fragment stability changes associated with bond formation. The transfer energies for the N_2 fragments are found to be negative while the transfer energies of the X^+ cations are positive. Bonding in diazonium ions is achieved because the cations are stabilized more than the N_2 groups are destabilized and not because both fragments would benefit energetically from their association. The comparison of the heterosubstituted diazonium ions (X = F, OH, NH_2) with the RNN^+ ions shows that the XN bond stabilities largely depend on the charge transfer from N_2 onto X^+. Increasing X electronegativity leads to larger charge transfer and—since the electron affinity is larger than the ionization energy of N_2—to larger bond dissociation energies. Among the $(RNN)^+$ ions, the relation is more complicated and *not* solely determined by charge transfer.

The N_2 transfer energies are correlated with the N_2 charges in a way that is well approximated by a linear function. This finding supports the generally made assumption that fragment stabilities can be discussed by fragment charges. More importantly, the slope of the linear correlation between the N_2 charges and the transfer energies ΔE_1 defines an *average ionization energy of N_2 in molecules*. It is found that its value is roughly equal to the ionization energy of free N_2. This finding suggests that the (de)stabilization of an electron-depleted fragment in a molecule can be estimated via the determination of *its charge in the molecule* and with the knowledge of its ionization potential. Similarly, it might also be possible to relate the stabilization of an electron-enriched fragment in a molecule with its charge via the electron affinity of the free fragment. A method was outlined that allows one to test this hypothesis. Future research will have to examine the generality of these findings and it is hoped that the present work might stipulate such investigations.

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Registry No. CPD, 129523-44-0; DACPD, 129551-42-4; *cis*-CVD, 137946-13-5; *trans*-CVD, 137946-14-6; FNN^+, 33687-51-3; $HONN^+$, 76412-54-9; H_2NNN^+, 43422-90-8; H_3CNN^+, 20404-06-2; $H_5C_2NN^+$, 84027-64-5; $H_3C_2NN^+$, 64709-62-2; HC_2NN^+, 108561-02-0; $PhNN^+$, 2684-02-8; F^+, 14701-13-4; HO^+, 12259-29-9; H_2N^+, 15194-15-7; H_3C^+, 14531-53-4; $H_5C_2^+$, 14936-94-8; $H_3C_2^+$, 14604-48-9; HC_2^+, 16456-59-0; Ph^+, 17333-73-2; N_2, 7727-37-9; 1,2-propadiene-1,3-diylium, 99818-38-9; 1,3-diamino-1,2-propadiene-1,3-diylium, 137964-66-0; 1-carboxyethenylium, 137946-15-7.

Notes

On the Origin of the C_3 Framework of Yeast-Generated (*R*)-*S*-Benzyl Thioglycerate

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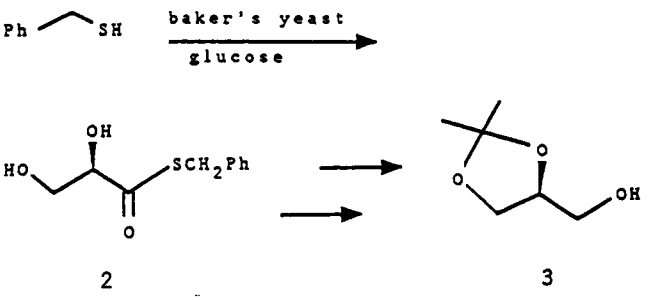
While there is an established knowledge on the predictable behavior of baker's yeast as a stereoselective reducing agent,[1] numerous reports are dedicated to "new capacities" attributed to this microorganism, which are observed when unnatural substrates are treated with fermenting yeast.[2]

In this context, we recently reported[3] on the formation of *S*-benzyl thioglycerate from baker's yeast (b.y.) incubation of benzyl mercaptan in the presence of glucose 1 as summarized in Scheme I. The product 2 of high optical purity was assigned the *R* absolute configuration, as proved by its conversion into isopropylideneglycerol (3) of *S* configuration and ≥98% ee. Starting from 20 g of $PhCH_2SH$, 1.6 g of thioglycerate 2 was obtained, 14 g of

