Synthesis and Antitumor Activities of 5′-*O*-Aminoacyl-3′-*O*-benzyl Derivatives of 2′-Deoxy-5-fluorouridine and Related Compounds

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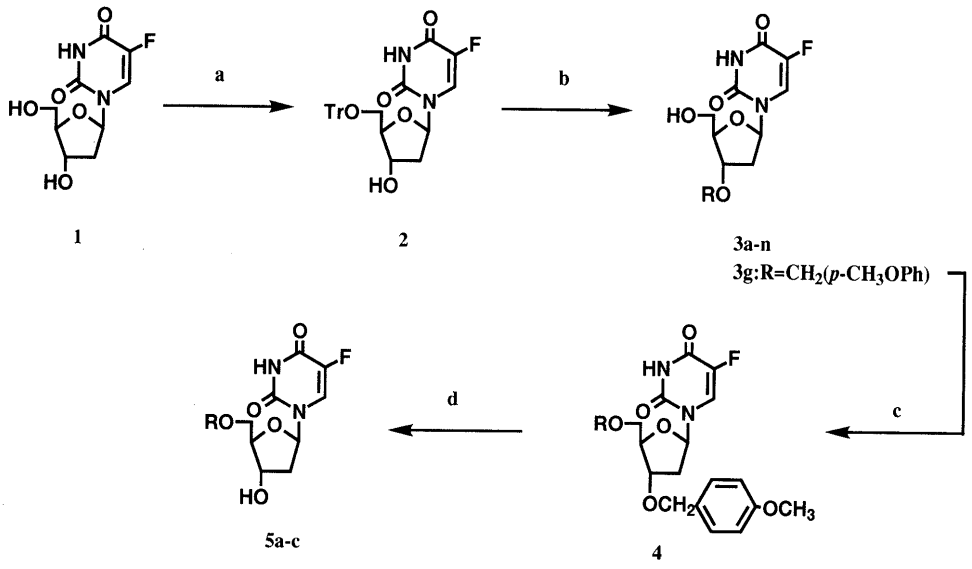
Various *O*-alkyl derivatives of 2′-deoxy-5-fluorouridine (FUdR) were synthesized and their antitumor activities in mice bearing sarcoma 180 (s.c.-*p.o.*) were evaluated in terms of the ED_{50} values (mg/kg/d). Most of these compounds were superior to FUdR in antitumor activity. In particular, the antitumor activity of 3′-*O*-(*p*-chlorobenzyl)-FUdR (3e) (ED_{50} = 0.87 mg/kg/d) was as much as 100 times that of FUdR (ED_{50} = 84 mg/kg/d). Further, various 5′-*O*-aminoacyl derivatives of 3e were synthesized and evaluated in terms of ED_{50} value and therapeutic index (T.I.). Both the ED_{50} value (0.41 mg/kg/d) and the T.I. (4.18) of 3′-*O*-(*p*-chlorobenzyl)-5′-*O*-glycyl-FUdR hydrochloride (6a) were significantly improved, compared with those of 3e and FUdR. FUdR plasma concentration after a single *p.o.* dosing of 6a was maintained for as long as 24 h.

Key words 2′-deoxy-5-fluorouridine; *O*-benzylation; *O*-aminoacylation; antitumor activity; blood concentration

5-Fluorouracil (5-FU)[1] and 2′-deoxy-5-fluorouridine (FUdR)[2] have been regarded as candidate drugs for the treatment of human solid cancers. FUdR exhibits much more potent cytotoxicity and a wider spectrum against various tumor cells than does 5-FU *in vitro*.[3] However, the *in vivo* activity of FUdR is not superior to that of 5-FU,[4] because of its rapid degradation by pyrimidine nucleoside phosphorylases.[5] In an attempt to minimize the degradation and retain the antitumor activity, FUdR was infused at a low dose on clinical trial[6] and many acyl and aroyl analogues of FUdR have also been synthesized.[7] Several *O*-acylated compounds showed an increase in antitumor activity over FUdR *in vivo*.[8] We considered that a depot form of FUdR, which would be resistant to degradation by pyrimidine nucleoside phosphorylases and be gradually activated to FUdR, would maintain an effective FUdR concentration in plasma for a longer time and result in greater antitumor activity.

Recently, our coworkers have reported the synthesis and antitumor activities of *O*-alkyl derivatives of 2′-deoxy-5-(trifluoromethyl)uridine (F_3Thd), which is a time-dependent agent, like FUdR.[9] The *O*-alkyl derivatives of F_3Thd are resistant to enzymatic degradation by pyrimidine nucleoside phosphorylases and are slowly activated by NADP-dependent microsomal drug-metabolizing enzymes after absorption, thus maintaining a higher concentration and a longer residence time of F_3Thd in plasma and showing improved antitumor activities.[10] On the basis of these data, we conjectured that *O*-alkylated derivatives of FUdR might show potent antitumor activity



a, TrCl, pyridine, 100°C
b, 1) RI or RCl/NaI, NaH/THF, 50°C
2) 0.5N HCl/MeOH, r.t.
c, RI or RCl/NaI, NaH/THF, 50°C
d, DDQ, CH_2Cl_2/H_2O, r.t.

