Synthesis and Biological Properties of Some Cyclic Phosphotriesters Derived from 2′-Deoxy-5-fluorouridine

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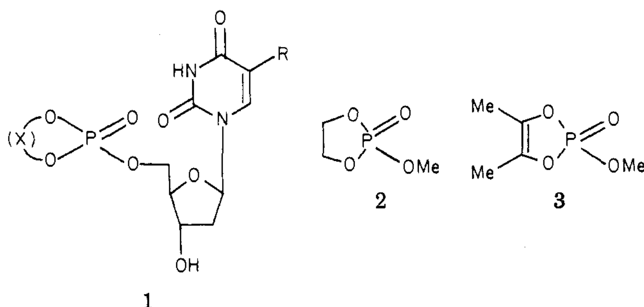
The following derivatives of 2′-deoxy-5′-*O*-1″,3″,2″-dioxaphosphacyclohex-2″-yluridine 2″-oxide have been synthesised: 5-fluoro (4), 5″-(benzyloxy)-5-methyl (6), 5″-(benzyloxy)-5-fluoro (7), 5″-hydroxy-5-methyl (8), 5-fluoro-5″-hydroxy (9), 5″,5″-difluoro-5-methyl (11), and 5,5″,5″-trifluoro (12). Compounds 4, 9, and 12 have been evaluated for their inhibitory effects on the growth and metabolism of murine leukemia L1210 cells. Compound 12 was nearly as potent as 2′-deoxy-5-fluorouridine in its inhibitory effect on these cells (ID_{50} = 0.003 and 0.001 μg/mL, respectively). Compounds 4 and 9 were about 300 times less active than 12. None of the compounds was an inhibitor of the cell-free thymidylate synthetase, although their antiproliferative effects were achieved by the inhibition of this enzyme.

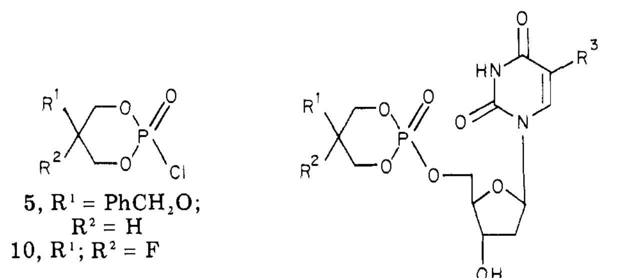
Many analogues of nucleosides act as antimetabolites, and a number of these are used or show promise as chemotherapeutic agents against viral or neoplastic diseases.[1] A vital step in the mode of action of these compounds is their conversion into their 5′-phosphates by cellular or virus-induced kinases. However, the corresponding nucleoside 5′-phosphates are not directly applicable as chemotherapeutic agents because of their poor penetration of cell membranes and rapid dephosphorylation to the parent nucleosides.[2] Derivatives of nucleoside phosphates have been synthesized in attempts to obtain compounds that could penetrate cells and then release the active nucleotide. Thus, esters of 2′-deoxy-5-fluorouridine 5′-phosphate have been synthesized for this purpose, but they do not appear to have shown the desired properties.[3]

In the present study, some cyclic phosphotriester derivatives of nucleosides, particularly of 2′-deoxy-5-fluorouridine, have been synthesized in order to obtain compounds of this type. By using a suitable cyclic phosphotriester group, we hoped to obtain a derivative that would slowly hydrolyze in vivo to give a nucleoside 5′-phosphate. The known difference in effective pH in certain tumor cells compared to normal cells[4] might be a basis for selective action against the tumor. The compounds studied were of the general structure 1, and the investigation was directed to obtaining a suitable group, X, that would confer the desired properties on the final product.



It is well known[5] that five-membered cyclic phosphodiesters, such as 2 and 3, are very easily hydrolyzed, i.e., in a few minutes at physiological pH at room temperature. Therefore, these systems were too unstable for our purposes. On the other hand, the analogous six-membered ring systems are known to be more stable to hydrolysis.[6] We obtained compound 4 by condensation of 2′-deoxy-5-fluorouridine with 2-chloro-1,3,2-dioxaphosphacyclohexane 2-oxide, which was prepared by a modification of Lanham's method.[7] (The mass spectrum of 4 has been reported,[8] but we have not traced any report of its synthesis or properties.) As expected, this compound was too resistant to hydrolysis for our purpose, but it appeared probable that the introduction of suitable substituents into the phosphorus-containing ring would give compounds that would be hydrolyzed more readily.

