PREPARATION OF 5′-O-PHOSPHONYLMETHYL ANALOGUES OF NUCLEOSIDE-5′-PHOSPHATES, 5′-DIPHOSPHATES AND 5′-TRIPHOSPHATES

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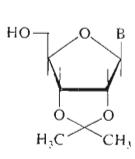
Reaction of a trialkyl phosphite (*VI*) or sodium salt of dialkyl phosphite (*V*) with bromomethyl acetate afforded dialkyl acetoxymethanephosphonates *VII* which were alcoholyzed to dialkyl hydroxymethanephosphonates *VIII*. Tosylation of the compounds *VIII* gave dialkyl *p*-toluenesulfonyloxymethanephosphonates *IX* which on reaction with 2′,3′-O-isopropylideneribonucleosides (*I*), 2′,3′-O-ethoxymethyleneribonucleosides (*XI*) or 3′-O-tetrahydropyranyl-2′-deoxyribonucleosides in the presence of sodium hydride, followed by acid hydrolysis, were converted into alkyl esters of 5′-O-phosphonylmethylnucleosides *XII*. Treatment of compounds *XII* with trimethylsilyl iodide led to 5′-O-phosphonylmethylnucleosides *II* and *XIV*. The ribo derivatives *II* were also obtained in low yields by reaction of compounds *I* with sodium chloromethanephosphonate in the presence of sodium hydride. Compound *II* reacted with morpholine in the presence of N,N′-dicyclohexylcarbodiimide to give morpholides *XV* which on treatment with phosphate afforded 5′-O-phosphorylphosphonylmethylribonucleosides *XVI*. Analogously, 5′-O-diphosphorylphosphonylmethylribonucleosides *XVII* were obtained from the derivatives *XV* by reaction with diphosphate. The compounds *II* are resistant towards *E. coli* alkaline phosphomonoesterase and snake venom and bull semen 5′-nucleotidase. *E. coli* alkaline phosphomonoesterase degrades the compounds *XVI* and *XVII* to compounds *II* and inorganic phosphate.

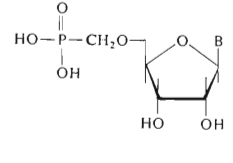
Nucleotide analogues whose phosphate residue would resist enzymatic hydrolysis are the object of continuous interest. Replacement of an ester-bonded phosphate moiety by other inorganic or organic phosphorus-containing acids (phosphites, alkanephosphonates or substituted alkanephosphonates) results in loss of one dissociable group. Some advance in this direction was achieved with esters of phosphothioic acid and with nucleoside-5′-triphosphates containing methanediphosphonic or iminodiphosphonic acid bonded to a natural 5′-nucleoside (for a review see ref.[1]). Nucleoside 5′-deoxy-5′-phosphonic acids, containing a phosphorus atom bonded directly to the carbon atom of the nucleoside sugar moiety, represent another alternative[2–4]. Although in these analogues the number of dissociable groups is the same as in the nucleotides, their molecular stereochemistry is markedly different and, moreover, their molecule lacks the 5′-hydroxyl oxygen whose electronegativity can play an important role in interactions with enzymes.

We studied therefore preparation of a new type of nucleoside analogues containing methanephosphonic acid bonded by an ether bridge to the sugar hydroxyl group of the nucleoside. These compounds – alkoxymethanephosphonic acids – contain a C—P bond which in all probability will be resistant towards phosphomonoester hydrolases. They have the same number of dissociable groups as the ester of phosphoric acid and have similar pK values; due to the insertion of an sp^3-hybridized carbon atom between the atoms of phosphorus and hydroxyl oxygen, these analogues are sufficiently conformationally flexible to adopt conformation similar to that of the natural nucleosides. This communication describes syntheses of 5′-O--phosphonylmethylnucleosides *II* and their P-phosphoryl and diphosphoryl derivatives, *i.e.* analogues of biologically important 5′-ribonucleotides, 5′-diphosphates and 5′-triphosphates. Some of the results have been already published in a preliminary form[5,6].

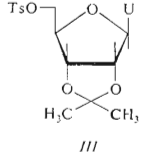
The first synthetic route was based on analogy of reaction of sodium alkoxides derived from 2′,3′-protected ribonucleosides *I* with sodium chloroacetate which afforded 5′-O-carboxymethyl ribonucleoside derivatives (see ref.[7] and references therein). Sodium salt, prepared *in situ* from chloromethanephosphonic acid with sodium hydride, on reaction with 2′,3′-O-isopropylideneribonucleoside *I* in the



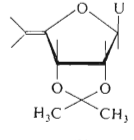
Ia, B = U
Ib, B = C
Ic, B = A



IIa, B = U
IIb, B = C
IIc, B = A



III



IV

In formulae *I*–*IV*; U = uracil-1-yl, C = cytosin-1-yl, A = adenin-9-yl, Ts = *p*-toluenesulfonyl residue.

SCHEME 1

presence of sodium hydride (its excess was necessary to bring about the alkoxide formation rather than the reaction at the heterocyclic nucleus), and subsequent acid hydrolysis, gave the compounds *II* of the expected properties. However, the yields of this reaction (Scheme 1) were unsatisfactory and improved neither by using an excess of chloromethanephosphonate (which leads to the mentioned side-reaction at the heterocyclic nucleus) nor by replacement of dimethyl sulfoxide by dimethylformamide, hexamethyl phosphotriamide or toluene as solvent.

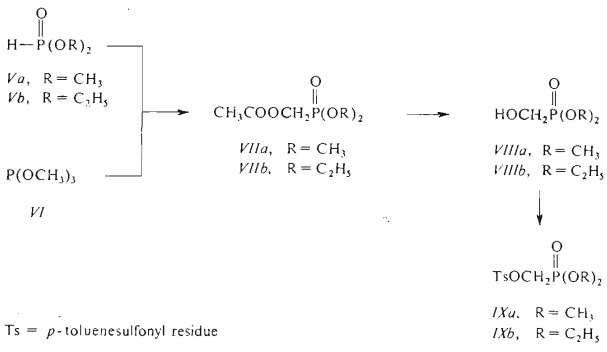
An alternative synthesis, consisting in the reaction of the activated nucleoside ester with alkoxide derived from an ester or salt of hydroxymethanephosphonic acid, was unsuccessful. Reaction of 5′-O-*p*-toluenesulfonyl-2′,3′-O-isopropylideneuridine (*III*) with diethyl hydroxymethanephosphonate (*VIIIb*) led to the known 4′,5-dehydro-2′,3′-O-isopropylideneuridine (*IV*) as the elimination product.