Scheme I



2 3

the mercaptan being recovered unreacted. Despite the modest chemical yield, we considered of interest the obtainment of a chiral compound in enantiomerically pure form which is available through a procedure similar to an extractive process from a natural source. In fact, the capture of a biosynthetic intermediate with a nonnatural reactant is of potential interest when the educt, as in the present case, is a highly functionalized chiral synthon complementary to those offered by nature as starting materials for the synthesis of chiral substances.[4] Attempts to extend the reaction leading to the thioglycerate 2 to other thiols gave different products,[5] thus suggesting that benzyl mercaptan is unique in its ability to be accepted in some metabolic transformation in yeast. We conceived that the product is obtained as the consequence of the action of benzyl mercaptan as a nucleophile in the glycolytic pathway of glucose. To obtain experimental support

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(2) See, for instance: Gibbs, D. E.; Barnes, D. *Tetrahedron Lett.* **1990**, *31*, 5555. Rama Rao, K.; Bhanumathi, N.; Sattur, P. B. *Tetrahedron Lett.* **1990**, *31*, 3201. Fadnavis, N. W.; Deshpande, A.; Chauhan, S.; Bhalerao, U. T. *J. Chem. Soc., Chem. Commun.* **1990**, 1548. Fronza, G.; Fuganti, C.; Grasselli, P.; Poli, G.; Servi, S. *J. Org. Chem.* **1988**, *53*, 6153. Buist, P. H.; Dallmann, H. G. *Tetrahedron Lett.* **1988**, *29*, 285.
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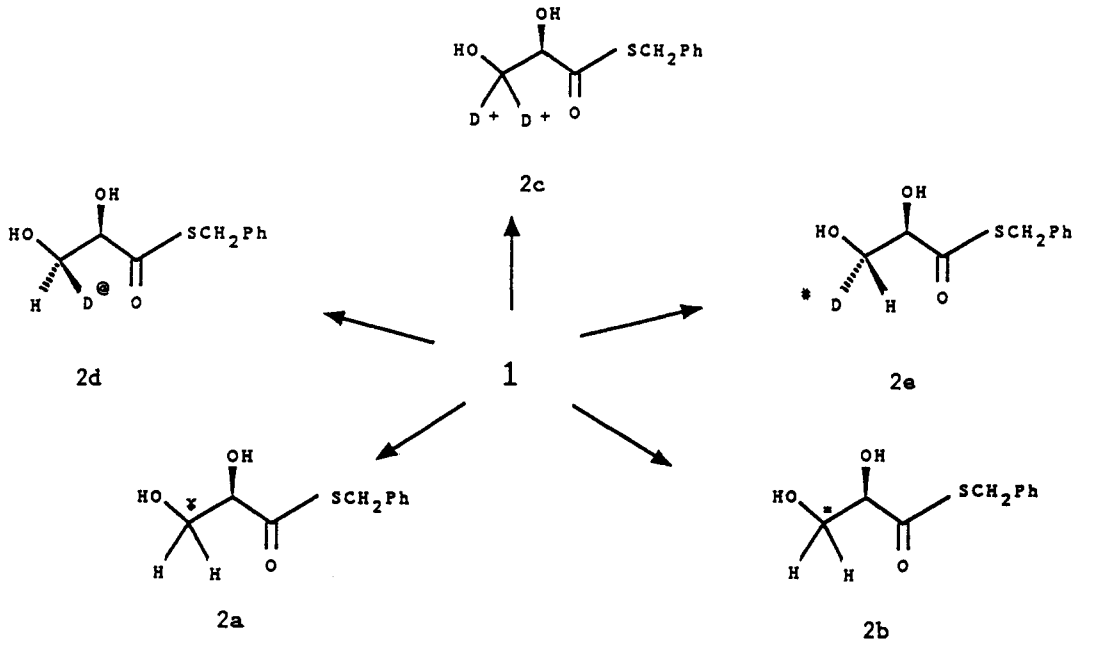
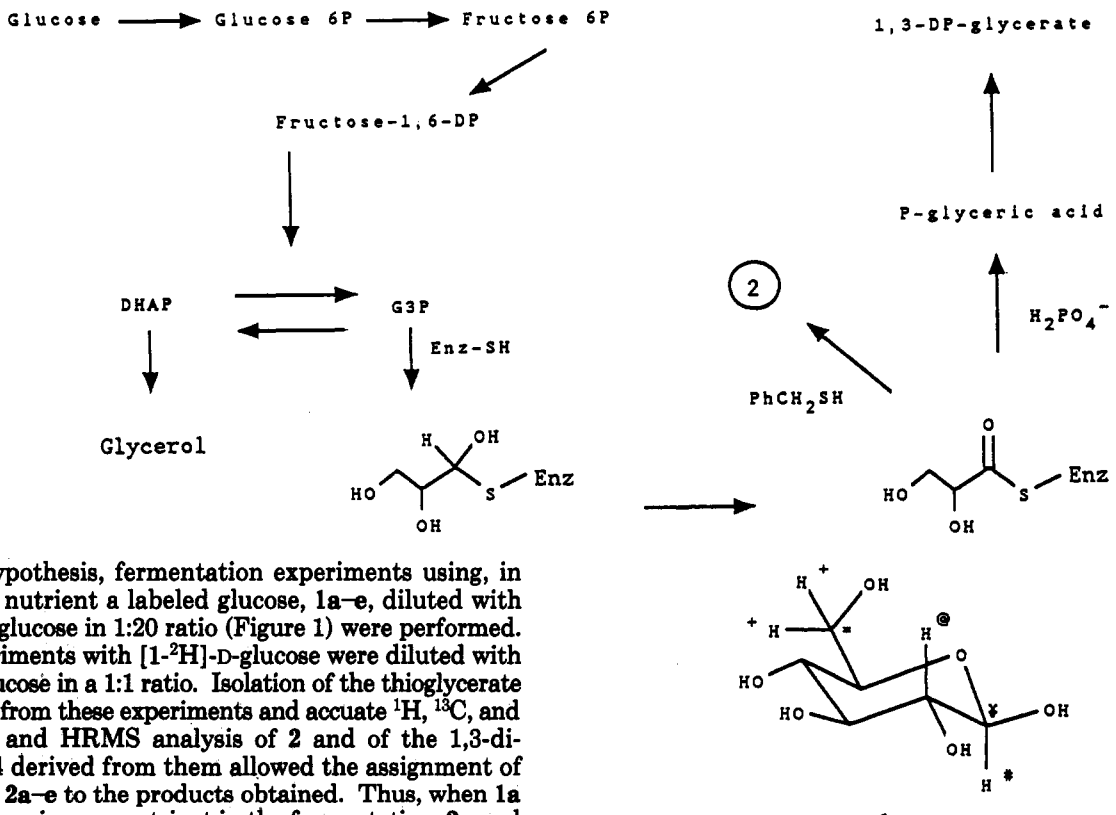
Scheme II



