

# Studies of Antitumor Agents. 1. Resolution of Racemic 1-(Tetrahydro-2-furanyl)-5-fluorouracil into the *R* and *S* Isomers and Examination of the Biological Activities of the Isomers

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1-(Tetrahydro-2-furanyl)-5-fluorouracil (Thf-FU), which is named Ftorafur or FT-207 and is used clinically as an antitumor agent, was conveniently synthesized by condensation of the trimethylsilyl derivative of 5-fluorouracil with 2-acetoxytetrahydrofuran using NaI as a catalyst. This optically inactive Thf-FU was resolved into optically active (*R*)-(+)- and (*S*)-(-)-Thf-FU in high optical purity and excellent yield by formation of diastereoisomers with brucine.  $^{13}\text{C}$  NMR data were obtained on Thf-FU and related compounds and the antibacterial activities and in vivo antitumor activities of these isomers were tested. The degradations of these isomers to 5-fluorouracil by liver microsomes were also examined. No significant differences were found in any of these properties of these isomers.

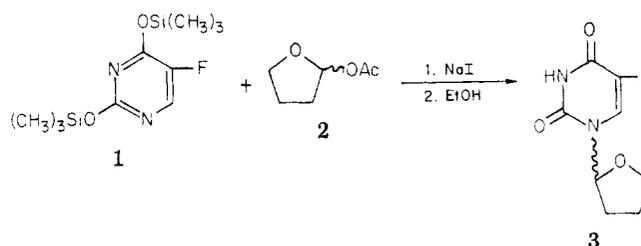
1-(Tetrahydro-2-furanyl)-5-fluorouracil (Thf-FU, **3**), named Ftorafur or FT-207, is a derivative of 5-fluorouracil (FU) which is used clinically as an antitumor agent.<sup>1-3</sup> Since Thf-FU contains one asymmetric carbon in the tetrahydrofuran ring, there are two stereoisomers, similar to the  $\alpha$ - and  $\beta$ -anomers in an ordinary nucleoside. Thf-FU was first synthesized by condensation of chloromercuri or bis(trimethylsilyl) derivatives of FU with 2-chlorotetrahydrofuran.<sup>4</sup> The product was a racemic mixture and it was used for biological studies and clinical purposes. Zemlička et al. synthesized (*R*)- and (*S*)-Thf-FU (**3a** and **3b**) starting from anomers of 5-fluoro-2'-deoxyuridine (FUdR) and reported that these stereoisomers show similar cytotoxicities and inhibitions of DNA synthesis in in vitro systems.<sup>5,6</sup> However, this procedure is not suitable for obtaining these isomers in large quantity.

This paper reports a simple and efficient method to prepare the *R* and *S* isomers of Thf-FU involving optical resolution of their racemic mixture. Some biological properties of these isomers, including their antitumor activities in vivo, were also described.

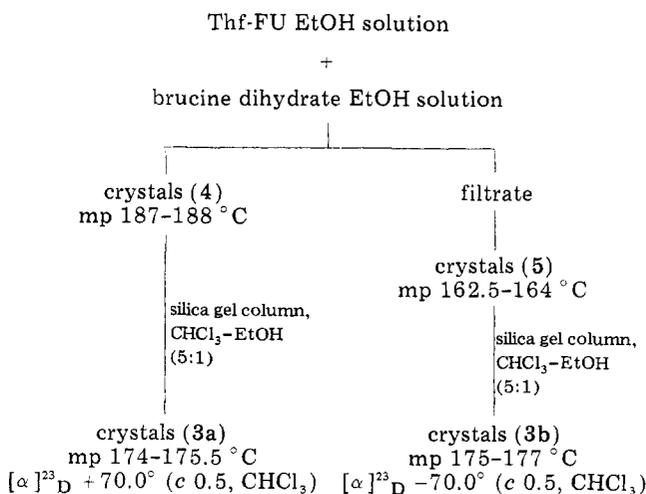
**Synthesis and Optical Resolution of Thf-FU (3).** A racemic mixture of Thf-FU (**3**) was synthesized by condensation of bis(trimethylsilyl)-5-fluorouracil (**1**) with 2-acetoxytetrahydrofuran (**2**) using sodium iodide as a catalyst (Scheme I). This type of condensation reaction, in which a trimethylsilylated base, an acetoxy sugar, and a Lewis acid such as stannous chloride are used, was developed by Vorbrüggen et al. for synthesis of nucleosides.<sup>7</sup> Use of sodium iodide as a catalyst made the work-up very simple and afforded the desired product in quite high yield (91%). The product (mp 166–168 °C) shows no optical activity.

