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Synthesis and Biological Activity of *O*-Alkyl-3-*N*-aminoacyloxymethyl-5-fluoro-2′-deoxyuridine Derivatives

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In an attempt to improve the effectiveness of action of 5-fluoro-2′-deoxyuridine (FUdR), various kinds of *O*-alkylated water-soluble analogues were synthesized. Antitumor activities against sarcoma 180 (solid) were also evaluated. Some compounds exhibited potent activities. In particular, 3′-*O*-*p*-chlorobenzyl-3-*N*-aminoacyloxymethylester derivatives were effective over a very wide range of dose and gave extremely large therapeutic ratios compared with known 5-fluorouracil (5-FU) derivatives.

Key words 5-fluoro-2′-deoxyuridine derivative; water-soluble; antitumor activity; sarcoma 180

5-Fluoro-2′-deoxyuridine (FUdR, **1**), an active metabolite of 5-fluorouracil (5-FU), frequently shows superior cytotoxicity to 5-FU *in vitro*, but has been reported to offer no advantage over 5-FU *in vivo*.[1] The reason for the low activity in animals has been considered to be its rapid phosphorylytic degradation to 5-FU, since the cytotoxicity of **1** is time-dependent or requires long retention *in vivo*. To overcome the above problems, many kinds of *O*-esters (3′,5′- or 3′,5′-di-) and *N*-amide derivatives of **1** have been synthesized with the aim of achieving long half-life in plasma and improved antitumor activity.[2] Some of these compounds having ester groups were found after clinical trials by oral administration to have severe side effects on digestive organs, such as diarrhea, because these esters are hydrolyzed easily to afford **1** in the digestive tract. Recently, it has been reported that *O*-alkyl derivatives of **1** and 2′-deoxy-5-trifluoromethyl uridine are synthesized and activated by NADP-dependent microsomal drug-metabolizing enzymes in the liver after absorption, and thus showed good activity.[3] We have recently synthesized water-soluble 6-*S*-aminoacyloxymethylmercaptopurine derivatives which showed significantly enhanced tumor immunity together with potent antitumor activities.[4]

In this paper, we describe the synthesis of water-soluble FUdR derivatives having an alkyl group on the 3′-*O* or 5′-*O* position and an aminoacyloxymethyl moiety on the 3-*N* position. They have higher lipophilicity than **1**, together with water solubility, and are expected to show a different distribution in the body and different pharmacokinetics. We also describe their potent antitumor activities.

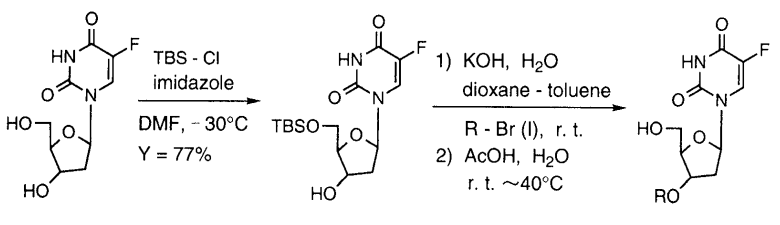
Chemistry

First, the water-soluble FUdR derivatives **6** having a 3′-*O*-benzyl group were prepared as shown in Charts 1 and 2.

Treatment of 5′-*O*-*tert*-butyldimethylsilyl (TBS)-FUdR **2** with various benzyl bromide (iodide) derivatives using a 5-fold molar excess of potassium hydroxide (KOH) in the presence of small amount of water in dioxane–toluene[5] and desilylation under mild acidic conditions gave predominantly 3′-*O*-benzyl-FUdR **3a**—**e**. Then, compounds **3** were allowed to react with chloromethyl esters[6] of *tert*-butyloxycarbonyl(Boc)-amino acid **4** in the presence of potassium carbonate (K_2CO_3) and sodium iodide (NaI) in acetone to give 3-*N*-substituted derivatives **5** in good yields. Removal of the Boc group from **5** proceeded readily in hydrogen chloride (HCl)–dioxane to give the HCl salts of 3-*N*-aminoacyloxymethyl-3′-*O*-benzyl derivatives **6a**—**j**. They were soluble to the extent of more than 50 mg/ml in water.

Similarly, **2** reacted with excess ethyl iodide (EtI) to afford the 3′-*O*-ethyl derivatives **3f**. Then **3f** reacted with **4** to give **5**, and deprotection afforded **6k**, **l** in good yields. On the other hand, **5b** reacted with some protected amino acids in acetonitrile in the presence of dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBT) and *N*,*N*-dimethylaminopyridine (DMAP) to give the ester **7**. After acidic deprotection, **8a**—**c** were obtained in good yields.

