because desolvation of deamino-T_4 costs about 10 kcal/mol less free energy than desolvation of L- or D-T_4.

Although TBPA is not a true drug receptor, acting only as a transport protein for T_4, the T_4-TBPA complex provides a simple working model of a drug-receptor interaction. The successful calculation of the experimental binding affinities to TBPA for analogues **1-4** illustrates a general method that may ultimately be useful for the design of new analogues and the prediction of their binding affinities.

Note Added in Proof. We have further refined the parameters and charges presented in Table III (S. J. Weines et al., to be submitted) and these are available from the authors on request.

Acknowledgment. We thank Professor R. Langridge for the use of the UCSF computer graphics laboratory, supported by Grant RR-1081. This work was supported by NIH Grants AM-17576 (E.C.J.), GM-29072 and CA-25644 (P.A.K.), the American Foundation for Pharmaceutical Education (J. M. Blaney), and the Medical Research Council of Great Britain. S.J.O. is a Mr. and Mrs. John Jaffé Donation Research Fellow of the Royal Society.

Registry No. 1, 51-48-9; **2**, 83208-10-0; **3**, 67-30-1; **4**, 83208-11-1.

Model Chemistry for a Covalent Mechanism of Action of Orotidine 5′-Phosphate Decarboxylase

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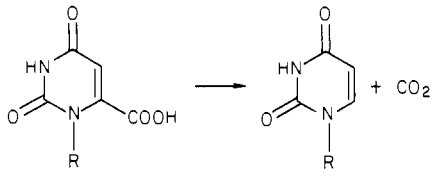
Contribution from the Department of Chemistry, Northwestern University, Evanston, Illinois 60201. *Received February 26, 1982*

Abstract: Orotidine 5′-phosphate decarboxylase (ODase) catalyzes the conversion of orotidylate to uridylate, the last step in the de novo biosynthesis of pyrimidine nucleotides. Model reactions are described that support a covalent catalytic mechanism for this enzyme in which, following protonation of the carboxyl group of orotidylic acid, an active-site nucleophile undergoes a Michael addition to the C-5 position. This covalent complex breaks down via an acid-base-catalyzed decarboxylative elimination reaction to give uridylate and CO_2 (Scheme II). The enzyme mechanism is modeled in two parts, the Michael addition reaction and the decarboxylative elimination. Bisulfite is shown to undergo a Michael addition to *N,N*-dimethylorotaldehyde and at room temperature to *N,N*-dimethyl-6-acetyluracil, both models for the activated form of orotidylate, the substrate for ODase (**6** → **7**). In a separate study, (±)-1,3-dimethyl-*r*-5-(methylthio)-5-methyl-*trans*-6-carboxyl-5,6-dihydrouracil (**15**) was prepared as a model for the ODase-orotidylate covalent complex. Activation by methylation of the sulfide (as a model for enzyme-catalyzed protonation) leads to instantaneous decarboxylative elimination at room temperature. When the corresponding ester (**9c**) is methylated, the dimethylsulfonium salt (**16b**) can be isolated, which upon ester hydrolysis gives the decarboxylative elimination product. These model studies support the Michael addition-decarboxylative elimination mechanism in favor of a noncovalent mechanism previously reported (Beak, P.; Siegel, B. *J. Am. Chem. Soc.* **1976**, *98*, 3601).

The final step in the de novo biosynthesis of pyrimidine nucleotides is the decarboxylation of orotidylic acid (**1**) to uridylic