We tried to resolve Thf-FU by formation of its diastereoisomers with optically active alkaloids such as cinchonine, cinchonidine, quinine, quinidine, and brucine and succeeded in resolving the diastereoisomers with brucine by recrystallization from ethanol, as illustrated in Chart I. Compound **4** was crystallized from a 1:1 mixture of the Thf-FU and brucine dihydrate in ethanol. Concentration of the mother liquor gave compound **5** as crystals. Elemental analysis showed that compounds **4** and **5** were a 1:1 complex of Thf-FU and brucine. On chromatography of these compounds on a silica gel column, the pure stereoisomer of Thf-FU was released with the *R* isomer (**3a**) from compound **4** and the *S* isomer (**3b**) from compound **5**. The isomers gave satisfactory values in elementary analysis and had the same properties (melting point,  $[\alpha]_D$ ,  $^1\text{H}$  NMR spectra, and  $^{13}\text{C}$  NMR spectra) as those of an authentic sample synthesized by the procedure of Zemlička et al.<sup>5,6</sup> Treatment of compounds **4** and **5** with dilute HCl gave **3a** and **3b**, respectively.

## Scheme I. Synthesis of Thf-FU



## Chart I



The  $^{13}\text{C}$  NMR data on the isomers and the intermediates in their alternative synthetic routes are summarized in Table I. The  $^{13}\text{C}$  signals were mainly assigned on the basis of the work of Jones et al.,<sup>8</sup> except that C-2' and C-3' were reversed as indicated by Mantsch et al.<sup>9</sup> Assignments of the  $^{13}\text{C}$  signals of 5-fluorouracil have been reported by Tarpley et al.<sup>10</sup> These nucleoside analogues all show nearly the same base carbon chemical shifts on each carbon as those of 5-fluorouracil.

The enantiomers of Thf-FU and 1-[3(*H*)-dihydrofuran-2-yl]-5-fluorouracil show identical shifts on each carbon, whereas the anomers of FUdR and 5-fluoro-2'-deoxyuridine-5'-carboxylic acid (FUdC) show considerable differences. It has been reported that a configurational change on C-1' of ribonucleosides produces a considerable shift of the C-2' signal.<sup>11</sup> However, with FUdR and FUdC, there is no chemical shift difference in C-2' of the anomers.

**Biological Activities of the *R* and *S* Isomers of Thf-FU.** Horwitz et al.<sup>6</sup> reported no significant difference in the effects of the isomers of Thf-FU, on DNA synthesis in murine L1210 leukemia cells or growth of cultured human fibroblasts. We examined the effects of the isomers

