(S)-2 from D-mannitol and the 94.4% of (R)-2 from L-serine.[11] Moreover, starting with deuterated D-glucose, stereospecifically labeled glycerols are available by simple chemical manipulation from 4c-e; further protection-deprotection technique would allow the preparation of glycerol labeled in the 2- or 3-position. Chirally deuterated glycerols are valuable intermediates for use in the determination of biosynthetic pathways. Their preparation has presented a rather complex synthetic problem as shown from recent examples in the literature.[12]

Experimental Section

The proton (300-MHz) and deuterium (46.1-MHz) spectra have been acquired on a Bruker CXP 300 spectrometer. The ^{2}H NMR experiments were performed with proton broad band decoupling. The ^{13}C spectra (62.9 MHz) were run on a Bruker AC250 spectrometer. In the case of enriched samples, the ^{13}C incorporation was determined using the inverse gated pulse sequence. With this technique the ^{1}H/^{13}C NOE contribution to the ^{13}C signal intensities is suppressed since the broad band proton decoupling is *on* during the acquisition and *off* during the relaxation delay (7 s).

GC/MS analyses were run on a triple-stage quadrupole mass spectrometer Finnigan MAT TSQ 70 equipped with a Varian 3400 gas chromatograph. The elution conditions were as follows: SE 54 capillary column, carrier gas He (1.2 psi), injector temperature 280 °C, transfer line temperature 270 °C, oven temperature programmed as follows: 100 °C, 2 min, 220 °C rate 12 °C/min, final isotherm 220 °C for 15 min. Isotopic enrichments were determined according to standard literature methods.[12] Mass spectra of compounds 4c-e were acquired in profile mode, with a scan range from m/z 95 to 105 and a total scan time of 0.30 s. The fragments monitored in this way correspond to $[C_5H_9O_2]^+$, $[C_5H_8DO_2]^+$, and $[C_5H_7D_2O_2]^+$, respectively.

Preparation of (*R*)-*S*-Benzyl Thioglycerate (2). In an open jar, 20 L of water at 35 °C was mixed with 1.5 kg of commercially available baker's yeast and 0.5 kg of glucose. After 30 min, 20 g of benzyl mercaptan in 20 mL of EtOH was added dropwise and the fermentation left under vigorous stirring at 25 °C for 18 h. Ethyl acetate (2 L) was poured into the reaction flask, and the organic phase was filtered through a Celite pad. The procedure was repeated three times. The organic phase was dried and the solvent evaporated under reduced pressure to yield 22 g of crude oil. Purification on silica gel gave, first, 14 g of unreacted benzyl mercaptan followed by 1.6 g of 2 as an oil which solidified on standing, $[\alpha]^{20}_D$ +69.5° (c 1, MeOH). Anal. Calcd for $C_{10}H_{12}O_3S$: C, 56.58; H, 5.70; S, 15.11. Found: C, 56.90; H, 5.85; S, 15.10. Experiments with labeled glucose were performed using the ^{13}C and ^{2}H containing compound in a 1:20 ratio with natural glucose. In feeding [1-^{2}H]glucose a 1:1 dilution was used. In these cases, a total of 20 g of glucose and 250 g of b.y. were used along with 10 g of benzyl mercaptan. The yield of compound 2 was 200 mg of purified compound from each run.

Determination of the Optical Purity of Benzyl Thioglycerate (2). Preparation of (4*R*)-2,2-Dimethyl-4-(benzyl(thiocarbonyl))-1,3-dioxolane (4) and (4*S*)-2,2-Dimethyl-1,3-dioxolane-4-methanol (3). The diol 2 (1 g, 4.7 mmol) was dissolved in 50 mL of dry acetone, and 0.1 g of *p*-TsOH and 1.2 mL (10 mmol) of dimethoxy propane were added in one portion at 25 °C. The solution was heated at reflux for 3 h, cooled, diluted with ethyl acetate, and washed with 5% aqueous solution of $NaHCO_3$. The organic phase was dried and the solvent evaporated under vacuum to yield an oil which was purified on silica, eluent hexane, so as to obtain (4*R*)-2,2-dimethyl-4-(benzyl(thiocarbonyl))-1,3-dioxolane (4, 1 g, 3.9 mmol, 83%, oil): $[\alpha]^{20}_D$ +41.7 (c 1, MeOH); GC/EI MS (SE 54 capillary column) m/z 252 M$^+$ (1), 234 (0.5), 194 (3), 166 (83), 121 (9), 101 (48), 91 (78), 73 (100), 65 (63). Anal. Calcd for $C_{13}H_{16}O_3S$: C, 61.88; H, 6.39; S, 12.76. Found: C, 61.76; H, 6.42; S, 12.80.

