Synthesis of some Nucleoside Cyclic Phosphoramidates and Related Compounds *via* Phosphoramidites

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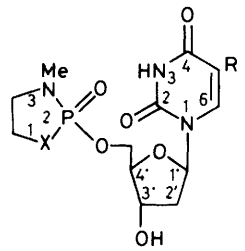
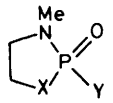
Reaction of 2-chloro-3-methyl-1-oxa-3-aza-2-phosphacyclopentane with thymidine and with 2'-deoxy-5-fluorouridine gave their 3',5'-bis-*O*-(3-methyl-1-oxa-3-aza-2-phosphacyclopentan-2-yl) derivatives (**5**) and (**6**). Reaction of these nucleosides with 2-chloro-1,3-dimethyl-1,3-diaza-2-phosphacyclopentane gave 3',5'-bis-*O*-(1,3-dimethyl-1,3-diaza-2-phosphacyclopentan-2-yl) derivatives (**7**) and (**8**). The phosphoramidites, (**7**) and (**8**) were oxidised with dinitrogen tetraoxide to the corresponding phosphoramidates (**9**) and (**10**). Attempts to oxidise (**5**) and (**6**) in a similar way resulted in opening of the phosphoramidate ring. Treatment of compounds (**5**)—(**8**) with sulphur gave the corresponding phosphorothioamidates (**11**)—(**14**). The hydrolysis of 3',5'-bis-*O*-(1,3-dimethyl-2-oxo-1,3-diaza-2-phosphacyclopentan-2-yl)thymidine (**9**) was studied using ^{31}P n.m.r. spectroscopy. At pH 7.0 and 25 °C the half-life was 21 h. Both phosphorus heterocyclic rings opened at the same rate. The hydrolysis of the second ring proceeded at *ca.* three times the rate of hydrolysis of the first. 2'-Deoxy-5-fluoro-3',5'-bis-*O*-(1,3-dimethyl-2-oxo-1,3-diaza-2-phosphacyclopentan-2-yl)-uridine (**10**) did not inhibit isolated purified thymidylate synthetase and was only a weak inhibitor of leukemia L1210 cell growth.

The synthesis of nucleoside cyclic phosphoramidates of the general structure (**1**) in which the nucleoside has been selectively substituted at the 5'-*O*- position has already been reported.[1] These compounds are slowly hydrolysed under physiological conditions and are potentially a source of nucleoside 5'-phosphates *in vivo*. The compounds were obtained by treating the deoxyribonucleoside with phosphoryl chloride under the conditions described by Yoshikawa *et al*[2,3] and then with the appropriate amine. The yields were not good, however, so

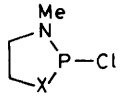


(**1**) R = Me or F
X = O or NMe

alternative routes were sought. It had already been ascertained that compounds of structure (**2**) did not react readily with nucleosides so attention was turned to the corresponding phosphorus(III) compounds (**3**) and (**4**) which were expected to be much more reactive. The cyclic phosphoramidites produced could then be oxidised to the required phosphoramidates.

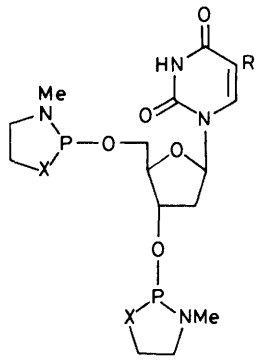


(**2**) X = O or NMe
Y = Cl or Br



(**3**) X = O
(**4**) X = NMe

2-Chloro-3-methyl-1-oxa-3-aza-2-phosphacyclopentane (**3**) was synthesised by the action of phosphorus trichloride on 2-methylaminoethanol as described in the literature[4] and allowed to react with thymidine in the presence of pyridine. The reaction was rapid and non-selective giving 3',5'-bis-*O*-(3-methyl-1-oxa-3-aza-2-phosphacyclopentan-2-yl)thymidine (**5**). Many attempts were made to obtain reaction only at the 5'-*O*-position but without success.