The third synthesis of the compounds *II* was based on the reversed starting combination, *i.e.* on reaction of nucleoside alkoxide with an activated dialkyl hydroxymethanephosphonate derivative. Since dialkyl hydroxymethanephosphonates *VIII* are prepared by a hardly controllable reaction of dialkyl phosphites *V* with paraformaldehyde in the presence of a base (see *e.g.* ref.[8]), we derived a new synthesis of these derivatives; alkali metal salts of dialkyl phosphites react with bromomethyl acetate, easily accessible by reaction of paraformaldehyde with acetyl bromide[9], to give dialkyl acetoxymethanephosphonates (*VII*) as the sole reaction products. Still more facile is the Arbuzov reaction of trialkyl phosphites with bromomethyl acetate, leading to the same products *VII*. Alcoholysis of the O-acetyl derivative *VII* afforded very pure dialkyl hydroxymethanephosphonate *VIII* in high yield.* Reaction with *p*-toluenesulfonyl chloride in the presence of a base gives smoothly O-*p*-toluenesulfonyl derivatives *IX* which can be crystallized or chromatographed on silica gel (Scheme 2).

Treatment of compound *Ia* with the *p*-toluenesulfonyl derivative *IXb* in the presence of 2 equivalents of sodium hydride in dimethylformamide resulted in the desired neutral diester of 5′-O-phosphonylmethyl-2′,3′-O-isopropylideneuridine *X* whose structure was unequivocally proved by its ^{1}H NMR spectrum. It was not necessary to isolate the intermediate *X*; on the contrary, its isolation was not advantageous. Optimal results were achieved when the *p*-toluenesulfonyl derivatives *IX* were allowed to react with the protected ribonucleosides *I* or *XI* in the presence of a larger excess of sodium hydride. Removal of the protecting group by acid hydrolysis afforded directly the 5′-O-phosphonylmethylribonucleoside monoester *XII* which was easily purified by chromatography on ion-exchange resins (Scheme 3). The overall reaction yield depended on the heterocyclic base; however in the presence of sufficient excess

* The high solubility of compounds *VIII* in water hindered their effective isolation in the previous preparations[8]; the conversion into acetyl derivatives *VII*, their purification by distillation and alcoholysis affords pure compounds *VIII*.

of sodium hydride no N-phosphonylmethyl derivatives of cytosine, adenine or guanine moieties were formed. Protection of these amino groups with N-benzoyl (*Id*) or N-dimethylaminomethylene (*XIb*) groups did not affect the yield of the compound *XII* (Table I).



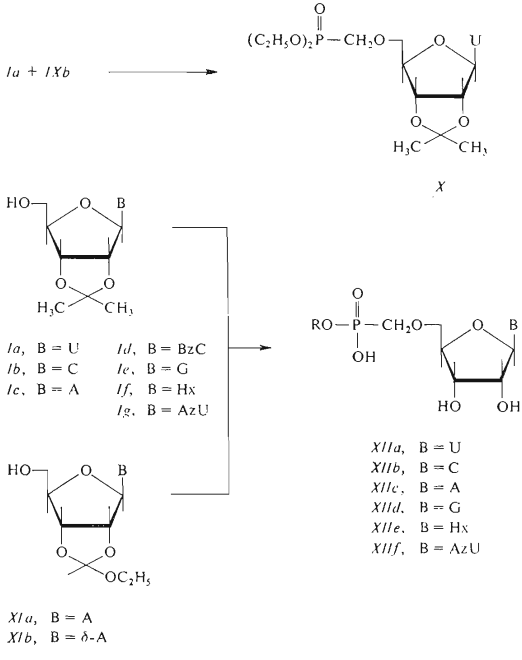
SCHEME 2

In analogy to alkyl esters of 5'-nucleotides, the alkyl esters of their methanephosphonyl analogues *XII* are also completely resistant toward alkaline as well as acid hydrolysis under conditions not destroying the nucleoside molecule. However, the methyl, as well as the ethyl, ester group can be quantitatively and without side-reactions removed by treatment with trimethylsilyl iodide in dimethylformamide at room temperature. This reaction transforms the compounds *XII* into completely pure 5'-O-phosphonylmethylribonucleosides *II* in high yields; their isolation consists only in de-salting. The reaction was carried out with natural pyrimidine and purine ribonucleosides (*IIa*–*IIe*) and proceeds smoothly also with the 6-azauracil derivative *IIf* which represents a nucleoside with an acidic heterocyclic moiety. It can be performed also with 2'-deoxyribonucleosides; since we could not exclude partial cleavage or migration in the reaction of the easily accessible 3'-O-acyl-2'-deoyribonucleosides with the compound *IX*, we chose as starting compounds the 3'-O--tetrahydropyranyl derivatives *XIII* which had been described already earlier[10]. Reaction of the compound *IXa* in the presence of sodium hydride afforded intermediates analogous to compounds *XII*; these were not characterized and were converted by reaction with trimethylsilyl iodide into 5'-O-phosphorylmethyl-2'-deoxyribonucleosides *XIV*. It is worth notice that the reaction of compounds *XIII* with the *p*-toluenesulfonyl derivative *IXa* was accompanied by a partial cleavage

of the nucleoside bond and formation of the corresponding heterocyclic base (Scheme 4).

Although the new synthesis of 5'-O-phosphonylmethylnucleosides by reaction of compound *IX* with protected nucleosides involves two steps, it is more advantageous then the preparation from chloromethanephosphonic acid. The *p*-toluenesulfonyl derivatives *IX* are well accessible and stable at room temperature.

5'-O-Phosphonylmethylribonucleosides *II* are the starting compounds for preparation of ribonucleoside 5'-diphosphate and 5'-triphosphate analogues (*XVI* and



In formulae *I*—*XII*: U = uracil-1-yl, C = cytosin-1-yl, A = adenin-9-yl, G = guanin-9-yl, Hx = hypoxanthin-9-yl, AzU = 6-azauracil-1-yl, BzC = N^4-benzoylcytosin-1-yl, δ-A = N^6-dimethylaminomethyleneadenin-9-yl residue.