Chart 1

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Table 1. Physical Properties and Antitumor Effects of *O*-Alkyl Derivatives of FUdR

Compound No.	R	mp (°C) (Recryst. sol.)	Yield[a] (%)	Formula	Analysis (%) Calcd (Found) C	H	N	ED_{50} (mg/kg/d)
3a	CH_3	141 (EtOH)	64	$C_{10}H_{13}FN_2O_5$	46.15 (46.13)	5.03 (5.01)	10.76 (10.79)	52
3b	C_2H_5	131—132 (AcOEt)	72	$C_{11}H_{15}FN_2O_5$	48.18 (48.18)	5.51 (5.61)	10.21 (10.31)	33
3c	CH_2Ph	140—142 (MeOH–pet. ether)	85	$C_{16}H_{17}FN_2O_5$	57.14 (57.20)	5.09 (5.21)	8.33 (8.21)	12
3d	CH_2(*p*-MePh)	190—191 (EtOH)	82	$C_{17}H_{19}FN_2O_5$	58.28 (58.32)	5.46 (5.74)	8.00 (7.85)	25
3e	CH_2 (*p*-ClPh)	202—203 (EtOH–DMA)	84	$C_{16}H_{16}ClFN_2O_5$	51.83 (51.90)	4.35 (4.37)	7.55 (7.59)	0.87
3f	CH_2(*p*-NO_2Ph)	139 (EtOH)	79	$C_{16}H_{16}FN_3O_7$	50.39 (50.31)	4.32 (4.11)	11.02 (11.54)	22
3g	CH_2(*p*-MeOPh)	174—175 (EtOH)	81	$C_{17}H_{19}FN_2O_6$	55.73 (55.84)	5.23 (5.33)	7.65 (7.48)	14
3h	CH_2(*p*-*tert*-BuPh)	Foam	79	$C_{20}H_{25}FN_2O_5$	61.21 (61.51)	6.42 (6.88)	7.13 (7.03)	75
3i	CH_2(*p*-FPh)	133—134 (EtOH–DMA)	89	$C_{16}H_{16}F_2N_2O_5$	54.24 (54.18)	4.55 (4.65)	7.91 (7.63)	19
3j	CH_2(*p*-CF_3Ph)	153—154 (EtOH)	85	$C_{17}H_{16}F_4N_2O_5$	50.50 (50.23)	3.99 (4.22)	6.93 (6.84)	3.0
3k	CH_2(*p*-BrPh)	194—195 (EtOH)	68	$C_{16}H_{16}BrFN_2O_5$	46.28 (46.31)	3.88 (3.94)	6.75 (6.47)	1.2
3l	CH_2(*o*-BrPh)	122—124 (EtOH)	72	$C_{16}H_{16}BrFN_2O_5$	46.28 (46.41)	3.88 (3.92)	6.75 (6.66)	5.8
3m	CH_2(*p*-EtPh)	Foam	82	$C_{18}H_{21}FN_2O_5$	59.33 (59.44)	5.81 (5.92)	7.69 (7.75)	>40
3n	CH_2(*m*,*p*-Cl_2Ph)	108—110 (MeOH–pet. ether)	73	$C_{16}H_{15}Cl_2FN_2O_5$	47.43 (47.02)	3.73 (3.59)	6.91 (6.89)	4.1
5a	CH_3	Foam	69	$C_{10}H_{13}FN_2O_5$	46.15 (45.71)	5.03 (5.41)	10.76 (9.99)	>40
5b	C_2H_5	Foam	48	$C_{11}H_{15}FN_2O_5$	48.17 (47.89)	5.51 (5.72)	10.21 (10.01)	39
5c	CH_2Ph	130—132 (EtOH)	51	$C_{16}H_{17}FN_2O_5$	57.14 (57.02)	5.09 (5.22)	8.33 (8.49)	16
FUdR								84

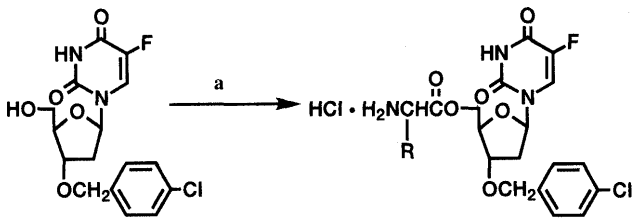
a) Yield from **2** or **3g**.

and *O*-aminoacylated derivatives of *O*-alkyl derivatives of FUdR, with improved water solubility, might have further enhanced antitumor activity.

In this paper, we describe the synthesis of *O*-alkyl derivatives of FUdR and *O*-aminoacyl derivatives of *O*-alkylated FUdR. Furthermore, we evaluated the *in vivo* antitumor activity against mice bearing sarcoma 180 (s.c.-*p.o.*) in terms of both the ED_{50} value and the therapeutic index (T.I.). In addition, we examined the plasma concentration of FUdR after administration of selected compounds to mice.