(**5**) R = Me, X = O
(**6**) R = F, X = O
(**7**) R = Me, X = NMe
(**8**) R = F, X = NMe

In the ^1H n.m.r. spectrum of (**5**) the multiplicity of the NMe signals shows the non-equivalence of the 3'- and 5'- phosphorus heterocycles and the presence of several isomers arising from the chirality at the phosphorus atoms. The presence of several isomers is confirmed by the ^{31}P n.m.r. spectrum. Eight signals would be expected; seven signals are actually observed indicating the coincidence of two signals.

A similar reaction was carried out with 2'-deoxy-5-fluorouridine to give 2'-deoxy-5-fluoro-3',5'-bis-*O*-(3-methyl-1-oxa-3-aza-2-phosphacyclopentan-2-yl)uridine (**6**). The NMe resonances in the ^1H n.m.r. spectrum are observed as only two sets of doublets, the signals for the different isomers being

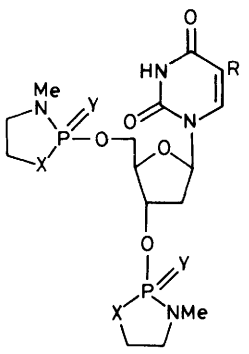
coincident. However in the 5-F decoupled spectrum the 6-H signal appears as four singlets of approximately equal intensity, corresponding to the four isomers. The ^{19}F n.m.r. spectrum consists of three sets of doublets with a chemical shift similar to that of 5-F in 2′-deoxy-5-fluorouridine. On changing the frequency from 56.4 to 94.1 MHz only two sets of doublets are observed and there is a significant change in line-shape. This is conclusive evidence for the presence of more than one molecular species.

Treatment of thymidine and 2′-deoxy-5-fluorouridine with 2-chloro-1,3-dimethyl-1,3-diaza-2-phosphacyclopentane (**4**) gave 3′,5′-bis-*O*-(1,3-dimethyl-1,3-diaza-2-phosphacyclopentan-2-yl)thymidine (**7**) and 2′-deoxy-5-fluoro-3′,5′-bis-*O*-(1,3-dimethyl-1,3-diaza-2-phosphacyclopentan-2-yl)uridine (**8**) respectively. The absence of chirality at phosphorus in these compounds meant that only one isomer was present.

Compounds (**5**)—(**8**) were very susceptible to hydrolysis, decomposing even in moist air. They were characterised by n.m.r. and u.v. spectroscopy but satisfactory elemental analyses were not obtained.

A number of reagents can be used to oxidise phosphites to phosphates. When applied to compounds (**5**)—(**8**) the following were inactive: oxygen, oxygen/Pt catalyst, mercury(II) oxide, and silver(II) oxide. Compounds (**6**) and (**8**) reacted rapidly with aqueous iodine at low temperature.[5] However, despite the numerous conditions tried, oxidation was always accompanied by opening of the phosphorus heterocycle. Oxidation of these cyclic phosphoramidites to cyclic phosphoramidates could be achieved by the use of *m*-chloroperbenzoic acid. However it was extremely difficult to remove *m*-chlorobenzoic acid from the products, so this was not a satisfactory procedure.

The best method for the oxidation of compounds (**7**) and (**8**) was to use dinitrogen tetraoxide under carefully controlled conditions which gave 3′,5′-bis-*O*-(1,3-dimethyl-2-oxo-1,3-diaza-2-phosphacyclopentan-2-yl)thymidine (**9**) and 2′-deoxy-5-fluoro-3′,5′-bis-*O*-(1,3-dimethyl-2-oxo-1,3-diaza-2-phosphacyclopentan-2-yl)uridine (**10**) respectively in good yield. The



(**9**) R = Me; X = NMe; Y = O
(**10**) R = F; X = NMe; Y = O
(**11**) R = Me; X = O; Y = S
(**12**) R = F; X = O; Y = S
(**13**) R = Me; X = NMe; Y = S
(**14**) R = F; X = NMe; Y = S

compounds had the expected characteristic u.v. and n.m.r. spectra. Attempts to oxidise (**5**) and (**6**) by the same procedure always resulted in opening of the phosphorus heterocycle, so no satisfactory method has been obtained.

