SYNTHESIS OF 2-DEOXY-β-D-RIBONUCLEOSIDES AND 2,3-DIDEOXY-β-D-PENTOFURANOSIDES ON IMMOBILIZED BACTERIAL CELLS*

Ivan VOTRUBA, Antonin HOLY**, Hana DVORAKOVA, Jaroslav GUNTER, Dana HOCKOVA, Hubert HREBABECKY, Tomas CIHLAR and Milena MASOJIDKOVA

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, The Czech Republic

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Alginate gel-entrapped cells of auxotrophic thymine-dependent strain of *E. coli* catalyze the transfer of 2-deoxy-D-ribofuranosyl moiety of 2′-deoxyuridine to purine and pyrimidine bases as well as their aza and deaza analogs. All experiments invariably gave β-anomers; in most cases, the reaction was regiospecific, affording N^9-isomers in the purine and N^1-isomers in the pyrimidine series. Also a 2,3-dideoxynucleoside can serve as donor of the glycosyl moiety. The acceptor activity of purine bases depends only little on substitution, the only condition being the presence of N^7-nitrogen atom. On the other hand, in the pyrimidine series the activity is limited to only a narrow choice of mostly short 5-alkyl and 5-halogeno uracil derivatives. Heterocyclic bases containing amino groups are deaminated; this can be avoided by conversion of the base to the corresponding *N*-dimethylaminomethylene derivative which is then ammonolyzed. The method was verified by isolation of 9-(2-deoxy-β-D-ribofuranosyl) derivatives of adenine, guanine, 2-chloroadenine, 6-methylpurine, 8-azaadenine, 8-azaguanine, 1-deazaadenine, 3-deazaadenine, 1-(2-deoxy-β-D-ribofuranosyl) derivatives of 5-ethyluracil, 5-fluorouracil, and 9-(2,3-dideoxy-β-D-pentofuranosyl)hypoxanthine, 9-(2,3-dideoxy-β-D-pentofuranosyl)-6-methylpurine, and other nucleosides.

2′-Deoxy-β-D-ribonucleosides of pyrimidine and purine bases are very important precursors in the biosynthesis of deoxyribonucleic acids. Natural deoxyribonucleosides are of importance as starting compounds for synthesis of oligodeoxyribonucleotides in gene manipulations or development of diagnostic probes. Many deoxynucleosides derived from chemically modified heterocyclic bases of both series are also significant registered or potential drugs (particularly antivirals and cancerostatics) or diagnostic tools.

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**The author to whom correspondence should be addressed.

Natural 2′-deoxyribonucleosides are usually prepared by enzymatic degradation of DNA or its precursors (nucleotides). Their chemical synthesis is of no advantage: of the four deoxyribonucleosides only thymidine and 2′-deoxycytidine can be easily prepared in bulk by conversion of the corresponding ribonucleosides[1]. Equally complicated is the synthesis of 2′-deoxyribonucleosides containing modified heterocyclic bases. For the chemical synthesis of this type of compounds many methods are available that consist in deoxyribosylation of suitable heterocyclic bases, transformation of the corresponding ribonucleosides, or modification of the heterocyclic system in preformed 2′-deoxyribonucleosides. Total synthesis by glycosylation is difficult because of limited accessibility and low stability of a suitable blocked derivative of 2-deoxy-D-ribose; recently, there appeared an alternative in deoxyribosylation by the phase-transfer method (see e.g. ref.[2] and references therein) but not all heterocyclic systems withstand the drastic conditions used in this reaction. However, the main problem with glycosidation techniques is the formation of mixtures of α- and β-anomers which in most cases are separable only with difficulty. It appears that (at least in some cases) the stereospecificity of this reaction is kinetically controlled and thus may be to a certain degree influenced[3] by suitable choice of conditions; nevertheless, the whole approach is still far from perfection.

For the transformation ribo → 2-deoxyribo, the corresponding ribonucleoside has to be synthesized first. Although in these cases it is not difficult to obtain uniform β-anomers[4], further conversion is possible without greater problems only in the pyrimidine series where one can utilize the easy formation of 2,2′-anhydronucleosides[5]. With purine derivatives, such attempts were feasible only when performed on a small scale because they require expensive procedures (use of triflates, silyl protecting groups etc., see e.g. ref.[6]).

Therefore, the enzyme-catalyzed transglycosylation of some synthetically easily accessible 2′-deoxyribonucleosides appears to be of advantage. As the most suitable we may consider thymidine or 2′-deoxyuridine, accessible by partial synthesis from uridine or even by total synthesis from D-arabinose[7]. The mentioned enzymatic method should be characterized by mild (physiological) conditions, the usually expected regiospecificity as well as stereospecificity of the transfer reaction, and simple scale-up ability.

The enzymatically catalyzed synthesis of deoxyribonucleosides has been described several times[8–11] making use of whole (procaryotic) cells, cell homogenates or enzyme preparations of various purity. Enzymes, catalyzing such transfer reactions, can be either nucleoside *N*-transdeoxyribosylases (e.g. from *Lactobacillus* sp.) or nucleoside phosphorylases. Whole cells or unpurified extracts contain also other enzymes metabolizing the bases as well as deoxynucleosides; any improvement is possible only at the expenses of effort exerted on purification of the given enzyme, lowering thus the effectivity of the way chosen.

We described recently a very effective and simple preparation of 2′-deoxy-β-D-ribonucleosides of heterocyclic bases, using immobilized cells of an auxotrophic thymine-dependent strain of *E. coli*[12]. The method consists in the transfer of 2-deoxyribofuranoside moiety from 2′-deoxyuridine (or other suitable donor) to the heterocyclic base:

$$\text{dUrd} + \text{base} \rightleftharpoons \text{BdRf} + \text{uracil}$$

As indicated by preliminary experiments, the reaction is stereospecific and purine and pyrimidine bases and their analogs afford "natural" isomers (i.e. the N^9-isomers from the purine and the N^1-isomers from the pyrimidine bases), whereas some imidazole derivatives give rise to both the possible regioisomers[13].

The mentioned catalyst contains immobilized bacterial cells harvested in the postlogarithmic growth phase and in comparison with the mentioned enzymatically catalyzed reactions offers the following advantages: (i) because of high immobilization capacity of the system chosen, it is possible to achieve a high content of the biomass (and high specific enzymatic activity) in the catalyst; (ii) not only the auxotroph used has high transdeoxyribosylation activity but also in the immobilized cells the undesired enzymatic side activities (phosphorylating, phosphorolytic, hydrolytic) are suppressed (except deamination); (iii) thanks to these properties, the syntheses can be performed in a dilute aqueous buffer and at relatively high concentration, to a considerable extent comparable with the methods of organic synthesis of these compounds.

We therefore decided to investigate in detail the applicability of this method to the preparation of 2-deoxy-β-D-ribofuranosides of modified heterocyclic bases. Special attention we focused to such bases whose deoxyribonucleosides already are of practical importance (e.g. in medicine), as well as to the investigation of regiospecificity of the transfer reaction in cases of bases for which we may expect an ambiguity of the transfer site. In the latter context, we were interested particularly in the deaza and aza analogs of purine bases. By isolation and characterization of their deoxyribonucleosides, of which some have not hitherto been prepared, we intend to illustrate the practical applicability of the method.

In the course of the last years, nucleosides modified on the sugar component received an extraordinary attention in connection with the quest for new antivirals (particularly against HIV) and cytostatics. Especially 3-azido-2,3-dideoxynucleosides (Retrovir), 2,3-dideoxyinosine (Didanosin), 2,3-dideoxycytidine (Zalcitabine) and other modified sugar nucleosides of natural bases are very important. It has been found that 2,3-dideoxynucleosides can be prepared in low yield by transglycosylation, catalyzed either with nucleoside-*N*-transdeoxyribosylase from *Lactobacillus* sp. or with nucleoside phosphorylase, i.e. with salvage pathway enzymes[14,15]. Since generally only sparse in-

formation exists about the donor specificity of transdeoxyribosylation reactions, we intended to extend our method to sugar-modified nucleosides.

RESULTS AND DISCUSSION

Basic Parameters

In the preliminary phase of our studies we have found that one of the best acceptors in the transdeoxyribosylation reaction is 6-methylpurine. Moreover, the exceptional properties of this base enabled a good HPLC and TLC separation of all the components of the reaction mixture to be made. Therefore, much of basic information on the course of the studied processes was obtained using this base as acceptor.

The first key problem is the stability of the catalyst and reproducibility of the procedures. The catalyst is prepared by dropping a suspension of bacterial cells in an alginate sol into a calcium chloride solution. We have verified that the catalyst is stable enough to afford practically the same conversion of model mixture 2′-deoxyuridine–6-methylpurine after storage for 1 week at +4 °C. In a series of experiments with the mentioned mixture, in which the catalyst was repeatedly used in 16-h cycles, the conversion practically did not change even after ninefold use of the same catalyst and amounted to 98.7 ± 0.8%. We also studied how significant is the amount of the compound withheld in the catalyst beads which swell considerably during the incubation. Upon disintegration of the beads and analysis of their content we found no significant presence of substrate, product or (which is more important) their possible metabolites (nucleotides).

The found pH optimum was 5.8, the conversion being not very different in the region pH 5 – 6 (Fig. 1). Acetate as well as phosphate buffer can be employed, however, after some time the phosphate buffer dissolved the catalyst beads. Therefore, we performed all the studies in an ammonium acetate buffer pH 5.8.

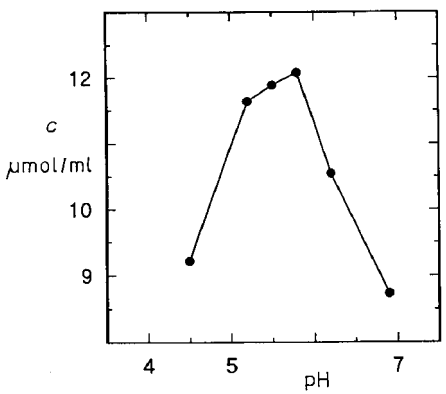


FIG. 1
Catalytic activity–pH profile of 2-deoxyriboside synthesis (Ca^{2+}-alginate entrapped *E. coli* SPT^- cells). *c*, Concentration of 6-methylpurine 2-deoxyriboside formed

We compared the donor ability of two pyrimidine deoxyribonucleosides, 2′-deoxyuridine and thymidine. It appeared that for the transfer of the sugar moiety to 6-methylpurine, after 6 h of incubation, the donor activity of thymidine was somewhat lower (by 28%) than of 2′-deoxyuridine (Fig. 2). In most experiments we employed 2′-deoxyuridine because of its good synthetic accessibility. In the system 2′-deoxyuridine – 6-methylpurine, the conversion of the heterocyclic base was proportional to the donor concentration only at lower donor/acceptor values, being practically the same for values higher than 2 (data not shown). For this reason we systematically performed our experiments with a twofold excess of the donor relative to the base.

Acceptor Activity of Heterocyclic Bases

In the discussion of our results we first of all compare the conversion of the heterocyclic bases into the corresponding nucleosides irrespectively of their isomeric or anomeric purity. This approach is an approximative one because it considers neither the possible differences between the UV-characteristics of the base and the nucleosides formed from it nor the possible distortion of the results due to low solubility of the base in the reaction medium. Consequently, we cannot consider the found conversion to be an absolute measure of the acceptor activity of the base and the numbers in Tables I and II are only of semiquantitative value. Of an equally little objectivity would be the evaluation by preparative experiments: the determination of conversion from the filtrate before separation of the mixture involves possible neglecting the undissolved base whereas values obtained after the separation relate to the isolated yield but not to the primary conversion of the heterocyclic base.

Another evaluating parameter that, according to our experience invariably coincided with the base conversion, was the concurrent conversion of 2′-deoxyuridine to uracil. In cases where no deoxyribonucleoside was formed from the base, we did not observe any uracil formation from 2′-deoxyuridine.

