SYNTHESIS OF 5'-O-PHOSPHONOMETHYL DERIVATIVES OF PYRIMIDINE 2'-DEOXYNUCLEOSIDES

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Reaction of sodium salt of 3-N,3'-O-bis(benzyloxymethyl)-2'-deoxyuridine (*X*) and 3-N,3'-O-bis-(benzyloxymethyl)-2'-deoxythymidine (*XI*) with diethyl *p*-toluenesulfonyloxymethanephosphonate in dimethylformamide afforded diesters of the respective 5'-O-phosphonomethyl* derivatives *XII* and *XVII*. Diethyl esters of 5'-O-phosphonomethyl-2'-deoxynucleosides *XV* and *XIX*, obtained after hydrogenolytic removal of the benzyloxymethyl groups, were converted into free 2'-deoxy-5'-O-phosphonomethyluridine (*XVI*) and a mixture of anomeric 1-(2-deoxy-5-O--phosphonomethyl-β-D-*erythro*-pentofuranosyl)thymines (*XXIIIa*, *XXIIIb*), respectively. Analogously, 2'-deoxy-5'-O-phosphonomethylcytidine (*XXXIV*) was prepared from 4-N-benzoyl-2'--deoxy-3'-O-(tetrahydro-2*H*-pyran-2-yl) cytidine (*XXX*) via diethyl ester of 2'-deoxy-5'-O--phosphonomethylcytidine (*XXXIII*). This compound reacted with bromotrimethylsilane to give compound *XXXIV* without anomerization and nucleoside bond cleavage. Condensation of the protected nucleosides *X* and *XI* with dibenzyl *p*-toluenesulfonyloxymethanephosphonate afforded dibenzyl esters of the corresponding 5'-O-phosphonomethyl derivatives *XIII* and *XVIII*. The free 5'-O-phosphonomethyl derivatives *XVI* and *XXIIIa* were obtained from *XIII* and *XVIII* by catalytic hydrogenation.

In the recent years, the search for novel types of antimetabolites of nucleic acids has also induced phosphonomethyl derivatives of nucleosides and nucleoside analogs, i.e. compounds with a methylene group between the phosphorus atom and an oxygen atom of the sugar ring[1-7]. Of particular importance are phosphonomethyl derivatives of acyclic nucleoside analogs which often exhibit high antiviral activity and which have been extensively studied (see e.g. refs[8-16] and references therein).

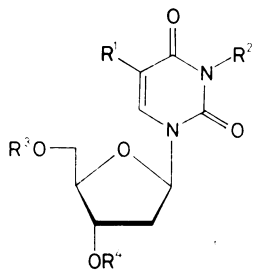
The most common preparation of 5'-O-phosphonomethyl nucleoside derivatives consists in the condensation of a dialkyl *p*-toluenesulfonyloxymethanephosphonate[2,17] with sodium salt of the nucleoside, followed by removal of the ester groups by treatment with bromotrimethylsilane and hydrolysis[1,2,4,7]. This general procedure, applied to nucleosides of the *ribo* series requires no protection of the base, and the phosphonates obtained in the first step as the diethyl (or dimethyl) esters can be converted without any problems into the free acids by the above-mentioned proce-

* Many authors use the name phosphonylmethyl.

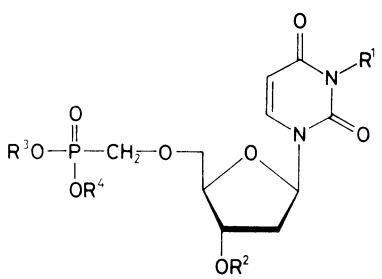
dure. However, attempts to apply this procedure to 5′-O-phosphonomethyl derivatives of 2′-deoxynucleosides resulted in only minute yields of products[1,2].

It appeared that for pyrimidine 2′-deoxynucleosides the increased risk of anomerization and cleavage of the nucleoside bond during the deblocking with bromotrimethylsilane represents a serious problem. For this reason, it was necessary to replace the usual bromotrimethylsilane treatment of esters by another method and to find suitable protection of the nucleoside, including its basic component.

For the protection of 2′-deoxyuridine and 2′-deoxythymidine we employed the benzyloxymethyl group* which is removable also by hydrogenolysis. The 5′-O-acyl derivatives *IV* and *V* reacted with benzyl chloromethyl ether in acetone in the presence of potassium carbonate under selective formation of the 3-N-benzyloxymethyl derivatives *VI* and *VII*. The benzyloxymethylation in position 3′ was effected by treatment with benzyl chloromethyl ether in ethyldiisopropylamine. After removal of the acyl groups from position 5′ by reaction with sodium methoxide in methanol we obtained 3-N,3′-O-bis(benzyloxymethyl)-2′-deoxyuridine *X* and 3-N,3′-O-bis-(benzyloxymethyl)-2′-deoxythymidine *XI* as starting compounds for the condensation with esters of *p*-toluenesulfonyloxymethanephosphonic acid. The direct benzyloxymethylation of the 5′-O-acyl derivatives in positions 3 and 3′ in ethyldiisopropylamine proceeded slowly and the yields were lower than in the above-described two-step procedure.



IV, $R^1 = R^2 = R^4 = H$; $R^3 = Bz$
V, $R^1 = CH_3$; $R^2 = R^4 = H$; $R^3 = Piv$
VI, $R^1 = R^4 = H$; $R^2 = BOM$; $R^3 = Bz$
VII, $R^1 = CH_3$; $R^2 = BOM$; $R^3 = Piv$; $R^4 = H$
VIII, $R^1 = H$; $R^2 = R^4 = BOM$; $R^3 = Bz$
IX, $R^1 = CH_3$; $R^2 = R^4 = BOM$; $R^3 = Piv$
X, $R^1 = R^3 = H$; $R^2 = R^4 = BOM$
XI, $R^1 = CH_3$; $R^2 = R^4 = BOM$; $R^3 = H$

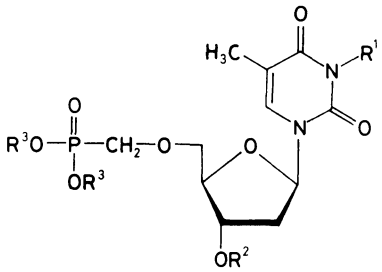


XII, $R^1 = R^2 = BOM$; $R^3 = R^4 = Et$
XIII, $R^1 = R^2 = BOM$; $R^3 = R^4 = Bn$
XIV, $R^1 = R^2 = BOM$; $R^3 = H$; $R^4 = Bn$
XV, $R^1 = R^2 = H$; $R^3 = R^4 = Et$
XVI, $R^1 = R^2 = R^3 = R^4 = H$

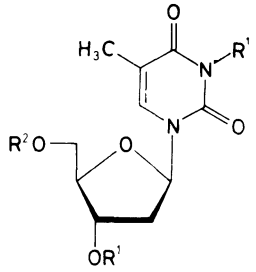
* The following abbreviations are used throughout this paper: Bn benzyl, BOM benzyloxymethyl, Bz benzoyl, Piv pivaloyl, THP tetrahydro-2*H*-pyran-2-yl, TBDPS tert-butyldiphenylsilyl, and Ts *p*-toluenesulfonyl.

Diethyl ester of 3-N,3'-O-bis(benzyloxymethyl)-2'-deoxy-5'-O-phosphonomethyluridine (*XII*) was prepared by reaction of sodium salt of 3-N,3'-O-bis(benzyloxymethyl)-2'-deoxyuridine (*X*) with diethyl *p*-toluenesulfonyloxymethanephosphonate (*IIIa*) in dimethylformamide. Removal of the benzyloxymethyl groups by catalytic hydrogenation afforded diethyl ester of 2'-deoxy-5'-O-phosphonomethyluridine (*XV*) which on treatment with bromotrimethylsilane in acetonitrile gave the desired 2'-deoxy-5'-O-phosphonomethyluridine (*XVI*) as pure β-anomer. Under these conditions, however, the reaction was accompanied by the concurrent cleavage of the nucleoside bond; the reaction mixtures contained uracil in amounts corresponding approximately to 50% cleavage of the starting diester *XV*. The described method afforded the free 2'-deoxy-5'-O-phosphonomethyluridine in about 30% yield.