Finally, 5′-*O*-alkyl derivatives **10a**, **b** were prepared



1 2 3

Chart 1

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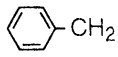
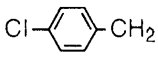
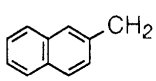
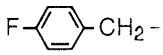
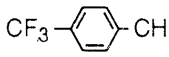
Table 1. Analytical and Physical Data for **3**

Compound	R	Yield (%) (from **2**)	Formula	*Anal.* Calcd C	Calcd H	Calcd N	Found C	Found H	Found N
3a		61	$C_{16}H_{17}FN_2O_5$	57.14	5.09	8.33	56.88	5.15	8.21
3b		78	$C_{16}H_{16}ClFN_2O_5$	51.83	4.35	7.56	51.69	4.37	7.47
3c		68	$C_{20}H_{19}FN_2O_5$	62.17	4.96	7.25	61.92	4.98	7.11
3d		91	$C_{16}H_{16}F_2N_2O_5$	54.24	4.55	7.91	53.98	4.50	7.68
3e		73	$C_{17}H_{16}F_4N_2O_5$	50.50	3.99	6.93	50.28	4.01	6.71
3f	Et	90	$C_{11}H_{15}FN_2O_5$	48.18	5.51	10.21	48.01	5.61	10.01

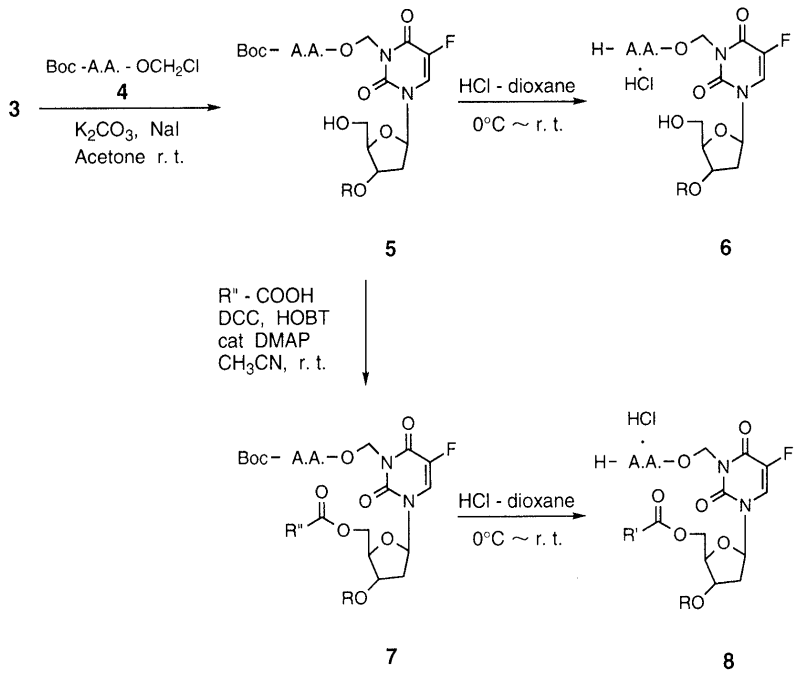


Chart 2

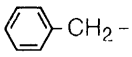
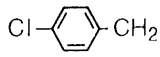
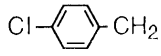
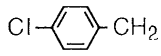
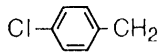
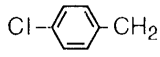
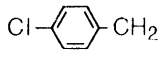
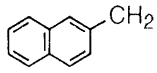
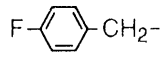
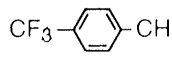
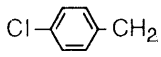
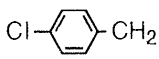
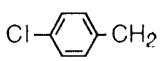
(Chart 3) to evaluate the biological activity of the regioisomers of **6e**, **k**. Treatment of 3′-*O*-TBS-FUdR **9** with *p*-chlorobenzyl iodide or EtI in the same manner afforded the 5′-*O*-*p*-chlorobenzyl or 5′-*O*-ethyl derivative. These compounds were allowed to react with the chloromethyl ester of Boc-L-valine and deprotected to provide **10a**, **b** in good yields.

Biological Activities and Discussion

Antitumor activities of the synthesized FUdR derivatives against sarcoma 180 (solid) in mice are summarized in Table 3, together with those of **1** and 5-FU. Many compounds showed very potent antitumor activities and larger therapeutic ratios (TRs) as compared with known compounds.