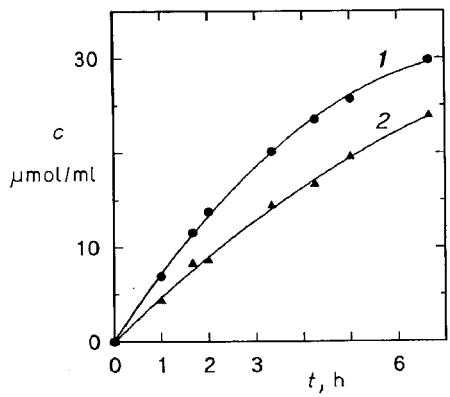


FIG. 2
Comparison of donor efficiency of 2′-deoxyuridine and thymidine (catalyzed by Ca^{2+}-alginate entrapped *E. coli* SPT⁻ cells, 5 beads/ml). *c*, Concentration of 6-methylpurine 2-deoxyriboside formed. *1* 74 mM 2′-Deoxyuridine, *2* 74 mM Thymidine. Concentration of 6-methylpurine (acceptor) was 37 mmol/l

TABLE I
Acceptor activity of purine bases in the reaction with 2′-deoxyuridine

Compound	Base	Conversion[a], %
I	Adenine	63[b]
II	2-Methyladenine	67
III	2-Methylthioadenine	72
IV	2,6-Diaminopurine	46
V	2-Chloroadenine	ND
VI	6-Methylpurine	55
VII	6-Chloropurine	63
VIII	6-Mercaptopurine	0
IX	6-Methylthiopurine	63
X	N^6-Methyladenine	64
XI	N^6-Dimethyladenine	70
XII	N^6-Furfuryladenine	90
XIII	2,6-Dichloropurine	33
XIV	Hypoxanthine	39
XV	Guanine	ND
XVI	N^2-Acetylguanine	37
XVII	N^6-Dimethylaminomethyleneadenine	75
XVIII	2-Chloro-N^6-dimethylaminomethyleneadenine	64
XIX	N^2-Dimethylaminomethyleneguanine	39
XX	8-Aminoadenine	21
XXI	4-Aminopyrazolo[3,4-*d*]pyrimidine	11
XXII	8-Azaadenine	68[c]
XXIII	N^6-Dimethylaminomethylene-8-azaadenine	68
XXIV	8-Aza-2,6-diaminopurine	45
XXV	8-Azahypoxanthine	4
XXVI	8-Azaguanine	ND
XXVII	N^6-Dimethylaminomethylene-8-azaguanine	68
XXVIII	1-Deazapurine	88
XXIX	1-Deazaadenine	93
XXX	1-Deaza-6-nitropurine	73
XXXI	3-Deazapurine	43
XXXII	6-Chloro-3-deazapurine	0
XXXIII	6-Azido-3-deazapurine	58
XXXIV	3-Deazaadenine	22
XXXV	3-Deaza-2,6-diaminopurine	traces
XXXVI	3-Deazaguanine	47
XXXVII	2-Azaadenine	26
XXXVIII	2-Azahypoxanthine	30

TABLE I
(*Continued*)

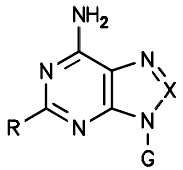
Compound	Base	Conversion, %
XL	1-Deazapurine-N^3-oxide	0
XLI	3-Deazapurine-N^1-oxide	0
XLII	4-Aminopyrrolo[2,3-*d*]pyrimidine	0
XLIII	4-Amino-2-mercaptopyrrolo-[2,3-*d*]pyrimidine	0
XLIV	4-Amino-2-methylthiopyrrolo[2,3-*d*]pyrimidine	0
XLV	2-Amino-4-hydroxypyrrolo[2,3-*d*]pyrimidine	ND
XLVI	N^1,N^6-Etheno-2-azaadenine	ND

[a] Prior to analysis, dimethylaminomethylene and acetyl derivatives were subjected to ammonolysis in 12.5% aqueous ammonia at room temperature overnight; ND not determined; [b] product 2′-deoxyinosine; [c] mixture of 2′-deoxy-8-azaadenosine and 2′-deoxy-8-azainosine.

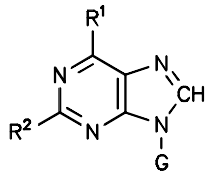
With purine bases and their analogs (Table I), as acceptors may serve derivatives of purine, 1-deazapurine, (imidazo[4,5-*b*]pyridine), 3-deazapurine (imidazo[4,5-*c*]pyridine), 8-azapurine (1,2,3-triazolo[4,5-*d*]pyrimidine) and 2-azapurine (imidazo[4,5-*d*]-1,2,3-triazine), the only condition being the presence of a nitrogen atom in position 7 of the purine system: derivatives of 7-deazapurine (pyrrolo[2,3-*d*]pyrimidine) (*XLIIa – XLVa*)* and pyrazolo[3,4-*d*]pyrimidine (*XXI*) are all inactive. Such a strict condition is not unusual even for other enzymes metabolizing purine derivatives. Another structural feature, clearly hindering interaction of the purine base with the enzyme, is the presence of a group affecting the π-electron distribution of the heteroaromatic system in the base: *N*-oxides of 1-deaza- and 3-deazapurine derivatives *XLa* and *XLIa* do not act as acceptors in the transglycosylation reaction.

Other substituents on the purine nucleous can affect the yields but have no principal influence. The position 6 in the parent purine system or its aza or deaza analogs can evidently be occupied by an atom of hydrogen or halogen, or an azido, nitro, alkyl, amino, alkyl(aryl)amino or dialkylamino group; the presence of a lactam–lactim tautomerism is detrimental to the acceptor activity and replacement of the oxo group in the position 6 of hypoxanthine by a thioxo group (*VIIIa*) resulted in complete failure, the only one observed in this series. However, *S*-alkylation (compound *IXa*) restores again the activity. Substitution in position 2 of the purine moiety with a halogen atom, or an alkyl or amino group has no principal effect on the susceptibility of the base toward the

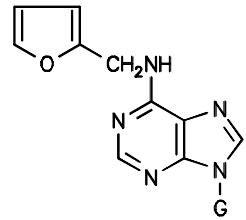
* In the formulae, *a* denotes heterocyclic base, *b* 2-deoxynucleoside, *c* 2,3-dideoxynucleoside.



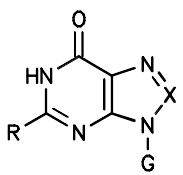
	R	X
I	H	CH
II	CH_3	CH
III	CH_3S	CH
IV	NH_2	CH
V	Cl	CH
XXII	H	N
XXIV	NH_2	N



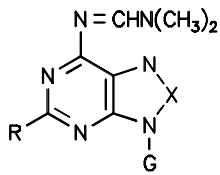
	R^1	R^2
VI	CH_3	H
VII	Cl	H
VIII	SH	H
IX	CH_3S	H
X	$NHCH_3$	H
XI	$N(CH_3)_2$	H
XIII	Cl	Cl



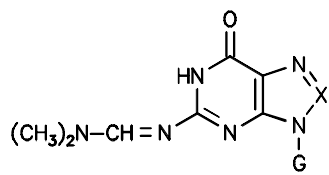
XII



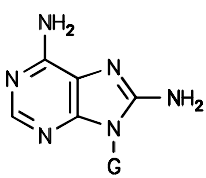
	R	X
XIV	H	CH
XV	NH_2	CH
XVI	CH_3CONH	CH
XXV	H	N
XXVI	NH_2	N



	R	X
XVII	H	CH
XVIII	Cl	CH
XXIII	H	N

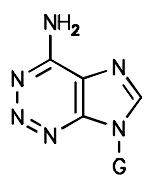


XIX, X = CH
XXVII, X = N

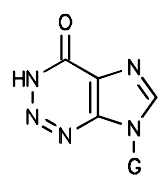


XX

XXI



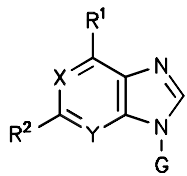
XXXVII



XXXVIII

glycosyl transfer. Although information in this respect is not exhaustive, it seems that the effect of this substitution is rather negative, similarly to the case of introduction of an amino group into position C-8 of adenine (compound *XXa*).

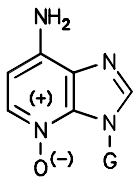
Evaluating the practical applicability of this method in the series of purine derivatives, we encounter two kinds of problems: (i) with adenine derivatives, the transglycosylation reaction may be considerably complicated by residual activity of adenosine aminohydrolase in the immobilized cells which in some cases leads to subsequent formation of the corresponding hypoxanthine derivatives and, which is no less important, (ii) the reaction is often limited by the very low solubility of the purine bases. The latter difficulty can be positively influenced by a technical modification (dimethyl sulfoxide can be added as cosolvent; up to final concentration 8% in the incubation mixture it presents no difficulties); however, we developed a procedure that simultaneously eliminates also the above-mentioned subsequent deamination: the amino group of adenine and guanine derivatives and their aza analogs was converted into an amidine group (the so called dimethylaminomethylene derivative) by reaction with dimethylformamide dineopentyl acetal (the corresponding dimethyl acetal might *N*-methylate the base). In all the cases studied (*XVII* – *XIX*, *XXIII*, *XXVII*), this group was sufficiently stable under the experimental conditions and could be easily removed by ammonolysis directly in the incubation mixture. Of course, it is possible, and sometimes because of



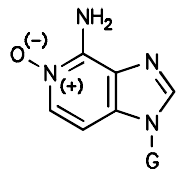
	R^1	R^2	X	Y
XXVIII	H	H	CH	N
XXIX	NH_2	H	CH	N
XXX	NO_2	H	CH	N
XXXI	H	H	N	CH
XXXII	Cl	H	N	CH
XXXIII	N_3	H	N	CH
XXXIV	NH_2	H	N	CH
XXXV	NH_2	NH_2	N	CH
XXXVI	OH	NH_2	N	CH

different chromatographic parameters advantageous, to isolate first the 2′-deoxynucleoside *N*-dimethylaminomethylene derivative and then to subject it to ammonolysis.

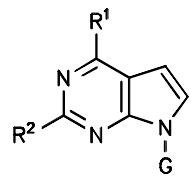
The practical applicability of the transdeoxyribosylation technique was demonstrated by the preparation of 2′-deoxyadenosine (*Ib*), 2′-deoxyguanosine (*XVb*), 2′-deoxyinosine (*XIVb*), 1-deaza-2′-deoxyadenosine (*XXIXb*), and 9-(2-deoxy-β-D-ribofuranosyl) derivatives of 1-deazapurine (*XXVIIIb*), 6-azido-3-deazapurine (*XXXIIIb*) and 1-deaza-6-nitropurine (*XXXb*). Using UV and NMR spectra[16], we ascribed the 9-(2-deoxy-β-D-ribofuranosyl) structure to all these compounds and thus the transfer reaction proceeds under formation of the "natural" N^9-isomers with both the purine and azapurine systems. The 1-deazaadenine derivative *XXIXb* was assigned structure by using the charac-



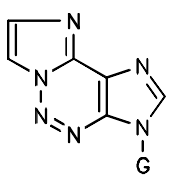
XL



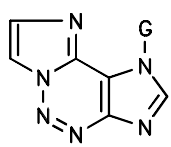
XLI



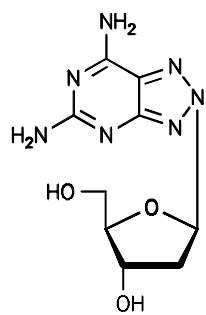
	R^1	R^2
XLII	NH_2	H
XLIII	NH_2	SH
XLIV	NH_2	SCH_3
XLV	OH	NH_2



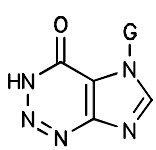
XLVI



XLVII



XLVIII



XXXIX

teristic spectrum of N^9-derivatives, the 6-nitro derivative *XXXb* was identified by comparison with the published ^{1}H NMR spectrum[17]. The structure of 3-deazapurine derivative *XXXIb* was established by comparison with the known N^9-substituted nucleoside analogs[16]. On the preparative scale we performed the synthesis of 2-chloro-2′-deoxyadenosine[18] (*Vb*), an important drug against hairy cell leukemia, and 9-(2-deoxy-β-D-ribofuranosyl)-6-methylpurine (*VIb*), a significant diagnostic of myco- plasma infection of eucaryotic cells[19].