SCHEME 3

XVII, respectively). For the reaction with phosphoric or diphosphoric acid, 5′-nucleotides can be activated by several described methods. For some of them we compared the yield and complexity of the arising reaction mixtures, using the HPLC technique. Of the methods chosen, *i.e.* activation with diphenylphosphoryl chloride[11], formation of an imidazolide[12] or a morpholide[13]; the most advantageous proved to be again the method, using reaction of morpholides with an inorganic phosphorus-containing acid; such intermediates were obtained by reaction of compounds *II* with morpholine in the presence of N,N′-dicyclohexylcarbodiimide and isolated as the N″-morpholino-N,N′-dicyclohexylguanidinium salts *XV*. Of the two variants of this method[13,14], 5′-O-phosphorylphosphonylribonucleosides *XVI* can be prepared in pyridine[13] whereas for preparation of the 5′-triphosphate analogues *XVII* dimethyl sulfoxide[14] was a better solvent, suppressing dismutation reactions. Despite

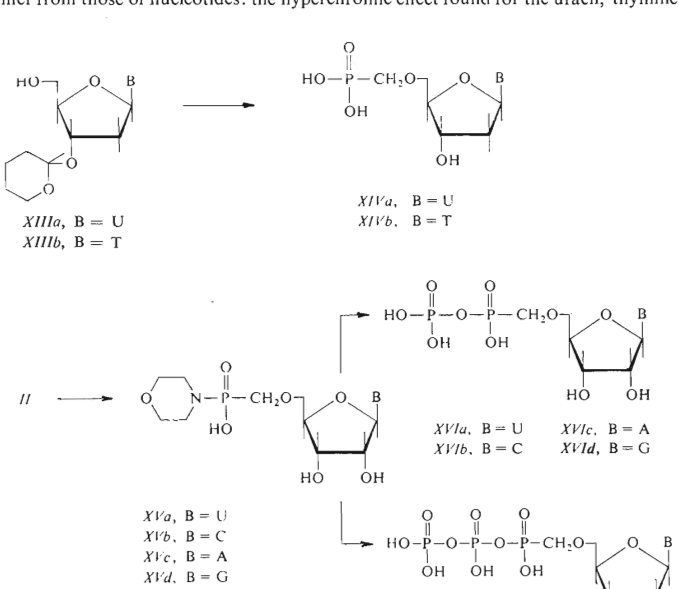
TABLE I
Methyl 5′-O-phosphonylmethylribonucleosides *XII*

Compound	Starting compound	Yield, %	R_F(S1) E_{Up}[a]	k[b]	Formula (M.W.)	N : P found (calc.)
XIIa[c]	*Ia*	60	0·45	—	$C_{12}H_{19}N_2O_9P$	2·53
	(10·0)		0·54		(366·3)	(2)
XIIb	*Ib*	41	0·25	1·92[d]	$C_{11}H_{18}N_3O_8P$	2·83
	(9·7)		0·57		(351·3)	(3)
	Id	43				
	(1·0)					
XIIc	*Ic*	27	—			
	XIa	39	0·43	1·04[e]	$C_{12}H_{18}N_5O_7P$	5·59
	(10·0)		0·51		(375·3)	(5)
	XIb	40	—			
	(2·0)					
XIId	*Ie*	47	0·27	1·72[f]	$C_{12}H_{18}N_5O_8P$	4·85
	(10·0)		0·32		(391·3)	(5)
XIIe	*If*	41	0·34	1·62[f]	$C_{12}H_{17}N_4O_8P$	
	(10·0)		0·83		(367·3)	(4)
XIIf	*Ig*	30	0·40	1·76[g]	$C_{10}H_{16}N_3O_9P$	
	(10·0)		0·96		(353·2)	(3)

[a] Electrophoretic mobility in E1 (referred to uridine 3′-phosphate); [b] retention (capacity) factor (HPLC) $k = (t_R - t_M)/t_M$ (t_R retention time, t_M hold-up time); [c] ethyl ester; [d] S6, [e] S8, [f] S7, [g] S4

warnings in the literature[14], chromatography on ion-exchange resin in strongly acidic medium was the method of choice for purification of compounds *XVI* and *XVII*: under these conditions the compounds did not decompose significantly.

Purity of the compounds *II*, *XVI* and *XVII*, described in this work, was checked by chromatography (paper chromatography and HPLC) and electrophoresis (the mobilities of compounds *II*, *XVI* and *XVII* correspond to those of their nucleotide partners, the alkyl esters *XII* and morpholides *XV* display mobilities, corresponding to one dissociation degree in a weakly alkaline medium). The UV spectra do not differ from those of nucleotides: the hyperchromic effect found for the uracil, thymine



In formulae *XIII*—*XVII*, U = uracil-1-yl, T = thymin-1-yl, C = cytosin-1-yl, A = adenin-9-yl, G = guanin-9-yl residue.

SCHEME 4

and 5-azauracil derivatives (*IIa*, *IIf*, *XIVa*,*b*) in alkaline media, as well as the position of the absorption maxima for the cytosine, adenine, hypoxanthine and guanine derivatives (*IIb*–*IIe*), represent a proof of their structure (or unchanged chromophore of the heterocyclic base).

The 5'-O-phosphonylmethylnucleosides *II* and *XIV* are stable in alkaline as well as acidic media. As expected, they are resistant toward three types of phosphomonoester hydrolases: *E. coli* non-specific alkaline phosphomonoesterase, snake venom (*Crotalus adamanteus*) 5'-nucleotidase and bull semen 5'-nucleotidase. However, *E. coli* alkaline phosphomonoesterase cleaves the P—O—P bonds in the 5'-diphosphate *XVI* and 5'-triphosphate *XVII* analogues, giving rise to 5'-O-phosphonylmethyl derivative *II* and inorganic phosphate.

The inhibition of 5'-nucleotidase by 5'-O-phosphonylmethyl derivatives of the type *II* has already been described in a preliminary communication[5,6]. Detailed studies of this and other biochemical aspects of this new type of nucleoside analogues will be published elsewhere.