We found that the substituent on the 3′-*O* position was the most influential on the activity *in vivo*. Among the 3′-*O*-substituents, the *p*-chlorobenzyl (**6b**—**g**) and *p*-trifluoromethylbenzyl (**6j**) derivatives showed potent antitumor activities and large TRs. However, the benzyl (**6a**), *p*-fluorobenzyl (**6i**) naphthylmethyl (**6h**) and ethyl (**6k**, **l**) derivatives exhibited weak activities and small TRs. On the other hand, 5′-*O*-alkyl derivatives (**10a**, **b**) showed strong activities, but small TRs. The amino acid moiety

Table 2. Water-Soluble Derivatives **6** and **8**

Compd.	R	A.A.	R′	Yield (%) (from **3**)
6a		β-Ala		47
6b		Gly		73
6c		L-Pro		77
6d		L-Phe		69
6e		L-Val		75
6f		D-Val		78
6g		β-Ala		66
6h		Gly		58
6i		L-Val		63
6j		L-Val		77
6k	Et	L-Val		70
6l	Et	L-Ala		71
8a		Gly	$HCl \cdot NH_2CH_2-$	74
8b		Gly	$HCl \cdot MeNHCH_2-$	68
8c		Gly	$HCl \cdot Me_2NCH_2-$	49

on the 3-*N* position had a moderate influence on the antitumor activity. Proline (**6c**) and valine derivatives (**6e**, **f**), which are bulkier than the others, seemed to have more potent antitumor activities than the others (**6b**, **d**, **g**). The absolute configuration of the amino acid moiety had little influence on the antitumor activity (**6e** *vs.* **6f**). The compounds having an amino acid moiety on the 5′-*O* position, especially **8c**, showed potent activity. Concerning administration route, **6c** showed stronger activity when it was administered orally (*p.o.*) than intraperitoneally (i.p.) or intravenously (i.v.). Among these compounds, **6c**, **8c**, having a *p*-chlorobenzyl group and **6j**, having a *p*-trifluoromethylbenzyl group at the 3′-*O* position gave very much larger TR values (63, 63 and 44) on oral administration than **1** or 5-FU (1.5 or 1.4) and therefore they seem to be very promising candidates as anticancer drugs.

Inhibition–dose curves of **6c**, **j** and **8c** are shown in Fig. 1 together with those of **1** and 5-FU. The compounds (**6c**, **j**, **8c**) gave very gentle slopes and were active below 0.01 mmol/kg, while **1** and 5-FU gave simple, steep curves and were inactive even at 0.1 mmol/kg. Consequently, these compounds (**6c**, **j**, **8c**) may act in a different manner from **1**. In general, when a high dose of **1** was administered, **1** was insufficiently anabolized to 5′-fluoro-2′-deoxyuridine-5′-phosphate (5-FdUMP) due to the short half-life in the plasma. On the other hand, when a small dose of **1** was administered continuously, a larger amount of **1** was anabolized to 5-FdUMP, which exhibited antitumor activity.[7] We assumed that the substituent on the 3-*N* position of these compounds (**6c**, **j**, **8c**) was first removed by enzymatic hydrolysis, followed by spontaneous decomposition to give the corresponding 3-NH compound, which would be accumulated in the liver. Then the substituent on the 3′-*O* position would be slowly metabolized oxidatively to afford **1**, which was anabolized to 5-FdUMP over a long period. This seems to be an ideal profile for an antitumor agent. However, the body weight of mice given these compounds decreased severely even at a moderate dose (for example, the body weight of mice given **8c** at 25 mg/kg was 5.0 g less than that of the control on day 7). This phenomenon implies an adverse influence on liver function.

In conclusion, we have synthesized water-soluble 3′-*O*-alkyl-3-*N*-aminoacyloxymethyl FUdR derivatives having potent antitumor activities. In particular, **6c**, **j** and **8c** showed extremely large TRs compared to **1** or 5-FU against sarcoma 180 in mice. But the body weight of mice administered these compounds decreased markedly, and

