Synthesis, Properties, and Biological Activity of some Nucleoside Cyclic Phosphoramidates

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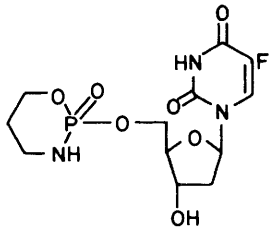
Reaction of the appropriate nucleoside with phosphoryl trichloride and then with *N*-methylethanolamine gave 2',3'-*O*-isopropylidene-5'-*O*-(3"-methyl-1"-oxa-3"-aza-2"-phosphacyclopentan-2"-yl)uridine 2"-oxide (**4**), 5'-*O*-(3"-methyl-1"-oxa-3"-aza-2"-phosphacyclopentan-2"-yl)thymidine 2"-oxide (**5**), and 2'-deoxy-5-fluoro-5'-*O*-(3"-methyl-1"-oxa-3"-aza-2"-phosphacyclopentan-2"-yl)uridine 2"-oxide (**6**). A similar sequence of reactions, but using *N,N'*-dimethylethylenediamine, gave 5'-*O*-(1",3"-dimethyl-1",3"-diaza-2"-phosphacyclopentan-2"-yl)thymidine 2"-oxide (**7**) and 2'-deoxy-5'-*O*-(1",3"-dimethyl-1",3"-diaza-2"-phosphacyclopentan-2"-yl)-5-fluorouridine 2"-oxide (**8**).

Compounds (**4**)—(**8**) were hydrolysed readily in the pH range 6.0—7.0 at 25 °C, the kinetics being first order in hydrogen ions and in substrate. The 1-oxa-3-aza -2-phospha derivatives were hydrolysed more readily than the 1,3-diaza-2-phospha derivatives and the 2'-deoxy-5-fluorouridine derivatives more rapidly than the thymidine derivatives. In each case hydrolysis resulted in the fission of one P–N bond.

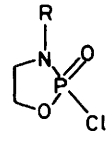
Compound (**8**) inhibited the growth of leukemia L1210 cells. It acted as a thymidylate synthetase inhibitor in the cell culture, but itself was not a substrate for the isolated, purified enzyme.

In a previous communication we reported the synthesis of 2'-deoxy-5-fluoro-5'-*O*-(1"-oxa-3"-aza-2"-phosphacyclohexan-2"-yl)uridine 2"-oxide (**1**).[1] It was hoped that this compound would enter living cells and then be metabolically converted into 2'-deoxy-5-fluorouridine 5'-phosphate by a similar oxidative and degradative pathway to that which activates the anticancer drug cyclophosphamide.[2–4] Compound (**1**) showed only slight activity against sarcoma 180 and no activity against leukemia L1210, however. It appeared, therefore, that the enzymes operative in the activation of cyclophosphamide were not active in this case.

An alternative approach to the problem of introducing a biologically active phosphate into a cell is to use a 'masking group' which will allow penetration into the cell and then by slow, non-enzymic hydrolysis release the required phosphate. The six-membered cyclic phosphoramidate (**1**) was too stable for this purpose; to obtain compounds which would be more readily hydrolysed, the synthesis of nucleoside derivatives containing five-membered phosphoramidate rings was undertaken.

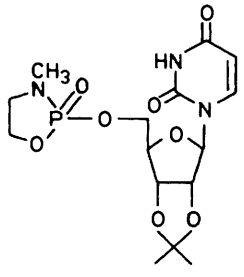


(**1**)