EXPERIMENTAL

Unless stated otherwise, the solutions were taken down at 40°C/2 kPa and the compounds were dried at 10–15 Pa over phosphorus pentoxide. Paper chromatography was performed on a Whatman No 1 paper in 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2) (S1), paper electrophoresis on a Whatman No 3MM paper in 0·05M triethylammonium hydrogen carbonate, pH 7·5 (E1) at 20 V/cm (1 h). Chromatography on silica gel was carried out on Silufol UV 254 plates (Kavalier, Czechoslovakia) in chloroform (S2) and in chloroform–ethanol (9 : 1) (S3). Preparative chromatography on silica gel was performed on loose layers (50 × 18 × 0·3 cm) of indicator-containing silica gel (prepared in the Service Laboratories of this Institute). Chromatography on cellulose was executed on a column (80 × 4 cm) of microcrystalline cellulose (Macherey and Nagel) in the system S1 (elution rate 20 ml/h, 20 ml fractions). Chromatography on DMAB-Separon H-40 (10–15 μ; ref.[15]) was carried out on a 1·7 × 50 cm column in triethylammonium hydrogen carbonate, pH 7·5 (linear gradient, 2 × 2 l, end concentration: 0·5M for *XIVa*, 1M for *XIVb*), elution rate 4 ml/min, detection at 260 nm.

HPLC analyses were performed *A*) on a glass column (0·8 × 10 cm) of DMAB-Separon H-40 (10–15 μ; ref.[15]) in 0·1M H_3BO_3-NaOH, pH 8·65 (2 ml/min); *B*) on a glass column (3·3 × × 150 mm) filled with Separon SI C 18 (5 μ) (Laboratorní přístroje, Prague, Czechoslovakia) in 0·1M triethylammonium hydrogen carbonate, pH 7·5, containing the following amount of methanol (% vol): S4 0% methanol, S5 2·5% methanol, S6 5% methanol, S7 10% methanol, S8 20% methanol. Elution rate 0·4 ml/min at room temperaure. The UV spectra were taken in aqueous solutions on a Specord UV-VIS spectrometer (Carl Zeiss, Jena); for quantitative determination the tabulated values[16] of molar extinction coefficients at pH 2 were used. The ^{1}H NMR spectra were measured on a Varian 100 instrument in deuteriochloroform (chemical shifts in ppm, coupling constants in Hz).

Dimethylformamide and dimethyl sulfoxide were distilled from phosphorus pentoxide. 2',3'-O-Isopropylideneribonucleosides and 2',3'-O-ethoxymethyleneribonucleosides were synthesized according to ref.[17] and ref.[18], respectively. Chloromethanephosphonic acid was prepared according to ref.[19].

5′-O-Phosphonylmethyluridine (*IIa*)

Sodium hydride (0·24 g; 10 mmol) was added to a stirred solution of chloromethanephosphonic acid[19] (0·65 g; 5 mmol) in dimethyl sulfoxide (20 ml). After the reaction had subsided, the resulting solution was added to a mixture of *Ia* (1·42 g; 5 mmol) and sodium hydride (0·24 g; 10 mmol) in dimethyl sulfoxide (30 ml). After stirring in a stoppered flask at room temperature for 100 h, the mixture was poured into water (200 ml), acidified with hydrochloric acid to pH 3·5 and the product was adsorbed on charcoal. The sorbent was collected by centrifugation, washed with water (3 × 100 ml) and stirred with a 2·5% ammonia–methanol mixture (4 : 1; 200 ml). The suspension was filtered through Celite which was then washed with the above solvent mixture (100 ml). The filtrate was taken down and the residue was applied on a column of Amberlite IR 4B (acetate; 100–200 mesh; 200 ml). The column was washed with formic acid (linear gradient 0–2M; 2 l each) and then with 0·5M ammonium formate in 2M formic acid (elution rate 3 ml/min, fraction 10 min), the product being eluted only with the salt solution. The eluate was taken down, the residue was codistilled with water (3 × 100 ml), and dissolved in water (50 ml). The solution was applied on a column of Dowex 50X8 (H^+, 300 ml) and the product was eluted with water. The eluate was taken down, the residue was codistilled with water (5 × 50 ml), applied on a 50 ml column of Dowex 50X8 (Li^+) and again eluted with water. After evaporation, the residue was

TABLE II

5′-O-Phosphonylmethylnucleosides *II* (lithium salts)

Compound	Starting compound (mmol)	Yield, %	R_F(S1) E_{Up}[a]	k[b]	Formula (FW)	N : P found (calc.)
IIa	*XIIa* (5·97)	91	0·12 0·95	1·08[c]	$C_{10}H_{13}Li_2N_2O_9P$ (350·1)	1·95 (2)
IIb[d]	*XIIb* (3·70)	81	0·10 0·93	0·62[c]	$C_{10}H_{16}N_3O_8P$ (337·2)	2·93 (3)
IIc[d]	*XIIc* (7·50)	70	0·11 0·82	2·68[e]	$C_{11}H_{16}N_5O_7P$ (361·3)	4·94 (5)
IId	*XIId* (4·10)	86	0·06 0·72	1·96[c]	$C_{11}H_{14}Li_2N_5O_8P$ (389·1)	4·94 (5)
IIe	*XIIe* (1·16)	72	0·10 0·83	0·74[e]	$C_{11}H_{13}Li_2N_4O_8P$ (374·1)	4·15 (4)
XIVa	*XIIIa* (2·40)	8	0·18 0·91	0·66[e]	$C_{10}H_{13}Li_2N_2O_8P$ (334·1)	— (2)
XIVb	*XIIIb* (3·40)	8	0·12 0·92	1·72[e]	$C_{11}H_{15}Li_2N_2O_8P$ (348·1)	1·95 (2)

[a] Electrophoretic mobility in E1 (referred to uridine 3′-phosphate); [b] retention (capacity) factor (HPLC) $k = (t_R - t_M b)\, t_M$ (t_R retention time, t_M hold-up time); [c] S5; [d] free acid, [e] S6;.

codistilled with ethanol (2 × 25 ml) and the product was precipitated with ether (100 ml) from ethanol (10 ml). Filtration, washing with ether and drying afforded 180 mg (8·5%) of *IIa* as the lithium salt (content 90%), identical (S1. E1 and k) with the product obtained by another route (Table II).

5'-O-Phosphonylmethylcytidine (*IIb*)

The reaction was carried out with 10 mmol of *Ib* in the same manner as described for *IIa*. After standing at room temperature for 3 days, the mixture was diluted with water (100 ml), neutralized with acetic acid, taken down *in vacuo* (finally at 80°C/13 Pa) and the residue was refluxed with 80% acetic acid (150 ml) for 2 h. After evaporation, the residue was applied on a column of Dowex 50X8 (H^+; 100 ml). The column was eluted with water till the UV absorption dropped and then with 2·5% ammonia. The ammonia eluate was taken down and the residue was chromatographed on a column of Dowex 1X2 (acetate, 200 ml). After washing with water till the absorption dropped, the column was washed with acetic acid (linear gradient 0—1M, 2 l each; 3 ml/min, fractions 10 min). The product was eluted with 0·6—0·8M acetic acid and after evaporation and codistillation with water (4 × 20 ml) it was precipitated with ether (100 ml) from ethanol (20 ml). Yield 0·77 g (23·5%) of *IIb*, identical (E1, S1, k and UV spectra) with a product obtained by another route (Table II). For $C_{10}H_{16}N_3O_8P$ (337·3) calculated: 35·60% C, 4·78% H, 12·46% N, 9·20% P; found: 35·49% C, 5·36% H, 12·45% N, 9·03% P.

