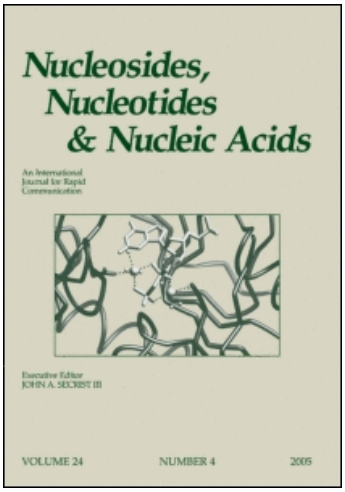

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The Cyclic Dimer of 5-Fluoro-2'-Deoxyuridylic Acid: A Potent Anticancer Agent

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THE CYCLIC DIMER OF 5-FLUORO-2'-DEOXYURIDYLIC ACID : A POTENT ANTICANCER AGENT

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Abstract

As part of a synthetic program on cyclic oligomers of DNA, the cyclic dimer of 5-fluoro-2'-deoxyuridylic acid (FdUMP) was synthesized. The fully protected dimer 5 was obtained following Catlin and Cramer's phosphotriester strategy. Autocondensation and deprotection then afforded the title compound 9 [cyclo(5FdUp5FdUp)] in excellent yield. In vitro, 9 proved slightly less active than FdUrd in inhibiting the proliferation of various murine and human tumor cells, but, in vivo, 9 was equally effective, and less toxic than 5-FdUrd in inhibiting adenocarcinoma tumor growth in mice.

INTRODUCTION

5-Fluorouracil (FUra) and 5-fluoro-2'-deoxyuridine (FdUrd) are well-known anticancer agents, FUra being used extensively in the management of carcinomas of the colon, breast and ovary. The cellular pharmacology of FUra and FdUrd is complex, and their cytotoxic and/or cytostatic activity may be achieved by several mechanisms, including (i) incorporation into RNA, with subsequent alterating of posttranscriptional processing or translation, (ii) incorporation into DNA and excision which may cause DNA fragmentation, and (iii) depletion of dTTP through inhibition of thymidylate synthase (ref. 1 and references cited therein). FdUrd 5'-monophosphate (FdUMP) is an exquisitely potent inhibitor of dTMP synthase,[2] and this enzyme may well be the principal, if not the sole, target for the inhibitory activity of FdUrd on tumor cell growth in vitro.[3,4]

However, in vivo FdUrd is rapidly catabolized by pyrimidine nucleoside phosphorylases to FUra, which then enters the pyrimidine degradation

pathway via dihydrothymine dehydrogenase. To prevent the premature degradation of FdUrd and/or FUra, and, hence, to enhance their antitumor potential, various strategies have been envisaged, including combination of FUra with other drugs [i.e. (E)-5-(2-bromovinyl)uracil (BVUra)][5] which inhibit the degradation of FUra, as well as the use of masked or prodrug form of FUra or FdUrd, such as 1-hexylcarbamoyl-5-fluorouracil,[6,7] 1-(2-tetrahydrofuryl)-5-fluorouracil (tegafur),[8] 5'-deoxy-5-fluorouridine,[9,10] and 3',5'-dioctanoyl-5-fluoro-2'-deoxyuridine.[11] Also, various derivatives of FdUMP have been synthesized, i.e. cyclic phosphotriesters[12,13] and methylphosphonates[14], all with the aim to stabilize the phosphate group of FdUMP.

We now describe the synthesis and antitumor properties of cyclo-(5FdUp5FdUp), the cyclic dimer of FdUMP, which, according to the biological effects shown, behaves as a prodrug of FdUrd.

