Studies on Antitumor Agents. 2. Syntheses and Antitumor Activities of 1-(Tetrahydro-2-furanyl)-5-fluorouracil and 1,3-Bis(tetrahydro-2-furanyl)-5-fluorouracil[1,2]

Mitsugi Yasumoto,* Ichiro Yamawaki, Teruyoshi Marunaka, and Sadao Hashimoto

Research Laboratory, Taiho Pharmaceutical Company, Tokushima, 771-01, Japan. *Received February 13, 1978*

Convenient and efficient methods were developed for preparing 1-(tetrahydro-2-furanyl)-5-fluorouracil (Thf-FU, **3**) [trade name, Futraful (Ftorafur) or FT-207], which is used clinically as an antitumor agent, and 1,3-bis(tetrahydro-2-furanyl)-5-fluorouracil (Thf_2-FU, **4**). For the syntheses, 2,4-bis(trimethylsilyl)-5-fluorouracil (Me_3Si-FU, **1**) and 2-acetoxytetrahydrofuran (Thf-OAc, **2**) were condensed in the presence of Friedel–Crafts catalysts, such as $SnCl_4$ and BF_3–Et_2O in dichloromethane, or in the presence of NaI in acetonitrile to give Thf-FU or Thf_2-FU depending on the reaction conditions and workup procedure. A trace of 3-(tetrahydro-2-furanyl)-5-fluorouracil (3-Thf-FU, **5**) was formed in these reactions. Thf_2-FU was easily hydrolyzed to Thf-FU. 2-Methoxytetrahydrofuran can be used instead of Thf-OAc for preparation of Thf-FU under similar conditions. The optimal ratios of Me_3Si-FU, Thf-OAc, and $SnCl_4$ or NaI for preparation of Thf-FU and Thf_2-FU were determined. In all cases, 2–2.5 equiv of Thf-OAc with respect to Me_3Si-FU gave the best results. The yields of Thf-FU and more especially of Thf_2-FU were greatly dependent on the relative amount of $SnCl_4$, and 0.01–0.1 equiv of the catalyst with respect to Me_3Si-FU gave the best results. Thf_2-FU was found to be effective against murine solid tumors and it was less toxic than Thf-FU when given orally. The antitumor activity of 3-Thf-FU is also reported.

5-Fluorouracil (5-FU), first synthesized by Duschinsky and Pleven,[3] has been used clinically as an antitumor agent. A derivative of 5-FU, 1-(tetrahydro-2-furanyl)-5-fluorouracil (Thf-FU, **3**), named Futraful (Ftorafur) or FT-207, was first synthesized by Hiller et al.[4] and it was also found to be an effective antitumor agent which can be given orally and which has low toxicity.[5-7] There are several reports on the synthesis of Thf-FU, including the syntheses of the pure stereoisomers.[4,8-11] Hiller et al. used chloromercuri- or 2,4-bis(trimethylsilyl)-5-fluorouracil (Me_3Si-FU, **1**) and 2-chlorotetrahydrofuran (Thf-Cl) and reported that the condensation should be carried out at low temperature (−20 to −40 °C) because Thf-Cl is unstable and that use of excess Thf-Cl caused a degradation reaction which reduced the yield of Thf-FU.[4a] Earl and Townsend also used Thf-Cl and 2,4-bis(trimethylsilyl)uracil to prepare 1-(tetrahydro-2-furanyl)uracil and fluorinated the product with trifluoromethyl hypofluorite.

This paper reports condensation reactions between Me_3Si-FU and 2-acetoxytetrahydrofuran[12] (Thf-OAc, **2**) or 2-methoxytetrahydrofuran[13] (Thf-OMe) using Friedel–Crafts catalysts or NaI. Optimal conditions are described for preparing Thf-FU and 1,3-bis(tetrahydro-2-furanyl)-5-fluorouracil (Thf_2-FU, **4**),[14] which showed similar activity to that of Thf-FU against murine solid tumors but had lower toxicity.

Condensation Using Various Catalysts. In the absence of catalyst, a high temperature and long reaction time were required for condensation of Me_3Si-FU with Thf-OAc in dichloromethane and the yield of Thf-FU was only moderate. Therefore, the effects of catalysts were investigated.