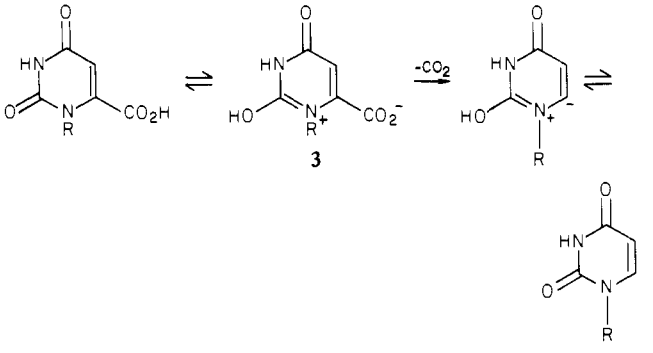


1 **2**

R = ribose 5′-phosphate

acid (**2**), a reaction catalyzed by orotidine 5′-phosphate decarboxylase (ODase; E. C. 4.1.1.23).[1] Uridylic acid can be metabolized further to cytidine and thymidine nucleotides and thus is a precursor to both RNA and DNA.[1] Malfunctioning of ODase has been shown to be responsible for the metabolic disease known as hereditary orotic aciduria.[2] The mechanism of action of this enzyme has not been delineated, but model studies[3] and enzyme inhibition studies[4] previously reported were interpreted to suggest that decarboxylation may occur from a noncovalent enzyme-stabilized zwitterion of the substrate (**3**, Scheme I) and that inductive stabilization of the resultant sp^2 carbanion is provided by the adjacent quaternary ammonium atom. Most of

Scheme I. Noncovalent Mechanism for ODase[3] (R = Ribose 5′-Phosphate)



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the model reactions,[3] however, were carried out at very high temperatures (180-220 °C), and although the mechanism for thermal decarboxylation of 1,3-dimethylorotic acid may proceed as was suggested, it did not seem likely to us to be an efficient enzyme-catalyzed mechanism. An alternative mechanism is the

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‡ Present address: Department of Pharmacology, The University of Michigan, Ann Arbor, MI 48109.

(1) Hartman, S. C. In "Metabolic Pathways", 3rd ed.; Greenberg, D. M., Ed.; Academic Press: 1970; Vol. IV.

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(3) Beak, P.; Siegel, B. *J. Am. Chem. Soc.* **1976**, *98*, 3601.

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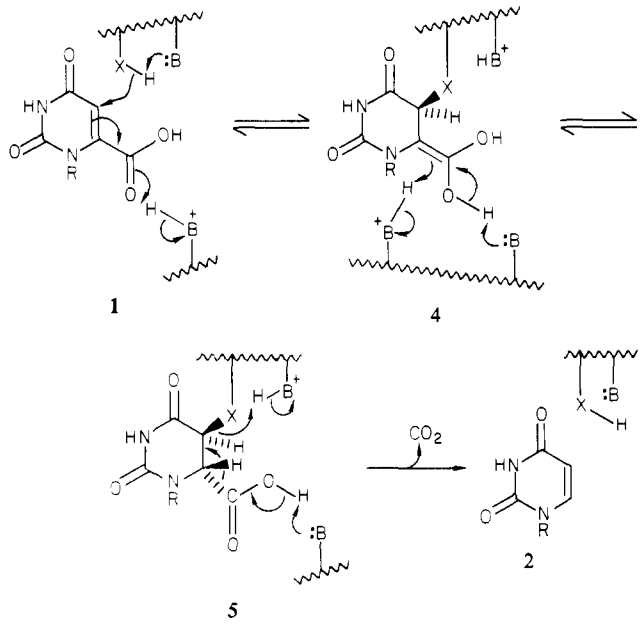
Scheme II. Covalent Catalytic Mechanism for ODase (R = Ribose 5'-Phosphate)