The first compounds synthesized contained a hydroxyl group at position 5 of the 1,3,2-dioxaphosphacyclopentane ring. To obtain these, we condensed 2-benzylglycerol with phosphoryl chloride to give 5-(benzyloxy)-2-chloro-1,3,2-dioxaphosphacyclohexane 2-oxide (5). The latter was



4, R^1, R^2 = H; R^3 = F
6, R^1 = H; R^2 = $PhCH_2O$; R^3 = CH_3
7, R^1 = H; R^2 = $PhCH_2O$; R^3 = F
8, R^1 = H; R^2 = HO; R^3 = CH_3
9, R^1 = H; R^2 = HO; R^3 = F
11, R^1, R^2 = F; R^3 = CH_3
12, R^1, R^2, R^3 = F

condensed with thymidine and with 2′-deoxy-5-fluorouridine to give 6 and 7, respectively. Hydrogenolysis of these compounds with hydrogen and palladium on charcoal gave the corresponding hydroxy compounds 8 and 9. These compounds could exist in two isomeric forms, depending upon the orientation of the substituent in the 1,3,2-dioxaphosphacyclopentane ring. We did not establish

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Table I. Inhibitory Effects of 5′-Substituted Derivatives of F-dUrd on the Proliferation of Murine Leukemia L1210 Cell Lines

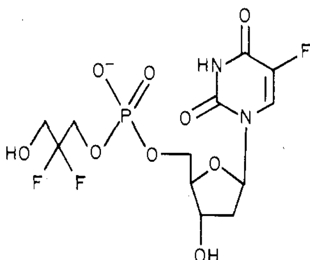
compd	ID_{50},[a] μg/mL, for cell growth: L1210/0	ID_{50},[a] μg/mL, for cell growth: L1210/BdUrd[b]	ratio of ID_{50} for L1210/0 cell growth/ ID_{50} for L1210/ BdUrd cell growth
F-dUrd	0.001 (±0.0001)[c]	1.75 (±0.40)[c]	1750
F-dUMP	0.002 (±0.0002)	2.43 (±0.43)	1215
1	0.275 (±0.027)	197 (±7.5)	716
9	0.320 (±0.066)	165 (±5.5)	516
12	0.003 (±0.0006)	2.42 (±0.40)	807

[a] Dose inhibiting cell proliferation by 50%. [b] L1210/BdUrd is a murine leukemia L1210 cell line selected from the parental L1210/0 cell line by its ability to grow in the presence of 5-Br-dUrd (260 μg/mL). This cell line was shown to be deficient for thymidine kinase.[10] [c] Data are taken from ref 10.

the orientation in our compounds or whether they were, in fact, mixtures of diastereomers. The hydrolysis of compound **9** in the pH range 3–10 was studied qualitatively. Only slight hydrolysis at pH 3 and 10 after 48 h at room temperature was observed.

In order to obtain a compound that could be more easily hydrolyzed, 2,2-difluoropropane-1,3-diol was condensed with phosphoryl chloride to give 2-chloro-5,5-difluoro-1,3,2-dioxaphosphacyclohexane 2-oxide (**10**). This was condensed with thymidine and with 2′-deoxy-5-fluorouridine to give compounds 11 and 12, respectively. The structures of compounds **4**, **6–9**, 11, and 12 were established from analytical data and NMR spectroscopy. In particular, the presence of a signal for the 3′-hydroxyl group and a downfield shift for the signals due to the 5′-protons indicated that substitution had occurred selectively on the 5′-hydroxyl group of the nucleoside.

Hydrolysis of compound 12 in the pH range 2.5–10 showed that it was easily hydrolyzed at room temperature, particularly under alkaline conditions. Electrophoresis at various pH values of the product of hydrolysis showed that it was singly negatively charged; i.e., it ran slower than thymidine 5′-phosphate at pH 6.7, but at about the same rate at pH 3.0. The most probable structure for this product is the acyclic phosphodiester, **13**.



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From the three 5′-substituted derivatives of 2′-deoxy-5-fluorouridine (F-dUrd) that were examined for their inhibitory effects on L1210/0 proliferation, compound **12** emerged as the most potent inhibitor (Table I). Its ID_{50} was 0.003 μg/mL. A slightly lower ID_{50} was recorded for F-dUrd and for its 5′-phosphate (F-dUMP).[9] The order of (decreasing) inhibitory activity was F-dUrd > F-dUMP > **12** > **4** > **9** (Table I).

