5-Fluorouracil Derivatives. XX.[1] Synthesis and Antitumor Activity of 5′-*O*-Unsaturated Acyl-5-fluorouridines

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Various kinds of 5′-*O*-unsaturated acyl 5-fluorouridines were synthesized to obtain 5-fluorouridine derivatives with low toxicity and high antitumor activity. Antitumor activity of the compounds against L-1210 leukemia in mice was examined, and the 5′-*O*-4-pentenoyl derivative showed the highest antitumor activity.

Keywords 5′-*O*-acyl-5-fluorouridine; 5-fluorouracil; antitumor activity; 5-fluorouridine; L-1210 leukemia; synthesis

5-Fluorouridine (FUR) (**1**), which was first synthesized by Heidelberger and his co-workers in 1957 as one of the 5-fluorouracil (5-FU) derivatives,[2] attracted considerable attention due to its high antitumor activity.[3] FUR showed excellent antitumor activity in a preclinical study,[4] and was superior to 5-FU and 5-fluoro-2-deoxyuridine (FUDR), particularly in mice with methotrexate-resistant leukemia.[3] However, FUR also showed leukopenia and thrombosis with slight gastrointestinal toxicity as side effects, and consequently it is not in clinical use. To reduce the toxicity while maintaining its high antitumor activity, various kinds of FUR derivatives have been synthesized, and modified FUR derivatives such as 5′-deoxy-5-fluorouridine[5] and 5′-*O*-(L-valyl)-5-fluorouridine[6] have been reported.

In our laboratory, as part of a continuing synthetic program designed to develop antitumor agents with high antitumor activity and low toxicity, we have synthesized various kinds of 5-FU derivatives such as 1-carbamoyl-,[7] 1-acyloxyalkyl-,[8] and 1-alkylthiocarbamoyl-,[9] 1-alkoxyalkyl-5-fluorouracils,[10] and also functional polymers containing 5-FU as a side chain.[1,11] In a previous paper,[12] we reported syntheses of 5′-*O*-acyl-5-fluorouridine, which showed high antitumor activity against L-1210 leukemia in mice. Encouraged by these results, we further synthesized other 5′-*O*-acyl FUR derivatives, and this time prepared 5′-*O*-unsaturated acyl FUR derivatives (**2**) as masked FUR derivatives. Ten kinds of aliphatic acyl groups with olefinic bond were introduced at the 5′-*O*-position of 5-fluorouridine (Chart 1, Table III). They showed higher antitumor activity than their saturated analogues. This paper describes the synthesis of 5′-*O*-unsaturated acyl-5-fluorouridines and antitumor activity of these compounds

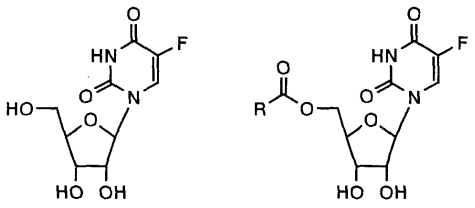
TABLE I. 2′,3′-*O*-Isopropylidene-5′-*O*-acyl-5-fluorouridines **4**[a]

