Chemical Industries Limited, Tokyo, Japan.

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Registry No. 1, 106343-59-3; 2, 85336-86-3; 3, 106344-30-3; 4, 106343-49-1; 5, 106343-54-8; 6, 106343-51-5; 7, 106343-52-6; 8, 49792-58-7; 9, 117269-11-1; 10, 99466-88-3; 11, 106343-50-4; 12, 117251-14-6; 13, 117251-15-7; 14, 117251-17-9; 15, 117251-18-0; 16, 75659-46-0; 17, 117251-19-1; 18, 117307-55-8; 19, 117251-20-4; 20, 117269-12-2; 21, 117307-56-9; 22, 117307-58-1; 23, 98064-87-0; 24, 117251-22-6; 25, 117251-24-8; 26, 117228-71-4; 27, 117228-73-6; 28, 117228-75-8; 29, 117228-77-0; 30, 117228-79-2; 31, 117228-81-6; 32, 117228-83-8; 33, 117228-85-0; 34, 117306-65-7; ^{195}Pt, 14191-88-9; *cis*-[Pt($^{15}NH_3$)$_2$Cl$_2$], 78017-69-3; *cis*-[Pt($^{15}NH_3$)$_2$(O1-DMF)Cl](NO$_3$), 117228-87-2; *cis*-[Pt($^{15}NH_3$)$_2$(NO$_3$)Cl], 117228-88-3; *cis*-[Pt($^{15}NH_3$)$_2$(O1-DMF)$_2$](NO$_3$)$_2$, 117228-90-7; *cis*-[Pt($^{15}NH_3$)$_2$(NO$_3$)(O1-DMF)](NO$_3$), 117228-92-9; *cis*-[Pt($^{15}NH_3$)$_2$(NO$_3$)$_2$], 117228-93-0; *cis*-[Pt($^{15}NH_3$)$_2$(H$_2$O)Cl](NO$_3$), 78039-63-1; *cis*-[Pt($^{15}NH_3$)$_2$(H$_2$O)$_2$](NO$_3$)$_2$, 78022-63-6; *trans*-[Pt(NH$_3$)$_2$Cl$_2$], 14913-33-8; [Pt(en)Cl$_2$], 14096-51-6; [Pt(dach)Cl$_2$], 52691-24-4; *cis*-[Pt(*i*-PrNH$_2$)$_2$Cl$_2$], 41637-05-2; [Pt(*trans*-(*R*,*R*)-dach)Cl$_2$], 61848-66-6; *cis*-[Pt(NH$_3$)$_2$Br$_2$], 15978-91-3; *cis*-[Pt(NH$_3$)$_2$Cl$_2$], 15663-27-1; *cis*-[Pt(NH$_3$)$_2$(O1-DMF)Cl](NO$_3$), 79084-71-2; *cis*-[Pt(NH$_3$)$_2$(NO$_3$)Cl], 117228-94-1; *cis*-[Pt(NH$_3$)$_2$(O1-DMF)$_2$](NO$_3$)$_2$, 79084-73-4; *cis*-[Pt(NH$_3$)$_2$(NO$_3$)(O1-DMF)](NO$_3$), 117228-96-3; *cis*-[Pt(NH$_3$)$_2$(NO$_3$)$_2$], 41575-87-5; *cis*-[Pt(NH$_3$)$_2$(H$_2$O)Cl](NO$_3$), 117228-97-4; *cis*-[Pt(NH$_3$)$_2$(H$_2$O)$_2$](NO$_3$)$_2$, 52241-26-6.

Supplementary Material Available: X-ray crystallographic data on compound **10** (6 pages); structure factor tables for **10** (7 pages). Ordering information is given on any current masthead page.

