_3.

4-*O*-Methyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)isobiuret (**23**)

A stirred mixture of powdered 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (3.18 g, 10 mmol) and dry toluene (50 ml) was saturated with dry hydrogen chloride for 4 h. The solution was kept at room temperature overnight with exclusion of moisture, evaporated and the residue co-evaporated with toluene (4 × 30 ml) to give syrupy 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride. A stirred mixture of this product with dry toluene (50 ml) and silver cyanate (5.25 g, 35 mmol) was heated within 30 min to 100 °C and kept at this temperature for 1 h. The silver salts were filtered off and the filtrate evaporated to give syrupy 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl isocyanate. A solution of this product in dry acetone (20 ml) was added dropwise at 0 °C with exclusion of moisture to a stirred solution of freshly prepared *O*-methylisourea (0.66g, 9 mmol) in acetone (10 ml). The reaction mixture was kept at room temperature for 30 min and evaporated to give crude **23** as thick syrup. A solution of this product in benzene (30 ml) was shaken with water (30 ml), kept at room temperature for 1 h and the crystalline precipitate filtered off with suction to give 3.16 g (80%) of the monohydrate of **23**, m.p. 70–76 °C, R_F 0.92 (B, D_3), $[\alpha]_D$ –190 (*c* 0.35, DMF). UV (EtOH), λ_{max} (log ε): 221 (4.25). CD (MeOH–H_2O, 9:1), λ_{max} ($[\Theta]_{max}$): 225 (–3450). ^{1}H NMR: 8.10 br, 1 H and 7.70 br, 1 H (NH_2); 7.87 d, 1 H, *J*(NH,1′) = 9.9 (NH); 5.42 dd, 1 H, *J*(1′,2′) = 6.0,

J(1′,NH) = 9.9 (H-1′); 5.21 dd, 1 H, *J*(3′,4′) = 4.1, *J*(3′,2′) = 5.6 (H-3′); 5.11 t, 1 H, *J* = 5.8 (H-2′); 4.20 m, 1 H (H-5′a); 4.03 m, 2 H (H-4′ and H-5′b); 3.68 s, 3 H (OCH_3); 2.05 s, 6 H and 2.045 s, 3 H (3 × OAc). FAB-MS: 376 (45) $[M_{23} + H]^+$. For $C_{14}H_{21}N_3O_9 \cdot H_2O$ (393.4) calculated: 42.75% C, 5.89% H, 10.68% N; found: 43.02% C, 5.75% H, 10.75% N. The anhydrous product was obtained in amorphous form on dissolution of the crystalline hydrate in chloroform (100 ml), drying of the solution with anhydrous sodium sulfate, evaporation of chloroform and drying of the syrupy residue at 40 °C *in vacuo*. For $C_{14}H_{21}N_3O_9$ (375.3) calculated: 44.80% C, 5.64% H, 11.20% N; found: 45.10% C, 5.72% H, 10.90% N. The amorphous water-free product is a more convenient intermediate for the synthesis of 5-azacytidine than the crystalline monohydrate due to formation of side-products during condensation with ethyl orthoformate[1].

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

A mixture of isobiuret **22** (0.249 g, 1 mmol), methanol (5 ml) and DMF dimethyl acetal (0.13 ml, 1 mmol) was stirred at room temperature for 24 h and kept overnight in a refrigerator. The product was filtered off by suction and recrystallized from ethanol to give 0.130 g (45%) of the hydrate of the α-D anomer **24**, m.p. 117–128 °C (dec.), R_F 0.38 (C, D_1), $[\alpha]_D$ –77 (*c* 0.3, water). ^{1}H NMR: 8.23 s, 1 H (H-6); 6.04 d, 1 H, *J*(1′,2′) = 4.1 (H-1′); 5.52 d, 1 H, *J*(OH,2′) = 4.9 (OH); 5.14 d, 1 H, *J*(OH,3′) = 6.1 (OH); 4.80 dd, 1 H, *J*(OH,5′) = 5.0 and 6.3 (OH); 4.12 brq, *J* = 4.5 (H-2′); 4.07 ddd, 1 H, *J*(3′,2′) = 4.6, *J*(3′,OH) = 6.0, *J*(3′,4′) = 6.8 (H-3′); 4.02 ddd, 1 H, *J*(4′,5′a) = 2.7, *J*(4′,5′b) = 4.1, *J*(4′,3′) = 6.8 (H-4′); 3.60 ddd, *J*(5′a,4′) = 2.7, *J*(5′a,OH) = 4.9, *J*(gem) = 12.2 (H-5′a); 3.43 ddd, 1 H, *J*(5′b,4′) = 4.1, *J*(5′b,OH) = 6.4, *J*(gem) = 12.2 (H-5′b); 3.16 s, 3 H and 3.04 s, 3 H (Me_2N, CH_3). FAB-MS: 295 (1) $[M_{24} + Na]^+$, 273 (35) $[M_{24} + H]^+$, 163 (2) $[aglycone_{24} + Na]^+$, 141 (100) $[aglycone_{24} + H]^+$. For $C_{10}H_{16}N_4O_5 \cdot H_2O$ (290.3) calculated: 41.38% C, 6.25% H, 19.30% N; found: 41.51% C, 5.98% H, 19.59% N.

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