(**2**) R = H
(**3**) R = CH_3

The most direct method appeared to be to condense 2-chloro-1-oxa-3-aza-2-phosphacyclopentane 2-oxide (**2**) with a nucleoside. However, attempts to synthesize (**2**) by the procedure reported by Bersin *et al.*[5] were unsuccessful as were a number of other attempts to obtain this compound. It was considered that this failure was probably due to the reactivity of the NH group in the product which led to polymerisation. The synthesis of the *N*-methyl derivative, (**3**), presented no problems, however, and was achieved in 86% yield by the reaction of *N*-methylethanolamine with phosphoryl trichloride. However, compound (**3**) and the corresponding bromo compound failed to react with nucleosides under a variety of conditions. In order to obtain a compound of the required structure, 2',3'-*O*-isopropylideneuridine was treated with phosphoryl trichloride in the presence of *N*-methylmorpholine and the intermediate so obtained was treated *in situ* with *N*-methylethanolamine to give 2',3'-*O*-isopropylidene-5'-*O*-(3"-methyl-1"-oxa-3"-aza-2"-phosphacyclopentan-2"-yl)uridine 2"-oxide (**4**). This compound was characterised by u.v. and n.m.r. spectroscopy and elemental analysis. The NCH_3 region of the ^1H n.m.r. spectrum consists of a pair of doublets with a phosphorus coupling constant of 10 Hz. The further splitting of these signals is ascribed to the presence of two isomers in the sample resulting from the presence of a chiral phosphorus centre. The hydrolysis of this compound was studied over a range of pH values. The half-life

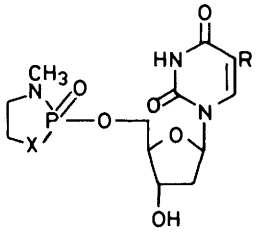


(**4**)

of decomposition at pH 6 and pH 7 at 19 °C was 1—2 h and 24 h respectively. The product of hydrolysis was more polar than (**4**), migrated toward the anode upon paper electrophoresis at pH 10.5, but did not migrate at pH 6.5. The compound showed similar ^1H n.m.r. resonances to compound (**4**) for the pyrimidine and sugar protons and for the 5"-H_2 protons. However the 4"-H_2 multiplet of (**4**) became a triplet in the hydrolysis product and the NCH_3 double doublet became a singlet. These observations are consistent with the scission of the P–N bond of (**4**) to give a product of structure ROP-$(O_2^-)OCH_2CH_2N^+H_2CH_3$ (R = nucleoside residue).

To obtain a similar derivative from thymidine the initial

reaction with phosphoryl trichloride was carried out in the presence of triethyl phosphate as described by Yoshikawa.[6,7] This gives selective reaction at the 5′-hydroxy group. The resulting intermediate was then treated with *N*-methylethanolamine to give 5′-*O*-(3″-methyl-1″-oxa-3″-aza-2″-phosphacyclopentan-2″-yl)thymidine 2″-oxide (**5**). The ^{1}H n.m.r. spectrum of this product is similar in the NCH_3 region to that of (**4**). Again the presence of two diastereoisomers is shown by this and by the presence of two 6-H resonances. Two signals (δ 21.9 and 21.8 p.p.m.) observed in the ^{31}P n.m.r. spectrum are assigned to the two diastereoisomers which are present in the ratio 2:1. Two less intense signals (δ 21.3 and 21.0 p.p.m.) were also observed. These are probably due to the presence of *ca.* 7% of the 3′-*O*-substituted isomers.



(**5**) R = CH_3, X = O
(**6**) R = F, X = O
(**7**) R = CH_3, X = NCH_3
(**8**) R = F, X = NCH_3

Application of similar reactions to 2′-deoxy-5-fluorouridine gave 2′-deoxy-5-fluoro-5′-*O*-(3″-methyl-1″-oxa-3″-aza-2″-phosphacyclopentan-2″-yl)uridine 2″-oxide (**6**). In this case in order to obtain the best yield it was necessary to use a small amount of water in the initial reaction with phosphoryl trichloride in triethyl phosphate as recommended by Yoshikawa for some cases.[6,7] The ^{1}H and ^{31}P n.m.r. spectra of the product indicated a similar situation to that with (**4**) and (**5**); the proportion of 3′-*O*-substituted compound in the product was higher, however (*ca.* 14%). A study of the structure of the products obtained upon hydrolysis of (**5**) and (**6**) gave similar results to those found with (**4**).

By carrying out similar reactions but using *N*,*N*′-dimethylethylenediamine instead of *N*-methylethanolamine, 5′-*O*-(1″,3″-dimethyl-1″,3″-diaza-2″-phosphacyclopentan-2″-yl)thymidine 2″-oxide (**7**) and 2′-deoxy-5′-*O*-(1″,3″-dimethyl-1″,3″-diaza-2″-phosphacyclopentan-2″-yl)-5-fluorouridine 2″-oxide (**8**) were obtained. The ^{1}H and ^{31}P n.m.r. spectra of these products were consistent with the assigned structures and showed the presence of diastereoisotopic NCH_3 groups and an achiral phosphorus centre. The signals for 4″-H_2 and 5″-H_2 were coincident.

