Pergamon

Bioorganic & Medicinal Chemistry, Vol. 3, No. 3, pp. 235–243, 1995

0968-0896/95 $9.50 + .00

0968-0896(95)00003-8

Nucleosides and Nucleotides—CXXXVII. Antitumor Phospholipids with 5-Fluorouridine as a Cytotoxic Polar-Head: Synthesis of 5'-Phosphatidyl-5-Fluorouridines by Phospholipase D-Catalyzed Transphosphatidylation [1]

Satoshi Shuto,[a] Hiromichi Itoh,[b] Atsushi Sakai,[b] Keishi Nakagami,[b] Shigeyuki Imamura[c] and Akira Matsuda*,[a]

[a]*Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan*
[b]*Institute for Life Science Research and* [c]*Diagnostic Division, Asahi Chemical Industry Co., Ltd, Mifuku, Ohito-cho, Shizuoka 410-23, Japan*

Abstract—5'-Phosphatidyl-5-fluorouridines, with the same backbone structure as that of natural phospholipids, in which a polar-head group of usual phospholipids is replaced by 5-fluorouridine, were designed to be potent antitumor agents. 5'-Phosphatidyl-5-fluorouridines with a variety of diacyl or dialkyl residues in the glycerol moiety, were synthesized by phospholipase D-catalyzed transphosphatidylation from the corresponding phosphatidylcholine and 5-fluorouridine. These new compounds were evaluated in mice with experimental tumors by ip and po administration. Dipalmitoyl and distearoyl derivatives **1b** and **1c** had the greatest antitumor activity against both P388 leukemia and Meth A fibrosarcoma in mice.

Introduction

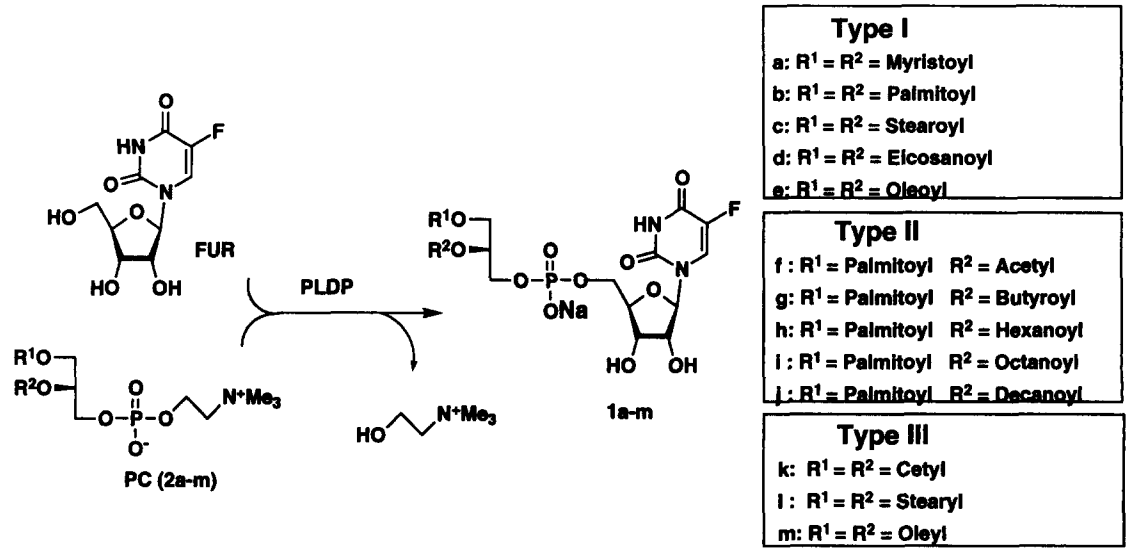
Nucleoside and nucleobase derivatives (e.g. 5-fluorouracil,[2] 1-β-D-arabinosylcytosine[3,4] and 6-mercaptopurine[5]) are widely used for the chemotherapy of cancer patients, but they have several disadvantages: the antitumor spectra are not broad enough; normal cells are affected, causing toxic side effects; tumors can develop resistance to these drugs; the drugs are often inactive against metastases. More effective antitumor agents, especially against solid tumors, are needed.

Much attention has been focused on the conversion of antitumor nucleosides to their phospholipid derivatives,[6–14] because of their possible advantages compared with the parent compounds: readier permeation through membranes,[12] resistance to enzymes that can inactivate biologically active nucleosides,[9b] improved pharmacokinetic properties due to high lipophilicity,[13] and the possibility of gradual intracellular release of an already phosphorylated agent.[8,9a,c,14] In addition, these compounds can form lipid-bilayers and be incorporated into liposomes, which could be used in drug-delivery systems.[15]

We have synthesized various 5'-phosphatidyl nucleosides with a backbone the same as that of natural phospholipids, in which the usual polar-head group, such as choline, serine, or inositol, is replaced by an antitumor nucleoside analogue.[11] Of these compounds, a 5'-phosphatidyl derivative of 5-fluorouridine **1b** with two palmitoyl groups as fatty acid residues in the glycerol moiety has potent activity against P388 leukemia by ip-administration in mice.[11b]

On the other hand, phospholipids in food are absorbed from the intestinal tract through a specific manner coupling with the deacylation–reacylation cycle;[16] a fatty acyl group at the *sn*-2-position of the phospholipid is hydrolyzed by phospholipase A_2 (PLA_2) from pancreatic juice to form a lysophospholipid that permeates into epithelial cells, where it is reacylated by acyltransferase at the *sn*-2-position with a fatty acid residue. Then the reacylated compound, after its incorporation into chylomicrons, is transported specifically to the lymphatic system but not to the blood circulatory system.[16] We have demonstrated that a 5'-phosphatidyl-5-fluorouridine **1b** given orally could be absorbed via a specific pathway for natural phospholipids to be transported to the lymph.[13] This finding suggests that compound **1b** could be used clinically in targeting chemotherapy of lymphoma or lymphatic metastases of certain tumors.[17]

From these results, the relationship between antitumor potency and the structure of the glycerol moiety of 5'-phosphatidyl-5-fluorouridines is of interest, and which route of administration would affect the antitumor effects because of the characteristic patterns in absorption and distribution of these compounds. The phosphatidyl-5-fluorouridines synthesized and evaluated here were classified as shown in Scheme 1 into three types depending on the substituents bound at the *sn*-1- and *sn*-2-positions of the glycerol moiety: Type I with the same fatty acyl residue at both positions; Type II



Scheme 1.

with a palmitoyl residue and a much lower acyl residue at the *sn*-1- and *sn*-2-positions, respectively; Type III with the same fatty alkyl residue at both positions.

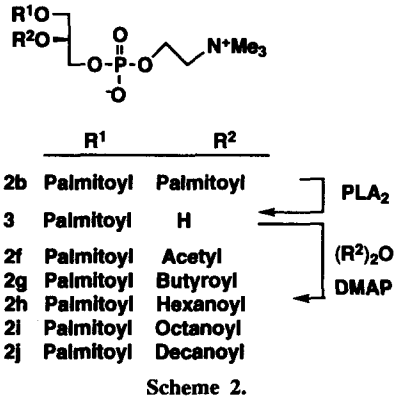
Chemistry

Phosphatidylnucleosides have multifunctional structures in which a polar 5'-nucleotide group and a lipophilic diacyl glycerol group coexist. Because of the characteristic structure, these are labile and have surface-active properties that make their synthesis difficult. The chemical synthetic methods of 5'-phosphatidylnucleosides reported so far are not general, nor are they practical,[18-21] limiting studies of phosphatidylnucleosides in spite of their biological interest. In particular, systematic studies of their structure–activity relationships have not been reported.

