ALIPHATIC ANALOGUES OF NUCLEOSIDES, NUCLEOTIDES, AND OLIGONUCLEOTIDES*

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Condensation of 1-O-*p*-toluenesulfonyl-2,3-O-isopropylidene-D-glycerol (*V*) with sodium salts of uracil, thymine, adenine, and 2-pyrimidinone and acidic hydrolysis afforded the (*S*)-2,3-dihydroxypropyl derivatives *IV*. The (*R*)-enantiomeric derivatives *XI* were prepared by condensation of methyl 5-O-*p*-toluenesulfonyl-2,3-O-isopropylidene-D-ribofuranoside (*VIII*) with the sodium salt of the base, removal of the isopropylidene group, oxidation with sodium periodate, and sodium borohydride reduction. (*RS*)-1-(3,4-Dihydroxybutyl)thymine (*XVIII*) and (*RS*)-(3,4-dihydroxybutyl)adenine (*XXIV*) were obtained from 1,2-O-isopropylidene-4-*p*-toluenesulfonyl-1,2,4-butanetriol (*XXI*). (*RS*)-1-(2-Hydroxypropyl)adenine (*XXV*) resulted from the condensation of 1-*p*-toluenesulfonyl-1,2-propanediol with the sodium salt of adenine. The adenine derivative *IVe* was converted to the 3′-phosphate *XXVI* and this acetylated to afford the acetyl derivative *XXVII*. Condensation of the 3′-O-trityl derivative of compound *IVe* with the acetate *XXVII* and removal of protecting groups afforded the ApA analogue *XXIXb*. Repetition of this procedure led to the ApApA aliphatic analogue *XXX*. (*S*)-9-(2,3-Dihydroxypropyl)adenine-2′-O-phosphoryl-5′--adenosine (*XXXII*) and adenyl-3′-yl-3-(*S*)-9-(2,3-dihydroxypropyl)adenine (*XXXIV*) were also synthesized. The analogues of GpUpU, GpCpU, and GpApA triplets and the ApApA aliphatic analogue do not stimulate the aminoacyl-tRNA bond to ribosomes.

Considerable attention has been recently paid to a novel group of nucleoside and nucleotide analogues, the sugar moiety of which is replaced by an aliphatic chain, since such compounds may be used as models in various physicochemical investigations on intra- or intermolecular interactions of nucleic acid components. Furthermore, simple polymeric matrices could be prepared on the basis of these analogues and investigated with respect to interactions at the level of polynucleotides. Some naturally occurring nucleoside-like substances are known, containing an aliphatic chain instead of the sugar moiety such as aristeromycin[1] (*I*; a carbocyclic analogue isosteric with adenosine), eritadenine[2,3] (*II*; an adenine derivative substituted at position 9 by a four-carbon aliphatic dihydroxy acid residue), and willardiin (*III*). These substances do not appear to be products of nucleic acid catabolism but more probably are formed by independent biochemical processes; their function in biochemical transformations in living cells is not quite clear.

In an earlier paper[4] of this Laboratory there has been reported preparation of some 2,3-dihydroxypropyl derivatives of pyrimidine bases and their esters with phosphoric acid. Some ribo-

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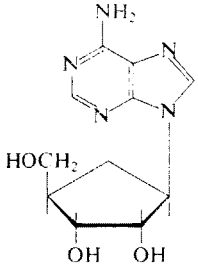
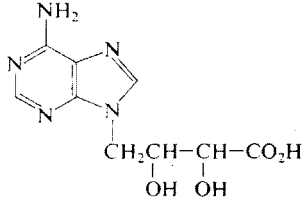
nucleases are able to cleave 2′,3′-cyclic phosphate of type *IV* when they possess the (*S*)-configuration; as shown by molecular models, only the (*S*)-derivatives can imitate the conformation of the naturally occurring β-D-ribonucleosides. The 2,3-dihydroxypropyl analogues of nucleosides may thus under certain conditions occur in conformations very similar to those of nucleosides in spite of the fact that the aliphatic residue does not exhibit the restricted rotation (due to the cyclic structure) and is attached to the heterocyclic base through a carbon atom with the sp^3 hybridisation. The chirality of compounds *IV* asserts itself in interactions with chiral molecules of proteins. Proposals of compounds *IV* for the preparation of polymeric matrices of nucleic acid type[5–10] are hardly justified since the racemic derivatives of the above types do not represent suitable models of nucleosides. We have shown[11] the impossibility of an interaction between the complementary oligonucleotides of the D- and L-series or the D- and DL-series. With racemates of the type *IV* and the related polymeric oligonucleotide analogues, there can be hardly expected the corresponding interaction with polymeric homo- or heteronucleotides.



I



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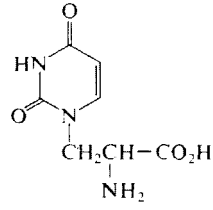


III

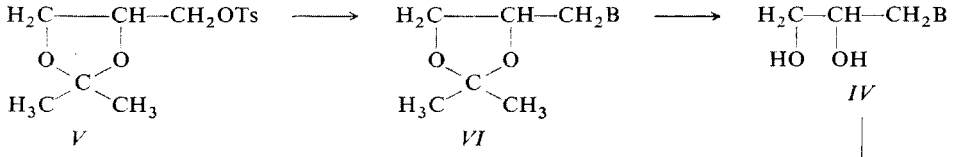
The aminoacyl derivatives of the 2,3-dihydroxypropyl analogue in the adenine series are efficient competitive inhibitors of puromycin in its influence on the dipeptide formation ("the puromycin reaction", *cf.*[12]) on the level of both the aminoacyl-tRNA[13,14] and the acylaminoacyl-tRNA hexanucleotide fragment[14]. All the three above mentioned aspects, namely, the behaviour of aliphatic-type analogues towards enzymes of nucleic acid metabolism, the possibility to prepare the enantiomeric oligonucleotide analogues and to examine their physical, chemical, and biochemical (*in vitro*) interactions with natural oligo- or polynucleotides, and finally the biochemical activity of aminoacyl esters of these substances stimulated the preparation of aliphatic analogues of nucleosides and nucleotides as reported in the present paper.

In the earlier paper[4], there was described the preparation of the racemic derivative as well as of the (*S*)-isomer („D-*glycero*") of the uracil and thymine series by reaction of the sodium salt of the corresponding 5-substituted 4-methoxy-2-pyrimidinone with 1-O-*p*-toluenesulfonyl-2,3-O-isopropylidene-D-glycerol (*V*). The same (*S*)-isomers have been now obtained by heating compound *V* with the sodium salt of uracil or thymine in dimethylformamide. The reaction affords a mixture of isomeric 2,3-O-iso-

propylidene derivatives *VI*, the pyrimidine nucleus of which is substituted by the aliphatic chain at position N^1 or N^3; the formation of a N^1,N^3-disubstituted derivative has not been observed. The isomers *VIa* and *VIc* or *VIb* and *VId* may be readily separated by chromatography on silica gel; their acidic hydrolysis afforded the corresponding (*S*)-1-(2,3-dihydroxypropyl) and (*S*)-3-(2,3-dihydroxypropyl) derivatives of uracil and thymine (*IVa*, *c* and *IVb*, *d*, resp.). Compounds *IVa* and *IVb* were identical with substances reported in the earlier paper[4] including the CD spectra; the structure of all these compounds was confirmed by NMR spectra.

The adenine derivative has been prepared analogously (Scheme 1). In accordance with the literature[5], an exclusive substitution at position N^9 takes place in the reaction of compound *VI* with the sodium salt of adenine. Acidic hydrolysis of the isopropylidene derivative *VIe* affords compound *IVe* which is deaminated with nitrous acid with the formation of the corresponding hypoxanthine derivative *IVf*. The UV spectra of all these substances corresponded to the appropriate 9-substituted purine derivatives. The racemic derivative *IVe* was prepared similarly.

The cytosine derivative *IVh* was prepared indirectly. The free hydroxyls of the uracil derivative *IVa* were protected by benzoylation and the resulting 2',3'-di-O-benzoyl derivative *VIIa* was transformed by thiation with phosphorus pentasulfide to the thiouracil derivative *VIIb*. By ammonolysis with methanolic ammonia and the simultaneous deblocking, compound *VIIb* afforded the cytosine derivative *IVh*. The attempted analogous thiation of the 2',3'-O-isopropylidene derivative *VIa* with phosphorus pentasulfide in dioxane was accompanied by a rapid removal of the protecting isopropylidene group. Even under these conditions, the thiation of the uracil nucleus is quantitative and the ammonolysis of the crude product proceeds with the exclusive formation of the cytosine derivative. The reaction is accompanied by phosphorylation of the aliphatic chain (the phosphorylated product was obtained in about 10% yield)

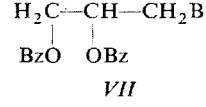


V *VI* *IV*

Ts = *p*-toluenesulfonyl

In formulae *IV*, *VI*

a, B = uracil-1-yl
b, B = thymin-1-yl
c, B = uracil-3-yl
d, B = thymin-3-yl
e, B = adenin-9-yl
f, B = hypoxanthin-9-yl
g, B = 2-pyrimidon-1-yl
h, B = cytosin-1-yl



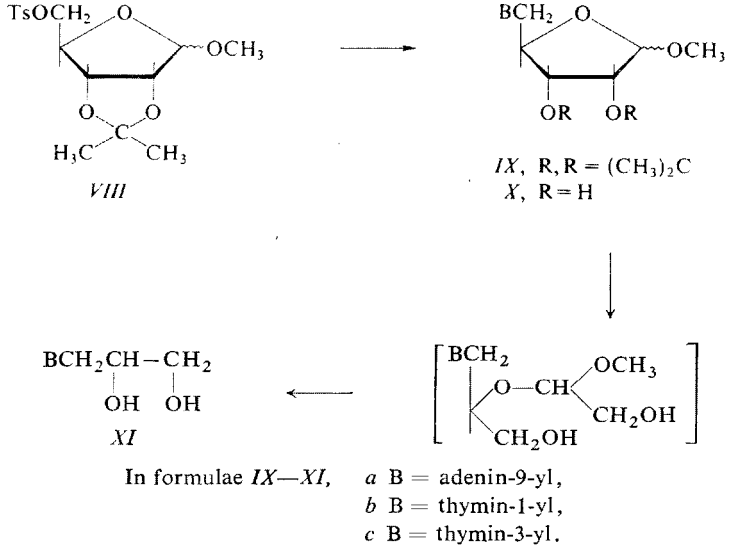
VII

a, B = uracil-1-yl
b, B = 4-thiouracil-1-yl
Bz = benzoyl group

SCHEME 1

and partial racemisation due to the absence of the protecting group. On the other hand, the route through the dibenzoate *VIIa* affords the (*S*)-enantiomer *IVh* without any racemisation.

In the preparation of (*R*)-isomers of compounds *IV*, there were used syntheses starting from D-ribose since the derivatives of the L-*glycero* series are less accessible (Scheme 2). D-Ribose was converted to methyl 5-O-*p*-toluenesulfonyl-2,3-O-isopropylidene-D-ribofuranoside[15] (*VIII*), the reaction of which with the sodium salt of adenine afforded methyl 5-(adenin-9-yl)-5-deoxy-2,3-O-isopropylidene-β-D-ribofuranoside (*IXa*), *cf.* ref.[15]. As indicated by NMR spectrum, the configuration of the glycosidic bond is exclusively β. The isopropylidene protecting group of compound *IXa* was removed by the action of methanolic hydrogen chloride without affecting the glycosidic bond at position 1. The thus-obtained derivative *Xa* was oxidised with sodium periodate and the resulting dialdehyde reduced *in situ* with sodium borohydride to afford an unstable intermediate which was quantitatively converted to the (*R*)-isomer *XIa* during the work-up of the reaction mixture under acidic conditions. The (*R*)-isomer of thymine derivative was prepared analogously. In the reaction of the *p*-toluenesulfonate *VIII* with the sodium salt of thymine, there are obtained (analogously to the reaction of compound *VI*) two isomers substituted at position N^1 (*IXb*) or N^3 (*IXc*) of the thymine ring. The separation is performed by chromatography on silica gel. The structure of compound *IXb* may be inferred from the whole reaction sequence and was confirmed by NMR spectrum. By an analogous procedure as applied with compound *IXa*, the derivative *IXb* was converted into (*R*)-1-(2,3-di-

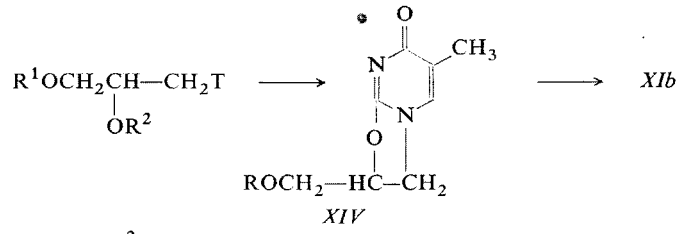


In formulae *IX*—*XI*, *a* B = adenin-9-yl, *b* B = thymin-1-yl, *c* B = thymin-3-yl.