In the method of glycosidation reported by Niedballa and Vorbrüggen,[15,16] a trimethylsilylated base and a 1-acyloxy sugar are condensed using a Friedel–Crafts catalyst. Therefore, we tested the effects of the Friedel–Crafts catalysts, BF_3–Et_2O,[17] $SnCl_4$, $TiCl_4$, $SiCl_4$, and $SbCl_5$. The results of reactions with Me_3Si-FU and Thf-OAc in a ratio of 1.0:1.5 are shown in Table I. From the yields and rates of condensation, BF_3–Et_2O and $SnCl_4$ seem to be the most promising catalysts, and further investigations were made mainly on the reactions catalyzed by $SnCl_4$.

Next, the condensation reaction was examined using various ratios of Me_3Si-FU, Thf-OAc, and $SnCl_4$. Yields were better with a large excess of Thf-OAc (1.5–2 equiv to Me_3Si-FU) and a smaller amount of $SnCl_4$ (0.01–0.1 equiv to Me_3Si-FU). The best yield (91–93%) was obtained with a ratio of base to furan to $SnCl_4$ of 1.0:2.0:0.1 or 0.01. Reduction of the amount of $SnCl_4$ below 0.005 equiv with respect to Me_3Si-FU reduced the yield of Thf-FU. Niedballa and Vorbrüggen reported that the change in the solvent from dichloroethane to acetonitrile altered the site of glycosidation.[18] During investigations on reaction conditions in acetonitrile, we found that NaI, which has been used as a catalyst in condensation of pyrimidine bases with tetrahydro-2-furanylmethyl halide,[19] was a good catalyst. The reaction containing base, furan, and NaI in a ratio of 1.0:1.5:0.05–2.0 at room temperature for 13–18 h gave Thf-FU in about 80% yield, though the reaction without catalyst gives a poor yield, even at high temperature. When KI is used instead of NaI, the yield is poor. When the relative amount of Thf-OAc was increased to 2.5 equiv the yield was almost quantitative. The yield of Thf-FU was not affected by the amount of NaI. However, increase in the amount of NaI reduced the reaction time and lowered the reaction temperature.

As described above, NaI was proved to be a good catalyst for the condensation in acetonitrile. Use of NaI is more convenient than use of $SnCl_4$ in dichloromethane, because it is easier to handle, and workup of the reaction mixture

Table I. Yields of Thf-FU and Thf_2-FU in Condensation Reactions of Me_3Si-FU with Thf-OAc in the Presence of Various Catalysts at Room Temperature[a]

molar ratio					
Me_3Si-FU	Thf-OAc	Catalyst	solvent	time, h	yield, %
1.0	1.5	BF_3–Et_2O, 1.0	CH_2Cl_2	1.0	70[b]
1.0	1.5	$TiCl_4$, 1.0	CH_2Cl_2	0.5	60[b]
1.0	1.5	$SbCl_5$, 1.0	CH_2Cl_2	0.1	8[b]
1.0	1.5	$SnCl_4$, 0.1	CH_2Cl_2	1.5	81[b]
1.0	1.0	$SnCl_4$, 0.01	CH_2Cl_2	8	61[b]
1.0	2.0	$SnCl_4$, 0.01	CH_2Cl_2	3	93[b]
1.0	1.0	NaI, 1.0	CH_3CN	23	69[b]
1.0	2.0	NaI, 1.0	CH_3CN	9	91[b]
1.0	2.0	$SnCl_4$, 0.01	CH_2Cl_2	3	96[c]
1.0	2.5	NaI, 1.0	CH_3CN	8	92[c]
1.0	2.5	BF_3–Et_2O, 0.02	CH_2Cl_2	3	79[c]

[a] The reaction course was followed by TLC after treating samples with aqueous NaOH (for Thf-FU) or Et_3N-EtOH (for Thf_2-FU), and when formation of Thf-FU or Thf_2-FU stopped the whole reaction mixture was worked up. [b] Yield of Thf-FU. [c] Yield of Thf_2-FU.

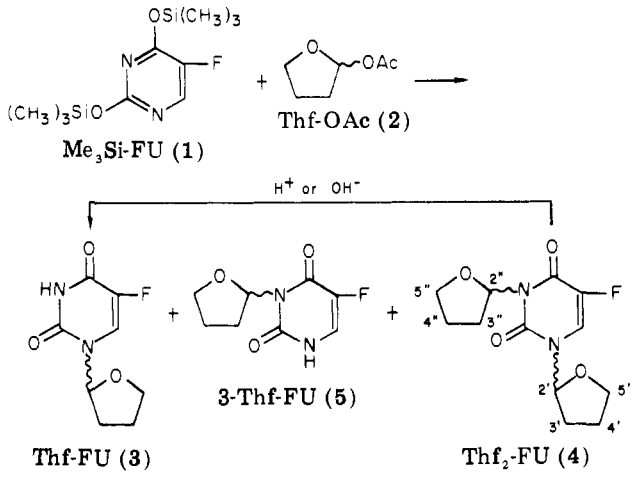
0022-2623/78/1821-0738$01.00/0

Table II. LD_{50} Values after Oral Administration of Thf_2-FU and Thf-FU[a]

