PREPARATION OF ANALOGUES OF CYTOSINE AND 2-PYRIMIDINONE NUCLEOSIDES AND THEIR EFFECT ON BACTERIAL (*Escherichia coli* A19) CYTIDINE AMINOHYDROLASE

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The set of compounds investigated as substrates and inhibitors of bacterial cytidine aminohydrolase (EC 3.5.4.5) consists of cytidine analogues modified in the heterocyclic base or the sugar moiety and analogues of the similar type derived from 1-(β-D-ribofuranosyl)-2-pyrimidone (*I*) and its isomers. The latter group of compounds includes also open-chain derivatives of neutral and acidic character. These compounds were prepared by novel synthetic procedures.

Minimum necessary conditions for the structure of an inhibitor of cytidine aminohydrolase from *E. coli* A19 include: a heterocyclic system containing an Rf—N—CO—N(H) fragment of a basic character in which Rf denotes a β-D-aldopentafuranoside with a 3-hydroxy group of *ribo*-configuration; the 5-hydroxy group of the sugar moiety may bear a substituent, except a phosphomonoester function. The heterocyclic base may also bear substituents in positions other than α to the nucleoside bond which do not reduce substantially the basicity of the system and do not change the conformation of the nucleoside molecule.

2-Pyrimidone nucleosides belong to interesting targets of investigation in the field of analogues of nucleic acid components. They may be regarded as parent compounds of both cytosine and uracil nucleosides and they display some of their characteristic properties without, however, exhibiting effects connected with the presence of an amino or hydroxy group in the position 4. Among such characteristic properties belongs also the ability to participate in hydrogen bond formation in biological (enzymatic as well as non-enzymatic) systems. For this reason considerable attention was paid *e.g.* to oligodeoxynucleotides containing these analogues[1,2]. 1-(β-D-Ribofuranosyl)-2-pyrimidone (*I*) which was prepared by us some time ago[3], is attractive mainly because of its specific antibacterial activity[4]. The mechanism of its action consists in the *in vivo* transformation of compound *I* into 1-(2-deoxy-β-D-ribofuranosyl)-2-pyrimidone 5'-phosphate which is a potent reversible inhibitor of thymidylate synthetase[5,6]. According to the present relatively detailed knowledge of the chemical

principles of inhibition of this enzyme, it seems that this activity of the 2-pyrimidone derivative is caused by the exceptionally high susceptibility of the 5,6-double bond towards addition reactions. This view is supported also *e.g.* by the easy intramolecular cyclization of the compound *I* into a 5',6-anhydro-6-hydroxy-1,2,3,6-tetrahydro--2-pyrimidone derivative[7]. However, the compound *I* which mediates the suppression of *de novo* synthesis of bacterial DNA by the mentioned mechanism, does not exhibit other important biological effects *in vivo*: it has no antiviral effect against the vesicular stomatitis virus, vaccinia virus or HSV-1 (ref.[8]) in concentrations up to $2 . 10^{-3}$ mol l^{-1} and, although its inhibitory effect on both L-1210 mouse leukemic cells and P-388 leukemia in mice *in vivo* at a high dosage (400 mg/kg) was reported[9], our results did not confirm these observations.

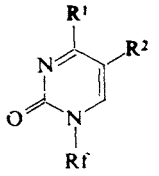
Recently it has been found that the compound *I*, as well as its 5-halogeno derivatives, is a significant inhibitor of cytidine deaminases of mouse kidney, rhesus monkey liver and yeast[9]. Cytidine deaminases (cytidine aminohydrolases, EC 3.5.4.5) play an important role in the catabolism of cytosine nucleosides and nucleotides and in the processes of the salvage pathway which reutilize uracil derivatives. Although these reactions can hardly be regarded as the targets for antimetabolic effects of nucleoside analogues they are nevertheless important, causing an undesired degradation of cytosine antimetabolites (*e.g.* cytosine arabinoside) used in chemotherapy. Therefore, a search for new effective inhibitors of these enzymes appears to be very desirable. Of the known compounds of this character, tetrahydrouridine[10] is the most effective inhibitor; its *in vitro* activity is one or two orders of magnitude higher than that of the compound *I*. However, it is not easily accessible, less stable and more toxic.

In the past years, our studies on antibacterial activity of 2-pyrimidone nucleoside resulted in a detailed elaboration of synthetic methods leading to analogues of *I* modified in the heterocyclic base as well as in the sugar moiety[11–13]. We have now performed a structure-activity study of 2-pyrimidone derivatives, aimed at determination of structural features required for cytidine deaminase inhibitors. This investigation was performed with an enzyme isolated from *Escherichia coli*[14]; the inhibitory activity of the parent compound *I* and of its analogues against this enzyme has not been determined as yet. The series comprises pyrimidine nucleosides with a modified heterocyclic or sugar component as well as analogues of *I* including novel compounds in which the sugar moiety is replaced by an aliphatic chain bearing hydroxy or carboxy groups. The inclusion of these "open-chain" analogues was motivated by the fact that some compounds of this type (*e.g.* purine derivatives), when interacting with enzymes, can simulate the structure (conformation) of the nucleoside molecule and form EI-complexes[15]. Since such 2-pyrimidone derivatives are chemically and enzymatically relatively stable (contrary to 2-pyrimidone nucleosides whose nucleoside bond is easily cleaved chemically as the result of the above-mentioned intramolecular cyclization, or by the action of enzymes under *in vivo* conditions),

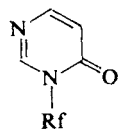
the open-chain derivatives might be particularly useful as ligands for purification of cytidine deaminases by affinity chromatography.

Synthesis of Compounds

The β-D-ribofuranosides of 2-pyrimidone and related pyrimidine bases (*I*–*XI*) were prepared by procedures described in our previous communications (see Experimental). Instead of the published condensation of mercury salt[16], the 4-methyl--2-pyrimidone derivative *III* was prepared by nucleosidation according to Vorbrüggen[17] which consists in the reaction of 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose with the trimethylsilyl derivative *XIIb* in the presence on tin tetrachloride. Methanolysis of the obtained perbenzoyl nucleoside *XIII* afforded the compound *III* whose constants agree with those of an authentic compound[16].



I; $R^1 = R^2 = H$
II; $R^1 = H$, $R^2 = CH_3$
III; $R^1 = CH_3$, $R^2 = H$



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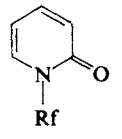
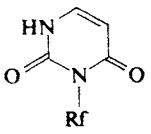


V



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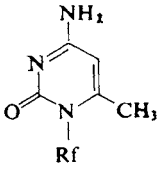
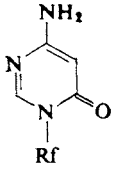
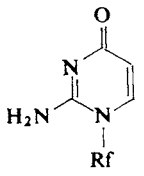


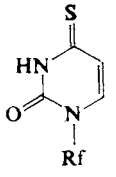
VII



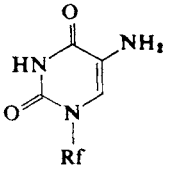
VIII



IX



X



XI

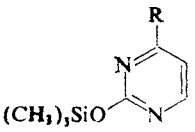
In formulae *I*–*XI*, Rf = β-D-Ribofuranosyl residue.

In this connection, we prepared also 6-methylcytidine (*VII*) by transformation of 6-methyluridine which makes use of the tribenzoate *XIV*, accessible by a previously described stereospecific synthesis[18]. The compound *XIV* was first converted into the 4-thiouracil derivative *XV* by reaction with phosphorus pentasulfide followed by methylation which afforded the compound *XVI*. This product was ammonolyzed to give 6-methylcytidine (*VII*), identical with authentic samples prepared by independent syntheses[19,20].

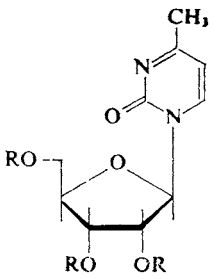
The group of 2-pyrimidone derivatives with modified sugar moiety (formulae *XVII*–*XXII*) includes the previously described derivatives of isomeric aldopentoses[12,13]; for the study of the effect of the phosphomonoester group we prepared the 5'-nucleotide *XXII* derived from the compound *I* by the previously described procedure[11], *i.e.* by treatment of the compound *I* with phosphorus oxychloride in triethyl phosphate[21]. The isolation of the pure product *XXII* from the reaction mixture is difficult because of the necessity to remove a great excess of inorganic salts which causes large losses. An efficient purification can be achieved by chromatography on a strongly acid ion-exchange resin (the compound *I* is basic enough to be completely retained whereas the phosphomonoester *XXII* is only retarded; it can be easily separated both from the inorganic salts and the unreacted compound *I*). The product obtained was isomerically as well as chemically pure according to HPLC To verify the effect of the 5'-hydroxy group (which is involved in the above-mentioned interaction in *I*) on the inhibitory activity we prepared also the 5'-O-methyl derivative *XXI*. It was synthesized from the sugar component *XXVII* obtained from methyl 2,3-O-isopropylidene-D-ribofuranoside *XXIII* (ref.[22]) in the following way: The compound *XXIII* was first treated with methyl iodide in the presence of sodium hydride to give the 5-O-methyl derivative *XXIV* and the protecting groups in positions 1, 2 and 3 were removed by acid hydrolysis. Re-glycosidation with methanol afforded methyl 5-O-methyl-D-ribofuranoside (*XXV*) which was subsequently converted into the 2,3-dibenzoate *XXVI* and the 1-O-acetate *XXVII*. The structure of the above intermediates and of the final synthone *XXVII* was confirmed by ^{1}H NMR. spectra. Condensation of compound *XXVII* with 2-pyrimidone O-trimethylsilyl derivative *XIIa* (ref.[13]) in the presence of tin tetrachloride in acetonitrile afforded the pure crystalline dibenzoyl derivative *XXVIII*. Its ^{1}H NMR spectrum proved its structure and anomeric homogeneity (the formation of the β-anomer was predetermined by the presence of the 2-O-benzoyl group in compound *XXVII*). Methanolysis of compound *XXVIII* gave finally the pure nucleoside *XXI*.