We paid particular attention to 8-azapurine derivatives where the N^8-isomer was invariably the principal product of alkylation reactions[20,21]. Isolation of the product aris-

TABLE II
Acceptor activity of pyrimidine bases in reaction with 2′-deoxyuridine

Compound	Base	Conversion, %
XLIX	Thymine	67
L	5-Ethyluracil	35
LI	5-Propyluracil	0
LII	5-(2-Propyl)uracil	0
LIII	5-(3-Butenyl)uracil	0
LIV	5-Cyclopropyluracil	39
LV	5-Cyclobutyluracil	0
LVI	5-Fluorouracil	64
LVII	5-Iodouracil	57
LVIII	5-Cyanouracil	0
LIX	5-Nitrouracil	0
LX	5-Carboxyuracil	0
LXI	5-Acetyluracil	31
LXII	6-Methyluracil	0
LXIII	2-Pyrimidone	0
LXIV	4-Methyl-2-pyrimidone	0
LXV	4-Methoxy-2-pyrimidone	0
LXVI	Cytosine	0
LXVII	N^4-Dimethylaminomethylenecytosine	22
LXVIII	6-Azauracil	0
LXIX	2-Thiouracil	0
LXX	2-Thiocytosine	0
LXXI	2-Pyridone	0
LXXII	4,6-Dihydroxypyrimidine	0

Incubation mixture contained: 0.85 mmol base, 1.7 mmol 2′-deoxyuridine in 50 ml 50 mM ammonium acetate, pH 5.8 and 150 catalyst beads (diameter 5 mm); incubation time 6 h at 37 °C

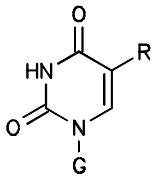
ing from N^6-dimethylaminomethylene-8-azaadenine (*XXIIIa*) has shown that also in this case the reaction afforded exclusively the N^9-isomer *XXIIIb*. Analogously, we prepared 8-aza-2′-deoxyguanosine (*XXVIb*) by reaction of N^6-dimethylaminomethylene-8-azaguanine (*XXVIIa*) followed by ammonolysis. In neither case we detected any other isomers. Surprisingly, the reaction of 8-aza-2,6-diaminopurine (*XXIVa*) gave the 8-isomer *XLVIII* as the only product with no other isomers being detected. We have no interpretation for this unexpected reversal of regiospecificity. Proof of all the structures was convincingly given by UV spectra characteristic of these isomers, by ^{1}H NMR spectra, and particularly by ^{13}C NMR spectra.

The isomer structure of 2′-deoxyribosides of 8-azaadenine (*XXIIb*), 8-aza-2,6-diaminopurine (*XXIVb*) and 8-azaguanine (*XXVIb*) was determined by ^{13}C NMR (proton-decoupled) spectra. The N^9-structure of compounds *XXIIb* and *XXVIb* was confirmed by the characteristic chemical shifts of the carbon atoms of the base as well as the sugar part. These data (see Experimental) are in accord with the literature values[22,23]. On the other hand, in the spectrum of derivative *XXIVb* we observed a marked downfield shift of the signal due to C-4 of the base (8.4 ppm) and of the anomeric C-1′ carbon signal (8.9 ppm) compared with the N^9-substituted derivatives of this base, other signals being affected only negligibly. These changes are characteristic of a substitution of 8-aza-2,6-diaminopurine in N^8-position.

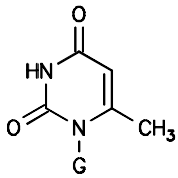
The regiospecific ambiguity was encountered in the case of 2-azapurine derivatives: from 2-azahypoxanthine (*XXXVIIIa*) we obtained the N^7- and N^9-isomers *XXXVIIIb* and *XXXIXb* in equimolar ratio, the tricyclic derivative N^1,N^6-etheno-2-azaadenine *XLVIa* afforded a mixture of N^9-isomer *XLVIb* and N^7-isomer *XLVIIb*, in which the former predominated (9 : 2). It is, however, worth notice that NMR analysis did not prove glycosylation of the triazine ring of the 2-azapurine system although alkylations into this position proceed easily. For the structural assignment we used the same characteristic shifts of the carbon atoms of the base[24]: whereas for compound *XXXVIIIb* the chemical shifts correspond to N^9-substitution, the spectrum of compound *XXXIXb* exhibits a characteristic downfield shift of the C-4 (9.4 ppm), C-8 (1.3 ppm) and C-1′ (1.5 ppm) signals, and an upfield shift of the C-5 signal (10 ppm). According to our experience, these changes are characteristic of the N^7-isomer. The structures of compounds *XLVIb* and *XLVIIb* were assigned analogously.

The applications of the transglycosylation reaction to the purine and analogous systems are thus wide and in majority of cases lead to the expected N^9-isomers. A substantially different situation was found in the pyrimidine series. As seen from Table II, the scope of modifications is generally very limited. From the failure of the acceptor activity of 2-pyrimidinone (*LXIIIa*), its 4-methyl derivative *LXIVa*, and 4-methoxy-2-pyrimidinone (*LXVa*) it is evident that for the acceptor activity a complete uracil or cytosine system is irreplaceable. Since with cytosine the results are distorted by residual cytosine aminohydrolase activity of the immobilized cells (the transdeoxyribosylation with

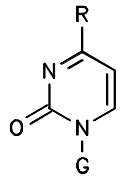
2′-deoxyuridine of the formed uracil is not discernible), also in this case we successfully made use of N^4-dimethylaminomethylene derivative *LXVIIa*. Of the whole studied group of derivatives, only a limited number of 5-alkyluracils are transglycosylation acceptors: the reaction was realized with thymine and its homolog 5-ethyluracil (*La*) but not with the propyl and 2-propyl uracil derivatives *LIa* and *LIIa* and the higher



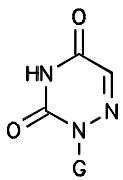
XLIX, R = CH_3
L, R = C_2H_5
LI, R = C_3H_7
LII, R = $(CH_3)_2CH$
LIII, R = $CH_2CH_2CH{=}CH_2$
LIV, R = cyclopropyl
LV, R = cyclobutyl
LVI, R = F
LVII R = I
LVIII, R = CN
LIX, R = NO_2
LX, R = COOH
LXI, R = CH_3CO



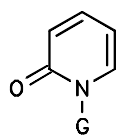
LXII



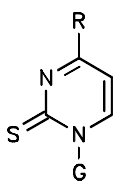
LXIII, R = H
LXIV, R = CH_3
LXV, R = OCH_3
LXVI, R = NH_2
LXVII, R = $N{=}CHN(CH_3)_2$



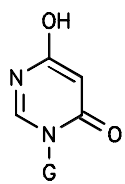
LXVIII



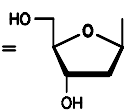
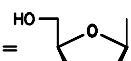
LXXI



LXIX, R = OH
LXX, R = NH_2



LXXII

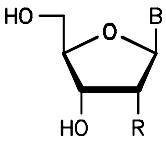
In formulae *I* – *LXXII* : *a*, G = H; *b*, G =  ; *c*, G = 

alkyl derivative *LIII*. Only the perhaps sterically less demanding 5-cyclopropyluracil (*LIVa*) proved to be good substrate, contrary to its homolog, 5-cyclobutyluracil (*LVa*). Whereas the reaction was rendered impossible by some electronegative substituents in position C-5 (cyano, nitro or carboxy groups), with other electronegative substituents (fluorine or iodine atoms) it proceeded normally. Also 5-acetyluracil (*LXIa*) reacted as acceptor of the deoxyribosyl moiety. A substitution of the uracil ring in position 6 is not acceptable (compound *LXIIa*) and the same holds for the oxo–thioxo change in position C-2 (compounds *LXIXa* and *LXXa*). In contrast to the purine series, replacement of –CH= grouping with –N= is not acceptable (*LXVIII*). We cannot make any general conclusions; obviously neither the apparent p*K* of the base nor the bulk of the substituent in position 5 are the decisive factors. It is clear, however, that the reaction, if proceeds, affords the N^1-regioisomer. Such considerable limitation of structural variability indicates that the catalysis may be due to another enzyme than that in the transdeoxyribosylation of purine bases. Also in the pyrimidine series we illustrated the potentialities of the method by preparing biologically active nucleosides: 5-ethyl-2′-deoxyuridine (*Lb*, antiherpetic Aedurid) and 5-fluoro-2′-deoxyuridine (*LVIb*, cytostatic Floxuridine).

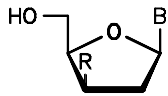
It is important to stress that the transdeoxyribosylation activities operating in immobilized cells need not be necessarily all activities of this type in the corresponding live bacterial cell. As an evident case we can mention the discrepancy between the repeatedly confirmed absence of acceptor activity of 2-pyrimidone (*LXIIIa*) in the transglycosylation catalyzed by immobilized cells and the high conversion of this base into its 2-deoxy-β-D-ribofuranoside *LXIIIb* which we observed in the live cells of *E. coli* in vivo as well as in their cell-free extract in vitro[25].

Donor Activity of Uracil and Thymine Nucleosides Modified in the Sugar Moiety

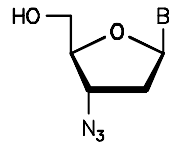
The donor activity was studied on two acceptor models: 6-methylpurine, mentioned above, and hypoxanthine. The results in Table III prove that the hydrogen atom in position 2 of deoxyribose cannot be replaced by hydroxy, alkoxy or amino groups or by a halogen atom in the *ribo* configuration. Also a hydroxyl group in *arabo* configuration in uracil arabinoside (*LXXIX*) has a prohibitive effect whereas an atom of fluorine in this configuration (compound *LXXXI*) is to a certain degree acceptable. The hydroxy group itself in position 2 of 2-deoxy-D-ribose is not indispensable, although the donor activity, if present in compounds without this group, is reduced by several orders of magnitude: in spite of this, we succeeded in performing the transfer of "2,3-dideoxyribofuranosyl" moiety to 6-methylpurine under formation of hitherto undescribed nucleoside *VIc*, as well as to hypoxanthine from dideoxyuridine and dideoxythymidine (*LXXVII*) (Fig. 3) (the isolated 2′,3′-dideoxyinosine (*XIVc*) is the antiviral Didanosin, used in treatment of AIDS). Also 3′-fluoro-2′,3′-dideoxyuridine (*LXXVIII*) proved to be a weakly active donor, whereas 3′-azido-2′,3′-dideoxythymidine (*LXXXII*) is inactive.



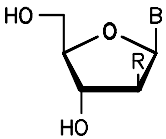
LXXIII, R = H
LXXIV, R = NH_2
LXXV, R = Cl
LXXVI, R = OCH_3



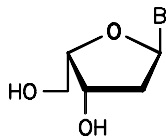
LXXVII, R = H
LXXVIII, R = F



LXXII, B = thymin-1-yl



LXXIX, R = OH
LXXXI, R = F



LXXX

In formulae *LXXIII* – *LXXXI* : B = uracil-1-yl

TABLE III
Donor activity of sugar-modified pyrimidine nucleosides

Compound	Donor	Activity
LXXIII	2′-Deoxyuridine	+
LXXIV	2′-Amino-2′-deoxyuridine	–
LXXV	2′-Chloro-2′-deoxyuridine	–
LXXVI	2′-*O*-Methyluridine	–
LXXVII	2′,3′-Dideoxyuridine	+
LXXVIII	1-(2,3-Dideoxy-3-fluoro-D-*threo*-pentofuranosyl)uracil	±[a]
LXXIX	1-(D-Arabinofuranosyl)uracil	–
LXXX	1-(2-Deoxy-2-L-*erythro*-pentofuranosyl)uracil	–
LXXXI	1-(2-Deoxy-2-fluoro-D-arabinofuranosyl)uracil	+
LXXXII	3′-Azido-2′,3′-dideoxythymidine	–

[a] Traces of product

In this respect, the 5′-hydroxy functionality seems to be of key importance and its relative position to the heterocyclic base system is decisive: 1-(2-deoxy-α-L-*erythro*-pentofuranosyl)uracil (*LXXX*), in which the configuration at C-4′ is opposite to that in 2′-deoxyuridine, is not a transglycosylation donor. We can thus conclude that, provided long reaction time, the transfer, catalyzed with immobilized cells described in this work, can be used also for the preparation of 2,3-dideoxynucleosides, particularly in the purine series. Continuation of this investigation, isolation of the possible modified compounds that might be accessible by this method, as well as utilization of the transglycosylation reaction for other types of nucleoside donors (hexofuranosides etc.) reaches already beyond the scope of this paper.