compd	LD_{50}, mg/kg (mmol/kg)			
	3 days	1 week	2 weeks	3 weeks
Thf_2-FU	2895 (10.7)	2780 (10.3)	2664 (9.9)	2664 (9.9)
Thf-FU	900 (4.5)	860 (4.3)	820 (4.1)	820 (4.1)
5-FU		200 (1.5)	145 (1.1)	115 (0.88)

[a] Drugs were administered orally to mice and LD_{50} values were determined by the "up and down" method at the times indicated.

Scheme I. Condensation Reaction of Me_3Si-FU with Thf-OAc



is simpler, although the reaction rate is slower. 2-Methoxytetrahydrofuran (Thf-OMe) can be used in the condensation instead of Thf-OAc in the presence of either catalyst.

Formation of 1,3-Bis(tetrahydro-2-furanyl)-5-fluorouracil.[14] The reaction of stoichiometric amounts of Me_3Si-FU and Thf-OAc using $SnCl_4$ gave Thf-FU in poor yield (Table I). To elucidate the reason for this poor yield, we examined the products of the reaction of base, furan, and $SnCl_4$ in a ratio of 1.0:1.0:0.01, using triethylamine in EtOH instead of aqueous NaOH solution to destroy $SnCl_4$. In addition to the spots of 5-FU and Thf-FU, a major spot having a higher R_f value (0.65) than that of Thf-FU (0.38) and a minor spot having a lower R_f value (0.21) were detected on a TLC plate developed with $CHCl_3$–dioxane (4:1, v/v). A mixture containing base, furan, and $SnCl_4$ in a ratio of 1.0:2.0:0.01, which gave the best yield of Thf-FU when worked up with aqueous NaOH, mainly gave the spot with a high R_f value under the same conditions. The compound in the spot of high R_f and the compound in the minor spot of low R_f value were identified as 1,3-bis(tetrahydro-2-furanyl)-5-fluorouracil (Thf_2-FU, **4**) and 3-(tetrahydro-2-furanyl)-5-fluorouracil (3-Thf-FU, **5**), respectively, by UV absorption, mass and NMR spectroscopies, and elemental analysis (see Scheme I and Experimental Section).

Thf_2-FU is readily hydrolyzed and in aqueous alkali it is rapidly and quantitatively hydrolyzed to Thf-FU. This means that the tetrahydrofuranyl group at N(3) is specifically removed. The time course of the reaction of base, furan, and $SnCl_4$ in a ratio of 1.0:2.0:0.01 was examined by the TLC extraction method and the results are illustrated in Figure 1. Thf-FU was rapidly formed at an early stage and was then converted more slowly to Thf_2-FU. 3-Thf-FU also appeared at an early stage and seemed to be converted to Thf_2-FU gradually. After 3 h at room temperature, the percentage yields were 3.7% Thf-FU and 96.1% Thf_2-FU. No 5-FU was detected. These results suggest that 5-FU, Thf-FU, and 3-Thf-FU compete for

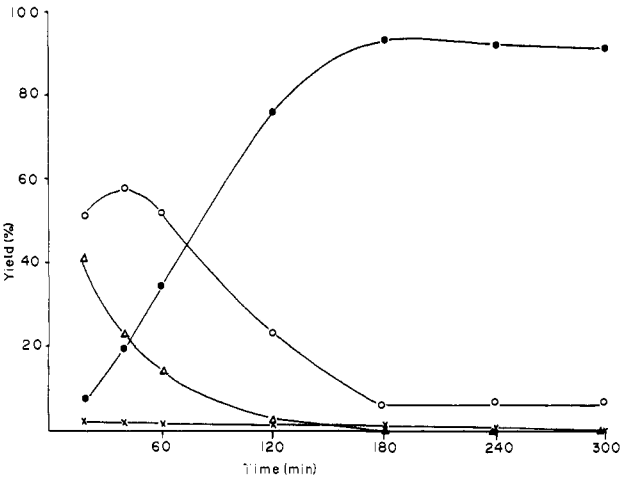


Figure 1. Time courses of the reactions of Me_3Si-FU, Thf-OAc, and $SnCl_4$ (1.0:2.0:0.01) in dichloromethane at room temperature. The yields of the products were determined by TLC extraction: O—O, Thf-FU; ●—●, Thf_2-FU; ×—×, 3-Thf-FU; Δ—Δ, 5-FU.