The synthone *XXVII* was used also in the preparation of additional two compounds: its reaction with 2,4-bis(trimethylsilyloxy)pyrimidine (*XIIc*) and tin tetrachloride gave the pure 2',3'-di-O-benzoyl-5'-O-methyluridine (*XXIX*), whose structure and anomeric purity was again proved by its ^{1}H NMR spectrum. 5'-O-Methyluridine (*XXXII*) was obtained from the dibenzoate *XXIX* by direct methanolysis. The reaction of compound *XXIX* with phosphorus pentasulfide, followed by methylation

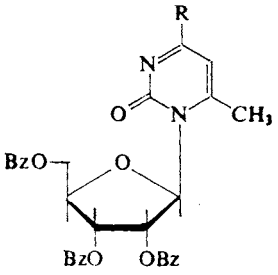
of the intermediate *XXX* and ammonolysis of the thus-obtained 4-methylthio--2-pyrimidone nucleoside *XXXI* afforded finally 5'-O-methylcytidine (*XXXIII*). After purification by chromatography on an ion-exchange resin the product was identical with the authentic compound prepared by another route[23,24]. Although the described method of preparation of 5'-O-methyl derivatives of uridine and cytidine requires the preparation of the synthone *XXVII*, it affords isomerically completely pure products and is more suitable for preparation of larger quantities than the earlier methods based on methylation of protected cytidine derivatives followed by their deamination.



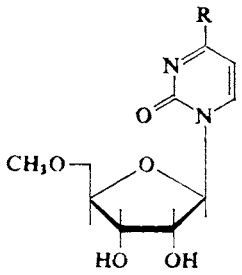
XIIa, R = H
XIIb, R = CH_3
XIIc, R = $OSi(CH_3)_3$



III, R = H
XIII, R = C_6H_5CO

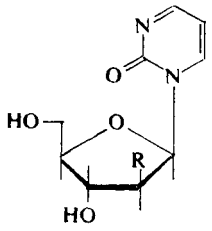


XIV, R = OH
XV, R = SH
XVI, R = SCH_3
Bz = C_6H_5CO

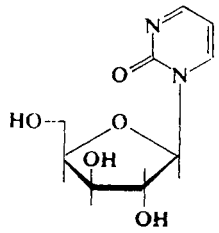


XXI, R = H
XXII, R = OH
XXIII, R = NH_2

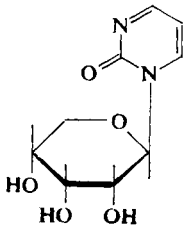
The group of the open-chain analogues (formulae *XXXIV–XLIV*) comprizes neutral 2-pyrimidone N-hydroxyalkyl derivatives (*XXXVII–XL*) which are suitable for study of the importance of an intact sugar moiety in the molecule of *I* for its inhibitory activity, and compounds of acidic character (and their derivatives) which



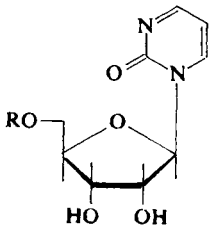
XVII, R = H
XVIII, R = OH



XIX



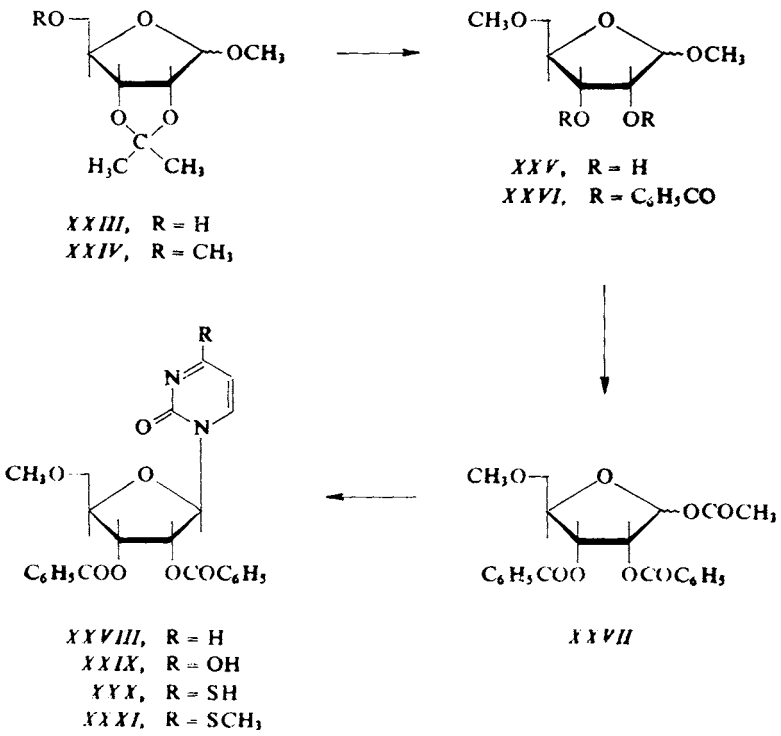
XX



I, R = H
XXI, R = CH_3
XXII, R = $P(O)(OH)_2$

might be useful for binding to activated polymeric carriers. All these derivatives were invariably prepared by reactions analogous to those used for synthesis of similar purine and pyrimidine compounds. The condensations of sodium salt of 2-pyrimidone (*XLV*) with suitable aliphatic synthones containing a primary *p*-toluenesulfonyloxy group were always accompanied by formation of the corresponding undesirable O-isomers (*e.g. LI* or *LV*). We did not succeed in suppression of their formation by changing the experimental conditions such as solvent, base, reaction temperature *etc.* An optimal amount of the desired N-isomers was obtained in dimethylformamide as a solvent. On the other hand, an exclusive formation of the O-isomers was observed on replacement of the sodium salt of the compound *XLV* by its trimethylsilyl derivative *XIIa* in the reactions with synthones containing a halogen atom instead of the *p*-toluenesulfonyl group. Since the attempted O→N migration in these compounds in the presence of Lewis acids in acetonitrile[3] was unsuccessful (*e.g.* treatment with tin tetrachloride resulted in a profound destruction of the O-isomer), no further attention was paid to these products. The O-isomers have completely different chromatographic and UV spectral properties than the N-isomers from which they can be easily separated.

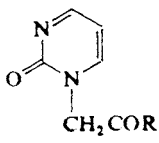
1-(*RS*)-(2,3-Dihydroxypropyl)-2-pyrimidone (*XXXVII*) was described already previously[25]; its homologue, 1-(*RS*)-*erythro*-(2,3-dihydroxybutyl)-2-pyrimidone



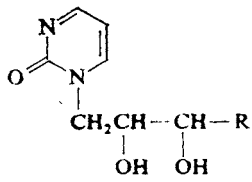
(*XXXVIII*), was prepared by reaction of the *p*-toluenesulfonyl derivative *XLVI* (ref.[26]) with the salt *XLV*, followed by acid hydrolysis of the intermediate *XLVII*. Similarly was synthesized 1-(*S*)-(3,4-dihydroxybutyl)-2-pyrimidone (*XL*) from the asymmetric synthone *LII* (ref.[26]). Condensation of the sodium salt *XLV* with another asymmetric synthone *XLVIII* (ref.[27]) afforded 1-(2*S*,3*S*)-*threo*-(2,3,4-trihydroxybutyl)-2-pyrimidone (*XXXIX*). In this case we isolated the fully protected intermediate *XLIX* which on methanolysis gave the debenzoylated derivative *L*; the acid hydrolysis of the latter compound gave the free triol *XXXIX*. (In this synthesis we analyzed and characterized also the O-isomer *LI*.) The above group which includes two racemic compounds *XXXVII* and *XXXVIII*, two homologues of the same absolute configuration 3*S*, and the pair of *erythro*- and *threo*-isomers of the 2,3-dihydroxybutyl derivatives *XXXVIII* and *XXXIX*, comprizes all the combinations required for evaluation of the effects of diastereoisomerism and absolute configuration in this series of compounds.

Purine ω-carboxyalkyl derivatives are very potent inhibitors[28] of certain enzymes and effective ligands for their purification by affinity chromatography[29]. Therefore, we prepared such compounds also in the 2-pyrimidone series: 1-(carboxymethyl)-

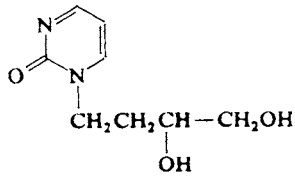
-2-pyrimidone (*XXXIV*), its esters and amides (*e.g. XXXV* or *XXXVI*) have been recently described in one of our communications[30]. The α-hydroxy acid *XLI* was synthesized by a reaction elaborated for the purine derivatives[31]: sodium salt of compound *XLV* reacted with bromoacetaldehyde acetal to give the N-isomer *LIV* (this reaction gave a relatively good yield of the N-isomer whereas the silyl derivative *XIIa* reacted with the bromoacetal under exclusive formation of the O-isomer *LV*). The compound *LIV* was transformed in an acid solution into the free aldehyde which *in situ* reacted with sodium cyanide in a neutral medium. Acid hydrolysis gave the racemic acid *XLI* which was isolated by chromatography on a column of anion exchange resin. The methyl ester *XLII*, prepared by acid-catalyzed esterification of *XLI*, was converted by ammonolysis into the amide *XLIII*. The homologous (2*R*,3*R*)-*erythro*-4-(2-pyrimidon-1-yl)-2,3-dihydroxybutanoic acid *XLIV* which is an analogue of D-eritadenine was obtained by one of the procedures used for the



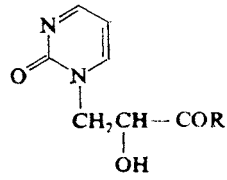
XXXIV, R = OH
XXXV, R = OCH_3
XXXVI, R = $NHC(CH_2OH)_3$



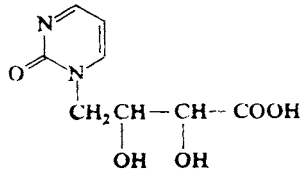
XXXVII, R = H
XXXVIII, R = CH_3
XXXIX, R = CH_2OH



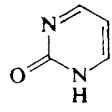
XL



XLI, R = OH
XLII, R = OCH_3
XLIII, R = NH_2



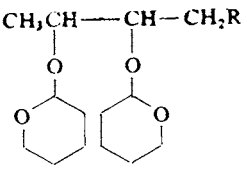
XLIV



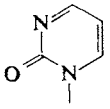
XLV

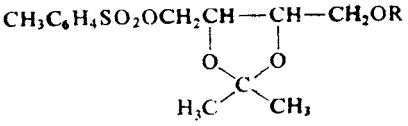
preparation of this purine antibiotic[22], *i.e.* by condensation of 2,3-O-cyclohexylidene-D-erythronolactone (*LVI*) with sodium salt of the base *XLV* followed by acid hydrolysis.