In view of this it was decided to treat the cyclic phosphoramidites with sulphur to give phosphorothioamidates which it was hoped might have the required hydrolytic properties. Compounds (**5**)—(**8**) reacted readily to give the phosphorothioamidates, (**11**)—(**14**). The n.m.r. spectra of (**11**) and (**12**) show similar characteristics to those of (**5**) and (**6**) in that they indicate the presence of stereoisomers owing to the chirality of the phosphorus centres. These features are absent from the spectra of (**13**) and (**14**). The mass spectra of (**11**)—(**14**) show weak molecular ion peaks and characteristic satellite peaks for some fragments due to the presence of ^{34}S.

The hydrolysis of compound (**11**) at room temperature in the pH range 3—8 was studied. After 1h approximately 50 and 10% hydrolysis occurred at pH 3 and 5 respectively. The compound was effectively stable to hydrolysis in the pH range 6—8. These properties made it unlikely that this type of compound would act as a source of organic phosphate in living cells.

A study of the hydrolysis of compound (**9**) was made using ^{31}P n.m.r. spectroscopy. At 25 °C and pH 7.0 the half-life was 21 h. The phosphoramidate rings were cleaved at one P–N bond and those at the 3′-*O*- and 5′-*O*-positions were cleaved at about equal rates. The intermediates with one ring opened were then hydrolysed further to give the product with both rings opened. Hydrolysis of the second ring occurred at about three times the rate of hydrolysis of the first. These hydrolytic reactions all showed first-order kinetics.

Compound (**10**) was inactive as an inhibitor of isolated, purified thymidylate synthetase from L1210 cells and only very weakly active as an inhibitor of the growth of leukemia L1210 cells.

Experimental

N.m.r. spectra were recorded on 100 MHz spectrometers (Perkin-Elmer R14 or Varian XL100) with $CDCl_3$ as solvent unless otherwise stated. Studies on the rate of hydrolysis were carried out using a Bruker WH400 highfield 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 solution, inorganic phosphate. U.v. spectra were measured in ethanol. Column chromatography was carried out on silica gel 60, 70—230 mesh, 0.2—0.063 mm, type 7734 (E. Merck A. G., Darmstadt, W. Germany). All experiments were carried out under scrupulously dry conditions unless otherwise stated.

2-Chloro-3-methyl-1-oxa-3-aza-2-phosphacyclopentane (**3**).[4]—A solution of phosphorus trichloride (10.3 g, 6.6 ml, 75 mmol) in chloroform (20 ml) and a solution of 2-methylaminoethanol (5.6 g, 5.25 ml, 75 mmol) and triethylamine (7.6 g, 10.5 ml, 75 mmol) in chloroform (20 ml) were added separately but simultaneously with vigorous stirring to chloroform (20 ml) under nitrogen at −25 to −35 °C. A solution of triethylamine (7.6 g, 10.5 ml, 75 mmol) in chloroform was then added dropwise with vigorous stirring and the solution stirred at room temperature for 15 min. The solvent was removed by evaporation under reduced pressure at 20—25 °C and the residue extracted with diethyl ether (2 × 75 ml) in an atmosphere of nitrogen. The ether was distilled off under reduced pressure and the residual oil distilled. The product (3 g, 33% yield) was collected as a colourless oil, b.p. 45—55 °C (0.1—0.5 mmHg); δ_H 2.73 (3 H, d, NMe, *J* 16 Hz), 2.9—3.4 (2 H, m, 4-H), and 4.3—4.7 (2 H, m, 5-H); $\nu_{max.}$ 2 900, 1 010, 925, and 780 cm^{-1}.