Like F-dUrd and F-dUMP, compounds **12**, **4**, and **9** were considerably less effective against a mutant L1210 cell line (designated as L1210/BdUrd), which has been shown to be deficient for thymidine kinase (TK).[10] This is reflected by the differences in the ID_{50} values of the compounds for L1210/0 and L1210/BdUrd cells. The ID_{50} values recorded for the L1210/BdUrd cells were many hundreds of times higher than those for the parent L1210/0 cells. These data tend to suggest that the compounds must be phosphorylated by the cellular TK to exert their antiproliferative effects. This would imply that the compounds are first hydrolyzed to release the parent compound F-dUrd, which is subsequently phosphorylated by the cellular TK (or possibly degraded to 5-fluorouracil, which is then subsequently glycosylated). Even F-dUMP was 1000-fold less inhibitory toward L1210/BdUrd cells than toward L1210/0 cells. This means that the 5′-phosphate group of F-dUMP is removed before the compound is taken up by the cells. The same observations have been made for 5-Et-dUMP[11] and 5-NO_2-dUMP,[10] two nucleotide analogues that are also highly dependent on the cellular TK activity to exert their antiproliferative effects.[10] However, although this cell line has shown to be deficient in, or to have an altered specificity of, its TK, it is possible that this "TK^-" cell line has other properties that it does not share with the parental cell line [see, for example, its susceptibility to (*E*)-5-(2-bromovinyl)-2′-deoxyuridine].[10] Thus, the large differences in cytotoxicity between compounds **4** and **12** are also compatible with the theory that the compounds penetrate into the cell and this is followed by a slow release of F-dUMP from **12**, whereas compound **4** is much more stable.

Upon phosphorylation by the cellular TK to the corresponding 5′-monophosphates, the compounds interact with the dTMP synthetase. This mode of action is suggested by the differences observed in the reversing effects of dUrd, dThd, and dCyd on the inhibition of tumor cell proliferation (Table II). As we have pointed out previously,[9] one may postulate that for those compounds that specifically act at the thymidylate synthetase level, the cytotoxic action should be reversed more efficiently upon addition of dThd than of dUrd. Indeed, dUrd, even if added in excess, may be able only to reverse partially the inhibitory action of the nucleoside analogues, since it is itself blocked at the dTMP synthetase level. This dTMP synthetase step is circumvented in the pathway that leads to the incorporation of dThd into DNA, and, therefore, dThd may readily overcome a blockade at the dTMP synthetase level.

The inhibitory effects of the compounds on dTMP synthetase in intact cells are clearly demonstrated by the high ratio of their ID_{50} upon addition of dThd to their ID_{50} upon addition of dUrd (Table II). This ratio for compound **12** was comparable to that for the reference compounds, F-dUrd, and F-dUMP. It amounted to more than 1000, thus indicating a strong interaction of **12** with dTMP

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Table II. Influence of dUrd, dThd, and dCyd on the Inhibitory Effects of 5′-Substituted Derivatives of F-dUrd on the Proliferation of L1210/0 Cells

	ID_{50},[a] μg/mL, for cell growth				ratio of ID_{50} upon addition of dThd/ID_{50} upon addition of dUrd
compd	as such	upon addition of dUrd (125 μg/mL)[b]	upon addition of dThd (5 μg/mL)[b]	upon addition of dCyd (500 μg/mL)[b]	
F-dUrd	0.001 (±0.0001)[c]	0.02 (±0.005)[c]	34 (±14.6)[c]		1700
F-dUMP	0.001 (±0.0001)	0.044 (±0.001)	>100		>2273
4	0.275 (±0.027)	13.2 (±2.66)	>100	1.38 (±0.418)	>7.58
9	0.321 (±0.056)	3.87 (±0.917)	>1000	2.04 (±1.32)	>258
12	0.003 (±0.0005)	0.048 (±0.007)	270 (±59.6)	0.036 (±0.027)	5637

[a] Dose inhibiting cell proliferation by 50%. [b] The doses employed for dUrd, dThd, and dCyd corresponded to the maximum concentration of dUrd, dThd, and dCyd, which were themselves not inhibitory to L1210 cell growth. [c] Data are taken from ref 9.

Table III. Inhibition of L1210/0 dTMP Synthetase by 5′-Substituted Derivatives of F-dUrd

compd	K_i/K_m[a]
F-dUrd	66.5
F-dUMP	0.0099[b]
4	>110
9	>60
12	>110

[a] K_m values for the individual experiments ranged from 1.27 to 3.12 μM. The average K_m value was 1.84 μM for F-dUMP and 2.03 μM for the other compounds. [b] Data taken from ref 12.

synthetase. The reversing effect of dCyd on the antiproliferative activities of the compounds was similar to that shown by dUrd (Table II). This is not surprising, since most of the dCyd added to the cells is deaminated to dUrd within the cell (at either the nucleoside or nucleotide level). Hence, its further action will be identical with that of dUrd and its metabolites.