The described ω-carboxyalkyl derivatives can be bound to polymeric carriers with terminal hydroxyalkyl or aminoalkyl groups; similarly, 2-pyrimidone N-(aminoalkyl) derivatives might be bound to carboxyalkyl polymers[29]. We tried to prepare a derivative of this type by the following reaction sequence: The symmetric *threo*-ditosyl derivative *LVII* (ref.[27]) was condensed with sodium salt of the base *XLV* to give the compound *LVIII*. Although the nucleophilic substitution by the azide ion for the primary tosyloxy group proceeded smoothly, the azido derivative *LIX* could not be converted into the amino compound *LX*. The hydrogenolysis on a palladium catalyst afforded the amino derivative (as evidenced by positive ninhydrine reaction) but a simultaneous reduction of the heterocyclic system took place during the procedure (as witnessed by the disappearance of the 2-pyrimidone chromophore). This observation is in accord with the previous report on easy reduction of 2-pyrimidone system[11].



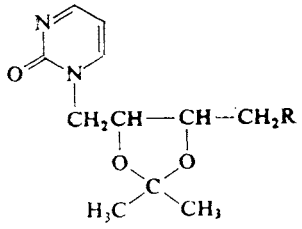
XLVI, R = $OSO_2C_6H_4CH_3$-*4*

XLVII, R = 



XLVIII, R = C_6H_5CO

LVII, R = $SO_2C_6H_4CH_3$-*4*



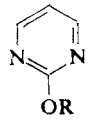
XLIX, R = C_6H_5COO

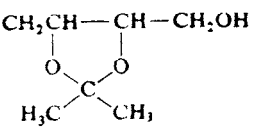
L, R = OH

LVIII, R = $OSO_2C_6H_4CH_3$-*4*

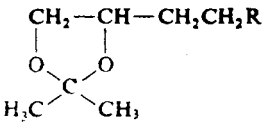
LIX, R = N_3

LX, R = NH_2



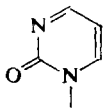
LI, R = 

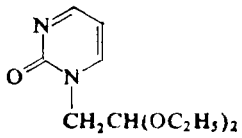
LV, R = $CH_2CH(OC_2H_5)_2$



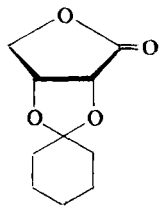
LII, R = $-OSO_2C_6H_4CH_3$-4

LIII, R =





LIV



LVI

All the newly synthesized compounds were extensively purified by usual procedures until they were homogeneous. The final purification of the products was done by chromatography on microcrystalline cellulose in the system 2-propanol–aqueous ammonia; this procedure was very effective in the purification of not only 2-pyrimidone derivatives but also of 6-methylcytidine (*VII*), isocytidine (*IX*), 4-thiouridine (*X*) and 5-aminouridine (*XI*). Compounds derived from 2-pyrimidone and its methyl derivatives were invariably accompanied with yellow impurities which were difficult to remove. The purification can be effectively achieved by filtration of their aqueous solutions through octadecyl-silica gel (N-alkyl-2-pyrimidones were only slightly retained). In addition to the analytical data, the compounds were characterized by their mass spectra and UV spectra; 2-pyrimidone derivatives exhibit characteristic absorption maxima at 300–310 nm.

Enzymatic Studies

Cytidine deaminase used in this study was a partially purified preparation from *E. coli* A19; we used this material because highly purified cytidine deaminase preparations are known to be very unstable[14]. To prevent the loss of the catalytic activity of this preparation on storage, it was supplemented by the addition of serum albumin or glycerol. We studied first the substrate activity of some cytidine analogues for which these data were not known (compounds *VII*–*XI*, *XXXIII*); this test was carried out at higher concentration of the substrates and an excess of the enzyme and the mixture was analyzed by HPLC. In this context, the most important finding is the resistance of cytidine isomers, 6-amino-4-pyrimidone *VIII*, and isocytidine *IX* towards the enzyme. The uridine derivative *XI* in which the amino group is in the position 5, *i.e.* neighbouring to the amino group of cytidine, is also stable against deamination. These findings confirm the high specificity of the enzyme towards the heterocyclic base of the substrate. Equally important is the structure of the sugar moiety and the presence of its functional groups in the substrate molecule. We found that 5'-O-methylcytidine (*XXXIII*) is not affected by bacterial cytidine deaminase; thus the 5'-hydroxy group is decisive for the substrate binding and one may feel justified to ask whether the known resistance of CMP against the bacterial enzyme

(as well as against cytidine deaminases from other sources) is due to the presence of the phosphate group or rather to the absence of the free 5'-hydroxy group. Since the structure of both the base and sugar component is important, it is not surprizing that also 6-methylcytidine (*VII*) is resistant against the action of the enzyme; the bulky methyl group in position 6 (in the neighbourhood of the nucleoside linkage) enforces the *syn*-conformation[32] the transition of which into conformation corresponding to the topochemistry of the active site is obviously energetically prohibitive.

However, the main objective of our investigations was a search for new cytidine deaminase inhibitors and determination of minimal structural requirements of such inhibitors. For this reason we determined the initial rates of deamination of [^{14}C]--cytidine for substrate: inhibitor ratio 2 : 1. The obtained values of the relative rates, v_i/v_0 (related to the uninhibited reaction) are listed in Table I.

A) *Effect of structure of the heterocyclic base.* This investigation was performed with a group of compounds, all of which contained the β-D-ribofuranoside unit. Since the cytidine isomer *VIII*, which is not a substrate, does not even inhibit the enzyme, it is obvious that under the conditions employed it is not bound to the

TABLE I
Inhibition of bacterial cytidine deaminase

Compound	v_i/v_0[a]	Compound	v_i/v_0[a]
I	0·07	*XX*	0·34
II	0·18	*XXI*	0·50
III	0·16	*XXII*	0·83
IV	1·00	*XXXIII*	0·84
V	1·08	*XXXIV*	1·02
VI	1·10	*XXXV*	1·01
VII	1·13	*XXXVI*	0·96
VIII	0·87	*XXXVII*	1·16
IX	0·78	*XXXVIII*	1·18
X	1·06	*XXXIX*	1·10
XI	0·96	*XL*	1·16
XVII	0·14	*XLI*	0·76
XVIII	0·16	*XLII*	1·05
XIX	1·01	*XLIII*	1·08
		XLIV	0·95

[a] Initial rates of [^{14}C]cytidine deamination in the presence (v_i) and absence (v_0) of compound tested.

enzyme. Also 4-thiouridine (*X*) or 5-aminouridine (*XI*) are not inhibitors and the effect of isocytidine (*IX*) on the deamination of cytidine is only marginal. As expected, 1-(β-D-ribofuranosyl)-2-pyrimidone (*I*) inhibits bacterial cytidine deaminase equally efficiently as the above-mentioned enzymes from liver, kidney or yeast. A somewhat weaker effect is exhibited by its 5-methyl derivative *II*. The position 5 of the pyrimidine nucleus is not decisive for the interaction with the enzyme (as demonstrated[9] also by the effect of 5-halogeno derivatives of compound *I*). Also the inhibitory effect of the isomeric 4-methyl derivative *III* is almost as strong as that of the 5-isomer *II*. The presence of a substituent in position 4 of the heterocyclic ring is thus of no great importance.

A comparison of the compound *I* with its isomer, 3-(β-D-ribofuranosyl)-4-pyrimidone (*IV*), and its deaza-analogue, 2-pyridone nucleoside *V*, shows that the structural fragment $-N_{(1)}-C(=O)-N_{(3)}-$ is necessary in the heterocyclic base of the inhibitor. Therefore. the cytidine isomer *VIII* cannot be an inhibitor (nor, apparently, a substrate). The nitrogen atom $N_{(3)}$ may be even protonated: *e.g.* the tetrahydro derivative of compound *I* and the 4-hydroxy derivative derived from it ("tetrahydrouridine") are very potent enzyme inhibitors[9], as well as the so-called nucleosides of cyclic ureas[7]. Naturally, the mentioned fragment is present also in other systems, *e.g.* in uridine or 5-aminouridine (*XI*) or even in 3-(β-D-ribofuranosyl)uracil (*VI*), which in one of its tautomeric forms is a 6-hydroxy-2-pyrimidone derivative; however, none of these compounds inhibits cytidine deaminase. The explanation may consist in basicity of the heterocyclic system (or its mentioned structural fragment): 2-pyrimidone and 4-methyl-2-pyrimidone N_1-methyl derivatives are basic (p*K* of the protonated form = 2·50 and 3·21, respectively) whereas 1-methyluracil and 3-methyluracil have an acidic —NH—CO—N— grouping (p*K* of the anion = 9·77 and 9·90, respectively). Although the N-methyl derivatives which correspond to the nucleosides *IV* and *VIII* are also derived from a basic system (p*K* of the protonated form = = 0·98 and 1·84, respectively), they do not contain the above-mentioned structural fragment (the p*K* values are taken from ref.[33]).

6-Methylcytidine (*VII*) is neither a substrate nor an inhibitor: this proves the importance of the conformation of the substrate molecule as a whole (including the sugar part) for the binding to the enzyme (*vide infra*).

B) *Effect of structure of the sugar moiety*. To assess the importance of the sugar moiety and its functional groups we compared the inhibitory effect of various 2-pyrimidone nucleosides. Comparison of the aldopentafuranosides *I*, *XVII*–*XIX* shows that the presence and configuration of the hydroxy group in position 2′ is evidently of minor significance (the 2-deoxyribofuranoside *XVII* and the arabinofuranoside *XVIII* are effective inhibitors), whereas the *ribo* configuration of the 3′-hydroxy group is indispensable (the β-D-xylofuranoside *XIX* is completely inactive). Since the configuration of the β-D-ribopyranoside *XX* obviously does not

meet the steric requirements for a substrate/inhibitor, this compound is much weaker inhibitor than the isomeric ribofuranoside *I*.