3′,5′-Bis-O-(3-methyl-1-oxa-3-aza-2-phosphacyclopentan-2-yl)thymidine (**5**).—A suspension of thymidine (250 mg, 1.0 mmol) in chloroform (40 ml) and triethylamine (0.25 g, 0.35 ml, 2.5 mmol) was cooled to 0 °C and compound (**3**) (360 mg, 2.61 mmol) added with vigorous stirring. The suspension was stirred for 1 h at room temperature and then water (30 ml) was added. The chloroform layer was separated, dried ($MgSO_4$), and the

solvent removed by evaporation under reduced pressure. Trituration of the residue with diethyl ether (15 ml) gave the *product* as a hygroscopic white solid (250 mg, 55% yield); $\lambda_{max.}$ 266 nm (ε 10 750); $\lambda_{min.}$ 234 nm (ε 1 900); δ_H 1.94 (3 H, s, Me), 2.20 (2 H, m, 2-H'), 2.71—2.76 (6 H, 3 × d, NMe, *J* 12 Hz), 2.9—3.3 (4 H, m, CH_2N), 3.8—4.7 (8 H, m, 3'-H, 4'-H, 5'-H, CH_2CH_2N), 6.25 (1 H, t, 1'-H), and 7.58 (1 H, s, 6-H); δ_p −139 p.p.m. (m).

*2'-Deoxy-5-fluoro-3',5'-bis-*O*-(3-methyl-1-oxa-3-aza-2-phosphacyclopentan-2-yl)uridine* (**6**).—This compound prepared by the same procedure as for compound (**5**), was isolated as a white *solid* (53%); $\lambda_{max.}$ 269 nm (ε 9 000); $\lambda_{min.}$ 235 nm (ε 2 100); δ 2.30 (2 H, m, 2'-H), 2.75 (6 H, 2 × d, NMe, *J* 11 Hz), 2.9—3.2 (4 H, m, CH_2N), 3.8—4.7 (8 H, m, 3'-H, 4'-H, 5'-H, CH_2CH_2N), 6.30 (1 H, t, 1'-H), 8.03 (1 H, 4 × d, 6-H, *J* 7 Hz), and 8.85 (1 H, s, NH); φ_F 170 p.p.m. (m).

2-Chloro-1,3-dimethyl-1,3-diaza-2-phosphacyclopentane (**4**).—This compound was prepared essentially by the procedure described for compound (**3**) except that the amine used was *N,N'*-dimethylethylenediamine and the reaction was carried out at −40 to −50 °C; the final treatment with triethylamine was at −30 °C. The *product*, obtained by distillation, was a clear, colourless liquid (66%), b.p. 50—60 °C (0.1 mmHg) (Found: C, 31.8; H, 6.3; N, 18.6. $C_4H_{10}ClN_2P$ requires C, 31.5; H, 6.6; N, 18.4%); δ 2.76 (6 H, d, NMe, *J* 15 Hz) and 3.30 (4 H, d, CH_2, *J* 7.5 Hz); $\nu_{max.}$ 2 850, 1 020, 940, and 705 cm^{-1}.

*3',5'-Bis-*O*-(1,3-dimethyl-1,3-diaza-2-phosphacyclopentan-2-yl)thymidine* (**7**).—This compound, prepared by essentially the same procedure as compound (**5**) except that the thymidine was treated with compound (**4**), was isolated as a white *solid* (77%); $\lambda_{max.}$ 267.5 nm (ε 9 300); $\lambda_{min.}$ 237 nm (ε 3 200); δ 1.95 (3 H, s, Me), 2.20 (2 H, m, 2'-H), 2.70 (12 H, 2 × d, NMe, *J* 13 Hz), 3.20 (8 H, m, CH_2N, CH_2CH_2N), 3.78 (2 H, m, 5'-H), 3.96 (1 H, m, 4'-H), 4.50 (1 H, m, 3'-H), 6.30 (1 H, t, 1'-H), and 7.73 (1 H, s, 6-H); δ_P −132 (s), and −127 p.p.m. (s).