The yields of compounds (**4**)—(**8**) were comparatively low (10—20%). This was partly due to their loss by hydrolysis during the isolation procedure. Some improvement was achieved by the use of rapid flash chromatography instead of conventional chromatography [*e.g.* in the case of (**6**), improvement from 18% to 47% yield].

The hydrolysis of some of the compounds synthesized as described above was studied using ^{31}P n.m.r. spectroscopy taking advantage of the large shift which occurs in the spectrum upon ring opening. The results (Table 1) show that the rate of hydrolysis is first order in substrate and in hydrogen ions as is expected.[8-10] The 1-oxa-3-aza-2-phosphacyclopentane derivative (**5**) is hydrolysed more rapidly over the pH range 6.6—7.0 than the 1,3-diaza-2-phosphacyclopentane derivative (**7**), and the 2′-deoxy-5-fluorouridine derivative, (**6**), is hydrolysed about 1.5 times more rapidly than the thymidine derivative (**5**).

The compounds synthesized as described above had the desired properties in that they would be expected to be slowly hydrolysed under physiological conditions. It also appeared reasonable that they might enter living cells and be hydrolysed to give a phosphodiester or an acyclic phosphoramidate. Whether these would give the desired 5′-phosphomonoester would be dependent upon the action of cellular enzymes. The fact that these cyclic phosphoramidates are hydrolysed more rapidly at pH 6 than at pH 7 suggests that they might have preferential action against some tumour cells which are known to have a lower pH than normal cells.[11,12]

Compound (**1**), whose synthesis has been reported elsewhere,[1] and compound (**8**) were found to be inactive as inhibitors of thymidylate synthetase when tested against the isolated enzyme. When tested for antiproliferative activity against leukemia L1210 cells, (**1**) showed very weak activity but (**8**) was appreciably active (*ca.* 0.03 of the activity of 2′-deoxy-5-fluorouridine) (Table 2). The fact that this activity was reduced far more by the addition of thymidine than by 2′-deoxyuridine indicated that (**8**) was acting as a thymidylate synthetase inhibiter. However, compound (**8**) had low activity against a 5-bromo-2′-deoxyuridine-resistant L1210 cell line (which presumably lacks thymidine kinase). One interpretation

Table 1. Rate of hydrolysis of nucleoside cyclic phosphoramidates at 25 °C

	$t_{\frac{1}{2}}$ (min)		
Compound	pH 6.4	pH 6.6	pH 7.0
(**5**)	75	120	300
(**6**)	55	90	180
(**7**)		600	1 920

Table 2. Inhibitory effects of nucleoside cyclic phosphoramidates on the proliferation of murine leukemia L1210 cell lines

	I.D.$_{50}$(μg ml^{-1}) for cell growth			
	L1210			
Compound	Neat compound	Upon addition of 2′-deoxyuridine (125 μg ml^{-1})[a]	Upon addition of thymidine (5 μg ml^{-1})[a]	L1210/BrdU[b]
2′-Deoxy-5-fluorouridine	0.001	0.02	34	
(**1**)	0.265	3.27	720	112
(**8**)	0.037	0.67	426	31.7

[a] Maximum concentration which was not inhibitory to L1210 cell growth. [b] Strain of L1210 cells which was resistant to the inhibitory effect of 5-bromo-2′-deoxyuridine.

of these results is that (**8**) was being degraded to 2′-deoxy-5-fluorouridine which was then phosphorylated to 2′-deoxy-5-fluorouridine 5′-phosphate rather than compound (**8**) being transformed directly into the 5′-phosphate.