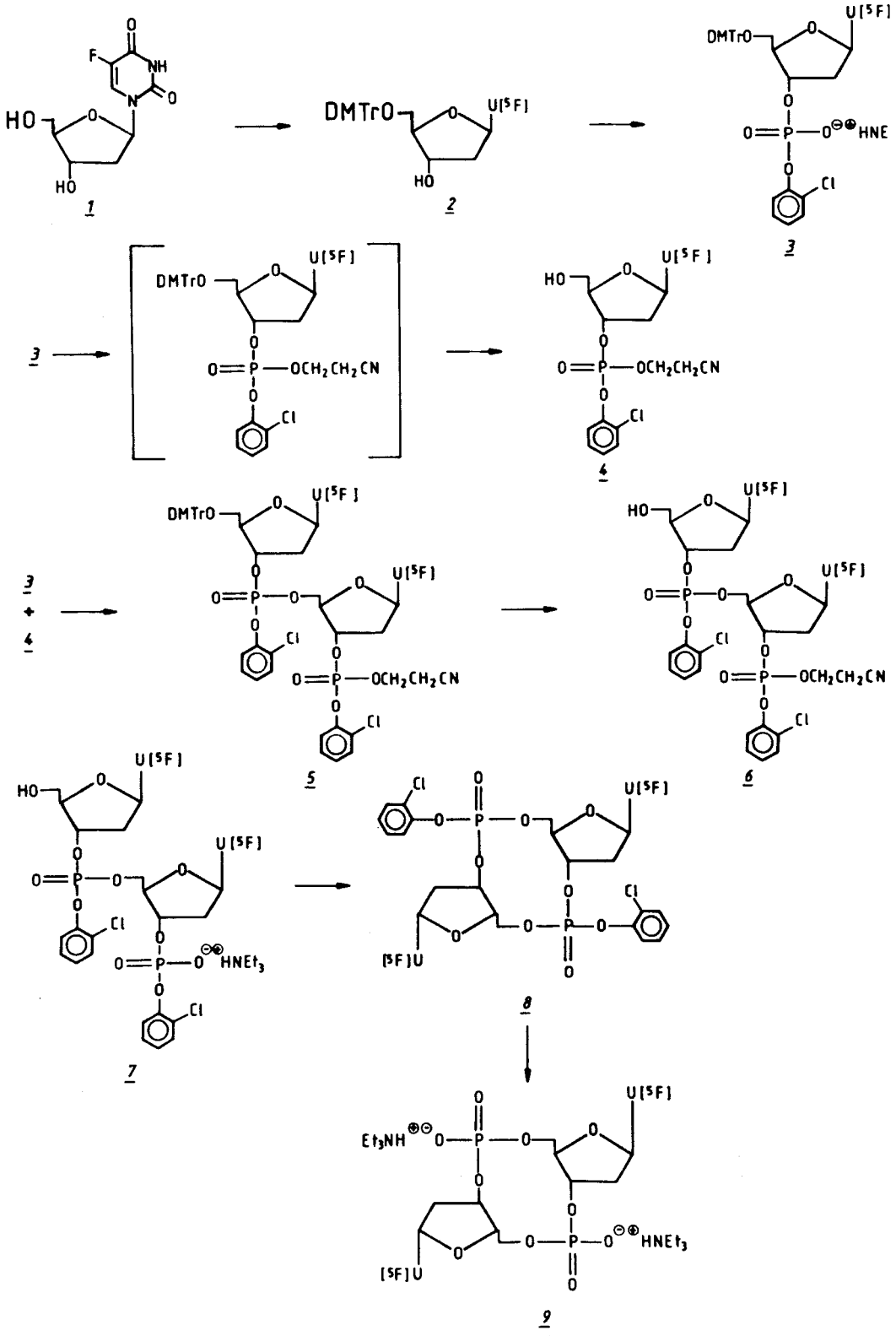
CHEMISTRY

The synthesis of compound 9 (Scheme 1) was part of a general synthetic program on macrocyclic oligomers of DNA. The conformation, cationic and molecular complexations, and biological properties of these macrocycles are indeed of interest. Particularly, the synthesis of 9 was undertaken because we assumed that it would be less easily metabolized than 5-fluorodeoxyuridine. Cyclo(pTpT), for example, is only slowly degraded by snake venom phosphodiesterase.[15]

The different steps involved in the synthesis of 9 are presented in Scheme 1. The route to the fully protected dimer 5 was as suggested by Catlin and Cramer's phosphotriester strategy of oligonucleotide synthesis in solution.[16] This phosphotriester route has also been used by C-Y.J. Hsu et al.[27] to obtain macrocyclic ribodinucleotides. To avoid side reactions on the way to 4, we coupled 3 with β-cyanoethanol in an extra step, instead of quenching the phosphorylating mixture of 2 by β-cyanoethanol. The difficulties encountered when using this last procedure have been described previously.[17]

The yields of the macrocyclization (7 to 8) were unexpectedly high (81 %). The reason is that cyclic dimers of DNA, being major products of autocondensation of nucleotides,[18-22] are conformationally favored.