SCHEME 2

hydroxypropyl)thymine (*XIb*), identical in every respect with the *S*-isomer except for the chiroptical properties (*vide infra*) (Scheme 2).

In addition to this route, an inversion has been developed of the (*S*)-isomers *IV* to the (*R*)-isomers *XI*. This inversion is analogous to the formation of $O^{2,2'}$-anhydronucleosides of the pyrimidine series and their hydrolysis to epimeric *arabino* derivatives (Scheme 3). Thus, the reaction of the thymine derivative *IVb* with triphenylmethyl chloride gave compound *XII* which was treated with methanesulfonyl chloride in pyridine to afford the derivative *XIII*. By the action of triethylamine in acetonitrile, compound *XIII* is converted into the cyclic intermediate *XIV*. The removal of the triphenylmethyl group in compound *XIV* by refluxing in 80% aqueous acetic acid is accompanied by a quantitative hydrolysis of the $O^{2,2'}$-anhydro bond with the formation of the (*R*)-isomer *XIb*. As indicated by the stereospecific course of the anhydro bond opening (the isomeric purity of the (*R*)-derivative *XIb* prepared by this route has been confirmed by comparison of CD spectra with those of an authentic specimen), the reaction proceeds analogously to that of $O^{2,2'}$-anhydrouridine, namely, by an attack of water at position 2 of the thymine ring of the anhydro derivative *XIV*.

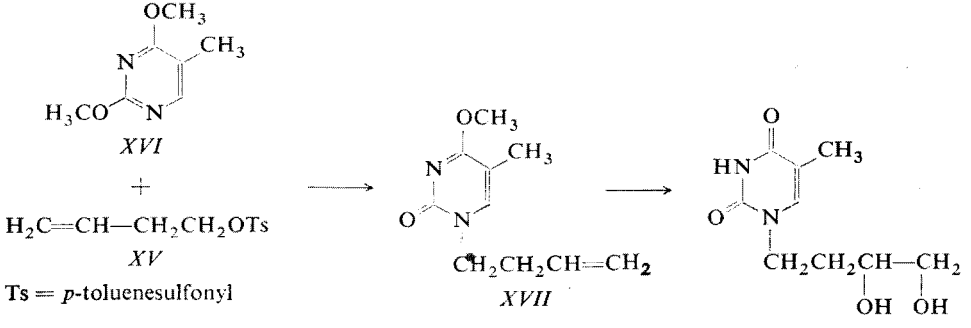


XII, $R^1 = (C_6H_5)_3C$, $R^2 = H$
XIII, $R^1 = (C_6H_5)_3C$, $R^2 = CH_3SO_2$
In formulae *XII*—*XIII* T = thymin-1-yl

SCHEME 3

None of the above compounds *IV* or *XI* inhibited the growth of *Escherichia coli* on a synthetic glucose-containing medium up to the concentration of 1 mg per 1 ml. Since these compounds could be cleaved and reutilised *in vivo*, it was of interest to prepare (*S*)-1-(2,3-dihydroxypropyl)-2-pyrimidinone (*IVg*) by reaction of compound *V* with the sodium salt of 2-pyrimidinone and the subsequent hydrolysis of the isopropylidene derivative *VIg*. In spite of the high bacteriostatic activity of the ribonucleoside derived from 2-pyrimidinone as inhibitor of DNA synthesis *de novo*[16], the aliphatic analogue *IVg* is inert. This result is in accordance with observations that either the (*S*)-isomers of compounds *IV* or the (*S*)-isomers do not interfere with the nucleoside transport into *E. coli*[17] or *B. subtilis*[18]. It may be thus indirectly inferred that also the aliphatic analogues *IV* do not penetrate the bacterial cells and do not affect the biochemical processes *in vivo*.

In the series of homologous 3,4-dihydroxybutyl derivatives of pyrimidine and purine bases, two synthetic methods have been developed analogous to the syntheses of compounds *IV* and *XI*. One method consists in reaction of homoallyl *p*-toluenesulfonate (*XV*) with 2,4-dimethoxy-5-methylpyrimidine (*XVI*) to give 1-(3-buten-1-yl)--4-methoxy-5-methyl-2-pyrimidinone (*XVII*) as expected from the knowledge on the Hilbert-Johnson reaction. *cis*-Hydroxylation of compound *XVII* with sodium chlorate in the presence of osmium tetroxide and the subsequent acidic hydrolysis (removal of the 4-methoxy group) afforded (Scheme 4) the racemic 1-(3,4-dihydroxybutyl)thymine (*XVIII*). An alternative route suitable for the synthesis of the enantiomeric derivatives of this type has been also worked out. 1,2,4-Butanetriol (*XIX*) was obtained by the reported procedure[19] from the inactive malic acid and then converted to the 1,2-O-isopropylidene derivative *XX* by reaction with 2,2-dimethoxypropane. Treatment of compound *XX* with *p*-toluenesulfonyl chloride in pyridine afforded the 4-O-*p*-toluenesulfonyl derivative *XXI*. In spite of mild conditions, compound *XXI* was obtained in a low yield and the 4-chloro derivative *XXII* was the principal product. The formation of compound *XXII* might be explained by the subsequent reaction of the *p*-toluenesulfonate *XXI* with pyridine hydrochloride or by elimination and addition of hydrogen chloride to the intermediary 1,2-dihydroxy-3-butene derivative.



SCHEME 4

$H_2C(R^1O)—CH(OR^1)—CH_2CH_2R^2$

XIX, $R^1 = H$, $R^2 = OH$
XX, $R^1,R^1 = (CH_3)_2C$, $R^2 = OH$
XXI, $R^1,R^1 = (CH_3)_2C$, $R^2 = 4\text{-}CH_3C_6H_4SO_2O$
XXII, $R^1,R^1 = (CH_3)_2C$, $R^2 = Cl$

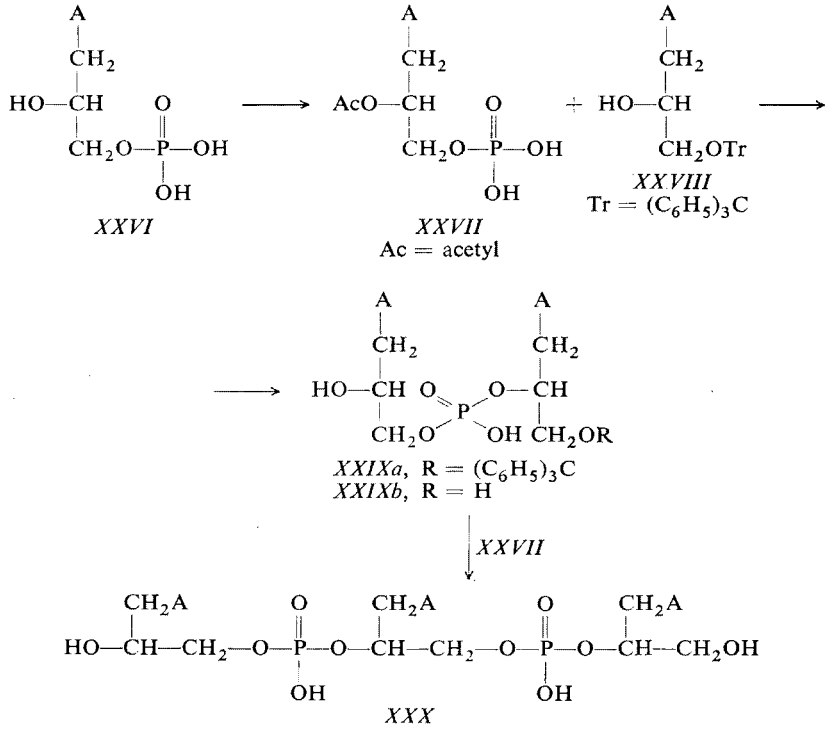
$H_2C(RO)—CH(OR)—CH_2CH_2A$

XXIII, R,R = $(CH_3)_2C$
XXIV, R = H
A = adenin-9-yl

The racemic adenine derivative *XXIII* was prepared from compound *XXI* by reaction with the sodium salt of adenine; acidic hydrolysis of *XXIII* led to the racemic 9-(3,4-dihydroxybutyl)adenine (*XXIV*). The key intermediate *XIX* may also be prepared from the optically active malic acid without loss of the original configuration.

The last analogue of the present set, namely, (*RS*)-9-(2-hydroxypropyl)adenine (*XXV*), was prepared from 1,2-propanediol by reaction with an equimolar amount of *p*-toluenesulfonyl chloride in pyridine and the condensation of the resulting *p*-toluenesulfonate with the sodium salt of adenine. The product *XXV* free of adenine was isolated by methanolysis of the peracetyl derivative. The structure of compound *XXV* was confirmed by analysis and NMR spectrum. This method is simpler than the procedure based on the ring closure of the adenine ring system[20].

The Cotton effect of the 2,3-dihydroxypropyl derivatives *IV* and *XI* is strikingly similar to that of the pyrimidine ribonucleosides and 2'-deoxyribonucleosides with respect to the position of extrema and values of extremum elipticities (Table I). This observation along with some other properties such as the ready formation of 2,2'-anhydro derivatives of type *XIV* or especially, acceptance of some derivatives *IV* by enzymes as substrates analogous to nucleosides[4], suggest similar steric relations of com-



SCHEME 5

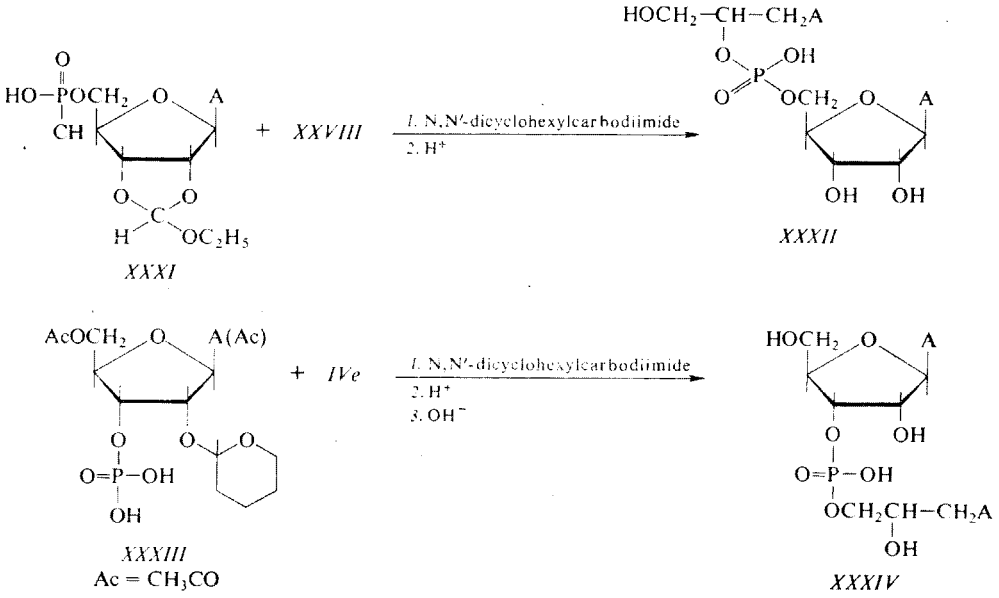
pounds *IV* or *XI* and the naturally occurring nucleosides possessing the cyclic furanose moiety. It was of interest to examine to what extent may be the conformations of oligomers of the above analogues similar to those of oligonucleotides with a special respect to the biological activity of the novel oligomers.