The described method may also be used for the preparation of isotopically labelled deoxyribonucleosides; the label can be present in the heterocyclic base and/or in the sugar component. In such cases, the mild conditions and easy isolation on the micro scale, as well as the possible recovery of both reaction components are of advantage.

EXPERIMENTAL

Unless stated otherwise, solutions were evaporated at 40 °C/2 kPa and compounds were dried at 13 Pa over phosphorus pentoxide. Melting points were determined on a Kofler block and are uncorrected. Thin-layer chromatography was performed on Silufol UV_{254} sheets in the solvent systems: S1, chloroform–methanol (3 : 1), S2, chloroform–methanol (4 : 1), S3, chloroform–methanol (7 : 3), S4, water–ethanol–acetone–ethyl acetate (1 : 2 : 2 : 12), S5, water–ethanol–acetone–ethyl acetate (1 : 1 : 1 : 4). Preparative chromatography on loose layers was carried out on 40 × 17 × 0.4 cm plates of silica gel containing a UV indicator (Kavalier, Votice, The Czech Republic). Paper chromatography was performed on a paper Whatman No. 1 in system S6, 2-propanol–concentrated aqueous ammonia–water (7 : 1 : 2), paper electrophoresis on a paper Whatman No. 3 MM at 40 V/cm for 1 h in 0.05 M triethylammonium hydrogen carbonate pH 7.5. The electrophoretical mobilities are referenced to uridine 3′-phosphate. Reversed-phase liquid chromatography was carried out on Separon SGX columns (150 × 3.3 mm) in 50 mM potassium dihydrogen phosphate pH 5.5, linear gradient of methanol (H1, 10 – 25% methanol during 20 min; H2, 10 – 45% methanol during 20 min; H3, 5 – 20% methanol

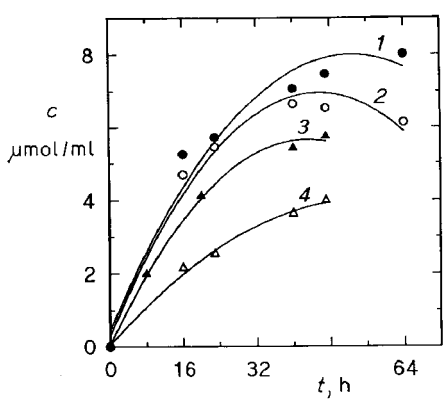


FIG. 3
Time course of 2′,3′-dideoxyribonucleoside synthesis catalyzed by Ca^{2+}-alginate entrapped *E. coli* SPT^- cells. *c*, concentration of 2′,3′-dideoxyribonucleoside formed. *1* 18.5 mM Hypoxanthine, 37 mM 2′,3′-dideoxyuridine and 10 beads/ml. *2* 18.5 mM Hypoxanthine, 37 mM 2′,3′-dideoxyuridine and 5 beads/ml. *3* 18.5 mM 6-Methylpurine, 37 mM 2′,3′-dideoxyuridine and 10 beads/ml. *4* 18.5 mM Hypoxanthine, 37 mM 2′,3′-dideoxythymidine and 5 beads/ml

during 15 min) or in 50 mM triethylammonium hydrogen carbonate pH 7.5 alone (H4) or with 6% of acetonitrile (H5). NMR spectra were measured on a spectrometer Varian UNITY-200 (^{1}H at 200 MHz, ^{13}C at 50.3 MHz) or Varian UNITY-500 (^{1}H at 500 MHz and ^{13}C at 125.7 MHz) in CD_3SOCD_3. Chemical shifts of protons were referenced to tetramethylsilane as internal standard whereas shifts of carbon atoms to the solvent signal and then calculated according to the relationship $\delta(CD_3SOCD_3) = 39.7$ ppm. In addition to the usual proton-decoupled ^{13}C NMR spectra we measured also J-modulated spectra ("attached proton test pulse sequence") or proton-coupled spectra, enabling carbon signals of C and CH_2 to be distinguished from those of CH and CH_3. UV absorption spectra were measured on a Beckmann DU-65 spectrometer; the wavelengths of extrema are given in nm. Mass spectra were taken on a ZAB-EQ spectrometer (VG Analytical) using EI (electron energy 70 eV) and FAB (ionization by *X*e, accelerating voltage 8 kV) techniques. Spectrophotometric determination of content of compounds was done in aqueous solutions (pH 2) and at wavelength of the absorption maximum, using published extinction coefficient values.

Materials

Thymidine, 2′-deoxyadenosine, 2′-deoxyguanosine, 2′-deoxyinosine, hypoxanthine and 5-fluorouracil were obtained from Calbiochem, adenine, guanine and thymine from Fluka. 2′-Deoxy-5-fluorouridine, 8-azaadenine, 8-aza-2,6-diaminopurine, 8-azaguanine and 2,6-dichloropurine were Sigma products. 2′-Deoxyuridine[1], dimethylformamide dineopentyl acetal[26] and 6-methylpurine[27] were prepared by published procedures. The pyrimidine bases were kindly given by Dr M. Ledvina of this Institute, other purine base analogs were prepared by published procedures. Bacterial cells were immobilized using alginate Protanal LF 10/60 (Protan, Norway).

Preparation of the Biocatalyst

Cultures of *Escherichia coli* SPT$^-$ (ref.[28]) were grown at 37 °C in a synthetic medium[29] supplemented with thymine (1 μg/ml) until the cell density has reached $8 \cdot 10^8$ cells/ml. The harvested cells were exhaustively washed by centrifugation (6 000 g, 35 min; 3 times) in the presence of 0.9% KCl. The resulting sediment of bacterial cells was suspended in 0.9% KCl (1 g of wet cells in 4 ml) and mixed with a water solution of alginate (1 g of the wet biomass in 4 ml of 0.9% KCl with 6 ml of 4.76% alginate). The ionotropic size-controlled gelation to entrap bacterial cells was achieved by dropping *E. coli* SPT$^-$-alginate suspension to 2% $CaCl_2$ (the precipitation bath)[30]. The resulting calcium-alginate beads (5 mm diameter) were then stirred in the precipitation bath for 4 h, washed 4 times with an excess of 0.9% KCl and stored in the same medium at 4 °C until used.

Stability of the Biocatalyst

The incubation mixture contained 6-methylpurine (158 mg, 1.18 mmol) and 2′-deoxyuridine (537 mg, 2.35 mmol) in 30 ml of 0.05 M ammonium acetate pH 5.8. After addition of the catalyst (90 beads, 370 mg of biomass), the mixture was incubated in a Dubnoff's incubator at 37 °C for 16 h. After decanting the supernatant, fresh solution of the same composition was added and the incubation procedure was repeated nine times (total). The samples were analyzed by HPLC on an SGX C18 column (150 × 3.3 mm); isocratic elution with 0.01 M potassium dihydrogen phosphate containing 13.5% of methanol. Molar absorption coefficients of 6-methylpurine and its deoxyriboside were taken as identical. The conversion in the last mixture achieved 99.6%, the average in all experiments was 98.7 ± 0.76%.

Synthesis of 2′-Deoxyribonucleosides

A) Analytical procedure. The incubation mixture contained the corresponding heterocyclic base (0.37 mmol) and 2′-deoxyuridine (0.74 mmol) in 50 mM ammonium acetate pH 5.7 (10 ml); 30 to 50 beads of the biocatalyst were added and the mixture was incubated for 16 h at 37 °C. The reaction was followed by HPLC (reversed phase Tessek-Separon^{TM} SCX C18, 7 μm, 15 cm × 3.3 mm) in an appropriate buffered solvent system. The data are given in Tables I and II.

B) Preparative procedure. Unless stated otherwise, the amount of reagents and solvents was scaled up 5 times with respect to procedure *A*, the reaction conditions and analytical procedure being identical. The supernatant was decanted, the catalyst washed by the same buffer as above (10 ml) and the combined supernatants were mebrane-filtered (nitrocellulose filters, 0.45 μm). The filtrate was further processed as indicated in specific cases.

Synthesis of 2′,3′-Dideoxyribonucleosides

The biocatalyst (50 to 100 beads) was added to a solution of the appropriate purine base (0.185 mmol) and 2′,3′-dideoxyuridine or 2′,3′-dideoxythymidine (0.37 mmol) in 50 mM ammonium acetate, pH 5.7 (10 ml). The reaction course (at 37 °C) was followed by HPLC.

2-Chloroadenine (*Va*)

A mixture of 2,6-dichloropurine (*XIIIa*, 1.5 g, 7.9 mmol) and 30% methanolic ammonia (60 ml) was heated at 100 °C for 12 h in an autoclave and then evaporated. The residue was dissolved in water and deionized on a column of Dowex 50 X 8 (H^+ form, 50 ml). The ion exchanger was washed with water to loss of UV absorption, suspended in dilute (1 : 1) aqueous ammonia (250 ml), stirred for 2 h, filtered, washed with water, and the filtrate was evaporated in vacuo. The dry residue was codistilled with ethanol (3 × 50 ml), mixed with acetone and filtered; yield 0.90 g (67%) of chromatographically homogeneous product *Va*, not melting up to 260 °C, R_F 0.42 (S4). For $C_5H_4ClN_5$ (169.6) calculated: 35.42% C, 2.38% H, 20.91% Cl, 41.30% N; found: 35.28% C, 2.44% H, 21.11% Cl, 41.06% N.

2-Chloro-N^6-dimethylaminomethyleneadenine (*XVIIIa*)

A mixture of compound *Va* (2.0 g, 11.8 mmol), dimethylformamide (30 ml) and dimethylformamide dineopentyl acetal (6 ml) was stirred at 80 °C to dissolution (calcium chloride protecting tube) and the stirring was continued at room temperature overnight. The solvent was evaporated at 40 °C/2 kPa to dryness, the residue was codistilled with toluene (2 × 25 ml) under the same conditions, and mixed with ethanol (20 ml). Ether (300 ml) was added, the suspension was filtered, washed with ether and dried in vacuo. Yield 2.0 g (75%) of compound *XVIIIa*, not melting up to 280 °C. For $C_8H_9ClN_6$ (224.7) calculated: 42.76% C, 4.04% H, 15.78% Cl, 37.41% N; found: 42.56% C, 4.24% H, 15.42% Cl, 37.38% N.

N^2-Dimethylaminomethyleneguanine (*XIXa*)

A mixture of guanine (3 g, 20 mmol), dimethylformamide (90 ml) and dimethylformamide dineopentyl acetal (15 ml) was stirred at 110 °C (calcium chloride protecting tube) to dissolution (2 h) and then evaporated at 60 °C/13 Pa. The residue was codistilled with toluene and ethanol (50 ml) was added together with small pieces of solid CO_2 (about 10 g). After standing at room temperature for 30 min, the mixture was evaporated in vacuo and the residue was recrystallized from water; yield 3.5 g (85%) of compound *XIXa* not melting up to 280 °C. For $C_8H_{10}N_6O$ (206.2) calculated: 46.60% C,

4.89% H, 40.76% N; found: 46.54% C, 4.95% H, 41.02% N. ^{1}H NMR spectrum: 12.70 brs, 1 H and 11.20 brs, 1 H (NH); 8.55 s, 1 H (H-8); 7.85 s, 1 H (N=CH); 3.00 s, 3 H and 3.10 s, 3 H (2 × CH_3).

8-Aza-N^6-dimethylaminomethyleneadenine (*XXIIIa*)

A mixture of 8-azaadenine (*XXIIa*, 5 g, 36.8 mmol), dimethylformamide (50 ml) and dimethylformamide dineopentyl acetal (25 ml) was stirred at 100 °C (calcium chloride tube) for 16 h and then evaporated at 40 °C/2 kPa. The dry residue was codistilled with toluene (2 × 25 ml) under the same conditions and then mixed with ethanol (100 ml) under simultaneous addition of solid CO_2 to maintain neutrality. After 30 min, the product was filtered, washed with ethanol and ether, and dried to give 5.6 g (79%) of compound *XXIIIa*, not melting up to 260 °C. The product was homogeneous according to HPLC in H5 (*k* 0.80). For $C_7H_9N_7$ (191.2) calculated: 43.97% C, 4.74% H, 51.28% N; found: 44.17% C, 4.46% H, 51.80% N.