The analogous reaction of sodium salt of 3-N,3'-O-bis(benzyloxymethyl)-2'-deoxythymidine (*XI*) with diethyl *p*-toluenesulfonyloxymethanephosphonate (*IIIa*)



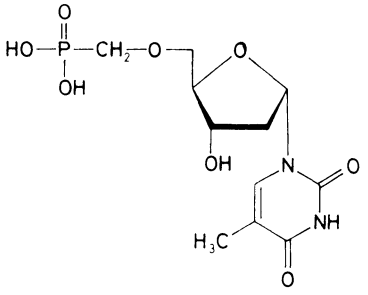
XVII, $R^1 = R^2 =$ BOM ; $R^3 =$ Et
XVIII, $R^1 = R^2 =$ BOM ; $R^3 =$ Bn
XIX, $R^1 = R^2 = H$; $R^3 =$ Et
XX, $R^1 = CH_2OH$; $R^2 = H$; $R^3 =$ Et
XXI, $R^1 = H$; $R^2 =$ Ac ; $R^3 =$ Et
XXII, $R^1 = CH_2OAc$; $R^2 =$ Ac ; $R^3 =$ Et
XXIIIa, $R^1 = R^2 = R^3 = H$



XXIV, $R^1 =$ BOM ; $R^2 =$ Ts
XXV, $R^1 =$ BOM ; $R^2 =$ Bn

$C_6H_5CH_2OCH_2P(O)(OCH_2C_6H_5)_2$

XXVI



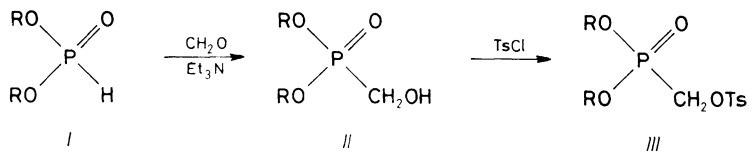
XXIII b

in dimethylformamide led to the desired diethyl ester of dibenzyloxymethyl-2'--deoxy-5'-O-phosphonomethylthymidine (*XVII*) in 40–45% yield. In the reaction mixture we identified 5'-O-*p*-toluenesulfonyl derivative *XXIV* as one of the side--products; moreover, the reaction gave also a mixture of polar compounds of oligomeric character (as shown by their mass spectra).

Catalytic hydrogenation of the diester *XVII* afforded diethyl ester of 2'-deoxy-5'--O-phosphonomethylthymidine (*XIX*). The hydrogenation intermediate, 3-N-hydroxymethyl derivative *XX*, has practically the same chromatographic mobility as the product *XIX*, making thus monitoring the hydrogenation course difficult. The presence of *XX* in the product *XIX* was proven by acetylation of the mixture, chromatographic separation of the acetates *XXI* and *XXII* and measurement of their ^{1}H NMR spectra.

Unlike the corresponding uracil derivative *XV*, the diethyl ester of 2'-deoxy-5'--O-phosphonomethylthymidine (*XIX*) reacted with bromotrimethylsilane in acetonitrile under formation of anomeric 2'-deoxy-5'-O-phosphonomethylthymidines *XXIIIa* and *XXIIIb* (in the ratio 2 : 3). Also in this case about 50% of the starting diethyl ester *XIX* underwent cleavage to thymine and the sugar derivative. An anomerization under these conditions was observed only with the 2'-deoxythymidine derivative. The anomerization of some 2'-deoxynucleosides in an acid medium is a known phenomenon and the different propensity of individual 2'-deoxynucleosides to anomerization (including the mechanism) is described[18].

Therefore, in addition to the mentioned method with intermediary format on of diethyl esters of phosphonomethyl derivatives, we elaborated an alternative way using the corresponding dibenzyl esters. Their catalytic hydrogenation afforded free 5'-O-phosphonomethyl derivatives without any anomerization or cleavage of the nucleoside bond. The required reagent, dibenzyl *p*-toluenesulfonyloxymethanephosphonate (*IIIb*), was synthesized using a procedure analogous to the preparation of diethyl ester *IIIa* (refs[2,17]), i.e. reaction of dibenzyl phosphite with paraformaldehyde, followed by reaction of the obtained dibenzyl hydroxymethanephosphonate (*IIb*) with *p*-toluenesulfonyl chloride in acetonitrile (Scheme 1).



I *II* *III*

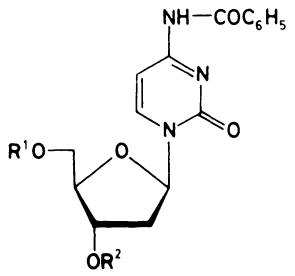
In formulae *I*–*III*: *a*, R = Et ; *b*, R = Bn

SCHEME 1

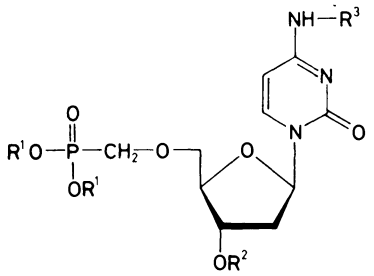
Reaction of the protected 2'-deoxyuridine derivative *X* with dibenzyl ester *IIIb* and sodium hydride in dimethylformamide afforded the expected dibenzyl ester of 3-N,3'-O-bis(benzyloxymethyl)-2'-deoxy-5'-O-phosphonomethyluridine (*XIII*) along with minor amount of the monobenzyl ester *XIV*. Catalytic hydrogenation of this mixture furnished pure 2'-deoxy-5'-O-phosphonomethyluridine (*XVI*) in a single step.

However, dibenzyl *p*-toluenesulfonyloxymethanephosphonate (*IIIb*) is much more reactive than the diethyl ester *IIIa* and its application is accompanied by a series of side reactions, e.g. O-benzylation of nucleosides in position 5'. Reaction of the 2'-deoxythymidine derivative *XI* with sodium hydride and dibenzyl ester *IIIb* afforded thus the desired dibenzyl ester of 3-N,3'-O-bis(benzyloxymethyl)-2'-deoxy--5'-O-phosphonomethylthymidine (*XVIII*), together with the 5'-O-benzyl derivative *XXV* as the side-product. Moreover, considerable amounts of polar products (molecular weight up to about 1 900) were formed and the reagent *IIIb* was to certain extent autobenzylated giving rise to dibenzyl benzyloxymethanephosphonate (*XXVI*). Although the overall yields of the phosphonate *XVIII* were about 20%, its catalytic hydrogenation afforded the pure β-anomer of 2'-deoxy-5'-O-phosphonomethylthymidine (*XXIIIa*). The reaction mixture after hydrogenation of *XVIII* contained 2'-deoxythymidine as a side-product (total yield up to 10%).

As the starting compound for preparation of 5'-O-phosphonomethyl-2'-deoxycytidine (*XXXIV*) we chose 4-N-benzoyl-2'-deoxy-3'-O-(tetrahydro-2*H*-pyran-2-yl)-cytidine (*XXX*) prepared from 4-N-benzoyl-2'-deoxycytidine (*XXVII*). 4-N-Benzoyl--2'-deoxycytidine was first converted into 4-N-benzoyl-2'-deoxy-5'-O-(tert-butyldiphenylsilyl)cytidine (*XXVIII*) by reaction with tert-butylchlorodiphenylsilane and



XXVII, $R^1 = R^2 = H$
XXVIII, R^1 = TBDPS ; $R^2 = H$
XXIX, R^1 = TBDPS ; R^2 = THP
XXX, $R^1 = H$; R^2 = THP



XXXI, R^1 = Et ; R^2 = THP ; R^3 = Bz
XXXII, R^1 = Et ; R^2 = THP ; $R^3 = H$
XXXIII, R^1 = Et ; $R^2 = R^3 = H$
XXXIV, $R^1 = R^2 = R^3 = H$

imidazole in dimethylformamide. Reaction of compound *XXVIII* with 3,4-dihydro--2*H*-pyran in the presence of trifluoroacetic acid gave 4-N-benzoyl-5'-O-(tert-butyl-

diphenylsilyl)-2′-deoxy-3′-O-(tetrahydro-2*H*-pyran-2-yl)cytidine (*XXIX*) in which the tert-butyldiphenylsilyl group was removed by treatment with tetrabutylammonium fluoride in dioxane to yield the desired 4-N-benzoyl-2′-deoxy-3′-O-(tetrahydro-2*H*-pyran-2-yl)cytidine (*XXX*).

Sodium salt of this compound, prepared in situ by treatment with sodium hydride, reacted with diethyl *p*-toluenesulfonyloxymethanephosphonate (*IIIa*) in dimethylformamide to give diethyl ester of 4-N-benzoyl-2′-deoxy-5′-O-phosphonomethyl-3′-O-(tetrahydro-2*H*-pyran-2-yl)cytidine (*XXXI*) in a yield of about 55%. After deprotection of the amino and the 3′-hydroxy groups by the action of ammonia in aqueous ethanol and acid hydrolysis, we obtained diethyl ester of 2′-deoxy-5′-O-phosphonomethylcytidine (*XXXIII*). Deblocking of the ester groups in compound *XXXIII* with bromotrimethylsilane in acetonitrile led to the pure β-anomer of 5′-O-phosphonomethylderivative *XXXIV*. The reaction yields were rather high (more than 80%) and, contrary to the analogous reaction of the 2′-deoxythymidine and 2′-deoxyuridine derivatives, the cleavage of the nucleoside bond was minimal (only 10% of the starting *XXXIII* was degraded to cytosine and the sugar derivative). This fact agrees with the generally known low propensity of cytosine nucleosides to anomerization and cleavage of the nucleoside bond[18].

A comparison of both these methods (diethyl ester and dibenzyl ester method) clearly shows that in the pyrimidine 2′-deoxynucleoside series the traditional approach, using ethyl esters and their deblocking with bromotrimethylsilane, is advantageous only in the preparation of the 2′-deoxycytidine phosphonomethyl derivative. Phosphonomethyl derivatives of 2′-deoxyuridine and 2′-deoxythymidine may be better prepared using the synthon *IIIb* which affords the intermediate dibenzyl esters in lower yields but does not require the treatment with bromotrimethylsilane that leads to considerable cleavage and anomerization of the nucleoside bond in the product.