For this purpose there have been synthesized some modified trinucleoside diphosphates containing analogues of the type *IV* and examined from the standpoint of their potential activity in stimulation of the aminoacyl-tRNA bond to ribosomes. Total synthesis was used in the preparation of the fully aliphatic analogue of the ApApA triplet where the adenosine residues are replaced by (*S*)-9-(2,3-dihydroxypropyl)adenine (*IVe*). Thus (Scheme 5), compound *IVe* was phosphorylated with phosphorus oxychloride in triethyl phosphate[21] under conditions analogous to those in the preparation of pyrimidine derivatives[4] to afford the phosphoric acid monoester *XXVI*. Acetylation of compound *XXVI* with acetic anhydride in pyridine yielded the 2′-O-acetyl derivative *XXVII*. The other reactant was prepared by tritylation of compound *IVe*. Condensation of the thus-obtained 3′-O-trityl derivative *XXVIII* with compound *XXVII* in the presence of N,N′-dicyclohexylcarbodiimide followed by deacetylation afforded compound *XXIXa*, *i.e.*, the trityl derivative of the dinucleoside phosphate analogue. Compound *XXIXa* was condensed under analogous conditions with an additional molecule of the acetate *XXVII* and the protecting groups were removed first by ammonolysis and then by refluxing in dilute acetic acid (there is no danger of isomerisation under acidic conditions in contrast to the synthesis of oligoribonucleotides. The product *XXX* was separated from the unreacted starting material *XXVI* by paper chromatography and isolated in the form of an ammonium salt. The structure of compound *XXX* was confirmed by degradation with *Penicillium brevicompactum* ribonuclease[4] with the formation of compounds *XXVI* and *IVe*.

Detritylation of the intermediate *XXIXa* with acetic acid afforded the dinucleoside phosphate analogue *XXIXb* which was quantitatively degraded by the above mentioned enzyme to a mixture of compounds *IVe* and *XVI*. Another dinucleoside phosphate analogue was prepared by condensation of the trityl derivative *XXVIII* with 2′,3′-O--ethoxymethyleneadenosine 5′-phosphate (*XXXI*) in the presence of N,N′-dicyclohexylcarbodiimide and the subsequent acidic removal of protecting groups. The thus--obtained (*S*)-9-(2,3-dihydroxypropyl)adenine-2′-phosphoryl-5′-adenosine (*XXXII*) affords a mixture of adenosine 5′-phosphate and compound *IVe* on the snake venom phosphodiesterase degradation, or a mixture of compound *XXVI* and adenosine by the action of *P. brevicompactum* ribonuclease (Scheme 6). An analogue with the reversed sequence of the two components was prepared from N^6,$O^{5'}$-diacetyl-$O^{2'}$--tetrahydropyranyladenosine 3′-phosphate[22] (*XXXIII*) by condensation with compound *IVe* in the presence of N,N′-dicyclohexylcarbodiimide followed by alkaline and acidic removal of protecting groups (Scheme 6); the resulting compound *XXXIV* yields a mixture of adenosine 3′-phosphate and compound *IVe* by the ribonuclease T2 degradation. In contrast to the successful use of N,N′-dicyclohexylcarbodiimide in

condensations of compound *IVe* or its phosphoric ester *XXVI*, the sulfonyl chloride type condensing agents were quite inefficient in this respect.

Further dinucleoside phosphate analogues were prepared by the transfer of the 3′-nucleotide residue from uridine 2′,3′-cyclic phosphate or cytidine 2′,3′-cyclic phosphate to (*S*)-1-(2,3-dihydroxypropyl)uracil (*IVa*) in the presence of pancreatic ribonuclease, namely, the Up-*IVa* and Cp-*IVa* doublets. A similar transfer of the 3′-guanylyl residue from guanosine 2′,3′-cyclic phosphate to the above dinucleoside phosphate analogues yielded the triplets shown in Table II. It may be inferred from data concerning the stimulation of the Lys-, Val-, Glu- and Ala-tRNA binding to ribosomes of *E. coli* under standard conditions and from comparison with corresponding functional standards that the replacement of the triplet codone pyrimidine or purine nucleoside by the aliphatic analogue *IV* results in all cases in a complete loss of the stimulation activity in the above system. Such a result does not surprise in the case of compound *XXX*, the formal analogue of the ApApA lysine codone, since compound *XXX* lacks hydroxylic groups in vicinal positions with respect to the phosphodiester bond and resembles thus d(ApApA) which is also inactive[23] in the above system; a similar explanation might be used with the Gp-IVe-pA triplet which could be more likely regarded as a GpdApA analogue and which is obviously also



SCHEME 6

inactive[23]. This argument can be hardly used in cases when the aliphatic analogue *IVa* or *IVe* is at the third place of the triplet. This place is poorly dependent upon the nature of the nucleoside or extent of the modification. When the replacement of the ribonucleoside at this place is accompanied by loss of the stimulative activity for the formation of the ternary complex, this effect might be ascribed to a disordered conformation of the molecule. Such a situation may be encountered when the naturally occurring nucleoside is replaced by a L-nucleoside[24] or when the modification of the nucleoside results in a changed conformation, *cf.* the substitution of uridine at position 6, ref.[25].

TABLE I

Ultraviolet and Circular Dichroism Spectra in Water

Wavelengths in nm; molar elipticities are given in parentheses.

Compound	Ultraviolet spectra			Circular dichroism spectra				
	λ_{max}	ε_{max}	λ_{min}	λ_I	λ_{II}	λ_{III}	Θ_{205}	$\lambda_{\Theta=0}$
IVa	260	9 800	233	267 (−1 700)	238 (780)	—	1 570	247
IVb	272	8 500	236	271·5 (−3 650)	240 (900)	216	−450	245·5 231·5
IVc	267	9 600	232	—	—	—	—	—
IVd	272	6 700	240	273·5 (−180)	243 (640)	s222 (−1 150)	−4 900	252·5 234·0
IVe	260	14 000	228	258·5 (950)	—	227 (850)	−2 950	221·5
IVf	250	10 600	223	—	—	—	—	—
IVg[a]	305	5 300	240	—	—	—	—	—
IVh	274	10 000	251	274 (−9 300)	s225 (1 500)	216 (1 950)	—	240·0 210·0
XIa[b]	260	14 200	228	258 (−900)	—	228 (−700)	6 100	224·0
XIb[b]	271	8 600	236	270·5 (3 650)	239 (−350)	s219 (1 500)	2 700	243·0 231·0
XIb[c]	272	8 700	236	270·5 (2 750)	238 (−250)	s218·5 (1 500)	2 500	243·5 231·0
XVIII	272	8 500	236	—	—	—	—	—
XXIV	261	14 500	228	—	—	—	—	—
XXV	261	14 200	228	—	—	—	—	—

[a] pH 2, λ_{max} 281 nm (ε_{max} 13000); [b] prepared from *VIII*; [c] prepared from *IVb*.

The above results are not encouraging with respect to the potential use of the polymeric derivatives of the aliphatic type *IV* as matrices analogous to nucleic acids. It cannot be however excluced that these polymers might function in some enzymatic systems as DNA-like matrices. Such enzymatic systems require almost exclusively chains of a sufficient length. In the polymerisation of 3′-phosphomonoesters of compounds *IV*, the recently reported method of Japanese investigators appeared as promising[8,9]. In our hands however, the above condensations of short oligonucleotide analogues afforded poor yields only and depended on the nature of the condensing agent in contrast to the extraordinarily ready polymerisation claimed[8,9]. In applications to the adenine and uracil analogues of type *IV* or *XXVI*, corresponding 2′,3′-cyclic phosphate *XXXVII* was always quantitatively isolated as the single reaction product despite a meticulous reproduction of the reported procedures or in prolonged reactions. In no case we could confirm the presence of any or shorter oligonucleotides on the basis of analogues *IV*. Curiously enough, the papers mentioned[8,9] do not contain satisfactory characterisations of the products or descriptions of the chromatographic analyses of the reaction mixtures. Our findings are in accordance with expectations since 2′,3′- or 3′,5′-cyclic phosphates are known to be single products in attempted polycondensations of nucleotides with vicinal *cis*-hydroxyls of both the furanose and pyranose series as well as in attempted polycondensation of analogous aliphatic phosphates. With compounds of type *XXXVII*, the autocondensation is not possible since the molecule lacks an additional hydroxylic function[26].

TABLE II

Aminoacyl-tRNA Binding to *E. coli* Ribosomes

Amino acid	Compound	[^{14}C]-Aminoacyl-tRNA bound	
		pmol	Δ pmol
Val	—	0·35	—
	GpUpU	2·79	2·44
	GpUp-*IVa*	0·30	−0·05
Ala	—	0·45	—
	GpCpU	2·28	1·83
	GpCp-*IVa*	0·43	−0·02
Glu	—	1·62	—
	GpApA	4·81	3·21
	Gp-*XXIXb*	1·64	0·02
	Gp-*XXXII*	1·57	−0·05
	Gp-*XXXIV*	1·78	0·14
Lys	—	2·20	—
	ApApA	3·42	1·22
	XXX	2·5	−0·05

The present work opens a route for the synthesis of chiral oligomers by similar methods as in the field of 2'-deoxyribonucleosides[27]. The constitutional analogy of aliphatic dihydroxy derivatives with nucleosides is obviously limited to compounds *IV* and *XI*. In homologous compounds of type *XXIII*, the *cis*-diol system is separated

TABLE III

Chromatography (R_F values in S_1-S_6) and Electrophoresis (in E_1)

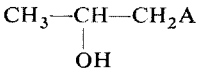
Compound	S1	S2	S3	S4	S5	S6
Uridine	0·45	0·35	—	—	—	—
Adenosine	0·52	0·47	—	—	—	—
IVa	0·53	0·47	—	—	0·20	0·38
IVb	0·67	0·53	—	—	0·21	0·45
IVc	0·63	0·47	—	—	0·33	0·47
IVd	0·69	0·56	—	—	0·10	0·22
IVe	0·53	0·50	—	—	0·12	0·20
IVf	0·41	0·40	—	—	—	—
IVg	0·67	—	—	—	0·15	0·34
IVh	0·52	0·47	—	—	—	0·05
VIa	0·72	—	0·28	0·54	—	—
VIb	0·78	—	0·32	0·76	—	—
VIc	0·80	—	0·16	—	—	—
VId	0·82		0·20	0·48	—	—
VIe	0·80	—	—	0·30	0·53	—
VIg	0·84	—	0·12	0·37	—	—
VIIa	—	—	0·34	—	—	—
VIIb	—	—	0·68	—	—	—
IXa	0·78	0·72	—	0·22	0·57	—
IXb	—	—	0·55	—	—	—
IXc	—	—	0·93	—	—	—
Xa	0·55	0·54	—	0·04	0·12	—
Xb	—	—	0·08	0·20	—	—
XIa	0·53	0·50	—	—	0·12	0·20
XIb	0·67	0·53	—	—	0·21	0·45
XII	—	—	—	—	0·27	0·49
XIII	—	—	—	—	0·28	0·56
XIV	—	—	—	—	0·08	0·13
XVII	—	—	0·20	0·32	0·53	—
XVIII	0·72	0·56	—	—	0·35	—
XXIII	0·85	—	—	0·37	0·62	—
XXIV	0·60	0·57	—	—	—	—
XXV	0·66	—	—	—	0·20	0·42

TABLE III
(*Continued*)

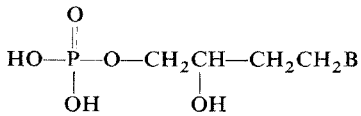
Compound	S1	S2	S3	S4	EI[a]
XXVI	0·10	0·21	—	—	0·91
XXVII	0·42	0·45	—	—	0·80
XXVIII	—	—	0·31	0·70	—
XXIXa	—	—	0·03	0·15	—
XXIXb	0·24	0·12	—	—	0·38
XXX	0·05	0·03	—	—	0·47
XXXII	0·23	0·12	—	—	0·32
XXXIV	0·24	0·12	—	—	0·32
XXXVa	0·18	0·24	—	—	0·92
XXXVb	0·24	0·30	—	—	0·52
XXXVIa	0·54	0·24	—	—	0·50
XXXVIb	0·57	0·30	—	—	0·52
XXXVIIa	0·50	0·26	—	—	0·50
XXXVIIb	0·52	0·22	—	—	0·48
Uridine 3′-phosphate	0·08	0·14	—	—	1·00
Adenosine 3′-phosphate	0·12	0·18	—	—	0·92
Uridine 2′,3′-cyclic phosphate	0·38	0·14	—	—	0·58
Adenosine 2′3′-cyclic phosphate	0·45	0·18	—	—	0·50

[a] Referred to uridine 3′-phosphate.