In a three-necked round-bottomed flask with nitrogen inlet, dropping funnel, and condenser was suspended $LiAlH_4$ (0.1 g, 2.6 mmol) in 30 mL of anhydrous ether, and 4 (1 g, 3.9 mmol) diluted in 5 mL of ether was added dropwise. The reaction mixture was stirred at 25 °C for 1 h, then ethyl acetate (5 mL) was added dropwise. The crude reaction solution was poured into ice and extracted with ether. The organic phase was dried and evaporated under reduced pressure so as to obtain crude 3. The above protected glycerol was converted, as reported in the literature,[10] into the (*R*)-(+)-MTPA ester and analyzed. Comparison with GLC mixtures of known composition allowed us to assign the *S* absolute configuration to compound 3 which is composed of more than 98.6% of one enantiomer.

NMR Data. Compound 2: ^{1}H NMR ($CDCl_3$) δ 3.89 (2 H, d, H-3, $J_{2,3}$ = 4.1 Hz), 4.15 (2 H, s, SCH_2), 4.32 (1 H, t, H-2), 4.40 (2 H, broad, 2 OH), 7.23 (5 H, m, C_6H_5); ^{13}C NMR ($CDCl_3$) δ, 32.84 (SCH_2), 64.29 (C-3), 78.16 (C-2), 201.79 (C-1).

Compound 2a (from feeding experiments with [1-^{13}C]glucose): ^{13}C NMR ($CDCl_3$) δ 32.93 (SCH_2, integration 3.74), 64.33 (C-3, 7.53), 78.09 (C-2, 3.66), 201.81 (C-1, 3.85); ca 60% of ^{13}C dilution at C-3.

Compound 2b (from feeding experiments with [6-^{13}C]glucose); ^{13}C NMR ($CDCl_3$) δ 32.79 (SCH_2, integration 12.07), 64.25 (C-3, 30.56), 78.21 (C-2, 11.40), 202.22 (C-1, 11.58); ca. 50% of ^{13}C dilution at C-3.

Compound 4: ^{1}H NMR (acetone-d_6) δ 1.34 (3 H, s, CH_3), 1.50 (3 H, s, CH_3), 4.02 (1 H, dd, H-5, $J_{5,5'}$ = 8.8 Hz, $J_{4,5}$ = 4.0 Hz), 4.10 (2 H, s, SCH_2), 4.28 (1 H, dd, H-5', $J_{4,5'}$ = 7.4 Hz), 7.20-7.35 (5 H, m, C_6H_5).

Compound 4c (from feeding experiments with [6,6-$^2H_2$]-glucose): ^{2}H NMR (acetone) δ 4.03 (^{2}H-5), 4.28 (^{2}H-5') (see Figure 3).

Compound 4d (from feeding experiments with [2-^{2}H]glucose): ^{2}H NMR (acetone) δ 4.03 (^{2}H-5) (see Figure 3).

Compound 4e (from feeding experiments with [1-^{2}H]glucose): ^{2}H NMR (acetone) δ 4.27 (^{2}H-5') (see Figure 3).

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Registry No. 2, 127812-04-8; D-glucose, 50-99-7; benzyl mercaptan, 100-53-8; glyceraldehyde 3-phosphate, 142-10-9; dihydroxyacetone phosphate, 57-04-5.