In recent years, we have undertaken the enzymatic synthesis of biologically important phospholipid derivatives.[6,7,11,22] Throughout the study, we have developed an enzymatic method for the preparation of 5'-phosphatidylnucleosides from a nucleoside and a 3-*sn*-phosphatidylcholine (PC) by a one-step reaction, in which phospholipase D-catalyzed transphosphatidylation, namely, the regiospecific transfer reaction of the phosphatidyl residue from PC to the 5'-hydroxyl group of a nucleoside, was utilized.[11] We planned to prepare various 5'-phosphatidyl-5-fluorouridines from the corresponding PCs and 5-fluorouridine (FUR), by this enzymatic method, as shown in Scheme 1.

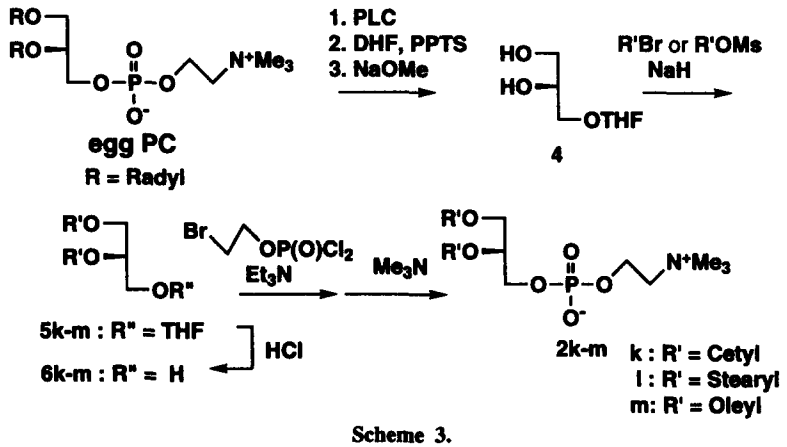
However, not every PC modified in the glycerol moiety, for phosphatidyl donors, has been available so far. Therefore, we developed practical methods for synthesizing Type II and III PCs (Type I PCs are commercially available).

PCs **2f–j**, which have a palmitoyl residue and a lower acyl residue (C2–C10) at the *sn*-1- and *sn*-2-positions, respectively, were synthesized from dipalmitoylphosphatidylcholine (DPPC, **2b**) as shown in Scheme 2. The palmitoyl group of DPPC at the *sn*-2-position was hydrolyzed regiospecifically with PLA_2 from *Streptomyces violaceoruber* in a two-phase system of glycine buffer (pH 9.8) and chloroform to give lysophosphatidylcholine **3** quantitatively. This enzyme was useful in the practical preparation of lysophosphatidylcholines because of its efficient catalytic potency and stability under the reaction conditions used. Treatment of **3** with an acid anhydride in the presence of dimethylaminopyridine (DMAP) in benzene afforded the desired PCs **2f–j** in good yields.



Scheme 2.

Dialkylether PCs have been recognized as minor phospholipids from natural source for a long time. In recent years, these have been noticed especially for their biological importance as the main kind of phospholipids of the membrane in archaebacteria.[23] The synthesis of dialkylether PCs has been reported[24,25] but the methods involved long reaction steps and the overall yields were low. In addition, the optical purity was often insufficient because a common intermediate, 1,2-*O*-isopropylidene-*sn*-glycerol, racemized easily during the work-up.[26] We developed a new chemoenzymatic method for the preparation of dialkylether



Scheme 3.

PCs from egg PC (which is readily available), as shown in Scheme 3. Egg PC was treated with phospholipase C (PLC) from *Bacillus cereus* in a two-phase system of PIPES buffer (pH 7.5) and chloroform for regiospecific hydrolysis at the *sn*-3-phosphodiester linkage, giving only 1,2-*O*-diacyl-*sn*-glycerol. The hydroxyl group at the *sn*-3-position of the diacylglycerol was protected with a tetrahydrofuranyl (THF) group, followed by treatment with sodium methoxide in methanol to afford 3-*O*-THF-*sn*-glycerol (**4**) in excellent overall yield from egg PC. The reaction of the *O*-sodium salt of **4**, prepared from **4** and sodium hydride, with alkyl bromide or methanesulfonate gave dialkylethers **5k–m**. The THF groups were hydrolyzed with HCl to furnish the desired 1,2-dialkylethers **6k–m** in high yields. Compounds **6k–m** were converted to the corresponding dialkylether PCs **2k–m** in excellent yields by a published procedure.[27] Thus, this method could provide optically pure dialkylether PCs practically.

These PCs **2f–m** synthesized as well as commercially available PCs **2a**, **2c** and **2d** were subjected to the phospholipase D-catalyzed transphosphatidylation with FUR as the phosphatidyl acceptor. The results are summarized in Tables 1 and 2. In the presence of excess FUR, PCs **2a**, **2c** and **2d**, which have the same saturated fatty acyl residues at both the *sn*-1- and *sn*-2-positions, were treated with phospholipase D from *Streptomyces* sp. AA 586 (PLDP) in a two-phase system of chloroform and acetate buffer (pH 5.6) at 45 °C. After the usual work-up and treatment with a cation-exchange resin, the desired phosphatidyl-FUR derivatives **1a**, **1c** and **1d**, were obtained as sodium salts in good yields, which are comparable to the earlier result for the synthesis of dipalmitoyl isostere **1b**.[11b] The synthesis of unsaturated phospholipid derivatives is usually troublesome due to their instability,[28,29] but the dioleoyl derivative **1e** was also synthesized readily by this enzymatic method. In the same way, Type II diacyl derivatives **1f–j** and Type III dialkylether derivatives **1k–m**, were also prepared with the corresponding PCs (**2f–j** and **2k–m**) as the phosphatidyl donors.

In this way, we used three phospholipases, PLA_2, PLC and PLDP to prepare a variety of phospholipids. These enzymes should be widely used for the preparation of compounds related to phospholipids because of their specific reactivity, availability by production in bulk from bacterial sources, and stability in both storage and reaction conditions, especially in organic solvents.