The fully protected cyclic dimer was a mixture of three diastereoisomers, because of the chirality of the phosphotriester functions (RR, SS and RS). We did not attempt to separate them, but proceeded directly onto deprotection. At the phosphodiester level, the molecule in solution revealed an axis of symmetry, as indicated by NMR spectroscopy (one type of



SCHEME 1

TABLE 1. Inhibitory effect of cyclo(5FdUp5FdUp) (compound 9) on the proliferation of tumor cells in vitro

Compound	50 % Inhibitory concentration[a] (μg/mL)				
	L1210/0	L1210/BdUrd	FM3A	Raji	Molt/4F
9	0.053 ± 0.015	2.9 ± 0.55	0.032 ± 0.002	0.22 ± 0.09	0.08 ± 0.01
FdUrd	0.0029 ± 0.0001	1.5 ± 0.11	0.0017 ± 0.0001	0.078 ± 0.028	0.042 ± 0.005
FUra	0.31 ± 0.08	0.33 ± 0.035	0.2 ± 0.04	12 ± 3	13 ± 5

[a]Required to reduce tumor cell proliferation by 50 % (values for 3-4 experiments, expressed as mean ± standard deviation). Tumor cell lines : murine leukemia (L1210/0), and a cell line (L1210/BdUrd) selected from the parental L1210/0 cell line by its ability to grow in the presence of 5-bromo-2'-deoxyuridine (260 μg/mL), murine mammary carcinoma (FM3A), human B-lymphoblast (Raji), human T-lymphoblast (Molt/4F).

phosphorus, one type of carbon for a given connectivity and one broadened fluorine doublet).

ANTITUMOR ACTIVITY

Compound 9 was evaluated for its inhibitory effect on tumor cell growth in vitro according to a well-established procedure.[4] FdUrd and FUra were included as the reference compounds. In all tumor cell systems, except for L1210/BdUrd, compound 9 proved more active than FUra, but less active than FdUrd (Table 1). FdUrd was more inhibitory to murine (L1210, FM3A) cells than human (Raji, Molt/4F), and so was 9. Moreover, L1210/-BdUrd cells which, because of their repeated passages in the presence of 5-bromo-2'-deoxyuridine, have a significantly reduced thymidine kinase (TK) activity,[3] were markedly less affected by FdUrd and 9 than the parental L1210/0 cell line. In contrast, L1210/0 and L1210/BdUrd were equally sensitive to FUra. These results suggest that under our in vitro assay conditions 9 acts as a prodrug of FdUrd, which implies the successive cleavage of the phosphodiester and phosphomonoester bonds.

In vivo, 9 was examined for its antitumor activity in BDF_1 mice inoculated subcutaneously with adenocarcinoma 755 (5×10^5 cells per mouse). This model has been amply described previously.[23-26] FUra and tegafur are effective in this tumor model and their antitumor activity is potentiated if combined with L-cysteine,[23] L-cystine[24] or (E)-5-(2-bromovinyl)-2'-deo-

TABLE 2. Inhibitory effect of cyclo(5FdUp5FdUp) (compound 9) on the growth of adenocarcinoma 755 tumors in BDF_1 mice