Chemistry

The synthesis of *O*-alkyl analogues of FUdR is outlined in Chart 1. Treatment of FUdR (**1**) with trityl chloride in pyridine at 100 °C afforded the tritylate **2**.[11] According to the previously reported method,[12] the reaction of **2** with alkyl iodide (or alkyl chloride with a catalytic amount of sodium iodide) using a 2-fold molar excess of sodium hydride in THF proceeded to give exclusively the 3′-*O*-alkyl-5′-trityl derivatives as intermediates. The detritylation of the 3′-*O*-alkyl derivatives by methanolic hydrogen chloride at room temperature gave 3′-*O*-alkyl



3e **6a-h**

a, 1) BocNHCHRCO$_2$H, $(CH_3)_3CCOCl$, DMAP/DMA, r.t. 2) 4N HCl-AcOEt/AcOEt, r.t.

Chart 2

compounds (**3a—n**) in satisfactory yields. 5′-*O*-Alkyl derivatives of FUdR (**5a—c**) were obtained by 3′-debenzylation with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) of the di-*O*-alkyl compounds (**4**), which were prepared by the above alkylation method from 3′-*O*-(*p*-methoxybenzyl)-FUdR (**3g**). The physical properties of **3a—n** and **5a—c** are listed in Table 1. The synthesis of compounds **6** is illustrated in Chart 2. The reaction of

Table 2. Physical Properties of **6a—h**

Compd. No.	R	mp (°C) (Recryst. sol.)	Yield[a] (%)	Formula	Analysis (%) Calcd (Found)		
					C	H	N
6a	H	103—106 (EtOH–2-PrOH)	93	$C_{18}H_{19}ClFN_3O_6 \cdot HCl \cdot H_2O$	44.82 (45.01	4.59 4.90	8.71 8.62)
6b	CH_3	145—147 (EtOH–2-PrOH)	89	$C_{19}H_{21}ClFNO_6 \cdot HCl \cdot H_2O$	45.98 (46.11	4.87 4.92	8.47 8.35)
6c	$CH(CH_3)_2$	98—100 (EtOH–2-PrOH)	88	$C_{21}H_{25}ClFN_3O_6 \cdot HCl \cdot H_2O$	48.10 (48.33	5.38 5.35	8.01 8.21)
6d	$CH_2CH(CH_3)_2$	88—91 (AcOEt–2-PrOH)	84	$C_{22}H_{27}ClFN_3O_6 \cdot HCl \cdot H_2O$	49.08 (49.42	5.62 5.58	7.80 7.88)
6e	$CH(CH_3)CH_2CH_3$	Foam	79	$C_{22}H_{27}ClFN_3O_6 \cdot HCl \cdot H_2O$	49.08 (48.79	5.62 5.87	7.80 7.56)
6f	$CH_2CH_2SCH_3$	Foam	85	$C_{21}H_{25}ClFN_3O_6S \cdot HCl$	46.85 (47.25	4.87 4.87	7.80 7.59)
6g	$CH_2CO_2CH_2Ph$	139—141 (EtOH–2-PrOH)	87	$C_{27}H_{27}ClFN_3O_8 \cdot HCl$	52.95 (52.72	4.44 4.52	6.86 6.67)
6h	CH_2Ph	125—128 (AcOEt–2-PrOH)	90	$C_{25}H_{25}ClFN_3O_6 \cdot HCl \cdot H_2O$	52.45 (52.56	4.93 4.92	7.34 7.11)

a) Yield from **3e**.

3′-*O*-(*p*-chlorobenzyl)-FUdR (**3e**) and mixed anhydrides with various *tert*-butoxycarbonyl (Boc) amino acids and pivaloyl chloride in the presence of dimethylaminopyridine (DMAP), followed by treatment with 4 N HCl/AcOEt for deprotection, gave 3′-*O*-(*p*-chlorobenzyl)-5′-*O*-aminoacyl FUdR hydrochloride derivatives (**6a—h**) as water-soluble compounds. The physical properties of **6a—h** are listed in Table 2.