Experimental

N.m.r. spectra were recorded on 100 MHz spectrometers (Perkin-Elmer R14 and Varian XL100) with $(CD_3)_2SO$ as the solvent unless otherwise stated. Studies of the rates of hydrolysis were carried out using a Brucker WH400 high-field n.m.r. spectrometer operating for ^{31}P at 162 MHz. Spectra for ^{31}P were all proton-decoupled. The standard was 50% H_3PO_4 or, in aqueous solutions, inorganic phosphate. U.v. spectra were all measured in ethanol on a Perkin-Elmer 552 spectrophotometer, and i.r. spectra on a Perkin-Elmer 257 spectrophotometer.

Column chromatography was carried out on silica gel 60, 230—400 mesh, 0.2—0.63 mm, type 7734 and flash chromatography on silica gel 60, 230—400 mesh, 0.063—0.04 mm (E. Merck A. G. Darmstadt, W. Germany). All experiments were carried out under scrupulously dry conditions unless otherwise indicated.

2-Chloro-3-methyl-1-oxa-3-aza-2-phosphacyclopentane 2-Oxide (**3**).—A mixture of phosphoryl trichloride (7.67 g, 50 mmol) and chloroform (20 ml) was added dropwise to vigorously stirred chloroform (15 ml) maintained at 0—5 °C. Separately, but simultaneously, a solution of *N*-methylethanolamine (3.75 g, 50 mmol) and triethylamine (5.05 g, 50 mmol) in chloroform (20 ml) was also added dropwise. The solution was then warmed to 20 °C, a solution of triethylamine (5.05 g, 50 mmol) in chloroform (15 ml) added, and the mixture was stirred at 25—30 °C for 30 min. The solvent was removed by evaporation under reduced pressure and the residue extracted with diethyl ether (4 × 40 ml); the combined extracts were evaporated under reduced pressure to give the product as a clear liquid (7.0 g, 86%); $\delta_H(CDCl_3)$ 2.8 (3 H, d, *J* 13 Hz, CH_3), 3.2—3.6 (2 H, m, 4-H_2), and 4.2—4.6 (2 H, m, 5-H_2); $\nu_{max.}$ 2 800—3 000, 1 300, 1 040, 1 020, 940, and 725 cm^{-1}. Reaction of this compound with methanol in the presence of triethylamine gave the known 2-methoxy derivative,[13] b.p. 98—110 °C (0.03—0.05 mmHg) (Found: C, 31.7; H, 6.4; P. 20.8. Calc. for $C_4H_{10}OP$: C, 31.8; H, 6.7; N, 9.3; P, 20.5%); $\delta_H(CDCl_3)$ 2.7 (3 H, d, *J* 10 Hz, NCH_3), 3.2—3.5 (2 H, m, 4-H_2), 3.7 (3 H, d, *J* 11 Hz, OCH_3), and 4.1—4.4 (2 H, m, 5-H_2); $\nu_{max.}$ 2 800—3 000, 1 260, 1 060, 1 040, 1 030, 940, and 810 cm^{-1}.

2′,3′-O-Isopropylidene-5′-O-(3″-methyl-1″-oxa-3″-aza-2″-phosphacyclopentan-2″-yl)uridine 2″-Oxide (**4**).—Phosphoryl trichloride (5.0 g, 32 mmol) was dissolved in 1,2-dimethoxyethane (15 ml) and *N*-methylmorpholine (2.4 g, 24 mmol) was added. The solution was cooled to 0 °C and vigorously stirred while a solution of 2′,3′-*O*-isopropylideneuridine (6.8 g, 24 mmol) and *N*-methylmorpholine (1.2 g, 12 mmol) in 1,2-dimethoxyethane (90 ml) was added. The mixture was stirred at 20 °C for 4 h and then cooled to 0 °C and a solution of *N*-methylethanolamine (4.5 g, 60 mmol) and *N*-methylmorpholine (6.2 g, 62 mmol) added dropwise to the vigorously stirred mixture. After being stirred for 1 h at 20 °C the resulting suspension was filtered and the precipitate washed with chloroform (2 × 50 ml). The combined filtrate and washings were evaporated under reduced pressure to give a pale orange oil. This was purified on a silica column which was eluted with ethanol–ethyl acetate (1:3). Pooling of the appropriate fractions and removal of the solvent under reduced pressure gave the *product* (**4**) as a white powder (1.0 g, 11%) (Found: C, 44.4; H, 5.5; N, 10.2; P, 7.7. $C_{15}H_{22}N_3O_8P$ requires C, 44.7; H, 5.5; N, 10.4; P, 7.7%); $\lambda_{max.}$ 259 nm (ε 11 200); $\lambda_{min.}$ 229 nm (ε 2 700); δ_H 1.32 (3 H, s, isopropylidene CH_3), 1.50 (3 H, s, isopropylidene CH_3), 2.55 (3 H, 2 d, J_{H-P} 10 Hz, NCH_3), 3.1—3.4 (2 H, m, 4″-H_2), 4.0—4.3 (5 H, m, 4′-H, 5-H_2, and 5″-H_2), 4.76 (1 H, m, 3′-H), 5.05 (1 H, m, 2′-H), 5.62 (1 H, d, *J* 8 Hz, 5-H), 5.80 (1 H, d, *J* 2 Hz, 1′-H), and 7.70 (1 H, d, *J* 8 Hz, 6-H).