Treatment	Tumor weight on day 11	
	Mean ± Standard deviation (mg)	T/C (%)[a]
Exp. I		
Control	2466 ± 607	
9 (30 mg/kg)[b]	2914 ± 518	118
9 (100 mg/kg)	799 ± 433	32
FdUrd (100 mg/kg)	810 ± 292	33
FdUrd (200 mg/kg)	Toxic (4/6)[c]	
FUra (10 mg/kg)	2127 ± 835	86
FUra (20 mg/kg)	2095 ± 1030	85
FUra (30 mg/kg)	330 ± 196	13
FUra (50 mg/kg)	Toxic (6/6)	
Exp. II		
Control	3477 ± 626	
9 (200 mg/kg)	923 ± 378	26
FdUrd (100 mg/kg)	1230 ± 432	35
FdUrd (200 mg/kg)	Toxic (3/6)	
Exp. III		
Control	2204 ± 1118	
9 (200 mg/kg)	585 ± 299	27
9 (3 mg/kg) + BVdUrd (100 mg/kg)	2009 ± 1011	91
9 (10 mg/kg) + BVdUrd (100 mg/kg)	1802 ± 1097	82
9 (30 mg/kg) + BVdUrd (100 mg/kg)	799 ± 355	36

[a]Ratio of average tumor weight in the treated group to that in the control group, expressed in %. Six mice per group.
[b]The compounds were administered intraperitoneally 5 times (days 1-5) at the indicated daily doses.
[c]Numbers in parentheses : dead mice/total number of mice.

xyuridine (BVdUrd).[25,26] Also, FdUrd proved effective in this system (Table 2). When administered at a dose of 100 mg/kg/day it reduced the average tumor weight to 33-35 %, but at 200 mg/kg/day FdUrd was toxic, as shown in two consecutive experiments (Exp. I, Exp. II : Table 2). Compound 9 compared favorably with FdUrd in that at a dose of 100 mg/kg/day it caused a similar reduction in tumor weight (to 32 %, see Exp. I : Table 2), but, unlike FdUrd, 9 was not toxic at a dose of 200 mg/kg/day, as again shown in two consecutive experiments (Exp. II, Exp. III : Table 2). This points to the superiority of 9 over FdUrd as an anticancer agent. From a comparison of the results obtained with 9 at 30 mg/kg/day in Exp. I and Exp. III, it is also obvious that, when 9 was combined with BVdUrd (100 mg/kg/day), its antitumor activity was markedly enhanced. This observation is in keeping with previous findings obtained for the combinations

of FUra (or tegafur) plus BVdUrd.[25,26] BVdUrd by itself does not show antitumor activity at 100 mg/kg/day.[25]

EXPERIMENTAL PART

Materials and Methods

Pyridine was refluxed on calcium hydride and distilled. 4,4'-Dimethoxytrityl chloride was recrystallized from cyclohexane containing acetyl chloride (5 %), and then from neat cyclohexane. Mesitylenesulfonyl chloride was recrystallized from hexane.

The t.l.c. were performed on plastic supported silicagel sheets, or, when stated, on glass supported silanized silicagel sheets from Merck (60 F_{254}).

Eluent A : methylene chloride/methanol (9:1, v/v)

B : acetone/water (6:4, v/v)

C : methylene chloride/methanol (20:1, v/v)

D : acetic acid/butanol/water (1:4:5, v/v/v, upper layer).

The spectra were recorded on a VARIAN XL-200 NMR spectrometer with TMS as internal reference for ^{1}H and ^{13}C spectra, phosphoric acid and trichlorofluoromethane as external reference for ^{31}P and ^{19}F spectra, respectively. The ^{1}H chemical shifts are reported in Table 3.

The UV spectra were recorded on a CARY 210 UV-Vis spectrophotometer.

Procedures

5-Fluoro-5'-O-di(p-methoxy)trityl-2'-deoxyuridine (2). Compound 1 (10.0 mmol) was dried by coevaporation with pyridine and dissolved in the same solvent (80 cc). 4,4'-Dimethoxytrityl chloride (12.8 mmol) was added and the mixture was stirred for three hours at r.t. When the reaction was complete (control by t.l.c., eluent A), the mixture was quenched with methanol. The residue after evaporation was partitioned between chloroform and sodium bicarbonate (5 %). The organic extracts were dried (magnesium sulfate), evaporated and the residue was chromatographed on silica (elution by an increasing percentage of methanol in methylene chloride, 0-5%). The yield was 83 % (Rf = 0.40, eluent A).