*2'-Deoxy-5-fluoro-3',5'-bis-*O*-(1,3-dimethyl-1,3-diaza-2-phosphacyclopentan-2-yl)uridine* (**8**).—This compound prepared by a similar procedure to that used for compound (**7**), was isolated as a white crystalline *solid* (83%); $\lambda_{max.}$ 268 nm (ε 7 300); $\lambda_{min.}$ 235 nm (ε 2 400); δ 2.20 (2 H, m, 2'-H), 2.71—2.75 (12 H, 2 × d, J_1 13 Hz, J_2 14 Hz), 3.0—3.3 (8 H, m, CH_2N, CH_2CH_2N), 3.83 (2 H, m, 5'-H), 4.03 (1 H, d, 4'-H), 4.50 (1 H, m, 3'-H), 6.32 (1 H, t, 1'-H), and 8.19 (1 H, d, 6-H, *J* 7 Hz); φ_F 164.5 p.p.m. (d, *J* 8 Hz); $\nu_{max.}$ 3 440, 2 920, 1 710, 1 470, 1 030, and 930 cm^{-1}.

*3',5'-Bis-*O*-(1,3-dimethyl-2-oxo-1,3-diaza-2-phosphacyclopentan-2-yl)thymidine* (**9**).—Dinitrogen tetraoxide was bubbled through a solution of compound (**5**) (150 mg, 0.32 mmol) in dichloromethane (40 ml) at −78 °C. After several minutes, a pale green colour developed and the flow of oxidant ceased. The colour was discharged by reducing the pressure to 0.1 mmHg at −70 °C for 30 min. The solvent was then removed by evaporation under reduced pressure at 0 °C and the residue dried to give the *product* as a white crystalline solid (150 mg, 94%), m.p. 75—85 °C (decomp.) (Found: C, 40.1; H, 6.0. $C_{18}H_{32}N_6O_7P_2 0.35CHCl_3$ requires C, 40.2; H, 6.0%); $\lambda_{max.}$ 264 nm (ε 8 000); $\lambda_{min.}$ 234 nm (ε 2 500); δ 2.00 (3 H, d, Me, *J* 1 Hz), 2.40 (2 H, m, 2'-H), 2.6—2.8 (12 H, m, NMe, *J* 10 Hz), 3.1—3.3 (8 H, 2 × d, CH_2N, CH_2CH_2N, *J* 10 Hz), 4.12 (2 H, m, 5'-H), 4.26 (1 H, m, 4'-H), 4.95 (1 H, m, 3'-H), 6.46 (1 H, q, 1'-H), 7.65 (1 H, d, 6-H, *J* < 1 Hz), and 9.26 (1 H, s, NH); δ_P (phosphate buffer, pH 7.0) 25.44 (s, intensity 83) and 26.05 p.p.m. (s, intensity 79); $\nu_{max.}$ 3 440, 2 940, 1 700, 1 470, 1 200—1 300, 1 035, 940, and 740 cm^{-1} [Found: (E.I., 70 eV): M^+, *m/z* 506.1808. $C_{18}H_{32}N_6O_7P_2$ requires *M*, 506.1807].