2′-Deoxyadenosine (*Ib*)

The catalyst (100 beads) was added to a solution of N^6-dimethylaminomethyleneadenine (*XVIIa*, 76.4 mg, 0.4 mmol) and 2′-deoxyuridine (182.4 mg, 0.8 mmol) in 0.05 M ammonium acetate pH 5.8 (10 ml) and the mixture was incubated in a Dubnoff's incubator at 37 °C for 18 h. After decanting, the catalyst was washed with water (10 ml), the combined portions were membrane-filtered (vide supra) and the filtrate was mixed with the same volume of concentrated aqueous ammonia. The mixture was heated rapidly to the boil, set aside for 30 min and concentrated in vacuo to about 10 ml. The concentrate was filtered through a small layer of Celite and applied onto a column of octadecylsilica gel (30 μm, 100 ml). After elution with water (2 ml/min) which washed out 2′-deoxyuridine and uracil, the column was further eluted with 10% aqueous methanol. The fractions containing 2′-deoxyadenosine (TLC, S3) were evaporated and the product was crystallized from ethanol with addition of ether; yield 44 mg of compound *Ib* (44% related to the base). The product was identical (HPLC) with an authentic material.

9-(2-Deoxy-β-D-ribofuranosyl)-2,6-diaminopurine (*IVb*)

The catalyst (250 beads) was added to a solution of 2′-deoxyuridine (0.925 g, 4.05 mmol) and 2,6-diaminopurine (*IVa*, 0.255 g, 1.7 mmol) in 0.05 M ammonium acetate buffer pH 5.8 (50 ml) and the mixture was incubated in a Dubnoff's incubator at 37 °C for 7 h. The mixture was decanted and the catalyst washed with water. After membrane-filtration, the mixture was separated by preparative thin-layer chromatography in system S5. The product-containing fraction was evaporated and the residue crystallized from ethanol–ether to give 0.20 g (41%) of 2′-deoxynucleoside *IVb*, m.p. 147 °C, R_F 0.33 (S5). For $C_{10}H_{14}N_6O_3$. H_2O (284.1) calculated: 42.26% C, 5.67% H, 29.56% N; found: 42.68% C, 5.38% H, 29.58% N. ^{1}H NMR spectrum: 7.92 s, 1 H (H-8); 6.77 brs, 2 H and 5.76 brs, 2 H (2 × NH_2); 6.17 dd, 1 H, *J*(1′,2a′) = 8.1, *J*(1′,2b′) = 6.0 (H-1′); 5.28 brs, 1 H and 5.25 brs, 1 H (5′-OH and 3′-OH); 4.35 brdt, 1 H, Σ*J* = 10.8 (H-3′); 3.84 td, 1 H, Σ*J* = 10.5 (H-4′); 3.59 dd, 1 H, *J*(5a′,4′) = 4.1, *J*(gem) = 12.0 (H-5a′); 3.51 dd, 1 H, *J*(5b′,4′) = 3.9, *J*(gem) = 12.0 (H-5b′); 2.58 ddd, 1 H, *J*(2a′,3′) = 5.6, *J*(2a′,1′) = 8.1, *J*(gem) = 13.2 (H-2a′); 2.18 ddd, 1 H, *J*(2b′,3′) = 2.7, *J*(2b′,1′) = 6.0, *J*(gem) = 13.2 (H-2b′). Mass spectrum, *m*/*z*: 267.2 (M + H). UV spectrum, λ_{max} (ε), pH 2: 290.0 (9 800), 253.0 (11 000); pH 7: 281.0 (12 000), 255.0 (9 300); pH 12: 280.0 (10 700), 256.0 (9 800).

9-(2-Deoxy-β-D-ribofuranosyl)-2-chloroadenine (*Vb*)

A solution of 2-chloro-N^6-dimethylaminomethyleneadenine (*XVIIIa*, 178 mg, 0.75 mmol) in a mixture of ethanol and dimethyl sulfoxide (3 : 2, 10 ml), together with the catalyst (160 beads), was added to a solution of 2′-deoxyuridine (370 mg, 1.5 mmol) in 0.05 M acetate buffer pH 5.8 (40 ml). The mixture was incubated at 37 °C for 16 h and worked up as described for 9-(2-deoxy-β-D-ribofuranosyl)-6-methylpurine. Crystallization from ethanol with addition of ether afforded 285 mg (67%) of product *Vb*, m.p. 232 °C. For $C_{10}H_{12}ClN_5O_3$ (285.7) calculated: 42.04% C, 4.23% H, 12.41% Cl, 24.51% N; found: 42.20% C, 4.11% H, 12.32% Cl, 24.47% N. ^{1}H NMR spectrum: 8.36 s, 1 H (H-8); 7.82 brs, 2 H (NH_2); 6.26 dd, 1 H, J(1′,2a′) = 7.3, J(1′,2b′) = 6.3 (H-1′); 5.32 br, 1 H (3′-OH); 4.97 brt, 1 H, J = 4.5 (5′-OH); 4.38 m, 1 H, J(3′,2b′) = J(3′,4′) = 3.2, J(3′,2a′) = 5.9 (H-3′); 3.85 brdt, 1 H, J(3′,4′) = 2.7, J(4′,5a′) = J(4′,5b′) = 4.6 (H-4′); 3.60 dt, 1 H and 3.51 dt, 1 H, J(5′,4′) = J(5′,OH) = 4.5, J(gem) = 11.7 (2 × H-5′); 2.64 ddd, 1 H, J(2a′,1′) = 7.5, J(2a′, 3′) = 5.9, J(gem) = 13.2 (H-2a′); 2.28 ddd, 1 H, J(2b′,1′) = 6.1, J(2b′,3′) = 3.2, J(gem) = 13.2, (H-2b′). Mass spectrum, *m/z*: 286 (M^+). UV spectrum, λ_{max} (ε), pH 2: 265 (14 500); pH 12: 265 (14 800).

9-(2-Deoxy-β-D-ribofuranosyl)-6-methylpurine (*VIb*)

The catalyst (600 beads, vide supra) was added to a solution of 2′-deoxyuridine (4.56 g, 20 mmol) and 6-methylpurine (*VIa*, 1.35 g, 10 mmol) in 0.05 M acetate buffer pH 5.7 (200 ml) and the mixture was incubated in a Dubnoff's incubator at 37 °C for 7 h. The supernatant was decanted, the catalyst washed with water (50 ml) and the combined portions were membrane filtered (Millipore, 0.45 μm). The filtrate was applied onto a column of octadecylsilica gel (30 μm, 190 ml), preequilibrated with water. The column was washed with water (2 ml/min) and the UV-absorbing fractions (20 ml) were analyzed by TLC in system S1. Fractions containing 2′-deoxyuridine were combined, taken down in vacuo and the residue was crystallized from ethanol with addition of ether to give 1.25 g (27%) of recovered 2′-deoxyuridine, identical with an authentic material. Further elution with 5% aqueous methanol and subsequent crystallization from ethanol with addition of light petroleum afforded 1.60 g (64%) of 9-(2-deoxy-β-D-ribofuranosyl)-6-methylpurine (*VIb*), m.p. 161 °C. For $C_{11}H_{14}N_4O_3$ (250.3) calculated: 52.79% C, 5.64% H, 22.39% N; found: 52.85% C, 5.56% H, 22.14% N. Mass spectrum, *m/z*: 250 (M^+). ^{1}H NMR spectrum: 8.77 s, 1 H (H-8); 8.71 s, 1 H (H-2); 6.44 dd, 1 H, J(1′,2a′) = 7.2, J(1′,2b′) = 6.3 (H-1′); 5.34 d, 1 H, J(3′,OH) = 4.0, (3′-OH); 4.99 t, 1 H, J(5′,OH) = 5.6 (5′-OH); 4.43 m, 1 H (H-3′); 3.88 td, 1 H, J(3′,4′) = 3.0, J(4′,5a′) = J(4′,5b′) = 4.5 (H-4′); 3.61 dq, 1 H, J(5a′,4′) = 4.5, J(5a′,OH) = 5.4, J(gem) = 12.0 (H-5a′); 3.51 dq, 1 H, J(5b′,4) = 4.5, J(5b′,OH) = 5.4, J(gem) = 12.0, (H-5b′); 2.77 dq, 1 H, J(2a′,1′) = 7.2, J(2a′,3′) = 5.8, J(gem) = 13.3 (H-2a′); 2.71 s, 3 H (6-CH_3); 2.32 dq, 1 H, J(2b′,1′) = 6.3, J(2b′,3′) = 3.5, J(gem) = 13.3 (H-2b′). UV spectrum, λ_{max} (ε), pH 2: 265 (7 000); pH 12: 263 (7 900).

9-(2-Deoxy-β-D-ribofuranosyl)-6-methylthiopurine (*IXb*)

The catalyst (100 beads, vide supra) was added to a solution of 2′-deoxyuridine (0.37 g, 1.6 mmol) and 6-methylthiopurine (*IXa*, 0.12 g, 0.7 mmol) in 0.05 M ammonium acetate buffer pH 5.8 (20 ml) and the mixture was incubated in a Dubnoff's incubator at 37 °C for 7 h. The supernatant was decanted and the catalyst washed with water. After membrane filtration, the mixture was separated by preparative thin-layer chromatography in system S2. The product fraction was evaporated and the residue crystallized from ethanol–ether to give 0.19 g (95%) of 2′-deoxynucleoside *IXb*, m.p. 159 °C, R_F 0.68 (S2). For $C_{11}H_{14}N_4O_3S$ (282.2) calculated: 46.81% C, 5.00% H, 19.84% N; found: 46.75% C, 5.00% H, 20.18% N. ^{1}H NMR spectrum: 8.73 s, 1 H and 8.66 s, 1 H (H-2 and H-8); 6.43 brt, 1 H, ΣJ = 13.4 (H-1′); 5.35 d, 1 H, J(3′,OH) = 4.4 (3′-OH); 5.00 t, 1 H, J = 5.6 (5′-OH); 4.44 m, 1 H,

(H-3′); 3.89 td, 1 H, *J*(4′,3′) = 3.2, *J*(4′,5a′) = *J*(4′,5b′) = 4.5 (H-4′); 3.62 dt, 1 H, *J*(5a′,4′) = *J*(5a′,OH) = 5.0, *J*(gem) = 11.7 (H-5a′); 3.52 ddd, 1 H, *J*(5b′,4′) = 4.6, *J*(5b′,OH) = 5.9, *J*(gem) = 11.7 (H-5b′); 2.76 ddd, 1 H, *J*(2a′,3′) = 5.9, *J*(2a′,1′) = 7.1, *J*(gem) = 13.2 (H-2a′); 2.66 s, 3 H (CH_3); 2.34 ddd, 1 H, *J*(2b′,3′) = 3.2, *J*(2b′,1′) = 6.3, *J*(gem) = 13.2 (H-2b′). Mass spectrum, *m*/*z*: 283.2 (M + H). UV spectrum, λ_{max} (ε), pH 2: 291.0 (19 100); pH 7: 291.0 (19 100), pH 12: 291.0 (24 000).