5′-O-(3″-Methyl-1″-oxa-3″-aza-2″-phosphacyclopentan-2″-yl)thymidine 2″-Oxide (**5**).—This was prepared by the same procedure as for (**4**), one mol equiv. of triethyl phosphate being included in the reaction mixture at the first stage. Purification was carried out on a silica column which was eluted with ethanol–chloroform (1:1). The *product* (**5**) was obtained as a white powder (21%), m.p. 58 °C (decomp.) (Found: C, 41.5; H, 5.8. $C_{13}H_{20}N_3O_7P\cdot H_2O$ requires C, 41.2; H, 5.8%); $\lambda_{max.}$ 267 nm (ε 9 000); $\lambda_{min.}$ 235 nm (ε 1 900); δ_H 1.80 (3 H, s, 5-CH_3), 2.13 (2 H, t, 2′-H_2), 2.58 (3 H, 2 d, J_{H-P} 10 Hz, NCH_3), 3.2—3.4 (2 H, m, 4″-H_2), 3.8—4.4 (6 H, m, 3′- and 4′-H, 5′- and 5″-H_2), 5.39 (1 H, OH), 6.19 (1 H, t, 1′-H), 7.48 (1 H, d, 6-H), and 11.2 (1 H, s, NH); δ_P(phosphate buffer; pH 7.0) 21.02 (s, intensity 8.5), 21.26 (s, intensity 9.7), 21.81 (s, intensity 79), and 21.91 p.p.m. (s, intensity 148).

Acidic Hydrolysis of (**4**) *and* (**5**).—A sample of the compound (120 mg) was dissolved in 10^{-3}M hydrochloric acid (25 ml) at room temperature. After 3 h the solution was evaporated to dryness under reduced pressure and the solid residue was dried by trituration with diethyl ether and then *in vacuo*. The ^1H n.m.r. spectra of the product was determined.

Hydrolysis product from (**4**). $\delta(D_2O)$ 1.40 (3 H, s, isopropylidene CH_3), 1.60 (3 H, s, isopropylidene CH_3), 2.78 (3 H, s, NCH_3), 3.25—3.4 (2 H, t, 4″-H_2), 4.0—4.2 (4 H, m, 5′- and 5″-H_2), 4.50 (1 H, m, 4′-H), 4.9—5.1 (2 H, m, 2′- and 3′-H), 5.8—5.9 (2 H, m, 1′- and 5-H), and 7.80 (1 H, d, 6-H).

Hydrolysis product from (**5**). $\delta(D_2O)$ 1.92 (3 H, s, CH_3), 2.38 (2 H, 2 d, 2′-H_2), 2.80 (3 H, s, NCH_3), 3.2—3.4 (2 H, t, 4″-H_2), 4.0—4.2 (5 H, m, 4′-H and 5′- and 5″-H), 4.50 (1 H, m, 3′-H), 6.28 (1 H, t, 1′-H), and 7.63 (1 H, s, 6-H).