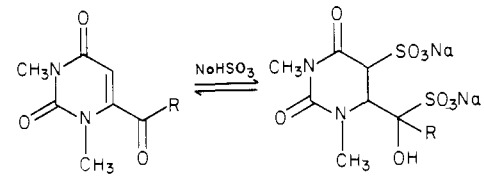


covalent catalytic pathway shown in Scheme II; variations of this mechanism have been hypothesized by others.[5] The mechanism involves a Michael addition of an active site nucleophile (X) to the α,β-unsaturated carboxylic acid, which is strongly activated by enzyme-catalyzed proton donation (**1** → **5**) followed by decarboxylative elimination of the trans intermediate (**5** → **2**). The mechanism in Scheme II is appealing for two major reasons. First, there is one covalent catalytic and six or more acid–base catalytic steps involved in this mechanism, all of which can be controlled and utilized for rate acceleration by the enzyme. Second, this covalent addition–elimination mechanism has precedents in other enzyme-catalyzed reactions, in particular, the conversion of deoxyuridylate to deoxythymidylate catalyzed by thymidylate synthetase.[6] The presence of an active site sulfhydryl has been demonstrated for yeast ODase,[7] and this may be involved in the covalent catalysis. In this paper we report model studies to support the Michael addition–decarboxylate elimination mechanism depicted in Scheme II.

Results and Discussion

Nucleophilic attack at the C-5 position of orotic acid analogues is precedented in the literaure.[8,9] Thus, cyanide ion was shown to undergo a Michael addition to the C-5 position of 1,3-dimethyl-6-cyanouracil[8a,b] and to 6-cyanouridines.[8c] Ureas condense with 5-haloorotic acid analogues, presumably via an intramolecular Michael addition to the C-5 position of the corresponding imide.[9] The most straightforward model study for the covalent catalytic mechanism of ODase would have been to show that nucleophiles add to the C-5 position of 1,3-dimethylorotate esters then, upon hydrolysis of the esters, decarboxylative elimination to 1,3-dimethyluracil occurs. Unfortunately, we were unable to obtain products of a Michael addition with a variety of nucleophiles[10] in both hydroxylic (EtOH, MeOH) and nonhydroxylic (DMF, THF, benzene) solvents to various esters (Me, Et, *t*-Bu) of 1,3-dimethylorotic acid. It is presumed that the additions occurred but that the equilibrium strongly favored starting materials. This may suggest that the activation of the carboxyl group by enzyme-catalyzed protonation (**1** → **4** in Scheme II) is important. Consequently, appropriate modifications in the models were incorporated that, we believe, were necessary because of the constraints involved in solution chemistry in contrast to the specificity of enzyme-catalyzed chemistry. The reactivities of the model compounds ultimately employed are closer in nature to the reactivities of the proposed intermediates depicted in Scheme II. The major modification was that the two principal half-reactions of the proposed enzyme-catalyzed mechanism (the Michael addition and decarboxylative elimination) had to be modeled separately.

Since the proposed ODase-catalyzed reaction (Scheme II) involved nucleophilic addition to a highly activated α,β-unsaturated carboxylic acid, it seemed reasonable to us that the corresponding α,β-unsaturated aldehyde or ketone would be more relevant models than an ester for the activated carboxylic acid. Oxidation of 1,3,6-trimethyluracil with SeO_2[11] afforded the desired 1,3-dimethylorotaldehyde (**6a**), purified as its diethyl acetal. When **6a** was treated with a large excess (up to 20-fold) of various nucleophiles,[10] only 1,2-adducts were observed by NMR spectroscopy. When 1 equiv of aqueous sodium bisulfite was used, a 1,2-bisulfite adduct formed immediately, which upon heating on a steam bath overnight, was equilibrated to a 1:1 mixture of **6a** and the bis-bisulfite adduct **7a**. Equilibration of **6a** (0.5 M) with



6a, R = H
b, R = CH_3

7a, R = H
b, R = CH_3

6 equiv of sodium bisulfite and 1.5 equiv of sodium sulfite[12] resulted in the formation of **7a** in a 95% yield by NMR spectroscopy. Since nucleophiles do not add to unactivated double bonds under neutral conditions, it must be assumed that **7a** is derived from an initial Michael addition to the C-5 position of **6a** followed by a 1,2-addition rather than vice versa. As has been reported by many others for bisulfite additions to pyrimidines[13] and for cysteine additions to α,β-unsaturated aldehydes,[14] we were unable to isolate the bisulfite addition product (**7a**) in pure form. All attempts to separate it from the excess bisulfite induced reversion to **6a**. Thus, chromatography on silica gel, cellulose, and paper produced only **6a**, as did treatment with acid or $BaCl_2$. Attempts at oxidation and reduction of **7a** also were fruitless; hydrogen peroxide gave decomposition to unknown substances, and sodium borohydride gave reduced **6a**. Evidence for the structure of **7a** is based on NMR spectra analysis (and the fact that the compound readily reverts to **6a**). As shown in Figure 1, treatment of **6a** (A, actually almost completely the hydrate)

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(7) Creasey, W. A.; Handschumacher, R. E. *J. Biol. Chem.* **1961**, *236*, 2058.