Thf-OAc and the combined yield of Thf-FU and Thf_2-FU, the yield of Thf-FU after treatment with aqueous alkali, is restricted by the amount of Thf-OAc added. This is why 2 equiv of Thf-OAc with respect to Me_3Si-FU is required for preparation of Thf-FU. Suitable conditions for synthesis of Thf_2-FU were examined. Reactions containing base, furan, and $SnCl_4$ in a ratio of 1.0:2.0–2.5:0.01–0.05 gave almost quantitative yields (94–96%) on TLC. Increase in the relative amount of $SnCl_4$ above 0.5 equiv reduced the yield of Thf_2-FU and reaction with 2.0 equiv of $SnCl_4$ gave no Thf_2-FU. Thus the yield of Thf_2-FU is more sensitive than that of Thf-FU to the amount of $SnCl_4$. Thf_2-FU can also be synthesized using NaI or BF_3–Et_2O for catalysis. The conditions for preparation of Thf-FU and Thf_2-FU in optimal yield are summarized and included in Table I.

Toxicity and Antitumor Activity of Thf_2-FU and 3-Thf-FU. The toxicity of Thf_2-FU on short-term administration was examined by giving the drug to mice orally.[20] The results are shown in Table II together with data on Thf-FU and 5-FU. The LD_{50} (mmol/kg) of Thf_2-FU was about three times that of Thf-FU for each period tested. So, Thf_2-FU is less toxic than Thf-FU, and Thf-FU in turn is much less toxic than 5-FU. Preliminary results for 3-Thf-FU showed that 3-Thf-FU has similar LD_{50} values to those of Thf-FU.

The antitumor activities of Thf_2-FU against murine solid tumors are shown in Table III.[20] The data on Thf-FU and 5-FU are included for comparison, Thf_2-FU was slightly more inhibitory than Thf-FU to murine solid tumors, Ehrlich carcinoma, sarcoma 180, Yoshida sarcoma, AH-130 carcinoma, and Walker 256 carcinosarcoma. None of these compounds had any significant activity against ascites tumor. The antitumor activity of 3-Thf-FU against AH-130 carcinoma is shown in Table IV. It seems that 3-Thf-FU is more inhibitory than Thf-FU at a dose of 0.15 mmol/kg. At a higher dose (0.45 mmol/kg) it strongly

Table III. Antitumor Effects of Thf_2-FU and Thf-FU on Murine Solid Tumors[a]

compd	dose, mmol/kg	tumor inhibn, % Ehrlich carcinoma[b]	sarcoma 180[b]	Yoshida sarcoma[c]	AH-130 carcinoma[c]	Walker 256 carcinosarcoma[c]
Thf_2-FU	0.15	50	45	43	32	27
	0.45	61	56	58	44	37
Thf-FU	0.15	38	38	32	22	27
	0.45	54	37	52	41	31
5-FU	0.08	42	48	41	36	24

[a] Mice or rats (ten animals/group) carrying solid tumors were given the drugs (0.15 and 0.45 mmol/kg) orally every day for 7 days from 1 day after tumor implantation. [b] Mice were used. [c] Rats were used.

Table IV. Antitumor Effects of 3-Thf-FU on AH-130 Carcinoma[a]

compd	dose, mmol/kg	body wt change, g	tumor wt, g	inhibn, %
3-Thf-FU	0.15	+47.3	5.4 ± 1.4	45
	0.45	+21.7	2.0 ± 0.8	80
Thf-FU	0.45	+40.9	6.4 ± 1.1	35
5-FU	0.08	+42.1	6.6 ± 1.8	33
control		+55.9	9.8 ± 1.4	

[a] Rats (ten animals/group) carrying solid tumors were given the drugs orally at the doses indicated every day for 7 days from 1 day after tumor implantation. On the 10th day after inoculation, the body weight and tumor weight were measured.