Table 1. Yields and physical data for 5'-(3-*sn*-phosphatidyl)-5-fluorouridines

Compound	Yield[a]	UV_{max} (nm)[b]	FAB-MS (m/z)	Formula[c]
1a	66	268	859(MNa_2-H)	$C_{40}H_{69}FN_2O_{13}PNa•2/3H_2O$
1c	68	268	d	$C_{48}H_{85}FN_2O_{13}PNa•H_2O$
1d	44	268	1027 (MNa)	$C_{52}H_{93}FN_2O_{13}PNa$
1e	64	268	d	$C_{48}H_{81}FN_2O_{13}PNa•H_2O$
1f	64	268	719 (MNa)	$C_{30}H_{49}FN_2O_{13}PNa$
1g	56	268	769 (MNa_2-H)	$C_{32}H_{53}FN_2O_{13}PNa$
1h	62	268	797 (MNa_2-H)	$C_{34}H_{57}FN_2O_{13}PNa$
1i	59	268	825 (MNa_2-H)	$C_{36}H_{61}FN_2O_{13}PNa$
1j	49	268	831 (MNa)	$C_{38}H_{65}FN_2O_{13}PNa$
1k	46	268	887 (MNa)	$C_{44}H_{81}FN_2O_{11}PNa•1/2H_2O$
1l	68	268	943 (MNa)	$C_{48}H_{89}N_5O_{11}PNa$
1m	58	268	939 (MNa)	$C_{48}H_{85}N_2O_{13}PNa•H_2O$

[a]Yields were based on phosphatidylcholine used. [b]Measured in MeOH. [c]Compounds were analyzed for C, H and N and were within ± 0.4% of the theoretical value. [d]A molecular-ion peak was not detected.

Table 2. ^{1}H NMR data for 5'-phosphatidyl-5-fluorouridines measured in $CDCl_3$:CD_3OD (3:1)

Compound	^{1}H-NMR data (δ)
1a[a]	7.97 (*d*, 1H, H-6, *J* = 6.3 Hz), 5.82 (*br d*, 1H, H-1'), 5.25 (*m*, 1H, glycerol CH), 4.5–3.9 (*m*, 9H, H-3', 4', 5', glycerol CH_2, glycerol CH_2OPO), 2.31 (*m*, 4H, $COCH_2$), 1.7–1.2 (*m*, 44H, myristoyl CH_2), 0.88 (*t*, 3H, CH_3).
1c[b]	8.03 (*d*, 1H, H-6, *J* = 6.4 Hz), 5.90 (*dd*, 1H, H-1', *J* = 1.5, 4.9 Hz), 5.25 (*m*, 1H, glycerol CH), 4.44–4.01 (*m*, 9H, H-3', 4', 5', glycerol CH_2, glycerol CH_2OPO), 2.32 (*m*, 4H, $COCH_2$), 1.60–1.27 (*m*, 60H, stearoyl CH_2), 0.89 (*t*, 3H, CH_3).
1d[a]	7.95 (*d*, 1H, H-6, *J* = 6.4 Hz), 5.80 (*br d*, 1H, H-1'), 5.24 (*m*, 1H, glycerol CH), 4.5–3.9 (*m*, 9H, H-3', 4', 5', glycerol CH_2, glycerol CH_2OPO), 2.31 (*m*, 4H, $COCH_2$), 1.7–1.2 (*m*, 68H, eicosanoyl CH_2), 0.88 (*t*, 3H, CH_3)
1e[b]	8.03 (*d*, 1H, H-6, *J* = 6.8 Hz), 5.90 (*dd*, 1H, H-1', *J* = 1.5, 4.9 Hz), 5.34 (*m*, 4H, oleoyl CH=), 5.25 (*m*, 1H, glycerol CH), 4.43–3.97 (*m*, 9H, H-3', 4', 5', glycerol CH_2, glycerol CH_2OPO), 2.32 (*m*, 4H, $COCH_2$), 2.02 (*m*, 8H, oleoyl CH_2CH=), 1.60–1.28 (*m*, 44H, oleoyl CH_2), 0.88 (*t*, 3H, CH_3).
1f[a]	7.99 (*d*, 1H, H-6, *J* = 6.6 Hz), 5.88 (*br d*, 1H, H-1'), 5.25 (*m*, 1H, glycerol CH), 4.5–3.9 (*m*, 9 H, H-3', 4', 5', glycerol CH_2, glycerol CH_2OPO), 2.33 (*m*, 4H, $COCH_2$), 2.08 (*s*, 3H, acetyl CH_3), 1.7–1.2 (*m*, 26H, palmitoyl CH_2), 0.88 (*t*, 3H, CH_3).
1g[a]	7.99 (*d*, 1H, H-6, *J* = 6.6 Hz), 5.85 (*br d*, 1H, H-1'), 5.25 (*m*, 1H, glycerol CH), 4.5–3.9 (*m*, 9H, H-3', 4', 5', glycerol CH_2, glycerol CH_2OPO), 2.32 (*m*, 4H, $COCH_2$), 1.7–1.2 (*m*, 28H, acyl CH_2), 0.94 (*m*, 6H, CH_3).
1h[a]	8.02 (*d*, 1H, H-6, *J* = 6.6 Hz), 5.90 (*br d*, 1H, H-1'), 5.24 (*m*, 1H, glycerol CH), 4.5–3.9 (*m*, 9H, H-3', 4', 5', glycerol CH_2, glycerol CH_2OPO), 2.34 (*m*, 4H, $COCH_2$), 1.7–1.2 (*m*, 32H, acyl CH_2), 0.90, 0.89 (each *t*, each 3H, CH_3).
1i[a]	7.99 (*d*, 1H, H-6, *J* = 6.6 Hz), 5.88 (*br d*, 1H, H-1'), 5.24 (*m*, 1H, glycerol CH), 4.5–3.9 (*m*, 9H, H-3', 4', 5', glycerol CH_2, glycerol CH_2OPO), 2.33 (*m*, 4H, $COCH_2$), 1.7–1.2 (*m*, 36H, acyl CH_2), 0.88 (*t*, 6H, CH_3).
1j[a]	7.98 (*d*, 1H, H-6, *J* = 6.6 Hz), 5.86 (*br d*, 1H, H-1'), 5.23 (*m*, 1H, glycerol CH), 4.5–3.9 (*m*, 9H, H-3', 4', 5', glycerol CH_2, glycerol CH_2OPO), 2.32 (*m*, 4H, $COCH_2$), 1.7-1.2 (*m*, 40H, acyl CH_2), 0.88 (*t*, 3H, CH_3).
1k[b]	8.03 (*d*,1H, H-6, *J* = 6.4 Hz), 5.87 (*dd*, 1H, H-1', *J* = 4.4 , 1.5 Hz), 4.24–4.11 (*m*, 5H, H-1', 2', 3', 4', 5'), 3.91 (*m*, 2H, glycerol CH_2OP), 3.64–3.43 (*m*, 7H, glycerol $CH(OCH_2)CH_2OCH_2$-), 1.55 (*m*, 4H, cetyl OCH_2CH_2-), 1.26 (*m*, 52H, cetyl CH_2), 0.88 (*t*, 3H, CH_3).
1l[b]	8.01 (*d*,1H, H-6, *J* = 6.4 Hz), 5.86 (*dd*, 1H, H-1', *J* = 4.4, 1.5 Hz), 4.25–4.12 (*m*, 5H, H-1', 2', 3', 4', 5'), 3.92 (*m*, 2H, glycerol CH_2OP), 3.63–3.43 (*m*, 7H, glycerol $CH(OCH_2)CH_2OCH_2$-), 1.54 (*m*, 4H, stearyl OCH_2CH_2-), 1.26 (*m*, 60H, stearyl CH_2), 0.88 (*t*, 3H, CH_3).
1m[b]	8.02 (*d*,1H, H-6, *J* = 6.8 Hz), 5.84 (*dd*, 1H, H-1', *J* = 4.4, 1.5 Hz), 5.34 (*m*, 4H, oleyl CH=), 4.25–4.12 (*m*, 5H, H-1', 2', 3', 4', 5'), 3.92 (*m*, 2H, glycerol CH_2OP), 3.63–3.43 (*m*, 7H, glycerol $CH(OCH_2)CH_2OCH_2$-), 2.01 (*m*, 8H, oleyl CH_2CH=), 1.55 (*m*, 4H, stearyl OCH_2CH_2-), 1.26 (*m*, 44H, oleyl CH_2), 0.88 (*t*, 3 H, CH_3).