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Facile Synthesis of 2',5'-Dideoxy-5-fluorouridine by Thymidine Phosphorylase

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Introduction

The antitumor agent 5-fluorouracil (5-FUra, 1) was first synthesized by Heidelberger et al. in 1957.[1] Many attempts have been made since then to prepare derivatives of 5-FUra in the search for compounds with greater selectivity against tumor tissues.[2-4] One of these compounds,

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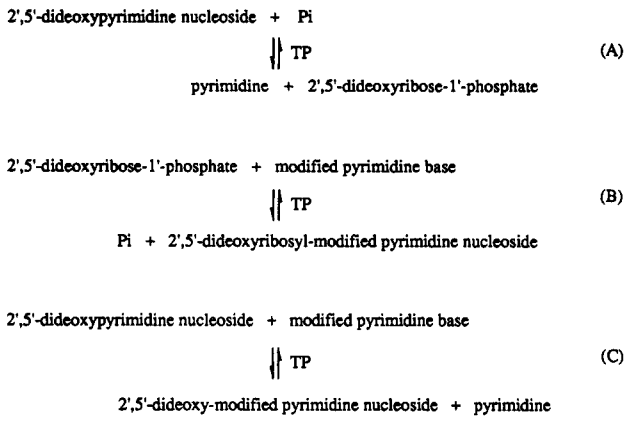
Scheme I. Reaction Catalyzed by Thymidine Phosphorylase (TP) in 2',5'-Dideoxyribosyl Transfer



5'-deoxy-5-fluorouridine (5'-dFU) was found to possess a better therapeutic index than 5-FUra in several murine tumors[5] and selective activity against human B tumor cells in culture.[2,3] We therefore decided to study 2',5'-dideoxy-5-fluorouridine (2',5'-ddFU, 2), another derivative of 5-FUra that could potentially show promising antitumor activity.

2',5'-Dideoxy-5-fluorouridine was originally synthesized by reduction of a 2',5'-dichloro intermediate with tributyltin hydride,[6] with an overall yield of 33% on the basis of uridine. It was also prepared from 2'-deoxy-5-fluorouridine by iodination using methyltriphenoxyphosphonium iodide followed by catalytic reduction (overall yield 46%).[7]

Here, we describe a facile synthesis of 2',5'-ddFU by an enzymatic dideoxyribosyl transfer reaction. Several nucleoside N-transfer reactions via phosphate esters of sugars, such as transribosylation,[8] transdeoxyribosylation,[9] transarabinosylation,[10] transaminoribosylation,[11] and trans-2',3'-dideoxyribosylation,[12,13] by nucleoside phosphorylases have been previously described, but this is the first report of a trans-2',5'-dideoxyribosylation reaction.

Results and Discussion

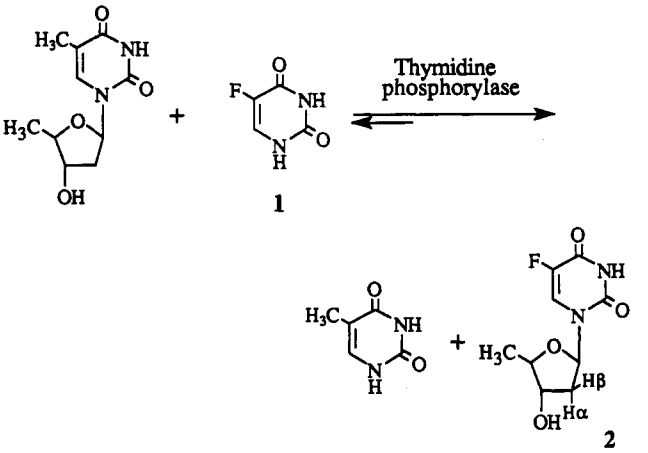
Synthesis of 2',5'-Dideoxy-5-fluorouridine. The deoxyribosyl transfer reaction catalyzed by nucleoside phosphorylases has been shown to involve the formation and utilization of deoxyribose-1'-phosphate.[11] Accordingly, the method for the enzymatic synthesis of 2',5'-dideoxyribonucleoside analogues involves a pair of coupled reactions. The first reaction (Scheme IA) is the phosphorolysis of a dideoxypyrimidine nucleoside catalyzed by thymidine phosphorylase to form 2',5'-dideoxyribose-1'-phosphate. The second reaction (Scheme IB) is the synthesis of the desired product from the 2',5'-dideoxyribose-1-phosphate ester generated in the first reaction and a modified pyrimidine base. The net result is the transfer of a dideoxyribosyl moiety from a pyrimidine nucleoside to a modified pyrimidine base (Scheme IC).