Studies on Antitumor Agents. 8.[1] Antitumor Activities of *O*-Alkyl Derivatives of 2′-Deoxy-5-(trifluoromethyl)uridine and 2′-Deoxy-5-fluorouridine

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O-Benzyl and *O*-ethyl derivatives of 2′-deoxy-5-(trifluoromethyl)uridine (F_3Thd) and 2′-deoxy-5-fluorouridine (FUdR) were synthesized. The oral antitumor activity of the compounds against sarcoma 180 in mice was examined. The 5′-*O*-ethyl (**3b**), 3′-*O*-ethyl (**3c**), 5′-*O*-benzyl (**3e**), and 3′-*O*-benzyl (**3f**) derivatives of F_3Thd were 4-fold more active than F_3Thd itself. Among the substituted-benzyl derivatives of F_3Thd, 3′-*O*-(*p*-chlorobenzyl)-F_3Thd (**3h**) showed the highest activity, with an ED_{50} less than one-tenth of that of F_3Thd. The activities of 5′-*O*-benzyl (**7c**) and 3′-*O*-benzyl (**7d**) derivatives of FUdR were equal to those of the effective *O*-alkyl derivatives of F_3Thd.

2′-Deoxy-5-(trifluoromethyl)uridine (F_3Thd) was first synthesized by Heidelberger and his co-workers in 1962.[2] It has shown considerable biological activity through the actions of its metabolites in a number of systems.[3-7] For example, the antitumor activity of F_3Thd against transplanted tumors such as adenocarcinoma 755 and L 1210 leukemia is equal to or higher than that of 2′-deoxy-5-fluorouridine (FUdR).[7]

However, F_3Thd showed unsatisfactory results in clinical cancer chemotherapy, because of its short half-life in plasma.[8] Rapid metabolic degradation by thymidine phosphorylase has been reported.[8,9] Thus, depot forms of F_3Thd which resist degradation by the enzyme would be expected to maintain higher concentrations of F_3Thd in plasma and thus show greater antitumor activities in vivo.

Recently, we have reported the synthesis and antitumor activity of acyl derivatives[10] and *O*-alkoxyalkyl derivatives[1] of F_3Thd. Acylation of F_3Thd has been shown to enhance antitumor activity, but the acyl derivatives were easily hydrolyzed to F_3Thd by intestinal homogenate. *O*-Alkoxyalkyl derivatives of F_3Thd resisted degradation by thymidine phosphorylase, were activated by NADP-dependent microsomal drug-metabolizing enzymes after absorption, and thus showed greater antitumor activity. O-Ethoxymethylation and O-benzyloxymethylation increased the in vivo antitumor activity of F_3Thd 6-fold. Closer studies of the metabolic pathway of these derivatives suggested that *O*-benzyl or *O*-ethyl derivatives of F_3Thd might also be activated by the NADP-dependent microsomal drug-metabolizing enzymes.

If the depot form of F_3Thd were activated slowly after absorption, it would be expected to decrease toxicity to the gastrointestinal tract and give rise to an increase in the area under the curve for the F_3Thd concentration in plasma. The overall result would be an improved therapeutic index. Since FUdR has a similar disadvantage in vivo,[11] because of its short half-life in plasma,[12] O-alkylation of FUdR might also be effective in enhancing the antitumor activity of FUdR in vivo.

On this basis, various *O*-benzyl and *O*-ethyl derivatives of F_3Thd and FUdR were synthesized, and their antitumor

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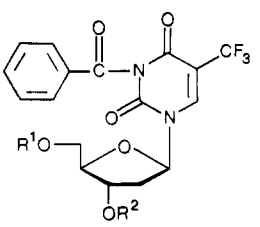
Table I. Physical Properties and Antitumor Effects of Alkyl Derivatives of F_3Thd and FUdR



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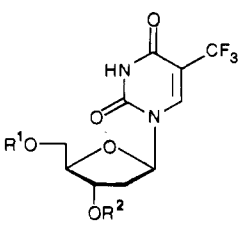

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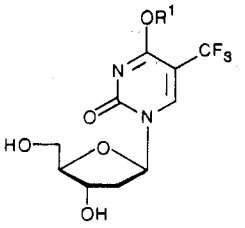

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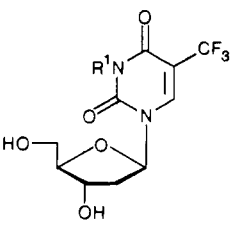
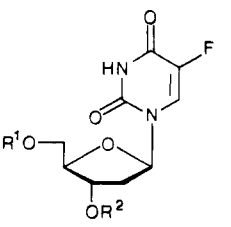