[a]100 MHz. [b]400 MHz.

Antitumor Activity

We evaluated the effects of the newly synthesized compounds against P388 leukemia implanted ip, by ip administration on days 1–5 (Table 3). The saturated diacyl derivatives **1a**, **1c** and **1d**, with the same saturated fatty acyl residues (myristoyl, stearoyl and eicosanoyl, respectively) at the *sn*-1- and *sn*-2-positions, increased the life span of the mice significantly. The distearoyl derivative **1c** gave the highest value for the percent increase in life span (ILS) at the optimum daily dose of 30 mg kg^{-1}. This result was superior to that with FUR (ILS_{max} 138% at 10 mg kg^{-1} day^{-1}), the parent compound, and was comparable to that of the dipalmitoyl derivative **1b** reported previously.[11b] The unsaturated dioleoyl derivative **1e** had an ILS_{max} of 112% (at 30 mg kg^{-1} day^{-1}), lower than that of the corresponding saturated derivative **1c**. Because of their having two lipophilic acyl residues, these Type I compounds were generally insoluble in aqueous medium, so they would not be suitable for administration by injection. For improving solubility, Type II compounds **1f–j**, which bear lower acyl residues at the *sn*-2-position, were synthesized. Solubility in water was improved (data not shown), but their antileukemic effects were only moderate (ILS_{max} <100%), as a rule. We designed dialkylether derivatives **1k–m** (Type III compounds) to be resistant to phospholipase A_1 and A_2 which hydrolyze the ester linkage of phospholipids at the *sn*-1- or *sn*-2-position. Of these compounds, dicetyl and dioleyl derivatives (**1k** and **1m**, respectively) had antileukemic effects (ILS 127% and 76%, respectively, at 30 mg kg^{-1} day^{-1}). However, the activity of distearyl derivative **1l** was slight, in spite of its structural similarity to the corresponding diacyl derivative **1c**. We also compared the antitumor effect of FUR 5'-cetylphosphate **7**,[6] a simple lipophilic analogue of FUR 5'-phosphate, with the effects of these 5'-phosphatidyl derivatives. However, cetylphosphate **7** had little effect in this

Table 3. Antitumor activities of 5'-(3-*sn*-phosphatidyl)-5-fluorouridines and related compounds against P388 leukemia implanted ip[a]

compound	ILS (%)[b]				wt change[c] (g/mouse) on day 7			
	dose, ip (mg kg^{-1} day^{-1})			dose, po (mg kg^{-1} day^{-1})	dose, ip (mg kg^{-1} day^{-1})			dose, po (mg kg^{-1} day^{-1})
	3	10	30	100	3	10	30	100
1a	58	109	133		+2.1	+1.6	−0.3	
1b[d]	31	130	206	21	+2.0	−0.6	−1.8	±0
1c	54	94	215	14	+3.6	+1.3	−3.2	−0.2
1d	30	70	151		+3.2	+2.6	−0.3	
1e	49	107	112	21	+3.6	+1.5	−2.6	−1.6
1f	40	59	29		+2.1	+1.0	−4.9	
1g	36	53	27		+3.0	+0.1	−4.3	
1h	45	80	32	21	+3.4	−0.3	−5.3	−1.5
1i	45	80	82		+2.7	−3.1	−5.3	
1j	61	122	51		+2.9	−2.8	−4.7	
1k	31	92	127	6	+2.9	+2.5	+0.1	
1l	16	24	31	2	+3.1	+3.4	+4.3	−0.3
1m	17	74	76	7	+3.2	+0.4	−3.4	+0.9
FUR	134	138	toxic[e]		+0.4	−3.3		
7	17	59	51		+0.9	−1.3	−4.1	

[a]Compounds were given on days 1–5. [b]Percent increase in life span. The control mice died in 7–10 days. [c] Weight changes for the control studies (50 mice) averaged +1.6 g/mouse. [d]Data were taken from Shuto *et al.*, (Ref. 11b). [e]All mice died by day 5.

system. This result showed the importance of the glycerol backbone for the antitumor potency.

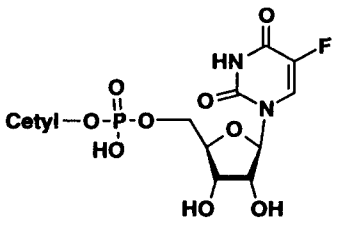
We investigated the antileukemic effects by po administration instead, and the results are also shown in Table 3. The treatment was on the same schedule as the ip injection, but none of the compounds tested had much effect (ILS < 30%).

The antitumor effects of phosphatidyl-FURs against solid tumor were tested with Meth A fibrosarcoma, implanted sc, in mice. The results are summarized in Table 4. When the compounds were administered ip for five consecutive days starting on the next day of the implantation, all of the compounds tested suppressed the proliferation of the tumor. The diacyl derivatives **1b**, **1c** and **1e** had strong effects (T/C < 30%). Compounds **1h**, **1k**, **1l** and **1m** also had good effects in this solid tumor system at 10 mg kg^{-1} day^{-1}, but daily doses of 30 mg kg^{-1} were toxic. FUR had only moderate effects on this tumor by either ip or po administration. The diacyl derivatives **1b**, **1c**, **1e** and **1h** also had antitumor effects clearly against the fibrosarcoma by po administration. On the same schedule, dialkylether derivatives **1k**, **1l** and **1m** were inactive when given po, in spite of their high activities against this fibrosarcoma when given ip.

Table 4. Antitumor activities of 5'-(3-*sn*-phosphatidyl)-5-fluorouridines and 5-fluorouridine against Meth A fibrosarcoma implanted sc in mice[a]

compound	T/C[b] (%)					wt change[c] (g/mouse) on day 7				
	dose, ip (mg kg^{-1} day^{-1})			dose, po (mg kg^{-1} day^{-1})		dose, ip (mg kg^{-1} day^{-1})			dose, po (mg kg^{-1} day^{-1})	
	3	10	30	30	100	3	10	30	30	100
1b	49[d]	38[d]	7[d]	42[d]	23[d]	+2.2	+1.3	−2.2	+3.3	−0.4
1c		29[d]	27[d]	34[d]		+0.4	−4.1		+1.7	
1e		29[d]	24[d]	34[d]		+1.2	−5.1		−1.5	
1h	45[d]	26[d]	toxic (5)[e]	23[d]	+3.3	−3.7			−3.5	
1k		28[d]	toxic (5)[e]	64		+1.4			+2.8	
1l		29[d]	toxic (5)[e]	84		+1.8			+3.1	
1m		36[d]	toxic (5)[e]	83		+1.9			+2.8	
FUR	44[d]	toxic (4)[e]		42[d]	toxic (5)[e]	−0.8			−5.3	toxic (5)[e]

[a]Compounds were given on days 1–5. [b]Antitumor activity was evaluated from the mean tumor volume of treated mice (T) over that of control mice (C). [c] Weight changes for the control studies (40 mice) averaged +3.1 g/mouse. [d]Significantly different from the control at $P < 0.01$. [e]Number in parenthesis indicates dead mice out of five mice by day 14.