Triethylammonium-5-fluoro-5'-O-di(p-methoxy)trityl-2'-deoxyuridine-3'-O-(o-chlorophenyl)phosphate (3). A solution of o-chlorophenyl phosphate ditriazolide was obtained by slow addition of o-chlorophenylphosphate dichloride (5.6 mmol) to a mixture of 1,2,4-triazole (18.4 mmol) and triethylamine (12.0 mmol) in dry THF (35.5 cc) at 0°C. The protected nucleoside 2 (2.0 mmol), dissolved in THF (2 cc), was then added. When the phosphorylation was complete (control by t.l.c., eluent A), the mixture

TABLE 3. 200 MHz - ^{1}H NMR spectra of the described compounds (δ, ppm)

Compound (Solvent)	6-H	1'-H	2'-H	3'-H	4'-H	5'-H	Others
2 $CDCl_3$	7.86 (d, J=6 Hz)	6.32 (pseudo t, Japp=7 Hz)	2.15 and 2.50	4.57	4.07	3.44	3.81 OCH_3 6.86-7.50 aryls 8.90 NH
3 $CDCl_3$	7.80 (d, J=6 Hz)	6.35 (pseudo t, Japp=7 Hz)	2.31 and 2.77	5.23	4.36	3.28 and 3.45	3.77 OCH_3 6.81-7.44 aryls 7.64 NH
4 $CDCl_3$	8.04 (d, J=6 Hz)	6.26 (m)	2.26-2.70	5.29	4.20-4.54	3.88	2.80 CH_2-CH_2-CN 7.10-7.52 aryls and NH
5 $CDCl_3$	7.63-7.88 (m, 2H)	6.32 (m, 2H)	2.24-2.92	5.38 (m, 2H)	3.34 - 4.64		2.24-2.92 CH_2-CH_2-CN 3.83 OCH_3 6.89-7.58 aryls 8.68 NH (2H)
6 CD_3OD	7.72 (m, 1H) and 8.13 (m, 1H)	6.07-6.31 (m, 2H)	2.18-2.72 (m, 4H)	5.24 (m, 2H)	3.71 - 4.62		2.90 CH_2-CH_2-CN 7.08-7.58 aryls
7 CD_3OD	7.70 (m, 1H) and 8.08 (m, 1H)	6.22 (m, 2H)	2.10-2.60 (m, 4H)	4.94 (m, 1H) and 5.20 (m, 1H)	3.70 - 4.54		6.90-7.56 aryls
8[a] $CDCl_3$ + CD_3OD	7.13-7.66	6.10 (pseudo t, Japp=7 Hz) and 6.25 (pseudo t, Japp=7 Hz)	2.48-2.72 (m, 4H)	5.39 (m, 2H)	4.23 - 4.59		7.13-7.66 aryls
9 D_2O	8.03 (d, J=6.4 Hz, 2H)	6.13 (m, 2H)	2.56 (m, 4H)	4.71 (m, 2H)	3.88 - 4.20 (m, 6H)		1.17 Et_3NH^+ (18H) 3.10 Et_3NH^+ (12H)

[a]Mixture of diastereoisomers.

was quenched with a triethylammonium bicarbonate buffer (TEAB, 1M, pH 9). The residue after evaporation was partitioned between methylene chloride and TEAB. The organic extracts were dried, concentrated, and added to dry diethyl ether. The obtained precipitate was pure 3. The yield was 88 % (Rf = 0.17, eluent A; Rf = 0.65 on silanized silica, eluent B).

5-Fluoro-2'-deoxyuridine-3'-O-(o-chlorophenyl, β-cyanoethyl)phosphate (4). Compound 3 (1.0 mmol) and tetrazole (6.0 mmol) were dried by coevaporation with pyridine and dissolved in a mixture of pyridine (3.8 cc) and 3-hydroxypropionitrile (3.0 mmol). A solution of mesitylenesulfonyl chloride (2.0 mmol) in pyridine (1.5 cc) was added dropwise. At the end of the reaction (3 hrs), water was added, followed by methylene chloride. The organic phase was washed with sodium bicarbonate (5 %), dried, and evaporated. The last traces of pyridine were removed by coevaporation with toluene.