5



8

compd	R^1	R^2	recryst solvent	mp, °C	yield, %	formula	ED_{50}, mg/kg per day
2b	C_2H_5	H		foam	46	$C_{19}H_{19}F_3N_2O_6$	23
2c	H	C_2H_5	EtOH	156–157	16	$C_{19}H_{19}F_3N_2O_6$	37
2e	CH_2Ph	H	benzene	153.5–155	10	$C_{24}H_{21}F_3N_2O_6$	26
2f	H	CH_2Ph	benzene	160.5–162.5	45	$C_{24}H_{21}F_3N_2O_6$	26
3a	C_2H_5	C_2H_5		foam	53	$C_{14}H_{19}F_3N_2O_5$	25
3b	C_2H_5	H	EtOH	188–189.5	43	$C_{12}H_{15}F_3N_2O_5$	14
3c	H	C_2H_5	EtOH	183–184	53	$C_{12}H_{15}F_3N_2O_5$	15
3d	CH_2Ph	CH_2Ph		foam	62	$C_{24}H_{23}F_3N_2O_5$	>40
3e	CH_2Ph	H	$CHCl_3$–EtOH	177–178	66	$C_{17}H_{17}F_3N_2O_5$	15
3f	H	CH_2Ph	EtOH–H_2O	159–160	54	$C_{17}H_{17}F_3N_2O_5$	13
3g	CH_2(*p*-ClPh)	H	EtOH	186–187.5	57	$C_{17}H_{16}ClF_3N_2O_5$	14
3h	H	CH_2(*p*-ClPh)		foam	62	$C_{17}H_{16}ClF_3N_2O_5$	<10
3i	CH_2(*o*-MePh)	H	EtOH–pet. ether	178–179	48	$C_{18}H_{19}F_3N_2O_5$	>40
3j	H	CH_2(*o*-MePh)		foam	57	$C_{18}H_{19}F_3N_2O_5$	40
3k	CH_2(*m*-MePh)	H	benzene–pet. ether	169–171	51	$C_{18}H_{19}F_3N_2O_5$	28
3l	H	CH_2(*m*-MePh)		foam	54	$C_{18}H_{19}F_3N_2O_5$	36
3m	CH_2(*p*-MeOPh)	H	benzene	174.5–176	43	$C_{18}H_{19}F_3N_2O_6$	>40
3n	H	CH_2(*p*-MeOPh)		oil	48	$C_{18}H_{19}F_3N_2O_6$	>40
4	CH_2Ph			foam	33	$C_{17}H_{17}F_3N_2O_5$	>40
5	CH_2Ph			foam	51	$C_{17}H_{17}F_3N_2O_5$	>40
F_3Thd							63
8a	C_2H_5	H		foam	53	$C_{11}H_{15}FN_2O_5$	39
8b	H	C_2H_5	AcOEt	131–132	42	$C_{11}H_{15}FN_2O_5$	>40
8c	CH_2Ph	H	EtOH	129–130	49	$C_{16}H_{17}FN_2O_5$	16
8d	H	CH_2Ph	MeOH–pet. ether	140–141.5	65	$C_{16}H_{17}FN_2O_5$	12
FUdR							84

activity by oral administration was evaluated to find suitable candidates for clinical use, especially for post-surgical maintenance therapy as has been done with other depot forms of 5-fluorouracil.[13]

This paper describes the synthesis of O-alkylated compounds of F_3Thd and FUdR and the oral antitumor activity of these compounds against sarcoma 180 in mice.

Chemistry

A number of 2′-O-methylated nucleosides have been detected as minor constituents of tRNA. 2′-O-Benzylated nucleosides are useful intermediates for the preparation of nucleotides or other modified nucleosides. Therefore, numerous methods have been reported for methylation or benzylation on the sugar portion of nucleosides.

Although selective 2′(3′)-O-benzylations of ribonucleosides using dibutyltin oxide and a benzyl halide[14] or stannous chloride and phenyldiazomethane[15] have been reported, these methods cannot be applied to the preparation of *O*-benzyldeoxyribonucleoside because of the lack of a 2′-hydroxyl group. Alkylation of uridine or thymidine by alkyl halides occurs at the N-3 position of the pyrimidine under neutral or mild basic conditions. In strong alkaline solutions, alkylations with alkyl halides have been reported to occur on the sugar hydroxyls.[16-18]

However, alkylations of F_3Thd or 5′-*O*-trityl-F_3Thd with alkyl halides under alkaline conditions gave mixtures of *O*-alkyl and *N*,*O*-dialkyl compounds or *N*-alkyl compounds exclusively. Therefore, N^3-benzoyl-F_3Thd (**1**) was used for the preparation of *O*-alkyl compounds of F_3Thd. Compound **1** can be easily obtained by the reaction of F_3Thd and benzoyl chloride in the presence of triethylamine in dimethylacetamide.[10] N^3-Benzoyl-FUdR (**6**) was similarly synthesized.