The presence of a free 5'-hydroxy group in the sugar part of the nucleoside is of considerable importance for some enzymes (*e.g.* adenosine aminohydrolase). O-Methylation in this position of compound *I* (compound *XXI*) results in a substantial reduction of the inhibitory activity. A still stronger effect is achieved by introduction of the polar phosphomonoester group to the 5'-hydroxy group: the 5'-nucleotide *XXII* shows practically no inhibition at all. This observation agrees with the resistance of CMP as substrate of cytidine deaminases (*vide supra*).

Replacement of the furanoside ring of the nucleoside by a carbon chain bearing primary or secondary hydroxy groups does not lead to any compounds of inhibitory character. This is in contrast to the adenine series, in which these derivatives can substitute adenosine as a substrate or product of the reaction (adenosine aminohydrolase, S-adenosyl-L-homocysteine hydrolase[28]). In cytidine deaminase, the sugar moiety (or its certain parts) must be evidently specifically bound to the enzyme; for such a binding the hydroxyalkyl chain is not sufficient.

Of the studied N-(ω-carboxyalkyl) derivatives of 2-pyrimidone only the α-hydroxy acid *XLI* has a weak inhibitory effect. Its ester *XLII* and amide *XLIII* are completely inactive; therefore we can hardly expect the supports containing a chemically bonded compound *XLI* (by means of an ester or amide bond) to have a significant affinity towards cytidine deaminase.

The results of our investigation on the above-mentioned compounds, together with literature data on cytidine deaminase inhibitors from other sources, may be summarized to give the following minimal structure of an inhibitor of this enzyme: such inhibitor should be a β-D-aldopentafuranoside with a 3'-hydroxy group in the *ribo*-configuration whose aglycone is a heterocyclic system containing an Rf—N—C—CO—N(H)— fragment (where Rf is the sugar moiety) of basic character. The molecule may be substituted in positions other than α to the nucleoside bond with groups which do not considerably reduce its basicity and do not affect substantially the molecular conformation. The sugar moiety of the inhibitor may be substituted in position 5' but not with a phosphomonoester group.

EXPERIMENTAL

Unless stated otherwise, the solutions were evaporated at 40°C/2 kPa and the compounds were dried over phosphorus pentoxide at 13 Pa. Melting points were determined on a Kofler block and are uncorrected. Paper chromatography was carried out on a Whatman paper No 1 in the system S1 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2). Thin-layer chromatography was carried out on Silufol UV_{254} plates in the system S2 chloroform–methanol (95 : 5), S3 chloroform–methanol (9 : 1), S4 chloroform–methanol (4 : 1), and S5 benzene–ethyl acetate (4 : 1) (Table II). Preparative chromatography on silica gel was performed on columns (usually 200 ml) or loose layers (45 × 15 × 0·4 cm) of Silpearl UV_{254} (Kavalier, Czechoslovakia).

Preparative chromatography on cellulose was done on an 80 × 4 cm column of microcrystalline cellulose Macherey & Nagel in the system S1 (elution rate 20 ml h^{-1}, detection with a Uvicord instrument). Basic compounds were deionized on 100 ml columns of Dowex 50X8 (H^+) which

TABLE II
Chromatography data (R_F)

Compound	S1	S2	S3	S4	S5
I	0·58	—	—	0·25	—
II	0·65	—	—	0·35	—
III	0·62	—	—	0·37	—
VII	0·64	—	—	0·11	—
XIII	—	0·50	—	—	—
XIV	—	0·27	—	—	—
XV	—	0·69	—	—	0·63
XVI	—	0·80	—	—	0·40
XXI	0·72	—	—	0·62	—
XXII	0·19	—	—	—	—
XXIII	—	—	—	—	0·26
XXIV	—	—	—	—	0·57
XXVII	—	—	—	—	0·50
XXVIII	—	0·35	—	—	—
XXIX	—	0·50	—	—	—
XXX	—	0·62	—	—	0·65
XXXI	—	0·68	—	—	0·20
XXXII	0·65	—	—	—	—
XXXIII	0·65	—	—	—	—
XXXVII	0·67	—	—	—	—
XXXVIII	0·73	—	—	—	—
XXXIX	0·65	—	—	—	—
XL	0·71	—	—	—	—
XLI	0·45[a]	—	—	—	—
XLII	—	—	—	0·67	—
XLIII	—	—	—	0·20	—
XLIV	0·45[b]	—	—	—	—
XLV	0·58	0·05	0·15	—	—
XLIX	—	0·30	0·68	—	—
L	—	0·06	0·26	—	—
LI	—	0·34	0·52	—	—
LIII	—	0·30	0·31	—	—
LIV	—	0·16	0·40	—	—
LVIII	—	0·22	0·52	—	—

[a] Electrophoretical mobility related to Up: 0·45, [b] 0·47.

after removal of salts with water were eluted with 2·5% aqueous ammonia (the UV-absorbing eluate was collected).

Ultraviolet absorption spectra were measured in aqueous solutions (unless stated otherwise) on a Specord UV—VIS spectrometer. ^{1}H NMR spectra were taken on a Tesla 100 MHz instrument in deuteriochloroform or hexadeuteriodimethyl sulfoxide with hexamethyldisilazane as internal standard; chemical shifts are given in ppm, coupling constants in Hz. High performance liquid chromatography (HPLC) was carried out on 3·3 × 150 mm columns of Separon SIX C18 in 0·1 mol l^{-1} triethylammonium hydrogen carbonate pH 7·5 (flow rate 0·4 ml/min), detection at 254 nm by an LCD 254 instrument with an EZ 11 recorder (Laboratorní přístroje, Prague), sensitivity 0·08 AUFS. Mass spectra were measured on an AEI 902 spectrometer, source temperature 120°C, 70 eV, direct inlet system. Chromatography on octadecyl-silica gel was performed on columns of the sorbent (20 μ, Laboratorní přístroje, Prague) in water (elution rate 3 ml/min, detection with a Uvicord instrument).

1-(β-D-Ribofuranosyl)-2-pyrimidone (*I*)

The title compound was prepared according to ref.[13]. After removal of the benzoyl groups by methanolysis and extraction of methyl benzoate with ether, the aqueous solution of crude compound *I* was concentrated *in vacuo* and chromatographed on a column of cellulose in the system S1. The product fractions were combined, taken down *in vacuo* and the residue dissolved in water (5 ml) and applied on a column of octadecyl-silica gel. After elution with water, the UV-absorbing fraction was taken down *in vacuo*, the residue codistilled with ethanol and dried, affording a chromatographically homogeneous colourless amorphous foam in 80—85% yield (based on the starting tribenzoate of the compound *I*).

1-(β-D-Ribofuranosyl)-4-methyl-2-pyrimidone (*II*)

Tin tetrachloride (6 ml) was added to a solution of 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose (36 mmol) and 2-trimethylsilyloxy-4-methylpyrimidine (b.p. 120°C/2 kPa; 40 mmol) in acetonitrile (300 ml). The mixture was stirred overnight at room temperature under calcium chloride protecting tube, taken down *in vacuo* and the residue was dissolved in chloroform (500 ml). A saturated solution of sodium hydrogen carbonate was gradually added with stirring until the reaction ceased. The mixture was filtered through Celite, the aqueous layer separated, the chloroform solution washed with water (2 × 100 ml), dried over magnesium sulfate and taken down. The residue was purified by chromatography on a column of silica gel (300 ml) in chloroform, affording 12·4 g (62%) of compound *XIII* as an amorphous foam, m.p. 83—85°C. For $C_{31}H_{26}N_2O_8$ (554·4) calculated: 67·14% C, 4·73% H, 5·05% N; found: 66·84% C, 4·69% H, 5·18% N. ^{1}H NMR spectrum (deuteriochloroform): 2·38 (s, 3 H) 4-CH_3; 4·62 (m, 1 H) $H_{4'}$; 4·80 (m, 2 H) 2 $H_{5'}$; 5·80 (br t, $J_{2',3'}$ = 5·5) $H_{2'}$; 5·90 (m, 1 H, $J_{3',4'}$ = 3·5) $H_{3'}$; 6·10 (d, 1 H, $J_{5,6}$ = 7·0) H_5; 6·47 (d, 1 H, $J_{1',2'}$ = 4·2) $H_{1'}$; 7·80 (d, 1 H) H_6; arom. signals 7·20—7·65 (m, 9 H) + 7·75—8·20 (m, 6 H).

A suspension of compound *XIII* (5·5 g; 10 mmol) in 0·05 mol l^{-1} methanolic sodium methoxide (250 ml) was stirred at room temperature overnight, neutralized with Dowex 50X8 (H^+), filtered and the solid was washed with methanol (100 ml). The filtrate was taken down *in vacuo*, the residue dissolved in water (100 ml), extracted with ether (3 × 50 ml) and the aqueous phase concentrated *in vacuo* to a small volume. Further purification was performed as described for compound *I*, affording 1·95 g (80%) of compound *II* as a yellowish amorphous foam. Mass spectrum: 242 (M^+), 212 (M—30), 211 (M—CH_2O), 153 (M—89), 139 (Base + 30), 110 (Base + H). For $C_{10}H_{14}N_2O_5$ (242·2) calculated: 49·58% C, 5·82% H, 11·57% N; found: 49·70% C, 5·88% H, 11·46% N. UV spectrum: (pH 2): λ_{max} 309 nm (ε_{max} 10 000) (agrees with ref.[16]).