The K_i/K_m values of L1210/0 dTMP synthetase for F-dUrd, F-dUMP, and the F-dUrd derivatives are presented in Table III. The K_m value of the enzyme for dUMP was 2.03 μM. Neither of the FDU derivatives nor FDU itself showed an appreciable inhibitory effect on cell-free dTMP synthetase activity. Their K_i/K_m values were very high (~100). In contrast, F-dUMP inhibited the cell-free dTMP synthetase at a 1000- to 10000-fold higher degree, its K_i/K_m value being 0.0099.[12] Thus, only F-dUMP appeared to be a potent inhibitor of the cell-free dTMP synthetase reaction. This again suggests that in the cell cultures the compounds first need to be hydrolyzed to F-dUrd (which then must be phosphorylated to F-dUMP), to 5-fluorouracil, which must be glycosylated and phosphorylated, or to F-dUMP.

Experimental Section

Chromatography. TLC was carried out with silica gel MN Kieselgel G/UV_{254}, and column chromatography was carried out with Kieselgel 60, 70–230 mesh ASTM type 7734 (unless otherwise stated) supplied by E. Merck AG, Darmstadt West Germany. All reactions were carried out under scrupulously anhydrous conditions unless otherwise indicated.

2-Chloro-1,3,2-dioxaphosphacyclohexane 2-Oxide.[7] A solution of propane-1,3-diol (3.8 mL, 3.8 g, 50 mmol) and triethylamine (14 mL, 100 mmol) in dichloromethane (25 mL) and a solution of phosphoryl chloride (4.6 mL, 7.67 g, 50 mmol) in dichloromethane (30 mL) were added separately but simultaneously, with stirring, to dichloromethane (30 mL) at 0 °C. The solution was warmed to 20 °C and stirred for a further 20 min. Removal of the solvent under reduced pressure gave a white solid. This was extracted with diethyl ether (2 × 50 mL), the solid was filtered off, and the solvent was removed from the filtrate by evaporation under reduced pressure. The residue was heated at 35 °C at 0.05 mmHg to evaporate off propane-1,3-diol that remained. The residue was then extracted with diethyl ether (3 × 40 mL), the residual solid was filtered off, and the filtrate was evaporated to dryness to give the white crystalline product (4.2 g, 54% yield): mp 39–42 °C; NMR ($CDCl_3$) δ 1.75–2.00 (1 H, m, H-5_{eq}, J_{P-H} = 15 Hz), 2.20–2.70 (1 H, m, H-5_{ax}), 4.3–4.8 (4 H, m, H-4, H-6).

2′-Deoxy-5-fluoro-5′-*O*-1″,3″,2″-dioxaphosphacyclohex-2″-yluridine 2″-Oxide (4). 2′-Deoxy-5-fluorouridine (500 mg, 2.03 mmol) was dissolved in pyridine (30 mL), freshly prepared 2-chloro-1,3,2-dioxaphosphacyclohexane 2-oxide (318 mg, 2.03 mmol) was added, and the solution was stirred at 20 °C for 17 h. Examination by TLC showed that the reaction was not complete, so more "phosphorylating agent" (160 mg, 1.02 mmol) was added, and the reaction was allowed to proceed for a further 24 h at 20 °C. The solvent was then evaporated off at 35 °C, the last traces being coevaporated with toluene (3 × 25 mL). The residue was washed with diethyl ether (2 × 10 mL), and the ether-insoluble residue was dissolved in a minimum volume of chloroform–ethanol (49:1). The solution was applied to a column of silica gel (150 g of Kieselgel 60, 230–400 mesh, packed dry), and the column was eluted under slight positive pressure. Pooling of the appropriate fractions and removal of the solvent by evaporation gave the product as a white crystalline solid (165 mg, 23% yield): mp 53–57 °C; UV (ethanol) λ_{max} 267 nm (ε 7600), λ_{min} 235 nm (ε 1750); NMR [$(CD_3)_2SO$] δ 1.0–1.5 (1 H, 2 t, H-5″$_{ax}$, J_{P-H} = 15 Hz), 1.65–1.85 (1 H, m, H-5″$_{eq}$, J_{P-H} = 15 Hz), 2.16 (2 H, t, H-2′), 3.95 (1 H, m, H-4′), 4.1–4.5 (7 H, m, H-4″, H-6″, H-3′, H-5′), 5.4 (1 H, s, OH-3′), 6.15 (1 H, t, H-1′), 7.92 (1 H, d, H-6, *J* = 7 Hz), 11.8 (1 H, s, NH). Anal. ($C_{12}H_{16}FN_2O_8P\cdot H_2O$) C, H, N.