Table I. Antiproliferative Activity against Human Tumor Cells (ED_{50}: μM)[a]

	A-549	MCF-7	HT-29	HeLa[b]	G361[b]
2',5'-ddFU	7×10^{-1}	3×10^{2}	2×10^{0}	4×10^{0}	6×10^{1}
5-FUra	4×10^{-1}	2×10^{1}	1×10^{0}	2×10^{0}	2×10^{0}

[a] The target cells were A-549 (human lung adenocarcinoma), MCF-7 (human breast adenocarcinoma) and HT-29 (human colon adenocarcinoma) cells. These assays were carried out at the Purdue Cell Culture Laboratory, following the microculture tetrazolium assay method. [b] Reference 4.

Quantitative and Qualitative Analysis of Reaction Mixture. The formation of 2',5'-dideoxy-5-fluorouridine was monitored simultaneously by ^{19}F NMR and HPLC for 8 h at room temperature. The identities of all the HPLC peaks were confirmed by comparing the complete UV spectra on the upslope, apex, and downslope of each peak with those of the authentic standard compounds, using a photodiode-array detector. ^{19}F NMR monitoring enables product quantitation directly from the reaction mixture, based on integration of the fluorine peaks from substrate (5-FUra) and product (2',5'-ddFU). Since these compounds have only one fluorine atom, the integration is directly proportional to the amount of 5-FUra left and of 2',5'-ddFU produced. The results obtained by the chromatographic analysis and by the direct ^{19}F NMR spectroscopic method are in good agreement, with 69% conversion.



Biological Activity of 2',5'-Dideoxy-5-fluorouridine. The cytotoxicity of 5-FUra is attributed to its nucleotides, 5-fluorouridine 5'-triphosphate and 5-fluorodeoxyuridine 5'-monophosphate, that competitively inhibit thymidylate synthetase.[14] 2',5'-Dideoxy-5-fluorouridine would not be expected to directly exhibit cytotoxicity due to the absence of the 5'-hydroxyl group. An intracellular activation to 5-FUra by thymidine phosphorylase would be required. It has been demonstrated that phosphorylase activity in human tumor tissue is significantly higher than in normal tissue from the same organ.[15,16] The degree of activation of 2',5'-ddFU to 5-FUra in tumor tissue will thus be higher and therefore the cytotoxicity stronger. Therefore, it is possible that 2',5'-dideoxy-5-fluorouridine will have more selective anticancer activity than 5-FUra itself. However, the previously reported studies indicated that 2',5'-ddFU did not inhibit RNA and DNA synthesis in cultured L1210 murine leukemia cells[6,17] and was inactive against sarcoma

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Table II. Observed ^1H–^1H NOEs (%) and ^1H–^1H Distances (in Å)[a]

protons	distance for S conformer (Å)	distance for N conformer (Å)	av distance for 62% S conformer (Å)	observed NOE (%)	NOE-derived distance (Å)
H6–H2′β	2.4	2.8	2.6	3.7	2.3
H6–H2′α	3.8	4.1	3.9	0.0	
H2′β–H2′α	1.8	1.8	1.8	14.7	(1.8)
H1′–H2′β	3.1	2.9	3.0	0.7	3.0
H1′–H2′α	2.4	2.3	2.4	4.0	2.2
H2′–H2′β	2.4	2.4	2.4	3.1	2.3
H2′–H2′α	2.7	3.0	2.8	2.0	2.5

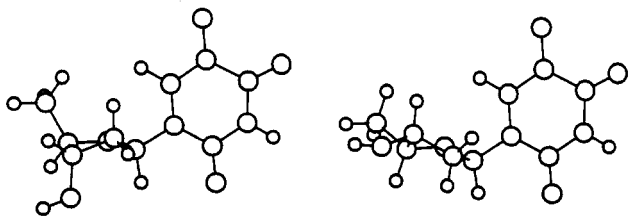
[a] The ^1H–^1H distances were measured from the two CHARMM minimum energy conformations of the dideoxyribose ring in 2′,5′-ddFU, i.e., the *S* and *N* conformers shown in Figure 1. The NOEs were calculated from the volume integrals in the 700-ms mixing time NOESY spectrum. The NOE-derived distances were calculated using the distance between H2′α and H2′β as the reference distance, as described in the text.