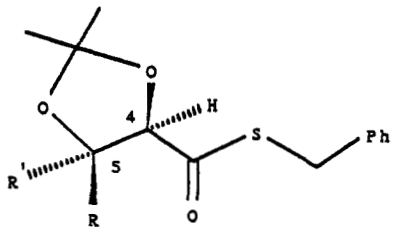
Scheme III



for the hypothesis, fermentation experiments using, in turn, as a nutrient a labeled glucose, **1a–e**, diluted with natural D-glucose in 1:20 ratio (Figure 1) were performed. The experiments with [1-^{2}H]-D-glucose were diluted with natural glucose in a 1:1 ratio. Isolation of the thioglycerate derivative from these experiments and accuate ^{1}H, ^{13}C, and ^{2}H NMR and HRMS analysis of **2** and of the 1,3-dioxolanes **4** derived from them allowed the assignment of structures **2a–e** to the products obtained. Thus, when **1a** and **1b** were given as nutrient in the fermentation, **2a** and **2b** were obtained, with the ^{13}C exclusively in position 3 of **2**. However, ^{13}C NMR studies showed that in **2** the ^{13}C label was diluted by ca. 60% and 50% with respect to the fed materials. When 2-deuterioglucose (**1d**) was used, the thioglycerate obtained, **2d**, contained about 20% of the label in position 3 (expected 25%). With 1-deuterioglucose (**1e**) as a precursor, **2e** was obtained, also containing deuterium in position 3 with a dilution of 75% (expected 50%) but with an absolute configuration at C3 opposite the one observed in **2d**. As summarized in Scheme II, this demonstrates that the C_3 unit found in *S*-benzyl thioglycerate **2** derives from D-glucose through insertion of benzyl mercaptan at some stage of the conventional glycolytic

Figure 1. Key: **a** = 1-^{13}C, **b** = 6-^{13}C, **c** = 6-dideuterio, **d** = 2-deuterio, **e** = 1-deuterio.

pathway. Part of the pathway is summarized in Scheme III. From the mechanism of glycolysis it is evident that all of the carbon atoms present in D-glucose are divided between the two 3C units dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (G3P), both possible precursors of the glycerate **2**: this would allow for at least 50% dilution of label. In the deuterium labeling experiments with [2-^{2}H]-D-glucose (**1d**) it can be expected that, since the glucose-6P ⇆ fructose-6P isomerization occurs through a well-established mechanism,[6] one hy-



4 R = R' = H

4c R = R' = D **4e** R = H, R' = D

4d R = D, R' = H

Figure 2.

drogen atom will be transferred, stereospecifically, from glucose C2 to fructose C1. Half of the label will be lost in this step, and a further 50% in the successive degradative step thus allowing a maximum deuterium recovery of 25%. From [1-^{2}H]-D-glucose (**1e**) the thioglycerate with deuterium in position 3 should be obtained with a maximum of 50% label, from the same degradative scheme to DHAP and G3P. The stereochemistry of C3 in **2d** and **2e** was determined from ^{1}H NMR studies of the derivatives **4** obtained from them. Compound **4** shows two vicinal coupling constants $J(4,5)$ and $J(4,5')$ by 4.0 and 7.4 Hz, respectively, where the value of ~7 Hz is characteristic of cis-oriented protons for the dioxolane ring.[7] We have confirmed this assignment performing a series of NOE experiments involving selective irradiation of the two geminal methyl groups. Irradiation of the high-field methyl (1.34 ppm) induces enhancements of H-4 (3.0%) and H-5' (2.8%), indicating that these two protons must lie on the same side of the ring. The saturation of the low-field methyl group (1.50 ppm) produces only a very small enhancement (0.3%) of the signal for H-5. Thus, the two geminal protons H-5 (4.02 ppm) and H-5' (4.27 ppm) can be assigned the *pro-R* and *pro-S* configuration, respectively. These observations are in agreement with the fact that the configuration in **2e** must be 3*S* as found in the starting glucose. The 3*R* configuration observed in **2d** is determined by the deuterium migration during glucose to fructose isomerization.[8] From these results it appears that the two compounds **2e** and **2d** are epimers at C3 and that their configuration is the one depicted in Scheme II. Comparison of ^{2}H NMR spectra of **4d** and **4e** with the one of **4c** obtained from the thioglycerate **2c** as the only deuterated product when 6-dideuterioglucose was used as a precursor showed that each of the two signals due to the two deuterium atoms is coincident with one of the signals from **4d** or **4e**, thus confirming that the two products must be two epimers and that no scrambling is occurring during the degradation from glucose. Figure 3 shows the deuterium spectra of the three compounds **4c–e**.