9-(2-Deoxy-β-D-ribofuranosyl)-N^6-furfuryladenine (*XIIb*)

The catalyst (250 beads, vide supra) was added to a solution of 2′-deoxyuridine (0.925 g, 4.05 mmol) and N^6-furfuryladenine (*XIIa*, 0.4 g, 1.85 mmol) in 0.05 M ammonium acetate buffer pH 5.8 (50 ml) and the mixture was incubated in a Dubnoff's incubator at 37 °C for 7 h. The supernatant was decanted and the catalyst washed with water. After membrane filtration, the mixture was separated by preparative thin-layer chromatography in system S2. The product fraction was evaporated and the residue on precipitation from ethanol with ether afforded 0.35 g (56%) of hygroscopic 2′-deoxynucleoside *XIIb*, R_F 0.72 (S2). ^{1}H NMR spectrum: 8.37 s, 1 H and 8.23 s, 1 H (H-8 and H-2); 8.29 br, 1 H (NH); 7.53 brdd, 1 H, *J*(5″,4″) = 2.0, *J*(5″,3″) = 1.0 (H-5″); 6.33 dd, 1 H, *J*(4″,3″) = 3.5, *J*(4″,5″) = 2.0 (H-4″); 6.22 brd, 1 H, *J*(3″,4″) = 3.5, *J*(3″,5″) = 1.0 (H-3″); 6.35 dd, 1 H, *J*(1′,2a′) = 7.8, *J*(1′,2b′) = 6.1 (H-1′); 5.31 d, 1 H, *J*(3′,OH) = 4.0 (3′-OH); 5.19 dd, 1 H, *J* = 4.9 and 5.6 (5′-OH); 4.68 br, 2 H (CH_2); 4.41 dq, 1 H, Σ*J* = 15.4 (H-3′); 3.88 td, 1 H, *J*(4′,3′) = 2.5, *J*(4′,5a′) = *J*(4′,5b′) = 4.2 (H-4′); 3.62 dt, 1 H, *J*(5a′,4′) = *J*(5a′,OH) = 4.6, *J*(gem) = 11.7 (H-5a′); 3.52 ddd, 1 H, *J*(5b′,4′) = 4.2, *J*(5b′,OH) = 6.6, *J*(gem) = 11.7 (H-5b′); 2.73 ddd, 1 H, *J*(2a′,3′) = 5.9, *J*(2a′,1′) = 7.8, *J*(gem) = 13.2 (H-2a′); 2.26 ddd, 1 H, *J*(2b′,3′) = 2.9, *J*(2b′,1′) = 6.1, *J*(gem) = 13.2 (H-2b′). $C_{15}H_{17}N_5O_4$ (331.3): mass spectrum, *m*/*z*: 332.1 (M + H). UV spectrum, λ_{max} (ε), pH 2: 268.0 (21 700); pH 7: 267.0 (23 400), pH 12: 268.0 (8 200). Spectrophotometrically determined content: >90%.

2′-Deoxyinosine (*XIVb*)

The incubation mixture contained hypoxanthine (204 mg, 1.5 mmol), 2′-deoxyuridine (684 mg, 3 mmol) and 400 beads of the catalyst in 0.05 M ammonium acetate buffer pH 5.8 (30 ml). After incubation at 37 °C for 16 h, the supernatant was decanted, the catalyst washed with water (10 ml) and the combined portions membrane filtered. After concentration in vacuo to about 10 ml, the mixture was separated by preparative HPLC, elution with a gradient water–methanol. The product fraction was evaporated in vacuo and the residue crystallized from ethanol–ether; yield 0.25 g (65%) of product *XIVb*, homogeneous according to HPLC, TLC and identical with an authentic sample.

2′-Deoxyguanosine (*XVb*)

A solution of N^2-dimethylaminomethylene-2′-deoxyguanosine (*XIXb*, 100 mg, 0.3 mmol) in a mixture of water (5 ml) and concentrated aqueous ammonia (2 ml) was allowed to stand at room temperature overnight and then taken down in vacuo, affording 2′-deoxyguanosine (*XVb*) in quantitative yield. The product was identical with an authentic sample according to paper chromatography in system S6 and to HPLC in system H4.

N^2-Dimethylaminomethylene-2′-deoxyguanosine (*XIXb*)

The title compound was prepared from N^2-dimethylaminomethyleneguanine (*XIXa*, 207 mg, 1.0 mmol) and 2′-deoxyuridine (456 mg, 2.0 mmol) in 0.05 M ammonium acetate buffer pH 5.8 (20 ml) in the same manner as described for 2′-deoxyadenosine (incubation for 30 h). After membrane filtration, the filtrate was applied onto a column of octadecylsilica gel (vide supra). Washing with water separated

uracil and 2′-deoxyuridine, 5% aqueous methanol eluted N^2-dimethylaminomethyleneguanine. Elution with 10% aqueous methanol afforded the deoxynucleoside. The product fractions were combined, the solvent evaporated in vacuo and the residue precipitated with ether from methanolic solution. Yield 161 mg (50%) of the title compound *XIXb*, m.p. 154 °C. For $C_{13}H_{18}N_6O_4$ (322.3) calculated: 48.44% C, 5.63% H, 26.08% N; found: 48.27% C, 5.46% H, 26.18% N. Mass spectrum, *m*/*z*: 322 (M^+). ^{1}H NMR spectrum: 8.62 s, 1 H (H-8); 8.35 s, 1 H (N=CH); 6.52 t, 1 H, *J* = 6.7 (H-1′); 4.32 m, 1 H (H-3′); 3.84 m, 1 H (H-4′); 3.55 m, 2 H (2 × H-5′); 3.15 s, 3 H and 3.02 s, 3 H (2 × NCH_3). UV spectrum, λ_{max} (ε), pH 7: 235 (17 300), 290 (23 700).

8-Aza-2′-deoxyadenosine (*XXIIb*)

The reaction was performed with 8-aza-N^6-dimethylaminomethyleneadenine (*XXIIIa*, 354 mg, 1.85 mmol) and 2′-deoxyuridine (844 mg, 3.7 mmol) in 0.05 M ammonium acetate buffer pH 5.8 (40 ml) in the same manner as described for 2′-deoxyadenosine (*Ib*) (incubation at 37°C for 16 h). After ammonolysis, the mixture was evaporated in vacuo and the product was isolated by preparative HPLC in a gradient methanol–water. The product fraction was evaporated and the residue crystallized from ethanol–ether; yield 120 mg (26% related to the base) of 8-aza-2′-adenosine (*XXIIb*), m.p. 196 – 199 °C. For $C_9H_{12}N_6O_3$ (252.2) calculated: 42.86% C, 4.80% H, 33.32% N; found: 42.57% C, 4.60% H, 33.50% N. ^{1}H NMR spectrum: 8.30 s, 1 H (H-2); 8.15 brs, 1 H and 8.30 brs, 1 H (NH_2); 6.61 t, 1 H, *J*(1′,2a′) = *J*(1′,2b′) = 6.35 (H-1′); 5.38 brs, 1 H (3′-OH); 4.89 brt, 1 H, *J* = 5.0 (5′-OH); 4.54 m, 1 H, (H-3′); 3.90 brq, 1 H, Σ*J* = 14.4, *J*(3′,4′) = 3.4 (H-4′); 3.57 dd, 1 H, *J*(5a′,4′) = 5.1, *J*(gem) = 11.7 (H-5a′); 3.40 dd, 1 H, *J*(5b′,4′) = 5.9, *J*(gem) = 11.7 (H-5b′); 3.03 dt, 1 H, *J*(2a′,1′) = *J*(2a′,3′) = 6.1, *J*(gem) = 13.4 (H-2a′); 2.41 ddd, 1 H, *J*(2b′,3′) = 4.4, *J*(2b′,1′) = 6.8, *J*(gem) = 13.4 (H-2b′). ^{13}C NMR spectrum: 157.14 (C-2); 156.42 (C-6); 148.97 (C-4); 124.50 (C-5); 88.66 (C-4′); 85.80 (C-1′); 71.09 (C-3′); 62.25 (C-5′); 38.32 (C-2′). UV spectrum, λ_{max} (ε), pH 2: 278 (10 600).

9-(2-Deoxy-β-D-ribofuranosyl)-8-azaguanine (*XXVIb*)

The catalyst (250 beads, vide supra) was added to a solution of 2′-deoxyuridine (0.925 g, 4.05 mmol) and 8-aza-N^2-dimethylaminomethyleneguanine (*XXVIIa*, 0.275 g, 1.3 mmol) in 0.05 M ammonium acetate buffer pH 5.8 (46 ml) and dimethyl sulfoxide (4 ml) and the mixture was incubated in a Dubnoff's incubator at 37 °C for 7 h. The supernatant was decanted and the catalyst washed with water. The mixture was worked up as described for 2′-deoxyadenosine (*Ib*). After membrane-filtration, the mixture was separated by preparative thin-layer chromatography in system S5. The product fraction was evaporated and the residue crystallized from ethanol–ether to give 0.05 g (15%) of 2′-deoxynucleoside *XXVIb*, not melting up to 280 °C, R_F 0.49 (S5). ^{1}H NMR spectrum: 10.25 br, 1 H (NH); 6.95 br, 2 H (NH_2); 6.29 t, 1 H, *J* = 6.4 (H-1′); 4.75 t, 1 H, *J* = 5.5 (5′-OH); 4.46 brpent, 1 H, Σ*J* = 17.6 (H-3′); 3.83 td, 1 H, *J*(4′, 3′) = 3.9, *J*(4′,5a′) = *J*(4′,5b′) = 5.8 (H-4′); 5.32 d, 1 H, *J*(3′,OH) = 4.4 (3′-OH); 3.50 dt, 1 H, *J*(5a′,4′) = *J*(5a′,OH) = 5.4, *J*(gem) = 11.7 (H-5a′); 3.37 dt, 1 H, *J*(5b′,4′) = 4.2, *J*(5b′,OH) = 6.1, *J*(gem) = 11.7 (H-5b′); 2.89 ddd, 1 H, *J*(2a′,3′) = *J*(2a′,1′) = 6.1, *J*(gem) = 13.2 (H-2a′); 2.32 ddd, 1 H, *J*(2b′, 3′) = 4.6, *J*(2b′,1′) = 6.7, *J*(gem) = 13.2 (H-2b′). ^{13}C NMR spectrum: 155.91 (C-6); 155.86 (C-2); 151.84 (C-4); 124.84 (C-5); 88.47 (C-4′); 84.32 (C-1′); 71.13 (C-3′); 62.42 (C-5′); 38.27 (C-2′). $C_9H_{12}N_6O_4$ (268.2): mass spectrum, *m*/*z*: 269.1 (M + H). UV spectrum, λ_{max} (ε), pH 2: 255.0 (10 500); pH 7: 255.0 (10 600), pH 12: 279.0 (9 600). Spectrophotometrically determined content: >90%.

9-(2-Deoxy-β-D-ribofuranosyl)-1-deazapurine (*XXVIIIb*)

The catalyst (135 beads, vide supra) was added to a solution of 2′-deoxyuridine (0.8 g, 3.4 mmol) and 1-deazapurine (*XXVIIIa*, 0.2 g, 1.7 mmol) in 0.05 M ammonium acetate buffer pH 5.8 (40 ml) and the mixture was incubated in a Dubnoff's incubator at 37 °C for 7 h. The supernatant was decanted and the catalyst washed with water. After membrane-filtration, the mixture was separated by preparative HPLC, elution with a gradient methanol–water. Upon mixing with dry ether, the product crystallized. Yield 0.20 g (51%) of deoxyribonucleoside *XXVIIIb*, m.p. 139 – 141 °C. For $C_{11}H_{13}N_3O_3$ (235.2) calculated: 56.17% C, 5.57% H, 17.86% N; found: 55.81% C, 5.39% H, 17.77% N. HPLC (H5, k = 6.54). ^{1}H NMR spectrum: 8.69 s, 1 H (H-8), 8.38 dd, 1 H, J(2,6) = 1.4, J(2,1) = 4.8 (H-2); 8.13 dd, 1 H, J(6,1) = 8.1, J(6,2) = 1.4 (H-6); 7.33 dd, 1 H, J(1,2) = 4.8, J(1,6) = 8.1 (H-1); 6.54 dd, 1 H, J(1′,2a′) = 7.6, J(1′,2b′) = 6.3 (H-1′); 5.32 d, 1 H, J(OH,3′) = 4.2 (3′-OH); 5.08 t, 1 H, J(OH,5′) = 5.6 (5′-OH); 4.47 m, 1 H (H-3′); 3.92 dt, 1 H, J(4′,3′) = 2.7, J(4′,5a′) = J(4′,5b′) = 4.5 (H-4′); 3.62 m, 2 H (H-5′); 2.81 ddd, 1 H, J(2a′,3′) = 5.8, J(2a′,1′) = 7.6, J(gem) = 15.4 (H-2a′); 2.32 ddd, 1 H, J(2b′,3′) = 3.2, J(2b′,1′) = 6.3, J(gem) = 15.4 (H-2b′). ^{13}C NMR spectrum: 146.80 (C-4); 144.40 (C-8); 144.39 (C-2); 136.90 (C-5); 128.36 (C-6); 119.20 (C-1); 88.54 (C-4′); 84.65 (C-1′); 71.57 (C-3′); 62.43 (C-5′); 40.08 (C-2′). UV spectrum, λ_{max} (ε), pH 2: 280.9 (8 600), 274.8 (9 600), 235.8 (4 800); pH 12: 280.8 (8 500), 242.8 (5 200).