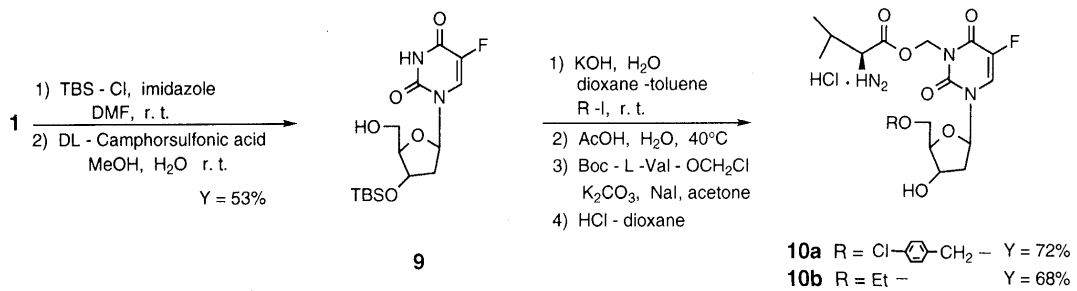


Chart 3

Table 3. Antitumor Activity against Sarcoma 180

No.	Admin. route	OD[a] (mg/kg/d)	Inhibition[b] (%)	TR[c]	No.	Admin. route	OD[a] (mg/kg/d)	Inhibition[b] (%)	TR[c]
6a	i.p.	200	62.7	1.5	**6j**	*p.o.*	400	88.9	>63
6b	i.p.	100	73.5	5.5	**6k**	*p.o.*	400	50.6	1.0
6c	i.p.	200	88.6	17.2	**6l**	*p.o.*	400	61.6	1.6
	p.o.	400	97.9	>63	**8a**	i.p.	100	74.9	>4
	i.v.	200	86.6	4.5	**8b**	*p.o.*	400	97.6	8.6
6d	i.p.	200	78.8	10.4	**8c**	*p.o.*	200	91.2	44.4
6e	*p.o.*	400	98.3	>16	**10a**	*p.o.*	200	96.5	1.1
6f	*p.o.*	200	90.8	17.7	**10b**	*p.o.*	400	84.4	2.6
6g	i.p.	100	88.1	>8	FUdR (**1**)	*p.o.*	200	65.1	1.5
6h	i.p.	100	49.1	—	5-FU	*p.o.*	50	64.0	1.4
6i	*p.o.*	100	60.0	1.6					

a) Optimal dose of drug. *b*) Inhibition (%) of tumor growth when treated at the optimal dose. Inhibition (%) = (1 − mean tumor weight of treated group/that of control group) × 100. *c*) Therapeutic ratio = OD/ED_{50}. ED_{50}; daily dose providing 50% inhibition of the tumor growth compared to the control.

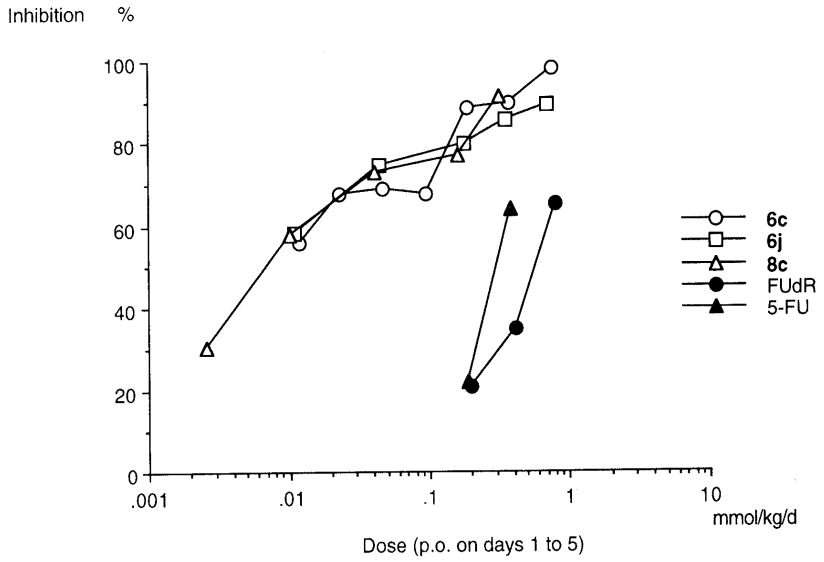


Fig. 1

therefore investigations on these compounds have been discontinued.

Experimental

Melting points were determined with a Buchi 535 digital melting point apparatus. All melting points are uncorrected. IR spectra were obtained with an Analect FX-6200 FT-IR spectrophotometer. ^{1}H-NMR were measured with a JEOL JNM-FX-200 spectrometer. Microanalyses were performed on a Perkin-Elmer 240B CHN analyzer. Silica gel 60K-230 (230—430 mesh) (Katayama) was used for column chromatography. In general, reactions were carried out in dry solvents under an argon atmosphere unless otherwise mentioned.

2′-Deoxy-5′-*O*-*tert*-butyldimethylsilyl-5-fluorouridine 2 Imidazole (20.83 g, 0.306 mmol) and *tert*-butyldimethylsilyl chloride (44.50 g, 0.295 mol) were added to a cold solution of **1** (50.13 g, 0.204 mmol) in dimethylformamide (DMF) (1 l) with stirring. The mixture was stirred for 5.5 h at −30 °C. Methanol (MeOH) was added, then the reaction mixture was brought to ambient temperature and concentrated *in vacuo*. The residue was dissolved in chloroform ($CHCl_3$). This solution was washed with aqueous $NaHCO_3$ and brine, and dried. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on SiO_2 using ethyl acetate (AcOEt)–hexane (2 : 1) as an eluent to give **2** (56.24 g, yield 77%) as a colorless powder. mp 203.0—204.5 °C (recrystallized from AcOEt–diisopropyl ether). IR (Nujol): 3560, 1715, 1690, 1660 cm^{-1}. FAB-MS *m/z*: 361 (MH^+). ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s), 0.92 (9H, s), 2.03 (1H, d, J=4 Hz, D_2O-exchangeable (exch.)), 2.14 (1H, m), 2.42 (1H, ddd, J=3, 6, 14 Hz), 3.85 (1H, dd, J=2, 11 Hz), 3.93 (1H, dd, J=2, 11 Hz), 4.07 (1H, m), 4.49 (1H, m), 6.35 (1H, m), 8.05 (1H, d, J=6 Hz), 8.63 (1H, br s, D_2O exch.). *Anal.* Calcd for $C_{15}H_{25}FN_2O_5Si$: C, 49.98; H, 6.99; N, 7.77. Found: C, 49.81; H, 7.14; N, 7.68.