180J in mice.[7] Recently, Miwa and Cook et al. reported that thymidine phosphorylase predominates in human tumors, whereas uridine phosphorylase predominates in murine tumors.[4] Thus, 2′,5′-ddFU could not be effectively activated to its nucleotide in a murine tumor. Table I shows that the in vitro antitumor cytotoxicity of 2′,5′-ddFU against human nonsmall cell lung adenocarcinoma (A-549) and human colon carcinoma (HT-29) is comparable to that of 5-FUra. Its in vivo antitumor activity remains to be evaluated.

Conformational Analysis of 2′,5′-Dideoxy-5-fluorouridine. The two most important factors to be considered in discussing the conformation of 2′,5′-ddFU are the rotation about the glycosyl N1–C1′ bond and the sugar pucker. ^1H–^1H 2-D NOE NMR spectroscopy was used for the conformational analysis of this nucleoside in DMSO solution, and these results were then compared with those obtained from molecular modeling studies. The ^1H NMR chemical shift and coupling constant data are summarized in the Experimental Section.

Pyrimidine nucleosides generally exist in the anti conformation about the glycosyl bond,[18] as defined by the torsion angle χ (O1′–C1′–N1–C2 in pyrimidines). Only the presence of bulky substituents at C6 of pyrimidines has been shown to induce a preference for the syn form.[19] In the 2-D NOE spectrum of 2′,5′-ddFU, the proton at C6 shows a strong cross-peak with H2′β only, suggesting that the preferred conformation is anti in DMSO solution. Also, in syn pyrimidine nucleosides, the H2′β NMR chemical shift is displaced downfield by about 0.6 ppm relative to that in anti nucleosides.[20] In this case, the value for $\delta_{H2'\beta}$ is 2.19 ppm, which is very similar to that in thymidine (2.10 ppm)[20] and in 5-iodo-5′-amino-2′,5′-dideoxyuridine (2.19 ppm),[21] both of which are in the anti conformation.

The conformation of the sugar ring (i.e., the dideoxyribose) is not fixed but is well-known to exist in dynamic equilibrium between the *S* (C2′-endo) and *N* (C3′-endo) conformers in solution[22] (Figure 1). The percentage of the 3′-endo conformer in the equilibrium mixture can be estimated with the formula % $N = 100J_{H3'H4'}/(J_{H1'H2'\beta} + J_{H3'H4'})$,[23] which yields a value of 38% *N* ($J_{H1'H2'\beta}$ = 6.7 Hz, $J_{H3'H4'}$ = 4.1 Hz). The values of the coupling constants for 2′,5′-ddFU (and hence the sugar pucker) are very similar to those for thymidine,[20] 5-iodo-5′-amino-2′,5′-dideoxyuridine,[21] and 2′-deoxyuridine.[24]



S *N*

Figure 1. *S* (C2′-endo) and *N* (C3′-endo) conformers of 2′,5′-ddFU from QUANTA. These two minimum energy conformations of the dideoxyribose ring were obtained as described in the text. The distances reported in Table II were measured from these two conformers.

The concept of pseudorotation, as defined by Altona and Sundaralingam,[22] allows an accurate description of ribose and deoxyribose ring conformations in nucleoside derivatives by means of only two parameters, τ_m (the amplitude puckering) and *P* (the phase angle). Both τ_m and *P* are functions of the five defined ring torsion angles, which are constrained in order to examine all the conformations of the ribose around the pseudorotational circle.[25] A less constrained path than pure pseudorotation was used in the present study: only one ring torsion angle, τ_3, was constrained as $\phi' = \tau_3 + 120°$, where ϕ' = O3′–C3′–C2′–C1′.[25,26] ϕ' was rotated at intervals of 10° from 60° to 170°, following the procedures of Orozco et al.[25] The glycosyl torsion angle χ was initially set at 240°, which lies in the anti range; it is similar to that found in other halogenated nucleosides[21] and identical to that in 5-fluoro-2′-deoxyuridine.[27]

After optimization at each stage, two minima were found at values of $\phi' = 80°$ (C2′-endo; *S*) and $\phi' = 147°$ (C3′-endo; *N*). Intramolecular ^1H–^1H distances were then measured for both these conformers. The resulting distances, when averaged according to the observed equilibrium populations in solution (i.e., 62% *S*, calculated earlier), correlated well with the observed ^1H–^1H NOEs in the 700-ms mixing time NOESY spectrum (Table II). In particular, the observed NOE between H6 and H2′β (3.7%) and that between H3′ and H2′α (2.0%) offer evidence that the 2′,5′-dideoxyribose ring in 2′,5′-ddFU mainly exists as the *S* conformer in DMSO solution. The ^1H–^1H internuclear distances calculated from the ratios of the enhancements using the known distance between H2′α and H2′β (1.8 Å) as the reference distance[28] are also shown in Table II.