5'-O-Phosphonylmethyladenosine (*IIc*)

The reaction was carried out with 10 mmol of compound *Ic* as described for *IIa* and the reaction mixture was processed as described for *IIb*. Chromatography on Dowex 1X2 (acetate) and precipitation with ether from methanol afforded 100 mg (2·8% based on *Ic*) of the compound *IIc* (free acid) which was identical (E1, S1, k and UV spectrum) with a sample prepared by another route (Table II). The same yield of product was also obtained starting from *XIb* (10 mmol) instead of *Ic*.

Dimethyl Acetoxymethanephosphonate (*VIIa*)

a) *From dimethyl phosphite* (Va): A solution of methanol (4 ml) in benzene (50 ml) was added dropwise to a stirred suspension of sodium hydride (2·4 g; 0·1 mol) in benzene (50 ml). After stirring for 30 min the mixture was cooled with ice-cold water and a solution of dimethyl phosphite (*Va*) (10 ml; 0·1 mol) in benzene (20 ml) was added dropwise. Stirring was continued for 20 min and the resulting mixture was added dropwise to a stirred solution of acetoxymethyl bromide (16·5 g; 0·11 mol) in benzene (50 ml). After stirring for 20 h at room temperature, the mixture was filtered, the solid washed with benzene (50 ml), the filtrate taken down *in vacuo* and the residue distilled, affording 7 g (38%) of compound *VIIa*, b.p. 92—95°C/2·5 Pa. For $C_5H_{11}O_5P$ (182·1) calculated: 32·97% C, 6·09% H, 17·00% P; found: 32·90% C, 6·08% H, 16·57% P. Mass spectrum: M^+ 182, 167 ($M-CH_3$), 152 ($M-CH_2O$), 140 ($M-CH_2CO$), 43 (CH_3CO).

b) *From trimethyl phosphite* (*VI*): A mixture of compound *VI* (24·8 g; 0·2 mol) and acetoxymethyl bromide[9] (38·2 g 0·25 mol) was refluxed at 100°C for 8 h (complete reaction) and distilled *in vacuo*, affording 29·3 g (78%) of compound *VIIa*, b.p. 108°C/13 Pa. For $C_5H_{11}O_5P$ (182·1) calculated: 32·97% C, 6·09% H, 17·00% P; found: 33·10% C, 6·18% H, 16·82% P.

Diethyl Acetoxymethanephosphonate (*VIIb*)

Diethyl phosphite (*Vb*) (12·8 ml; 0·1 mol) was added under stirring and cooling to a suspension of sodium hydride (2·4 g; 0·1 mol) in benzene (50 ml). After stirring for 30 min this mixture was added dropwise to a solution of acetoxymethyl bromide (16·5 g; 0·108 mol) in benzene (50 ml). The mixture was stirred overnight, filtered, the solid was washed with benzene (50 ml) and the filtrate was taken down. The residue was distilled *in vacuo*, giving 13·3 g (63%) of compound *VIIb*, b.p. 102—105°C/2·5 Pa. For $C_7H_{15}O_5P$ (210·2) calculated: 39·99% C, 7·19% H, 14·76% P; found: 38·92% C, 6·96% H, 14·76% P. Mass spectrum: M^+ 210, 168 (M—CH_2CO), 167 (M—CH_3.CO), 165 (M—OC_2H_5), 138 (P(OH)$(OC_2H_5)_2$), 43 (CH_3CO).

Dimethyl Hydroxymethanephosphonate (*VIIIa*)

A solution of sodium methoxide (1M) was added to a solution of compound *VIIa* (18·2 g; 0·1 mol) in methanol (50 ml) to alkaline reaction (moist indicator paper). After standing overnight in a stoppered flask the mixture was neutralized with Dowex 50X8 (H^+; washed with methanol). The ion exchange resin was filtered off and the filtrate was taken down *in vacuo*. Distillation of the residue gave 11·9 g (85%) of compound *VIIIa*, b.p. 96°C/6 Pa. For $C_3H_9O_4P$ (140·1) calculated: 25·72% C, 6·48% H, 22·11% P; found: 24·26% C, 6·34% H, 22·42% P. Mass spectrum: M^+ 140, 110 (M—CH_2O), 109 (M—CH_3O), 95, 80 (M—2 CH_2O), 79 (M—CH_3O—CH_2O).

Diethyl Hydroxymethanephosphonate (*VIIIb*)

A solution of compound *VIIb* (21 g; 0·1 mol) in ethanol (50 ml) was adjusted with 1M sodium ethoxide to an alkaline reaction (moist pH-paper) and worked up as described for compound *VIIIa*. Yield 15·1 g (90%) of compound *VIIIb*, b.p. 108—109°C/6 Pa. For $C_5H_{13}O_4P$ (168·2) calculated: 35·70% C, 7·79% H, 18·45% P; found: 36·18% C, 7·52% H, 18·22% P.