Run	Comp. No.	Yield (%)	^{1}H-NMR ($CDCl_3$–DMSO-d_6)
1	**4a**	90	0.85—1.0 (3H, m, CH_3), 1.0—1.4 (22H, m, $(CH_2)_{11}$), 1.35 (3H, s, CH_3), 1.46 (3H, s, CH_3), 1.8—2.1 (4H, m, $CH_2CH=CHCH_2$), 2.24 (2H, t, J=7 Hz, CH_2CO), 4.2—4.45 (3H, m, H4′, 5′), 4.7—5.0 (2H, m, H2′, 3′), 5.2—5.5 (2H, m, vinyl), 5.72 (1H, d, J=2 Hz, H1′), 7.42 (1H, d, J=6 Hz, H6), 9.5 (1H, br s, NH)
2	**4b**	92	1.1—1.4 (12H, m, $(CH_2)_6$), 1.36 (3H, s, CH_3), 1.57 (3H, s, CH_3), 1.8—2.1 (2H, m, $=CHCH_2$), 2.1—2.4 (2H, m, CH_2CO), 4.2—4.5 (3H, m, H4′, 5′), 4.7—5.1 (4H, m, H2′, 3′, $CH_2=$), 5.6—5.95 (2H, m, $CH_2=CH$, H1′), 7.46 (1H, d, J=6 Hz, H6), 9.34 (1H, br s, NH)
3	**4c**	61	0.8—1.0 (3H, m, CH_3), 1.2—1.5 (4H, m, $(CH_2)_2$), 1.36 (3H, s, CH_3), 1.59 (3H, s, CH_3), 1.9—2.1 (2H, m, allyl), 3.05 (2H, d, J=5.4 Hz, CH_2CO), 4.25—4.5 (3H, m, H4′, 5′), 5.4—5.7 (2H, m, CH=CH), 5.74 (1H, d, J=2 Hz, H1′), 7.43 (1H, d, J=6 Hz, H6), 8.96 (1H, br s, NH)
4	**4d**	91	1.32 (3H, s, CH_3), 1.52 (3H, s, CH_3), 4.23—4.50 (3H, m, H4′, 5′), 4.77—4.93 (1H, m, H3′), 5.05 (1H, dd, $J_{1'2'}$= 2 Hz, $J_{2'3'}$=16 Hz, H2′), 5.80 (1H, d, J=2 Hz, H1′), 6.48 (1H, d, J=16 Hz, =CHCO), 7.27—7.70 (5H, m, arom.), 7.65 (1H, d, J=16 Hz, $CH=CHCO$), 7.93 (1H, br s, NH)
5	**4e**	75	0.88 (3H, t, J=7 Hz, CH_3), 1.20—1.60 (2H, m, CH_3CH_2), 1.33 (3H, s, CH_3), 1.55 (3H, s, CH_3), 1.8—2.1 (2H, m, allyl), 3.06 (2H, d, J=5 Hz, CH_2CO), 4.2—4.5 (3H, m, H4′, 5′), 4.7—5.0 (2H, m, H2′, 3′), 5.4—5.7 (2H, m, vinyl), 5.73 (1H, d, J=2 Hz, H1′), 7.48 (1H, br s, NH)
6	**4f**	29 (87)[b]	0.91 (3H, t, J=7 Hz, CH_3), 1.0—1.8 (4H, m, $(CH_2)_2$), 1.33 (3H, s, CH_3), 1.54 (3H, s, CH_3), 2.13—2.27 (2H, m, allyl), 4.21—4.46 (3H, m, H4′, 5′), 4.72—4.85 (1H, m, H3′), 4.88—5.00 (1H, m, H2′), 5.75 (1H, d, J=2 Hz, H1′), 5.8—5.9 (1H, m, vinyl), 6.92—7.06 (1H, m, vinyl), 7.57 (1H, d, J=7 Hz, H6), 11.72 (1H, br s, NH)
7	**4g**	82	1.00 (3H, t, J=7 Hz, CH_3), 1.35 (3H, s, CH_3), 1.57 (3H, s, CH_3), 1.75—2.16 (2H, m, allyl), 3.05 (2H, d, J= 6 Hz, CH_2CO), 4.2—4.5 (3H, m, H4′, 5′), 4.61—4.93 (2H, m, H2′, 3′), 5.4—5.6 (2H, m, vinyl), 5.70 (1H, d, J= 2 Hz, H1′), 7.46 (1H, d, J=7 Hz, H6), 10.8 (1H, s, NH)
8	**4h**	20 (82)[b]	1.06 (3H, t, J=7 Hz, CH_3), 1.30 (3H, s, CH_3), 1.52 (3H, s, CH_3), 2.15—2.31 (2H, m, CH_2), 4.18—4.39 (3H, m, H4′, 5′), 4.71—4.80 (1H, m, H3′), 4.90—5.03 (1H, m, H2′), 5.69—5.84 (2H, m, H1′, vinyl), 6.92—7.08 (1H, m, vinyl), 7.86 (1H, d, J=7 Hz, H6), 11.9 (1H, s, NH)
9	**4i**	88	1.34 (3H, s, CH_3), 1.54 (3H, s, CH_3), 2.2—2.5 (4H, m, $(CH_2)_2$), 4.31 (3H, br s, H4′, 5′), 4.6—5.2 (3H, m, H2′, 3′, vinyl), 5.5—6.0 (3H, m, H1′, vinyl), 7.62 (1H, d, J=6 Hz, H6), 10.9 (1H, br s, NH)
10	**4j**	40	1.35 (3H, s, CH_3), 1.56 (3H, s, CH_3), 1.94 (3H, br s, $CH_3CH=$), 4.23—4.46 (3H, m, H4′, 5′), 4.66—4.96 (2H, m, H2′, 3′), 5.5—5.8 (2H, m, vinyl), 6.12 (1H, m, H1′), 7.36 (1H, d, J=7 Hz, H1′), 10.7 (1H, br s, NH)