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(9) Kodama, Y.; Saikawa, I.; Kodama, T.; Hori, T.; Imaizumi, H.; Maeda, T. Japanese Patent 75 101 391; *Chem. Abstr.* **1976**, *84*, 74296g.

(10) The following nucleophiles were used with each orotidylate model: cysteine, CH_3COSH, $HSCH_2CO_2H$, PhSH, $PhCH_2SH$, KOCN, NaN_3, KSCN.

(11) (a) Zee-Cheng, K.; Cheng, C. C. *J. Heterocycl. Chem.* **1967**, *4*, 163. (b) Mikhailopulo, I. A.; Gunar, V. I.; Zav'yalov, S. I. *Izv. Akad. Nauk SSSR, Ser. Khim* **1967**, *6*, 1388.

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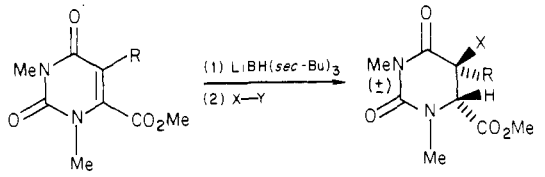
(14) Esterbauer, H.; Ertl, A.; Scholz, N. *Tetrahedron* **1976**, *32*, 285.

in H_2O with bisulfite gives the hexahydropyrimidine spectrum (B). The C-6 proton resonance is a multiplet at 4.79 ppm and the C-5 proton is a doublet at 5.18 ppm. When the bisulfite addition was carried out in D_2O, deuteration at C-6 occurred; the C-6 proton resonance does not appear, and the C-5 proton collapses to a singlet (C). This supports a C-5, rather than C-6, addition to the pyrimidine. Similar results were obtained with the corresponding methyl ketone (**6b**), which was prepared by the treatment of the phenyl thioester of 1,3-dimethylorotic acid with lithium dimethylcuprate.[15] As in the case of the aldehyde, the bis-adduct **7b** could not be isolated in pure form. The reaction of **6b** (1.0 M) to **7b**, however, was essentially complete (ca. 95%) after incubation with only 2 equiv of aqueous sodium bisulfite *at room temperature* for 1 h. The accelerated rate of this reaction relative to that with the aldehyde (**6a**) may be because, unlike **6a**, the ketone (**6b**) does not appear to exist as the hydrate in water. The structure of **7b** also was determined by NMR spectroscopy. As with **7a**, when the reaction was carried out in D_2O, the C-6 proton resonance vanished and the C-5 proton resonance collapsed to a singlet. The facility with which **6** undergoes a Michael addition of a sulfur nucleophile to the C-5 position of an activated orotic acid analogue in solution suggests that a similar ODase-catalyzed reaction is quite feasible. The active-site nucleophile of ODase could be positioned so that exclusive 1,4-addition to the C-5 carbon would occur, allowing this process to be very efficient.