Biological Results and Discussion

The antitumor activities of various *O*-alkyl derivatives of FUdR in mice bearing sarcoma 180 after oral administration are shown in Table 1. *O*-Alkylation of FUdR increased the *in vivo* antitumor activity of FUdR. The ED_{50} values of 3′-*O*-ethyl (**3b**), 3′-*O*-benzyl (**3c**), 5′-*O*-ethyl (**5b**) and 5′-*O*-benzyl (**5c**) derivatives of FUdR and FUdR were 33, 12, 39, 16 and 84 mg/kg per day, respectively. *O*-Benzylation of FUdR was favorable for high antitumor activity compared with *O*-methylation or *O*-ethylation of FUdR. The 3′-*O*- benzyl compound (**3c**) was slightly more active than the 5′-*O*-benzyl compound (**5c**). Several 3′-*O*-substituted benzyl derivatives of FUdR (**3d—n**) were therefore examined. 3′-*O*-Substituted benzyl compounds with electron-withdrawing gruops such as Cl, CF_3, Br (**3e**, **3j**, **3k**) were more active than those with electron-donating groups such as Me, *tert*-Bu and Et (**3d**, **3h**, **3m**). In particular, 3′-*O*-(*p*-chlorobenzyl)-FUdR (**3e**) showed the highest activity, with an ED_{50} approximately one-hundredth of that of FUdR. Furthermore, the antitumor activities of aminoacyl derivatives of **3e** were evaluated in terms of both the ED_{50} value and the therapeutic index (Table 4). The activities of aminoacyl derivatives of **3e** were similar to or slightly greater than that of **3e**. Among various aminoacyl derivatives with good water solubility (**6a—h**), **6a** possessed not only the lowest ED_{50} value (0.41 mg/kg/d), which was less than a two-hundredth of that of FUdR, but also the highest T.I. (4.8). Thus, **6a** was superior to **3e** and to FUdR itself from the viewpoint of balance of antitumor activity and toxicity. In addition, the plasma concentration of FUdR was measured as a function of time following a single *p.o.* dose of [6-^{3}H]-**6a** or [6-^{3}H]FUdR at a dose of 1 mg/kg (Fig. 1). In the case of FUdR itself, a very rapid decrease of the FUdR plasma concentration was observed within the first 2 h. Thereafter the plasma concentration fell to an unmeasurable level (less than 0.002 μg/ml). On the other hand, **6a** was metabolized to **3e** by enzymatic or spontaneous hydrolysis within 0.5 h after administration, and subsequently, a high plasma concentration of **3e** was maintained for 24 h (1.67—0.13 μg/ml). Compound **3e**, the metabolite of **6a**, was gradually activated and the plasma FUdR concentration remained in the range of 0.005 to 0.010 μg/ml for as long as 24 h after a single dose, though the plasma FUdR concentration at 1 h was lower than that in the case of FUdR itself. The plasma concentration of 5-FU, the metabolite of FUdR, was unmeasurably low. These results suggest that **6a** may be a favorable depot form of FUdR and a useful candidate for a clinical agent. The biological effects of **6a** are being further evaluated.

Experimental

Melting points were determined with a Yanagimoto MP-3 micro melting point apparatus and are uncorrected. ^{1}H-NMR spectra were obtained with a JEOL JNM-FX90Q spectrometer using tetramethylsilane as an internal standard and coupling constants are given in hertz. All new compounds were characterized by melting point determination, ^{1}H-NMR measurement and elemental analyses.

5′-*O*-Trityl-2′-deoxy-5-fluorouridine (2) The title compound was prepared by the reported procedure.[11] A mixture of FUdR (2.46 g, 10 mmol) and trityl chloride (2.8 g, 10 mmol) in pyridine (20 ml) was stirred for 30 min at 100 °C, then extracted with $CHCl_3$, and the $CHCl_3$ layer was washed with water and concentrated. The residue was crystallized from EtOH, giving 3.9 g (80%) of **2**. mp 195—196 °C. *Anal.* Calcd for $C_{28}H_{25}FN_2O_5$: C, 68.84; H, 5.16; N, 5.73. Found: C, 69.32; H, 5.49; N, 5.24.

3′-*O*-Ethyl-2′-deoxy-5-fluorouridine (3b) A solution of 5′-*O*-trityl FUdR (**2**) (2.93 g, 6 mmol) in THF (12 ml) was treated with NaH (60%, 480 mg, 12 mmol), and the mixture was stirred for 30 min at 50 °C. Ethyl iodide (1.13 g, 7.2 mmol) was added and the reaction mixture was stirred for 2 h at the same temperature. The reaction mixture was cooled down and neutralized with saturated ammonium chloride solution, then the