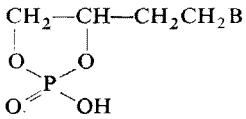
from the heterocyclic ring by a C—C bond with sp^3 hybridisation; the free rotation of the aliphatic residue is consequently as high that it interferes with the formation of a conformation analogous to that of a naturally occurring nucleoside. Thus *e.g.*,



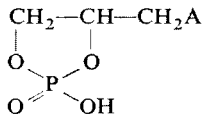
A = adenin-9-yl
XXV



XXXVa, B = adenin-9-yl
XXXVb, B = thymin-1-yl



XXXVIa, B = adenin-9-yl
XXXVIb, B = thymin-1-yl



XXXVII
A = adenin-9-yl

the 3',4'-cyclic phosphate *XXVI* (obtained from compound *XXIII* by phosphorylation with phosphorus oxychloride in triethyl phosphate and cyclisation of the resulting 4'-O-phosphoryl derivative *XXXV* with N,N'-dicyclohexylcarbodiimide) is quite resistant towards the *P. brevicompactum* ribonuclease while the one carbon atom shorter (*S*)-enantiomer *XXXVII* is cleaved quantitatively (the racemate of compound *XXXVII* is degraded by 50%). In this respect it does not appear desirable to extend the investigations on aliphatic analogues by examinations of further higher homologues. On the other hand, the behaviour of the related tri-, tetra, or polyhydroxy derivatives could be considerably different.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). The m.p.'s and b.p.'s were not corrected. Unless stated otherwise, the solutions were taken down on a rotatory evaporator at 35°C/15 Torr and the analytical samples were dried at 0·1 Torr over phosphorus pentoxide.

Methods

Paper chromatography was performed by the descending technique on paper Whatman No 1 in the solvent systems S_1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), and S_2, 1-butanol–glacial acetic acid–water (5 : 2 : 3). Thin-layer chromatography was carried out on ready-for-use Silufol UV_{235} (Kavalier Glassworks, Votice, Czechoslovakia) silica gel sheets in the solvent systems S_3, chloroform–ethanol (95 : 5); S_4, chloroform–ethanol (9 : 1); S_5, chloroform–ethanol (8 : 2); S_6, chloroform–ethanol (7 : 3), and S_7, benzene—ethyl acetate (6 : 4). Paper electrophoresis was performed on paper Whatman No 3 MM at 20 V/cm for 1 h in 0·1M triethylammonium hydrogen carbonate (pH 7·5). For the R_F values and electrophoretical mobilites see Table III. Preparative chromatographies on silica gel were performed either on a column of the Pitra silica gel (particle size, 30—60 micron) or on loose layers (40 × 16 × 0·3 cm) of the fluorescent-indicator-containing silica gel (produced by Service Laboratories of this Institute in Prague-Suchdol). The UV spectra were taken in aqueous solutions on a Zeiss Specord apparatus. The CD spectra were measured in water on a Jouan Dichrograph. The NMR spectra were recorded in deuteriochloroform or hexadeuteriodimethyl sulfoxide on a Varian 100 apparatus (hexamethyldisiloxane as internal standard). Chemical shift values are expressed in δ (p.p.m.); the coupling constants are given in Hz. Enzymatic degradation with *P. brevicompactum* ribonuclease was performed with 2—3 μmol of the test substance in 100 μl of a 0·05M-Tris buffer solution (pH 7·8) containing 30 e.u. of the enzyme[4] (4 h at 37°C). The snake venom (*Crotalus terr. terr.*) phosphodiesterase (Boehringer, German Federal Republic) degradation was performed analogously (10 μg of the protein).

(*S*)-1-(2,3-Dihydroxypropyl)uracil (*IVa*) and (*S*)-3-(2,3-Dihydroxypropyl)uracil (*IVc*)

A mixture of 1-O-*p*-toluenesulfonyl-2,3-O-isopropylidene-D-glycerol[4] (*V*; 29·0 g; 0·1 mol). the sodium salt of uracil[28] (16 g; 0·12 mol), and dimethylformamide (100 ml) was stirred at 100°C for 12 h under exclusion of atmospheric moisture, cooled down, and filtered through Celite. The filtrate was evaporated at 40°C/0·1 Torr and the residue coevaporated under the same conditions with three 20 ml portions of toluene to remove the residual dimethylformamide. The final residue

was extracted with hot chloroform (200 ml), the whole filtered through Celite, and the material on the filter washed with chloroform (200 ml). The filtrate and washings were combined, concentrated to a small volume, and the concentrate applied to a column of silica gel (200 g) packed in chloroform. Elution with chloroform afforded 3·4 g (15%) of compound *VIc* as an amorphous foam. Elution with 9 : 1 chloroform–ethanol, evaporation of the eluate, and crystallisation of the residue from ethanol yielded 7·9 g (35%) of compound *VIa*, m.p. 159—160°C. For $C_{10}H_{14}N_2O_4$ (226·2) calculated: 53·09% C, 6·24% H, 12·38% N; found: 53·11% C, 6·25% H, 12·39% N.

A mixture of compound *VIa* (3·0 g; 13·3 mmol) and 80% aqueous acetic acid (50 ml) was refluxed for 30 min, evaporated under diminished pressure, the residue coevaporated with ethanol, and finally crystallised from ethanol (150 ml) to yield 1·3 g (69%) of compound *IVa*, m.p. 167 to 168°C. For $C_7H_{10}N_2O_4$ (186·2) calculated: 45·15% C, 5·41% H, 15·05% N; found: 45·38% C, 5·56% H, 15·09% N. The product *IVa* is identical with an authentic material on the m.p. determination and chromatography in the solvent systems S_1, S_2, and S_5.

The 3-isomer *IVc* was prepared analogously from compound *VIc* in 54% yield. The acetic acid was evaporated, the residue coevaporated with three 20 ml portions of ethanol and finally precipitated from ethanol (5 ml) with ether (100 ml). The precipitate was collected with suction, washed with ether, and dried under diminished pressure. For $C_7H_{10}N_2O_4$ (186·2) found: 45·43% C 5·62% H, 15·40% N.

(*S*)-1-(2,3-Dihydroxypropyl)thymine (*IVb*) and (*S*)-3-(2,3-Dihydroxypropyl)thymine (*IVd*)

A mixture of compound *V* (0·1 mol) and the sodium salt of thymine[28] (0·12 mol) was heated in dimethylformamide (100 ml) at 100°C for 8 h under stirring and exclusion of atmospheric moisture and processed analogously to the preparation of compounds *IVa* and *IVc*. Yield, 8·5 g (35·4%) of compound *VId* as an amorphous foam. For $C_{11}H_{16}N_2O_4$ (240·2) calculated: 55·00% C, 6·71% H, 11·66% N; found: 55·12% C, 6·78% H, 11·80% N. An additional elution with chloroform and crystallisation from ethyl acetate–light petroleum yielded 10·3 g (42·7%) of compound *VIb*, m.p. 165—167°C. For $C_{11}H_{16}N_2O_4$ (240·2) calculated as above; found: 55·36% C, 6·74% H, 11·71% N.

A solution of compound *VIb* (8·5 g; 35·4 mmol) in 80% aqueous acetic was refluxed for 30 min, evaporated *in vacuo*, the residue coevaporated with three 20 ml portions of water and three 20 ml portions of ethanol, and the final residue crystallised from ethanol. Yield, 4·3 g (57%) of compound *IVb*, m.p. 115°C (sublimation from 70°C), $[\alpha]_D^{25}$ −54·3° (*c* 1, water). For $C_8H_{12}N_2O_4$ (200·2) calculated: 48·02% C, 6·03% H, 14·00% N; found: 47·98% C, 6·45% H, 13·97% N.

Compound *VId* (8·5 g; 35·4 mmol) afforded similarly 59% (after crystallisation from ethanol) of compound *IVd*, m.p. 136—137°C, $[\alpha]_D^{25}$ −61·4° (*c* 1; water). Found: 48·13% C, 6·68% H, 13·28% N.

(*S*)-9-(2,3-Dihydroxypropyl)adenine (*IVe*)

A mixture of compound *V* (11·4 g; 40 mmol) and the sodium salt of adenine[28] (7·8 g; 50 mmol) in dimethylformamide (100 ml) was heated at 100°C for 8 h under exclusion of atmospheric moisture, evaporated at 40°C/0·1 Torr, and the residue crystallised from a little 90% aqueous methanol. The solid was collected with suction, washed with methanol, and dried under diminished pressure to yield 6·0 g (60%) of the chromatographically homogeneous compound *VIe*, m.p. 210—211°C. For $C_{11}H_{15}N_5O_2$ (249·3) calculated: 52·99% C, 6·06% H, 28·09% N; found:

53·00% C, 6·10% H, 28·50% N. NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·27 (s, 3 H) and 1·31 (s, 3 H) CH_3—C—CH_3; 3·89 (m, 2 H, $J_{1',2'} = 6·0$, $J_{1'',2'} = 5·5$, $J_{gem} = 9·0$) 2 $H_{1'}$; 4·29 (m, 2 H) 2 $H_{3'}$; 4·49 (m, 1 H) $H_{2'}$; 7·10 (br s, 2 H) NH_2; 8·05 + 8·15 (2 s, 2 H) H_2 + H_8. Compound *VIe* (4·2 g; 17·1 mmol) was then refluxed in 80% aqueous acetic acid (60 ml) for 30 min, the mixture evaporated, the residue coevaporated with 50% aqueous ethanol and finally crystallised from methanol to yield 3·0 g (84%) of compound *IVe*, m.p. 202—203°C, $[\alpha]_D^{25}$ −35·4° (*c* 1; water). For $C_8H_{11}N_5O_2$ (209·2) calculated: 45·92% C, 5·30% H, 33·48% N; found: 45·88% C, 5·49% H, 32·95% H. NMR spectrum (hexadeuteriodimethyl sulfoxide): 3·45 (m, 2 H) 2 $H_{1'}$; 3·82 (m, 1 H) $H_{2'}$; 4·14 (m, 2 H, $J_{2',3'} = 3·5$, $J_{gem} = 12·5$) 2 $H_{3'}$; 7·06 (br s, 2 H) NH_2; 8·01 + + 8·20 (2 s, 2 H) H_2 + H_8.

From the racemic derivative[4] *VI*, there was analogously prepared the racemic derivative *IVe*, m.p. 207—208°C (reported[5], m.p. 205—206°C), homogeneous on chromatography and identical (except for the optical activity) with the (*S*)-enantiomer *IVe*.