9-(2-Deoxy-β-D-ribofuranosyl)-1-deazaadenine (*XXIXb*)

The catalyst (135 beads, vide supra) was added to a solution of 2′-deoxyuridine (0.8 g, 3.4 mmol) and 1-deazaadenine (*XXIXa*, 0.23 g, 1.7 mmol) in 0.05 M ammonium acetate buffer pH 5.8 (40 ml) and the mixture was incubated in a Dubnoff's incubator at 37 °C for 7 h. The supernatant was decanted and the catalyst washed with water. After membrane-filtration, the mixture was separated by preparative HPLC, elution with a gradient methanol–water. Crystallization from ethanol–ether–light petroleum afforded 0.1 g (27%) of product *XXIXb*, m.p. 224 – 226 °C. For $C_{11}H_{14}N_4O_3$ (250.2) calculated: 52.80% C, 5.64% H, 22.38% N; found: 52.57% C, 5.60% H, 22.50% N. HPLC (H5, k = 5.48). ^{1}H NMR spectrum: 8.26 s, 1 H (H-8); 7.78 d, 1 H, J(1,2) = 5.6 (H-2); 6.44 brs, 2 H (NH_2); 6.38 d, 1 H, J(2,1) = 5.6 (H-1); 6.38 dd, 1 H, J(1′,2a′) = 8.4, J(1′,2b′) = 5.9 (H-1′); 5.77 dd, 1 H, J = 4.3 and 7.5 (5′-OH); 5.28 d, 1 H, J(OH,3′) = 3.7 (3′-OH); 3.91 brq, 1 H, ΣJ = 9.2 (H-4′); 3.64 dt, 1 H, ΣJ = 20.0 (H-5a′); 3.53 m, 1 H (H-5b′); 2.79 ddd, 1 H, J(2a′,1′) = 8.4, J(2a′,3′) = 5.5, J(gem) = 13.4 (H-2a′); 2.20 ddd, 1 H, J(2b′,1′) = 5.9, J(2b′,3′) = 2.5, J(gem) = 13.4 (H-2b′). UV spectrum: λ_{max} (ε), pH 2: 280.6 (17 100), 263.0 (12 400); pH 12: 261.8 (13 800), 275.0 (10 100).

9-(2-Deoxy-β-D-ribofuranosyl)-6-nitro-1-deazapurine (*XXXb*)

The catalyst (135 beads, vide supra) was added to a solution of 2′-deoxyuridine (0.8 g, 3.4 mmol) and 6-nitro-1-deazapurine (*XXXa*, 0.268 g, 1.6 mmol) in 0.05 M ammonium acetate buffer pH 5.8 (40 ml) and the mixture was incubated in a Dubnoff's incubator at 37 °C for 7 h. The supernatant was decanted and the catalyst washed with water. After membrane-filtration, the mixture was separated by preparative HPLC, elution with a gradient methanol–water. Standing with dry ether in a refrigerator afforded 0.13 g (27%) of yellow crystalline product *XXXb*, m.p. 122 – 124 °C. For $C_{11}H_{12}N_4O_5 \cdot H_2O$ (298.2) calculated: 44.30% C, 4.73% H, 18.78% N; found: 44.05% C, 4.50% H, 18.81% N. HPLC (H5, k = 9.01). ^{1}H NMR spectrum: 9.01 s, 1 H (H-8); 8.67 d, 1 H, J(1,2) = 5.4 (H-2); 8.01 d, 1 H, J(2,1) = 5.4 (H-1); 6.58 t, 1 H, J = 6.6 (H-1′); 5.35 brd, 1 H and 4.97 brt, 1 H, J = 4.0 and 5.0 (3′-OH and 5′-OH); 4.44 m, 1 H (H-3′); 3.91 dt, 1H (H-4′); 3.63 dt, 1 H and 3.53 dt, 1 H (H-5a′ and H-5b′); 2.78 brpent, 1 H, J(2a′,1′) = 6.8, J(2a′,3′) = 6.2, J(gem) = 13.4 (H-2a′); 2.40 ddd, 1 H, J(2b′,1′)

= 6.4, *J*(2b′,3′) = 3.8, *J*(gem) = 13.4 (H-2b′). UV spectrum, λ_{max} (ε), pH 2: 296.9 (5 600); pH 12: 322.4 (4 900).

9-(2-Deoxy-β-D-ribofuranosyl)-6-azido-3-deazapurine (*XXXIIIb*)

The catalyst (135 beads, vide supra) was added to a solution of 2′-deoxyuridine (0.43 g, 1.88 mmol) and 6-azido-3-deazapurine (*XXXIIIa*, 0.150 g, 0.94 mmol) in 0.05 M ammonium acetate buffer pH 5.8 (40 ml) and the mixture was incubated in a Dubnoff's incubator at 37 °C for 7 h. The supernatant was decanted and the catalyst washed with water. After membrane-filtration, the mixture was separated by preparative HPLC, elution with a gradient methanol–water. The product was crystallized from ethanol–ether–light petroleum; yield 0.09 g (35%) of white crystals of compound *XXXIIIb*, m.p. 177 – 178 °C. HPLC (H5, *k* = 6.68). For $C_{11}H_{12}N_6O_3$ (276.2) calculated: 47.83% C, 4.38% H, 30.42% N; found: 47.92% C, 4.45% H, 30.56% N. ^{1}H NMR spectrum: 9.10 d, 1 H, *J*(2,3) = 7.3 (H-2); 8.87 s, 1 H (H-8); 7.76 d, 1 H, *J*(3,2) = 7.3 (H-3); 6.84 t, 1 H, *J* = 6.6 (H-1′); 5.45 d, 1 H and 5.08 t, 1 H, *J* = 4.5 and 5.5 (3′-OH and 5′-OH); 4.47 m, 1 H (H-3′); 3.97 q, 1 H, Σ*J* = 12.0 (H-4′); 3.69 dt, 1 H and 3.58 dt, 1 H (H-5a′ and H-5b′); 2.76 ddd, 1 H, *J*(2a′,1′) = 7.2, *J*(2a′,3′) = 6.0, *J*(gem) = 13.2 (H-2a′); 2.50 ddd, 1 H, *J*(2b′,1′) = 6.0, *J*(2b′,3′) = 3.5, *J*(gem) = 13.2 (H-2b′). Mass spectrum, *m/z*: 276 (M + H). UV spectrum, λ_{max} (ε), pH 2: 263.2 (4 500); pH 12: 263.5 (4 400).

9-(2-Deoxy-β-D-ribofuranosyl)-3-deazaguanine (*XXXVIb*)

The catalyst (250 beads, vide supra) was added to a solution of 2′-deoxyuridine (0.925 g, 4.05 mmol) and 3-deazaguanine (*XXXVIa*, 0.250 g, 1.66 mmol) in 0.05 M ammonium acetate buffer pH 5.8 (50 ml) and the mixture was incubated in a Dubnoff's incubator at 37 °C for 7 h. The supernatant was decanted and the catalyst washed with water. After membrane-filtration, the mixture was separated by preparative thin-layer chromatography in system S5. The product fraction was evaporated and the residue crystallized from ethanol–ether to give 0.06 g (13%) of 2′-deoxynucleoside *XXXVIb*, m.p. 165 °C (decomp.), *k* = 1.79 (H4). For $C_{11}H_{14}N_4O_4 . H_2O$ (284.2) calculated: 46.48% C, 5.67% H, 19.70% N; found: 46.07% C, 5.26% H, 19.31% N. ^{1}H NMR spectrum: 10.30 br, 1 H (NH); 7.86 s, 1 H (H-8); 5.94 dd, 1 H, *J* = 6.1 and 7.3 (H-1′); 5.59 brs, 2 H (NH_2); 5.43 s, 1 H (H-3); 5.32 brs, 1 H and 4.91 brs, 1 H (5′-OH and 3′-OH); 4.37 dt, 1 H, *J*(3′,2b′) = *J*(3′,4′) = 3.2, *J*(3′,2a′) = 6.1 (H-3′); 3.80 td, 1 H, *J*(4′,3′) = 3.2, *J*(4′,5a′) = *J*(4′,5b′) = 4.7 (H-4′); 3.52 dd, 1 H, *J*(5a′,4′) = 4.7, *J*(gem) = 11.7 (H-5a′); 3.47 dd, 1 H, *J*(5b′,4′) = 4.7, *J*(gem) = 11.7 (H-5b′); 2.44 ddd, 1 H, *J*(2a′,3′) = 6.1, *J*(2a′,1′) = 7.3, *J*(gem) = 13.2 (H-2a′); 2.21 ddd, 1 H, *J*(2b′,3′) = 3.2, *J*(2b′,1′) = 6.1, *J*(gem) = 13.2 (H-2b′). Mass spectrum, *m/z*: 267.1 (M + H). UV spectrum, λ_{max} (ε), pH 2: 281.0 (10 800); pH 7: 300.0 (8 200), 270.0 (10 600); pH 12: 276.0 (10 500).

9-(2-Deoxy-β-D-ribofuranosyl)-2-azahypoxanthine (*XXXVIIIb*) and
7-(2-Deoxy-β-D-ribofuranosyl)-2-azahypoxanthine (*XXXIXb*)

The catalyst (250 beads, vide supra) was added to a solution of 2′-deoxyuridine (0.925 g, 4.0 mmol) and 2-azahypoxanthine (*XXXVIIIa*, 0.255 g, 1.9 mmol) in 0.05 M ammonium acetate buffer pH 5.8 (50 ml) and the mixture was incubated in a Dubnoff's incubator at 37 °C for 7 h. The supernatant was decanted and the catalyst washed with water. After membrane-filtration, the mixture was separated by preparative HPLC, elution with a gradient methanol–water. The product was crystallized from ethanol–ether; yield 0.38 g (79%) of a crystalline mixture of 7- and 9-isomers *XXXVIIIb* and *XXXIXb* in the ratio 1 : 1 (NMR); repeated separation of this mixture by HPLC under the same conditions afforded samples of both isomers for identification. *XXXVIIIb*: ^{1}H NMR spectrum: 8.53 s, 1 H (H-8); 6.47 t, 1 H, *J* = 6.6 (H-1′); 5.30 br, 2 H (OH); 4.44 dt, 1 H, *J*(3′,2b′) = *J*(3′,4′) = 3.5, *J*(3′,2a′) = 6.1

(H-3′); 3.92 td, 1 H, Σ*J* = 11.2 (H-4′); 3.63 dd, 1 H, *J*(5a′,4′) = 4.1, *J*(gem) = 12.0 (H-5a′); 3.54 dd, 1 H, *J*(5b′,4′) = 4.4, *J*(gem) = 12.0 (H-5b′); 2.74 ddd, 1 H, *J*(2a′,1′) = 7.1, *J*(2a′,3′) = 6.1, *J*(gem) = 13.2 (H-2a′); 2.38 ddd, 1 H, *J*(2b′,1′) = 6.3, *J*(2b′,3′) = 3.7, *J*(gem) = 13.2, (H-2b′). ^{13}C NMR spectrum: 157.50 (C-6); 145.48 (C-4); 141.48 (C-8); 125.37 (C-5); 88.50 (C-4′); 85.08 (C-1′); 70.75 (C-3′); 61.71 (C-5′); 40.14 (C-2′). *XXXIXb*: ^{1}H NMR spectrum: 8.72 s, 1 H (H-8); 6.58 t, 1 H, *J* = 6.6 (H-1′); 5.60 br, 2 H (OH); 4.39 dt, 1 H, *J*(3′,2b′) = *J*(3′,4′) = 3.4, *J*(3′,2a′) = 5.9 (H-3′); 3.90 brq, 1 H, Σ*J* = 11.2 (H-4′); 3.63 dd, 1 H, *J*(5a′,4′) = 4.2, *J*(gem) = 12.0 (H-5a′); 3.56 dd, 1 H, *J*(5b′,4′) = 4.2, *J*(gem) = 12.0 (H-5b′); 2.57 pent, 1 H, *J*(2a′,1′) = *J*(2a′,3′) = 6.5, *J*(gem) = 13.2 (H-2a′); 2.38 ddd, 1 H, *J*(2b′,1′) = 6.1, *J*(2b′,3′) = 3.7, *J*(gem) = 13.2 (H-2b′). ^{13}C NMR spectrum: 154.90 (C-4); 154.20 (C-6); 142.75 (C-8); 115.37 (C-5); 88.47 (C-4′); 86.64 (C-1′); 70.30 (C-3′); 61.38 (C-5′); 41.81 (C-2′).