7

Chart 1.

The high activity of diacyl compounds against the solid tumor by po administration can be explained; they would be absorbed through the specific absorption pathway for natural phospholipids from the intestinal tract as described above, which would transport the compounds specifically to the lymph.[13] These compounds might be used clinically in cases of lymphatic metastases of tumors, because antitumor agents known to date do not have such lymphatropic properties as these diacyl compounds. The inactivity of these dialkylether derivatives by oral administration against Meth A fibrosarcoma in mice suggests that they are not absorbed by this route because they cannot be digested by PLA_2 in the pancreatic juice due to their ether linkage at the *sn*-2-position. Diacyl derivatives were inactive against P388 leukemia when given po, in spite of their strong effects against the same leukemia when given ip. These compounds seem not to enter into the abdominal cavity when administered orally because of their characteristic absorption and distribution properties. These results suggested that the distribution of these agents in mice can be changed depending on the route of administration.

5-Fluorouracil is one of the most effective anticancer agents in clinical use. Although FUR has strong *in vitro* antitumor activity, greater than that of 5-fluorouracil,[30] its high toxicity to the intestine,[31] especially in oral administration, has restricted its clinical use. The serious intestinal toxicity of FUR could be avoided by present derivatization, because the compounds would be absorbed orally in a masked form,[13] which would then show the therapeutic effect as antitumor agents after metabolic activation.

Antitumor nucleobase or nucleoside analogues, including 5-fluorouracil and FUR, are known to show the activity only after metabolic conversion to 5'-nucleotides in cells, and drug resistance to such analogues can often result from the deletion of enzymes such as kinases or phosphoribosyltransferases that synthesize nucleotides.[32-34] 5'-Phosphatidyl-FURs may have antitumor effects against cells resistant to the parent compounds, because the derivatives can be hydrolyzed in the cells by phospholipase C into a phosphorylated polar-head, namely, FUR 5'-phosphate and a diacylglycerol.[35]

In conclusion, the derivatization of FUR to the corresponding 5'-phosphatidyl analogues resulted in increased antitumor effects of the compounds. In particular the di-saturated acyl derivatives such as **1b** and **1c** had the greatest effects. These compounds are promising candidates for the clinical treatment of lymphatic metastases as well as primary tumors because of their characteristics of absorption and distribution. The antitumor effects of 5'-phosphatidylnucleosides would be dependent on their being recognized as phospholipids by the body. Therefore, a fatty diacylglycerol structure which is the usual structure of natural glycerophospholipids would be important in this derivatization.

Experimental

Melting points were determined on a Yanagimoto MP-3 micro-melting point apparatus and are uncorrected. The NMR spectra were recorded with a JEOL FX-100 or GSX-400 spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), *m* (multiplet), or *br* (broad). All exchangeable protons were detected by the addition of D_2O or CD_3OD. UV absorption spectra were recorded with a Shimadzu UV-240 spectrophotometer. Mass spectra were measured on a JEOL JMS-D300 spectrometer. Thin-layer chromatography was carried out on Merck coated plate $60F_{254}$. Flash chromatography was conducted with Merck silica gel 9385. PLDP, PLA_2 and PLC were products of the Diagnostic Division of Asahi Chemical Industry Co., Ltd. Synthetic PCs (**2a–e**) were gifts of Nippon Fine Chemical Co., Ltd. Egg PC was purchased from QP Co., Ltd.

General procedure for the synthesis of 5'-(3-sn-phosphatidyl)-5-fluorouridine (1)

A $CHCl_3$ solution (20 mL) of 3-*sn*-phosphatidylcholine (**2**, 1.0 mmol) was added to a solution of PLDP (10 mg, 1860 units, for **1a** and **1c–e**; 30 mg, 5580 units, for **1f–m**) and FUR (1.32 g, 5.0 mmol) in sodium acetate buffer (200 mM, pH 5.7, 8 mL) containing $CaCl_2$ (250 mM). The mixture was stirred at 45 °C for 6 h, then a mixture of 2 N HCl (5 mL), MeOH (20 mL) and $CHCl_3$ (20 mL) was added, and the mixture was shaken. The separated organic layer was washed with H_2O (2 × 10 mL) and evaporated to dryness. The residue was purified by flash chromatography (silica gel, $CHCl_3$:MeOH, 10:1 followed by 6:1), and fractions containing the desired product were evaporated. The residue was dissolved in a mixture of 2 N HCl (5 mL), MeOH (10 mL), and $CHCl_3$ (20 mL), and the resulting mixture was partitioned. The organic layer was washed with water (2 × 5 mL) and evaporated. The residue was dissolved in a mixture of $CHCl_3$:MeOH:H_2O (10:5:1, 15 mL), and the solution was put on a column of Diaion WK-20 resin (2 × 8 cm, Na^+ form). The column was developed with the same solvent, and the eluate was concentrated, giving **1** as a sodium salt. Yields and

physical data are summarized in Table 1. ^{1}H NMR data are listed in Table 2.

*1-O-Palmitoyl-*sn*-glycero-3-phosphocholine (3)*

A $CHCl_3$ solution (100 mL) of DPPC (**2b**, 3.67 g, 5.0 mmol) was added to a solution of PLA_2 (18 mg, 100 units) in glycine–Na buffer (200 mM, pH 9.8, 10 mL) containing 1 M $CaCl_2$ (10 mL). The mixture was stirred at 37 °C for 18 h, then a mixture of MeOH (50 mL) and $CHCl_3$ (20 mL) was added, and the mixture was shaken and partitioned. The aqueous layer was extracted with $CHCl_3$:MeOH (2:1, 200 mL). The organic layers were combined and evaporated to dryness. The residue was purified by flash chromatography (silica gel; $CHCl_3$:MeOH, 10:1, 3:1, 1:1 and 1:2), and fractions containing the desired product were evaporated. The residue was dissolved in a mixture of MeOH (20 mL), $CHCl_3$ (40 mL) and H_2O (12 mL), and the resulting mixture was partitioned. The organic layer was evaporated to dryness to give **3** (2.30 g, 93%) as a white solid: ^{1}H NMR ($CDCl_3$:CD_3OD = 3:1, 100 MHz), 4.3–3.7 (*m*, 9H, glycerol CH, CH_2 and choline CH_2), 3.32 (*s*, 9H, choline CH_3), 2.30 (*m*, 2H, $COCH_2$), 1.4–1.2 (*m*, 26H, palmitoyl CH_2), 0.88 (*t*, 3H, palmitoyl CH_3); FAB-MS (negative); m/z 496 (M^-).