(*S*)-9-(2,3-Dihydroxypropyl)hypoxanthine (*IVf*)

To a mixture of compound *IVe* (1·0 g; 4·8 mmol) and sodium nitrite (2·5 g) in water (20 ml) there was added with stirring acetic acid (20 ml) and the stirring was continued at room temperature for 5 h. The mixture was then evaporated under diminished pressure, the residue applied in water (20 ml) to a column of Dowex 50 (H^+) ion exchange resin (100 ml), and the column washed with water until the UV absorption and conductivity dropped to the original value. The product was then eluted with dilute (1 : 10) aqueous ammonia, the UV-absorbing portion of the eluate evaporated, and the residue crystallised from 80% aqueous ethanol. Yield, 878 mg (87%) of the chromatographically (solvent system S_1) homogeneous compound *IVf*, m.p. 244—246°C (reported[5], 251—252°C), $[\alpha]_D^{25}$ −14·8° (*c* 0·5; water). For $C_8H_{10}N_4O_3$ (210·2) calculated: 45·70% C, 4·79% H, 26·65% N; found: 44·46% C, 4·75% H, 23·31% N.

(*S*)-1-(2,3-Dihydroxypropyl)-2-pyrimidinone (*IVg*)

To a solution of 2-pyrimidinone[29] (20 mmol) in 1M methanolic sodium methoxide (22 ml) there was added with stirring ether (200 ml). The crystalline precipitate of the sodium salt was collected with suction, washed with ether under exclusion of atmospheric moisture, and dried under diminished pressure. A suspension of this salt, compound *V* (5·7 g; 20 mmol), and dimethylformamide (20 ml) was stirred under exclusion of atmospheric moisture 5 h at room temperature and then 7 h at 100°C. The mixture was evaporated at 40°C/0·1 Torr, the residue extracted with hot chloroform (100 ml), the extract filtered through Celite, and the filtrate evaporated under diminished pressure. The residue was chromatographed on two layers of loose silica gel (see above) in the solvent system S_3. Bands of the product were eluted with methanol, the eluate evaporated, and the residue crystallised from ethanol, ether being added until the solution was turbid. Yield, 2·24 g (53·5%) of compound *VIg*, m.p. 113—114°C. For $C_{10}H_{14}N_2O_3$ (210·2) calculated: 57·13 C, 6·71% H, 13·33% N; found: 57·02% C, 6·71% H, 13·40% N.

Compound *VIg* (1·65 g; 7·8 mmol) was refluxed in 80% aqueous acetic acid (20 ml) for 30 min, the mixture evaporated, the residue coevaporated with ethanol, and the residue applied to one layer of loose silica gel. The band of the product was eluted with methanol, and the residue crystallised from a mixture of ethanol and light petroleum. Yield, 1·0 g (75·5%) of compound *IVg*. For $C_7H_{10}N_2O_3$ (170·2) calculated: 49·39% C, 5·92% H, 16·46% N; found: 47·73% C, 5·83% H, 16·20% N.

(*S*)-1-(2,3-Dibenzoyloxypropyl)uracil (*VIIa*)

To a mixture of compound *IVa* (3·7 g; 20 mmol), benzoyl cyanide (6·5 g; 50 mmol), and acetonitrile (30 ml) there was added with stirring triethylamine (2 ml) and the stirring continued for 30 min. The originally clear solution spontaneously deposited crystals. The mixture was diluted with ether, the crystals collected with suction, washed with ether, and recrystallised from acetonitrile (100 ml) to yield 6·2 g (79%) of compound *VIIa*, m.p. 140–141°C. For $C_{21}H_{18}N_2O_6$ (394·4) calculated: 63·94% C, 4·60% H, 7·10% N; found: 64·02% C, 4·75% H, 7·02% N.

(*S*)-1-(2,3-Dibenzoyloxypropyl)-4-thiouracil (*VIIb*)

A mixture of compound *VIIa* (5·9 g; 15 mmol), phosphorus pentasulfide (7·2 g; 33·5 mmol), and dioxane (250 ml) was refluxed for 1 h under exclusion of atmospheric moisture (calcium chloride tube), filtered while hot, and the material on the filter washed with dioxane (50 ml). The filtrate and washings were combined and evaporated under diminished pressure. The residue was dissolved in chloroform (300 ml), the solution washed with three 100 ml portions of saturated aqueous sodium hydrogen carbonate and water, dried over anhydrous magnesium sulfate, and evaporated. The residue was crystallised from acetonitrile to yield 5·5 g (88%) of compound *VIIb*, m.p. 138°C. For $C_{21}H_{18}N_2O_5S$ (410·4) calculated: 61·45% C, 4·42% H, 6·82% N, 7·81% S; found: 60·72% C, 4·45% H, 7·03% N, 8·16% S.

(*S*)-1-(2,3-Dihydroxypropyl)cytosine (*IVh*)

A solution of compound *VIIb* (5·0 g; 12 mmol) in 30% methanolic ammonia (70 ml) was heated in an autoclave at 100°C for 12 h, evaporated under diminished pressure, and distributed between water (100 ml) and ether (50 ml). The aqueous phase was washed with two 25 ml portions of ether, evaporated, the residue adjusted to pH 3 with conc. hydrochloric acid after dilution with water (20 ml), and the solution applied to a column of Dowex 50 X 8 ion exchange resin (150 ml). The column was eluted with water to the drop of the UV absorption and conductivity, and then with dilute (1 : 10) aqueous ammonia. The UV-absorbing ammonia eluate was evaporated, the residue coevaporated three times with ethanol, the final residue dissolved in hot methanol (20 ml) and the solution added dropwise into ether (200 ml). The precipitate was collected with suction, washed with ether, and dried. Yield, 1·2 g (54%) of the chromatographically and electrophoretically homogeneous product *IVh*, m.p. 165–168°C. For $C_7H_{11}N_3O_3$ (185·2) calculated: 45·40% C, 5·99% H, 22·69% N; found: 45·64% C, 6·03% H, 23·01% N.

(*R*)-1-(2,3-Dihydroxypropyl)thymine (*XIb*)

A. A mixture of methyl 5-O-*p*-toluenesulfonyl-2,3-O-isopropylidene-D-ribofuranoside[15] (*VIII*; 7·2 g; 20 mmol), the sodium salt of thymine[28] 3·7 g; 25 mmol), and dimethylformamide (70 ml) was heated at 100°C for 14 h with stirring and under exclusion of atmospheric moisture. The mixture was then evaporated at 0·1 Torr/40°C, the residue extracted with hot chloroform (200 ml), the extract filtered through Celite, the filtrate evaporated under diminished pressure, and the residue chromatographed on a column of silica gel (120 g) in chloroform. Elution with chloroform afforded first 2·3 g (37%) of the 3-isomer *IXc* as an amorphous foam and then 2·5 g (40%) of the amorphous compound *IXa*. For $C_{14}H_{20}N_2O_6$ (312·3) calculated: 53·83% C, 6·45% H, 8·97% N; found: 54·38% C, 6·38% H, 8·45% N. NMR spectrum ($CDCl_3$): 1·32 + 1·48 (2 s, 2 × 3 H) CH_3—C—CH_3; 1·91 (s, 3 H) C_5—CH_3; 3·42 (s, 3 H) OCH_3; 3·49 (q, 1 H, $J_{5'',4'} = 8·0$, $J_{gem} = 14·0$) $H_{5''}$; 4·19 (q, 1 H, $J_{5',4'} = 6·5$) $H_{5'}$; 4·50 (q, 1 H, $J_{4',1'} = 0$, $J_{4',5'} = 6·5$, $J_{4',5''} = 8·0$) $H_{4'}$; 4·70 (m, 2 H) $H_{2'} + H_{3'}$; 5·01 (s, 1 H) $H_{1'}$ (β-anomer).

A solution of compound *IXa* (2·3 g; 7·4 mmol) in 3·5M methanolic hydrogen chloride was kept at room temperature overnight and evaporated under diminished pressure. The residue was coevaporated with five 20 ml portions of methanol and the final residue chromatographed on one layer of loose silica gel in the solvent system S_4. The band of the product was eluted with methanol, the eluate evaporated, and the residue dried under diminished pressure. Yield, 1·2 g (59·5%) of the amorphous compound *Xb*, chromatographcically homogeneous in S_4. A mixture of this product, sodium periodate (1·2 g; 5·6 mmol), and 80% aqueous methanol (25 ml) was stirred at 0°C for 1 h, treated with ethylene glycol (0·2 ml), stirred at 0°C for additional 10 min, and diluted with methanol (25 ml). The suspension was filtered off and the material on the filter washed with methanol (20 ml). The filtrate and washings were combined and stirred with sodium borohydride (1·5 g) for 1 h at room temperature. The mixture was adjusted to pH 6 with acetic acid, evaporated under diminished pressure, the residue dissolved in a small amount of water, the solution applied to a column of Amberlite IR 4B (acetate cycle) ion exchange resin (150 ml) and the column eluted with water. The UV-absorbing eluate portion was evaporated, the residue applied to a column of Dowex 50 (H^+) ion exchange resin (100 ml), and the column eluted with water. The UV-absorbing portion of the eluate was evaporated, the residue coevaporated with three 20 ml portions of ethanol, and the final residue chromatographed on one layer of loose silica gel in the solvent system S_5. The product was eluted in a column with methanol (500 ml). The eluate was evaporated under diminished pressure and the residue was crystallised from ethanol, ether being added until the solution was turbid. Yield, 0·6 g (68%, referred to compound *Xb*) of the (*R*)-enantiomer *XIb*, m.p. 118—120°C. For $C_8H_{12}N_2O_4$ (200·2) calculated: 48·02% C, 6·03% H, 14·00% N; found: 48·15% C, 6·15% H, 14·23% N.

B. A mixture of compound *IVb* (1·0 g; 5 mmol), triphenylmethyl chloride (1·7 g; 6 mmol), and pyridine (10 ml) was stirred at room temperature for 2 days, diluted with ethyl acetate (100 ml), washed with two portions of water (25 ml each), three 50 ml portions of dilute (1 : 10) hydrochloric acid, two 25 ml portions of water, and two 25 ml portions of saturated aqueous sodium hydrogen carbonate, dried over anhydrous magnesium sulfate, and filtered. The filtrate was evaporated in vacuo and dried under diminished pressure to afford 2·1 g (95%) of the chromatographically homogeneous (in solvent systems S_3 and S_7) compound *XII*. This product was dissolved in pyridine (20 ml), the solution treated at 0°C with methanesulfonyl chloride (0·8 ml; 1·18 g; 10·3 mmol) under stirring, the mixture stirred at room temperature overnight, diluted with water (100 ml), and extracted with ethyl acetate (100 ml). The extract was washed with four 50 ml portions of dilute (1 : 100) hydrochloric acid, water (50 ml), and saturated aqueous sodium hydrogen carbonate (50 ml), dried over anhydrous magnesium sulfate, filtered, the filtrate evaporated, and the residue (compound *XIII*, homogeneous on chromatography in the solvent system S_7) dried under diminished pressure. A mixture of the dry compound *XIII*, acetonitrile (70 ml), and triethylamine (30 ml) was then refluxed under exclusion of atmospheric moisture (calcium chloride safe-guard tube). As shown by chromatography in S_7, the reaction was complete after 3·5 h. The mixture was evaporated under diminished pressure and the residue refluxed in 80% aqueous acetic acid (50 ml) for 30 min. The mixture (containing exclusively the compound *XIb* according to chromatography in S_5) was evaporated under diminished pressure, the residue diluted with water (100 ml), washed with two 25 ml portions of ether, and the aqueous phase evaporated. The residue was chromatographed on one layer of loose silica gel in the solvent system S_5. The band of the product was eluted with methanol, the eluate evaporated, and the residue crystallised from ethanol–ether. Yield, 0·5 g (50%, referred to compound *IVb*) of the chromatographically homogeneous (in S_1, S_2, and S_5) compound *XIb*, m.p. 118—120°C. As indicated by CD spectrum (Table I), both the specimens of compound *XIb* prepared by procedure *A* or *B* are identical (*R*)-enantiomers.