EXPERIMENTAL

Unless stated otherwise, the solvents were evaporated at 40°C/2 kPa and the compounds were dried over phosphorus pentoxide at 13 Pa. The melting points were determined on a Kofler block and are uncorrected. Thin-layer chromatography was performed on Silufol sheats UV 254 (Kavalier, Czechoslovakia). The solvent systems are given in the text. Spots were detected with UV light at 254 nm or by spraying with 0·5% solution of 4-(4-nitrobenzyl)pyridine in ethanol followed by heating and exposure to ammonia vapours[19,20]. Preparative column chromatography was carried out on silica gel (30—60 μm; Service Laboratories of the Institute). Reversed-phase chromatography was performed on octadecyl-silica gel (20 μm; Laboratorní přístroje, Prague) in water; detection on Uvicord 4701 A (LKB, Sweden) at 254 nm. Electrophoreses were carried out on a Whatman No 3MM paper in 0·1M triethylammonium hydrogen carbonate for 1 h at 20 V/cm. The electrophoretic mobilities given in the text (E_{Up}) are related to uridine 5′-phosphate.

UV spectra were taken on a Pye Unicam 8800 instrument, mass spectra (*m/z*) on a ZAB-EQ (VG Analytical) spectrometer using the EI (electron energy 70 eV), FAB (xenone, 8 kV) and

SIMS (Cs^+, 35 kV) techniques. Glycerol (G) and thioglycerol (TG) were used as matrices. Proton NMR spectra were obtained with a Varian XL-200 (200 MHz) instrument in hexadeuteriodimethyl sulfoxide with tetramethylsilane as internal standard, or in deuterium oxide with sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS). IR spectra were recorded on a Zeiss UR 20 spectrophotometer, the wavenumbers are given in cm^{-1}.

N,N-Dimethylformamide was distilled in vacuo from phosphorus pentoxide and then from calcium hydride. The 5'-O-acyl derivatives *IV* and *V* were prepared from 2'-deoxyuridine and 2'-deoxythymidine, respectively, using standard acylation procedures[21-23].

Dibenzyl *p*-Toluenesulfonyloxymethanephosphonate (*IIIb*)

Triethylamine (4·9 ml) was added to a stirred mixture of compound *Ib* (92 g; 351 mmol) and paraformaldehyde (12·3 g; 409 mmol) and the reaction mixture was heated to 80°C for 15 min (reflux condenser). The triethylamine was evaporated, the residue was dissolved in ethyl acetate (100 ml) and the excess paraformaldehyde was removed by filtration through a short column of silica gel (5 cm). After evaporation, the filtrate afforded 92 g (90%) of compound *IIb*. This material was dissolved in acetonitrile (450 ml) and mixed with *p*-toluenesulfonyl chloride (64·6 g; 339 mmol), triethyl amine (50 ml) and 4-(dimethylamino)pyridine (410 mg; 3·4 mmol). The mixture was stirred at 20°C for 1 h and the reaction was monitored by TLC (toluene–ethyl acetate (1 : 1); detection with 4-(4-nitrobenzyl)pyridine[19]). After 1 h 2M triethylammonium hydrogen carbonate (300 ml) was added and the mixture was set aside at 20°C for 1 h and then in a refrigerator for 12 h. After evaporation, the residue was shaken with ether (3 × 200 ml). The combined ethereal extracts were extracted with saturated sodium hydrogen carbonate solution (200 ml) and with saturated sodium chloride solution and dried over magnesium sulfate. The ethereal solution was filtered, the solvent evaporated and the crude syrupy product *IIIb* crystallized from tetrachloromethane. The crystals were collected on filter, washed with ether and air-dried; yield 75 g (55%), m.p. 64–65°C, R_F 0·74 (toluene–acetone 1 : 1). For $C_{22}H_{23}O_6PS$ (446·4) calculated: 59·18% C, 5·19% H, 6·94% O, 7·18% S; found: 59·08% C, 5·26% H, 7·01% P, 7·20% S. Mass spectrum (EI): 355 ($M - C_6H_5CH_2$). IR spectrum (CCl_4): 3 040, 3 070, 3 091 (C–H arom.); 1 599 (ring arom.); 1 460, 1 470 (CH_2); 1 384 (SO_2 asym.); 1 273 (P–O); 1 181, 1 192 (SO_2 sym.); 977, 1 014, 1 052, 825 (P–O–C). ^1H NMR spectrum (hexadeuteriodimethyl sulfoxide): 2·41 s, 3 H (CH_3); 4·46 d, 2 H (P–CH_2, J(P, CH_2) = 9·4); 5·02 d, 4 H (2 × $POCH_2$, J = 8·7); 7·26–7·40 m, 10 H (H-arom.); 7·45 and 7·77 2 × d, 4 H (H-arom., J = 8·6).

5'-O-Benzoyl-3-N-benzyloxymethyl-2'-deoxyuridine (*VI*)

Potassium carbonate (30 g) was added to a solution of 5'-O-benzoyl-2'-deoxyuridine (*IV*; 20·0 g; 60 mmol) in dry acetone (600 ml) and the mixture was stirred at 56°C for 10 min. Benzyl chloromethyl ether (15 g; 90 mmol) was added and the mixture was stirred at 56°C under reflux condenser. After 2 h another portion of benzyl chloromethyl ether (4·7 g; 30 mmol) was added and the heating was continued for additional 3 h. The potassium carbonate was filtered off, washed with acetone, the filtrate was evaporated and the residue was dissolved in chloroform (500 ml). The chloroform solution was dried over magnesium sulfate, the solvent was evaporated and the remaining crude 5'-O-benzoyl-3-N-benzyloxymethyl-2'-deoxyuridine *VI* (60 g) was used in the preparation of compound *X* without further purification. For characterization, the crude product *VI* (300 mg) was purified by chromatography on silica gel in toluene–acetone (2 : 1; R_F 0·45). The compound was isolated as an amorphous residue (130 mg). For $C_{24}H_{24}N_2O_7$ (452·5) calculated: 63·71% C, 5·35% H, 6·19% N; found: 63·71% C, 5·52% H, 5·80% N. Mass spectrum (SIMS; matrix TG + G 3 : 1; TFA): 453 (M + H). IR spectrum ($CHCl_3$): 3 490 (OH

bonded); 3 617 (OH free); 3 068, 3 091, 703, 716 (C–H arom.); 1 720, 1 676 (C=O); 1 603, 1 586 (ring arom.); 1 462 (CH_2); 1 280 (C–O benzoate); 1 096 (C–O–C). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 2·26 t, 2 H (2 × H-2′, J = 6·1); 4·09 q, 1 H (H-4′, $\sum J$ = 14·0); 4·35 m, 1 H (H-3′); 4·50 2 × dd, 2 H (H-5′, H-5″, J(5′, 4′) = 4·0; J(5″, 4′) = 5·0); 4·59 s, 2 H (OCH_2Ph); 5·31 s, 2 H (NCH_2O); 5·51 d, 1 H (OH, J(3′, OH) = 4·2); 5·66 d, 1 H (H-5, J(5, 6) = = 8·2); 6·20 t, 1 H (H-1′, J(1′, 2′) = 6·7); 7·30 s, 5 H (H-arom.); 7·50–7·70 m, 3 H (H-arom.); 7·70 d, 1 H (H-6, J(6, 5) = 8·2); 7·90–8·05 m, 2 H (H-arom.); exchange with CD_3COOD: 4·36 q, 1 H (H-3′, J(3′, 4′) = 4·5).

3-N-Benzyloxymethyl-2′-deoxy-5′-O-pivaloylthymidine (*VII*)

The title compound was prepared from 2′-deoxy-5′-O-pivaloylthymidine (17 g; 52 mmol) in the same manner as described for the uracil derivative *VI*. The obtained crude product *VII* (32 g) was used without purification in the further reactions. Pure compound *VII* was obtained by chromatography of the crude product (300 mg) in toluene–acetone (2 : 1; R_F 0·46). It was isolated as a syrup (216 mg) which crystallized on standing in the cold. Mass spectrum (SIMS; TG): 447 (M + H), 339 (M−benzyl−CH_3). IR spectrum (CCl_4): 3 620 (OH free); 3 490 (OH intermol. bonded); 3 038, 3 070, 3 093, 700 (C–H arom.); 1 734 (C=O ester); 1 715, 1 672 (C=O); 1 466 (CH_2); 1 384, 1 395, 1 365 (CH_3); 1 283 (C–O) ester; 1 102 (ether). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·16 s, 9 H (tert-butyl): 1·84 d, 3 H (CH_3, J(CH_3, 6) = 1·2); 2·18 m, 2 H (2 × H-2′); 3·97 q, 1 H (H-4′, $\sum J$ = 13·0); 4·25 m, 1 H (H-3′); 4·26 d, 2 H (2 × H-5′, J(5′, 4′) = 4·3); 4·59 s, 2 H (OCH_2Ph); 5·34 s, 2 H (NCH_2O); 5·42 d, 1 H (OH, J(OH, 3′) = = 4·7); 6·20 t, 1 H (H-1′, J(1′, 2′) = 6·8); 7·30 s, 5 H (H-arom.); 7·44 d, 1 H (H-6, J(6, CH_3) = = 1·2); exchange with CD_3COOD: 4·23 d, 3 H (H-3′, H-5′).