2′-Deoxy-3′-*O*-*tert*-butyldimethylsilyl-5-fluorouridine 9 Imidazole (8.17 g, 120 mmol) and *tert*-butyldimethylsilyl chloride (13.6 g, 90 mmol) were added to a cold solution of **1** (4.92 g, 20 mmol) in DMF (100 ml) with stirring, and the mixture was stirred for 1 h at room temperature. Then MeOH was added, and the reaction mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt. This solution was washed with 1% HCl, aqueous $NaHCO_3$ and brine, and dried. The solvent was removed *in vacuo*, and the residue was dissolved in MeOH–water (9 : 1, 200 ml). DL-10-Camphorsulfonic acid (800 mg, 3.4 mmol) was added to the solution, and the mixture was stirred for 6 h at room temperature. After addition of $NaHCO_3$ (3.0 g), the reaction mixture was diluted with AcOEt and water. The organic layer was separated, washed with brine, and dried. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on SiO_2 using AcOEt–hexane (2 : 1) as an eluent to give **9** (3.83 g, yield 53%) as a colorless powder. mp 172.0—173.0 °C (recrystallized from AcOEt–hexane). IR (Nujol): 3310, 3200, 1715, 1660 cm^{-1}. FAB-MS *m/z*: 361 (MH^+). ^{1}H-NMR ($CDCl_3$) δ: 0.09 (6H, s), 0.89 (9H, s), 2.1—2.5 (3H, m), 3.6—4.2 (3H, m), 4.3—4.6 (1H, m), 6.22 (1H, m), 7.94 (1H, d, J=7 Hz), 9.19 (1H, br s, D_2O-exch.). *Anal.* Calcd for $C_{15}H_{25}FN_2O_5Si$: C, 49.98; H, 6.99; N, 7.77. Found: C, 49.78; H, 6.94; N, 7.59.

General Procedure for the Synthesis of 3 A suspension of **2** (10.0 g, 27.7 mmol) and KOH (8.11 g, 139 mmol) in toluene–dioxane (1 : 3, 200 ml) and H_2O (0.5 ml) was stirred at room temperature for 2.5 h, then benzyl bromide (55.4 mmol) was added. The reaction mixture was stirred at room temperature for 6 h. The solvent was removed *in vacuo*,