*General procedure for synthesis of 3-*sn*-phosphatidylcholine with a lower acyl group at the* sn*-2-position (2f–j)*

A mixture of **3** (993 mg, 2.0 mmol), acid anhydride (10 mmol), and DMAP (366 mg, 3.0 mmol) in benzene (100 mL) was stirred at room temperature for 20 h. MeOH (10 mL) was added to the solution and the resulting mixture was evaporated. The residue was dissolved in a mixture of 2 N HCl (12 mL), MeOH (20 mL), and $CHCl_3$ (40 mL), and the resulting mixture was partitioned. The organic layer was washed with H_2O (2 × 10 mL) and evaporated. The residue was purified by flash chromatography (silica gel; $CHCl_3$:MeOH, 10:1, 3:1, 1:1 and 1:2), and fractions containing the desired product were evaporated. The residue was dissolved in a mixture of MeOH (20 mL), $CHCl_3$ (40 mL) and H_2O (12 mL), and the resulting mixture was partitioned. The organic layer was evaporated to give **2**. **2f**: yield 95%; FAB-MS (positive) m/z 538 (MH^+); ^{1}H NMR ($CDCl_3$, 100 MHz). 5.15 (*m*, 1H, glycerol CH), 4.39–3.70 (*m*, 8H, glycerol CH_2 and choline CH_2), 3.67 (*s*, 3H, acetyl CH_3), 3.21 (*s*, 9H, choline CH_3), 2.30 (*m*, 2H, $COCH_2$), 1.61–1.26 (*m*, 26H, palmitoyl CH_2), 0.88 (*t*, 3H, palmitoyl CH_3). **2g**: yield 85%; FAB-MS (positive) m/z 566 (MH^+); ^{1}H NMR ($CDCl_3$, 100 MHz), 5.18 (*m*, 1H, glycerol CH), 4.40–3.77 (*m*, 8H, glycerol CH_2 and choline CH_2), 3.21 (*s*, 9H, choline CH_3), 2.29 (*m*, 4H, $COCH_2$), 1.72–1.26 (*m*, 28H, acyl CH_2), 0.94, 0.88 (each *t*, each 3H, butyroyl and palmitoyl CH_3). **2h**: yield 96%; FAB-MS (positive) m/z 594 (MH^+); ^{1}H NMR ($CDCl_3$, 100 MHz), 5.20 (*m*, 1H, glycerol CH), 4.48–3.63 (*m*, 8H, glycerol CH_2 and choline CH_2), 3.21 (*s*, 9H, choline CH_3), 2.30 (*m*, 4H, $COCH_2$), 1.64–1.25 (*m*, 32H, acyl CH_2), 0.88 (*t*, 6H, acyl CH_3). **2i**: yield 84%; FAB-MS (positive) m/z 622 (MH^+); ^{1}H NMR ($CDCl_3$, 100 MHz), 5.20 (*m*, 1H, glycerol CH), 4.44–3.66 (*m*, 8H, glycerol CH_2 and choline CH_2), 3.21 (*s*, 9H, choline CH_3), 2.30 (*m*, 4H, $COCH_2$), 1.70–1.26 (*m*, 36H, acyl CH_2), 0.88 (*t*, 6H, acyl CH_3). **2j**: yield 82%; FAB-MS (positive) m/z 649 (MH^+); ^{1}H NMR ($CDCl_3$, 100 MHz), 5.17 (*m*, 1H, glycerol CH), 4.45–3.70 (*m*, 8H, glycerol CH_2 and choline CH_2), 3.21 (*s*, 9H, choline CH_3), 2.30 (*m*, 4H, $COCH_2$), 1.65–1.26 (*m*, 40H, acyl CH_2), 0.88 (*t*, 6H, acyl CH_3).

*3-O-Tetrahydrofuranyl-*sn*-glycerol (4)*

A $CHCl_3$ solution (400 mL) of egg PC (**2n**, 34.5 g) was added to a solution of PLC (100 mg, 8700 units) in PIPES buffer (200 mM, pH 7.5, 40 mL). The mixture was stirred at room temperature for 18 h, saturated NaCl (100 mL) was added, and the mixture was shaken and partitioned. The aqueous layer was extracted with a mixture of $CHCl_3$ and MeOH (2:1, 800 mL). The organic layers were combined and evaporated to dryness. The residue was dissolved in $CHCl_3$ (300 mL), dried with Na_2SO_4, and evaporated. A mixture of the residual oil (23.9 g), 2,3-dihydrofuran (DHF, 19.0 mL, 25.2 mmol), and pyridinium *p*-toluenesulfonate (PPTS, 2.12 g, 4.2 mmol) in benzene (500 mL) was stirred at room temperature for 4 h. Hexane (200 mL) was added to the mixture to give an insoluble material. The supernatant was obtained by decanting and concentrated. The residue was purified by flash chromatography (silica gel; hexane:AcOEt, 100:1, 80:1, and 20:1). Fractions containing the desired product were evaporated. An NaOMe solution (1 M in MeOH, 95 mL) was added to a mixture of the residue in MeOH (300 mL) and benzene (200 mL), and the resulting mixture was stirred at room temperature for 0.5 h. The mixture was neutralized with AcOH:benzene (1:9) and evaporated. The residue was purified by flash chromatography (silica gel; $CHCl_3$ and $CHCl_3$:MeOH, 20:1). Fractions containing the desired product were evaporated to give **4** (5.9 g) as a pale yellow oil: CIMS m/z 163 (MH^+); ^{1}H NMR (CD_3OD, 100 MHz), 5.14 (*m*, 1H, THF CH), 4.02–3.51 (*m*, 7H, glycerol CH and CH_2, THF CH_2O), 1.95 (*m*, 4H, THF CH_2).

*1,2-Di-O-alkyl-*sn*-glycerols (6k–m); general procedure*

A mixture of sodium hydride (55%, 2.09 g, 48 mmol) and **4** (1.95 g, 12 mmol) in DMF (50 mL) was stirred at room temperature for 5 min under an argon atmosphere, an alkylating reagent (48 mmol, cetyl bromide for **6k**, stearyl bromide for **6l** and oleyl methanesulfonate for **6m**) was added, and the mixture was stirred at 60 °C for 18 h. The resulting solution was evaporated and the residue was dissolved in a mixture of $CHCl_3$ (100 mL) and water (50 mL). The mixture was neutralized with AcOH and partitioned. The organic layer was dried with Na_2SO_4 and evaporated, and the residue was purified by flash chromatography (silica gel; hexane:AcOEt, 100:1 and 50:1) to give 1,2-di-*O*-alkyl-3-*O*-tetrahydrofuranyl-*sn*-glycerols **5k–m** as a colorless oil. Concentrated HCl