a) 2 eq of carboxylic acid, 2 eq of DCC and a catalytic amount of DMAP were employed. *b*) 2 eq of carboxylic acid, 2 eq of DCC and 2 eq of DMAP were employed.

against intraperitoneally implanted L1210 in mice.

2′,3′-*O*-Isopropylidene-5-fluorouridine (**3**)[13] was treated with unsaturated carboxylic acid (2 eq) in the presence of dicyclohexylcarbodiimide (DCC) (2 eq) and a catalytic amount of 4-dimethylaminopyridine (DMAP) in pyridine to afford 5′-*O*-acyl derivatives (**4**) as shown in Table I. In the case of 2-pentenoyl and 2-heptenoyl derivatives, 2 eq of DMAP was necessary to obtain 5′-*O*-acylated compounds in good yields (runs 6, 8). The isopropylidene moiety was removed by methanolysis with 80% trifluoroacetic acid in methanol to obtain 5′-*O*-unsaturated acyl-5-fluorouridines (**2**) (Table II).



FUR 1

Chart 1

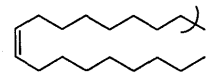
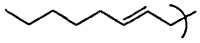
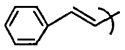
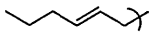
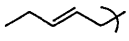
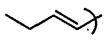
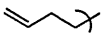
The antitumor activity of these compounds was tested against L-1210 leukemia by intraperitoneal administration in mice, and the *ILS* (increase in life span) value and *TR* (therapeutic ratio) value are shown in Table III. All the compounds showed moderate to high antitumor activity. 2-Nonenoyl (**2c**), 3-heptenoyl (**2e**), 3-hexenoyl (**2g**), 4-pentenoyl (**2i**), and methacryloyl (**2j**) derivatives showed excellent *ILS* values at doses of 10 mg/kg/d far superior to FUR, though they are slightly inferior in terms of *TR* values. The most active compound in this series proved to be **2i**. No correlation between carbon number of the acyl group and antitumor activity was observed in this screening system, but it is noted that these unsaturated derivatives showed higher *ILS* values than their saturated analogues which we reported in a previous paper.[12] The reason antitumor activity was improved by the introduction of