6-Methylcytidine (*VII*)

Phosphorus pentasulfide (4 g) was added to a boiling and stirred solution of 2',3',5'-tri-O-benzoyl--6-methyluridine (*XIV*; see[18]; 5·55 g, 9·7 mmol) in dioxane (200 ml) and the stirred mixture was refluxed for further 30 min (quantitative conversion). The hot suspension was filtered through Celite, the layers were separated and the chloroform solution was washed with water (2 × 50 ml), dried over magnesium sulfate, filtered and taken down *in vacuo*. The residue was purified by chromatography on two layers of silica gel in chloroform, the product bands were eluted with methanol and the eluate was taken down affording the amorphous product *XV* (5·55 g; 98%), m.p. 106 to 108°C. For $C_{31}H_{26}N_2O_8S$ (586·6) calculated: 63·47% C, 4·47% H, 4·78% N, 5·47% S; found: 63·16% C, 4·44% H, 4·75% N, 5·25% S. ^{1}H NMR spectrum (deuteriochloroform): 2·24 (s, 3 H) 6-CH_3; 4·60—4·86 (m, 3 H) $H_{4'}$ + 2 $H_{5'}$; 5·71 (br s, 1 H, $J_{1',2'} \approx 1·0$) $H_{1'}$; 6·06—6·16 (m, 2 H) $H_{2'}$ + $H_{3'}$; 6·33 (d, 1 H, $J_{5,NH} = 1·8$) H_5; 9·78 (br, 1 H) NH; arom. signals 7·80—8·10 (m, 6 H) and 7·20—7·60 (m, 9 H).

To a solution of the compound *XV* (5·3 g; 9 mmol) in dichloromethane (60 ml) was added methyl iodide (10 ml), followed by 1 mol l^{-1} sodium methoxide in methanol (8·7 ml). After standing for 1 h at room temperature, the mixture was diluted with water (200 ml) and the organic layer was washed with water (50 ml), dried and taken down *in vacuo*. The residue (compound *XVI*) was heated with 30% methanolic ammonia (200 ml) to 100°C for 8 h in an autoclave. The mixture was taken down *in vacuo*, the residue was dissolved in water (200 ml) and extracted with ether (3 × 100 ml). The aqueous layer was concentrated *in vacuo* to about 50 ml, Dowex 50X8 (H^+) was added to acid reaction and the suspension was applied on a column (100 ml) of the same ion-exchange resin. After washing with water to disappearance of the UV absorption, the column was washed with 2·5% aqueous ammonia and the UV-absorbing ammonia eluate was taken down *in vacuo*. The thus-obtained dry crude compound *VII* was purified by chromatography on cellulose in the system S1, the product-containing fractions were combined, taken down *in vacuo* and the residue was crystallized from ethanol–ether, yielding 1·05 g (46%) of compound *VII*, homogeneous according to chromatography and HPLC and identical with an authentic sample[20]. UV Spectrum (pH 2): λ_{max} 281 nm (ε_{max} 11 000), (pH 7): λ_{max} 273 nm (ε_{max} 8 400).

1-(5-O-Methyl-β-D-ribofuranosyl)-2-pyrimidone (*XXI*)

A solution of methyl 2,3-O-isopropylidene-D-ribofuranoside (*XXIII*, ref.[22]; 42·9 g, 0·21 mol) in dimethylformamide (100 ml) was added to an ice-cooled and stirred suspension of sodium hydride (5·1 g; 0·213 mol) in dimethylformamide (200 ml). After stirring at 0°C for 1 h, methyl iodide (25 ml; 0·4 mol) was added and the mixture was stirred under reflux condenser with a calcium chloride tube at 0°C for 5 h and then at room temperature overnight. The suspension was taken down at 50°C/2 kPa, water (200 ml) was added and the mixture was extracted with ether (4 × 150 ml). This extract was washed with water (3 × 100 ml), dried over magnesium sulfate and the solvent was evaporated *in vacuo*. Distillation of the residue afforded 37·6 g (81%) of compound *XXIV*, b.p. 84—86°C/13 Pa. For $C_{10}H_{18}O_5$ (218·2) calculated: 55·03% C, 8·31% H; found 55·27% C, 8·24% H.

Compound *XXIV* (37·5 g; 0·17 mol) was dissolved in 0·1 mol l^{-1} sulfuric acid in 25% aqueous methanol (200 ml), the solution was heated to 70°C for 4 h, cooled, diluted with water (200 ml) and neutralized (to pH 7·0) with a saturated solution of barium hydroxide. The suspension was taken to the boil with charcoal (1 g) and was filtered through Celite. The filtrate was taken down, the residue codistilled with dioxane *in vacuo* (2 × 100 ml) and dried over phosphorus pentoxide *in vacuo* overnight. A solution of this product in methanol (500 ml) was stirred at 0°C with magnesium sulfate (25 g) and conc. sulfuric acid (2·5 ml) for 6 h and left in a refrigerator

overnight. Calcium carbonate (50 g) was added, the mixture was stirred for 1 h, filtered through Celite which was then washed with methanol and the filtrate taken was down *in vacuo*. After drying at 13 Pa the residue (20·85 g; 68%) did not reduce the Fehling solution. This crude compound *XXV* (0·12 mol) was dissolved in pyridine (150 ml), cooled to 0°C, and benzoyl chloride (35 ml; 0·3 mol) was added dropwise under stirring. The mixture was stirred at 0°C for 4 h and then at room temperature overnight. Water (20 ml) was added and, after stirring for 30 min, the mixture was concentrated *in vacuo* to half of its original volume. The residue was taken up in ethyl acetate (500 ml) and the solution washed with water (4 × 200 ml). The organic layer was taken down *in vacuo*, the residue codistilled with toluene (3 × 100 ml) and dissolved in ether (300 ml). After washing with saturated sodium hydrogen carbonate solution (2 × 50 ml) and water (2 × 50 ml), the solution was dried over magnesium sulfate, filtered and the solvent was evaporated *in vacuo*. The residue in benzene (25 ml) was filtered through a column of alumina (100 g; activity II), eluted with benzene and taken down *in vacuo*. The obtained chromatographically pure compound *XXVI* was dried over phosphorus pentoxide *in vacuo*. ^{1}H NMR spectrum (deuteriochloroform): 3·45 + 3·48 (2 × s, 6 H) OCH_3; 3·67 (2 dd, 2 H; $J_{5,4} = 2{\cdot}4$, $J_{5',4} = 6{\cdot}3$, $J_{gem} = 10{\cdot}6$) 2 H_5; 4·52 (m, 1 H, $J_{3,4} = 6{\cdot}0$) H_4; 5·15 (br s, 1 H, $J_{1,2} = 1{\cdot}0$) H; 5·60 (m, 2 H) H_2 + H_5; arom. protons 7·15–8·17 (for the β-anomer of *XXVI*; β : α = 3 : 1).

The compound *XXVI* obtained above was dissolved in a mixture of acetic acid (500 ml) and acetic anhydride (100 ml). Concentrated sulfuric acid (30 ml) was added dropwise to this solution under stirring and ice-cooling at a temperature below 15°C. After stirring at 0°C for 2 h and standing overnight at room temperature, the mixture was poured into 2 litres of ice-cold water and extracted with chloroform (3 × 200 ml). The extract was washed with water (3 × × 200 ml) and stirred with a saturated solution of sodium hydrogen carbonate (500 ml) to alkaline reaction of the aqueous phase. The chloroform solution was separated, washed with water (2 × 50 ml), dried over magnesium sulfate and taken down *in vacuo*. The residue was chromatographed on a column of silica gel (500 ml) in benzene and the fraction, containing compound *XXVII*, was taken down *in vacuo* and dried, affording 35·2 g (72·6% based on *XXV*) of the glassy product *XXVII*. For $C_{22}H_{22}O_8$ (414·4) calculated: 63·76% C, 5·35% H; found: 63·90% C, 5·32% H. ^{1}H NMR spectrum (deuteriochloroform): (α : β-isomers about 1 : 1) : α-isomer : 2·10 (s, 3 H) $OCOCH_3$; 3·42 (s, 3 H) OCH_3; 3·70 (br s, 2 H) 2 H_5; 4·61 (br s, 1 H) H_4; 5·75 (br. s, 2 H) H_2 + + H_3; 6·43 (d, 1 H, $J_{1,2} = 1{\cdot}0$) H_1. β-Isomer: 2·12 (s, 3 H) $OCOCH_3$; 3·42 (s, 3 H) OCH_3; 3·68 (2 × dd, 2 H, $J_{5,4} = 3{\cdot}0$, $J_{5',4} = 3{\cdot}1$) 2 H_5; 4·58 (dd, 1 H, $J = 3{\cdot}5$) H_4; 5·80 (m, 1 H) H_3; 5·56 (dd, 1 H, $J_{2,3} = 6{\cdot}1$) H_2; 6·69 (d, 1 H, $J_{1,2} = 4{\cdot}4$) H_1. Arom. signals (for both isomers): 7·80–8·20 (m, 4 H) + 7·15–6·65 (m, 6 H).

A solution of the compound *XXVII* (13·1 g; 31·6 mmol) in acetonitrile (300 ml) was mixed with compound *XIIa* (6·3 g; 37·5 mmol). Tin tetrachloride (5 ml) was added rapidly and the mixture was stirred under exclusion of moisture for 30 min and then set aside at room temperature for 2 days. After evaporation of the solvent *in vacuo*, the residue was mixed with chloroform (500 ml) and water (300 ml) and solid sodium hydrogen carbonate was added unter stirring until the aqueous layer had faintly alkaline reaction. The mixture was then filtered through Celite which was washed with chloroform (200 ml), the layers were separated and the chloroform extract was washed with water (3 × 100 ml). Upon drying over magnesium sulfate, the filtered extract was taken down *in vacuo* and the residue chromatographed on a column of silica gel (500 ml). Elution with chloroform afforded a chromatographically homogeneous fraction of the product *XXVIII* which was purified by crystallization from ethanol; yield 7·5 g (45%) of *XXVIII*, m.p. 167°C. For $C_{24}H_{22}N_2O_7$ (450·4) calculated: 63·99% C, 4·92% H, 6·22% N; found: 63·83% C, 4·83% H, 6·22% N. ^{1}H NMR spectrum (deuteriochloroform): 3·52 (s, 3 H) OCH_3; 3·73 + 3·89 (dd, 2 H, $J_{4',5'} = 2{\cdot}1$, $J_{4',5''} = 2{\cdot}4$, $J_{gem} = 10{\cdot}8$) 2 $H_{5'}$; 4·56 (br q, 1 H) $H_{4'}$; 5·82 (dd, 1 H, $J_{2',3'} = 5{\cdot}4$, $J_{3',4'} = 3{\cdot}3$) $H_{3'}$; 5·69 (t, 1 H, $J_{2',1'} = J_{2',3'} = 5{\cdot}4$) $H_{2'}$; 6·67 (d, 1 H, $J_{1',2'} = 5{\cdot}4$)

$H_{1'}$; 6·40 (dd, 1 H, $J_{5,6}$ = 6·8, $J_{5,4}$ = 3·8) H_5; 8·36 (dd, 1 H, $J_{6,5}$ = 6·8, $J_{6,4}$ = 2·8) H_6; 8·60 (br, 1 H) H_4; arom. signals 7·90—8·05 (4 H) + 7·25—7·70 (6 H). Conformation of the sugar 2-*endo*.