Table 3. ^{1}H-NMR Data for FUdR Derivatives

Compd. No.	^{1}H-NMR (DMSO-d_6) δ (ppm)
3a	11.83 (1H, s, N^3-H), 8.19 (1H, d, J=7 Hz, H-6), 6.05 (1H, t, J=7 Hz, H-1′), 5.02 (1H, t, J=5 Hz, HO-5′), 3.93 (2H, br s, H-3′, H-4′), 3.65—3.55 (2H, m, H-5′), 3.25 (3H, s, OCH_3), 2.30—2.05 (2H, m, H-2′)
3b	11.85 (1H, s, N^3-H), 8.21 (1H, d, J=7 Hz, H-6), 6.08 (1H, t, J=7 Hz, H-1′), 5.32 (1H, t, J=5 Hz, HO-5′), 4.20—3.90 (2H, m, H-3′, 4′). 3.80—3.50 (2H, m, H-5′), 3.47 (2H, q, $C\underline{H}_2CH_3$), 2.40—2.10 (2H, m, H-2′)
3c	11.88 (1H, s, N^3-H), 8.69 (1H, d, H-6), 7.37 (5H, s, Ph-H), 6.10 (1H, m, H-1′), 5.30 (1H, t, HO-5′), 4.54 (2H, s, CH_2), 4.30—4.0 (2H, m, H-3′, 4′), 3.70—3.50 (2H, H-5′), 2.50—2.10 (2H, m, H-2′)
3d	11.84 (1H, s, N^3-H), 8.20 (1H, d, J=7 Hz, H-6), 7.30—7.10 (4H, m, Ph-H), 6.11 (1H, t, J=7 Hz, H-1′), 5.20 (1H, t, J=5 Hz, HO-5′), 4.48 (2H, s, CH_2), 4.20—4.01 (2H, m, H-3′, 4′), 3.65—3.60 (2H, m, H-5′), 2.29—2.12 (5H, m, H-2′ and CH_3)
3e	11.82 (1H, s, N^3-H), 8.20 (1H, d, J=7 Hz, H-6), 7.38 (4H, s, Ph-H), 6.14 (1H, t, J=7 Hz, H-1′), 5.20 (1H, t, J=5 Hz, HO-5′), 4.53 (2H, s, CH_2), 4.23—4.14 (1H, m, H-3′), 4.09—4.02 (1H, m, H-4′), 3.70—3.55 (2H, m, H-5′), 2.40—2.02 (2H, m, H-2′)
3f	11.85 (1H, br s, N^3-H), 8.18 (1H, d, J=7 Hz, H-6), 8.15 (2H, d, J=8 Hz, Ph-H), 7.62 (2H, d, J=8 Hz, Ph-H), 6.14 (1H, t, J=7 Hz, H-1′), 5.24 (1H, t, J=5 Hz, HO-5′), 4.70 (2H, s, CH_2), 4.25—4.05 (2H, m, H-3′, 4′), 3.70—3.60 (2H, m, H-5′), 2.40—2.10 (2H, m, H-2′)
3g	11.81 (1H, br s, N^3-H), 8.19 (1H, d, J=7 Hz, H-6), 7.27 (2H, d, J=8 Hz, Ph-H), 6.91 (2H, d, J=8 Hz, Ph-H), 6.12 (1H, t, J=6 Hz, H-1′), 5.19 (1H, t, J=5 Hz, HO-5′), 4.45 (2H, s, CH_2), 4.19—4.02 (2H, m, H-3′, 4′), 3.70—3.50 (2H, m, H-5′), 2.31—2.13 (2H, m, H-2′)
3h	11.80 (1H, br s, N^3-H), 8.18 (1H, d, J=7 Hz, H-6), 7.48 (2H, d, J=8 Hz, Ph-H), 7.30 (2H, d, J=8 Hz, Ph-H), 6.12 (1H, t, J=6 Hz, H-1′), 5.18 (1H, br s, J=5 Hz, HO-5′), 4.47 (2H, s, CH_2), 4.29—4.02 (2H, m, H-3′, 4′), 3.76—3.60 (2H, m, H-5′), 2.25—2.05 (2H, m, H-2′), 1.27 (9H, s, *tert*-butyl)
3i	11.87 (1H, br s, N^3-H), 8.22 (1H, d, J=7 Hz, H-6), 7.50—7.0 (4H, m, Ph-H), 6.13 (1H, t, J=7 Hz, H-1′), 5.25 (1H, t, J=5 Hz, HO-5′), 4.52 (2H, s, CH_2), 4.21—4.00 (2H, m, H-3′, 4′), 3.65—3.62 (2H, m, H-5′)
3j	11.87 (1H, br s, N^3-H), 8.20 (1H, d, J=7 Hz, H-6), 7.74 (2H, d, J=8 Hz, Ph-H), 7.65 (2H, d, J=8 Hz, Ph-H), 6.14 (1H, t, J=7 Hz, H-1′), 5.20 (1H, br, HO-5′), 4.65 (2H, m, CH_2), 4.28—4.05 (2H, m, H-3′, 4′), 3.65—3.60 (2H, m, H-3′, 4′), 2.50—2.10 (2H, m, H-2′)
3k	11.85 (1H, br s, N^3-H), 8.20 (1H, d, J=7 Hz, H-6), 7.55 (2H, d, J=8 Hz, Ph-H), 7.30 (2H, d, J=8 Hz, Ph-H), 6.11 (1H, t, J=6 Hz, H-1′), 5.19 (1H, t, J=5 Hz, HO-5′), 4.51 (2H, s, CH_2), 4.23—4.02 (2H, m, H-3′, 4′), 3.73—3.60 (2H, m, H-5′), 2.36—2.07 (2H, m, H-2′)
3l	11.80 (1H, br s, N^3-H), 8.20 (1H, d, J=7 Hz, H-6), 7.65—7.30 (4H, m, Ph-H), 6.15 (1H, t, J=6 Hz, H-1′), 4.57 (2H, s, CH_2), 4.25—4.05 (2H, m, H-3′, 4′), 3.69—3.52 (2H, m, H-5′), 2.41—1.93 (2H, m, H-2′)
3m	11.84 (1H, s, N^3-H), 8.21 (1H, d, J=7 Hz, H-6), 7.40—7.0 (4H, m, Ph-H), 6.12 (1H, t, J=6 Hz, H-1′), 5.23 (1H, t, J=5 Hz, HO-5′), 4.54 (2H, s, CH_2), 4.23—4.21 (1H, m, H-3′), 4.07—4.02 (1H, m, H-4′), 3.62—3.59 (2H, q, $C\underline{H}_2CH_3$), 2.40—2.20 (2H, m, H-2′), 1.17 (3H, t, $CH_2C\underline{H}_3$)