Table 4. Analytical and Physical Data for **6**, **8**, **10**

Compd. No.	Formula	*Anal.* Calcd (Found) C	H	N	FAB-MS MH^+	^{1}H-NMR (DMSO-d_6) δ
6a	$C_{20}H_{24}FN_3O_7 \cdot HCl \cdot 1.5H_2O$	47.96 (47.94	5.63 5.42	8.39 8.37)	438	2.22 (1H, m), 2.39 (1H, m), 2.72 (2H, t, J=7 Hz), 3.02 (2H, br m), 3.5—3.8 (2H, m), 4.09 (1H, m), 4.22 (1H, m), 4.55 (2H, s), 5.33 (1H, br s, D_2O exch.), 5.84 (2H, s), 6.17 (1H, m), 7.2—7.5 (5H, m), 7.98 (3H, br s, D_2O exch.), 8.40 (1H, d, J=7 Hz).
6b	$C_{19}H_{21}ClFN_3O_7 \cdot HCl \cdot 0.3H_2O$	45.67 (45.77	4.56 4.57	8.41 8.21)	458	2.2—2.5 (2H, m), 3.64 (2H, m), 3.84 (2H, s), 4.09 (1H, m), 4.22 (1H, m), 4.54 (2H, s), 5.32 (1H, br, D_2O exch.), 5.93 (2H, s), 6.17 (1H, dd, J=6, 7 Hz), 7.37 (2H, m), 7.44 (2H, m), 8.42 (1H, d, J=6 Hz), 8.44 (3H, br s, D_2O exch.).
6c	$C_{22}H_{25}ClFN_3O_7 \cdot HCl \cdot 0.5H_2O$	48.63 (48.89	5.01 4.94	7.73 7.52)	498	1.9—2.4 (6H, m), 3.20 (2H, m), 3.64 (2H, m), 4.09 (1H, d, J=2 Hz), 4.23 (1H, m), 4.41 (1H, dd, J=7, 8 Hz), 4.54 (2H, s), 5.3 (1H, br s, D_2O exch.), 5.91 (1H, d, J=12 Hz), 5.97 (1H, d, J=12 Hz), 6.17 (1H, t, J=6 Hz), 7.37 (2H, m), 7.43 (2H, m), 8.42 (1H, d, J=7 Hz), 9—10 (2H, br s, D_2O exch.).
6d	$C_{26}H_{27}ClFN_3O_7 \cdot HCl \cdot 0.3H_2O$	52.95 (52.82	4.89 4.82	7.12 6.93)	548	2.22 (1H, m), 2.38 (1H, m), 3.00 (1H, dd, J=8, 14 Hz), 3.21 (1H, dd, J=6, 14 Hz), 3.67 (2H, m), 4.12 (1H, d, J=2 Hz), 4.24 (1H, m), 4.33 (1H, dd, J=5, 8 Hz), 4.56 (2H, s), 5.39 (1H, br, D_2O exch.), 5.68 (1H, d, J=10 Hz), 5.99 (1H, d, J=10 Hz), 6.18 (1H, m), 7.23 (5H, m), 7.38 (2H, dd, J=2, 9 Hz), 7.44 (2H, dd, J=2, 9 Hz), 8.41 (1H, d, J=7 Hz), 8.69 (3H, br s, D_2O exch.).
6e	$C_{22}H_{27}ClFN_3O_7 \cdot HCl \cdot 0.5H_2O$	48.45 (48.70	5.36 5.41	7.70 7.44)	500	0.93 (3H, d, J=7 Hz), 0.96 (3H, d, J=7 Hz), 2.0—2.5 (3H, m), 3.64 (2H, m), 3.93 (1H, d, J=4 Hz), 4.09 (1H, d, J=2 Hz), 4.23 (1H, m), 4.54 (2H, s), 5.38 (1H, br, D_2O exch.), 5.88 (1H, d, J=10 Hz), 5.95 (1H, d, J=10 Hz), 6.17 (1H, t, J=6 Hz), 7.37 (2H, dd, J=2, 9 Hz), 7.43 (2H, dd, J=2, 9 Hz), 8.43 (1H, d, J=7 Hz), 8.56 (3H, br s, D_2O exch.).
6f	$C_{22}H_{27}ClFN_3O_7 \cdot HCl$	49.26 (49.04	5.26 5.22	7.83 7.61)	500	0.94 (6H, d, J=7 Hz), 2.2 (2H, m), 2.4 (1H, m), 3.64 (2H, m), 3.93 (1H, d, J=4 Hz), 4.09 (1H, d, J=2 Hz), 4.23 (1H, m), 4.54 (2H, s), 5.35 (1H, br, D_2O exch.), 5.86 (1H, d, J=10 Hz), 5.98 (1H, d, J=10 Hz), 6.17 (1H, m), 7.37 (2H, dd, J=2, 9 Hz), 7.43 (2H, dd, J=2, 9 Hz), 8.44 (1H, d, J=7 Hz), 8.56 (3H, br s, D_2O exch.).
6g	$C_{20}H_{23}ClFN_3O_7 \cdot HCl \cdot H_2O$	45.64 (45.81	4.98 5.19	7.98 7.94)	472	2.22 (1H, m), 2.38 (1H, ddd, J=3, 6, 13.5 Hz), 2.72 (2H, t, J=7 Hz), 3.01 (2H, t, J=7 Hz), 3.4—3.8 (2H, m), 4.08 (1H, m), 4.21 (1H, m), 4.54 (2H, s), 5.34 (1H, t, J=5 Hz), D_2O exch.), 5.83 (2H, s), 6.17 (1H, m), 7.39 (2H, d, J=9 Hz), 7.41 (2H, d, J=9 Hz), 8.01 (3H, br s, D_2O exch.), 8.40 (1H, d, J=7 Hz).
6h	$C_{23}H_{25}ClFN_3O_7 \cdot HCl \cdot 0.5H_2O$	53.24 (53.16	5.05 5.12	8.10 7.88)	474	2.25 (1H, m), 2.46 (1H, m), 3.5—3.8 (2H, m), 3.86 (2H, s), 4.15 (1H, m), 4.28 (1H, m), 4.72 (2H, s), 5.35 (1H, br, D_2O exch.), 5.93 (1H, d, J=12 Hz), 5.94 (1H, d, J=12 Hz), 6.21 (1H, m), 7.4—7.7 (3H, m), 7.8—8.1 (4H, m), 8.35 (3H, br s, D_2O exch.), 8.43 (1H, d, J=7 Hz).