(2 mL) was added to the resulting oil in a mixture of MeOH (100 mL) and benzene (100 mL), and the mixture was stirred at room temperature for 1 h and evaporated. From the resulting residue, **6k** was obtained as a white crystal from EtOH (4.87 g, 75%): mp 48.5–49.5 °C (lit. 48.5–49.5 °C[36]); $[\alpha]_D$ = –8.4 (*c* 0.83, $CHCl_3$; lit. –7.5[36]); CIMS m/z 541 (MH^+); ^1H NMR ($CDCl_3$, 100 MHz), 3.71–3.36 (*m*, 9H, glycerol CH, CH_2, cetyl CH_2O), 2.16 (*t*, 1H, OH), 1.58–1.25 (*m*, 56H, cetyl CH_2), 0.88 (*t*, 6H, cetyl CH_3). Anal. ($C_{35}H_{57}O_3$) C, H, N. From the resulting residue, **6l** was obtained as a white crystal from EtOH (6.09 g, 85%): mp 56.5–57.0 °C (lit. 53.5–54.5 °C[36]). $[\alpha]_D$ = –7.0 (*c* 0.85, $CHCl_3$; lit. –6.9[36]); CIMS m/z 597 (MH^+); ^1H NMR ($CDCl_3$, 100 MHz), 3.71–3.36 (*m*, 9H, glycerol CH, CH_2, stearyl CH_2O), 2.16 (*t*, 1H, OH), 1.57–1.25 (*m*, 64H, stearyl CH_2), 0.88 (*t*, 6H, cetyl CH_3). Anal. ($C_{39}H_{80}O_3$) C, H, N. From the resulting residue, **6m** was obtained as a colorless oil (5.83 g, 82%), by purification by flash chromatography (silica gel; hexane:AcOEt, 100:1 and 20:1); $[\alpha]_D$ = –6.5 (*c* 0.87, $CHCl_3$); CIMS m/z 593 (MH^+); ^1H NMR ($CDCl_3$, 100 MHz), 5.35 (*m*, 4H, CH=CH), 3.70–3.37 (*m*, 9H, glycerol CH, CH_2, cetyl CH_2O), 2.15 (*t*, 1H, OH), 2.01 (*m*, 2H, $C\underline{H}_2CH$=), 1.58–1.27 (*m*, 48H, oleoyl CH_2), 0.88 (*t*, 6H, cetyl CH_3).

*1,2-Di-O-alkyl-*sn*-glycero-3-phosphocholines (2k–2m); general procedure*

A mixture of **6k–m** (9.0 mmol), 2-bromoethylphosphorodichloridate (3.05 g, 12.6 mmol) and Et_3N (2.5 mL, 18 mmol) in $CHCl_3$ (150 mL) was stirred for 20 h at room temperature under an argon atmosphere. Then 0.1 M KCl (150 mL) was added to the mixture and the whole was stirred for 3 h at room temperature. The organic layer was separated, dried (Na_2SO_4), and evaporated. A mixture of Me_3N and MeOH (1:3, 30 mL) was added to a solution of the residue in benzene (10 mL). The solution was heated at 60 °C for 20 h in a stainless steel tube and the solvent was removed. The residue was dissolved in a mixture of MeOH (60 mL), $CHCl_3$ (120 mL) and H_2O (30 mL) and the whole was shaken. The organic layer was separated and evaporated. AgOAc (12.0 g, 80 mmol) was added to a solution of the residue in aqueous MeOH (95%, 200 mL) and the mixture was stirred at 40 °C for 18 h in the dark. Insoluble materials were removed by filtration, and the filtrate was evaporated to dryness. The residue was purified by flash chromatography (silica gel; $CHCl_3$:MeOH, 10:1, and then $CHCl_3$:MeOH:H_2O, 100:10:1 and 50:10:1), and fractions containing the desired product were evaporated. The residue was dissolved in a mixture of hot $CHCl_3$/acetone and then cooled in an ice-bath to give a white precipitate of **2. 2k** (5.52 g, 87%); FAB-MS (positive) m/z 706 (MH^+); ^1H NMR ($CDCl_3$:CD_3OD, 3:1, 100 MHz), 4.22 (*m*, 2H, glycerol CH_2), 3.90 (*m*, 2H, choline CH_2OP), 3.71–3.34 (*m*, 9H, glycerol CH, CH_2, cetyl CH_2O), 3.21 (*s*, 9H, choline CH_3), 1.56–1.26 (*m*, 56H, cetyl CH_2), 0.88 (*t*, 6H, cetyl CH_3). Anal. ($C_{40}H_{84}NO_6P$•5/2H_2O) C, H, N. **2l** (5.76 g, 84%); FAB-MS (positive) m/z 762 (MH^+); ^1H NMR ($CDCl_3$:CD_3OD, 3:1, 100 MHz), 4.22 (*m*, 2H, glycerol CH_2), 3.90 (*m*, 2H, choline CH_2OP), 3.70–3.37 (*m*, 9H, glycerol CH, CH_2, stearyl CH_2O), 3.21 (*s*, 9H, choline CH_3), 1.58–1.26 (*m*, 64H, stearyl CH_2), 0.88 (*t*, 6H, stearyl CH_3). Anal. ($C_{44}H_{92}NO_6P$•3/2H_2O) C, H, N. **2m** (5.59 g, 82%); FAB-MS (positive) m/z 758 (MH^+); ^1H NMR ($CDCl_3$:CD_3OD, 3:1, 100 MHz), 5.40 (*m*, 4H, CH=CH), 4.26 (*m*, 2H, glycerol CH_2), 3.90 (*m*, 2H, choline CH_2OP), 3.68–3.37 (*m*, 9H, glycerol CH, CH_2, oleoyl CH_2O), 3.21 (*s*, 9H, choline CH_3), 1.99 (*m*, $C\underline{H}_2CH$=), 1.56–1.27 (*m*, 48H, oleoyl CH_2), 0.88 (*t*, 6H, oleoyl CH_3). Anal. ($C_{44}H_{88}NO_6P$•2H_2O) C, H, N.

Antitumor assays in mice

Mice were purchased from Charles River Japan, Inc. P388 leukemia: P388 lymphocytic leukemia cells were maintained by serial transplantation in C57BL/6xDBA/2F_1 (B6D2F1) male mice. The leukemic cells (1×10^6) were inoculated ip into B6D2F1 male mice (mean weight 20 g). After 24 h, mice were randomized into groups of five and housed in shoebox cages. For administration to animals, compounds were sonicated in Tris-buffered saline, and the preparations were given ip or po on days 1 through 5 (i.e. treatment was started 24 h after tumor inoculation). Meth A fibrosarcoma: Meth A fibrosarcoma cells were maintained by serial transplantation in BALB/c male mice. Fibrosarcoma cells (1×10^6) were inoculated subcutaneously into BALB/c male mice (mean weight 22 g). After 24 h, mice were randomized into groups of five and housed in shoebox cages. Treatment with compounds was done as described for P388 leukemia. On day 14 the major axis and minor axis of the tumor were measured and the volume was calculated by the following equation: tumor volume (mm^3) = [minor axis (mm)]^2 × major axis (mm)/2. Antitumor activities were evaluated from the mean tumor volume of treated mice (T) over that of control mice (C) and the data were indicated with T/C (%); T/C (%) = T/C × 100.

Acknowledgment

We are grateful to Dr Y. Nanba of Nippon Fine Chemical Co., Ltd for a gift of phosphatidylcholines.

References and Notes

1. Part 136: Matsuda, A; Azuma, A. *Nucleosides Nucleotides* in press.

2. Heidelberger, C. *Adv. Enzymol.* **1983**, *54*, 57.

3. Keating, M. J.; McCreadie, K. B.; Bodey, G. P.; Smith, T. L.; Gehan, E.; Friedreich, E. J. *JAMA* **1982**, *248*, 2481.