3n	11.85 (1H, s, N^3-H), 8.20 (1H, d, J=7 Hz, H-6), 7.60—7.20 (3H, m, Ph-H), 6.12 (1H, t, J=7 Hz, H-1′), 5.24 (1H, t, J=7 Hz, HO-5′), 4.54 (2H, s, CH_2), 4.22—4.10 (1H, m, H-4′), 3.65—3.60 (2H, m, H-5′), 2.40—2.00 (2H, m, H-2′)
5a	11.79 (1H, s, N^3-H), 8.11 (1H, d, J=7 Hz, H-6), 6.09 (1H, m, H-1′), 5.38 (1H, br, HO-3′), 4.10 (2H, m, H-3′, 4′), 3.60—3.45 (2H, m, H-5′), 3.28 (3H, s, OCH_3), 2.30—2.10 (2H, m, H-2′)
5b	11.84 (1H, br, N^3-H), 8.12 (1H, d, H-6), 6.12 (1H, m, H-1′), 5.35 (1H, br, HO-3′), 4.23 (1H, m, H-3′), 3.87 (1H, m, H-4′), 3.58 (2H, m, H-5′), 5.30 (2H, q, $O\underline{C}H_2CH_3$), 2.30—2.10 (2H, m, H-2′), 1.15 (3H, m, $OCH_2C\underline{H}_3$)
5c	11.88 (1H, s, N^3-H), 8.38 (1H, d, H-6), 6.09 (1H, m, H-1′), 5.40 (1H, d, HO-3′), 4.55 (2H, s, CH_2), 4.27 (1H, m, H-3′), 3.98 (1H, m, H-4′), 3.80—3.40 (2H, m, H-5′), 2.24 (2H, t, H-2′)
6a	11.92 (1H, s, N^3-H), 8.56 (3H, s, N^+H_3), 8.02 (1H, d, J=7 Hz, H-6), 7.45—7.32 (4H, m, Ph-H), 6.14 (1H, t, J=6 Hz, H-1′), 4.55 (2H, s, CH_2), 4.40—4.00 (4H, m, H-3′, 4′ and $COCH_2$), 3.90—3.75 (2H, m, H-5′), 2.50—2.10 (2H, m, H-2′)
6b	11.91 (1H, br s, N^3-H), 8.45 (3H, s, N^+H_3), 8.01 (1H, d, J=7 Hz, H-6), 7.40 (4H, s, Ph-H), 6.12 (1H, t, J=7 Hz, H-1′), 4.55 (2H, s, CH_2), 4.40—3.90 (5H, m, H-3′, 4′, 5′ and COCH), 2.50—2.10 (2H, m, H-2′), 1.43 (3H, d, J=8 Hz, $CHC\underline{H}_3$)
6c	11.30 (1H, br s, N^3-H), 8.45 (3H, s, N^+H_3), 7.95 (1H, d, J=7 Hz, H-6), 7.35 (4H, s, Ph-H), 6.10 (1H, t, J=7 Hz, H-1′), 4.50 (2H, s, CH_2), 4.40—4.00 (4H, m, H-3′, 4′, 5′), 3.80 (1H, br, COCH), 2.50—2.10 (2H, m, H-2′), 1.90—1.50 (1H, br, $C\underline{H}(CH_3)_2$), 1.10—0.70 (6H, m, $CH(C\underline{H}_3)_2$)
6d	11.70 (1H, br s, N^3-H), 8.38 (3H, s, N^+H_3), 7.96 (1H, d, J=7 Hz, H-6), 7.40 (4H, s, Ph-H), 6.15 (1H, t, J=7 Hz, H-1′), 4.53 (2H, s, CH_2), 4.20—3.60 (4H, m, H-3′, 4′, 5′), 3.25 (1H, t, J=11 Hz, COCH), 2.50—2.12 (2H, m, H-2′), 1.90—1.20 (3H, m, CH_2CH), 1.00 (3H, s, CH_3), 0.90 (3H, s, CH_3)
6e	11.70 (1H, br s, N^3-H), 8.51 (3H, s, N^+H_3), 8.00 (1H, d, J=7 Hz, H-6), 7.40 (4H, s, Ph-H), 6.15 (1H, t, J=7 Hz, H-1′), 4.60 (2H, s, CH_2), 4.42—4.01 (4H, m, H-3′, 4′, 5′), 3.85 (1H, d, J=6 Hz, COCH), 2.40—2.20 (2H, m, H-2′), 2.20—1.80 (1H, br, $C\underline{H}CH_3$), 1.80—0.70 (8H, m. $CH(C\underline{H}_3)C\underline{H}_2C\underline{H}_3$)
6f	11.90 (1H, br s, N^3-H), 8.44 (3H, s, N^+H_3), 7.98 (1H, d, J=7 Hz, H-6), 7.38 (4H, s, Ph-H), 6.10 (1H, t, J=7 Hz, H-1′), 4.52 (2H, s, CH_2), 4.45—3.95 (4H, m, H-3′, 4′, 5′), 3.40 (1H, t, COCH), 2.90—2.85 (2H, m, CH_2S), 2.40—2.20 (2H, br, H-2′), 2.19 (3H, s, SCH_3), 1.60—1.50 (2H, m, $C\underline{H}_2CH_2S$)
6g	11.90 (1H, br s, N^3-H), 8.65 (3H, s, N^+H_3), 8.00 (1H, d, J=7 Hz, H-6), 7.39 (4H, s, Ph-H), 7.35 (5H, s, Ph-H), 6.11 (1H, t, J=7 Hz, H-1′), 5.11 (2H, s, CH_2), 4.52 (2H, s, CH_2), 4.40—3.95 (4H, br, H-3′, 4′, 5′), 2.40—2.10 (2H, br, H-2′)
6h	11.90 (1H, br, N^3-H), 8.38 (3H, s, N^+H_3), 8.00 (1H, d, J=8 Hz, H-6), 7.40 (4H, s, Ph-H), 7.23 (5H, s, Ph-H), 6.10 (1H, t, J=8 Hz, H-1′), 4.50 (2H, s, CH_2), 4.40—4.00 (5H, br, H-3′, 4′, 5′ and COCH), 3.19 (2H, s, CH_2Ph), 2.15—2.00 (2H, br, H-2′)