6i	$C_{22}H_{27}F_2N_3O_7 \cdot HCl \cdot 0.2H_2O$	50.47 (50.43	5.29 5.18	8.03 7.82)	484	0.94 (3H, d, J=7 Hz), 0.95 (3H, d, J=7 Hz), 2.1—2.4 (3H, m), 3.64 (2H, m), 3.92 ((1H, d, J=4 Hz), 4.09 (1H, m), 4.23 (1H, m), 4.53 (2H, s), 5.35 (1H, br s, D_2O exch.), 5.88 (1H, d, J=10 Hz), 5.95 (1H, d, J=10 Hz), 6.16 (1H, m), 7.18 (2H, m), 7.40 (2H, m), 8.43 (1H, d, J=7 Hz), 8.57 (3H, br s, D_2O exch.).
6j	$C_{23}H_{27}F_4N_3O_7 \cdot HCl \cdot 0.5H_2O$	47.71 (47.79	5.05 5.02	7.26 7.08)	534	0.94 (3H, d, J=7 Hz), 0.95 (3H, d, J=7 Hz), 2.1—2.4 (3H, m), 3.66 (2H, m), 3.93 (1H, d, J=4 Hz), 4.13 (1H, m), 4.27 (1H, m), 4.66 (2H, s), 5.39 (1H, br s, D_2O exch.), 5.88 (1H, d, J=10 Hz), 5.96 (1H, d, J=10 Hz), 6.19 (1H, m), 7.58 (2H, d, J=8 Hz), 7.74 (2H, d, J=8 Hz), 8.44 (1H, d, J=7 Hz), 8.56 (3H, br s, D_2O exch.).
6k	$C_{17}H_{26}FN_3O_7 \cdot HCl \cdot 0.5H_2O$	45.49 (45.21	6.29 6.20	9.36 9.28)	404	0.94 (6H, d, J=7 Hz), 1.13 (3H, t, J=7 Hz), 2.1—2.4 (3H, m), 3.48 (2H, q, J=7 Hz), 3.60 (2H, m), 3.92 (1H, d, J=4 Hz), 3.97 (1H, d, J=3 Hz), 4.10 (1H, m), 5.35 (1H, br s, D_2O exch.), 5.88 (1H, d, J=10 Hz), 5.95 (1H, d, J=10 Hz), 6.12 (1H, dd, J=5, 6 Hz), 8.43 (1H, d, J=7 Hz), 8.58 (3H, br s, D_2O exch.).
6l	$C_{15}H_{22}FN_3O_7 \cdot HCl \cdot H_2O$	41.92 (41.81	5.86 5.70	9.77 9.94)	376	1.13 (3H, t, J=7 Hz), 1.38 (3H, d, J=7 Hz), 2.1—2.2 (2H, m), 3.47 (2H, q, J=7 Hz), 3.4—3.8 (2H, m), 3.97 (1H, m), 4.0—4.2 (2H, m), 5.35 (1H, br s, D_2O exch.), 5.92 (2H, s), 6.12 (1H, m), 8.42 (1H, d, J=7 Hz), 8.58 (3H, br s, D_2O exch.).
8a	$C_{21}H_{24}ClFN_4O_8 \cdot 2HCl \cdot 0.5H_2O$	42.26 (42.50	4.56 4.68	9.39 9.19)	515	2.4 (2H, m), 3.83 (4H, s), 4.2—4.5 (4H, m), 4.57 (2H, s), 5.93 (2H, s), 6.20 (1H, t, J=6 Hz), 7.38 (2H, m), 7.45 (2H, m), 8.22 (1H, d, J=7 Hz), 8.55 (6H, br s, D_2O exch.).
8b	$C_{22}H_{26}ClFN_4O_8 \cdot 2HCl \cdot H_2O$	42.63 (42.51	4.88 5.01	9.04 8.98)	529	2.4 (2H, m), 2.57 (3H, s), 3.83 (2H, s), 3.99 (2H, s), 4.2—4.4 (4H, m), 4.57 (2H, s), 5.93 (2H, s), 6.20 (1H, dd, J=6, 7 Hz), 7.39 (2H, dd, J=2, 9 Hz), 7.45 (2H, dd, J=2, 9 Hz), 8.23 (1H, d, J=7 Hz), 8.55 (3H, br s, D_2O exch.), 9.58 (2H, br s, D_2O exch.).
8c	$C_{23}H_{28}ClFN_4O_8 \cdot 2HCl \cdot 1.5H_2O$	42.97 (42.99	5.17 5.07	8.71 8.54)	543	2.4 (2H, m), 2.85 (6H, s), 3.83 (2H, br s), 4.26 (4H, m), 4.43 (2H, d, J=5 Hz), 4.57 (2H, s), 5.93 (2H, s), 6.20 (1H, t, J=6 Hz), 7.39 (2H, m), 7.45 (2H, dd, J=3, 9 Hz), 8.23 (1H, d, J=7 Hz), 8.54 (3H, br s, D_2O exch.), 10.93 (1H, br s, D_2O exch.).
10a	$C_{22}H_{27}ClFN_3O_7 \cdot HCl \cdot 0.3H_2O$	48.77 (48.88	5.32 5.33	7.76 7.54)	500	0.94 (3H, d, J=7 Hz), 0.95 (3H, d, J=7 Hz), 2.0—2.3 (3H, m), 3.65 (1H, dd, J=3, 11 Hz), 3.73 (1H, dd, J=3, 11 Hz), 3.91 (1H, d, J=7 Hz), 3.97 (1H, m), 4.27 (1H, m), 4.55 (2H, s), 5.50 (1H, br s, D_2O exch.), 5.87 (1H, d, J=10 Hz), 5.93 (1H, d, J=10 Hz), 6.17 (1H, m), 7.37 (2H, m), 7.42 (2H, m), 8.12 (1H, d, J=7 Hz), 8.60 (3H, br s, D_2O exch.).
10b	$C_{17}H_{26}FN_3O_7 \cdot HCl \cdot H_2O$	44.59 (44.87	6.38 6.45	9.18 8.90)	404	0.94 (3H, d, J=7 Hz), 0.95 (3H, d, J=7 Hz), 1.16 (3H, t, J=7 Hz), 2.19 (3H, m), 3.4—3.7 (4H, m), 3.92 (2H, m), 4.25 (1H, m), 5.4 (1H, br s, D_2O exch.), 5.87 (1H, d, J=9 Hz), 5.95 (1H, d, J=9 Hz), 6.16 (1H, m), 8.32 (1H, d, J=7 Hz), 8.58 (3H, br s, D_2O exch.).