3-N,3′-O-Bis(benzyloxymethyl)-2′-deoxy-5′-O-pivaloylthymidine (*IX*)

The crude product *VII* (32 g; 37 mmol, calculated for the pure substance) was codistilled with toluene (100 ml) and stirred with ethyldiisopropylamine (25 ml) and benzyl chloromethyl ether (9·9 g; 63 mmol) at 50°C for 1 h. After cooling, the reaction mixture was diluted with chloroform (500 ml), the solution was washed with 2% hydrochloric acid until the aqueous layer was acid, then with saturated solution of sodium hydrogen carbonate to alkaline reaction of the aqueous phase, and then with water (300 ml). After drying over anhydrous magnesium sulfate and filtration, the solvent was evaporated and the residue (compound *IX*; 35 g) was used in the preparation of *XI* without further purification. For characterization, a sample of the crude product *IX* (300 mg) was purified by chromatography on silica gel in toluene–acetone (3 : 1; R_F 0·55); affording 144 mg of the amorphous substance. Mass spectrum (SIMS; TG + G 3 : 1): 567 (M + H). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·13 s, 9H (tert-butyl); 1·83 d, 3 H (CH_3); 2·26 ddd, 1 H (H-2″, J(2″, 1′) = 7·4; J(2″, 3′) = 6·4; J(2″, 2′) = 14·0); 2·38 ddd, 1 H (H-2′, J(2′, 1′) = 6·4; J(2′, 3′) = 3·2); 4·10–4·26 m, 3 H (H-4′, 2 × H-5′); 4·36 m, 1 H (H-3′, $\sum J$ = = 12·8; J(3′, 4′) = 3·2); 4·59 s, 4 H (2 × CH_2Ph); 4·82 s, 2 H (OCH_2O); 5·33 s, 2 H (NCH_2O); 6·18 t, 1 H (H-1′, $\sum J$ = 13·8); 7·29 s, 5 H (H-arom.); 7·33 s, 5 H (H-arom.); 7·46 q, 1 H (H-6, J(6, CH_3) = 1·4).

3-N,3′-O-Bis(benzyloxymethyl)-2′-deoxyuridine (*X*)

The crude product *VI* (60 g; 57·5 mmol, calculated for the pure substance) was treated in the same manner as described for compound *IX*. A solution of compound *VIII* in 0·2M methanolic sodium methoxide (100 ml) was stirred at room temperature for 1 h. The reaction mixture was

neutralized with Dowex 50X8 (H^+ form), the ion exchanger was filtered off, washed with methanol and the filtrate was taken down. The liquid residue was shaken with light petroleum (2 × 100 ml). The light petroleum layer was separated from the product which was chromatographed on silica gel in toluene–acetone (2 : 1; R_F 0·42). Fractions containing the pure product *X* were combined and the solvents were evaporated; yield 18·7 g (66% related to compound *VI*) of the amorphous material. For $C_{25}H_{28}N_2O_7$ (468·5) calculated: 64·09% C, 6·02% H, 5·98% N; found: 64·50% C, 6·15% H, 5·80% N. Mass spectrum (SIMS; TG + G 3 : 1; DMF): 469·4 (M + H). IR spectrum (4% CCl_4): 3 037, 3 070, 3 094 (C–H arom.); 2 893, 2 950 (CH_2); 1 679, 1 717 (C=O); 1 455, 1 465, 1 472 (CH_2); 1 051, 1 069, 1 108 (C–O); (CCl_4, dilute solution): 3 639, 3 631 (OH free); 3 492, 3 388 (OH bonded). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 2·20 m, 2 H (2 × H-2′); 3·60 m, 2 H (2 × H-5′), 4·01 q, 1 H (H-4′, $\sum J$ = 10·0); 4·37 m, 1 H (H-3′, $\sum J$ = 14·0); 4·59 s, 2 H (OCH_2Ph); 4·60 s, 2 H (OCH_2Ph); 4·82 s, 2 H (OCH_2O); 5·14 t, 1 H (OH, J(5′, OH) = 5·0); 5·33 s, 2 H (NCH_2O); 5·79 d, 1 H (H-5, J(5, 6) = 8·2); 6·17 t, 1 H (H-1′, J(1′, 2′) = 6·3); 7·31 s, 5 H (H-arom.); 7·35 s, 5 H (H-arom.); 7·93 d, 1 H (H-6, J(6, 5) = 8·2); exchange with CD_3COOD: 2·24 m, 2 H (H-2′, J(2′, 3′) = 5·8); 3·60 d, 2 H (H-5′, J(5′, 4′) = 3·8); 4·01 q, 1 H (H-4′, $\sum J$ = 10·0; J(4′, 3′) = 2·4).

3-N,3′-O-Bis(benzyloxymethyl)-2′-deoxythymidine (*XI*)

The crude product *IX* (35 g; 29·6 mmol, calculated for the pure compound), prepared analogously as described for compound *VIII*, was stirred with 0·2M methanolic sodium methoxide (100 ml) for 1 h at room temperature. The reaction mixture was neutralized with Dowex 50X8 (H^+ form), the Dowex was filtered off, washed with methanol and the filtrate was taken down. The liquid residue was washed with light petroleum (2 × 100 ml), the petroleum layer was removed and traces of the solvent were removed in vacuo. The residue was chromatographed on silica gel in toluene–acetone (2 : 1) affording the product as a syrup (10·3 g; 72%), R_F 0·49 (toluene–acetone 2 : 1); 0·34 (toluene–acetone 3 : 1). For $C_{26}H_{30}N_2O_7$ (482·5) calculated: 64·72% C, 6·27% H, 5·81% N; found: 64·62% C, 6·26% H, 5·71% N. Mass spectrum (SIMS; TG + G 3 : 1; TFA): 483 (M + H). IR spectrum (CCl_4; dilute solution): 3 478 (OH bonded); 3 630 (OH free); (CCl_4, 4%): 3 488 (OH bonded); 3 037, 3 071, 3 090 (C–H arom.); 1 711, 1 671, 1 643 (C=O); 1 468 (CH_2); 1 106, 1 051 (C–O–C); 702 (C–H arom.). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·83 d, 3 H (CH_3, J(6, CH_3) = 1·2); 2·24 m, 2 H (2 × H-2′); 3·62 m, 2 H (2 × H-5′); 3·99 q, 1 H (H-4′, $\sum J$ = 10·0); 4·38 p, 1 H (H-3′, $\sum J$ = 14·0); 4·59 s, 4 H (2 × OCH_2Ph); 4·81 s, 2 H (OCH_2O); 5·14 t, 1 H (OH, J(5′, OH) = 5·4); 5·33 s, 2 H (NCH_2O); 6·19 t, 1 H (H-1′, J(1′, 2′) = 6·3); 7·30 s, 5 H (H-arom.); 7·34 s, 5 H (H-arom.); 7·78 d, 1 H (H-6, J(6, CH_3) = 1·2); exchange with CD_3COOD: 2·27 m, 2 H (H-2′, J(2′, 3′) = = 5·6); 3·63 d, 2 H (H-5′, J(5′, 4′) = 3·7); 4·00 q, 1 H (H-4′, $\sum J$ = 10·0; J(4′, 3′) = 2·7).

Diethyl Ester of 3-N,3′-O-Bis(benzyloxymethyl)-2′-deoxy--5′-O-phosphonomethyluridine (*XII*)

A solution of compound *X* (2 g; 4 mmol) in a mixture of dimethylformamide and toluene (2 : 1; 40 ml) was concentrated to half of the original volume and a 60% suspension of sodium hydride in paraffin oil (480 mg; 12 mmol) was added under argon. The mixture was stirred at room temperature for 30 min, diethyl *p*-toluenesulfonyloxymethanephosphonate (*IIIa*; 1·9 g; 6 mmol) was added, the stirring at room temperature was continued for 2 h, and then the mixture was allowed to stand overnight in a refrigerator. The reaction was monitored by TLC in ethyl acetate (R_F of *X*: 0·62; R_F of *XII*: 0·25). The mixture was neutralized with acetic acid (0·5 ml) and then codistilled with toluene (50 ml). The residue was coevaporated with xylene (2 × 30 ml), dissolved in ethyl acetate (120 ml) and washed with water (80 ml). The organic phase was separated,

dried over magnesium sulfate, the solvent was evaporated and the remaining material (4·6 g) was chromatographed on silica gel (800 ml). After elution of the unreacted starting compound *X* (320 mg; 16%) with ethyl acetate, the product was eluted with ethyl acetate–acetone (4 : 1); yield 840 mg (33%) of the amorphous substance, R_F 0·25 (ethyl acetate). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·22 t, 6 H (2 × CH_3); 2·28 m, 2 H (2 × H-2′); 3·74 m, 2 H (2 × H-5′); 3·88 d, 2 H (P–CH_2, *J*(P, CH) = 8·7); 4·04 dq, 4 H (2 × $POCH_2$, *J*(P, OCH) = 8·2; *J*(CH_2, CH_3) = 7·0); 4·12 m, 1 H (H-4′); 4·35 dt, 1 H (H-3′, $\sum J$ = 12·0); 4·59 s, 4 H (OCH_2Ph); 4·81 s, 2 H (OCH_2O); 5·32 s, 2 H (NCH_2O); 5·75 d, 1 H (H-5, *J*(5, 6) = 8·0); 6·18 t, 1 H (H-1′, *J*(1′, 2′) = 7·0; *J*(1′, 2″) = 6·4); 7·30 s, 5 H (H-arom.); 7·35 s, 5 H (H-arom.); 7·85 d, 1 H (H-6, *J*(6, 5) = 8·0).