organic layer was washed with brine and concentrated. A mixture of the residue and 0.5 N methanolic hydrogen chloride (25 ml) was stirred for 1.5 h at room temperature, then neutralized with $NaHCO_3$ and filtered. The filtrate was concentrated and the residue was purified by silica gel column chromatography with $CHCl_3$–EtOH (10 : 1). Recrystallization from AcOEt gave **3b** (1.18 g, 72%). Compound **3a** was synthesized by the same procedures as employed in the preparation of **3b**. Physicochemical and ^{1}H-NMR spectral data for these compounds are given in Tables 1 and 3, respectively.

2′-Deoxy-3′-*O*-(*p*-methoxybenzyl)-5-fluorouridine (3g) A solution of **2** (4.88 g, 10 mmol) in THF (25 ml) was treated with NaH (60%, 1.0 g, 25 mmol), and the mixture was stirred for 30 min at 50 °C. Then *p*-methoxybenzyl chloride (1.88 g, 12 mmol) and NaI (300 mg, 2 mmol) were added and the reaction mixture was stirred overnight at same

Table 4. Effect of FUdR Derivatives on Sarcoma 180 (s.c.-*p.o.*) in Male ICR Mice

Compd. No.	ED_{50}[a] (mg/kg/d)	IB_{50}[b] (mg/kg/d)	T.I.[c] (IB_{50}/ED_{50})
3e	0.87	1.42	1.64
6a	0.41	1.97	4.80
6b	2.41	2.82	1.17
6c	1.12	2.42	2.16
6d	1.65	3.10	1.87
6e	1.92	2.85	1.48
6f	2.61	3.77	1.44
6g	1.97	2.75	1.39
6h	1.44	1.98	1.37
FUdR	84	70	0.84

a) Dose for 50% inhibition of growth of sarcoma 180 (s.c.-*p.o.*). *b*) Dose for 50% inhibition of body weight increase (days 0—11). *c*) Therapeutic index.

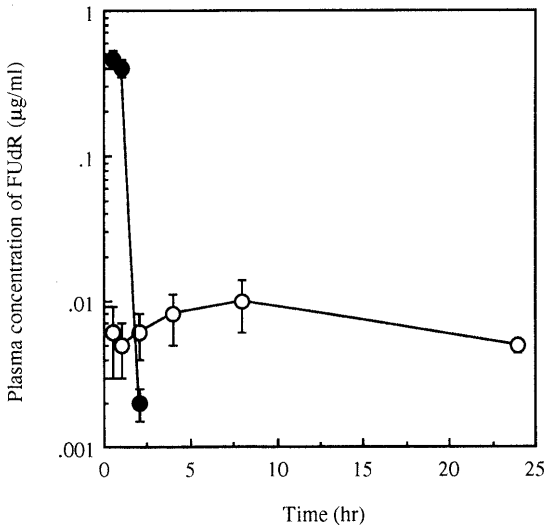


Fig. 1. Plasma Concentrations of FUdR after Oral Administration of FUdR and **6a** to Mice

FUdR and **6a** were administered at 1.0 mg/kg. At the indicated time after administration, plasma concentrations were determined by a bioassay method. —●—, FUdR; —○—, **6a**. Each point represents the mean ± S.D. of 5 mice.

temperature. It was neutralized with saturated ammonium chloride solution and the THF layer was concentrated. The residue was stirred for 2 h at room temperature in 0.5 N methanolic hydrogen chloride solution, then neutralized with $NaHCO_3$ and the precipitates were filtered off. The filtrate was concentrated and the residue was purified by silica gel column chromatography with $CHCl_3$–EtOH (10 : 1). Recrystallization from $CHCl_3$ gave **3g** (2.96 g, 81%). Compounds **3c—f** and **3h—n** were synthesized by the same procedures as employed in the preparation of **3g**. Physicochemical and ^{1}H-NMR spectral data for these compounds are given in Tables 1 and 3, respectively.