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Because of the inherent flexibility of this molecule and the r^{-6} dependence of the NOE, however, these NOE-derived time-averaged distances merely represent the minimum allowable internuclear distances. The conformer population with the shortest distance between a particular pair of protons will contribute a disproportionate amount to the internuclear relaxation.[28]

It appears that the conformation of 2′,5′-ddFU is closely related to the known conformations of other pyrimidine 2′-deoxynucleosides.[23,25] This similarity in conformation may partially account for its ready trans-2′,5′-dideoxyribosylation by thymidine phosphorylase despite the fact that it is not a natural substrate for this enzyme.

In summary, we developed an enzymatic method for the facile synthesis of 2′,5′-dideoxy-5-fluorouridine by a 2′,5′-dideoxyribosyl transfer reaction, which provides a new and attractive alternative to existing procedures for the synthesis of 2′,5′-ddFU and other modified nucleosides.

Experimental Section

General. The melting point is uncorrected. ^{1}H NMR and ^{19}F NMR spectra were obtained at ambient temperature at 500 MHz for ^{1}H and 470 MHz for ^{19}F. The DMSO-d_5 peak in the NMR solvent was used as the internal reference for all the ^{1}H NMR spectra and was referenced at 2.49 ppm relative to TMS. The chemical shifts for ^{19}F NMR were measured relative to external trifluoroacetic acid. The 2-D NOE spectra were obtained on approximately 5 mg of material and were recorded in the phase-sensitive absorption mode using the hypercomplex method.[29] The sweep width was set to 4000 Hz in both dimensions; 300–350 increments were acquired in the t_1 dimension with eight transients for each FID, and 2048 points were collected in the t_2 dimension. The recycle time was 5 s, and the mixing times were 500 and 700 ms. For data processing, zero-filling to 2K by 2K was carried out, and base-line corrections and sine-bell windows were used in both dimensions. The fast atom bombardment (FAB) mass spectral data were obtained in DTT/DTE (dithiothreitol:dithioerythritol = 3:1) as the sample matrix.

Analytical HPLC utilized an Alltech Econosphere RP-C18 (150 × 4.6 mm, 3 μm) column, eluted at 1 mL/min with a linear gradient of 4–10% CH_3CN in 50 mM $HCOONH_4$, for 5 min. Preparative HPLC was done on an Alltech Econosphere RP-C18 (250 × 22.5 mm, 10 μm) column, eluted at 5 mL/min with a mobile phase of 15% CH_3CN in water isocratically.

Molecular modeling studies on 2′,5′-ddFU were carried out using the program QUANTA (v 3.0, Polygen Corp.). Energy minimizations were done with the standard CHARMM minimizers (steepest descents and adopted-basis Newton–Raphson methods) within QUANTA. The standard force-field parameter set supplied with the program was employed. A distance-dependent dielectric constant was used to mimic solvent effects, since the solvent was not explicitly included.