Dibenzyl Ester of 3-N,3′-O-Bis(benzyloxymethyl)-2′-deoxy-5′-O--phosphonomethyluridine (*XIII*)

The title compound was prepared from dibenzyloxymethyl derivative *X* (10·0 g; 21 mmol) and compound *IIIb* (14·3 g; 32 mmol) analogously as described for compound *XII*. The reaction time was 3 h and the reaction was monitored by TLC in ethyl acetate–toluene (3 : 1; R_F of *X*: 0·52; R_F of product *XIII*: 0·56). Yield 5·4 g (34%) of amorphous compound *XIII*. For $C_{40}H_{43}N_2O_{10}P$ (742·7) calculated: 64·68% C, 5·84% H, 3·77% N, 4·17% P; found: 64·98% C, 6·01% H, 3·87% N, 3·97% P. Mass spectrum (SIMS; TG + G 3 : 1): 743 (M + H). IR spectrum (CCl_4): 3 038, 3 071, 3 095 (C–H arom.); 2 873, 2 953 (CH_2); 1 720, 1 679 (C=O); 1 461, 1 470 (CH_2); 1 261, 1 278 (P=O); 1 168, 1 048, 1 101, 1 001, 972 (C–O and P–O–C). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 2·07 m, 1 H (H-2″, *J*(2″, 1′) = 7·2; *J*(2″, 3′) = 6·0); 2·29 ddd, 1 H (H-2′, *J*(2′, 1′) = 6·0; *J*(2′, 3′) = 3·0; *J*(2′, 2″) = 14·0); 3·71 2 × dd, 2 H (H-5′, H-5″, *J*(5′, 4′) = 3·1; *J*(5″, 4′) = 3·8; *J*(5, 5″) = 10·8); 3·97 d, 2 H (P–CH_2O, *J*(P, CH) = 8·6); 4·10 q, 1 H (H-4′, $\sum J$ = 9·4; *J*(4′, 3′) = 2·5); 4·29 p, 1 H (H-3′, $\sum J$ = 11·5; *J*(3′, 4′) = 2·5); 4·58 s, 4 H (2 × $PhCH_2O$); 4·78 s, 2 H (OCH_2O); 5·03 d, 2 H ($POCH_2$); 5·07 d, 2 H ($POCH_2$, *J*(P, OCH) = 1·2); 5·30 s, 2 H (NCH_2O); 5·63 d, 1 H (H-5, *J*(5, 6) = 8·2); 6·15 t, 1 H (H-1′, *J*(1′, 2′) = 6·0); *J*(1′, 2″) = 7·0); 7·32 s, 20 H (H-arom.); 7·77 d, 1 H (H-6, *J*(6, 5) = 8·2).

In addition to the product *XIII*, the chromatography in ethyl acetate–toluene (3 : 1) afforded dibenzyl benzyloxymethanephosphonate *XXVI* (R_F 0·90) as an amorphous substance (1·3 g; 16%). For $C_{22}H_{23}O_4P$ (382·4) calculated: 69·10% C, 6·06% H, 8·10% P; found: 69·83% C, 5·79% H, 8·02% P. Mass spectrum (SIMS; TG + G 3 : 1): 383 (M + H), 291 (M — benzyl). IR spectrum (CCl_4): 3 038, 3 071, 3 094 (C–H arom.); 2 957, 2 868 (CH_2); 1 459, 1 471 (CH_2); 1 265 (P=O); 1 014, 1 043, 978 (P–O–C); 1 110 (C–O–C). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 3·91 d, 2 H (PCH_2O, *J*(P, CH) = 8·4); 4·56 s, 2 H (OCH_2Ph); 5·04 d, 2 H ($POCH_2$, *J*(P, OCH) = 1·3); 5·08 d, 2 H (P–OCH_2, *J*(P, OCH) = 1·6); 7·36 s, 15 H (H-arom.). Further elution with ethyl acetate–acetone–ethanol–water (15 : 3 : 4 : 3) afforded 2·3 g (16%) of amorphous monobenzyl ester of 3-N,3′-O-bis(benzyloxymethyl)-2′-deoxy-5′-O-phosphonomethyluridine (*XIV*). Mass spectrum (SIMS; TG + G 3 : 1; TFA): 654 (M + H). Electrophoretic mobility corresponded to 1 negative charge; hydrogenation gave compound *XVI*.

Diethyl Ester of 2′-Deoxy-5′-O-phosphonomethyluridine (*XV*)

Compound *XII* (700 mg; 1·1 mmol) was hydrogenated in methanol (50 ml) over 10% Pd on active carbon (150 mg) for 24 h at 20°C and under atmospheric pressure. The catalyst was filtered through a layer of Celite which was then washed with methanol and the filtrate was taken down. Chromatography of the residue on silica gel (50 g) in ethyl acetate–acetone–ethanol–water (18 : 3 : 2 : 2) afforded 325 mg (76%) of amorphous *XV* (R_F 0·35). Mass spectrum (SIMS; TG):

379 (M + H). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·24 t, 6 H (2 × CH_3, $J(CH_3CH_2)$ = 7·0); 2·09 m, 2 H (2 × H-2′); 3·71 m, 2 H (2 × H-5′); 3·87 d, 2 H (P–CH_2, J(P, CH) = 8·0); 3·88 m, 1 H (H-4′); 4·05 dq, 4 H (2 × $POCH_2$, J(P, OCH) = 8·4; $J(CH_2, CH_3)$ = = 7·0); 4·25 m, 1 H (H-3′); 5·34 d, 1 H (OH, J(3′, OH) = 4·7); 5·59 dd, 1 H (H-5, J(5, 6) = 8·0); J(5, NH) = 2·0); 6·17 t, 1 H (H-1′, J(1′, 2′) = 6·8; J(1′, 2″) = 7·0); 7·78 d, 1 H (H-6, J(6, 5) = = 8·2); 11·28 bs, 1 H (NH).

2′-Deoxy-5′-O-phosphonomethyluridine (*XVI*)

A) Bromotrimethylsilane (98% purity; 0·46 ml; 3·5 mmol) was added to a solution of *XV* (300 mg; 0·8 mmol) in acetonitrile (13 ml) at 0°C under argon and the mixture was stirred for 24 h at room temperature. After addition of 2M solution of triethylammonium hydrogen carbonate (TEAB; 1 ml) the solvent was evaporated and the residue was dissolved in water (2 ml). The solution was made alkaline with triethylamine and applied onto a column of DEAE Sephadex (HCO_3^- form; 150 ml). The products were eluted with a gradient of TEAB (0–0·4 mol l^{-1}; total volume 900 ml). Evaporation of the UV-absorbing fractions furnished two products: uracil (35 mg; 39·4%) and crude *XVI*, containing sugar component not absorbing in the UV (300 mg). The product was purified by chromatography on a column of octadecyl-silica gel (reversed phase; 250 ml); elution with water. Fractions, containing pure product, were evaporated and the product was dried in a desiccator over phosphorus pentoxide; yield 104 mg (41%), m.p. 183–185°C; R_F 0·20 (ethyl acetate–acetone–ethanol–water 13 : 3 : 4 : 5), E_{Up} 0·89. For $C_{10}H_{15}N_2PO_8$ (322·2) calculated: 37·27% C, 4·69% H, 8·69% N, 9·61% P; found: 37·16% C, 4·96% H, 8·88% N, 9·10% P. UV spectrum (pH 7): λ_{max} 261 nm, ε_{max} 9 900. Mass spectrum (SIMS; TG + G 3 : 1): 323 (M + H). IR spectrum (KBr): 3 263, 3 130 (OH, NH); 2 935 (CH_2); 1 660–1 715 (C=O); 1 471 (CH_2); 1 195 (P=O); 1 071 (C–O). ^{1}H NMR spectrum (D_2O): 2·41 dd, 2 H (2 × H-2′, $\sum J$ = 11·7; J(2′, 3′) = 4·7); 3·67 d, 2 H (P–CH_2, J(P, CH) = 8·4); 3·81 m, 2 H (2 × H-5′); 4·15 q, 1 H (H-4′, $\sum J$ = 11·5); 4·54 q, 1 H (H-3′, $\sum J$ = 13·5; J(3′, 4′) = 4·0); 5·93 d, 1 H (H-5, J(5, 6) = 8·0); 6·32 t, 1 H (H-1′, J(1′, 2′) = 7·0); 7·99 d, 1 H (H-6, J(6, 5) = 8·0).