5′-*O*-Benzyl-2′-deoxy-5-fluorouridine (5c) A solution of **3g** (1.83 g, 5 mmol) in THF (150 ml) was treated with NaH (60%, 500 mg, 12.5 mmol), and the mixture was stirred for 30 min at 80 °C. Then benzyl bromide (1.02 g, 6 mmol) was added. The reaction mixture was stirred overnight at 50 °C, then saturated ammonium chloride solution was added and the whole was stirred for 2 h at room temperature. The organic layer was separated and concentrated. To the residue, CH_2Cl_2 (100 ml), water (5 ml) and then DDQ (1.14 g, 5 mmol) were added. The mixture was stirred for 1 h at room temperature, then washed with water, and the organic layer was separated and concentrated. The residue was purified by silica gel column chromatography with $CHCl_3$–EtOH (10 : 1). Recrystallization from EtOH gave **5c** (857 mg, 51%). Compounds **5a—b** were synthesized by the same procedures as employed in the preparation of **5c**. Physicochemical and ^{1}H-NMR spectral data for these compounds are given in Tables 1 and 3, respectively.

2′-Deoxy-3′-*O*-(*p*-chlorobenzyl)-5′-*O*-glycyl-5-fluorouridine (6a) Pivaloyl chloride (434 mg, 3.6 mmol) was added dropwise to a solution of *N*-(*tert*-butoxycarbonyl)glycine (631 mg, 3.6 mmol) and triethylamine (631 mg, 3.6 mmol) in dimethylacetamide (20 ml) with stirring at 0 °C. A solution of **3e** (1.33 g, 3.6 mmol) in DMA (20 ml) was further added dropwise to the reaction mixture. Stirring was continued at room temperature for 30 min and then the mixture was poured into a saturated $NaHCO_3$ solution mixed with ice. The whole was extracted with AcOEt. The organic solution was washed with 0.1 N HCl and water, dried over $MgSO_4$, and concentrated *in vacuo*. A solution of the residue in AcOEt (5 ml) was treated with 4 N HCl–AcOEt (5 ml) at 5 °C, and concentrated *in vacuo* at below 30 °C. The residue was recrystallized from EtOH–2-PrOH to give **6a** (1.3 g, 93%). Compounds **6b—h** were synthesized by the same procedure as employed in the preparation of **6a**. Physicochemical and ^{1}H-NMR spectral data for these compounds are given in Tables 2 and 3, respectively.

Antitumor Test Mice of the ICR strain were used. Five-week-old male ICR mice were inoculated subcutaneously in the axillary region with 5×10^6 sarcoma 180 cells and given test compounds orally once a day for 7 consecutive days beginning 24 h after inoculation of the tumor cells. Groups of seven mice were used for each dose, and the test compounds were suspended in 0.5% (carboxymethyl)cellulose (CMC) solution containing 0.1% Tween 80. On day 10, the tumors were excised and weighed. The inhibitory effects of test compounds were calculated from the ratio of the tumor weight in the test group to that in the control group. The results are given Tables 1 and 4.

Therapeutic Evaluation The therapeutic index (IB_{50}/ED_{50}), which represents the balance between the antitumor effect and the toxicity of a drug, was calculated for each compound as the ratio of the dose required to achieve 50% growth inhibition in the sarcoma 180 test (ED_{50}) to the dose producing a 50% inhibition of body-weight increase in the host mice (IB_{50}). The IB_{50} values were determined from the average body-weight gain recorded for the treated mice during the test period (days 0—11) with that noted for the control animals.

Assay of the Plasma Concentration of FUdR [6-^{3}H]-**6a** was synthesized from [6-^{3}H]-2′-deoxy-5-fluorouridine ([6-^{3}H]FUdR; Daiichi Pure Chemicals Co., Ltd.) according to the above method and had a radioactivity of 44.4 MBq/mg. Male ICR mice (Clea Japan, 5 weeks old) bearing sarcoma 180 were orally administered with [6-^{3}H]-**6a** (1.0 mg/44.4 MBq/kg body weight) or [6-^{3}H]FUdR (1.0 mg/2.1 GBq/kg body weight). Groups of 5 mice each were evaluated at the desired time points. At the indicated period after drug administration, 5 mice were killed. Blood samples were collected and centrifuged to separate the plasma. Samples of 200 μl of plasma were extracted with an equal volume of 0.1 M HCl/CH_3OH. The extracts were supplemented with 20 μl of authentic standard solution containing 0.2 mg of non-radiolabeled **6a**, FUdR and 5-FU, respectively, for thin layer chromatographic (TLC) analysis. Samples were concentrated to dryness under nitrogen gas at 25 °C. The residues were dissolved in 50 μl of CH_3OH and subjected to TLC analysis.[13]

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