TABLE II. 5′-*O*-Acyl-5-fluorouridines **2**

Comp. No.	Yield (%)	mp[a] (°C)	Formula	Analysis (%) Found (Calcd) C	H	N	^{1}H-NMR ($CDCl_3$–DMSO-d_6)
2a	39	Oil	$C_{27}H_{43}FN_2O_7$	61.10 (61.58	8.03 8.23	5.30 5.32)	0.7—1.0 (3H, brs, CH_3), 1.30 (20H, s, $(CH_2)_{10}$), 1.5—1.8 (2H, m, CH_2CH_2CO), 1.8—2.1 (4H, m, allyl), 2.43 (2H, t, J=6 Hz, CH_2CO), 2.9 (2H, brs, OH), 4.0—4.2 (3H, m, H4′, 5′), 4.34 (2H, s, H2′, 3′), 5.3—5.5 (2H, m, vinyl), 5.81 (1H, s, H1′), 7.71 (1H, d, J=6 Hz, H6), 10.8 (1H, brs, NH)
2b	87	106—107	$C_{20}H_{29}FN_2O_7$	56.24 (56.07	6.87 6.82	6.48 6.54)	1.29 (8H, s, $(CH_2)_4$), 1.4—1.5 (2H, m, CH_2CH_2C=), 1.6—1.7 (2H, m, CH_2CH_2CO), 1.9—2.1 (2H, m, CH_2CO), 2.3—2.5 (2H, m, CH_2CH=), 3.6 (2H, brs, OH), 4.0—4.5 (5H, m, H2′, 3′, 4′, 5′), 4.75—5.15 (2H, m, CH_2=CH), 5.6—5.85 (2H, m, H1′, CH_2=CH), 7.80 (1H, d, J=6 Hz, H6), 10.6 (1H, brs, NH)
2c	74	106—107	$C_{18}H_{25}FN_2O_7$	53.74 (53.99	6.25 6.29	7.04 7.00)	0.75—1.00 (3H, m, CH_3), 1.1—1.5 (6H, m, $(CH_2)_3$), 1.9—2.2 (2H, m, CH_2CH=), 2.9—3.3 (4H, m, CH_2CO, OH), 4.0—4.5 (5H, m, H2′, 3′, 4′, 5′), 5.45—5.7 (2H, m, vinyl), 5.78 (1H, s, H1′), 7.74 (1H, d, J=6 Hz, H6), 10.8 (1H, s, NH)
2d	79	190—191	$C_{18}H_{17}FN_2O_7$	55.26 (55.10	4.44 4.47	6.98 7.14)	3.0 (2H, brs, OH), 4.07—4.35 (3H, m, H4′, 5′), 4.43—4.6 (2H, H2′, 3′), 5.74—5.91 (1H, m, H1′), 6.46 (1H, d, J=16 Hz, =CHCO), 7.18—7.90 (5H, m, arom.), 7.68 (1H, d, J=16 Hz, CH=CHCO), 7.75 (1H, d, J=7 Hz, H6), 10.8 (1H, s, NH)
2e	74	110—111	$C_{16}H_{21}FN_2O_7$	51.15 (51.61	5.65 5.68	7.49 7.52)	0.87 (3H, t, J=7 Hz, CH_3), 1.36 (sixtet, 2H, J=7 Hz, CH_3CH_2), 1.8—2.1 (2H, m, CH_2CH=), 2.9 (2H, brs, OH), 3.0—3.2 (2H, m, CH_2CO), 4.0—4.6 (5H, m, H2′, 3′, 4′, 5′), 5.3—5.6 (2H, m, vinyl), 5.81 (1H, s, H1′), 7.74 (1H, d, J=6 Hz, H6), 10.7 (1H, s, NH)
2f	78	Oil	$C_{16}H_{21}FN_2O_7$	51.46 (51.61	5.67 5.68	7.12 7.52)	0.88 (3H, t, J=7 Hz, CH_3), 1.13—1.63 (4H, m, $CH_3CH_2CH_2$–), 2.00—2.40 (2H, m, CH_2CH=), 2.9 (2H, brs, OH), 4.0—4.6 (5H, m, H2′, 3′, 4′, 5′), 5.86 (1H, brs, H1′), 5.90 (1H, d, J=15 Hz, CH=CHCO), 7.04 (1H, dt, J=15, 7 Hz, CH=CHCO), 7.77 (1H, brs, NH)
2g	85	109	$C_{15}H_{19}FN_2O_7$	50.68 (50.28	5.43 5.34	7.78 7.82)	0.97 (3H, t, J=7 Hz, CH_3), 1.87—2.18 (2H, m, CH_2CH=), 2.9 (2H, brs, OH), 3.06 (2H, d, J=5 Hz, CH_2CO), 3.91—4.15 (3H, m, H4′, 5′), 4.21—4.32 (2H, m, H2′, 3′), 5.41—5.60 (2H, m, vinyl), 5.69—5.81 (1H, m, H1′), 7.76 (1H, d, J=6 Hz, H6), 10.92 (1H, brs, NH)
2h	80	138—139	$C_{14}H_{17}FN_2O_7$	48.73 (48.84	5.04 4.98	8.07 8.14)	1.09 (3H, t, J=7 Hz, CH_3), 2.20—2.34 (2H, m, CH_2), 2.8 (2H, brs, OH), 4.1—4.5 (5H, m, H2′, 3′, 4′, 5′), 5.78 (1H, s, H1′), 5.85 (1H, d, J=15 Hz, vinyl), 7.0—7.15 (1H, m, vinyl), 7.75 (1H, d, J=6 Hz, H6), 11.7 (1H, brs, NH)
2i	73	96—97	$C_{14}H_{17}FN_2O_7$	48.79 (48.84	4.95 4.98	8.14 8.14)	2.3—2.5 (4H, m, $(CH_2)_2$), 2.9 (2H, brs, OH), 3.95—4.45 (5H, m, H2′, 3′, 4′, 5′), 4.88—5.18 (2H, m, vinyl), 5.74 (1H, s, H1′), 5.6—6.0 (1H, m, vinyl), 7.77 (1H, d, J=7 Hz, H6), 10.85 (1H, brs, NH)
2j	72	47—49.5	$C_{13}H_{15}FN_2O_7$	46.88 (47.28	4.96 4.58	8.08 8.48)	1.92 (3H, s, CH_3), 3.0 (2H, brs, OH), 3.9—4.6 (5H, m, H2′, 3′, 4′, 5′), 5.59 (1H, brs, vinyl), 5.75 (1H, brs, vinyl), 6.03 (1H, s, H1′), 7.62 (1H, d, J=6 Hz, H6), 10.7 (1H, brs, NH)