inhibits tumor growth, but at this dose it is also toxic to normal body growth of rats. It is known that Thf-FU is a masked compound and that it is hydrolyzed gradually to an active compound, 5-FU, in liver microsomes, thus having a prolonged effect.[21-23] Biological studies on Thf_2-FU have shown that it acts in a similar manner to Thf-FU but keeps the concentrations of 5-FU in the blood and tissues at higher levels when it is administered orally to animals.[24,25] Fujita et al. reported that when Thf_2-FU is given orally to rabbits or mice, the concentrations of 5-FU in the blood and tissues from an early stage are much higher (three to five times) than after administration of Thf-FU, and they remain higher for up to 8 h after the administration.[24] Furthermore Kawaguchi et al. have shown that when Thf_2-FU is given orally to rats, the concentrations of 5-FU are five to seven times higher in the blood and normal tissues and eight to twelve times higher in tumor tissue than when Thf-FU is given by the same route and that they remain high for longer periods.[25] Thus they suggested that the activation of Thf_2-FU in vivo involves mainly enzymatic hydrolysis of Thf_2-FU to 3-Thf-FU and nonenzymatic hydrolysis of the latter to 5-FU.

Experimental Section

$SnCl_4$, NaI (special grade), and BF_3–Et_2O (ca. 47%) were products of Wako Pure Chemicals Co. Ltd. (Osaka, Japan). $SbCl_5$ (99%), $SiCl_4$ (ultrapure grade), and $TiCl_4$ (99.9%) were products of Alfa Chemical Co. (Danvers, Mass.). Thin-layer chromatography (TLC) was performed on Kiesel gel 60 F_{254} (0.25 mm) plates, product of Merck (Darmstadt, Germany). ^1H NMR spectra were recorded in a Hitachi R-22 spectrometer using tetramethylsilane as an internal standard. Mass spectra were obtained with a JEOL JMS-01G-2 spectrometer. UV spectra were recorded on a Hitachi 124 spectrophotometer. Elemental analyses were carried out with a Yanagimoto CHN Corder MT-2 analyzer. Melting points were determined in a Yanagimoto micromelting point apparatus and are reported as uncorrected values. Room temperature was 20–25 °C.

Synthesis of 1-(Tetrahydro-2-furanyl)-5-fluorouracil (Thf-FU, 3). (1) Without Catalyst. A mixture of 2,4-bis(trimethylsilyl)-5-fluorouracil[4] [Me_3Si-FU (1), 5.48 g, 0.02 mol] and 2-acetoxytetrahydrofuran[11] [Thf-OAc (2), 3.9 g, 0.03 mol] was heated at 95–100 °C for 12 h. $CHCl_3$ and water were added to the reaction mixture and the resulting mixture was shaken thoroughly. The $CHCl_3$ layer was separated, and aqueous 1 N NaOH solution was added to adjust the pH to 10–12. The mixture was kept at room temperature for 2 h, maintaining the pH of the aqueous layer at 10–12 with vigorous stirring. The aqueous layer was then adjusted to pH 4–6 with dilute HCl and the $CHCl_3$ layer was separated. The aqueous fraction was extracted further with $CHCl_3$, and the $CHCl_3$ fractions were combined and evaporated to dryness. Crystallization of the residue from EtOH gave 2.52 g (63%) of Thf-FU: mp 165–167 °C (lit.[4] 164–165 °C); UV λ_{max}^{pH2} 271 nm (ε 9000), λ_{max}^{pH12} 270 nm (ε 6900), $\lambda_{max}^{95\%EtOH}$ 270.5 nm (ε 8800). Anal. ($C_8H_9FN_2O_3$) C, H, N.

(2) Using $SnCl_4$. $SnCl_4$ solution in CH_2Cl_2 (5.2% w/v, 1 mL, 0.2 mmol) and Thf-OAc (2, 9 g, 0.03 mol) were added to a solution of Me_3Si-FU (1, 5.48 g, 0.02 mol) in CH_2Cl_2 (70 mL), and the resulting solution was kept at room temperature for 6 h. After addition of 1 N NaOH solution to the mixture, the same workup as described in the first experiment gave 3.33 g (83.3%) of Thf-FU.

(3) Using BF_3–Et_2O. BF_3–Et_2O (2.84 g, 0.02 mol) in ether (ca. 47%) and Thf-OAc (2, 3.9 g, 0.03 mol) were added to a solution of Me_3Si-FU (1, 5.48 g, 0.02 mol) in CH_2Cl_2 (70 mL), and the resulting solution was kept at room temperature for 1 h. After addition of 1 N NaOH solution to the mixture, the same workup as described in the first experiment gave 2.82 g (70.4%) of Thf-FU

(4) Using NaI. NaI (1.5 g, 0.01 mol) and Thf-OAc (2, 3.9 g, 0.03 mol) were added to a solution of Me_3Si-FU (1, 5.48 g, 0.02 mol) in CH_3CN (70 mL), and the resulting solution was kept at room temperature for 18 h. The reaction mixture was evaporated to dryness and the residue was shaken with a $CHCl_3$–water mixture. The $CHCl_3$ layer was mixed with 1 N NaOH and the same workup of the mixture as described in the first experiment gave 3.23 g (80.8%) of Thf-FU.