Table I. <sup>13</sup>C NMR Spectral Data in Me<sub>2</sub>SO-d<sub>6</sub>

	Chemical shift, ppm <sup>a</sup>									Coupling constant, Hz		
	C-2	C-4	C-5	C-6	C-1'	C-2'	C-3'	C-4'	C-5'	J <sub>C<sub>2</sub>-F</sub>	J <sub>C<sub>3</sub>-F</sub>	J <sub>C<sub>5</sub>-F</sub>
FU	150.0	157.9	140.0	126.3						25	230	31
β-FUR <sup>b</sup>	150.0	157.5	140.8	125.5	88.5	73.7	69.5	85.0	60.1	25	232	35
β-FuDR	149.7	157.5	139.5	125.2	84.8	39.6	70.0	87.8	60.9	27	231	34
α-FuDR	150.1	158.2	140.5	126.3	86.3	39.6	70.2	89.9	61.6	27	231	35
β-FUDC <sup>c</sup>	148.7	156.6	140.5	124.4	84.8	38.1	73.8	86.5	173.2	25	230	38
α-FUDC	149.7	157.5	140.8	126.2	86.0	38.1	71.8	86.0	172.0	25	230	33
(R)-(+)-Dhf-FU <sup>d</sup>	149.4	157.2	140.8	124.7	85.0	35.0	100.2	145.3		24	230	35
(S)-(-)-Dhf-FU	149.4	157.2	140.8	124.7	84.8	35.0	100.2	145.2		24	230	35
(R)-(+)-Thf-FU	150.0	158.2	139.9	125.3	86.7	31.3	23.4	69.1		25	230	34
(S)-(-)-Thf-FU	150.0	158.2	139.9	125.3	86.7	31.1	23.4	69.1		25	230	34

<sup>a</sup> Relative to internal Me<sub>4</sub>Si. <sup>b</sup> 5-Fluorouridine. <sup>c</sup> 5-Fluoro-2'-deoxyuridine-5'-carboxylic acid. <sup>d</sup> 1-[3(H)-Dihydrofuran-2-yl]-5-fluorouracil.

Table II. MIC<sup>a</sup> Data on FU, Racemic Thf-FU, and the Thf-FU Isomers

	FU	(R)-(+)-Thf-FU	(S)-(-)-Thf-FU	Thf-FU
<i>Staphylococcus aureus</i> ATCC 6538P	0.7230	120.0	120.0	120.0
<i>Sarcina lutea</i> ATCC 9341	0.7230	60.0	120.0	120.0
<i>Micrococcus flavus</i> ATCC 10240	0.0425	7500	3750	7500
<i>Bacillus subtilis</i> ATCC 6633	46.20	7800	>10 <sup>4</sup>	7800
<i>Escherichia coli</i> NIHJ	186.0	>10 <sup>4</sup>	>10 <sup>4</sup>	>10 <sup>4</sup>
<i>Klebsiella pneumoniae</i> ATCC 10031	6010	>10 <sup>4</sup>	>10 <sup>4</sup>	>10 <sup>4</sup>
<i>Proteus vulgaris</i> IFO 3045	185.0	>10	>10 <sup>4</sup>	>10 <sup>4</sup>
<i>Pseudomonas diminute</i> IAM 1513	>10 <sup>4</sup>	>10 <sup>4</sup>	>10 <sup>4</sup>	>10 <sup>4</sup>

<sup>a</sup> Minimum inhibitory concentration (10<sup>-4</sup> mM).

Table III. Antitumor Effects of Racemic Thf-FU and the Thf-FU Isomers on Experimental Tumors in Rats<sup>a</sup>

	Body wt, g	Tumor wt, g	Inhibn, %
AH-130 Carcinoma			
(R)-(+)-Thf-FU	+40.2	1.82 ± 0.97	61.5
(S)-(-)-Thf-FU	+33.0	1.33 ± 0.67	71.9
Thf-FU	+33.0	2.30 ± 0.80	51.4
Control	+64.1	4.73 ± 1.19	
Yoshida Sarcoma			
(R)-(+)-Thf-FU	+42.0	2.42 ± 0.44	33.7
(S)-(-)-Thf-FU	+39.3	2.17 ± 0.43	40.6
Thf-FU	+33.2	2.23 ± 0.56	38.9
Control	+53.0	3.65 ± 0.60	

<sup>a</sup> Rats (six animals per group) carrying carcinoma were treated daily with a drug (90 mg/kg) for 7 days from one day after tumor implantation. On the tenth day after inoculation, the body weight and tumor weight were measured.