a) Recrystallized from 2-propanol.

TABLE III. Antitumor Activity of 5′-*O*-Acyl-5-fluorouridines **2**

Compound	R	*ILS*[a] (%) Dose (mg/kg/d) 0.3	1	3	10	30	100	ILS_{30}[a] (mg/kg/d)	ILS_{max}[a] (mg/kg/d)	*TR*[a]
2a		14	18	48	75	98		1.5	30	20
2b		25	46	58	98	85	13	0.40	10	25
2c		21	38	81	131	61	−11	0.56	10	18
2d		20	39	59	91	20		0.56	10	18
2e		25	46	72	115	26	34	0.40	10	25
2f				17	35	67	56	7.1	30	4.2
2g			35	75	131			0.86	10	12[b]
2h				46	108	35		2.2	10	4.5[b]
2i		20	45	109	150	38	13	0.48	10	21
2j		19	35	71	123	53		0.67	10	15
5-FU				38	73	60		2.3	10	4[b]
FUR		46	62	91	33			0.1	3	30[b]

a) See Experimental. *b*) Estimated by extrapolation.

olefinic bond to the side chain of FUR has not been clarified but we speculated that the hydrolysis of the 5′-*O*-acyl group was retarded and the slow release of 5-FU reduced the toxicity, thus improving the *ILS* values. Further screening tests are necessary but these unsaturated analogues will be a potent antitumor agent.

Experimental

Melting points were determined on a Yamato melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL JNM FX-100S or Varian EM-390 with tetramethylsilane as an internal standard.

General Procedure for the Acylation of 2′,3′-*O*-Isopropylidene-5-fluorouridine (3) To a pyridine solution (3 ml) of 2′,3′-*O*-isopropylidene-5-fluorouridine (**3**) (1 mmol), DCC (2 mmol), and a catalytic amount of DMAP was added unsaturated carboxylic acid (2 mmol) at room temperature. The mixture was stirred at that temperature overnight, then 10% HCl solution was added. The mixture was extracted with CH_2Cl_2 and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated to leave an oil, which was purified by column chromatography (SiO_2, CH_2Cl_2–CH_3OH; 15 : 1) to give **4**.

General Procedure for the Synthesis of 5′-*O*-Acyl-5-fluorouridines (2) A solution of **4** (1.0 mmol) in an 80% methanolic solution of trifluoroacetic acid (2.0 ml) was stirred at room temperature for 10 min and concentrated *in vacuo* to leave an oil, which was purified by column chromatography (SiO_2, CH_2Cl_2–CH_3OH; 10 : 1) to give **2**.

Animals and Tumor System Male BDF_1 mice weighing 20±2 g were used. Six mice in each group, either test or control, were implanted intraperitoneally with 1×10^5 L-1210 leukemia cells. The compound to be tested was injected intraperitoneally once daily for 5 d, starting 24 h after tumor implantation.

Evaluation of Antitumor Activity The *ILS* was calculated by using the following formula:

$$ILS(\%) = (T - C)/C \times 100$$

where T is the average number of days before death in the test group and C is the average number of days before death in the control group.[13]

$$TR = ILS_{max}/ILS_{30}$$

ILS_{max} = dose amount (mg/kg/d) showing highest *ILS*

ILS_{30} = dose amount (mg/kg/d) showing 30% *ILS*

Acknowledgement The authors wish to thank the Advanced Center for Chemical Analysis, Ehime University, for elemental analyses.

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