The DMTr protection was cleaved by dissolution in a 2 % solution of benzenesulfonic acid in chloroform/methanol (7:3, v/v, 12.0 cc). The deprotection was complete after about 10 min (control by t.l.c., eluent A). Pyridine (2 cc) was added. The mixture was diluted by methylene chloride and washed with sodium bicarbonate (5 %). The organic extracts were dried, evaporated and coevaporated with toluene. The residue was chromatographed on silica (eluent : methylene chloride/methanol, 98:2, v/v). The yield was 62 % (Rf = 0.82, eluent A).

5-Fluoro-5'-O-di(p-methoxy)trityl-2'-deoxyuridylyl-[3'-O-(o-chlorophenyl)-5']-5-fluoro-2'-deoxyuridine-3'-O-(o-chlorphenyl, β-cyanoethyl)-phosphate (5). Compounds 3 (1.4 mmol), 4 (1.0 mmol), and tetrazole (8.0 mmol) were dried by coevaporation with pyridine, and then dissolved in the same solvent (12.0 cc). Mesitylenesulfonyl chloride (1.6 mmol) was added. Compound 4 completely disappeared in about 4 hrs, as checked by t.l.c. (eluent A). Water was added, followed by methylene chloride. The organic phase was washed with sodium bicarbonate (5 %), dried, evaporated, and coevaporated with toluene. The residue was chromatographed on silica (elution by an increasing percentage of methanol in methylene chloride, 0-5 %). The product was dissolved in methylene chloride and precipitated from a mixture of petroleum ether and diethyl ether (1:1, v/v). The yield was 79 % (Rf = 0.44, eluent C).

5-Fluoro-2'-deoxyuridylyl-[3'-O-(o-chlorophenyl)-5']-5-fluoro-2'-deoxyuridine-3'-O-(o-chlorophenyl, β-cyanoethyl)phosphate (6). The DMTr protection was cleaved as described for compound 4. The product was purified by column chromatography (elution by an increasing percentage of methanol in methylene chloride, 0-5 %). The yield was 74 % (Rf = 0.70, eluent D).

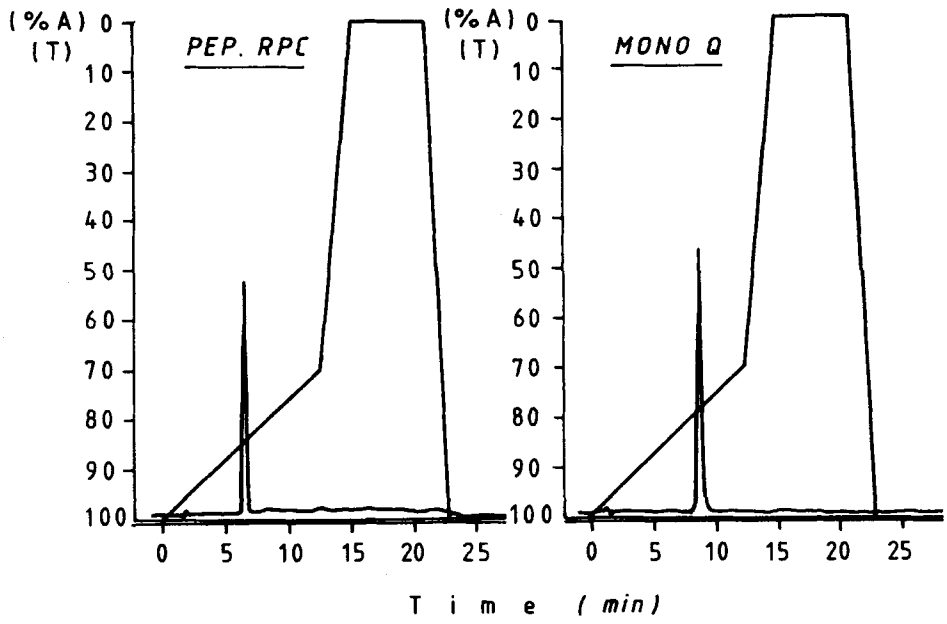


FIGURE 1

Reversed phase chromatography	Anion exchange chromatography
Buffer A : TEAA (100 mM) pH 7	Buffer A : KH_2PO_4 (20 mM) CH_3CN (20 %) pH 7
Buffer B : A/CH_3CN (1:1, v/v)	Buffer B : A + KCl (1 M)
$t_{retention}$ = 6.7 min	$t_{retention}$ = 8.5 min