*2'-Deoxy-5-fluoro-3',5'-bis-*O*-(1,3-dimethyl-2-oxo-1,3-diaza-2-phosphacyclopentan-2-yl)uridine* (**10**).—A solution of compound (**6**) (460 mg, 0.96 mmol) in dichloromethane (35 ml) was cooled to −78 °C and to it was added a standardised solution of dinitrogen tetraoxide in dichloromethane (6.7 ml, sufficient to oxidise 1.90 mmol of phosphite) with vigorous stirring. After 10 min at −78 °C an additional amount of the solution of dinitrogen tetraoxide (0.1 ml) was added and the solution stirred at −78 °C for a further 15 min. The solution was then evaporated to dryness under reduced pressure at 25 °C to give the *product* as a crystalline white solid (484 mg, 95%), m.p. 88—92 °C (Found: C, 38.4; H, 6.2; N, 16.1. $C_{17}H_{29}FN_6O_7P_2 \cdot H_2O$ requires C, 38.6; H, 5.9; N, 15.9%); $\lambda_{max.}$ 267 nm (ε 8 500); $\lambda_{min.}$ 235.5 nm (ε 2 400); δ 2.30 (2 H, m, 2'-H), 2.65 (12 H, 2 × d, NMe, *J* 10 Hz), 3.17 (8 H, d, CH_2N, CH_2N, *J* 10 Hz), 4.20 (3 H, m, 4'-H, 5'-H), 4.95 (1 H, m, 3'-H), 6.36 (1 H, t, 1'-H), 7.90 (1 H, d, 6-H, *J* 7 Hz), and 10.4 (1 H, s, NH); δ_P ($CDCl_3/CD_3OD$) 25.4 p.p.m. (d); φ_F ($CDCl_3/CD_3OD$) 165.1 p.p.m. (d, *J* 7 Hz).

*3',5'-Bis-*O*-(3-methyl-2-thioxo-1-oxa-3-aza-2-phosphacyclopentan-2-yl)thymidine* (**11**).—To a solution of compound (**5**) (200 mg, 0.44 mmol) in chloroform (20 ml), sulphur (70 mg, 2.2 mmol) was added and the suspension stirred at room temperature for 1 h. It was then filtered, the filtrate evaporated to dryness under reduced pressure, and the residue washed with diethyl ether (10 ml) and then carbon tetrachloride (30 ml). The solid residue was purified by chromatography on a column of silica gel which was eluted with ethanol–chloroform (1:9). Appropriate fractions were collected and the solvent evaporated under reduced pressure to give the *product* as a white powder (50 mg, 22%), m.p. 160—163 °C (Found: C, 37.3; H, 5.1; N, 10.7. $C_{16}H_{26}N_4O_7P_2S_2$ requires C, 37.5; H, 5.1; N. 10.9%); $\lambda_{max.}$ 265 nm (ε 9 400); $\lambda_{min.}$ 234 nm (ε 2 400); δ 2.00 (3 H, s, Me) 2.2—2.4 (2 H, m, 2'-H), 2.6—2.8 (12 H, 2 × d, NMe, *J* 12.5 Hz), 3.3—3.5 (4 H, m, CH_2N), 4.2—4.4 (7 H, m, 4'-H, 5'-H, CH_2CH_2N), 5.20 (1 H, m, 3'-H), 6.41 (1 H, q, 1'-H), 7.54 (1 H, m, 6-H), and 8.80 (1 H, s, NH); δ_P −70.6 p.p.m. (m); *m/z* (E.I., 70 eV) 512 (M^+, < 1%), 387 (4%), 359 (6%), 306 (6%), 233 (40%), 206 (10%), 136 (20%), 81 (100%), 57 (12%), and 42 (8%).

*2'-Deoxy-5-fluoro-3',5'-bis-*O*-(3-methyl-2-thioxo-1-oxa-3-aza-2-phosphacyclopentan-2-yl)uridine* (**12**).—This compound, prepared from compound (**6**) by the same procedure as described above for compound (**11**), was isolated as a white *powder* (30%), m.p. 138—142 °C (Found: C, 34.9; H, 4.7; N, 10.5; $C_{15}H_{23}FN_4O_7P_2S_2$ requires C, 34.9; H, 4.5; N, 10.9%); $\lambda_{max.}$ 268 nm (ε 8 000), $\lambda_{min.}$ 239 nm (ε 3 500); δ 2.20 (2 H, m, 2'-H), 2.78—2.80 (6 H, 2 × d, NMe, *J* 12 Hz), 3.3—3.5 (4 H, m, CH_2N), 4.2—4.5 (7 H, m, 4'-H, 5'-H, CH_2CH_2N), 6.40 (1 H, m, 1'-H), 7.90 (1 H, 4 × d, 6-H, *J* 7 Hz), and 9.40 (1 H, s, NH); δ_P −70 p.p.m. (m); φ_F 165.0 p.p.m. (m); *m/z* (E.I., 12 eV) 517 (M^+) 363, 306, 233 (base peak), 210, 154, 138, 81, and 57.