2′-Deoxy-5-fluoro-5′-O-(3″-methyl-1″-oxa-3″-aza-2″-phosphacyclopentan-2″-yl)uridine 2″-Oxide (**6**).—Phosphoryl trichloride (0.61 g, 4 mmol) was dissolved in triethyl phosphate (5 ml) and the solution was cooled to 0 °C. 2′-Deoxy-5-fluorouridine (0.5 g, 2 mmol) was added followed by water (10 μl, 0.5 mmol) and the solution was stirred at 4 °C for 38 h. A further portion of phosphoryl trichloride (0.33 g, 2.1 mmol) was added and the solution was stirred at 0 °C for 3 h. The solution was then evaporated under reduced pressure at 40 °C for 30 min to remove any water which may have remained, and was then cooled to −15 °C. A mixture of *N*-methylethanolamine (0.86 g, 11 mmol), *N*-methylmorpholine (1.59 g, 16 mmol), and triethyl phosphate (1 ml) was then added dropwise to the vigorously stirred mixture. After being stirred for 1 h at 0 °C the solution was filtered and the filtrate was evaporated under reduced pressure at 50—60 °C The residue was then purified by silica gel column chromatography using ethanol–ethyl acetate (1:1) as eluant. The *product* (**6**) was obtained as a white powder (18% yield using 'conventional' chromatography; 47% when 'flash chromatography' was used), m.p. 66—73 °C (Found: C, 39.3; H, 4.9; N, 11.2. $C_{12}H_{17}FN_3O_7P$ requires C, 39.5; H, 4.7; N, 11.5%); $\lambda_{max.}$ 270 nm (ε 8 500); $\lambda_{min.}$ 235 nm (ε 1 900); δ_H 2.12 (2 H, m, 2′-H_2), 2.56 (3 H, d, J_{H-P} 10 Hz, NCH_3), 3.2—3.4 (2 H, m, 4″-H_2), 3.9—4.3 (6 H, m, 3′- and 4′-H, 5′- and 5″-H_2), 5.40 (1 H, s, OH), 6.12 (1 H, m, 1′-H), and 7.87 (1 H, 2 d, J_{H-F} 7 Hz, 6-H); δ_P (phosphate buffer: pH 7.0) 21.0 (s, intensity 19), 21.1 (s, intensity 18), 21.8 (s, intensity 113), and 22.0 p.p.m. (s, intensity 118); ϕ_F 166.3 p.p.m. (m, 5-F).

5′-O-(1″,3″-Dimethyl-1″,3″-diaza-2″-phosphacyclopentan-2″-yl)thymidine 2″-Oxide (**7**).—Thymidine (2.2 g, 9 mmol) was suspended in 1,2-dimethoxyethane (3.5 ml) and *N*-methyl-

morpholine (1.2 g, 12 mmol) and triethyl phosphate (1.5 ml) were added. The suspension was cooled to 0 °C and vigorously stirred while a solution of phosphoryl trichloride (1.7 g, 10.8 mmol) in 1,2-dimethoxyethane (10 ml) was added dropwise. After being stirred for 3 h at room temperature, the suspension was cooled to 0 °C and a solution of *N,N'*-dimethylethylenediamine (0.87 g, 10 mmol) and *N*-methylmorpholine (2.1 g, 21 mmol) in 1,2-dimethoxyethane (10 ml) was added dropwise to the vigorously stirred mixture. The suspension was stirred at room temperature for 2 h, the solvent was evaporated off under reduced pressure, and the residue was extracted with ethanol–chloroform (1:9) (2 × 35 ml). The extracts were evaporated under reduced pressure and the resulting yellow oil purified on a column of silica gel which was eluted with ethanol–chloroform (1:9) to give the *product* (**7**) as a white solid (500 mg, 15%), m.p. 58 °C (Found: C, 42.9; H, 6.1. $C_{14}H_{23}N_4O_6P$ requires C, 42.9; H, 6.4%); $\lambda_{max.}$ 267 nm (ε 9 300); $\lambda_{min.}$ 235 nm (ε 1 100); $\delta_H(CDCl_3)$ 1.94 (3 H, s, 5-CH_3), 2.30 (2 H, m, 2'-H_2), 2.62 (6 H, 2 d, *J* 10 Hz, NCH_3), 3.16 (4 H, d, *J* 10 Hz, 4''- and 5''-H_2), 4.0—4.2 (3 H, m, 4'-H and 5'-H_2), 4.45 (1 H, m, 3'-H), 6.33 (1 H, t, 1'-H), 7.50 (1 H, s, 6-H), and 9.20 (1 H, s, NH); δ_P (phosphate buffer; pH 7.0) 22.8 (s, intensity 20), 25.9 (s, intensity 9), and 26.5 p.p.m. (s, intensity 144).