on various bacteria and experimental tumors in rats and their degradation by the microsomal fraction of mouse liver (Tables II-IV). The antibacterial activities of FU, the R and S isomers of Thf-FU, and racemic Thf-FU were tested in defined medium by the plate dilution technique. No significant difference was observed in the minimum inhibitory concentrations (MIC) of the isomers of Thf-FU on the bacteria. In most cases, FU was much more active than Thf-FU. Next, the antitumor activities of the isomers were tested in vivo on AH-130 carcinoma and Yoshida sarcoma in mice (Table III). Again, no difference was observed in the inhibitory effects of the Thf-FU isomers on tumor growth. Fujita suggested that Thf-FU is the

Table IV. Degradations of (R)-(+)- and (S)-(-)-Thf-FU by the Liver Microsomal Fraction<sup>a</sup>

Substrate	NADPH	Released FU, μg/mL
(R)-(+)-Thf-FU	+	10.32
	-	0.51
(S)-(-)-Thf-FU	+	13.16
	-	0.72

<sup>a</sup> The complete reaction mixture (1 mL) contained 0.8 mL of microsomal fraction, 0.1 mL of 50 mM NADPH, and the Thf-FU isomer (200 μg). After incubation at 37 °C for 4 h, the unchanged isomer was removed by extraction with CHCl<sub>3</sub> and the amount of FU was determined by assaying its antibacterial activity against *Staphylococcus aureus* 209P.

masked form of FU and that it is converted to active FU in the liver.<sup>12</sup> Our results and those of Horwitz et al.<sup>6</sup> strongly support this idea. Fujii et al. suggested that activation of Thf-FU in the liver is due to a drug-metabolizing enzyme, which is dependent on NADPH, in liver microsomes.<sup>13</sup> We examined the in vitro degradation of Thf-FU isomers in the microsomal fraction of mouse liver (Table IV). The NADPH-dependent enzyme attacked both isomers equally well releasing FU. The involvement of this type of nonspecific enzyme in degradation of Thf-FU may account for the equal antitumor activities of the Thf-FU isomers in vivo.

## Experimental Section

Bucine dihydrate and silica gel for column chromatography (silica gel 60, 70-230 mesh) were products of Merck (Darmstadt, Germany). NADPH (Lot No. N1630) was a product of Sigma Chemical Co. (St. Louis, Mo.). <sup>1</sup>H NMR spectra were recorded on a Hitachi R-22 spectrometer using Me<sub>4</sub>Si as an internal standard. <sup>13</sup>C NMR spectra were recorded on a Hitachi R-26 spectrometer operating in the FT mode using Me<sub>4</sub>Si as an internal standard. UV spectra were recorded on a Hitachi 124 spectrophotometer. Specific rotations were measured with a Jasco DIP-180 polarimeter. Melting points were determined in a Yanagimoto micro-melting point apparatus and are reported as uncorrected values.

**Synthesis of 1-(Tetrahydro-2-furanyl)-5-fluorouracil (3, Thf-FU).** To a solution of 2,4-bis(trimethylsilyl)-5-fluorouracil<sup>4</sup> (1, 13.7 g, 0.05 mol) in acetonitrile (100 mL), NaI (3.7 g, 0.025 mol) and 2-acetoxytetrahydrofuran<sup>14</sup> (2, 11.0 g, 0.085 mol) were added and the mixture was heated at 60-65 °C for 7 h. Then the solvent was evaporated off and the residue was treated with water (40 mL) and CHCl<sub>3</sub> (100 mL). The CHCl<sub>3</sub> layer was separated and the CHCl<sub>3</sub> was evaporated off. The residue was dissolved in 50% EtOH and refluxed with stirring for 1.5 h. The solvent was evaporated off and the residue was crystallized from EtOH to yield 9.1 g (91%) of Thf-FU (3): mp 166-168 °C; [α]<sub>D</sub><sup>23</sup> 0°, [α]<sub>D</sub><sup>23</sup> 436 0° (c 0.5, CHCl<sub>3</sub>); UV λ<sub>max</sub><sup>PH2</sup> 271 nm (ε 9000); UV λ<sub>max</sub><sup>PH7</sup> 270 nm (ε 8800); UV λ<sub>max</sub><sup>PH12</sup> 270 nm (ε 6900); <sup>1</sup>H NMR (pyridine-d<sub>5</sub>) δ 3.72 and 4.09 [m(2), 2, C<sub>4H</sub>], 6.12 [qd, 1, C<sub>1H</sub>], 7.72