Treatment of compound **1** and 3 equiv of iodoethane in the presence of an excess of silver oxide in acetone gave a mixture of N^3-benzoyl-3′,5′-di-*O*-ethyl- (**2a**), N^3-benzoyl-5′-*O*-ethyl- (**2b**), and N^3-benzoyl-3′-*O*-ethyl-F_3Thd (**2c**), which were separated by silica gel column chromatography. Benzylation of **1** was carried out with benzyl bromide in 2-butanone, giving a mixture of N^3-benzoyl-3′,5′-di-*O*-benzyl- (**2d**), N^3-benzoyl-5′-*O*-benzyl- (**2e**), and N^3-benzoyl-3′-*O*-benzyl-F_3Thd (**2f**).

Other substituted-benzyl derivatives of **1** and the *O*-alkyl compounds of **6** were similarly prepared. Deacylation of the N^3-benzoyl-*O*-alkyl compounds with aqueous ammonia in ethanol or a cosolvent of ethanol and acetone gave the *O*-alkyl compounds **3a–n** and **8a–d**, respectively. O^4-

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Benzyl-F_3Thd (**4**) was synthesized by treatment of F_3Thd with benzyl bromide and silver oxide in 2-butanone. N^3-Benzyl-F_3Thd (**5**) was obtained by treatment of F_3Thd with benzyl bromide in the presence of potassium carbonate in dimethylformamide.

The structures of the *O*-alkyl derivatives of F_3Thd and FUdR are summarized in Table I.

Biological Results and Discussion

As shown in Table I, O-ethylation and O-benzylation increased the in vivo antitumor activity of F_3Thd. The ED_{50} values of 5′-*O*-ethyl (**3b**), 3′-*O*-ethyl (**3c**), 5′-*O*-benzyl (**3e**), and 3′-*O*-benzyl (**3f**) derivatives of F_3Thd were 14, 15, 15, and 13 mg/kg per day, respectively, and were equal to those of *O*-ethoxymethyl or *O*-benzyloxymethyl derivatives[1] of F_3Thd. As expected from the results obtained with acyl derivatives[10] of F_3Thd, N^3-benzoylation decreased the activities of the corresponding *O*-alkyl derivatives of F_3Thd (**2b**, **2c**, **2e**, **2f** versus **3b**, **3c**, **3e**, **3f**).

3′,5′-Di-O-substituted compounds **3a** and **3d** were less active than the mono-*O*-alkyl compounds, in contrast to the *O*-alkoxyalkyl derivatives[1] of F_3Thd. O^4-Benzyl-F_3Thd (**4**) and N^3-benzyl-F_3Thd (**5**) showed no activity at the same dose as F_3Thd.

Several substituted-benzyl compounds of F_3Thd (**3g–n**) were next examined for the effect of substituents on antitumor activity. 3′-*O*-(*p*-Chlorobenzyl)-F_3Thd (**3h**) showed the highest activity, with the ED_{50} less than one-tenth of the ED_{50} of F_3Thd. The ED_{50} values of *O*-alkyl compounds of FUdR (**8a–d**) are summarized in Table I. The activities of *O*-benzyl derivatives of FUdR (**8c** and **8d**) were almost equal to those of the effective *O*-alkyl derivatives of F_3Thd. In contrast, unlike the results with *O*-ethyl derivatives of F_3Thd, *O*-ethyl derivatives of FUdR (**8a** and **8b**) were only slightly more effective than FUdR.

Finally, it should be noted that O-alkylated F_3Thd and FUdR were resistant to degradation by thymidine phosphorylase and showed no activity in vitro against HeLa cells.[19] Further biochemical pharmacology of the derivatives will be reported elsewhere.