(*R*)-9-(2,3-Dihydroxypropyl)adenine (*XIa*)

A mixture of compound[15] *VIII* (7·2 g; 20 mmol), the sodium salt of adenine (3·9 g; 25 mmol), and dimethylformamide (70 ml) was heated at 100°C for 8 h under stirring and exclusion of atmospheric moisture, diluted while hot with toluene (100 ml), kept at room temperature overnight, filtered off, and the material on the filter washed with toluene (100 ml). The filtrate and washings were combined, evaporated at 50°C/0·1 Torr, and the residue extracted with two 100 ml portions of hot chloroform. The extract was filtered through Celite and the material on the filter washed with chloroform (100 ml). The filtrate and washings were combined and evaporated under diminished pressure to afford another crop of the crystalline product. This crop was dissolved in chloroform (200 ml) together with the crop obtained directly from the reaction mixture (on dilution with toluene). The chloroform solution was washed with two 50 ml portions of water, dried over anhydrous magnesium sulfate, and evaporated. The residue was crystallised from methanol (100 ml) with the addition of water to obtain a clear hot solution. Yield, 4·0 g (63%) of compound *IXa*, m.p. 252–254°C. For $C_{14}H_{19}N_5O_4$ (321·3) calculated: 52·33% C, 5·96% H, 21·80% N; found: 52·83% C, 6·05% H, 22·14% N.

A suspension of compound *IXa* (3·2 g; 10 mmol) in 5% methanolic hydrogen chloride was stirred at room temperature overnight. As indicated by chromatography in the solvent system S_5, the reaction was complete. The mixture was adjusted to pH 9 (moistened pH-paper) by the addition of 30% methanolic ammonia and stirred for 20 min. The precipitate of ammonium chloride was filtered off and washed with methanol (50 ml). The filtrate and washings were combined, evaporated, and the residue crystallised from methanol to afford 2·35 g (80%) of the chromatographically homogeneous (in S_6) compound *Xa*, m.p. 195°C (decomp.). For $C_{11}H_{15}N_5O_4$ (281·3) calculated: 49·96% C, 5·37% H, 24·90% N; found: 50·50% C, 5·42% H, 25·25% N.

To a solution of compound *Xa* (2·0 g; 7·1 mmol) in 40% aqueous methanol (50 ml) there was added sodium periodate (2·6 g; 12 mmol), the whole stirred at 0°C for 1 h, treated with ethylene glycol (1·0 ml), kept for 10 min at 0°C, diluted with ethanol (50 ml), filtered off, and the material on the filter washed with 80% aqueous ethanol (50 ml). The filtrate and washings were combined and treated at 0°C with sodium borohydride (1·5 g). The mixture was stirred at 0°C for 1 h, adjusted to pH 6 with acetic acid, and concentrated under diminished pressure to the volume of about 25 ml. The concentrate was applied to a column of Dowex 50 X 8 (H^+) ion exchange resin (150 ml) and the column washed with water until the conductivity dropped to the original value and the UV absorption disappeared. The column was then eluted with dilute (1 : 10) ammonia, the UV-absorbing portion of the eluate evaporated under diminished pressure, and the residue crystallised from ethanol. Yield, 1·2 g (81%) of compound *XIa*, m.p. 203–204°C, homogeneous on chromatography in the solvent systems S_1, S_2, and S_6, and identical with the (*S*)-enantiomer. For $C_8H_{11}N_5O_2$ (209·2) calculated: 45·92% C, 5·30% H, 33·48% N; found: 45·48% C, 5·25% H, 33·57% N.

(*RS*)-1-(3,4-Dihydroxybutyl)thymine (*XVIII*)

A mixture of 1-*p*-toluenesulfonyloxy-2-butene[30] (*XV*), the sodium salt of thymine[28] (3·5 g; 25 mmol), methanol (20 ml), and 1M methanolic sodium methoxide (26 ml) was refluxed for 18 h, neutralised with acetic acid, evaporated under diminished pressure, and the residue dissolved in water (20 ml). The aqueous solution was applied to a column of Amberlite IR 4B (acetate cycle) ion exchange resin (100 ml) and the UV-absorbing material eluted with water. The eluate was concentrated under diminished pressure to the volume of about 50 ml, the concentrate applied to a column of Dowex 50 X 8 (H^+) ion exchange resin, the UV-absorbing material eluted with water, the eluate evaporated under diminished pressure, the residue coevaporated with ethanol

(20 ml), and finally chromatographed on 4 layers of loose silica gel in the solvent system S_3. Bands of the product were eluted with methanol (500 ml), the eluate evaporated under diminished pressure, and the residue crystallised from ethanol (20 ml) and cyclohexane (200 ml) to afford 1·76 g (36·4%) of compound *XVII*, m.p. 127–129°C. For $C_{10}H_{14}N_2O_2$ (194·2) calculated: 62·35% C, 7·26% H, 14·42% N; found: 63·30% C, 6·71% H, 15·05% N. NMR spectrum (deuteriochloroform): 1·93 (d, 3 H, $J_{CH_3,H} = 1·2$) 5-CH_3; 2·44 (m, 2 H, $J_{2',1'} = J_{2',1''} = 6·5$, $J_{2',3'} = 6·5$) 2 $H_{2'}$; 3·77 (t, 2 H, $J_{1',2'} = J_{1',2''} = 6·5$) 2 $H_{1'}$; 5·10 (m, 2 H) 2 $H_{4'}$; 5·77 (m, 1 H) $H_{3'}$; 6·95 (q, 1 H) H_6; 9·51 (br s, 1 H) NH.

To a solution of compound *XVII* (1·25 g; 6·5 mmol) and sodium chlorate (0·9 g; 8·4 mmol) in 50% aqueous methanol there was added osmium tetroxide (20 mg) and the whole was stirred at room temperature for 24 h. The reaction was then complete as indicated by chromatography in S_5. The mixture was filtered through Celite and the filtering agent washed with water (50 ml). The filtrate and washings were combined and applied to a column of Amberlite IR 4B (acetate) ion exchange resin (100 ml) and the UV-absorbing material was eluted with water. The eluate was concentrated under diminished pressure and the concentrate applied to a column of Dowex 50 X 8 (H^+) ion exchange resin (60 ml). The column was eluted with water, the UV-absorbing portion of the eluate evaporated under diminished pressure, and the residue chromatographed on two layers of loose silica gel in the solvent system S_5. The product was eluted with methanol (300 ml), the eluate evaporated under diminished pressure and the residue crystallised from ethanol–ether. Yield, 0·86 g (62%) of compound *XVIII*, homogeneous on chromatography (in S_1 and S_5). For $C_8H_{12}N_2O_4$ (200·2) calculated: 48·00% C, 6·02% H, 14·00% N; found: 47·87% C, 6·77% H, 13·70% N.

1,2-O-Isopropylidene-1,2,4-butanetriol[19] (*XX*)

A mixture of 1,2,4-butanetriol[19] (*XIX*; 0·4 mmol), acetone (100 ml), 2,2-dimethoxypropane (100 ml), and *p*-toluenesulfonic acid hydrate (2·5 g) was kept at room temperature for 2 days, neutralised with 1M methanolic sodium methoxide, evaporated under diminished pressure, the residue triturated with ether (200 ml), the mixture filtered off, and the material on the filter washed with ether (100 ml). The filtrate and washings were combined, evaporated, and the residue distilled under diminished pressure to afford 46 g (80%) of compound *XX*, b.p. 107–109°C/16 Torr. For $C_7H_{14}O_3$ (146·2) calculated: 57·50% C, 9·65% H; found: 58·28% C, 9·61% H. NMR spectrum (deuteriochloroform): 1·35 and 1·40 (2 s, 2 × 3 H) isopropylidene group; 1·81 (q, 2 H, $J_{3,2} = J_{3,4} = 6·0$) 2 H_3; 2·43 (s, 1 H) OH; 3·58 (t, 1 H, $J_{1,1'} = J_{1,2} = 8·0$) H_1; 3·79 (t, 2 H, $J_{4,3} = 6·0$) 2 H_4; 4·08 (q, 1 H, $J_{1',2} = 6·0$, $J_{1,1'} = 8·0$) $H_{1'}$; 4·30 (m, 1 H) H_2.

4-O-*p*-Toluenesulfonyl-1,2-O-isopropylidene-1,2,4-butanetriol (*XXI*) and 2,2-Dimethyl-4-(2-chloroethyl)dioxolane (*XXII*)

To a solution of compound *XX* (45 g; 0·31 mol) in pyridine (100 ml) there was added with stirring at −40°C *p*-toluenesulfonyl chloride (72·5 g; 0·38 mol) and the temperature was allowed to rise to 0°C in the course of 1 h. The mixture was then kept overnight at 0°C and for additional 24 h at room temperature. Ice was then added (300 g), the product extracted with three 100 ml portions of ether and two 100 ml portions of ethyl acetate, the extracts combined, washed with two 100 ml portions of saturated aqueous sodium hydrogen carbonate and water (100 ml), dried over anhydrous magnesium sulfate, and evaporated. The residue was dissolved in ether (50 ml), the solution diluted with light petroleum (300 ml), and cooled down to −70°C to deposit crystals. The supernatant was decanted and the solid recrystallised analogously once more. The material

after decantation of the supernatant was allowed to warm to room temperature; the resulting melt was dried over phosphorus pentoxide at 0·1 Torr. Yield, 23·2 g (25%) of compound *XXI*, homogeneous on chromatography in the solvent system S_4; this substance was directly used in the next step without any further purification.

The decantates after the crystallisation of compound *XXI* were evaporated and the residues distilled to afford 14·0 g (27%) of the oily compound *XXII*, b.p. 74°C/14 Torr. For $C_7H_{13}ClO_2$ (164·6) calculated: 51·07% C, 7·95% H, 21·54% Cl; found: 51·62% C, 7·95% H, 21·08% Cl. NMR spectrum (deuteriochloroform): 1·29 and 1·34 (2 × s, 2 × 3 H) CH_3—C—CH_3; 1·95 (m, 2 H) 2 $H_{1'}$; 3·60 (q, 2 H, J = 6·0, J = 7·4) 2 $H_{2'}$; 3·52 (t, 1 H, $J_{5,4}$ = 7·2, J_{gem} = 7·6) H_5; 4·03 (q, 1 H, $J_{5',4}$ = 6·0, J_{gem} = 7·6) $H_{5'}$; 4·22 (m, 1 H) H_4.

(*RS*)-9-(3,4-Dihydroxybutyl)adenine (*XXIV*)

A mixture of compound *XXI* (23·2 g; 77·5 mmol), the sodium salt of adenine[28] (14·0 g; 90 mmol), and dimethylformamide (50 ml) was heated at 80°C for 5 h under stirring and exclusion of atmospheric moisture, and evaporated under diminished pressure. The residue was extracted with three 200 ml portions of hot chloroform, the extracts combined, filtered through Celite, and the filtrate evaporated. The residue was dissolved in chloroform (50 ml) and the solution was applied to a column of silica gel (150 g) packed in chloroform. Elution with chloroform, evaporation of fractions containing the compound *XXIII*, and crystallisation of the residue from a mixture of ethanol and light petroleum yielded 10·3 g (50·5%) of compound *XXIII*, m.p. 218°C. For $C_{12}H_{17}N_5O_2$ (263·3) calculated: 54·73% C, 6·51% H, 26·60% N; found: 54·92% C, 6·51% H, 26·73% N. NMR spectrum (deuteriochloroform): 1·34 and 1·42 (2 s, 2 × 3 H) CH_3—C—CH_3; 2·10 (m, 2 H) 2 $H_{2'}$; 3·53 (m, 1 H) and 3·80—4·50 (m, 4 H) $H_{1'}$ + $H_{3'}$ + 2 $H_{4'}$; 6·40 (br, s, 2 H) NH_2; 7·86 (s, 1 H) H_2; 8·36 (s, 1 H) H_8.