Triethylammonium 5-fluoro-2'-deoxyuridylyl-[3'-O-(o-chlorophenyl)-5']-5-fluoro-2'-deoxyuridine-3'-O-(o-chlorophenyl)phosphate (7). Compound 6 (1.0 mmol) was dissolved in a mixture of acetonitrile and triethylamine (1:1, v/v, 24 cc). The removal of the ß-cyanoethyl protection was complete after 1 h 30, as checked by t.l.c. (eluent A). Evaporation gave a quantitative yield of pure 7 (Rf = 0.43, eluent D).

Cyclo-(5-fluoro-2'-deoxyuridylyl-[3'-O-(o-chlorophenyl)-5']-5-fluoro-2'-deoxyuridylyl-[3'-O-(o-chlorophenyl)-5']) (8). Mesitylenesulfonyl chloride (2.0 mmol) and tetrazole (10.0 mmol) were dried by coevaporation with pyridine, and dissolved in the same solvent (200 cc). A solution of compound 7 (0.2 mmol) in pyridine (5 cc) was added dropwise. The mixture was stirred at r.t. for 24 hrs. Water (10 cc) was added, and the mixture evaporated. The residue was partitioned between methylene chloride and sodium bicarbonate (5 %). The organic extracts were dried, evaporated, and the residue was chromatographed on silica. The mixture of diastereoisomers was

eluted with an increasing amount of methanol in methylene chloride (0-5 %). The yield was 81 % (Rf = 0.28, 0.34, 0.37, eluent A).

Cyclo-[5-fluoro-2'-deoxyuridylyl-(3',5')-5-fluoro-2'-deoxy-uridylyl-(3',5')], triethylammonium salt (9). The protected cyclic dimer 8 (0.1 mmol), syn-p-nitrobenzaldoxime (1.2 mmol) and N^1,N^1,N^3,N^3-tetramethylguanidine (1.2 mmol) were stirred for 24 hrs in a mixture of THF and water (1:1, v/v, 2.4 cc). The residue after evaporation was partitioned between water and methylene chloride. The water phase was neutralized by acetic acid, washed with ether and lyophilized. A 90 % pure product was obtained by low pressure reversed phase chromatography using a triethylammonium acetate buffer (TEAA, pH 7.0) containing 3 % acetonitrile.

This product was further purified by FPLC using a Pep-RPC HR 5/5 (Pharmacia) reversed phase column. The compound was eluted by an increasing amount of a mixture TEAA (100 mM)/acetonitrile (1:1, v/v) in TEAA (100 mM), and lyophilized. The lyophilization step was repeated several times in order to reduce the TEAA concentration to trace amounts (controlled by NMR). The yield was 69 % . The purity of the product was checked by FPLC, with both reversed phase and anion exchange columns (Figure 1).

^{13}C NMR (50 MHz, D_2O, δ (ppm)) (decoupled in ^1H).

11.07 (s, $\underline{CH_3}$-CH_2-N); 41.16 (broad s, C2'); 49.45 (s, CH_3-$\underline{CH_2}$-N); 64.75 (d, J_{C-P}=5.2Hz, C5'); 72.57 (d, J_{C-P}=5.2Hz, C3'); 84.96 (dd, J_{C-P}=10.4Hz, C4'); 87.56 (s, C1'); 128.55 (d, J_{C-F}=34.7Hz, C6); 143.42 (d, J_{C-F}=233.3Hz, C5); 153.00 (s, C2); 162.67 (d, J_{C-F}=25.4Hz, C4).

^{31}P NMR (81 MHz, D_2O, δ) -0.70 ppm.

^{19}F NMR (188 MHz, D_2O, δ) -162.81 ppm (broad d, J=6.4hz).

UV (H_2O) λ_{max} 269 nm (ε 8700), λ_{min} 245 nm (ε 4100).