Experimental Section

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 LMN-FX 100 spectrometer using tetramethylsilane as an internal standard. The structures of the compounds were confirmed by the elemental analyses as well as by ^{1}H NMR measurements. Elemental analyses were carried out at the section for instrumental analysis of Tokushima Institute and Center for Instrumental Analysis of Hokkaido University. Column chromatography was performed with Merck silica gel 60.

N^3-Benzoyl-2′-deoxy-5′-*O*-ethyl-5-(trifluoromethyl)uridine (2b). A mixture of N^3-benzoyl-2′-deoxy-5-(trifluoromethyl)uridine (**1**)[10] (2 g, 5 mmol), iodoethane (2.3 g, 15 mmol), and silver oxide (5.8 g, 25 mmol) in acetone (20 mL) was refluxed with stirring for 5 h. The reaction mixture was filtered, the filtrate was concentrated, and the residue was purified by silica gel column chromatography (silica gel, 50 g, 2.2 × 20 cm) with $CHCl_3$–EtOH (25:1) as an eluent. The later fractions (eluate from 120 mL to 140 mL) were combined and evaporated to leave 980 mg (46%) of **2b** as an amorphous foam: NMR (DMSO-d_6) δ 8.56 (1 H, d, H-6), 6.05 (1 H, t, H-1′), 5.39 (1 H, d, HO-3′), 4.26 (1 H, m, H-3′), 4.00 (1 H, m, H-4′), 3.68 (2 H, m, H-5′), 3.53 (2 H, q, *O*-C*H*$_2$CH$_3$), 2.30 (2 H, m, H-2′), 1.15 (3 H, t, *O*-CH$_2$C*H*$_3$). Anal. ($C_{19}H_{19}F_3N_2O_6$) C, H, N. From the earlier fractions (eluate from 90 mL to 115 mL and from 60 mL to 70 mL), 340 mg (16%) of **2c** and a small amount of **2a** were obtained, respectively.

N^3-Benzoyl-3′-*O*-benzyl-2′-deoxy-5-(trifluoromethyl)uridine (2f). A mixture of **1** (6 g, 15 mmol), benzyl bromide (7.7 g, 45 mmol), and silver oxide (8.7 g, 37.5 mmol) in 2-butanone (60 mL) was refluxed for 2 h. The reaction mixture was filtered and the filtrate was concentrated. The residue was purified by silica gel column chromatography (silica gel 80 g, 3 × 19 cm) with benzene–acetone (10:1) as an eluent. The fractions (eluate from 140 mL to 180 mL) were combined and evaporated. The residue was recrystallized from benzene to give 3.24 g of **2f** (44%): mp 160.5–162.5 °C; NMR (DMSO-d_6) δ 8.96 (1 H, d, H-6), 6.09 (1 H, t, H-1′), 5.41 (1 H, t, HO-5′), 4.55 (2 H, s, benzylic methylene), 4.1–4.4 (1 H, m, H-3′), 4.17 (1 H, t, H-4′), 3.5–3.9 (2 H, m, H-5′), 2.4–2.6 (2 H, m, H-2′). Anal. ($C_{24}H_{21}F_3N_2O_6$) C, H, N. From the earlier fractions (eluate from 120 mL to 135 mL), a small amount of **2d** was obtained. Similar treatment of the latter fractions (eluate from 190 mL to 210 mL) gave 0.7 g of **2e** (9.5%).

Other substituted-benzyl derivatives of **1** were synthesized similarly.

3′-*O*-Benzyl-2′-deoxy-5-(trifluoromethyl)uridine (3f). To a solution of **2f** (557 mg, 1.1 mmol) in EtOH–acetone (5:1, 12 mL) was added 30% NH_4OH (1.2 mL) and the mixture was stirred for 2.5 h at room temperature. The mixture was evaporated and the residue was purified by silica gel column chromatography (silica gel 15 g, 1.8 × 8 cm) with benzene–acetone (10:1) as an eluent. The appropriate fractions (from 60 mL to 75 mL) were combined and evaporated. The residue was recrystallized from EtOH–water, giving 235 mg of **3f** (54%); mp 159–160 °C; NMR (DMSo-d_6) δ 11.88 (1 H, s, N^3-He, 8.69 (1 H, d, H-6), 6.10 (1 H, t, H-1′), 5.30 (1 H, t, HO-5′), 4.54 (2 H, s, benzylic methylene), 4.0–4.3 (2 H, m, H-3′ and H-4′), 3.60 (2 H, br s, H-5′), 2.32 (2 H, t, H-2′). Anal. ($C_{17}H_{17}F_3N_2O_5$) C, H, N.