4. Albertok, P. *Fundamentals Cancer Chemother.* **1978**, *23*, 88.

5. Elion, G. B.; Burgi, E.; Hitchings, G. H. *J. Am. Chem. Soc.* **1952**, *74*, 411.

6. Shuto, S.; Ueda, S.; Imamura, S.; Fukukawa, K.; Ueda, T. *Chem. Pharm. Bull.* **1988**, *36*, 5020.

7. Shuto, S.; Itoh, H.; Endo, E.; Fukukawa, K.; Tsujino, M.; Matsuda, A.; Ueda, T. *Chem. Pharm. Bull.* **1987**, *35*, 3523.

8. Turcotte, J. G.; Srivastava, S. P.; Meresak, W. A.; Rizkalla, B. A.; Louzon, F.; Wunz, T. P. *Biochim. Biophys. Acta* **1980**, *619*, 604.

9. (a) Hong, C. I.; Nechaev, A.; Kirisits, A. J.; Buchheit, D. J.; West, C. R. *J. Med. Chem.* **1980**, *23*, 1343; (b) Matsushita, T.; Ryu, E. K.; Hong, C. I.; MacCoss, M. *Cancer Res.* **1981**, *41*, 2707; (c) Hong, C. I.; An, S.-H.; Buchheit, D. J.; Nechaev, A.; Kirisits, A. J.; West, C. R.; Berdel, W. E. *J. Med. Chem.* **1986**, *29*, 2038.

10. Herrmann, R.; Berdel, W. E. *Cancer Res.* **1992**, *52*, 1865.

11. (a) Shuto, S.; Itoh, H.; Obara, T.; Nakagami, K.; Yaso, M.; Yaginuma, S.; Tsujino, M.; Saito, T.; Matsuda, A.; Ueda, T. *Nucleosides Nucleotides* **1992**, *11*, 437; (b) Shuto, S.; Itoh, H.; Ueda, S.; Imamura, S.; Fukukawa, K.; Tsujino, M.; Matsuda, A.; Ueda, T. *Chem. Pharm. Bull.* **1988**, *36*, 209; (c) Shuto, S.; Ueda, S.; Imamura, S.; Fukukawa, K.; Tsujino, M.; Matsuda, A.; Ueda, T. *Tetrahedron Lett.* **1987**, *28*, 199.

12. (a) Neumann, J.-M.; Herve, M.; Debouzy, J.-C; Iglesias, G. F.; Gouyette, C.; Dupraz, B.; Huynh-Dinh, T. *J. Am. Chem. Soc.* **1989**, *111*, 4270; (b) Debouzy, J.-C.; Gerve, M.; Neumann, J.-M.; Gouyette, C.; Dupraz, B.; Huynh-Dinh, T. *Biochem. Pharmacol.* **1990**, *39*, 1657.

13. Sakai, A.; Mori, N.; Shuto, S.; Suzuki, T. *J. Pharm. Sci.* **1993**, *82*, 575.

14. (a) van Wijk, G. M. T.; Hostetler, K. Y.; Schlame, M.; van den Bosch, H. *Biochim. Biophys. Acta* **1991**, *1086*, 99; (b) Hostetler, K. Y.; Parker, S.; Sridhar, C. N.; Martin, M. J.; Li., J.-L.; Stuhmiller, L. M.; van Wijk, G. M. T.; van den Bosch, H.; Gardner, M. F.; Aldern, K. A.; Richman, D. D. *Proc. Natl Acad. Sci. U.S.A.* **1993**, *90*, 11835.

15. Doi, K.; Oku, N.; Toyota, T.; Shuto, S.; Sakai, A.; Itoh, H.; Okada, S. *Biol. Pharm. Bull.* **1994**, *17*, 1414.

16. Lands, W. E. M.; Hart, P. *J. Biol. Chem.* **1965**, *240*, 1905.

17. Recently, the inhibitory effect of compound **1b** on experimental lymphatic metastases in mice was observed. Hayashi, M.; Itoh, H.; Koshio, T.; Nakagami, K.; Komiyama, K. *Biol. Pharm. Bull.* **1993**, *16*, 778.

18. van Wijk, G. M. T.; Suurmeijer, C. N. S. P.; van den Bosch, H. *Biochim. Biophys. Acta* **1992**, *1165*, 45.

19. (a) Ramirez, F.; Mandal, S. B.; Marecek, J. F. *Synthesis* **1982**, 402; (b) Ramirez, F.; Mandal, S. B.; Marecek, J. F. *J. Am. Chem. Soc.* **1982**, *104*, 5483.

20. Oltvoort, J. J.; van der Marel, G. A.; van Boeckel, C. A. A.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1983**, *102*, 523.

21. Hostetler, K. Y.; Stuhmiller, L. M.; Lenting, H. B. M.; van den Bosch, H.; Richman, D. D. *J. Biol. Chem.* **1990**, *265*, 6112.

22. Shuto, S.; Imamura, S.; Fukukawa, K.; Sakakibara, H.; Murase, J. *Chem. Pharm. Bull.* **1987**, *37*, 447.

23. For an example see Kakinuma, K.; Yamagishi, M.; Fujimoto, Y.; Ikekawa, N.; Oshima, T. *J. Am. Chem. Soc.* **1990**, *112*, 2740.

24. Stannacey, M. Z.; Baer, E. *J. Biol. Chem.* **1964**, *239*, 410.

25. Chen, J.-S.; Barton, P. G. *Can. J. Biochem.* **1970**, *48*, 584.

26. Baldwin, J. J.; Mensler, R. K.; Alison, B. H.; McClure, D. E. *J. Org. Chem.* **1978**, *43*, 4876.

27. Hirt, V. R.; Berchtold, R. *Pharm. Acta Helvetiae* **1958**, *33*, 349.

28. Hermetter, A.; Paltauf, F.; Hauser, H. *Chem. Phys. Lipids* **1982**, *30*, 35.

29. Browning, J.; Seelig, J. *Chem. Phys. Lipids* **1979**, *24*, 103.

30. Umeda, M.; Heidelberger, C. *Cancer Res.* **1968**, *28*, 2529.

31. Houghton, J. A.; Houghton, P. J.; Wooten, R. S. *Cancer Res.* **1979**, *39*, 2406.

32. Lepage, G. A.; Hersh, E. M. *Biochem. Biophys. Res. Commun.* **1972**, *46*, 1918.

33. Kanzawa, F.; Hoshi, A.; Kuretani, K. *J. Pharmacobio-Dyn.* **1980**, *3*, 390.

34. Inaba, M.; Nagashima, K.; Tsukagoshi, S.; Sakurai, Y. *Cancer Res.* **1986**, *46*, 1063.

35. In preliminary experiment, **1b** showed antileukemic activity against 5-FU resistant P388 leukemia implanted ip in mice; compound **1b** showed an ILS of 93%, when administered ip at 30 mg kg^{-1} day^{-1} on days 1–5; 5-FU had an ILS_{max} of 26% at 30 mg kg^{-1} day^{-1} in this tumor system.

36. Kates, M.; Chen, T. H.; Stanacev, N. Z. *Biochemistry* **1963**, *2*, 394.

(*Received in Japan* 10 *November* 1994; *accepted* 12 *December* 1994)