5-(Benzyloxy)-2-chloro-1,3,2-dioxaphosphacyclohexane 2-Oxide (5). A solution of 2-*O*-benzylglycerol[13] (1.8 g, 10 mmol) in dry dichloromethane (10 mL) containing 2,6-lutidine (2.1 g, 20 mmol) was added dropwise with stirring over a period of 1 h to a solution of phosphoryl chloride (1.5 g, 10 mmol) in dry dichloromethane (10 ml) at −15 °C. The temperature was allowed to rise to 0 °C during the addition and then to room temperature, and the mixture was stirred for 18 h. It was then filtered, and the filtrate was evaporated to dryness. The residue was extracted with dry acetone (3 × 10 mL), and the combined acetone extracts were evaporated to dryness. The residue was dissolved in dry chloroform (5 mL), carbon tetrachloride (15 mL) was added, and the solution was allowed to stand at 0 °C. The crystals that formed were collected and dried to give the product (1.4 g, 53% yield): mp 88–90 °C; NMR ($CDCl_3$) δ 3.61 (1 H, m, H-5), 4.53 (4 H, m, H-4), 4.63 (2 H, s, $PhCH_2$), 7.34 (5 H, s, Ph); EIMS, *m/e* 264, 262 (M^+) 157, 155, 117, 107, 91 (base peak). Anal. ($C_{10}H_{12}O_4PCl$) C, H.

5″-(Benzyloxy)-5′-*O*-1″,3″,2″-dioxaphosphacyclohex-2″-ylthymidine 2″-Oxide (6). Thymidine (1.21 g, 5 mmol) was allowed to react with compound **5** (1.67 g, 6.5 mmol) in dry pyridine (25 mL) with shaking for 42 h at room temperature (TLC

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showed the presence of thymidine and the product in about equal amounts). The pyridine was removed by evaporation and coevaporation with methanol. The residue was fractionated on a column of silica gel (300 g) with chloroform–ethanol (4:1) as eluant. Evaporation of the solvent gave a residue (0.5 g, 22% yield), which crystallized from ethanol to give the product: mp 170 °C; UV (ethanol) λ_{max} 266 nm (ϵ 9880), λ_{min} 235 nm (ϵ 2194); NMR (CD_3OD) δ 1.89 (3 H, s, CH_3), 2.30 (2 H, t, *H*-2'), 3.67 (1 H, m, H-5''), 4.11 (1 H, m, H-4'), 4.31 and 4.49 (7 H, m, H-5', H-4'', H-6'', H-3'), 4.63 (2 H, s, $PhCH_2$), 6.25 (1 H, t, H-1'), 7.33 (5 H, s, Ph), 7.50 (1 H, s, H-6). Anal. ($C_{20}H_{25}N_2O_9P$) C, H, N.

5''-(Benzyloxy)-2'-deoxy-5-fluoro-5'-*O*-1'',3'',2''-dioxaphosphacyclohex-2''-yluridine 2''-Oxide (7). 2'-Deoxy-5-fluorouridine (2.46 g, 10 mmol) was treated in the same manner as described above for thymidine. The mixture of products was fractionated on a column of silica gel (1 kg) with chloroform–ethanol (5:1) as eluant. Evaporation of the required fractions gave a white powder (1.75 g, 37% yield), which was crystallized from propan-2-ol to give the product: mp 94–96 °C; UV (ethanol) λ_{max} 269 nm (ϵ 8230), λ_{min} 235 nm (ϵ 1750); ^1H NMR [$(CD_3)_2SO$] δ 2.16 (2 H, t, H-2'), 3.66 (1 H, m, H-5''), 3.95 (1 H, m, H-4'), 4.20 (3 H, m, H-5', H-3'), 4.39 (4 H, m, H-4'', H-6''), 4.61 (2 H, s, $PhCH_2$), 7.15 (1 H, t, H-1'), 7.35 (5 H, s, Ph), 7.92 (1 H, d, H-6, J_{H-F} = 7 Hz), 11.78 (1 H, d, NH, J_{NH-F} = 5 Hz); ^{19}F NMR [$(CD_3)_2SO$] ϕ 165.9 (m, F-5). Anal. ($C_{19}H_{22}FN_2O_9P$) C, H, N.