(d, 1, C<sub>6H</sub>, J<sub>H-F</sub> = 7.0 Hz). Anal. (C<sub>8</sub>H<sub>9</sub>FN<sub>2</sub>O<sub>3</sub>) C, H, N.

**Optical Resolution of Thf-FU (3).** (1) **Formation of Diastereoisomers with Brucine.** A saturated solution of the Thf-FU (10 g, 0.05 mol) in EtOH at 70 °C and a saturated solution of brucine dihydrate (21.5 g, 0.05 mol) in EtOH at 70 °C were mixed and the resulting solution stood at room temperature until crystallization was complete. The solid was recrystallized from EtOH at 70 °C to give 12.5 g (42.1%) of a pure diastereoisomer (4): mp 187–188 °C; [α]<sub>D</sub><sup>23</sup> -46.0° (c 0.5, CHCl<sub>3</sub>). Anal. (C<sub>31</sub>H<sub>35</sub>FN<sub>4</sub>O<sub>7</sub>) C, H, N. Concentration of the mother liquor gave a solid, which was recrystallized from EtOH at 70 °C to give 10.7 g (36.0%) of another pure diastereoisomer (5): mp 162.5–164 °C; [α]<sub>D</sub><sup>23</sup> -89.6° (c 0.5, CHCl<sub>3</sub>). Anal. (C<sub>31</sub>H<sub>35</sub>FN<sub>4</sub>O<sub>7</sub>) C, H, N.

(2) **Recovery of R and S Isomers from the Diastereoisomers.** (a) **Treatment with Silica Gel.** The crystals (4, 11.9 g) were dissolved in CHCl<sub>3</sub>-EtOH (5:1, v/v) and applied to a column of silica gel (500 g) equilibrated with the same solvent. Elution with the same solvent gave 3.8 g (95%) of the R-(+) isomer of Thf-FU (3a): mp 174–175.5 °C; [α]<sub>D</sub><sup>23</sup> +70.0°, [α]<sub>D</sub><sup>23</sup><sub>436</sub> +182.0° (c 0.5, CHCl<sub>3</sub>); UV λ<sub>max</sub><sup>pH<sup>12</sup></sup> 271 nm (ε 9100); UV λ<sub>max</sub><sup>pH<sup>7</sup></sup> 270 nm (ε 8800); UV λ<sub>max</sub><sup>pH<sup>12</sup></sup> 270 nm (ε 7000); <sup>1</sup>H NMR (pyridine-d<sub>5</sub>) δ 3.73 and 4.10 [m(2), 2, C<sub>4H</sub>], 6.13 (qd, 1, C<sub>1H</sub>), 7.72 (d, 1, C<sub>6H</sub>, J<sub>H-F</sub> = 7.0 Hz). Anal. (C<sub>8</sub>H<sub>9</sub>FN<sub>2</sub>O<sub>3</sub>) C, H, N. The same treatment of crystals of 5 gave 3.7 g (92.5%) of the S-(-) isomer of Thf-FU (3b): mp 175–177 °C; [α]<sub>D</sub><sup>23</sup> -70.0°, [α]<sub>D</sub><sup>23</sup><sub>436</sub> -187.0° (c 0.5, CHCl<sub>3</sub>); UV λ<sub>max</sub><sup>pH<sup>2</sup></sup> 271 nm (ε 9000); UV λ<sub>max</sub><sup>pH<sup>7</sup></sup> 270 nm (ε 8700); UV λ<sub>max</sub><sup>pH<sup>12</sup></sup> 270 nm (ε 6900); <sup>1</sup>H NMR (pyridine-d<sub>5</sub>) δ 3.73 and 4.10 [m(2), 2, C<sub>4H</sub>], 6.12 (qd, 1, C<sub>1H</sub>), 7.72 (d, 1, C<sub>6H</sub>, J<sub>H-F</sub> = 7.0 Hz). Anal. (C<sub>8</sub>H<sub>9</sub>FN<sub>2</sub>O<sub>3</sub>) C, H, N.