B) Compound *XIII* (5 g; 6·7 mmol) was hydrogenated in methanol (120 ml) over 10% Pd on active carbon (1·4 g) at 20°C and under atmospheric pressure. After 8 h another portion of the catalyst (0·3 g) was added and the mixture was hydrogenated for 2 h. The catalyst was filtered off, washed with methanol and the filtrate was evaporated. The crude product (2·32 g) was purified by chromatography on silica gel in a mixture of ethyl acetate–acetone–ethanol–water (13 : 3 : 4 : 5). The combined product-containing fractions were evaporated, the amorphous residue was dissolved in aqueous methanol and precipitated with ethanol. The crystalline product was collected and dried in vacuo over phosphorus pentoxide; yield 1·47 g (67·6%).

C) The crude product *XIV* (2 g) was dissolved in methanol (50 ml) and hydrogenated over 10% Pd on active carbon (0·6 g) for 72 h at 20°C and atmospheric pressure. The reaction mixture was processed as described in the procedure *B*); yield 318 mg (32%).

Diethyl Ester of 3-N,3′-O-Bis(benzyloxymethyl)-2′-deoxy-
-5′-O-phosphonomethylthymidine (*XVII*)

Compound *XVII* was prepared from 3-N,3′-O-bis(benzyloxymethyl)-2′-deoxythymidine (*XI*; 1·6 g; 3·2 mmol) in the same manner as described for compound *XII*. The reaction course was monitored by TLC in the system toluene–acetone 3 : 1 (R_F: product *XVII* 0·14; starting *XI* 0·34). The same solvent system was used for the chromatographic isolation of the product by column chromatography on silica gel (200 g). Yield 1·2 g (56%) of amorphous *XVII*. For $C_{31}H_{41}N_2O_{10}P$

(632·6) calculated: 58·85% C, 6·53% H, 4·43% N, 4·90% P; found: 58·52% C, 6·76% H, 4·40% N, 4·83% P. Mass spectrum (SIMS; TG + G 3 : 1; TFA): 633 (M + H). IR spectrum (CCl_4): 3 036, 3 072, 3 090 (C–H arom.); 1 714, 1 675 (C=O); 1 465 (CH_2); 1 263 (P=O); 1 167 (P–OEt); 1 101 (C–O–C); 1 055, 1 037 (C–O–C and P–O–C); 975 (P–O–C); 700 (C–H arom.). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·21 t, 6 H (2 × CH_3, $J(CH_3, CH_2)$ = 7·0); 1·87 d, 3 H (5-CH_3); 2·16 m, 1 H (H-2″, $J(2'', 3')$ = 6·2; $J(2'', 2')$ = 14·0); 2·32 m, 1 H (H-2′, $J(2', 3')$ = = 3·0); 3·75 m, 2 H (2 × H-5′); 3·89 d, 2 H (P–CH_2, J(P, CH) = 8·2); 4·03 2 × dq, 4 H (2 × $POCH_2$, $J(CH_2, CH_3)$ = 7·0; J(P, OCH) = 8·3); 4·09 m, 1 H (H-4′); 4·35 m, 1 H (H-3′, $\sum J$ = 12·0; $J(3', 4')$ = 2·8); 4·57 s, 2 H (3′-O–CH_2O–CH_2Ph); 4·59 s, 2 H (3-CH_2O–CH_2Ph); 4·82 s, 2 H (OCH_2O); 5·34 s, 2 H (NCH_2O); 6·21 dd, 1 H (H-1′, $J(1', 2')$ = 6·2; $J(1', 2'')$ = 7·8); 7·29 s, 5 H (H-arom.); 7·35 s, 5 H (H-arom.); 7·55 q, 1 H (H-6, J(6, CH_3) = 1·4).

In addition to *XVII*, the chromatography gave 3-N,3′-O-bis(benzyloxymethyl)-2′-deoxy-5′-O--(*p*-toluenesulfonyl)thymidine (*XXIV*) (200 mg; 10%) as a syrupy side-product. R_F 0·80 (toluene–acetone 3 : 1). For $C_{33}H_{36}N_2O_9S$ (636·7) calculated: 62·25% C, 5·70% H, 4·40% N, 5·04% S; found: 61·92% C, 5·55% H, 4·52% N, 4·85% S. Mass spectrum (SIMS; TG + G 3 : 1; TFA): 637 (M + H).

Dibenzyl Ester of 3-N,3′-O-Bis(benzyloxymethyl)-2′-deoxy-5′-O--phosphonomethylthymidine (*XVIII*)

The title compound was prepared from compounds *XI* (10·1 g; 21 mmol) and *IIIb* (14·02 g; 31·4 mmol) as described for compound *XII*. Chromatography of the crude product on a column of silica gel (600 g) in 3 : 1 mixture of toluene and acetone (R_F 0·32) afforded 3·7 g (23%) of pure amorphous *XVIII*. For $C_{41}H_{45}N_2O_{10}P$ (756·8) calculated: 65·07% C, 5·99% H, 3·70% N, 4·09% P; found: 65·02% C, 6·16% H, 3·57% N, 3·96% P. Mass spectrum (SIMS; TG + G 3 : 1; H_2O): 758 (M + H). IR spectrum (CCl_4): 3 038, 3 070, 3 092 (C–H arom.); 2 957, 2 895 (CH_3); 1 713, 1 673 (C=O); 1 465 (CH_2); 1 262 (P=O); 1 106 (C–O); 1 049 (C–O and P–O); 1 011, 976 (P–O). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·77 d, 3 H (CH_3, J = 1·2); 2·07 m, 1 H (H-2″, $J(2'', 3')$ = 6·2; $J(2'', 2')$ = 13·4); 2·24 m, 1 H (H-2′, $J(2', 3')$ = 3·0); 3·67 dd, 1 H (H-5″, $J(5'', 4')$ = 4·3; $J(5'', 5')$ = 10·8); 3·77 dd, 1 H (H-5′, $J(5', 4')$ = 3·2); 3·98 d, 2 H (P–CH_2, J(P, CH) = 8·3); 4·08 q, 1 H (H-4′, $\sum J$ = 10·0); 4·30 m, 1 H (H-3′, $\sum J$ = 11·5; $J(3', 4')$ = 2·5); 4·58 s, 4 H (2 × $PhCH_2$); 4·78 s, 2 H (OCH_2O); 5·03 d, 2 H (P–OCH_2, J(P, OCH) = 1·3); 5·07 d, 2 H (P–OCH_2, J(P, OCH) = 1·6); 5·32 s, 2 H (NCH_2O); 6·18 dd, 1 H (H-1′, $J(1', 2')$ = 6·0; $J(1', 2'')$ = 8·0); 7·29 s, 5 H (H-arom.); 7·33 s, 15 H (H-arom.); 7·49 q, 1 H (H-6, J(6, CH_3) = 1·4).

Side-products *XXVI* (1·9 g; 16%) and *XXV* (2·3 g; 19%) were also obtained. The latter (*XXV*, 5′-O-benzyl-3-N,3′-O-bis(benzyloxymethyl)-2′-deoxythymidine) was isolated as an amorphous substance, R_F 0·65 (toluene–acetone 3 : 1). For $C_{33}H_{36}N_2O_7$ (572·6) calculated: 69·21% C, 6·34% H, 4·89% N; found: 69·16% C, 6·11% H, 4·81% N. Mass spectrum (SIMS; TG + G 3 : 1; H_2O): 573 (M + H). IR spectrum (CCl_4): 3 034, 3 068, 3 089 (C–H arom.); 2 954, 2 891 (CH_3); 2 930, 2 864 (CH_2); 1 712, 1 674 (C=O); 1 464 (CH_2); 1 052, 1 079, 1 108 (C–O). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·58 d, 3 H (CH_3, J = 1·2); 2·21 m, 1 H (H-2″, $J(2'', 1')$ = 7·0; $J(2'', 3')$ = 6·2; $J(2'', 2')$ = 14·0); 2·35 m, 1 H (H-2′, $J(2', 1')$ = 6·2; $J(2', 3')$ = = 3·4); 3·63 dd, 1 H (H-5″, $J(5'', 4')$ = 3·6; $J(5'', 5')$ = 10·7); 3·74 dd, 1 H (H-5′, $J(5', 4')$ = 3·4); 4·14 q, 1 H (H-4′, $\sum J$ = 10·2); 4·43 m, 1 H (H-3′, $\sum J$ = 12·8; $J(3', 4')$ = 3·2); 4·55 s, 2 H (5′-O–CH_2Ph); 4·58 s, 2 H (3-CH_2–O–CH_2Ph); 4·57 s, 2 H (3′-O–CH_2–CH_2Ph); 4·82 s, 2 H (OCH_2O); 5·32 s, 2 H (NCH_2O); 6·20 t, 1 H (H-1′, $\sum J$ = 13·2); 7·28 s, 5 H (H-arom.); 7·35 s, 10 H (H-arom.); 7·58 q, 1 H (H-6, J(6, CH_3) = 1·4).