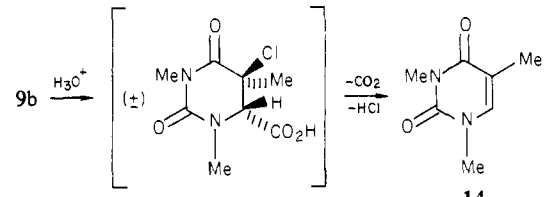
Since the Michael addition products (**7a** and **7b**) could not be used to show decarboxylative elimination, a stable preformed model compound for **5** (Scheme II) was sought. Lithium tri-*sec*-butylborohydride (L-Selectride), is known to reduce the 5,6-double bond of orotic ester analogues and to produce the corresponding hexahydropyrimidines after aqueous quench.[16] This reagent, therefore, was used to add a hydride to C-6 of methyl 1,3-dimethylorotate (**8a**) and to produce an enolate that was



8a, R = H
b, R = CH_3

9a, R = H; X = CH_3
b, R = CH_3; X = Cl
c, R = CH_3; X = SCH_3

quenched with appropriate electrophilic reagents, thus introducing a leaving group at C-5 of the hexahydropyrimidine ring (**9**). These compounds, then, are models for **5**. In order to determine that L-Selectride added a hydride at C-6, we quenched the enolate generated with methyl iodide. The NMR spectrum of the isolated material (**9a**) clearly showed a C-5 methyl doublet (1.36 ppm), a C-5 proton doublet of quartets (3.03 ppm), and a C-6 proton doublet (3.67 ppm). Deuterium incorporation at C-5, resulting from a D_2O quench, also indicated the generation of a C-5 carbanion. When Cl_2, Br_2, or CH_3SCl were used to quench the enolate, however, the corresponding 5-chloro, 5-bromo, and 5-methylthio analogues were not isolated. After chromatography, a mixture of **8a** and another compound was obtained that, by spectral analysis, appeared to have lost its hexahydropyrimidine integrity. Apparently, the acidity of the C-5 proton increased sufficiently when an electron-withdrawing group was incorporated at C-5 that, under the conditions of the reaction or during the workup procedure, decomposition by eliminative ring opening may have occurred. Dihydroorotic acid derivatives are known to be susceptible to this sort of elimination.[17] In order to prevent this ring cleavage reaction resulting from an acidic C-5 proton, we introduced a minor modification in the structure of the model compound, namely, a C-5 methyl group. Consequently, methyl 1,3,5-trimethylorotate (**8b**) was reduced with L-Selectride, and the resulting enolate was quenched by addition to a solution of chlorine, thereby yielding a 1:1 mixture of **8b** and the desired **9b**. The stereochemical assignment for **9b** in which the leaving group and carbomethoxyl group are trans is based on its chemical reactivity (vide infra) and the invariable production of **8b** in its preparation. It is believed that quenching of the enolate is a nonstereospecific process and that **8b** results, under the reaction conditions, by elimination of HX from the diastereomer in which the leaving group and the C-6 proton are trans (none of this diastereomer ever was isolated). This ester (**9b**) then was subjected



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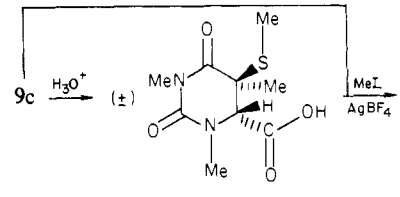
to hydrolytic conditions. All attempts at base hydrolysis of **9b** led to the formation of **8b**, presumably via an E1cB reaction. However, hydrolysis of **9b** in 1 N HCl on a steam bath for 2 h gave, in quantitative yield, 1,3-dimethylthymine (**14**), the product of ester hydrolysis followed by decarboxylative elimination (a model for **5** → **2**, Scheme II). The intermediate carboxylic acid (**13**) was not detected by TLC or NMR, suggesting that, under these conditions, decarboxylative elimination is fast relative to ester hydrolysis. The same conversion directly to **14** occurred when **9b** was allowed to stand in 6 N HCl at room temperature for 24 h. Other methods of ester hydrolysis of **9b** (e.g., phenyl selenide in THF–HMPA[18] and NaI in refluxing acetone) were unsuccessful at revealing intermediate **13**.