Dimethyl *p*-Toluenesulfonyloxymethanephosphonate (*IXa*)

Triethylamine (31·5 ml; 0·225 mol) was added dropwise to a solution of compound *VIIIa* (30·8 g; 0·22 mol) in ether (300 ml). The mixture was cooled to —10°C and a solution of *p*-toluenesulfonyl chloride (42·9 g; 0·225 mol) in ether (300 ml) was added dropwise at —10°C with stirring which was continued (with exclusion of moisture) for 3 h at 0°C and overnight at room temperature. The mixture was filtered, the solid washed with ether (50 ml) and the filtrate taken down *in vacuo*. The mixture was stirred with light petroleum (200 ml) at 0°C for 2 h, the separated crystals were collected on filter, washed with light petroleum and dried *in vacuo*, yielding 38·8 g (60%) of *IXa*, m.p. 55—56°C (light petroleum). For $C_{10}H_{15}O_6PS$ (294·3) calculated: 40·81% C, 5·14% H, 10·55% P, 10·89% S; found: 41·06% C, 4·99% H, 11·24% P, 10·97% S. Mass spectrum: M^+ 294, 263 (M—OCH_3), 230 (M—SO_2), 200 (M—SO_2—CH_2O), 91 (BP)($C_6H_5CH_2$). IR spectrum ($CHCl_3$): (ν, cm^{-1}): P—O 1 260; P—O-Alkyl 1 042, 815; (SO_2) sym 1 190, 1 177; (SO_2) assym 1 374; ring 1 598, 1 494, 1 449. ^1H NMR spectrum ($CDCl_3$): 2·46 (s, 3 H) CH_3-phenyl; 3·69 + 3·87 (2 s, 2 × 3 H) P—OCH_3; 4·23 (d, 2 H, $J_{P,H}$ = 9·85) P—CH_2; 7·35 (d, 2 H, J = 8·0) + 7·80 (d, 2 H) arom.

Diethyl *p*-Toluenesulfonyloxymethanephosphonate (*IXb*)

The reaction was performed with compound *VIIIb* (37 g; 0·22 mol) in the same manner as described for compound *IXa*. After dissolution of the reaction mixture in ether, filtration and evaporation, the residue was chromatographed on a column (200 g) of silica gel (according

to Pitra; 30—40 μ) in chloroform. The product fractions (R_F 0·15; S2) were combined, taken down and dried *in vacuo*; yield 56·7 (80%) of a yellowish oil *IXb*. For $C_{12}H_{19}O_6PS$ (322·4) calculated: 44·70% C, 5·94% H, 9·63% P, 9·94% S; found: 45·08% C, 5·84% H, 9·83% P, 9·64% S.

Reaction of *III* with Sodium Salt of *VIIIa*

Sodium hydride (0·24 g; 10 mmol) was added to a solution of compound *VIIIa* (1·4 g; 10 mmol) in dimethylformamide (20 ml). After stirring for 10 min under exclusion of moisture, 5'-O-*p*-toluenesulfonyl-2',3'-O-isopropylideneuridine^{20} (*III*) (0·81 g; 1·85 mmol) in dimethylformamide (10 ml) was added. The mixture was stirred overnight, filtered, the solid was washed with dimethylformamide (10 ml) and the filtrate was taken down at 40°C/13 Pa. The residue was mixed with chloroform (50 ml), filtered through Celite, the filtrate was taken down and the residue was chromatographed on 1 plate of silica gel in S3. The product (R_F 0·20, S3) was eluted with methanol and after evaporation of the solvent precipitated with ether from ethanol; yield 240 mg (48·7%) of the amorphous product *IV* with positive reaction with potassium permanganate. For $C_{12}H_{14}N_2O_5$ (266·2) calculated: 54·13% C, 5·30% H, 10·52% N; found: 54·36% C, 5·30% H, 10·08% N.

Reaction of Compound *Ia* with *p*-Toluenesulfonyl Derivative *IXb*

Sodium hydride (0·24 g; 10 mmol) was added to a solution of compound *Ia* (1·42 g; 5 mmol) in dimethyl sulfoxide (20 ml) and after stirring for 15 min a solution of compound *IXb* (1·62 g; 5 mmol) in dimethyl sulfoxide (2 ml) was added. The mixture was stirred for 3 days in a stoppered flask, mixed with 0·3 ml (5 mmol) of acetic acid and taken down at 60°C/13 Pa. The residue was codistilled with dimethylformamide (3 × 10 ml) under the same conditions, the residue was extracted with hot chloroform (3 × 50 ml), the extract was filtered through Celite and taken down. The residue was chromatographed on two layers of silica gel in the system S3. The bands of the starting compound *Ia* (R_F 0·35; S3) and of the product (R_F 0·56; S3) were eluted (à 200 ml) with methanol. The eluates after evaporation and drying *in vacuo* afforded 0·70 g (32%) of compound *X* as an amorphous, chromatographically homogeneous foam. For $C_{17}H_{27}N_2O_9P$ (434·5) calculated: 46·99% C, 6·26% H, 6·45% N, 7·14% P; found: 47·35% C, 6·81% H, 6·22% N, 6·81% P. ^{1}H NMR spectrum ($CDCl_3$): 1·35 + 1·58 (S s, 2 × 3 H), $(CH_3)_2C$; 1·34 (t, J = 7·0) + + 4·20 (q) OC_2H_5; 3·83 (2 dd, 2 H, $J_{5',4'}$ = 2·0, $J_{5'',4'}$ = 3·0, $J_{5',5''}$ = 11·0) 2 $H_{5'}$; 4·39 (d, 2 H, J = 6·0) P—CH_2; 4·29 (m, 1 H) $H_{4'}$; 4·83 (dd, 1 H, $J_{2',1'}$ = 2·9, $J_{2',3'}$ = 6·3) H_2, 4·91 (dd, 1 H) $H_{3'}$; 5·76 (d, 1 H) H_5; 5·83 (d, 1 H, $J_{1',2'}$ = 2·8) $H_{1'}$; 7·68 (d, 1 H, $J_{5,6}$ = 8·1) H_6. ^{31}P-Decoupling: P—CH_2O— d 4·39, J_{P-CH_2} = 12·7; dec.: s 4·39; P—OCH_2-pent 4·17, J_{CH_2O-P} = = 7·0, $J_{CH_3CH_2}$ = 7·0; dec.: q 4·17, $J_{CH_3CH_2}$ = 7·0.

Alkyl Esters of 5'-O-Phosphonylmethylribonucleosides *XII*

Sodium hydride (30 mmol) was added to a suspension of compound *I* or *XI* (10 mmol) in dimethylformamide (100 ml). After stirring at room temperature for 30 min with exclusion of moisture (calcium chloride tube), compound *IX* (10 mmol) was added. The mixture was stirred in a stoppered flask for 60 h at room temperature, mixed with acetic acid (1·8 ml; 30 mmol) and taken down at 40°C/13 Pa. The residue was dissolved in 1M-H_2SO_4 (100 ml), the solution was set aside at 40°C for 15 h and neutralized with barium hydroxide to pH 7·0. The suspension was centrifuged, the supernatant was filtered through Celite, evaporated and the residue chromatographed on a column (30 × 3 cm) of Dowex 1X2 (acetate; 200—400 mesh). After removal of the neutral UV-absorbing materials by washing the column with water, the product was eluted with

linear gradient of 2 l of water and 2 l of formic acid of the following concentrations: 1M (*XIIa,d,e*), 2M (*XIIf*), 0·5M (*XIIc*), 0·1M (*XIIb*). Fractions, containing the product *XII*, were taken down, the residue was codistilled with water (3 × 50 ml) and ethanol (2 × 50 ml) and the product was precipitated with ether from methanolic solution. The yields and properties of thus-prepared compounds *XII* are given in Table I.