A suspension of compound *XXVIII* (3·0 g; 6·7 mmol) in 0·03 mol l^{-1} methanolic sodium methoxide (150 ml) was stirred until it dissolved and the solution was set aside at room temperature overnight under exclusion of moisture. The reaction mixture was neutralized with Dowex 50 X 8 (H^+), made alkaline with triethylamine, filtered and the solid was washed with methanol (100 ml). After evaporation *in vacuo*, the residue was dissolved in water and extracted with ether (3 × 50 ml). The aqueous phase was taken down *in vacuo*, the dry residue was taken up in water, the solution was filtered through a column of octadecyl-silica and the UV-absorbing eluate was again taken down *in vacuo*. Codistillation of the residue with ethanol (3 × 50 ml) and drying *in vacuo* gave 1·40 g (86%) of *XXI* as an amorphous foam. For $C_{10}H_{14}N_2O_5$ (242·2) calculated: 49·58% C, 5·82% H, 11·56% N; found: 49·98% C, 5·78% H, 11·26% N. UV spectrum (pH 2): λ_{max} 315 nm (ε_{max} 7 000). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 3·37 (s, 3 H) OCH_3; 3·56 + 3·72 (dd, 2 H, $J_{4',5'}$ = 2·4, $J_{4',5''}$ = 3·3, J_{gem} = 11·0) 2 $H_{5'}$; 4·00 (m, center, 3 H) $H_{2'}$ + $H_{3'}$ + $H_{4'}$; 5·19 (d, 1 H, $J_{3',OH}$ = 5·0) 3'-OH; 5·63 (d, 1 H, $J_{2',OH}$ = 4·0) 2'-OH; 5·77 (d, 1 H, $J_{1',2'}$ = 2·2) $H_{1'}$; 6·53 (dd, 1 H, $J_{5,6}$ = 6·8, $J_{5,4}$ = 4·2) H_5; 8·35 (dd, 1 H, $J_{6,5}$ = 6·8, $J_{6,4}$ = 2·9) H_6; 8·58 (br, 1 H) H_4.

1-(β-D-Ribofuranosyl)-2-pyrimidone 5'-Phosphate (*XXII*)

Compound *I* (1·1 g; 5 mmol) was dissolved in warm triethyl phosphate (15 ml) and the solution was cooled with ice. Phosphorus oxychloride (1 ml, 11 mmol) was added with stirring and ice-cooling and the mixture was stirred at 0°C for 2 h and without cooling 2 h. The clear reaction mixture was poured into water (200 ml) and adjusted rapidly to pH 10·0 with 4 mol l^{-1} lithium hydroxide, the pH being rendered at 9·5—10·0 with the mentioned hydroxide until it remained constant (45 min). The mixture was stirred for further 30 min, neutralized with Dowex 50X 8 (H^+) to pH 7, filtered, the resin washed with water (25 ml) and the filtrate taken down. The residue was codistilled with ethanol (3 × 50 ml), dissolved in ethanol (20 ml) and added dropwise under stirring into ether (300 ml). The separated crude lithium salt of *XXII* was filtered, washed with ether and dried *in vacuo*, dissolved in water (20 ml), acidified by addition of Dowex 50 X 8 (H^+) to pH 2—3, applied on a column (150 ml) of the same ion-exchange resin and eluted with water. The UV-absorbing aqueous eluate was neutralized with lithium hydroxide, concentrated *in vacuo* and filtered through a column of Dowex 50X8 (Li^+; 20 ml), which was then washed with water. The eluate was taken down *in vacuo*, the residue codistilled with ethanol (3 × 25 ml) and the lithium salt of compound *XXII* was precipitated from ethanol (5 ml) with ether (100 ml). Filtration, washing with ether and drying *in vacuo* afforded 0·90 g (56%) of the product. For $C_9H_{11}N_2O_8PLi_2$ (320·1) calculated: 8·75% N, 9·70% P; found: 9·01% N, 9·67% P. UV spectrum (pH 2): λ_{max} 315 nm (ε_{max} 7 300).

5'-O-Methyluridine (*XXXII*)

To a stirred solution of compound *XXVII* (40 mmol) in acetonitrile (300 ml) was added freshly distilled (b.p. 110°C/2 kPa) 2,4-bis(trimethylsilyloxy)pyrimidine (*XIIc*; 22·4 g; 87·5 mmol) followed by tin tetrachloride (8 ml) in one portion. The mixture was stirred without cooling for 30 min and set aside overnight under exclusion of moisture. After evaporation *in vacuo* the mixture was processed in the same manner as described for the compound *XXI*. Chromatography on a column of silica gel (400 ml) afforded 16·1 g (86%) of the amorphous product *XXIX*. For $C_{24}H_{22}N_2O_8$ (466·4) calculated: 61·80% C, 4·75% H, 6·01% N; found: 61·75% C, 4·56% H, 6·21% N. ^{1}H NMR spectrum (deuteriochloroform): 3·53 (s, 3 H,) OCH_3; 3·78 (2 dd, 2 H,

$J_{5',4'} = 2{\cdot}1$, $J_{5'',4'} = 2{\cdot}3$, $J_{gem} = 10{\cdot}4$) 2 $H_{5'}$; 4·48 (br q, 1 H, $J = 2{\cdot}0$) $H_{4'}$; 5·63 (dd, 1 H, $J_{2',1'} = 7{\cdot}0$, $J_{2',3'} = 5{\cdot}7$) $H_{2'}$; 5·80 (dd, 1 H, $J_{3',2'} = 5{\cdot}7$, $J_{3',4'} = 2{\cdot}2$) $H_{3'}$; 5·85 (dd, 1 H, $J_{5,6} = 8{\cdot}2$, $J_{5,NH} = 2{\cdot}3$) H_5; 6·57 (d, 1 H, $J_{1',2'} = 7{\cdot}0$) $H_{1'}$; 8·86 (br, 1 H) NH; arom. + H_6 7·75—8·10 (m, 5 H); 7·25—7·60 (m, 6 H).

A solution of the compound *XXIX* (2·4 g; 5·1 mmol) in 0·05 mol l^{-1} methanolic sodium methoxide (100 ml) was set aside at room temperature overnight in stoppered bottle. The mixture was neutralized with Dowex 50X8 (H^+), made alkaline with triethylamine, filtered, the resin was washed with methanol (100 ml) and the filtrate taken down *in vacuo*. The residue was dissolved in water (100 ml), the solution extracted with ether (3 × 25 ml), the aqueous phase evaporated *in vacuo* and the residue codistilled with ethanol (2 × 20 ml) and crystallized from ethanol–ether to give 0·85 g (65%) of *XXXII*, m.p. 135°C (reported[24] m.p. 134—135°C). For $C_{10}H_{14}N_2O_6$ (258·2) calculated: 46·51% C, 5·46% H, 10·85% N; found: 46·21% C, 5·18% H, 11·12% N. ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 3·31 (s, 3 H) OCH_3; 3·53 (m, 2 H) 2 $H_{5'}$; 3·93 (m, 3 H) $H_{2'}$ + $H_{3'}$ + $H_{4'}$; 5·15 (br, 1 H) 3'-OH; 5·40 (br, 1 H) 2'-OH; 5·66 (d, 1 H, $J_{5,6} = 8{\cdot}2$) H_5; 5·75 (d, 1 H, $J_{1',2'} = 5{\cdot}0$) $H_{1'}$; 7·69 (d, 1 H, $J_{6,5} = 8{\cdot}2$) H_6; 11·23 (br, 1 H) NH. UV spectrum (pH 2): λ_{max} 263 nm (ε_{max} 10 700).

5'-O-Methylcytidine (*XXXIII*)

Phosphorus pentasulfide (2 g) was added to a boiling and stirred solution of compound *XXIX* (3·9 g; 6·2 mmol) in dioxane (100 ml) and the suspension was refluxed with stirring for 1 h under exclusion of moisture. The hot mixture was filtered through Celite which was then washed with boiling dioxane (50 ml) and the solvent was evaporated *in vacuo*. The residue was dissolved in chloroform (500 ml) and the solution stirred with a solution of sodium hydrogen carbonate (200 ml, solid sodium hydrogen carbonate was gradually added) until the aqueous phase became slightly alkaline. The mixture was filtered through Celite which was washed with chloroform (100 ml) and the combined chloroform layers were washed with water (2 × 100 ml). After drying and evaporation of the solvent the residue was dried *in vacuo*, yielding 2·70 g (90%) of compound *XXX* as an amorphous yellow foam. For $C_{24}H_{22}N_2O_7S$ (482·4) calculated: 59·75% C, 4·60% H, 5·81% N, 6·65% S; found: 59·70% C, 4·48% H, 5·91% N, 6·75% S. ^{1}H NMR spectrum (deuteriochloroform): 3·52 (s, 3 H) OCH_3; 3·78 (2 dd, 2 H, $J_{5',4'} = J_{5'',4'} = 2{\cdot}1$, $J_{gem} = 10{\cdot}6$) 2 $H_{5'}$; 4·50 (br q, 1 H, $J = 2{\cdot}1$) $H_{4'}$; 5·64 (dd, 1 H, $J_{2',1'} = 6{\cdot}8$, $J_{2',3'} = 5{\cdot}7$) $H_{2'}$; 5·80 (dd, 1 H, $J_{3',2'} = 5{\cdot}7$, $J_{3',4'} = 2{\cdot}2$) $H_{3'}$; 6·48 (dd, 1 H, $J_{5,NH} = 2{\cdot}1$, $J_{5,6} = 7{\cdot}8$) H_5; 6·52 (d, 1 H, $J_{1',2'} = 6{\cdot}8$) $H_{1'}$; 7·69 (d, 1 H, $J_{6,5} = 8{\cdot}0$) H_6; 9·40 (br, 1 H) NH; 7·85—8·10 (m, 4 H) + 7·25—7·70 (m, 6 H) aromatic protons.