Enzymatic Synthesis of 2′,5′-Dideoxy-5-fluorouridine. A solution of 39 mg (0.3 mmol) of 5-fluorouracil (Sigma Chemical Co.) and 22.6 mg (0.1 mmol) of 2′,5′-dideoxythymidine (Sigma Chemical Co.) in 5 mL of 5 mM sodium phosphate buffer, pH 7.4 (D_2O:H_2O = 3:1) was prepared. To this solution was added 23.8 units of thymidine phosphorylase from *E. coli* (Sigma Chemical Co.). A part of this solution (0.7 mL) was taken in an NMR tube for ^{19}F NMR monitoring at 20 °C for 8 h. The rest was analyzed simultaneously by HPLC. After 8 h of reaction at room temperature, the reaction mixture was filtered through an Amicon filter to remove the enzyme and to stop the reaction. The filtrate was loaded on a preparative HPLC column by consecutive injections of 1.0 mL each. The fractions corresponding to 2′,5′-dideoxy-5-fluorouridine were collected, combined, and finally freeze-dried, yielding 13.5 mg (0.059 mmol, 59%) of 2′,5′-dideoxy-5-fluorouridine as a white powder: mp 168 °C (lit.[7] mp 171–173 °C); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.825 (H_6, 1 H, $^3J_{HF}$ = 7.0 Hz), 6.057 ($H_{1'}$, td, 1 H, $^3J_{1'2'\alpha}$ = $^3J_{1'2'\beta}$ = 6.7 Hz, $^5J_{1'5F}$ = 1.8 Hz), 3.943 ($H_{3'}$, dt, 1 H, $^3J_{3'2'\beta}$ = 6.7 Hz, $^3J_{3'2'\alpha}$ = $^3J_{3'4'}$ = 4.1 Hz), 3.765 ($H_{4'}$, qd, 1 H, $^3J_{4'5'}$ = 6.4 Hz, $^3J_{4'3'}$ = 4.1 Hz), 2.191 ($H_{2'\beta}$, dt, 1 H, $^2J_{2'\beta2'\alpha}$ = 13.5 Hz, $^3J_{2'\beta1'}$ = $^3J_{2'\beta3'}$ = 6.7 Hz), 2.039 ($H_{2'\alpha}$, ddd, 1 H, $^2J_{2'\alpha2'\beta}$ = 13.5 Hz, $^3J_{2'\alpha1'}$ = 6.7, $^3J_{2'\alpha3'}$ = 4.1 Hz), 1.233 ($H_{5'}$, d, 3 H, $^3J_{5'4'}$ = 6.4 Hz); ^{19}F NMR (470 MHz, D_2O:H_2O = 3:1) δ 89.847 (d, 1 F, $^3J_{5FH}$ = 7.0 Hz); FAB–MS *m/z* (relative intensity) 231.0766 (231.0781, calcd for $C_9H_{11}N_2O_4F$; MH^+, 49) and 131 (BH^+, 100).

Cytotoxicity Assay. The cytotoxicity assay using the human solid tumor cell line systems, following the microculture tetrazolium assay method,[30] was performed at the Purdue Cell Culture Laboratory. The target cells were HT-29 (human colon carcinoma), A-549 (human lung adenocarcinoma), and MCF-7 (human breast adenocarcinoma) cells.

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Registry No. 1, 51-21-8; 2, 61168-97-6; 5′-deoxythymidine, 3458-14-8; thymine, 65-71-4; thymidine phosphorylase, 9030-23-3.

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Chirality as a Probe in β-Keto Ester Tautomerism[1]

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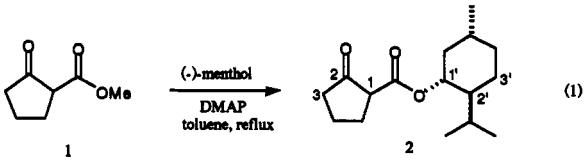
Over the past two decades, significant advances in the development of asymmetric synthetic methods have been achieved.[2] A direct consequence of these advances is a substantial increase in our understanding of reaction pathways at a very fundamental level. In this paper, the use of chirality as a probe to study equilibria in chiral β-keto esters is detailed. This analysis provides a direct method to study keto–enol tautomerism[3] utilizing NMR spectroscopy and details a ramification of having remote chiral centers in readily enolizable systems.

In a related investigation, we required a quantity of chiral keto ester **2**. As outlined in eq 1, (−)-menthyl 2-



1 2 (1)

oxocyclopentanecarboxylate (**2**) was prepared in 55% yield from **1**, following the method of Taber.[4,5] While an unequal mixture of epimers at C1 was expected, we were intrigued to find that the ^{1}H and ^{13}C NMR spectra indicated a *single diastereomer* of **2** was present in $CDCl_3$ solution at 20 °C (enol undetected by NMR). When **2** was analyzed in C_6D_6 at different times following dissolution, the emergence of a second product (an epimer at C1) was observed. Figure 1 is a composite of ^{1}H NMR spectra of **2** (in C_6D_6) in the region of 1.8 to 5 ppm,[6] taken over 5 h. The dd at 2.78 and the ddd at 4.90 ppm were identified as the protons on C1 (α-enolizable H) and C1′, respectively. Note that a second overlapping dd appears at 2.78 and that

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