In the case of compound *Id* the mixture after evaporation of dimethylformamide was dissolved in methanol (100 ml), made alkaline with 1M sodium methoxide solution (moist pH-paper) and set aside for 6 h at 40°C. After neutralization with dry Dowex 50X8 (H^+), the mixture was made alkaline with triethylamine, filtered and the filtrate was taken down *in vacuo*. The residue was dissolved in water (100 ml), extracted with ether (2 × 20 ml), the aqueous phase was taken down *in vacuo* and the residue was worked up with 1M-H_2SO_4 (*vide supra*).

Preparation of 5′-O-Phosphonylmethylribonucleosides (*II*) from Compounds *XII*

Trimethylsilyl iodide (50 mmol) was added dropwise to a stirred suspension of compound *XII* (5 mmol) in dimethylformamide (50 ml) and the homogeneous solution was set aside in the dark at room temperature overnight. After addition of 0·2M triethylammonium hydrogen carbonate, pH 7·5, (300 ml) the mixture was stirred at room temperature for 2 h and extracted with chloroform (5 × 100 ml). The aqueous phase was taken down *in vacuo*, the residue was codistilled with ethanol (3 × 50 ml), applied on a column (100 ml) of Dowex 50X8 (Li^+) and the UV-absorbing material was eluted with water. The eluate was taken down, the residue codistilled with ethanol (2 × 50 ml) and stirred with an ethanol–acetone (1 : 1) mixture (100 ml) for 1 h. After centrifugation, the solid was washed with the same mixture (3 × 100 ml), finally with ether, and dried *in vacuo*. The yields and properties of thus-obtained lithium salts of compounds *II* are listed in Table II.

The lithium salts of *IIb* and *IIc* were converted into the free acids on a column of Dowex 1X2 (acetate; 50 ml). After washing the column with water, the acid was eluted with 0·5M formic acid. The eluate was taken down, the residue codistilled with water (4 × 50 ml) and ethanol (2 × 25 ml) and precipitated from methanol with ether. The product was collected on filter, washed with ether and dried *in vacuo*. The yields and properties are given in Table II.

5′-O-Phosphonylmethyl-2′-deoxyuridine (*XIVa*) and
5′-O-Phosphonylmethyl-2′-deoxythymidine (*XIVb*)

The reaction was performed according to the general method of preparation of compounds *XII*, starting from 2·4 mmol of compound *XIIIa* or 3·4 mmol of compound *XIIIb* (ref.[10]). After neutralization with barium hydroxide filtration and evaporation the residue was dissolved in water (100 ml) acidified with sulfuric acid to pH 3·5 and treated with Norit till the UV-absorption at 260 nm disappeared. After centrifugation the solid was washed with water (3 × × 100 ml) and the product was eluted with 20% aqueous pyridine (2 × 100 ml). The eluate was made alkaline with ammonia, taken down, the residue was codistilled with 2% ammonia and the product *XII* was isolated by chromatography on a cellulose column. The residue after evaporation was dried, dissolved in dimethylformamide (25 ml) and stirred with trimethylsilyl iodide (25 mmol). The mixture was then worked up as described in the general preparation of compounds *II*. The crude compounds *XIVa,b* were purified by chromatography on DMAB-Sephadex H-40 (*cf.* above), the eluates were taken down *in vacuo* and the residue was codistilled with water (2 × 25 ml). The product was converted into the lithium salt on a column of Dowex

50X8 (Li^+; 20 ml). The aqueous UV-absorbing eluate was taken down, the residue was co-distilled with ethanol (2 × 25 ml) and the product was precipitated with ether from methanol. Yields and properties of the lithium salts of compounds *XIVa,b* are given in Table II.

Morpholidates of 5'-O-Phosphonylmethylribonucleosides *XV*

Morpholine salt of compound *II* was prepared by neutralization of a solution of the free acid *II* with morpholine or from lithium salt of *II* on a column of Dowex 50X8 (pyridinium form) by elution with 20% pyridine, treatment of the eluate with excess morpholine and evaporation *in vacuo*.

A solution of N,N'-dicyclohexylcarbodiimide (1·8 g; 8·8 mmol) in tert-butyl alcohol (50 ml) was added dropwise during 3·5 h to a stirred and refluxing solution of morpholine salt of *II* (2 mmol) and morpholine (0·8 ml; 9 mmol) in a mixture of tert-butyl alcohol and water (60 ml; 1 : 1). After stirring under reflux for 5 h, morpholine (0·8 ml) and N,N'-dicyclohexylcarbodiimide (1·8 g) were added and the stirring under reflux was continued for additional 5 h. The mixture was evaporated *in vacuo* and the residue was taken up in water (50 ml). After filtration and washing the solid with water (50 ml), the filtrate was extracted with ether (5 × 50 ml) and the aqueous phase was taken down. The residue was codistilled with ethanol (2 × 50 ml) and precipitated with ether from methanol. The thus-obtained compounds *XV* (N''-morpholino N,N'-dicyclohexylguanidinium salts, content >95%) were chromatographically and electrophoretically homogeneous; their yields and properties are given in Table III.