Water (30 ml) and methyl iodide (3 ml) were added to a solution of compound *XXX* (2·4 g; 5 mmol) in methanol (100 ml). Methanolic sodium methoxide (1 mol l^{-1}; 5 ml) was then added dropwise during 30 min, the mixture was stirred for 10 min, diluted with chloroform (400 ml), washed with water (2 × 100 ml), dried over magnesium sulfate, filtered and taken down *in vacuo*. The dry residue (compound *XXXI*; 2·5 g) was heated to 110°C with 30% methanolic ammonia (70 ml) in an autoclave for 8 h. After evaporation of the solvent *in vacuo*, the residue was dissolved in water (100 ml) and the solution extracted with ether (3 × 50 ml). The aqueous layer was concentrated *in vacuo* to about 20 ml, mixed with Dowex 50X8 (H^+) to an acid reaction and the suspension was applied on a column of the same ion-exchange resin (100 ml). The column was washed with water to final disappearance of the UV absorption (2 UV-absorbing peaks) and then with 2·5% ammonia. The ammonia UV-absorbing eluate was taken down *in vacuo*, the residue dissolved in water (5 ml), filtered through a column of octadecyl-silica gel and eluted with water. The UV absorbing aqueous eluate was again taken down *in vacuo* and the residue was codistilled with ethanol (2 × 25 ml). Crystallization of the residue from ethanol–ether gave

0·95 g (74%) of compound *XXXIII*, m.p. 172–173°C. For $C_{10}H_{15}N_3O_5$ (257·2) calculated: 46·68% C, 5·88% H, 16·34% N; found: 46·64% C, 5·79% H, 16·27% N. Mass spectrum: 258 (M + 1), 257 (M^+) 240 (M–OH), 239 (M–H_2O), 222 (240–H_2O) 212 (M–CH_3OCH_2), 140 (B+30), 112 (BH_2), 11 (BH). UV spectrum (pH 2): λ_{max} 279 nm, (ε_{max} 13 200).

1-(*RS*)-*erythro*-(2,3-Dihydroxybutyl)-2-pyrimidone (*XXXVIII*)

Sodium hydride (20 mmol) was added to a solution of 2-pyrimidone (1·92 g; 20 mmol) in dimethylformamide (50 ml) and the mixture was heated to 80°C for 1 h. Compound *XLVI* (ref.[26]; 7 g; 16·4 mmol) was added and the mixture was heated to 100°C for 13 h under exclusion of moisture. After evaporation of the solvent *in vacuo* the residue was extracted with hot chloroform (500 ml total), the extract filtered through Celite and taken down. Chromatography of the residue on a silica gel column (200 ml) in chloroform gave 1·64 g (4·7 mmol; 28·4%) of compound *XLVII* as a yellow oil. This product was heated to 60°C with 0·25 mol l^{-1} sulfuric acid (40 ml) for 5 h, diluted with water (100 ml) and neutralized with barium hydroxide. The suspension was heated to the boil and filtered through Celite while hot. The filtrate was taken down, the residue was chromatographed on octadecyl-silica gel in water and the product-containing fraction was taken down. The residue was chromatographed on a silica gel plate in the system S4. Elution of the product band with methanol, followed by evaporation and drying *in vacuo*, gave 0·30 g (1·63 mmol, 35% based on *XLVII*) of the amorphous product *XXXVIII*. For $C_8H_{12}N_2O_3$ (184·2) calculated: 52·16% C, 6·57% H, 15·21% N; found: 51·88% C, 6·37% H, 15·49% N. Mass spectrum: 184 (M^+), 183 (M–H), 166 (M–H_2O), 139 (M–CH(OH)–CH_3), 109 (Base+CH_2), 97 (Base+2 H), 96 (Base+H), UV spectrum (pH 2): λ_{max} 307 nm, (ε_{max} 4 900).

1-(2*S*,3*S*)-*threo*-(2,3,4-Trihydroxybutyl)-2-pyrimidone (*XXXIX*)

A solution of sodium salt of 2-pyrimidone (12·5 mmol) in dimethylformamide (30 ml) was prepared as described for the compound *XXXVIII*. Compound *XLVIII* (ref.[27]; 5·25 g; 12·5 mmol) was added to this solution and the mixture was stirred at 100°C for 15 h under exclusion of moisture. The work-up was the same as for the compound *XXXVIII*. Chromatography on a column *I* of silica gel in chloroform–methanol (95 : 5) afforded 3·94 g of a crude product which was crystallized from ethyl acetate–light petroleum to give 2·1 g (49%) of the compound *XLIX*, m.p. 150°C. For $C_{18}H_{20}N_2O_5$ (344·4) calculated: 62·78% C, 5·85% H, 8·14% N; found: 62·74% C, 5·98% H, 8·06% N.

A mixture of the compound *XLIX* (2·0 g; 5·8 mmol) and 0·1 mol l^{-1} methanolic sodium methoxide (50 ml) was stirred until dissolution and after standing for 24 h at room temperature Dowex 50 X 8 (H^+) was added to neutrality. The suspension was made alkaline with triethylamine, filtered, the resin washed with methanol and the filtrate taken down *in vacuo*. A solution of the residue in water (100 ml) was extracted with ether (3 × 25 ml) and water was evaporated *in vacuo*. Chromatography of the residue on one plate of silica gel in the system S3 followed by crystallization from ethyl acetate–light petroleum afforded 0·82 g (3·4 mmol), 59%) of compound L, m.p. 85–86°C. For $C_{11}H_{16}N_2O_4$ (240·3) calculated: 54·99% C, 6·71% H, 11·66% N; found: 54·92% C, 6·69% H, 11·50% N. Mass spectrum: 251 ($C_{11}H_{17}N_2O_4$, M+1), 225 (M–CH_3), 222 (M–H_2O), 210 (M–30), 110 (Base+CH_3), 97 (Base+2 H). UV spectrum (methanol: λ_{max} 312 nm, (ε_{max} 5 300).

A boiling solution of the compound L (0·80 g) in 80% methanol (100 ml) was stirred with Dowex 50 X 8 (H^+; 10 ml) for 5 h, made alkaline with ammonia, filtered, the Dowex washed with methanol and the filtrate taken down. The residue was chromatographed on a column of octadecyl–silica gel in water. The crystalline residue was triturated with ether, filtered and dried *in vacuo*, yielding 0·60 g (90%) of compound *XXXIX*, m.p. 110–111°C. For $C_8H_{12}N_2O_4$ (200·2) calculated:

47·99% C, 6·04% H, 14·00% N; found: 47·92% C, 6·25% H, 14·08% N. Mass spectrum: 200 (M^+), 199 (M−H), 182 (M−H_2O), 169 (M−CH_2OH), 152 (169−OH), 151 (169−H_2O), 139 (M−−$CH(OH)CH_2OH$), 110 (Base+CH_3), 109 (Base+CH_2), 97 (Base+2 H). UV spectrum (water): λ_{max} 307 nm, (ε_{max} 5 200).

Chromatography of the crude reaction mixture on silica gel afforded, in addition to the product *XLIX*, an oil (1·10 g) which on methanolysis and chromatography on a silica gel column (100 ml) in the system S3 afforded 0·67 g (2·8 mmol; 22%) of the O-isomer *LI*. Mass spectrum: 225 (M−CH_3), 210 (M−CH_2O), 209 (M−CH_2OH), 122 (base+OCH_2CH), 97 (base+2 H). UV spectrum (methanol): λ_{max} 267 nm, ε_{max} 3 700.

1-(*S*)-(3,4-Dihydroxybutyl)-2-pyrimidone (*XL*).

A solution of sodium salt of 2-pyrimidone (25 mmol) in dimethylformamide (80 ml) was prepared as described for compound *XXXVIII*. After addition of compound *LII* (ref.[26]; 7·5 g, 25 mmol) the mixture was stirred under exclusion of moisture at 100°C for 9 h and worked up as described for compound *XXXVIII*. The residue after evaporation of the chloroform extract was chromatographed on a column of octadecyl-silica gel in water. The UV absorbing eluate was taken down, the residue codistilled with ethanol and the product precipitated with ether (200 ml) from ethanol (10 ml). The ethereal supernatant was taken down *in vacuo* and the residue was purified by chromatography on one plate of silica gel in the system S3. Elution of the main UV-absorbing band afforded 1·43 g (6·38 mmol, 25·5%) of compound *LIII*. Mass spectrum: 209 (M−CH_3), 166 (M−$(CH_3)_2CO$), 149, 110 (base + CH_3), 109 (base + CH_2), 97 (base + 2H), 95 (base). This product was heated with 0·25 mol l^{-1} sulfuric acid (10 ml) to 60°C for 3h. After dilution with water (50 ml) the mixture was neutralized with barium hydroxide, heated to 80°C and filtered while hot through Celite. The filtrate was taken down and the residue chromatographed on cellulose in the system S1 and then on octadecyl-silica gel in water. Purification by chromatography on one plate of silica gel in the system S4, followed by elution with methanol, evaporation *in vacuo* and drying, afforded 0·43 g (37%, based on *LIII*) of the amorphous product *XL*. Mass spectrum: 184 ($C_8H_{12}N_2O_3$, M+), 183 (M−H), 166 (M−H_2O), 153 (M−CH_2OH), 109 (base + CH_2), 97 (base + 2 H). UV spectrum (water): λ_{max} 307 nm, ε_{max} 5 200.