5''-Hydroxy-5'-*O*-1'',3'',2''-dioxaphosphacyclohex-2''-ylthymidine 2''-Oxide (8). A solution of compound **6** (150 mg, 0.32 mmol) in dry ethanol (20 ml) was shaken in an atmosphere of hydrogen in the presence of 10% Pd on charcoal at room temperature for 16 h. The catalyst was filtered off, and the filtrate was evaporated to dryness to give a white solid of almost pure product (45 mg, 37% yield), mp 150–155 °C. Purification of this by column chromatography on silica gel gave the pure product: mp 182 °C; UV (ethanol) λ_{max} 266 nm (ϵ 8300), λ_{min} 235 nm (ϵ 1800); NMR (CD_3OD) δ 1.89 (3 H, s, CH_3), 2.28 (2 H, t, H-2'), 3.83 (1 H, m, H-5''), 4.07 (1 H, m, H-4'), 4.27 (3 H, m, H-5', H-3'), 4.50 (4 H, m, H-4'', H-6''), 6.25 (1 H, t, H-1'), 7.51 (1 H, s, H-6). Anal. ($C_{13}H_{19}N_2O_9P$) C, H, N.

2'-Deoxy-5-fluoro-5''-hydroxy-5'-*O*-1'',3'',2''-dioxaphosphacyclohex-2''-yluridine 2''-Oxide (9). A solution of compound **7** (300 mg, 0.66 mmol) in glacial acetic acid (30 mL) was shaken in an atmosphere of hydrogen in the presence of 5% Pd on charcoal (400 mg) for 1 h. The catalyst was filtered off, and the filtrate was evaporated to dryness. The residue was crystallized from ethanol to give the product (105 mg, 42% yield): UV (ethanol) λ_{max} 268 nm (ϵ 6000), λ_{min} 237 nm (ϵ 1400); NMR [$(CD_3)_2SO$] δ 2.15 (2 H, t, H-2'), 3.6 (1 H, m, H-5''), 3.72 (1 H, m, H-4'), 4.0–4.2 (3 H, m, H-5', H-3'), 4.3–4.4 (4 H, m, H-4'', H-6''), 5.0–6.0 (2 H, m, OH-3', OH-5''), 6.17 (1 H, t, H-1'), 7.93 (1 H, d, H-6, J_{H6-F5} = 7 Hz). Anal. ($C_{12}H_{16}FN_2O_9P$) C, H, N.

Compound **9** (1-mg samples in 0.10 mL) was treated with buffers of pH 3.0, 7.0, and 10.0, respectively, at room temperature. The reactions were monitored by TLC in acetonitrile and in ethanol. The possible formation of a charged product was monitored by paper electrophoresis at pH 7. After 24 h there was no evidence for hydrolysis, but after 48 h, at pH 3.0 and 10.0 there was present a trace of a hydrolysis product.

2-Chloro-5,5-difluoro-1,3,2-dioxaphosphacyclohexane 2-Oxide (10). A solution of 2,2-difluoropropane-1,3-diol (2.42 g, 20 mmol)[14] in 2,6-lutidine (4.64 mL, 40 mmol) in dry dichloromethane (10 mL) was added dropwise, with stirring, to a solution of phosphoryl chloride (3.67 g, 20 mmol) in dry dichloromethane (10 mL) at -15 °C over a period of 1 h, the mixture being allowed to warm up to 0 °C. After the addition, the resulting suspension was warmed to room temperature and stirred for 18 h. The suspension was filtered, and the filtrate was evaporated to dryness. The resulting solid residue was extracted with dry acetone (4 × 12 mL), and the combined extracts were evaporated under reduced pressure to give a solid residue (4 g). This was dissolved in dry chloroform (5 mL), dry carbon tetrachloride (5 mL) was added, and the solution was cooled to 0 °C. The crystals that formed were collected and dried to give a substance (2 g, 52% yield), mp 78 °C, which was shown to be mainly the required product (contaminated with a small amount of 2,6-lutidine hydrochloride) and sufficiently pure to use in the following reactions.

5'',5''-Difluoro-5'-*O*-1'',3'',2''-dioxaphosphacyclohex-2''-ylthymidine 2''-Oxide (11). Thymidine (1.21 g, 5 mmol) and **10** (1 g, 5.2 mmol) were dissolved in pyridine (25 mL), and the mixture was shaken at room temperature for 18 h. An additional quantity of **10** (0.38 g, 2 mmol) was added, and the mixture was shaken for a further 24 h. The solvent was removed by evaporation and coevaporation with methanol and the residue was fractionated on a column of silica gel (400 g) with chloroform–ethanol (4:1) as eluant to give a white amorphous solid (0.5 g, 25% yield) which was crystallized from ethanol–chloroform to give the product: mp 116 °C; UV (ethanol) λ_{max} 266 nm (ϵ 9650), λ_{min} 234 nm (ϵ 2160); ^1H NMR [$(CD_3)_2SO$] δ 1.77 (3 H, s, CH_3), 2.16 (2 H, t, H-2'), 3.96 (1 H, m, H-4'), 4.27 (3 H, m, H-5', H-3'), 4.76–4.46 (4 H, m, H-4'', H-6''), 5.40 (1 H, d, OH-3'), 6.18 (1 H, t, H-1'), 7.47 (1 H, s, H-6), 11.25 (1 H, s, NH); ^{19}F NMR [$(CD_3)_2SO$] ϕ 120.0 (1 F, d, F_{eq}, $J_{F_{ax}-F_{eq}}$ = 265 Hz), 111.8 (1 F, d, F_{ax}, $J_{F_{ax}-F_{eq}}$ = 265 Hz). Anal. ($C_{13}H_{17}FN_2O_8P\cdot0.5H_2O$) C, H, N.