(5) Using NaI and Thf-OMe. NaI (3.0 g, 0.02 mol) and 2-methoxytetrahydrofuran[12] (Thf-OMe, 2.04 g, 0.02 mol) were added to a solution of Me_3Si-FU (1, 5.48 g, 0.02 mol) in CH_3CN (60 mL), and the resulting mixture was kept at 75–80 °C for 12 h and then evaporated to dryness. The residue was shaken with a $CHCl_3$–water mixture, and the $CHCl_3$ layer was washed with water and evaporated. Crystallization of the residue from EtOH gave 2.04 g (51%) of Thf-FU.

Synthesis of 1,3-Bis(tetrahydro-2-furanyl)-5-fluorouracil (Thf_2-FU, 4). (1) Using $SnCl_4$. $SnCl_4$ solution in CH_2Cl_2 (5.2% w/v, 1 mL, 0.2 mmol) and Thf-OAc (2, 5.2 g, 0.04 mol) were added to a solution of Me_3Si-FU (1, 5.48 g, 0.02 mol) in CH_2Cl_2 (70 mL) below 10 °C using a reaction flask dried with a stream of N_2. The reaction mixture was kept at room temperature for 3 h and an Et_3N (0.6 mL)–EtOH (10 mL) mixture was added below 10 °C. Volatile materials were evaporated off below room temperature under reduced pressure, and the residue was shaken with a $CHCl_3$–ice water mixture. The $CHCl_3$ layer was washed with water, dried over anhydrous Na_2SO_4, and filtered. Then the $CHCl_3$ solution was evaporated off below room temperature. The residue was applied to a column of silica gel (Merck kieselgel 60, 70–230 mesh, 400 g) and the product was eluted with a benzene–acetone mixture (5:4, v/v). The solvent was evaporated off. The residue gave a single spot on TLC in benzene–acetone (5:4, v/v). This material was crystallized from 90% EtOH, giving 4.35 g (80.6%) of Thf_2-FU: mp 110–112 °C; UV λ_{max}^{pH2} 275 nm (ε 9000). λ_{max}^{pH10} 275 nm (ε 8900), λ_{max}^{EtOH} 274 nm (ε 8300); ^1H NMR (pyridine-d_5) δ 3.50–4.60 (m, 4, C_5-H and $C_{5'}$-H), 6.04 (m, 1, C_2-H), 6.84 (m, 1, $C_{2'}$-H), 7.63 (d, 1, C_6H, J_{H-F} = 6.4 Hz) (the numbering system of the furan rings is shown in Scheme I); $[\alpha]^{23}_D$ 0° (c 0.5, $CHCl_3$);

mass spectrum m/e 270 (M^+). Anal. ($C_{12}H_{15}FN_2O_4$) C, H, N.

(2) Using BF_3-Et_2O. BF_3-Et_2O (57 mg, 0.4 mmol) in ether (1 mL) and Thf-OAc (2, 6.5 g, 0.05 mol) were added to a solution of Me_3Si-FU (1, 5.48 g, 0.02 mol) in CH_2Cl_2 (100 mL), using a reaction flask dried with a stream of N_2. The resulting solution was kept at room temperature for 3 h. Workup of the mixture as described in the first experiment gave 3.62 g (67%) of Thf_2-FU.

(3) Using NaI. NaI (3.0 g, 0.02 mol) and Thf-OAc (2, 6.5 g, 0.05 mol) were added to a solution of Me_3Si-FU (1, 5.48 g, 0.02 mol) in CH_3CN (50 mL), and the resulting solution was kept at 60 °C for 0.5 h and then evaporated below room temperature. The residue was shaken with a $CHCl_3$–ice water mixture. The $CHCl_3$ layer which separated was washed with water, dried over anhydrous Na_2SO_4, and evaporated. Silica gel column chromatography of the residue in the same manner as described in the first experiment and crystallization gave 4.2 g (77.8%) of Thf_2-FU.