9-(2-Deoxy-β-D-ribofuranosyl)-1,N^6-etheno-2-azaadenine (*XLVIb*) and
7-(2-Deoxy-β-D-ribofuranosyl)-1,N^6-etheno-2-azaadenine (*XLVIIb*)

The catalyst (250 beads, vide supra) was added to a solution of 2′-deoxyuridine (0.925 g, 4.0 mmol) and 1,N^6-etheno-2-azaadenine (*XLVIa*, 0.305 g, 1.9 mmol) in 0.05 M ammonium acetate buffer pH 5.8 (50 ml) and the mixture was incubated in a Dubnoff's incubator at 37 °C for 7 h. The supernatant was decanted and the catalyst washed with water. After membrane-filtration, the mixture was separated by preparative HPLC, elution with a gradient methanol–water. The product was crystallized from ethanol–ether; yield 0.430 g (82%) of unseparable crystalline mixture of both isomers in the ratio 2 : 9 (NMR). *XLVIIb*: ^{1}H NMR spectrum: 8.89 s, 1 H (H-8); 8.81 d, 1 H, *J*(11,10) = 1.7 (H-11); 7.85 d, 1 H, *J*(10,11) = 1.7 (H-10); 6.70 t, 1 H, *J* = 6.3 (H-1′); other signals not assigned. ^{13}C NMR spectrum: 145.21 (C-4); 133.39 (C-10); 129.26 (C-6); 118.95 (C-5); 115.45 (C-11); 88.80 (C-4′); 70.46 (C-3′); 61.40 (C-5′); 40.98 (C-2′). *XLVIb*: ^{1}H NMR spectrum: 8.96 s, 1 H (H-8); 8.76 d, 1 H, *J*(11,10) = 1.7 (H-11); 7.85 d, 1 H, *J*(10,11) = 1.7 (H-10); 6.67 t, 1 H, *J* = 6.3 (H-1′); 5.40 br, 2 H (OH); 4.49 brdt, 1 H, *J*(3′,4′) = 3.5, (H-3′); 3.95 brq, 1 H, Σ*J* = 12.7 (H-4′); 3.66 dd, 1 H, *J*(5a′,4′) = 4.6, *J*(gem) = 11.7 (H-5a′); 3.57 dd, 1 H, *J*(5b′,4′) = 4.6, *J*(gem) = 11.7 (H-5b′); 2.84 dt, 1 H, *J*(2a′,1′) = 6.1, *J*(2a′,3′) = 6.3, *J*(gem) = 13.4 (H-2a′); 2.51 ddd, 1 H, *J*(2b′,1′) = 6.6, *J*(2b′,3′) = 4.4, *J*(gem) = 13.4 (H-2b′). ^{13}C NMR spectrum: 137.79 (C-4); 133.66 (C-10); 133.27 (C-6); 126.55 (C-5); 115.45 (C-11); 88.46 (C-4′); 85.28 (C-1′); 70.36 (C-3′), 61.38 (C-5′); 40.16 (C-2′).

8-(2-Deoxy-β-D-ribofuranosyl)-8-aza-2,6-diaminopurine (*XLVIIIb*)

The catalyst (250 beads, vide supra) was added to a solution of 2′-deoxyuridine (0.925 g, 4.05 mmol) and 8-aza-2,6-diaminopurine (*XLVIIIa*, 0.280 g, 1.8 mmol) in 0.05 M ammonium acetate buffer pH 5.8 (50 ml) and the mixture was incubated in a Dubnoff's incubator at 37 °C for 7 h. The supernatant was decanted and the catalyst washed with water. After membrane-filtration, the mixture was separated by preparative thin-layer chromatography in system S5. The product fraction was evaporated and the residue crystallized from ethanol–ether; yield 0.03 g (6%) of 2′-deoxynucleoside *XLVIIIb*, not melting up to 280 °C, R_F 0.31 (S5). ^{1}H NMR spectrum: 7.70 br, 1 H and 7.50 br, 1 H (NH_2); 6.13 brs, 2 H (NH_2); 6.31 dd, 1 H, *J*(1′,2a′) = 4.6, *J*(1′,2b′) = 6.8 (H-1′); 5.40 br, 1 H and 4.78 br, 1 H (5′-OH and 3′-OH); 4.49 brq, 1 H, Σ*J* = 15.6 (H-3′); 3.87 brq, 1 H, Σ*J* = 15.6 (H-4′); 3.52 dd, 1 H, *J*(5a′,4′) = 5.1, *J*(gem) = 11.7 (H-5a′); 3.38 dd, 1 H, *J*(5b′,4′) = 6.3, *J*(gem) = 11.7 (H-5b′); 2.80 ddd, 1 H, *J*(2a′,3′) = 6.3, *J*(2a′,1′) = 4.6, *J*(gem) = 13.4 (H-2a′); 2.38 ddd, 1 H, *J*(2b′,3′) = 5.1, *J*(2b′,1′) = 6.8, *J*(gem) = 13.4 (H-2b′). ^{13}C NMR spectrum: 162.80 (C-2); 159.92 (C-4); 157.06 (C-6); 123.06 (C-5); 93.09 (C-1′); 88.85 (C-4′); 71.05 (C-3′); 62.49 (C-5′); 39.52 (C-2′). $C_9H_{13}N_7O_3$ (267.2): mass spectrum, *m*/*z*: 268.2 (M + H). UV spectrum, λ_{max} (ε), pH 2: 280.0 (8 000), 263.0 (8 800); pH 7: 309.0 (5 000), 259.0 (3 900); pH 12: 311.0 (5 200), 258.0 (4 100). Spectrophotometrically determined content: >90%.

Thymidine (*XLIXb*)

The incubation mixture contained 2′-deoxyuridine (228 mg, 1 mmol), thymine (63 mg, 0.5 mmol), 100 catalyst beads and 50 ml of 0.05 M ammonium acetate pH 5.8. After incubation at 37 °C for 16 h, the supernatant was decanted, the catalyst washed with water (5 ml) and the combined filtrates were membrane-filtered. The filtrate was concentrated in vacuo to about 5 ml, filtered through a layer of Celite and applied onto a column of octadecylsilica gel (180 ml). Elution with water (2 ml/min, fractions 20 ml, followed by TLC in S1) washed out successively uracil, 2′-deoxyuridine (100 mg, 46%) and thymidine (*XLIXb*, 70 mg, 61%, after crystallization from ethanol–ether), m.p. 125 °C. The product was identical with an authentic material (HPLC, TLC (S1) and paper chromatography (S6)).

5-Ethyl-2′-deoxyuridine (*Lb*)

The incubation mixture contained 2′-deoxyuridine (2.28 g, 10 mmol), 5-ethyluracil (*La*, 0.70 g, 5 mmol), about 1 000 beads of the catalyst and 0.05 M ammonium acetate pH 5.8 (500 ml). After 18 h of incubation at 37 °C, the supernatant was decanted, the catalyst washed with water (50 ml) and the combined filtrates were membrane-filtered. The filtrate was concentrated in vacuo to about 50 ml, filtered through a layer of Celite and applied onto a column of octadecylsilica gel (180 ml). Elution with water (2 ml/min, fractions 20 ml, monitored by TLC in S4) washed out uracil and then 2′-deoxyuridine (1.7 g, 75%). Elution with 5% aqueous methanol afforded 0.87 g (68%) of 5-ethyl-2′-deoxyuridine (*Lb*), m.p. 156 °C (ethanol–ether). For $C_{11}H_{16}N_2O_5$ (256.2) calculated: 51.56% C, 6.29% H, 10.93% N; found: 52.12% C, 5.72% H, 11.15% N. UV spectrum, λ_{max} (ε), pH 2: 268 (9 600).

2′-Deoxy-5-fluorouridine (*LVIb*)

The incubation mixture contained 5-fluorouracil (*LVIa*, 65 mg, 0.5 mmol), thymidine (245 mg, 1 mmol), 100 beads of the catalyst and 0.05 M ammonium acetate pH 5.8 (50 ml). After incubation at 37 °C for 16 h, the supernatant was decanted, the catalyst washed with water (25 ml) and the combined filtrates were concentrated in vacuo at 30 °C to about 20 ml. The mixture was filtered through Celite and the filtrate was made alkaline with ammonia (pH 9). This solution was applied onto a column of Sephadex A-25 (HCO_3^- form, 200 ml), preequilibrated with 0.01 M triethylammonium hydrogen carbonate pH 7.5. The column was washed with the same buffer (2 ml/min) to drop of UV absorption (thymine and thymidine). Subsequent elution with 0.1 M triethylammonium hydrogen carbonate afforded a mixture of 5-fluorouracil and the product. After evaporation of solvent in vacuo, the residue was applied onto a column of octadecylsilica gel (100 ml). Elution with water, evaporation of the product fractions (monitored by TLC in S1) and crystallization from ethanol with addition of ether afforded 75 mg (62%) of 2′-deoxy-5-fluorouridine (*LVIb*), identical with an authentic material (HPLC, TLC in S4, paper chromatography in S6 and paper electrophoresis (mobility 0.56 related to uridine-3′-phosphate)), m.p. 150 – 151 °C (reported[31] m.p. 150 °C). UV spectrum, λ_{max}, pH 2: 269.

9-(2,3-Dideoxy-β-D-ribofuranosyl)-6-methylpurine (*VIc*)

The catalyst (420 beads, vide supra) was added to a solution of 2′,3′-dideoxyuridine (0.6 g, 2.8 mmol) and 6-methylpurine (*VIa*, 0.2 g, 1.3 mmol) in 0.05 M ammonium acetate buffer pH 5.8 (80 ml) and the mixture was incubated in a Dubnoff's incubator at 37 °C for 7 h. The supernatant was decanted and the catalyst washed with water. After membrane-filtration, the mixture was separated by preparative thin-layer chromatography in system S2. The product fraction was evaporated and the residue crystallized from ethanol–ether; yield 0.06 g (20%) of 2′,3′-dideoxynucleoside *VIc*, m.p. 107 °C, R_F 0.70 (S2). For $C_{11}H_{14}N_4O_2$ (234.2) calculated: 56.40% C, 6.03% H, 23.91% N; found: 56.60% C, 6.14% H, 23.52% N. ^{1}H NMR spectrum: 8.77 s, 1 H and 8.74 s, 1 H (H-2 and H-8); 6.34 dd, 1 H,

J(1′,2a′) = 6.1, J(1′,2b′) = 3.9 (H-1′); 4.97 t, 1 H, J = 5.5 (5′-OH); 4.14 m, 1 H, (H-4′); 3.63 ddd, 1 H, J(5a′,4′) = 4.1, J(5a′,OH) = 5.6, J(gem) = 11.7 (H-5a′); 3.51 dt, 1 H, J(5b′,4′) = J(5b′,OH) = 5.0, J(gem) = 11.7 (H-5b′); 2.71 s, 3 H (CH_3); 2.48 m, 2 H (2 × H-2′); 2.01 – 2.14 m, 2 H (2 × H-3′). Mass spectrum, m/z: 235.1 (M + H). UV spectrum, λ_{max} (ε), pH 2: 263.0 (7 500); pH 7: 261.0 (7 800), pH 12: 261.0 (8 400).

2′,3′-Dideoxyinosine (*XIVc*)

The title compound was prepared from 2′,3′-dideoxyuridine and hypoxanthine in the yield of 18%; m.p. 188 – 189 °C. For $C_{10}H_{12}N_4O_3$ (236.2) calculated: 50.84% C, 5.12% H, 23.72% N; found: 51.01% C, 5.04% H, 23.88% N. ^{1}H NMR spectrum: 12.35 brs, 1 H (NH); 8.33 s, 1 H and 8.07 s, 1 H (H-2 and H-8); 6.20 dd, 1 H, J(1′,2′) = 6.8 (H-1′); 5.00 br, 1 H (5′-OH); 4.10 m, 1 H, J(1′,2′) = 3.4 and 6.8 (H-4′); 3.62 dd, 1 H, J(5a′,4′) = 3.9, J(gem) = 11.7 (H-5a′); 3.51 dd, 1 H, J(5b′,4′) = 4.6, J(gem) = 11.7 (H-5b′); 2.44 m, 1 H, ΣJ = 38.1 (H-2a′); 2.33 m, 1 H, ΣJ = 28.3 (H-2b′).

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