2'-Deoxy-5,5'',5''-trifluoro-5'-*O*-1'',3'',2''-dioxaphosphacyclohex-2''-yluridine 2''-Oxide (12). A solution of 2'-deoxy-5-fluorouridine (2.46 g, 10 mmol) and **10** (2 g, 10.4 mmol) in dry pyridine (50 ml) was shaken at room temperature for 18 h. A further quantity of **13** (0.38 g, 2 mmol) was added, and the mixture was shaken for a further 24 h. The solvent was then removed by evaporation and coevaporation with methanol under reduced pressure to give a residue, which was separated on a column of silica gel (750 g) with chloroform–ethanol (1:4) as eluant. The required fractions were pooled and evaporated to dryness to give a white powder (1.23 g, 30% yield), which was crystallized from propan-2-ol to give the product: mp 138 °C; UV (ethanol) λ_{max} 268 nm (ϵ 7600); λ_{min} 235 nm (ϵ 1560); ^1H NMR [$(CD_3)_2SO$] δ 2.68 (2 H, t, H-2'), 3.98 (1 H, q, H-4'), 4.30 (3 H, m, H-5', H-3'), 4.58 (4 H, m, H-4'', H-6''), 5.36 (1 H, br s, OH-3'), 6.16 (1 H, t, H-1'), 7.94 (1 H, d, H-6, J_{H-F} = 7 Hz), 11.70 (1 H, br s, NH). Anal. ($C_{12}H_{14}F_3N_2O_8P$) C, H, N.

The hydrolysis of compound **12** was determined in aqueous solution at 20 °C at various pH values by running samples selected at appropriate times on TLC and determining the degree of hydrolysis from the UV absorption of the hydrolysis product relative to that of the starting material. The "half-life" of the compound at the various pH values is as follows:

pH:	2.5	7.0	8.0	9.0	10.0
$t_{1/2}$, h:	48	72	3.0	2.5	0.25

The product of hydrolysis was run on paper electrophoresis at 5 kV with thymidine 5'-phosphate as reference. At pH 6.7 the mobility relative to thymidine 5'-phosphate was 0.7, whereas at pH 3.0 it was 0.97.

Biological Assays. Cells. Murine leukemia L1210 cells were grown in 75-cm^2 tissue-culture flasks (Falcon 3024F; Becton Dickinson France S.A., Grenoble, France) in Eagle's minimal essential medium, supplemented with 10% (v/v) inactivated fetal calf serum (Gibco Bio-Cult, Glasgow, Scotland), 2 mM L-glutamine (Flow Laboratories, Irvine, Scotland), 0.075% (w/v) $NaHCO_3$ (Flow Laboratories), and 25 units/mL of nystatine (S.A. Labaz N.V., Brussels, Belgium). The subline L1210/BdUrd has been selected from the parental L1210/0 cells by its ability to grow in the presence of 5-Br-dUrd (260 μg/mL). This cell line is deficient for thymidine kinase.[10] The L1210 cell lines used were found to be *Mycoplasma* free.

Inhibition of Tumor Cell Growth. All assays were performed in Linbro microplates (Model FB-48-TC, Linbro Chemical Company, New Haven, CT). To each well were added 5×10^4 L1210 cells and a given amount of the test compound. In those assays that were aimed at reversing the inhibitory effects of the test compounds, dUrd (125 μg/mL), dThd (5 μg/mL), or dCyd (500 μg/mL) was added to the cells, together with varying amounts of the test compound. The doses employed for dUrd, dThd, and dCyd corresponded to the maximal concentrations of dUrd, dThd, and dCyd, which were themselves not inhibitory to L1210 cell proliferation. The cells were allowed to proliferate for 48 h at 37 °C in humidified, CO_2-controlled atmosphere. The growth of the cells was linear during this 48-h incubation period. At the end of the incubation period, the cells were counted in a Coulter

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counter (Coulter Electronics Ltd., Harpenden, Herts, England). The ID_{50} (50% inhibitory dose) was defined as the concentration of compound that reduced the number of living cells by 50%.