A mixture of compound *XXIII* (8·0 g; 30·4 mmol) and 80% aqueous acetic acid (100 ml) was refluxed for 40 min, evaporated, the residue coevaporated with four 50 ml portions of ethanol, and the final residue crystallised from 90% aqueous ethanol to yield 4·2 g (62%) of compound *XXIV*, m.p. 217—218°C, homogeneous on chromatography in the solvent systems S_1 and S_2. For $C_9H_{13}N_5O_2$ (223·3) calculated: 48·42% C, 5·87% H, 31·38% N; found: 48·60% C, 6·07% H, 31·07% N. NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·60—2·00 (m, 2 H) 2 $H_{2'}$; 3·30 (m, 3 H) 2 $H_{4'}$ + $H_{3'}$; 3·35 (br s, 1 H) OH; 4·23 (br t, 2 H, J_{gem} = 7·0) 2 $H_{1'}$; 7·05 (br s, 2 H) NH_2; 8·02 (s, 1 H) H_8; 8·11 (s, 1 H) H_2.

(*RS*)-9-(2-Hydroxypropyl)adenine (*XXV*)

To a stirred solution of 1,2-propanediol (38·0 g; 0·5 mol) in pyridine (200 ml) there was added at 0°C *p*-toluenesulfonyl chloride (95·2 g; 0·5 mol), the mixture stirred at 0°C for 1 h, kept at room temperature overnight, and filtered off. The filtrate was evaporated under diminished pressure, the residue treated with ice (300 g), and extracted with three 200 ml portions of ether. The extract was washed with 100 ml portions of water, dilute (1 : 10) aqueous sulfuric acid (3 portions), water, saturated aqueous sodium hydrogen carbonate, and water, dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. The residue was dissolved in ether (50 ml), the solution treated with light petroleum (300 ml), the whole cooled to —70°C, and the supernatant decanted. The residual oil was dried over phosphorus pentoxide at 0·1 Torr. Yield, 68 g (59%) of the crude *p*-toluenesulfonyl derivative.

A mixture of the above *p*-toluenesulfonyl derivative (11·5 g; 50 mmol), the sodium salt of adenine[28] (7·9 g; 50 mmol), and dimethylformamide (50 ml) was heated with stirring at 80°C

for 3 h and evaporated at 50°C/0·1 Torr. The residue was dissolved in water (200 ml), the solution washed with two 50 ml portions of ether, the aqueous phase (free of ether which was removed under diminished pressure) adjusted to pH 3 by the addition of Dowex 50 (H^+) ion exchange resin, applied to a column of the same resin (250 ml), and the column washed with water to the drop of conductivity and the UV absorption. The elution was performed with dilute (1 : 10) aqueous ammonia, the UV-absorbing eluate portion evaporated, and the residue coevaporated with one 50 ml portion of ethanol and three 50 ml portions of pyridine. A mixture of the final residue with pyridine (50 ml) and acetic anhydride (50 ml) was stirred at room temperature overnight and methanol (100 ml) was then added with ice-cooling. After 30 min, the mixture was evaporated and the residue was coevaporated with four 50 ml portions of toluene. The final residue was diluted with chloroform (100 ml), the mixture filtered with suction, and the material on the filter washed with additional chloroform (100 ml). The filtrate and washings were combined, concentrated under diminished pressure to the volume of about 50 ml, and the concentrate applied to a column of silica gel (100 g) packed in chloroform. The main component (R_F value 0·50 in the solvent system S_3) of the mixture was eluted with chloroform and the eluate evaporated under diminished pressure to yield 4·0 g of the peracetate of compound *XXV*. This product was kept in 0·1M methanolic sodium methoxide (50 ml) overnight, the mixture evaporated, the residue coevaporated with ethanol, and the final residue crystallised from boiling acetonitrile to yield 1·5 g (15·4%, referred to adenine) of the chromatographically (S_1, S_2, and S_6) homogeneous compound *XXV*, m.p. 200°C (decomposition). For $C_8H_{11}N_5O$ (193·2) calculated: 49·73% C, 5·74% H, 36·25% N; found: 50·23% C, 5·85% H, 37·00% N. NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·10 (d, 3 H) CH_3; 3·35 (br s, 1 H) OH; 3·90—4·25 (m, 3 H) 2 $H_{1'}$ + $H_{2'}$; 6·72 (br s, 2 H) NH_2; 7·92 (s, 1 H) H_2; 8·15 (s, 1 H) H_8.

(*S*)-9-(2,3-Dihydroxypropyl)adenine 3′-Phosphate (*XXVI*)

To a suspension of compound *IVe* (4·2 g; 20 mmol) in triethyl phosphate (30 ml) there was added phosphorus oxychloride (5 ml). The mixture was stirred at room temperature for 6 h, decomposed with water (50 ml), adjusted to pH 7·5 by the addition of 10% aqueous lithium hydroxide, and concentrated under diminished pressure to the volume of about 50 ml. A mixture (500 ml) of ethanol and acetone (1 : 1) was added to the concentrate, the solid collected with suction, washed with the same solvent mixture, and dried. This crude lithium salt of compound *XXVI* was dissolved in water (50 ml) and the aqueous solution applied to a column of Dowex 50 X 8 (H^+) ion exchange resin (100 ml). The column was eluted with water, the UV-absorbing fractions of the eluate evaporated, and the residue crystallised from aqueous ethanol to yield 4·05 g (70%) of the free acid *XXVI*, m.p. 246—247°C. For $C_8H_{12}N_5O_5P$ (289·3) calculated: 33·21% C, 4·17% H, 24·21% N, 10·72 P; found: 33·28% C, 4·48% H, 23·62% N, 10·60% P.

(*S*)-9-(2,3-Dihydroxypropyl)adenine 2′,3′-Cyclic Phosphate (*XXXVII*)

A mixture of compound *XXVI* (1·0 g; 3·46 mmol), water (30 ml), dimethylformamide (25 ml), conc. aqueous ammonia (6 ml), tert-butyl alcohol (25 ml), and N,N′-dicyclohexylcarbodiimide (12 g) was refluxed for 4 h, cooled down, diluted with water (200 ml), washed with three 100 ml portions of ether, and the aqueous phase taken down under diminished pressure. The residue was dissolved in water (20 ml), the aqueous solution applied to a column of Dowex 50 X 8 (Li^+ cycle) ion exchange resin (20 ml), and the column eluted with water. The UV-absorbing fractions of the eluate were evaporated and the residue was purified by precipitation from water (10 ml) with ethanol (100 ml) and acetone (100 ml). The precipitate was collected with suction,

washed with acetone and ether, and dried under diminished pressure. Yield, 0·69 g (72·5%) of the lithium salt of compound *XXXVII*, homogeneous on chromatography (S_1) and electrophoresis (E_1); content (spectrophotometry): 97%. The *P. brevicompactum* ribonuclease degradation of this salt affords quantitatively compound *XXVI*.

Attempted Polycondensation of Compound *XXVI* (*cf.*[9])

A mixture of compound *XXVI* (0·5 g; 1·7 mmol), dimethylformamide (9 ml), water (1 ml), and N,N′-dicyclohexylcarbodiimide (5 g) was refluxed for 8 h, treated with additional 5 g of N,N′-dicyclohexylcarbodiimide, and refluxed for 8 h more. Water (200 ml) was then added, the mixture washed with two 50 ml portions of ether, the aqueous phase evaporated, the residue coevaporated with three 50 ml portions of water, the final residue triturated with 20 ml of water, collected with suction, and washed with water. This procedure was repeated twice; the precipitate did not contain any UV-absorbing components. The aqueous solution was evaporated again and the residue was coevaporated with two 20 ml portions of ethanol. The residual substance was homogeneous on chromatography (S_1 and S_2) and electrophoresis (E_1) and identical with compound *XXXVII*.

(*RS*)-9-(3,4-Dihydroxybutyl)adenine 4′-Phosphate (*XXXVa*)

A mixture of compound *XXIV* (0·67 g; 3 mmol), phosphorus oxychloride (0·8 ml; 8·7 mmol), and triethyl phosphate (5 ml) was stirred at room temperature for 3 h, diluted with water (25 ml), heated at 70°C for 1 h, neutralised with 10% aqueous lithium hydroxide, and evaporated under diminished pressure. The residue was dissolved in water (20 ml), the aqueous solution applied to a column of Dowex 50 X 8 (H^+) ion exchange resin (200 ml), and the column washed with water to the drop of the UV-absorption. The elution was then carried out with 2M acetic acid, the UV-absorbing portion of the eluate was evaporated and the residue was crystallised from aqueous acetone to yield 0·55 g (60·5%) of the free acid *XXXVa*. For $C_9H_{14}N_5O_5P$ (303·3) calculated: 36·00% C, 4·67% H, 23·34% N, 10·35% P; found: 36·15% C, 4·92% H, 23·12% N, 10·60% P.

(*RS*)-1-(3,4-Dihydroxybutyl)thymine 4′-Phosphate (*XXXVb*)

A mixture of compound *XVIII* (0·34 g; 1·70 mmol), phosphorus oxychloride (0·5 ml; 5·5 mmol), and triethyl phosphate (5 ml) was stirred at room temperature for 80 min, diluted with water (20 ml), the whole heated at 70°C for 1 h, neutralised with triethylamine, and evaporated. The residue was chromatographed on 6 sheets of paper Whatman No 3 MM in the solvent system S_1, bands of the product eluted with dilute (1 : 50) aqueous ammonia, the eluates evaporated, the residue coevaporated with methanol, and finally purified by precipitation from methanol (5 ml) with ether (100 ml). The precipitate was collected with suction, washed with ether, and dried under diminished pressure to yield 460 mg (92%) of the ammonium salt of compound *XXXVb*, homogeneous on chromatography (S_1) and electrophoresis (E_1); content (spectrophotometry): 97%. For $C_8H_{16}N_3O_7P$ (297·3) calculated: 14·18% N, 10·47% P; found: 13·87% N, 10·51% P.

(*RS*)-9-(3,4-Dihydroxybutyl)adenine 3′,4′-Cyclic Phosphate (*XXXVIa*)

A mixture of the acid *XXXVa* (364 mg; 1·2 mmol), 2M aqueous ammonia (10 ml), dimethylformamide (8 ml), tert-butyl alcohol (8 ml), and N,N′-dicyclohexylcarbodiimide (4 g) was refluxed for 4 h, cooled down, diluted with water (100 ml), washed with two 25 ml portions of ether, and

the aqueous phase evaporated under diminished pressure. The residue was chromatographed on four sheets of paper Whatman No 3 MM in the solvent system S_1, bands of the product eluted with dilute (1 : 50) aqueous ammonia, the eluates evaporated, and the residue purified by precipitation from methanol (5 ml) with ether (100 ml). The precipitate was collected by centrifugation, washed with ether, and dried under diminished pressure. Yield, 300 mg (83%) of the ammonium salt of compound *XXXVIa*, homogeneous on chromatography (S_1) and electrophoresis (E_1), and resistant towards the *P. brevicompactum* ribonuclease degradation.

(*RS*)-1-(3,4-Dihydroxybutyl)thymine 3′,4′-Cyclic Phosphate (*XXXVIb*)

The title compound (in the form of the ammonium salt) was obtained in 76% yield from 1·2 mmol of compound *XXXVb* analogously to the preparation of compound *XXXVIa*. The product *XXXVIb* was homogeneous on chromatography (S_1) and electrophoresis (E_1) and resistant to the degradation with the *P. brevicompactum* ribonuclease. When kept at 50°C in 50% aqueous acetic acid for 2 h, the product *XXXVIb* recovers compound *XXXVb*.

N^6,$O^{2'}$-Diacetyl-(*S*)-9-(2,3-dihydroxypropyl)adenine 3′-Phosphate (*XXVII*)

A mixture of compound *XXVI* (1·306 g; 4·52 mmol), pyridine (13 ml), and acetic anhydride (6·5 ml) was stirred at room temperature overnight, the solution evaporated, the residue coevaporated with three 50 ml portions of toluene, three 50 ml portions of 50% aqueous ethanol, and two 25 ml portions of ethanol, washed twice by decantation with ether, and dried under diminished pressure. Yield, 1·427 g (77%) of the amorphous pyridinium salt of compound *XXVII*, homogeneous on chromatography (S_2) and electrophoresis (E_1). Content (by spectrophotometry, after removal of the pyridine): 94% for the molecular weight of 410·3.