Not all of the above feeding experiments show a percentage of label dilution consistent with the expected

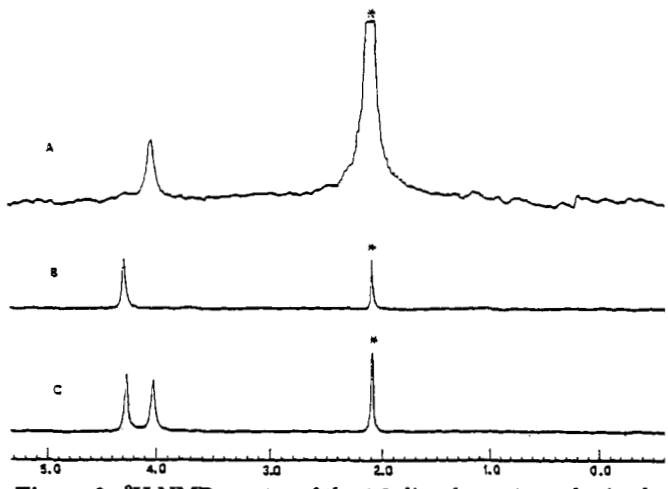


Figure 3. ^{2}H NMR spectra of the 1,3-dioxolanes **4c–e** obtained from different feeding experiments using as precursors (A) [2-^{2}H]glucose (yielding (5*R*)-[^{2}H]-**4d**), (B) [1-^{2}H]glucose (yielding (5*S*)-[^{2}H]-**4e**), (C) [6,6-$^2H_2$]glucose (yielding 5,5'-[$^2H_2$]-**4c**). Asterisks denote the natural abundance deuterium signals of the solvent (acetone).

values. Thus, while levels of dilution are as expected for **2b** and in reasonable agreement for **2d**, **2a**, **2c**, and **2e** show a measurably lower label than expected. This can be tentatively explained by taking into account the fact that the K_{eq} for DHAP ⇆ G3P is such that the equilibrium is shifted toward DHAP. However, G3P is the more reactive intermediate and can undergo further degradation to ethanol through pyruvate. Similarly, DHAP can undergo reduction to glycerol.[9] In summary, the labels located in glucose in the incorporation into **2** are diluted by 50% when they are in position 6 and at higher extent when in 1 and 2. However, whereas the label of [1-^{13}C]glucose is diluted by 60%, those in [1-^{2}H]- and [2-^{2}H]glucose are diluted by 75%.[10] Since not all of the parameters affecting product formation can be controlled in complex enzymatic systems like b.y., differences in the composition of the products mixture and, thus, incorporation of glucose from one run to another are acceptable. Moreover, qualitative identification of labels incorporated into isolated fragments is usually considered sufficient to support a metabolic pathway. A further example of the involvement of this equilibrium (DHAP ⇆ G3P) in the formation of **2** is seen in the fact that, when dihydroxyacetone (DHA) is used as unique carbon source in the fermentation instead of glucose, the yield of benzylthioglycerate is three times higher. This suggests a more prompt use of DHA by the enzymic system. Insertion of benzyl mercaptan into this metabolic pathway can be envisaged as occurring in the step in which G3P is transformed into 1,3-DP-glycerate. In fact, oxidation of G3P is known to proceed via the intermediate formation of an activated thioester which is displaced by a phosphate unit or, in our case, by benzyl mercaptan (Figure 2). Apart from the interest in the transformation catalyzed by the b.y. enzymic system, from a synthetic point of view it is noteworthy that (*S*)-**2** can be obtained with an ee which is intermediate between the 99.4% of

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(10) One of the reviewers suggests to take into account as an explanation for the observed lower presence of deuterium the participation of glucose-6-phosphate and mannose-6-phosphate isomerase whose action might labilize deuterium from position 2 and 1 of hexose-6-phosphate before it is drained off into triosephosphates by aldolase. He also suggests that the fact that deuterium from 6-dideuterioglucose labels both methylene hydrogens of **2c** in an equal extent indicates that the resynthesis of hexosophosphates from triosephosphates by aldolase does not play a significant role under the experimental conditions.

(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).

Acknowledgment. We thank Dr. G. Allegrone for some MS measurements and Piano Finalizzato CNR Chimica Fine e Secondaria 2 for financial support.

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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