Compounds **3a–e**, **3g–n**, and **8a–d** were synthesized similarly.

O^4-Benzyl-2′-deoxy-5-(trifluoromethyl)uridine (4). A mixture of F_3Thd (4.5 g, 15 mmol), benzyl bromide (7.7 g, 45 mmol), and silver oxide (8.7 g, 37.5 mmol) in 2-butanone (60 mL) was stirred for 5 h at 65–70 °C, and the product was purified by silica gel column chromatography (benzene–acetone = 10:1) as described above: yield, 1.9 g (33%); NMR (DMSO-d_6) δ 8.98 (1 H, d, H-6), 6.03 (1 H, t, H-1′), 5.43 (2 H, s, benzylic methylene), 5.26 (1 H, d, HO-3′), 5.24 (1 H, t, HO-5′), 4.24 (1 H, m, H-3′), 3.86 (1 H, m, H-4′), 3.65 (2 H, m, H-5′), 2.25 (2 H, m, H-2′). Anal. ($C_{17}H_{17}F_3N_2O_5$) C, H, N.

N^3-Benzoyl-2′-deoxy-5-(trifluoromethyl)uridine (5). A mixture of F_3Thd (1.7 g, 5.6 mmol), benzyl bromide (0.96 g, 5.6 mmol), and K_2CO_3 (0.77 g, 5.6 mmol) in DMF (7 mL) was stirred for 6 h at 80 °C. The reaction mixture was filtered and the filtrate was evaporated. The residue was purified by silica gel column chromatography ($CHCl_3$–EtOH = 10:1) to yield 1.1 g (51%) of amorphous **5**: NMR (DMSO-d_6) δ 8.82 (1 H, s, H-6), 6.10 (1 H, t, H-1′), 5.27 (1 H, d, HO-3′), 5.25 (1 H, t, HO-5′), 4.96 (2 H, s, benzylic methylene), 4.25 (1 H, m, H-3′, 3.84 (1 H, m, H-4′), 3.63 (2 H, m, H-5′), 2.25 (2 H, t, H-2′). Anal. ($C_{17}H_{17}F_3N_2O_5$) C, H, N.

N^3-Benzoyl-2′-deoxy-5-fluorouridine (6). Benzoyl chloride (8.6 g, 61 mmol) was added dropwise to a solution of FUdR (15 g, 61 mmol) and triethylamine (9 mL) in dimethylacetamide (45 mL) below 10 °C, and the mixture was stirred for 12 h at room temperature. The reaction mixture was filtered and filtrate was evaporated. The residue was suspended in water and extracted with ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4, and concentrated. The residue was recrystallized from EtOH, giving 10.5 g of **6** (49%): mp 126–127 °C. Anal. ($C_{16}H_{15}FN_2O_6$) C, H, N.

N^3-Benzoyl-2′-deoxy-5′-*O*-ethyl-5-fluorouridine (7a). A mixture of **6** (3.5 g, 10 mmol), iodoethane (4.7 g, 30 mmol), and silver oxide (5.8 g, 25 mmol) in 2-butanone (40 mL) was stirred for 9 h at 65–70 °C, and the product was purified by silica gel column chromatography (silica gel 100 g, 3 × 21 cm) with benzene–acetone (10:1). The later fractions (eluate from 200 mL to 240 mL) were combined and treated to obtain crystals of **7a** (1.48 g, 39%, from benzene): mp 143–144 °C; NMR (DMSo-d_6) δ 8.40 (1 H, d, H-6), 6.10 (1 H, t, H-1′), 5.39 (1 H, d, HO-3′), 4.1–4.4 (1 H, m, H-3′), 3.8–4.0 (1 H, m, H-4′), 3.5–3.7 (2 H, m, H-5′), 3.54 (2 H, q, *O*-C*H*$_2$CH$_3$), 2.1–2.4 (2 H, m, H-2′), 1.19 (3 H, t, *O*-

(19) Unpublished results: On growth inhibition tests against HeLa cells (day 0, cell seeding 1 × 10^4/plate; day 1, drug applying; day 7, cell counting; the IC_{90} value of F_3Thd was 0.25 μg/mL), the IC_{90} values of 5′-*O*-ethyl (**3b**), 3′-*O*-ethyl (**3c**), 5′-*O*-benzyl (**3e**), and 3′-*O*-benzyl (**3f**) derivatives of F_3Thd were all over 10 μg/mL.