In an effort to slow down the decarboxylative elimination step and, at the same time, to design a more appropriate model for **5** (Scheme II), the methylthio analogue (**9c**) was prepared by trapping the enolate formed from L-Selectride reduction of **8b** with methanesulfenyl chloride. It is interesting to note that when dimethyl disulfide was used to quench the enolate, only **8b** was isolated, presumably because of the basicity of the methylthiolate ion generated, which may promote E1cB elimination of the product. Compound **9c** not only has a poorer leaving group at C-5 than does **9b** and therefore should undergo decarboxylative elimination more slowly than **9b** but, after ester hydrolysis, it would be an excellent model for the proposed covalent enzyme–substrate complex, with the assumption that an active-site sulfhydryl residue is involved. In contrast to **9b**, acid hydrolysis of **9c** did indeed give the desired model compound, the carboxylic acid intermediate (**15**), in a quantitative yield. According to the mechanism in



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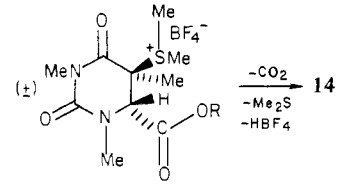

16a, R = H
b, R = Me

(15) Anderson, R. J.; Henrick, C. A.; Rosenblum, L. D. *J. Am. Chem. Soc.* **1974**, *96*, 3654.
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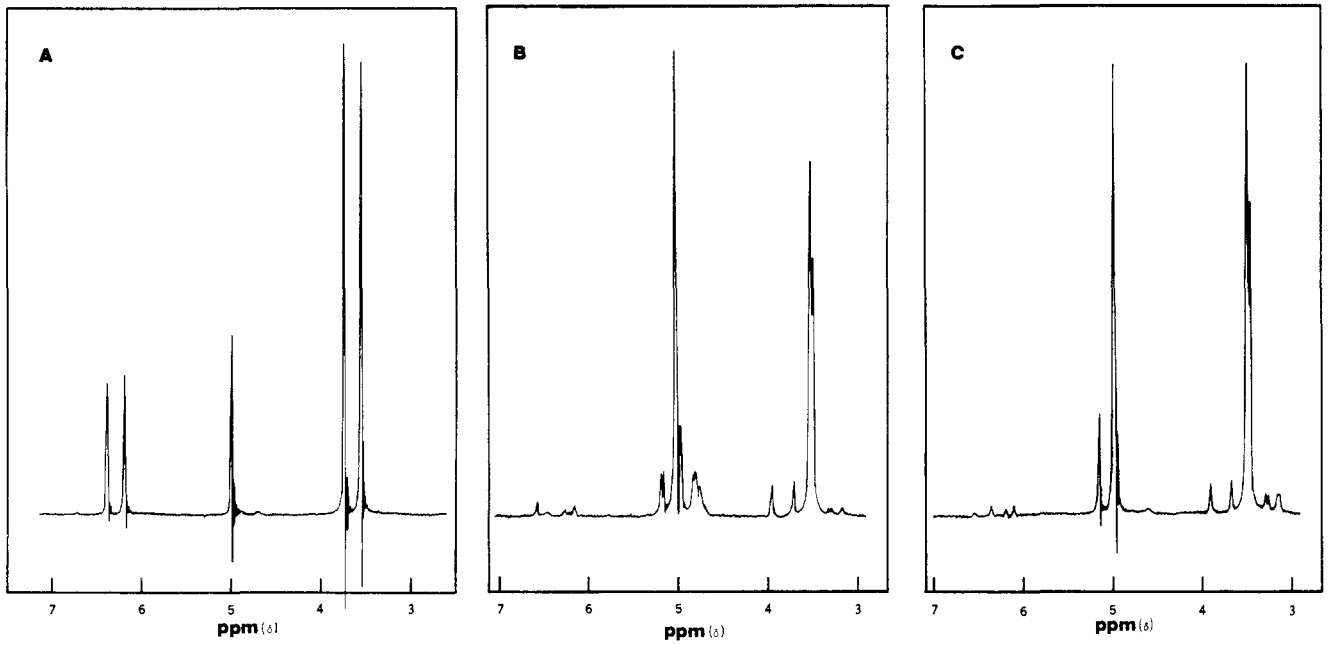