Diethyl Ester of 2'-Deoxy-5'-O-phosphonomethylthymidine (*XIX*)

Compound *XVII* (1 g; 1·6 mmol) was hydrogenated in methanol (50 ml) over 10% Pd on active carbon (0·2 g) at room temperature and atmospheric pressure for 24 h. The catalyst was removed by filtration through a layer of Celite, the filtrate was taken down and the residue was purified by chromatography on silica gel in the system ethyl acetate–acetone–ethanol–water (18 : 3 : 2 : 2). Yield of the amorphous crude product (R_F 0·46) was 0·44 g (71%). According to the ^{1}H NMR spectrum, the product contained about 10% of 3-hydroxymethyl derivative *XX* and was therefore characterized as the acetyl derivative *XXI*.

Acetylation of Mixture of Diethyl Ester of 2'-Deoxy-5'-O-phosphonomethylthymidine (*XIX*) and Diethyl Ester of 2'-Deoxy-3-hydroxymethyl-5'-O-phosphonomethylthymidine (*XX*)

N,N-Dimethylaminopyridine (20 mg) and acetic anhydride (0·4 ml) were added to a solution of the crude compound *XIX* (150 mg, obtained by hydrogenation of diester *XVII*) in acetonitrile (4 ml). The mixture was stirred at room temperature for 3 h, the solvent was evaporated, the residue was codistilled with toluene (2 × 5 ml) and chromatographed on a column of silica gel (20 g) in toluene–acetone (1 : 1). Compound *XXI* (R_F 0·23) was obtained as an amorphous residue (120 mg; 92%). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·22 t, 6 H (2 × CH_3, $J(CH_3, CH_2)$ = 7·1); 1·82 d, 3 H (CH_3, $J(CH_3, 6)$ = 1·1); 2·06 s, 3 H (CH_3CO); 2·26 m, 2 H (2 × H-2'); 3·76 d, 2 H (2 × H-5', $J(5', 4')$ = 3·5); 3·91 d, 2 H (P–CH_2, J(P, CH) = 8·4); 4·05 dq, 4 H (2 × POCH_2, $J(CH_2, CH_3)$ = 7·1; J(P, OCH) = 8·4); 4·12 m, 1 H (H-4'); 5·20 m, 1 H (H-3', $J(3', 4')$ = 2·0); 6·20 t, 1 H (H-1', $J(1', 2')$ = $J(1', 2'')$ = 7·3); 7·53 q, 1 H (H-6, $J(6, CH_3)$ = 1·1); 11·33 bs, 1 H (NH).

Compound *XXII* (R_F 0·49; 11 mg; 8%) was isolated as further component of the reaction mixture. ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide + CD_3COOD): 1·23 t, 6 H (2 × CH_3, $J(CH_3, CH_2)$ = 7·1); 1·90 d, 3 H (CH_3, $J(CH_3, 6)$ = 1·2); 2·01 s, 3 H (3-CH_2O–$COCH_3$); 2·07 s, 3 H (3'-O–$COCH_3$); 2·32 m, 2 H (2 × H-2'); 3·78 m, 2 H (2 × H-5', $J(5', 4')$ = 3·0; $J(5'', 4')$ = = 4·0); 3·92 d, 2 H (P–CH_2, J(P, CH) = 8·2); 4·05 dq, 4 H (2 × P–OCH_2, $J(CH_2, CH_3)$ = 7·1; J(P, OCH) = 8·5); 4·14 m, 1 H (H-4'); 5·22 m, 1 H (H-3', $J(3', 4')$ = 2·0); 5·80 s, 2 H (NCH_2O); 6·22 t, 1 H (H-1', $J(1', 2')$ = $J(1', 2'')$ = 7·3); 7·65 q 1 H (H-6, $J(6, CH_3)$ = 1·2).

2'-Deoxy-5'-O-phosphonomethylthymidine (*XXIIIa*) and
1-(2-Deoxy-5-O-phosphonomethyl-α-D-*erythro*-pentofuranosyl)thymine (*XXIIIb*)

Bromotrimethylsilane (0·65 ml; 5 mmol) was added at 0°C under argon to a solution of *XIX* (365 mg; 0·93 mmol) in acetonitrile (15 ml) and the mixture was stirred at room temperature for 20 h. The reaction was monitored by electrophoresis on paper and by TLC in ethyl acetate–acetone–ethanol–water (13 : 3 : 4 : 5); R_F of the product 0·20. The reaction mixture was mixed with 2M triethylammonium hydrogen carbonate (TEAB; 1 ml) and taken down. The dry residue was dissolved in water (2 ml), the solution made alkaline with triethylamine and applied onto a column of DEAE Sephadex (HCO_3^- form; 150 ml). After elution with water (100 ml) and a gradient of TEAB (0–0·4 mol l^{-1}, total volume 900 ml), the UV absorbing fractions were combined, the water was evaporated and the residue was several times coevaporated with water to decompose TEAB. The remaining crude product (0·75 g) was dissolved in water (2 ml) and chromatographed on a column of octadecyl-silica gel (100 ml). The column was washed with water at a rate of 3 ml/min and the UV absorbing eluate was collected. After evaporation, the product was converted into the lithium salt by passing through a column of Dowex 50 (Li^+ form; 100 ml) and elution of the lithium salt with water. The eluate was evaporated to give 200 mg (62%)

of a mixture of the lithium salts of *XXIIIa* and *XXIIIb*. According to the ^{1}H NMR spectrum, the ratio of the α and β anomer in the mixture was 3 : 2. ^{1}H NMR spectrum (D_2O), α-anomer: 1·91 d, 3 H (CH_3, $J(CH_3, 6)$ = 1·5); 2·16 m, 1 H (H-2″, $J(2'', 3')$ = 3·0; $J(2'', 2')$ = 14·5); 2·79 m, 1 H (H-2′, $J(2', 3')$ = 7·0); 6·20 q, 1 H (H-1′, $J(1', 2')$ = 7·2; $J(1', 2'')$ = 3·6); 7·76 q, 1 H (H-6, $J(6, CH_3)$ = 1·5). β-Anomer: 1·92 d, 3 H (CH_3, $J(CH_3, 6)$ = 1·3); 2·37 m, 2 H (2 × H-2′); 6·31 t, 1 H (H-1′, $J(1', 2')$ = $J(1', 2'')$ = 6·8); 7·62 q, 1 H (H-6, $J(6, CH_3)$ = 1·3). Other signals, not assigned to the individual α and β anomers: 3·60–3·85 m (H-4′, 2 × H-5′, P–CH_2); 4·50 m (H-3′).

2′-Deoxy-5′-O-phosphonomethylthymidine (*XXIIIa*)

Compound *XVIII* (1·2 g; 1·59 mmol) was hydrogenated in methanol (30 ml) over 10% Pd on active carbon (0·4 g) at room temperature and atmospheric pressure for 24 h. The catalyst was removed by filtration through Celite, the solvent was evaporated and the product purified by chromatography on silica gel (50 g) in ethyl acetate–acetone–ethanol–water (13 : 3 : 4 : 5) (R_F 0·24). The product fractions were combined, the solvent was evaporated and the residue was stirred with methanol. The product was collected and dried in vacuo over phosphorus pentoxide, yield 330 mg (62·5%), E_{Up} 0·82. For $C_{11}H_{17}N_2O_8P$ (336·2) calculated: 8·33% N, 9·21% P; found: 8·44% N, 9·19% P. UV spectrum (pH 7): λ_{max} 267 nm, ε_{max} 9 200. Mass spectrum (FAB; G): 337 (M + H). IR spectrum (KBr): 3 270 (OH, NH); 1 707 (C=O); 1 645–1 663 (C–C + C–O); 1 368 (CH_3); 1 196 (P=O); 1 058 (C–O–C); 946 (P–O–H). ^{1}H NMR spectrum corresponded to the above-described one.

4-N-Benzoyl-2′-deoxy-3′-O-(tetrahydro-2*H*-pyran-2-yl)cytidine (*XXX*)