Preparation of L1210/0 Cell Extracts. One-milliliter cell pellets were first washed with cold PBS (phosphate-buffered saline), and then 3 mL of suspension buffer (10 mM potassium phosphate buffer, pH 7.5, containing 0.01 M β-mercaptoethanol and 0.1 M KCl) was added. The cell suspension was sonicated two times for 10 s and cleared by centrifugation at 25000*g* for 30 min. The 30-70% $(NH_4)_2SO_4$ precipitate of the cell homogenate was resuspended in 1 mL of suspension buffer, dialyzed against the same buffer for 2-3 h at 4 °C, and stored in aliquots at -20 °C.

Thymidylate Synthetase Assay. The cell extracts were assayed for dTMP synthetase activity in a standard reaction mixture containing 0.26 mM tetrahydrofolate, 5.0 mM formaldehyde, 15 mM β-mercaptoethanol, 0.1 M NaF, 45 μM (0.025 μCi) [5-^{3}H]dUMP, and an appropriate amount of inhibitor (FdUrd or any of its derivatives) in a total volume of 30 μL of 0.05 M potassium phosphate buffer, pH 7.5. The reaction was initiated by addition of 10 μL of cell extract. The reaction mixture was incubated at 37 °C for 30 min, and the reaction was terminated by the addition of 160 μL of a charcoal suspension (at 100 mg/mL in 2% trichloroacetic acid). After centrifugation for 10 min at 1000*g*, 100 μL of supernatant was assayed for radioactivity in a toluene-based scintillant.

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Synthesis and Antitumor Activity of *cis*-Dichloroplatinum(II)-N-Aminated Nucleoside Complexes†

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N-Aminated nucleoside complexes of *cis*-dichloroplatinum(II) were synthesized, and their antitumor activities against L1210 cells in mice and in vitro were studied. While the native nucleosides failed to show any antitumor activity, the complexes exhibited high antitumor activity and had no nephrotoxicity in mice. Studies on their mode of action in vitro indicated that the ligands played characteristic roles in the appearance of antitumor activity; ribonucleoside complexes caused the inhibition of RNA synthesis, and deoxyribo- or arabinonucleoside complexes caused the inhibition of DNA synthesis.

cis-Diamminedichloroplatinum(II) (*cis*-DDP) is an inorganic platinum compound that has been shown to possess antitumor activity in a variety of animal tumor models.[2,3] Clinical studies with *cis*-DDP have shown it to be effective in the treatment of human solid tumors.[4,5] Furthermore, various platinum(II) complexes, such as that of 1,2-diaminocyclohexane, which has an altered structure in the ligand moiety, have been synthesized and tested on some tumor systems in mice.[6]

In our previous papers, we have described how SeG-Pt(II) and TG-Pt(II) complexes show antitumor activity against L1210 cells in mice and how that activity was mainly dependent upon the ligand SeG or TG.[7,8] We have also reported that SeG-Pt(II) had a direct cytotoxicity against L5178Y cells in vitro.[9] However, very few papers have supported the concept that the ligand plays a characteristic role in the antitumor action of the *cis*-DDP complexes.

The present study was conducted to synthesize the *cis*-dichloroplatinum(II)-N-aminated nucleoside complexes 3-aminocytidine-dichloroplatinum(II) (**8**), 3-amino-2'-deoxycytidine-dichloroplatinum(II) (**9**), 3-amino-1-β-D-arabinofuranosylcytosine-dichloroplatinum(II) (**10**), 3-aminotubercidin-dichloroplatinum(II) (**13**), and 5-amino-1-β-D-ribofuranosylimidazol-4-carboxamide-dichloroplatinum(II) (**15**) and to examine their antitumor effects against L1210 cells in mice and in vitro. In addition, the nephrotoxicity of these complexes on mice was examined and compared to that of *cis*-DDP.

Results and Discussion

Synthesis and Structure Elucidation of the Complexes. N-Amination reaction of nucleosides was carried out by using 2,4-dinitrophenoxyamine (**1**) as an aminating agent, which is directed toward the most basic endo nitrogen in a nucleoside. The reaction site in a nucleoside has been well established as position 3 in cytidine and position 1 in adenosine.[10]

It has been reported that 3-aminocytidine hydrochloride (**5**) reacts with orthoesters, such as ethyl orthoformate or ethyl orthoacetate, to give fused heterocyclic bases.[11] Also, 3-aminouridine reacts with acetic anhydride to afford the *N*-acetyl derivative.[11] Therefore, it is reasonable to assume

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