2'-Deoxy-5'-O-(1'',3''-dimethyl-1'',3''-diaza-2''-phosphacyclopentan-2''-yl)-5-fluorouridine 2''-Oxide (**8**).—Phosphoryl trichloride (0.62 g, 4 mmol) was dissolved in triethyl phosphate (5 ml) and the solution was cooled to 0 °C. 2'-Deoxy-5-fluorouridine (0.5 g, 2.03 mmol) and water (5 μl, 0.25 mmol) were added and the mixture was stirred at 0 °C for 48 h. More phosphoryl trichloride (0.33 g, 2.1 mmol) was then added and the reaction allowed to proceed for a further 2 h at 0 °C. The solution was evaporated under reduced pressure at 40 °C for 30 min to remove any remaining water and was then cooled to 0 °C. A solution of *N*-methylmorpholine (1.6 g, 16 mmol) and *N,N*-dimethylethylenediamine (0.87 g, 10 mmol) in triethyl phosphate (2 ml) was added dropwise to the stirred mixture which was then stirred for 17 h at *ca.* −10 °C. The mixture was then evaporated to dryness under reduced pressure at 40—50 °C and the residue purified on a silica column. The column was eluted with ethanol–chloroform (1:3), and appropriate fractions collected and evaporated to dryness to give the *product* (**8**) as a white powder (180 mg, 23%), m.p. 60—70 °C (decomp.) (Found: C, 40.6; H, 6.0; N, 14.2. $C_{13}H_{20}FN_4O_6P\cdot 0.5H_2O$ requires C, 40.3; H, 5.3; N, 14.5%); $\lambda_{max.}$ 269 nm (ε 7 200); $\lambda_{min.}$ 234 nm (ε 1 000); $\delta_H(CDCl_3)$ 2.40 (2 H, m, 2'-H_2), 2.63 (6 H, 2 d, *J* 10 Hz, NCH_3), 3.15 (4 H, d, *J* 10 Hz, 4''- and 5''-H_2), 3.90 (1 H, m, 4'-H), 4.10 (2 H, m, 5'-H_2), 4.50 (1 H, m, 3'-H), 4.90 (1 H, s, OH), 6.27 (1 H, m, 1'-H), and 7.86 (1 H, d, *J* 6.5 Hz, 6-H); $\phi_F(CDCl_3–CD_3OD)$ 166.0 p.p.m. (m, J_1 7 Hz).

Hydrolysis Studies.—The compounds synthesized as described above were dissolved in buffers of the appropriate pH at room temperature. At intervals samples were removed and fractionated by t.l.c. in acetonitrile. The areas of the plate occupied by starting material and by hydrolysis product were eluted and the amount of material in each determined by u.v. spectroscopy. Some of the compounds were examined in more detail by the use of ^{31}P n.m.r. spectroscopy as follows. Samples of the compounds (0.1 mmol) were dissolved in 0.5M KH_2PO_4–NaOH buffer (3.0 ml) at pH values in the range 6.4—7.0. The ^{31}P n.m.r. spectra were recorded at appropriate intervals at 162 MHz at 25 °C. The extent of ring opening was determined by measuring the decrease in intensity of the ^{31}P n.m.r. signal for the original compound (δ_P *ca.* 22—25 p.p.m.) and the increase in signal for the ring-opened compound (δ_P *ca.* −2 to +8 p.p.m.). The results are shown in Table 1.

Biological Tests.—The assays for thymidylate synthetase inhibition and for antiproliferative activity against leukemia L1210 cells were carried out as previously described.[14] Against purified thymidylate synthetase compounds (**1**) and (**8**) showed K_i/K_m values of 81 and 10 respectively (*cf.* 0.016 for 2'-deoxy-5-fluorouridine 5'-phosphate). The activities of these compounds against L1210 leukemia cells are shown in Table 2.

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