3′-O-Triphenylmethyl-(*S*)-9-(2,3-dihydroxypropyl)adenine (*XXVIII*)

A mixture of compound *IVe* (2·1 g; 10 mmol), triphenylmethyl chloride (4·0 g; 14·4 mmol), and pyridine (20 ml) was briefly heated to the boiling point and then stirred at room temperature for 3 days. Ethyl acetate (100 ml) was then added, the mixture washed with three 100 ml portions of 0·5M-H_2SO_4, saturated aqueous sodium hydrogen carbonate (100 ml), and water (100 ml), dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. Yield, 4·3 g (95·5%) of compound *XXVIII*, homogeneous on chromatography in the solvent system S_3. For $C_{27}H_{25}N_5O_2$ (451·5) calculated: 15·52% N; found: 15·98% N.

3′-O-Triphenylmethyl-(*S*)-9-(2,3-dihydroxypropyl)adenine-2′-phosphoryl-3′-(*S*)-9-(2,3-dihydroxypropyl)adenine (*XXIXa*)

A mixture of the pyridinium salt of compound *XXVII* (1·4 g; 3·42 mmol), compound *XXVIII* (3·2 g; 7·1 mmol), pyridine (10 ml), and N,N′-dicyclohexylcarbodiimide (5·2 g) was kept at room temperature for 5 days under exclusion of daylight and atmospheric moisture. Water (2 ml) was then added, the mixture stirred for 2 h, diluted with chloroform (200 ml), washed with two 50 ml portions of water, filtered through Celite, the filtrate evaporated, and the residue dissolved in 30% methanolic ammonia (100 ml). The mixture was allowed to stand at room temperature for 24 h, evaporated, and the residue in chloroform (50 ml) applied to a column of silica gel (50 g) packed in chloroform. The column was successively eluted with 1 l of chloroform, 1 l of chloroform–ethanol (9 : 1) to remove compound *XXVIII*, and finally with 1 l of methanol. The methanolic

eluate was evaporated and the residue chromatographed on two layers of loose silica gel in the solvent system S_5. Bands of the product *XXIXa* were eluted with methanol (500 ml), the eluates evaporated, the residue dried under diminished pressure, and then purified by precipitation from chloroform (20 ml) with light petroleum (200 ml). The precipitate was collected with suction, washed with light petroleum, and dried under diminished pressure to afford 944 mg (35% of compound *XXIXa*, homogeneous on chromatography in the solvent system S_4.

(*S*)-9-(2,3-Dihydroxypropyl)adenine-2′-phosphoryl-3′-(*S*)-9-(2,3-dihydroxypropyl)adenine (*XXIXb*)

A solution of compound *XXIXa* (181 mg; 0·25 mmol) in 80% aqueous acetic acid (10 ml) was refluxed for 30 min, cooled down, diluted with water (50 ml), and washed with two 25 ml portions of ether. The aqueous phase was evaporated under diminished pressure and the residue chromatographed on three sheets of paper Whatman No 3 MM in the solvent system S_1. Bands of the product were eluted with dilute (1 : 50) aqueous ammonia and the eluates freeze-dried. Yield, 0·18 mmol (72%) (by spectrophotometry) of compound *XXIXb*, homogeneous on chromatography (S_1) and electrophoresis (E_1). The *P. brevicompactum* ribonuclease degradation affords quantitatively a mixture of compounds *XXVI* and *IVe* in the ratio 1·00 : 0·94 (after chromatography in S_1). The UV spectrum (pH 2): λ_{max} 259 nm.

(*S*)-9-(2,3-Dihydroxypropyl)adenine-2′-phosphoryl-3′-(*S*)-9-(2,3-dihydroxypropyl)adenine-2′-phosphoryl-3′-(*S*)-9-(2,3-dihydroxypropyl)adenine (*XXX*)

A mixture of compound *XXIXa* (236 mg; 326 μmol), the pyridinium salt of compound *XXVII* (310 mg; 755 μmol), N,N′-dicyclohexylcarbodiimide (1·8 g), and pyridine (3 ml) was stirred at room temperature for 5 days, decomposed with water (1 ml), kept at room temperature for 1 h, treated with 30% methanolic ammonia (50 ml), and allowed to stand at room temperature overnight. The mixture was then evaporated under diminished pressure, the residue coevaporated with ethanol (20 ml), and the final residue refluxed in 80% aqueous acetic acid (20 ml) for 30 min. The solution was evaporated, the residue diluted with water (50 ml), washed with three 20 ml portions of ether, the aqueous phase evaporated, and the residue chromatographed on three sheets of paper Whatman No 3 MM in the solvent system S_1 for 3 days. Bands of the product were eluted with dilute (1 : 50) aqueous ammonia and the eluates (their content was determined by spectrophotometry) were freeze-dried. Yield, 2090 A_{260} at pH 2 (49 μmol *i.e.*15%) of compound *XXX*, homogeneous on chromatography (S_1) and electrophoresis (E_1). The *P. brevicompactum* ribonuclease degradation affords under standard conditions a mixture of compounds *XXVI* and *IVe* in the ratio 1·87 : 1·00. The UV spectrum (pH 2): λ_{max} 259 nm.

Adenyl-3′-yl-3′-(*S*)-9-(2,3-dihydroxypropyl)adenine (*XXXIV*)

A solution of the pyridinium salt of N^6,$O^{5'}$-diacetyl-2′-O-tetrahydropyranyladenosine 3′-phosphate[22] (*XXXIII*; 1 mmol) and compound *IVe* (418 mg; 2 mmol) in pyridine (25 ml) was evaporated at 35°C/0·1 Torr and the residue dried by coevaporation with five 20 ml portions of pyridine under analogous conditions. The final residue was dissolved in pyridine (10 ml), the solution treated with N,N′-dicyclohexylcarbodiimide (2·5 g), and the whole was stirred at room temperature for 5 days under exclusion of atmospheric moisture. Water (2 ml) was then added, the mixture stirred for 1 h, and treated with 30% methanolic ammonia (30 ml). The whole mixture was kept at room temperature overnight, evaporated, and the residue heated at 50°C in 50% aqueous

acetic acid (50 ml) for 30 min. The solution was evaporated under diminished pressure, the residue coevaporated with water (20 ml), and chromatographed on four sheets of paper Whatman No 3 MM in the solvent system S_1 for 2 days. Bands of the product were eluted with dilute (1 : 50) aqueous ammonia and the eluates (their content was determined spectrophotometrically) were freeze-dried. Yield, 7300 A_{260} (257 μmol *i.e.* 26%) of compound *XXXIV*, homogeneous on chromatography and electrophoresis. The degradation with the *P. brevicompactum* ribonuclease or ribonuclease T2 affords a mixture of 3'-AMP and compound *IVe* in ratios 1·00 : 0·98 and 1·00 : 1·05, resp. Compound *XXXIV* was resistant towards the snake venom phosphodiesterase under standard conditions. The UV spectrum (pH 2): λ_{max} 259 nm.

(*S*)-9-(2,3-Dihydroxypropyl)adenine-2'-phosphoryl-5'-adenosine (*XXXII*)

A mixture of adenosine 5'-phosphate (monohydrate of the free acid; 3 mmol), dimethylformamide (10 ml), triethyl orthoformate (5 ml), and 6M hydrogen chloride in dimethylformamide (0·5 ml) was stirred at room temperature overnight, treated with pyridine (10 ml), and evaporated. The residue was coevaporated with five 20 ml portions of pyridine at 40°C/0·1 Torr and the final residue was dissolved in pyridine (10 ml). This solution was stirred with compound *XXVIII* (1.2 g; 2·66 mmol) and N,N'-dicyclohexylcarbodiimide (4 g) for 5 days at room temperature under exclusion of atmospheric moisture. Water (2 ml) was then added, the mixture stirred at room temperature for 2 h, evaporated, the residue coevaporated with two 25 ml portions of toluene, and the final residue refluxed in 80% aqueous acetic acid (50 ml) for 30 min. The solution was diluted with water (100 ml), washed with three 25 ml portions of ether, the aqueous phase evaporated under diminished pressure, the residue coevaporated with two 20 ml portions of water, neutralised with aqueous ammonia, and applied to a 80 × 4 cm column of DEAE-cellulose (Cellex D, standard capacity, HCO_3^- form). The column was washed with water (2 l) and then eluted with the use of a linear gradient of triethylammonium hydrogen carbonate, pH 7·5, 2 l of water in the mixing chamber and 2 l of a 0·2M buffer solution in the reservoir (elution rate, 3 ml per min; the fractions were taken in 10 min intervals). The product was eluted in the 0·05—0·10M buffer fraction. The eluate was evaporated, the volatile buffer removed by coevaporation with three 20 ml portions of methanol, and the residue applied to a column of Dowex 50 X 8 (H^+) ion exchange resin (100 ml). The column was washed with water to the drop of conductivity and the UV absorption and then eluted with dilute (1 : 10) aqueous ammonia. The UV-absorbing fraction of the eluate was evaporated under diminished pressure and the residue chromatographed on four sheets of paper Whatman No 3 MM in the solvent system S_1 for 2 days. Bands of the product were eluted with dilute aqueous ammonia and the eluates were freeze-dried. Yield, 231 mg (16%) of the ammonium salt of compound *XXXII*, homogeneous on chromatography (S_1) and electrophoresis (E_1). The snake venom phosphodiesterase degradation of the product affords a mixture of 5'-AMP and compound *IVe* in the ratio 1·00 : 1·08. The *P. brevicompactum* ribonuclease degradation affords a mixture of compound *XXVI* and adenosine in the ratio 1·00 : 1·08. Compound *XXXII* is resistant towards ribonuclease T2.

Enzymatic Synthesis of Uridylyl-(3' → 3')-(*S*)-1-(2,3-dihydroxypropyl)uracil (Up-*IVa*) and Cytidylyl-(3' → 3')-(*S*)-1-(2,3-dihydroxypropyl)uracil (Cp-*IVa*)

A mixture of compound *IVa* (20 μmol) and the lithium salt of uridine 2',3'-cyclic phosphate or cytidine 2',3'-cyclic phosphate (20 μmol each) in 50% aqueous pyridine (200 μl) was incubated with 50 μg of pancreatic ribonuclease (Lachema, Czechoslovakia) at 0°C for 24 h and then chromatographed on a sheet of paper Whatman No 3 MM in the solvent system S_2. Bands of

the product were eluted with water, the eluates freeze-dried and the residue purified by electrophoresis in the buffer solution E_1. Bands of the product were eluted with water and the yield (18% of Up-*IVa* and 14% of Cp-*IVa*) was determined spectrophotometrically.

Enzymatic Synthesis of Trinucleoside Diphosphate Analogues

A mixture of guanosine 2',3'-cyclic phosphate (5 μmol) and the appropriate doublet (2 μmol) in 50 μl of a 0·1M Tris buffer solution (pH 7·0) was incubated with 2—3 e.u. of ribonuclease T1 (Sankyo) at 0°C for 24 h and then chromatographed on a sheet of paper Whatman No 3 MM analogously to Up-*IVa* and Cp-*IVa* to afford 3·5% of GpUp-*IVa*, 5% of GpCp-*IVa*, 4% of Gp-*XXIX*, 5% of Gp-*XXXIV*, and 7% of Gp-*XXXII*.

Assay of Aminoacyl-tRNA Binding to Ribosomes

The general procedure described elsewhere[11] was applied. The [^{14}C]-amino acids were obtained from the Institute for Research, Production, and Application of Radioisotopes, Prague, Czechoslovakia; specific activity (mCi per mmol): Lys 82, Val 146, Ala 71, Glu 80. The aminoacyl-tRNAs contained (pmoles of the amino acid per A_{260}): Val 35·2, Ala 33, Lys 29, Glu 35. The results are summarised in Table II.

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