(b) **Treatment with Dilute HCl.** A solution of the crystals (4, 5.9 g) in EtOH (150 mL) was mixed with dilute HCl keeping the pH at 3.5–4.5 at room temperature. The mixture was stirred for 1 h, then the solvent was removed in vacuo, and the residue was treated with water (30 mL)-CHCl<sub>3</sub> (90 mL). The CHCl<sub>3</sub> layer was separated, washed with water, and evaporated to dryness. Recrystallization of the residue from EtOH gave 1.8 g (90.1%) of the R isomer 3a. The same treatment of crystals of 5 gave 1.7 g (85.6%) of the S isomer 3b.

**Antibacterial Activity.** The minimum inhibitory concentrations (MIC) of the present compounds against various bacteria were determined in defined medium<sup>15</sup> (Muller Hinton medium) using the standard plate dilution method<sup>16</sup> with incubation at 37 °C for 24 h.

**Antitumor Activity in Vivo.** Male Donryu rats weighing 120 ± 5 g were inoculated subcutaneously in the inguinal region with 5 × 10<sup>6</sup> cells of AH-130 carcinoma or Yoshida sarcoma per rat. Intraperitoneal administration of a drug (90 mg/kg) was started 24 h later and continued daily for 7 days in test groups of six animals each. On the tenth day after the last inoculation the tumor was weighed and compared with tumors in the control group.

**Degradation of the Thf-FU Isomers by the Microsome Fraction of Mouse Liver.** The microsome fraction was prepared by a standard method from mouse liver and suspended in 10 mM

phosphate buffer (pH 7.4) containing 1.15% KCl at a concentration equivalent to that of a 25% liver homogenate. A mixture of 0.8 mL of this suspension, 0.1 mL of 50 mM NADPH, and the Thf-FU isomer (200 μg) was adjusted to 1 mL with the same buffer-salt mixture. This solution was incubated at 37 °C for 4 h and then extracted with CHCl<sub>3</sub> (10 vol × 2). The aqueous layer was separated and neutralized. FU was determined by assaying the antibacterial activity of the sample against *Staphylococcus aureus* 209P by the thin-layer-cup method.<sup>17</sup>

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## Carbon-13 Nuclear Magnetic Resonance Investigations into the Interactions of Bisulfite with Pyrimidine Nucleosides and Nucleotides

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Carbon-13 NMR is utilized to demonstrate the attack of bisulfite anion on uridine, 5-fluorouridine, and uridine 5'-monophosphate. The attack produces a pair of diastereomeric adducts similar in structure to those seen in the uracil series. Intensity data from the equilibrium system give an estimate for the individual equilibrium constants. Thymidine and thymidine 5'-monophosphate show no evidence of nucleophilic attack by bisulfite. This evidence indicates that bisulfite addition to nucleosides and nucleotides models the enzymatic methylation of uridine by the enzyme thymidylate synthetase better than the uracil bisulfite system.

Nucleophilic attack at carbon-6 of the uracil ring is postulated as an integral part of the mechanism of

pharmacologically important enzymatic reactions such as the conversion of 2'-deoxyuridine 5'-monophosphate to