Methyl (*RS*)-3-(2-Pyrimidon-1-yl)-2-hydroxypropanoate (*XLII*)
and (*RS*)-3-(2-Pyrimidon-1-yl)-2-hydroxypropanoic Acid (*XLI*)

A solution of sodium salt of 2-pyrimidone (50 mmol) in dimethylformamide (110 ml) was prepared in the same manner as described for compound *XXXVIII*. To this solution bromoacetaldehyde diethylacetal (12 ml) was added with stirring at 100°C during 1 h. After heating for 4 h to 100°C, another part of this reagent (3 ml) was added in one portion. The mixture was stirred at 100°C for 12 h under exclusion of moisture and processed as described for compound *XXXVIII*. Chromatography of the crude extract on a column of silica gel in chloroform–methanol (97·5 : 2·5) afforded 8·03 g (37·9 mmol; 75·8%) of compound *LIV* as a yellow oil; UV spectrum (methanol): λ_{max} 305 nm. This product was heated with 0·1 mol l^{-1} hydrochloric acid (200 ml) to 80°C for 3 h (quantitative reaction), the mixture was cooled with ice, sodium cyanide (10 g) was added with stirring and the mixture was rapidly neutralized with acetic acid. After stirring at 0°C for 7 h and room temperature overnight, the mixture was refluxed with hydrochloric acid (120 ml) for 4 h and taken down *in vacuo*. The residue was codistilled with water (3 × 100 ml), neutralized with ammonia and applied on a column of Dowex 50X8 (H^+; 300 ml). After washing with water to disappearance of UV absorption, the product was eluted with 2·5% aqueous ammonia. The UV-absorbing ammonia eluate was taken down *in vacuo*, the residue dissolved in water (20 ml), made alkaline with ammonia and applied on a column of Dowex 1X2 (acetate). The column was

washed with water to drop of UV absorption and then with a linear gradient of formic acid (0—1 mol l^{-1}; 2 litres each). The combined product fractions were taken down *in vacuo*, the residue was codistilled with water (3 × 100 ml) and ethanol (2 × 100 ml), dried *in vacuo* and refluxed under stirring with a mixture of methanol (100 ml) and sulfuric acid (0·5 ml) After cooling with ice and neutralization with triethylamine the solvent was evaporated and the residue chromatographed on a column of silica gel (200 g) in chloroform. Elution with chloroform–methanol (95 : 5) afforded after crystallization from light petroleum 1·05 g (5·3 mmol; 11%) of compound *XLII*, m.p. 128—128·5°C. For $C_8H_{10}N_2O_4$ (198·2) calculated: 48·48% C, 5·09% H, 14·14% N; found: 48·45% C, 4·86% H, 14·22% N. Mass spectrum: 198 (M^+), 167 ($M-OCH_3$), 139 ($M-COOCH_3$), 109 (base + CH_2), 97 (base + 2 H). UV spectrum (methanol): λ_{max} 312 nm, ε_{max} 5 500.

A solution of compound *XLII* (0·35 g; 1·76 mmol) in 0·1 mol l^{-1} sodium hydroxide (15 ml) was set aside at room temperature overnight, diluted with water (50 ml), neutralized with Dowex 50X8 (H^+) to pH 7·0, filtered and taken down *in vacuo*. The residue was codistilled with ethanol (2 × 25 ml), the product precipitated from methanol (5 ml) with ether (100 ml), filtered, washed with ether and dried *in vacuo*, affording 0·26 g (72%) of chromatographically and electrophoretically homogeneous sodium salt of compound *XLI*; UV spectrum (water): λ_{max} 307 nm, ε_{max} 5 300.

(*RS*)-3-(2-Pyrimidon-1-yl)-2-hydroxypropanoic Acid Amide (*XLIII*)

Compound *XLII* (0·35 g; 1·76 mmol) was heated with 30% methanolic ammonia (50 ml) in an autoclave to 110°C for 12 h. After evaporation *in vacuo*, the residue was codistilled with ethanol (2 × 25 ml), precipitated from ethanol (5 ml) with ether (100 ml), filtered, washed with ether and dried *in vacuo*, yielding 0·27 g of yellowish crude product which was chromatographed on a column of octadecyl-silica gel in water. The UV-absorbing eluate was taken down *in vacuo*, the residue codistilled with ethanol (10 ml) and the crystalline residue crystallized from ethanol–ether, giving 0·12 g (41%) of compound *XLIII*, m.p. 223—224°C. For $C_7H_9N_3O_3$ (183·2) calculated: 45·89% C, 4·95% H, 22·95% N; found: 45·57% C, 4·98% H, 22·96% N. Mass spectrum: 183 (M^+), 139 ($M-CONH_2$), 109 (base + CH_2), 97 (base + 2 H). UV spectrum (water): λ_{max} 306 nm, ε_{max} 5 200.

(2*R*,3*R*)-*erythro*-4-(2-Pyrimidon-1-yl)-2,3-dihydroxybutanoic Acid (*XLIV*)

Compound *LVI* (ref.[22]; 12·5 mmol) was added to a solution of sodium salt of 2-pyrimidone (12·5 mmol) in dimethylformamide (50 ml), prepared as described for *XXXVIII*. The mixture was stirred at 100°C for 14 h under exclusion of moisture. The solvent was evaporated *in vacuo* and the residue chromatographed on an octadecyl-silica gel column in water. The UV-absorbing eluate was evaporated to dryness *in vacuo*. The residue was heated with 0·25 mol l^{-1} sulfuric acid (100 ml) to 60°C for 6 h, neutralized with barium hydroxide, heated to 80°C and filtered through Celite while hot. The filtrate was concentrated *in vacuo* to about 20 ml, made alkaline with ammonia and applied on a column of Dowex 1X2 (acetate; 100 ml). After washing with water to disappearance of UV absorption, the product was eluted with a linear gradient of formic acid (0—0·5 ml l^{-1}, 1 litre each). The product fraction was taken down, the residue codistilled with water (3 × 50 ml) and chromatographed on a column of cellulose in the system S1. The product fractions were combined, evaporated, and the residue in water (5 ml) applied on a column of Dowex 50X8 (Li^+). The UV absorbing eluate was taken down, the residue codistilled with ethanol (2 × 25 ml), precipitated with ether (200 ml) from methanol (10 ml), filtered, washed with ether and dried *in vacuo*, yielding 0·60 g (22%) of lithium salt of compound *XLIV* which

was chromatographically and electrophoretically homogeneous. UV spectrum (water): λ_{max} 305 nm, ε_{max} 5 300.

4-(*p*-Toluenesulfonyloxymethyl)-5-(2-pyrimidon-1-yl)methyl-2,2-dimethyl-(4*S*,4*S*)--1,3-dioxolane (*LVIII*)

Compound *LVII* (ref.[27]; 4·95 g; 10·5 mmol) was added to a solution of sodium salt of 2-pyrimidone (10 mmol) in dimethylformamide (40 ml) prepared as described for *XXXVIII*, and the mixture was heated to 100°C for 12 h under exclusion of moisture. After evaporation *in vacuo* the mixture was extracted with boiling chloroform (300 ml total), the extract was taken down *in vacuo* and the residue purified by chromatography on silica gel column (200 g). Elution with chloroform, followed by evaporation of the solvent and crystallization from ethyl acetate–light petroleum, gave 0·60 g (15%) of compound *LVIII*, m.p. 139–140°C. For $C_{18}H_{22}N_2O_6S$ (394·4) calculated: 54·80% C, 5·62% H, 7·10% N, 8·13% S; found: 54·51% C, 5·62% H, 7·01% N, 8·07% S.

Reaction of Compound *LVIII* with Sodium Azide

A mixture of compound *LVIII* (0·60 g), sodium azide (0·60 g), and dimethylformamide (12 ml) was heated under stirring and exclusion of moisture to 100°C for 2 h, filtered while hot and the filtrate was taken down *in vacuo*. Extraction of the residue with hot chloroform gave compound *LIX* which was hydrogenated over 10% Pd/C (0·5 g) in acetic acid (50 ml) at room temperature. The mixture was filtered, taken down and the residue codistilled with water (3 × 100 ml). According to paper chromatography in S1 and TLC in S3 and S4, the product did not contain any UV-absorbing compounds and showed only ninhydrin-positive spots.

Reaction of Compound *XIIa* with Bromoacetaldehyde Diethyl Acetal

A mixture of compound *XIIa* (4·5 g; 26·8 mmol), acetonitrile (40 ml) and bromoacetaldehyde diethyl acetal (5 ml; 33·2 mmol) was set aside at room temperature overnight and then refluxed for 8 h under exclusion of moisture. After evaporation, water (100 ml), was added, the mixture filtered through Celite and the filtrate extracted with chloroform (6 × 100 ml). The chloroform extract was dried over magnesium sulfate, filtered, the solvent removed *in vacuo* and the residue chromatographed on one plate of silica gel in the system S3, affording 0·92 g (16%) of the oily product LV; UV spectrum (methanol): λ_{max} 260 nm.

Enzymatic Studies

Preparation of the enzyme. Cytidine deaminase was prepared from *E. coli* A19 and was partially purified according to ref.[14] up to the third purification step (fractionation on DEAE cellulose).

Determination of the enzymatic activity and inhibitory effect. The reaction mixture (total volume 0·25 ml) contained: 10^{-2} mol l^{-1} Tris-HCl, pH 7·4, $5 . 10^{-5}$ mol l^{-1} [^{14}C]-cytidine (0·74 MBq. μmol^{-1}), and the above cytidine deaminase preparation (65 μg). In the inhibitory experiments, the concentration of the tested compound was $2·5 . 10^{-5}$ mol l^{-1}. The mixtures were incubated at 37°C for 5 min, the aliquotes taken from the mixture were chromatographed on a paper Whatman No 3 MM in 1-butanol–acetic acid–water (10 : 1 : 3) with uridine and cytidine as markers. The spots of these compounds were cut out and the radioactivity was determined by scintillation on a Packard Tricarb® 300 instrument in toluene scintillation solution.

Determination of the substrate activity. The reaction mixture (total volume 0·25 ml) contained: 10^{-2} mol l^{-1} Tris-HCl pH 7·4, cytidine deaminase preparation (120 μg of the protein) and

$5 . 10^{-5}$ mol l^{-1} of the tested analogue; incubation at 37°C for 60 min. Samples (10 μl) were analyzed by HPLC (*vide supra*) in 10% aqueous methanol containing 10 mmol l^{-1} ammonium dihydrogen phosphate, pH 3·9.

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