*3',5'-Bis-*O*-(1,3-dimethyl-2-thioxo-1,3-diaza-2-phosphacyclopentan-2-yl)thymidine* (**13**).—This compound, prepared from compound (**7**) by the procedure described for compound (**11**) except that the reaction with sulphur was allowed to proceed for 3 h, was isolated in 88% yield, m.p. 198—201 °C (Found: C, 39.6; H, 5.9; N, 15.2. $C_{18}H_{32}N_6O_5P_2S_2$ requires C, 40.1; H, 6.0; N, 15.6%); $\lambda_{max.}$ 264 nm (ε 9 500); $\lambda_{min.}$ 233 nm (ε 2 400); δ 2.05 (3 H, d, CH_3, *J* 1.3 Hz), 2.2—2.3 (2 H, m, 2'-H), 2.6—2.8 (12 H, 3 × d, NMe, *J* 12.5 Hz), 3.1—3.4 (8 H, m, CH_2N, CH_2CH_2N), 3.9—4.2 (3 H, m, 4'-H, 5'-H), 5.07 (1 H, m, 3'-H), 6.46 (1 H, q, 1'-H), and 7.66 (1 H, d, 6-H, *J* 1.3 Hz); δ_P

– 68.4 (s), –68.0 p.p.m. (s); *m/z* (E.I., 70 eV) 538 (M^+ < 1%), 413 (1%), 372 (2%), 297 (3%), 243 (20%), 148 (30%), 102 (20%), 81 (100%), and 44 (88%).

2′-Deoxy-5-fluoro-3′,5′-bis-O-(1,3-dimethyl-2-thioxo-1,3-diaza-2-phosphacyclopentan-2-yl)uridine (**14**).—This compound, prepared from compound (**8**) as described above for the synthesis of (**13**), was isolated as a white *powder* (71%), m.p. 188—192 °C (Found: C, 37.3; H, 5.3; N, 15.2. $C_{17}H_{29}FN_6O_5P_2S_2$ requires C, 37.6; H, 5.4; N, 15.5%); $\lambda_{max.}$ 267 nm (ε 8 800); $\lambda_{min.}$ 235 nm (ε 2 400); δ 2.1—2.3 (2 H, m, 2′-H), 2.66—2.73 (12 H, 2 × d, NMe, *J* 12 Hz), 3.1—3.5 (8 H, m, CH_2N, CH_2CH_2N), 4.1—4.3 (3 H, m, 4′-H, 5′-H), 5.08 (1 H, m, 3′-H), 6.42 (1 H, t, 1′-H), and 7.98 (1 H, d, 6-H, *J* 7 Hz); δ_P –69.2 (s), and –68.4 p.p.m. (s); φ_F 163.9 p.p.m. (d, *J* 7 Hz) [Found (E.I., 70 eV): M^+ *m/z* 542.1092. $C_{17}H_{29}FN_5O_5P_2S_2$ requires *M*, 542.1096].

Hydrolysis Studies.—The rate of hydrolysis of compound (**9**) was determined at 25 °C in 0.5M-phosphate buffer, pH 7.0, by measuring the decrease in the ^{31}P n.m.r. signals at δ *ca.* 25 p.p.m. and the increase in those at δ *ca.* 8 p.p.m. It was possible to identify ring opening at the 5′-*O*- or 3′-*O* positions. The results are summarised in the discussion.

Biological Tests.—Compound (**10**) was examined for inhibitory activity against isolated purified thymidylate synthetase and against leukemia L1210 cells as previously described.[6] The compound was essentially inactive against the isolated enzyme and it inhibited the growth of L1210 cells by 50% at 0.320 μg/ml (*ca.* 0.003 the activity of 2′-deoxy-5-fluorouridine).

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