Figure 1. NMR spectra (in D_2O) of **6a**, **7a**, and **7a** prepared in D_2O. Compound **6a** (A) was treated with sodium bisulfite in H_2O at 100 °C for 4 h (B). The same reaction was carried out in D_2O (C). The peak at δ 5.0 is HDO; the small peaks in B and C at δ 3.66, 3.90, 6.06, and 6.50 are from the 1,2-bisulfite adduct. The minor peaks between δ 3.1 and 3.3 in B and C arise from an impurity of unknown structure.

Scheme II, decarboxylative elimination would be catalyzed by enzyme-catalyzed protonation of the leaving group. No reaction occurred, however, when **15** was heated in 6 N HCl or in trifluoroacetic acid. This most likely is a result of low protonation of the sulfide and lack of base catalysis at the carboxyl group. Consequently, **15** was methylated to the sulfonium salt in order to model enzyme-catalyzed protonation and to activate the leaving group. Treatment of a solution of **15** in methyl iodide *at room temperature* with 1 equiv of $AgBF_4$[19] produced an instantaneous reaction, and the decarboxylative elimination product **14** (presumably via **16a**) was isolated in a 55% purified yield. When the methylation reaction was carried out on the corresponding ester (**9c**), the methylsulfonium tetrafluoroborate salt (**16b**) was obtained in a 88% yield. This suggests that the decarboxylative elimination arising from methylation of **15** was the result of sulfonium ion formation. Acid hydrolysis of **16b** led to a mixture of **14** and **15**.

The model study described here suggests that ODase may act via a Michael addition to the C-5 position of orotidine 5'-phosphate followed by decarboxylative elimination to uridine-5'-phosphate (Scheme II). The elimination would be facilitated if the C-6 protonation were stereospecifically syn to the active-site nucleophile and if this nucleophile were protonated prior to or during the decarboxylation. Recent enzyme inhibition studies[4] have indicated the presence of a highly acidic residue at the active site of ODase, which may be involved in such a protonation. Unlike the high temperatures required in the previously reported model studies of ODase,[3] both the Michael addition and the decarboxylative elimination reactions described here occurred readily at room temperature. The models of Beak and Siegel,[3] however, are quite relevant to the mechanism supported in this paper. Initial enzyme-catalyzed quaternary ammonium formation at the N-1 position of orotidylate (i.e., protonated **3**, Scheme I) should further activate the substrate for Michael addition and for decarboxylative elimination. Also, the observation that 6-azauridylate is a potent inhibitor of ODase[4,20] would be consistent with the addition–elimination mechanism. Enzyme-catalyzed protonation of N-6 of 6-azauridylate (as suggested in Scheme II for activation of the carboxylic acid of orotidylate) would activate the C-5 position for nucleophilic attack, and a tightly bound readily reversible covalent enzyme complex with 6-azauridylate may form.

(19) For a similar alkylation reaction employing $AgClO_4$ see: Scartazzini, R.; Mislow, K. *Tetrahedron Lett.* **1967**, 2719.

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Experimental Section

General Methods. Melting points were obtained on a Thomas-Hoover Unimelt apparatus and are uncorrected. Proton magnetic resonance spectra were recorded on a Varian Model EM360A or a Perkin-Elmer Model R20B NMR spectrometer with internal tetramethylsilane as standard or with HDO at 5.00 ppm when D_2O was the solvent. Infrared spectra were obtained on a Perkin-Elmer Model 283 spectrophotometer. Elemental analyses were performed by Microtech Laboratories, Inc., Skokie, IL.