Isolation and Characterization of 3-(Tetrahydro-2-furanyl)-5-fluorouracil (3-Thf-FU, 5). 3-(Tetrahydro-2-furanyl)-5-fluorouracil (3-Thf-FU) was isolated by silica gel column chromatography of the products from reaction 1 for the Thf_2-FU synthesis described above. The products were eluted with $CHCl_3$–dioxane (4:1, v/v). The fractions containing 3-Thf-FU, which was eluted after Thf-FU, were pooled and evaporated to give 80 mg (2%) of 3-Thf-FU: mp 127.5–129 °C; UV λ_{max}^{pH2} 269 nm (ϵ 13 000), λ_{max}^{pH12} 301 nm (ϵ 17 400), λ_{max}^{MeOH} 269 nm (ϵ 12 900); $[\alpha]^{23}_D$ 0° (c 0.5, $CHCl_3$); mass spectrum m/e 200 (M^+). Anal. ($C_8H_9FN_2O_3$) C, H, N.

Hydrolysis of Thf_2-FU (4) to Thf-FU (3). (1) A mixture of Thf_2-FU (2.7 g) and 50% aqueous EtOH (150 mL) was stirred at 60–80 °C for 1.5–2 h. The mixture was then evaporated and crystallization of the residue from EtOH gave 1.88 g (94%) of Thf-FU.

(2) To a solution of Thf_2-FU (2.7 g) in EtOH (50 mL), aqueous 1 N NaOH solution was added and the mixture was stirred at room temperature for 2–2.5 h, maintaining the pH at 10–12. The EtOH was evaporated off and the residual solution was adjusted to pH 4–6 with dilute HCl solution and extracted with $CHCl_3$. The $CHCl_3$ layer separated was washed with water and evaporated. Crystallization of the residue from EtOH gave 1.82 g (91%) of Thf-FU.

Determination of Thf-FU, Thf_2-FU, and 3-Thf-FU by TLC Extraction. The reaction mixture after treatment with Et_3N–EtOH was centrifuged (2500 rpm, 10 min) and an aliquot of the supernatant was applied to a TLC plate. The plate was developed with $CHCl_3$–dioxane (4:1, v/v) and dried in air for 10 min. The spots (the R_f values of Thf_2-FU, Thf-FU, 3-Thf-FU, and 5-FU are 0.65, 0.38, 0.21, and 0.05, respectively) were located under a UV lamp, scraped off, and extracted with 95% EtOH (Thf-FU and Thf_2-FU) or MeOH (3-Thf-FU) for 10 min. The mixture was centrifuged (2500 rpm, 20 min) and the UV absorbance of the supernatant was measured at its λ_{max}: Thf-FU, 270.5 nm (ϵ 8800 in 95% EtOH); Thf_2-FU, 274 nm (ϵ 8200 in 95% EtOH); 3-Thf-FU, 269 nm (ϵ 12 900 in MeOH).

Toxicity Test. Compounds were administered orally to male mice (ddY strain, 5 weeks old) and LD_{50} values were determined by the "up and down" method[26] after 3 days, 1 week, 2 weeks, and 3 weeks.

Antitumor Activity Test. The animals used were ddY strain mice for Ehrlich carcinoma, ICR strain mice for sarcoma 180, Donryu strain rats for Yoshida sarcoma and AH-130 carcinoma, and Wistar strain rats for Walker 256 carcinosarcoma. Animals were inoculated subcutaneously with 5×10^6 tumor cells. Oral administration of a drug (0.15 and 0.45 mmol/kg) was started 24 h later and continued daily for 7 days in test groups of ten animals each. On the 10th day after tumor inoculation, the average weight of tumors was compared with that in the control group.

Acknowledgment. The authors wish to thank Professor M. Ikehara and Dr. S. Uesugi, Faculty of Pharmaceutical Sciences, Osaka University, and Professor S. Fujii, Institute of Protein Research, Osaka University, for helpful suggestions.