CH_2CH_3). Anal. ($C_{18}H_{19}FN_2O_6$) C, H, N. Treatment of the earlier fractions (eluate from 160 mL to 185 mL) gave 0.86 g (23%) of 3′-*O*-ethyl compound **7b**.

5′-*O*- and 3′-*O*-benzyl compounds (**7c** and **7d**) of **6** were synthesized similarly.

Antitumor Test. Mice of the ICR strain (Japan Clea Inc., Tokyo, Japan) 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 in Table I.

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Registry No. **1**, 95969-47-4; **2b**, 95969-74-7; **2c**, 95969-75-8; **2e**, 95969-77-0; **2f**, 95969-78-1; **3a**, 116953-91-4; **3b**, 96141-36-5; **3c**, 96141-38-7; **3d**, 116953-92-5; **3e**, 95969-49-6; **3f**, 96141-37-6; **3g**, 95969-61-2; **3h**, 95969-62-3; **3i**, 95969-63-4; **3j**, 95969-64-5; **3k**, 95969-65-6; **3l**, 95969-66-7; **3m**, 95969-67-8; **3n**, 95969-68-9; **4**, 116953-93-6; **5**, 117069-24-6; **8a**, 95969-43-0; **8b**, 95969-42-9; **8c**, 95969-45-2; **8d**, 95969-44-1; F_3Thd, 70-00-8; FUdR, 50-91-9.

Development of Phosphonate Derivatives of Gadolinium Chelates for NMR Imaging of Calcified Soft Tissues

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We have synthesized several classes of gadolinium (Gd) complexes for use as NMR contrast agents in the detection of soft-tissue calcification. Class I was made up of strongly chelated GdDTPA complexes with one carboxylate arm coupled to a phosphonate-containing molecule through an amide link. Class II complexes were formed by Gd with several aminophosphonates and phosphono carboxylic acids. Class III were Gd complexes of weak chelates containing no phosphonate. The calcium-seeking ability of each complex was assessed by in vivo bone uptake. Tissue distribution in normal rats showed that only the complexes of GdDTPA modified with a diphosphonate group and GdEDTMP (EDTMP is ethylenediaminetetrakis(methylenephosponate)) showed adequate bone localization at the concentrations required for NMR contrast enhancement (~20% of a 100 μmol/kg dose).

Despite the high sensitivity of magnetic resonance (MR) parameters to changes in tissue water, they are not, by themselves, disease specific.[1,2] Different pathologic conditions may share identical relaxation times and spin densities. More diagnostic specificity is available from magnetic resonance imaging (MRI) with the use of paramagnetic contrast agents, but these have not been fully exploited to date. GdDTPA has found widespread application in neuroradiology, in studying abnormalities of the GI tract, liver, and kidney, and also in visualizing heart infarcts, tumors, and other edematous lesions.[3-9] The success of GdDTPA is due to its uptake into extracellular fluid spaces,[10-14] and it is not diagnostically useful for lesions that do not involve significant changes in fluid distribution.

A variety of diseases cause changes that result in abnormal calcium accumulations in soft tissue. These include cerebral infarcts, myocardial infarcts, and some tumors (mammary tumors, hepatocellular carcinoma, and bone metastases).[10-14] Most can be visualized by radioisotope scanning techniques when ^{99m}Tc-labeled phosphonate complexes are used for localization.[15-17] When large amounts of calcium are deposited in these lesions, they can also become opaque to X-rays, providing important diagnostic information. Unfortunately MRI, which relies on tissue water, provides no information on tissue calcium concentration.

Diseased cells, because they are unable to keep extracellular free calcium ions out of the phosphate-rich intracellular medium, precipitate calcium phosphates irreversibly.[18] These amorphous precipitates, like bone material, can be identified by calcium-seeking agents carrying paramagnetic probes. Modifications of Gd chelates that

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