Imidazole (1·83 g; 26·8 mmol) and tert-butyldiphenylchlorosilane (3·56 ml; 13·4 mmol) were added to a solution of 4-N-benzoyl-2′-deoxycytidine[24] (*XXVII*; 4·45 g; 13·4 mmol) in dimethylformamide (45 ml) and the mixture was stirred at room temperature. After 3 h another portion of tert-butyldiphenylchlorosilane (0·5 ml) was added and stirring was continued for further 4 h. The stirred mixture was then poured into water (900 ml) and set aside in a refrigerator for 24 h. The separated material was collected, washed with water and dried on the air. The thus-obtained product *XXVIII* was stirred with 3,4-dihydro-2*H*-pyran (30 ml) and trifluoroacetic acid (0·8 ml) at room temperature for 10 h. After evaporation, the residue was coevaporated with toluene (2 × 30 ml), mixed with dioxane (40 ml) and stirred with 1M tetrabutylammonium fluoride in tetrahydrofuran (25 ml). After the end of the reaction (monitored by TLC in toluene–acetone 1 : 1) the solution was concentrated, the residue coevaporated with toluene (50 ml) and partitioned between chloroform (100 ml) and water (50 ml). The aqueous phase was extracted with chloroform (2 × 50 ml), the combined chloroform extracts were dried over sodium sulfate and the solvent was evaporated. Chromatography of the residue on silica gel (350 g) in toluene–acetone 1 : 1 (R_F of the product 0·30) afforded 2·8 g (50% related to 4-N-benzoyl-2′-deoxycytidine) of *XXX* as a white amorphous substance. For $C_{21}H_{25}N_3O_6$ (415·4) calculated: 60·71% C, 6·07% H, 10·12% N; found: 59·91% C, 5·92% H, 9·86% N. Mass spectrum (FAB; TG): 416 (M + H). IR spectrum (dilute solution in $CHCl_3$): 3 411 (NH); ($CHCl_3$, saturated solution): 3 414 (NH); 3 300–3 400 (OH bonded); 1 712, 1 701 (amide I); 1 667 (C=O); 1 627 (C=C); 1 521, 1 507 (amide II); 1 488, 1 416 (phenyl); 1 136, 1 115, 1 081, 1 038 (C–O). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·54 m, 6 H (3 × CH_2); 2·13 m, 1 H (H-2″, $J(2'', 1')$ = 7·0; $J(2'', 3')$ = 6·5; $J(2', 2'')$ = 14·0); 2·48 ddd, 1 H (H-2′, $J(2', 1')$ = 6·0; $J(2', 3')$ = 3·0); 3·65 m, 4 H (2 × H-5′, OCH_2); 4·05 m, 1 H (H-4′); 4·36 m, 1 H (H-3′); 4·70 s, 1 H (O–CH–O); 5·12 t, 1 H (OH, $J(5', OH)$ = 5·0); 6·12 t, 1 H (H-1′, $\sum J$ = 13·0); 7·30–8·10 m, 5 H (H-arom.); 7·33 d, 1 H (H-5, $J(5, 6)$ = 7·5); 8·38 d, 1 H (H-6, $J(6, 5)$ = 7·5); 11·22 bs, 1 H (NH).

Diethyl Ester of 4-N-Benzoyl-2'-deoxy-5'-O-phosphonomethyl-3'-O-(tetrahydro-2*H*-pyran-2-yl)cytidine (*XXXI*)

The title compound was prepared from compounds *XXX* (2·6 g, 6·3 mmol) and *IIIa* (2·9 g; 9 mmol) by the procedure described for the preparation of compound *XII*, the reaction time being 2 h. The crude product was purified by chromatography on a column of silica gel (450 g) in toluene–acetone (1 : 1) (R_F 0·25) and the pure product *XXXI* was crystallized from ethanol; yield 2 g (56%), m.p. 140–142°C. For $C_{26}H_{36}N_3O_9P$ (565·5) calculated: 55·22% C, 6·42% H, 7·43% N, 5·48% P; found: 55·11% C, 6·63% H, 7·67% N, 5·32% P. Mass spectrum (SIMS; TG): 566 (M + H). IR spectrum (KBr): 3 220 (NH); 1 696, 1 712 (amide I); 1 670, 1 683 (C=O); 1 635, 1 628 (C=C); 1 571 (C=N); 1 507, 1 521 (amide II); 1 491, 1 496 (phenyl ring); 1 307, 1 324 (C–N); 1 260 (P=O); 1 133, 1 122, 1 081, 1 030 (C–O, P–O–C); 977 (P–O–C); 711 (phenyl). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·25 t, 6 H (2 × CH_3, J = 7·1); 1·52 m, 6 H (H-pyranyl); 2·19 m, 2 H (2 × H-2'); 3·51 m, 2 H (O–CH_2); 3·79 m, 2 H (2 × H-5'); 3·93 d, 2 H (P–CH_2, J(P, CH) = 8·4); 4·07 dq, 4 H (2 × $POCH_2$, J(P, OCH) = 8·6; J(CH_2, CH_3) = 7·1); 4·15 m, 1 H (H-4'); 4·36 m, 1 H (H-3'); 4·71 m, 1 H (O–CH–O); 6·17 bt, 1 H (H-1', J(1', 2') = = 6·3); 7·40 d, 1 H (H-5, J(5, 6) = 7·5); 7·45–7·60 m, 3 H (H-arom.); 7·95–8·05 m, 2 H (H-arom.); 8·31 d, 1 H (H-6, J(6, 5) = 7·5); 11·23 bs, 1 H (NH).

Diethyl Ester of 2'-Deoxy-5'-O-phosphonomethylcytidine (*XXXIII*)

A solution of *XXXI* (1·84 g; 3·25 mmol) in a 1 : 1 mixture of ethanol and 12% aqueous ammonia (150 ml) was stirred at room temperature for 24 h. The solution was taken down in vacuo, the residue was codistilled with ethanol (50 ml) and then stirred with 70% acetic acid (60 ml) at 50°C for 1 h. The reaction mixture was again evaporated, the residue was codistilled with water (2 × 100 ml) and chromatographed on silica gel (180 g) in ethyl acetate–acetone–ethanol–water (15 : 3 : 4 : 3). Yield 1 g (82%) of *XXXIII*, m.p. 155–157°C, R_F 0·27. For $C_{14}H_{24}N_3O_7P$ (377·3) calculated: 11·14% N, 8·21% P; found: 11·32% N, 9·19% P. Mass spectrum (FAB; TG; TFA): 378 (M + H). IR spectrum (KBr): 3 370, 3 133 (NH_2, OH bonded); 1 658 (NH_2 and C=O); 1 633 (C=C); 1 244 (P=O); 1 127, 1 098, 1 064, 1 029, 976, 796 (C–O, P–O–C); 1 165 (P–O–C_2H_5); $CHCl_3$ (dilute solution): 3 531, 3 417 (NH_2). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1·23 t, 6 H (2 × CH_3, J(CH_2, CH_3) = 7·0); 1·91 m, 1 H (H-2'', J(2'', 1') = 7·2; J(2'', 3') = = 6·3; J(2'', 2') = 13·4); 2·13 m, 1 H (H-2', J(2', 1') = 6·2; J(2', 3') = 3·5); 3·69 m, 2 H (2 × H-5', J(5', 4') = 2·5; J(5'', 4') = 4·0; J(5', 5'') = 10·5); 3·87 d, 2 H (P–CH_2, J(P, CH) = 8·4); 3·90 m, 1 H (H-4'); 4·05 dq, 4 H (2 × $POCH_2$, J(P, OCH) = 8·4); 4·20 m, 1 H (H-3'); 5·27 d, 1 H (OH, J(3', OH) = 4·0); 5·75 d, 1 H (H-5, J(5, 6) = 7·5); 6·20 t, 1 H (H-1', J(1', 2'') = 7·2; J(1', 2') = 6·2); 7·12 bs, 2 H (NH_2); 7·75 d, 1 H (H-6, J(6, 5) = 7·5).

2'-Deoxy-5'-O-phosphonomethylcytidine (*XXXIV*)

Bromotrimethylsilane (1·55 ml; 12 mmol) was added to a suspension of compound *XXXIII* (0·91 g; 2·4 mmol) in acetonitrile (80 ml) and the mixture was stirred in an argon atmosphere at room temperature for 7 h. Toluene (50 ml) was added, the solvents were evaporated in vacuo at 30°C and the residue was twice coevaporated with toluene (2 × 50 ml). In the second coevaporation with toluene 2 drops of triethylamine were added. The residue was dissolved in water, (2 ml), the solution made alkaline with triethylamine and applied onto a column of Dowex 1X2 (acetate form; 230 ml). The ion exchanger was first washed with water (500 ml) and then with 0·3M acetic acid. The UV absorbing eluate was collected. The first fractions contained small amount of cytosine (about 30 mg) and monoethyl ester of the desired product (40 mg). Fractions, containing the free phosphonomethyl derivative *XXXIV* were combined and the solvent was

evaporated. Crystallization from water afforded 690 mg (82%) of the product *XXXIV* as a sesquihydrate, m.p. 155–157°C; R_F 0·10 (ethyl acetate–acetone–ethanol–water (13 : 3 : 4 : 5)), E_{Up} 0·81. For $C_{10}H_{16}N_3O_7P.1{\cdot}5\,H_2O$ (348·2) calculated: 34·49% C, 5·49% H, 12·07% N, 8·89% P; found: 34·20% C, 5·46% H, 11·86% N, 8·79% P. UV spectrum (pH 7): λ_{max} 272 nm ε_{max} 9 600; (pH 2): λ_{max} 278 nm, ε_{max} 13 900. Mass spectrum (FAB; TG + TFA): 322 (M + H). IR spectrum (KBr): 3 400–2 900 (NH_2, NH, C– OH); 2 500–2 800 (P–OH); 1 728 (C=O of the protonated base); 1 548 (NH); 1 682 (NH_2); 1 192 (P=O); 1 131, 1 073 (C–O); 937 (P–O–H). ^{1}H NMR spectrum (D_2O): 2·29 t, 2 H (2 × H-2′); 3·55 d, 2 H (P–CH_2, J(P, CH) = 9·0); 3·79 m, 2 H (2 × H-5′); 4·02 m, 1 H (H-4′); 4·32 q, 1 H (H-3′, J = 6·0); 6·08 d, 1 H (H-5, J(5, 6) = 8·0); 6·27 t, 1 H (H-1′, J(1′, 2′) = J(1′, 2″) = 6·9); 7·86 d, 1 H (H-6, J(6, 5) = 8·0).

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