References and Notes

(1) Presented in part at the Chugoku-Shikoku Branch Meeting of the Pharmaceutical Society of Japan, Kochi, Shikoku, Oct 1975.
(2) For part 1 of this series, see M. Yasumoto, A. Moriyama, N. Unemi, S. Hashimoto, and T. Suzue, *J. Med. Chem.*, **20**, 1592 (1977).
(3) R. Duschinsky and E. Pleven, *J. Am. Chem. Soc.*, **79**, 4559 (1957).
(4) (a) S. A. Hiller, R. A. Zhuk, and M. Yu. Lidak, *Dokl. Acad. Nauk USSR*, **176**, 332 (1967); (b) S. A. Hiller, R. A. Zhuk, M. Yu. Lidak, and A. A. Zidermane, British Patent 1 168 391 (1969).
(5) C. Konda, H. Niitani, N. Sakauchi, A. Suzuki, Y. Sakai, T. Sakano, M. Shimoyama, T. Kitahara, S. Kumaoka, and K. Kimura, *Jpn. J. Cancer Clin.*, **19**, 495 (1973).
(6) Y. Takemasa, M. Suehara, Y. Koyama, and T. Kimura, *Cancer Chemother. (Jpn.)*, **1**, 259 (1974).
(7) T. Saito, A. Wakui, M. Yokoyama, H. Takahashi, K. Takahashi, H. Ishigaki, K. Watanabe, and S. Tada, *J. Jpn. Soc. Cancer Ther.*, **9**, 395 (1974).
(8) R. A. Earl and L. B. Townsend, *J. Heterocycl. Chem.*, **9**, 1141 (1972).
(9) (a) J. Žemlička, R. Gasser, J. V. Freisler, and J. P. Horwitz, *J. Am. Chem. Soc.*, **94**, 3213 (1972); (b) J. P. Horwitz, J. J. McCormick, K. D. Philips, V. M. Maher, J. R. Otto, D. Kessel, and J. Žemlička, *Cancer Res.*, **35**, 1301 (1975).
(10) M. Yasumoto, A. Moriyama, N. Unemi, S. Hashimoto, and T. Suzue, *J. Med. Chem.*, **20**, 1592 (1977).
(11) T. Kametani, K. Kigasawa, M. Hiiragi, K. Wakisaka, O. Kusama, K. Kawasaki, and H. Sugi, *J. Heterocycl. Chem.*, **14**, 473 (1977).
(12) H. Normant, *C. R. Hebd. Seances Acad. Sci.*, **228**, 102 (1949).
(13) W. Reppe, *Justus Liebigs Ann. Chem.*, **596**, 182 (1955). Our Thf-OMe sample [bp 26–28.5 °C (29–30 mmHg)] was synthesized from 2,3-dihydrofuran, MeOH, and HCl by a procedure analogous to that described in ref 12 for the preparation of 2-ethoxytetrahydrofuran.
(14) (a) M. Yasumoto, T. Marunaka, S. Hashimoto, K. Harima, and T. Suzue, Japan Kokai 50 384 (1975); Japanese Patent 23 512 (1976) [*Chem. Abstr.*, **83**, 193366u (1975)]; (b) Japan Kokai 136 687 (1976).
(15) U. Niedballa and H. Vorbrüggen, *Angew. Chem., Int. Ed. Engl.*, **9**, 46 (1970).
(16) U. Niedballa and H. Vorbrüggen, *J. Org. Chem.*, **39**, 3654 (1974).
(17) T. Ogawa, M. Yasui, and M. Matsui, *Agric. Biol. Chem.*, **36**, 913 (1972).
(18) U. Niedballa and H. Vorbrüggen, *J. Org. Chem.*, **39**, 3660 (1974).
(19) J. Defaye, M. Naumberg, and J. Reyners, *J. Heterocycl. Chem.*, **6**, 229 (1969).
(20) N. Unemi, M. Yasumoto, K. Morita, M. Kajihara, and H. Toide, paper presented at the 35th Annual Meeting of the Japanese Cancer Association, Tokyo, Oct 1976.
(21) (a) H. Fujita, *Sogo Rinsho*, **20**, 1350 (1971); (b) H. Fujita, K. Ogawa, T. Sawabe, and K. Kimura, *Jpn. J. Cancer Clin.*, **18**, 911 (1972).
(22) S. Fujii, H. Okuda, H. Toide, N. Watanabe, K. Taira, and S. Hashimoto, *Oyo Yakuri*, **8**, 597 (1974).
(23) S. Ohira, S. Maezawa, K. Watanabe, and T. Saito, *Jpn. J. Cancer Clin.*, **22**, 856 (1976).
(24) H. Fujita, K. Ogawa, A. Kato, K. Kitazato, A. Yasuda, and K. Kimura, paper presented at the 35th Annual Meeting of the Japanese Cancer Association, Tokyo, Oct 1976.
(25) Y. Kawaguchi, Y. Nakamura, T. Sato, S. Takeda, T. Marunaka, and S. Fujii, unpublished results.
(26) K. A. Brownlee, *J. Am. Stat. Assoc.*, **48**, 262 (1953).