the residue was dissolved in AcOEt and water, and the solution was neutralized with 10% HCl. The organic layer was separated, washed with aqueous $NaHCO_3$ and brine, and dried. The solvent was removed *in vacuo*, and the residue was dissolved in acetic acid–water (2 : 1, 200 ml). The solution was stirred at 40 °C for 5 h, then the solvent was removed *in vacuo*, and the residue was purified by column chromatography on SiO_2 using AcOEt–hexane (4 : 1) as an eluent to give **3** as a powder.

General Procedure for the Synthesis of 6 A suspension of the chloromethylester **4** (3.0 mmol) and NaI (3.0 mmol) in acetone (15 ml) was stirred at room temperature for 1 h, then **3** (2.4 mmol) and K_2CO_3 (12.0 mmol) were added. The reaction mixture was stirred at room temperature for 20 h. The precipitate was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on SiO_2 using AcOEt–hexane (1 : 5—1 : 3) as an eluent to give **5**.

A cold solution of **5** in dioxane (5 ml) was treated with 15% HCl/dioxane (5 ml) with stirring on an ice bath, then the mixture was allowed to come to ambient temperature and further stirred for 1 h. After addition of Et_2O (100 ml), the reaction mixture was stirred vigorously for 1 h. The resulting precipitate was collected by filtration and washed with Et_2O to give **6** as an amorphous powder.

10a, **b** were each obtained as an amorphous powder in the same manner as described for the preparation of **6**.

General Procedure for the Synthesis of 8 A mixture of the glycine derivative (4.0 mmol), HOBT (540 mg, 4.0 mmol) and DCC (990 mg, 4.8 mmol) in CH_3CN (30 ml) was stirred at room temperature for 3 h, then **5b** (1.12 g, 2.0 mmol) and DMAP (50 mg, 0.4 mmol) were added. The mixture was stirred for 18 h. The precipitate was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was dissolved in AcOEt, then the solution was washed with 1% aqueous K_2CO_3, water and brine, and dried. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on SiO_2 using $CHCl_3$–AcOEt (2 : 1) as an eluent to give **7**.

This product was treated with HCl/dioxane in the same manner as described for the preparation of **6** to give **8** as an amorphous powder.

Analytical and physical data of **6**, **8**, **10** are summarized in Table 4.

Antitumor Activity against Sarcoma 180 Female ICR mice (5 weeks old) weighing 20—24 g were inoculated subcutaneously in the left inguinal region with sarcoma 180 cells (2×10^6 cells/body) on day 0. Each compound was administered daily, i.p., *p.o.*, or i.v. as a single injection (0.2 ml/body), from days 1 to 5. Compounds were dissolved in saline containing 1% NIKKOL HCO-60 (hydrogenated castor oil; Nikko Chemical, Japan). Each group except the control consisted of five mice; the control group consisted of 10 mice. On day 10, tumors were dissected and weighed. Inhibition of tumor growth was determined by comparing the mean weight of the tumor in the treated group with the mean weight of the tumor in the control group.

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