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REFERENCES

1. J.A. Houghton, K.D. Weiss, L.G. Williams, P.M. Torrance and P.J. Houghton, Biochem. Pharmacol., 35, 1351 (1986).

2. J. Balzarini, E. De Clercq, M.P. Mertes, D. Shugar and P.F. Torrence, Biochem. Pharmacol., 31, 3673 (1982).
3. J. Balzarini, E. De Clercq, P.F. Torrence, M.P. Mertes, J.S. Park, C.L. Schmidt, D. Shugar, P.J. Barr, A.S. Jones, G. Verhelst and R.T. Walker, Biochem. Pharmacol., 31, 1089 (1982).
4. E. De Clercq, J. Balzarini, P.F. Torrence, M.P. Mertes, C.L. Schmidt, D. Shugar, P.J. Barr, A.S. Jones, G. Verhelst and R.T. Walker, Mol. Pharmacol., 19, 321 (1981).
5. C. Desgranges, G. Razaka, E. De Clercq, P. Herdewijn, J. Balzarini, F. Drouillet and H. Bricaud, Cancer Res., 46, 1094 (1986).
6. M. Iigo, A. Hoshi, A. Nakamura and K. Kuretani, Cancer Treatment Reports, 63, 1895 (1979).
7. M. Iigo, K. Kuretani and A. Hoshi, J. Natl. Cancer Inst., 66, 345 (1981).
8. J.E. Byfield, C.L. Hornbeck, S.S. Frankel, T.R. Sharp and J.C. Griffiths, Cancer Treatment Reports, 69, 645 (1985).
9. R.D. Armstrong and R.B. Diasio, Cancer Res., 41, 4891 (1981).
10. E.A. de Bruijn, A.T. van Oosterom, U.R. Tjaden, H.J.E.M. Reeuwijk and H.M. Pinedo, Cancer Res., 45, 5931 (1985).
11. S. Fukushima, T. Kawaguchi, M. Nishida, K. Juni, Y. Yamashita, M. Takahashi and M. Nakano, Cancer Res., 47, 1930 (1987).
12. D. Farquhar, N.J. Kuttesch, M.G. Wilkerson and T. Winkler, J. Med. Chem., 26, 1153 (1983).
13. R.N. Hunston, A.S. Jones, C. McGuigan, R.T. Walker, J. Balzarini and E. De Clercq. J. Med. Chem., 27, 440 (1984).
14. W. Rode, T. Kulikowski, B. Kedzierska and D. Shugar, Biochem. Pharmacol., 36, 203 (1987).
15. G.M. Tener, H.G. Khorana, R. Markham and E.H. Pol, J. Am. Chem. Soc., 80, 6223 (1958).
16. J.C. Catlin and F. Cramer, J. Org. Chem., 38, 245 (1973).
17. P. Francois, G. Hamoir, E. Sonveaux, H. Vermeersch and Y. Ma, Bull. Soc. Chim. Belg., 94, 821 (1985).
18. A.F. Turner and H.G. Khorana, J. Am. Chem. Soc., 81, 4651 (1959).
19. H.G. Khorana and J.P. Vizsolyi, J. Am. Chem. Soc., 83, 675 (1961).
20. R.K. Ralph, W.J. Connors, H. Schaller and H.G. Khorana, J. Am. Chem. Soc., 85, 1983 (1963).
21. W. Falk and Ch. Tamm, Helv. Chim. Acta, 55, 1928 (1972).
22. E. Sherwood, A. Joshi and J. Oro, J. Mol. Evol., 10, 193 (1977).
23. M. Iigo and A. Hoshi, Biochem. Pharmacol., 35, 727 (1986).
24. M. Iigo, A. Hoshi and H. Kitagawa, J. Cancer Res. Clin. Oncol., 111, 187 (1986).
25. M. Iigo, Y. Nakajima, A. Hoshi and E. De Clercq, Eur. J. Cancer Clin. Oncol., 23, 773 (1987).
26. M. Iigo, Z. Yamaizumi, S. Nishimura, A. Hoshi and E. De Clercq, Jpn. J. Cancer Res. (Gann), 78, 409 (1987).
27. C-Y.J. Hsu, D. Don and R.A. Jones, Nucleosides & Nucleotides, 4, 377 (1985).