5'-O-Phosphorylphosphonylmethylribonucleosides *XVI*

Compound *XV* (1 mmol) was added to a 0·35M solution of tri-n-butylammonium dihydrogen phosphate (10 ml) in pyridine. The mixture was taken down at 30°C/13 Pa, the residue was co-distilled under the same conditions with pyridine (3 × 20 ml) and the remaining oil was dissolved in pyridine (10 ml). After standing at room temperature for 60 h in a stoppered flask, the mixture was taken down, the residue was mixed with water (100 ml), acidified with hydrochloric acid to pH 3·5 and mixed with Norit (5 g). After stirring for 5 h the mixture was centrifuged and the solid was washed with water (3 × 100 ml). The product was desorbed with 50%

TABLE III

Morpholidates of 5'-O-Phosphonylmethylribonucleosides *XV*

Compound	Starting compound (mmol)	Yield, %	R_F	E_{Up} [a]
XVa	*IIa* (3·76)	98	0·41	0·54
XVb	*IIb* (2·50)	100	0·40	0·43
XVc	*IIc* (0·69)	95	0·37	0·45
XVd	*IId* (2·00)	95	0·24	0·43

[a] Electrophoretic mobility in E1 (referred to uridine 3'-phosphate).

aqueous pyridine (2 × 50 ml). After adjusting with ammonia to pH 8·5—9, the supernatant was taken down *in vacuo*, the residue was codistilled with water (2 × 25 ml) and chromatographed on a 1·2 × 20 cm column of Dowex 1X2 (Cl^-). The column was eluted first with 0·01M-HCl and then subjected to gradient elution with 0·01M-HCl (2 l) and 0·01M-HCl containing lithium chloride (2 l) in concentrations 0·1M for *XVIb,c* and 0·5M for *XVIa,d*. The combined product-containing fractions were neutralized with LiOH, evaporated and the residue was extracted with acetone–ethanol (1 : 1; 100 ml). After centrifugation the solid was washed with the same mixture (2 × 100 ml), ether (50 ml) and dried. Yields and properties of the thus-prepared compounds *XVI* are given in Table IV.

5′-O-Diphosphorylphosphonylmethylribonucleosides *XVII*

Method A: Compound *XV* (1 mmol) was added to 0·35M bis-tributylammonium salt of diphosphoric acid in pyridine (10 ml). The mixture was taken down at 30°C/13 Pa and the residue was codistilled with pyridine (3 × 10 ml) under the same conditions. A solution of the residue in pyridine (10 ml) was set aside at room temperature for 24 h in a stoppered flask and taken down.

TABLE IV

5′-O-Phosphorylphosphonylmethyl- (*XVI*) and 5′-O-diphosphorylphosphonylmethylribonucleosides (*XVII*)

Compound	Starting compound	Method Yield, %	k^a	Formula (MW)
XVIa	*XVa* (1·00)	*A* 46	1·85[b]	$C_{10}H_{13}Li_3N_2O_{12}P_2$ (436·0)
XVIb	*XVb* (1·50)	*A* 26	1·00[b]	$C_{10}H_{14}Li_3N_3O_{11}P_2$ (435·0)
XVIc	— (0·69)[d]	*A* 50	3·49[c]	$C_{11}H_{14}Li_3N_5O_{10}P_2$ (459·0)
XVId	*XVd* (0·78)	*A* 56	3·00[b]	$C_{11}H_{14}Li_3N_5O_{11}P_2$ (475·0)
XVIIa	*XVa* (1·00)	*B* 46	3·48[b]	$C_{10}H_{14}Li_3N_2O_{15}P_3$ (516·0)
XVIIb	*XVb* (1·38)	*B* 19	1·62[b]	$C_{10}H_{15}Li_3N_3O_{14}P_3$ (515·0)
XVIIc	*XVc* (0·69)	*A* 42	5·11[c]	$C_{11}H_{15}Li_3N_5O_{13}P_3$ (539·0)
XVIId	*XVd* (1·00)	*A* 29	4·94[b]	$C_{11}H_{15}Li_3N_5O_{14}P_3$ (555·0)

[a] Retention (capacity) factor (HPLC) $k = (t_R - t_M)/t_M$ (t_R retention time, t_M hold-up time); [b] S5; [c] S6; [d] by-product from the synthesis of *XVIIc*.

The mixture was adsorbed on Norit, desorbed with 50% pyridine and then purified by chromatography on Dowex 1C2 (Cl^-) as described for compounds *XVI*. After washing with 0·01M-HCl, the product was obtained by linear gradient elution with 0·01M-HCl (2 l) and 0·01M-HCl containing lithium chloride (2 l) (0·2M for *XVIIc*, 0·5M for *XIIId*). The lithium salts were isolated as described for compounds *XVI*.

Method B: Compound *XV* (1 mmol) was added to 0·35M bis-tributylammonium salt of diphosphoric acid in dimethyl sulfoxide (10 ml). The mixture was stirred until it became homogeneous, set aside in a stoppered flask for 3 days, diluted with water (100 ml) and applied on a column (2·5 × 50 cm) of DEAE cellulose (Cellex D, std. capacity) in HCO_3^- cycle. The column was washed with water until the UV absorption of the eluate disappeared and then which 0·4M triethylammonium hydrogen carbonate, pH 7·5. The UV-absorbing eluate was taken down, the residue was codistilled with water and worked up with Norit and chromatography on Dowex 1X2 (Cl^-) as described for compounds *XVI*. Lithium chloride concentrations in 0·01M-HCl: for *XVIIa* 0·5M, for *XVIIb* 0·2M. The lithium salts of *XVIIa,b* were isolated in the same manner as described for compounds *XVI* (Table IV).

Stability toward Dephosphorylating Enzymes

a) Snake venom 5'-nucleotidase (*Crotalus adamanteus*): The reaction mixture consisted of 100 µl of $5·9 \times 10^{-4}$M substrate (*IIa*, *IIb*, *IIc*) in 0·1M TRIS-HCl, pH 9·0, and 10 µl of the enzyme solution (1 mg/ml, Sigma, Switzerland);

b) Bull semen 5'-nucleotidase: The reaction mixture consisted of 100 µl of $5·9 \times 10^{-4}$M substrate (*IIa*, *IIb*, *IIc*) in 0·1M TRIS-HCl, pH 8·0, and 10 µl of the enzyme solution (1 mg/ml);

c) *E. coli* alkaline phosphatase: The reaction mixture consisted of 100 µl of $5·9 . 10^{-4}$M substrate (*IIa*, *XVIa*, *XVIIa*) in 0·1M TRIS-HCl, pH 9·0, and 10 µl of the enzyme solution (1 mg/ml, Sigma, Switzerland).

For all the three enzymes the mixtures were incubated at 37°C, samples were taken after 20 min and 15 h and were analyzed by HPLC, method *A*(*B*). Under the described conditions compounds *II* were not cleaved even after 15 h with any of the three enzymes used. Compounds *XVI* and *XVII* afforded 100% of compound *II* after 20 minutes' treatment with alkaline phosphatase. Under comparable conditions both 5'-nucleotidases dephosphorylated 30% of UMP after 20 min (after 15 h the reaction was quantitative); with alkaline phosphatase UMP reacted quantitatively after 20 min.

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