Orotic acid, 6-methyluracil, dimethyl sulfate, dimethyl disulfide, bromoethane, dicyclohexylamine, silver tetrafluoroborate, lithium tri-*sec*-butylborohydride (L-Selectride) in THF, *n*-BuLi, and CH_3Li in ether were purchased from the Aldrich Chemical Co. Cl_2 was a product of Matheson. 5-Methylorotic acid was purchased from Pfaltz and Bauer, Inc. Tetrahydrofuran and ether were distilled from Na (benzophenone ketyl) under nitrogen immediately prior to use. Methanol was distilled from $Mg(OMe)_2$ and stored under argon. Dimethylformamide was distilled from BaO and stored over 4-Å molecular sieves. Benzene was freshly distilled and stored over 4-Å sieves. Acetone and $CHCl_3$ were distilled from P_2O_5 and stored under argon.

Preparative layer chromatography plates were obtained from Merck, and 100–200 mesh silica gel for column chromatography was from Grace Davison Chemicals. Flex-needle is a trade name of the Aldrich Chemical Co.

1,3-Dimethylorotic Acid. This was prepared according to a modified procedure of Curran and Angier.[21] A solution of orotic acid (30.0 g, 0.19 mol) and NaOH (30.8 g, 0.76 mol) in 800 mL of H_2O was cooled to 5 °C and treated with dimethyl sulfate (54 mL, 0.29 mol) dropwise over 2 h with vigorous stirring. The reaction mixture was stirred at 5–15 °C overnight and then acidified cold with concentrated HCl (120 mL). The resulting precipitate was collected by suction filtration and washed with a small amount of H_2O, and the combined filtrate and washing were rotary evaporated to 400 mL and then extracted with EtOAc (4 × 500 mL). The precipitate was dissolved in the combined organic extracts, and the solution was dried ($CaSO_4$) and rotary evaporated to dryness. Recrystallization from ether afforded, after drying over P_2O_5 at 60 °C, 26.5 g (75%) of the product as colorless needles: mp 151.5–153 °C (lit.[3] mp 153–154 °C); ^1H NMR (Me_2SO-d_6) δ 3.15 (s, 3, NCH_3), 3.35 (s, 3, NCH_3), 5.98 (s, 1, H-5), 13.40 (s, 1, CO_2H); IR (KBr) 1628, 1655, 1700, 1721, 1735, 3400 cm^{-1}.

Methyl 1,3-Dimethylorotate (8a). A suspension of 1,3-dimethylorotic acid (10.0 g, 54.4 mmol) in 100 mL of dry benzene under argon was treated with thionyl chloride (5.0 mL, 68 mmol) and 0.1 mL of dry

(21) Curran, W. V.; Angier, R. B. *J. Org. Chem.* **1966**, *31*, 201.

DMF. The reaction mixture was heated at reflux under argon overnight, allowed to cool to room temperature, and carefully treated with absolute MeOH (10 mL). After the solution was stirred for 10 min, the solvents were removed by rotary evaporation, and the residue was recrystallized from ether to afford 8.87 g (83%) of the product as colorless needles: mp 76–77 °C (lit.[3] mp 77-78 °C); ^{1}H NMR ($CDCl_3$) δ 3.30 (s, 3, NCH_3), 3.48 (s, 3, NCH_3), 3.92 (s, 3, CO_2CH_3), 6.17 (s, 1, H-5); IR (KBr) 1651, 1709, 1737 cm^{-1}.

Ethyl 1,3-Dimethylorotate. Substitution of absolute EtOH for MeOH in the the above procedure afforded, after recrystallization from hexane, the ethyl ester in 87% yield: mp 60–60.5 °C; ^{1}H NMR (CCl_4) δ 1.38 (t, J = 7 Hz, 3, CH_2CH_3), 3.22 (s, 3, NCH_3), 3.41 (s, 3, NCH_3), 4.33 (q, J = 7 Hz, 2, CH_2CH_3), 5.98 (s, 1, H-5); IR (KBr) 1663, 1712, 1736 cm^{-1}. Anal. ($C_9H_{12}N_2O_4$) C, H, N.

***tert*-Butyl 1,3-Dimethylorotate.** Substitution of a solution of 1 equiv of KO-*t*-Bu in *t*-BuOH for MeOH and stirring the resulting solution overnight afforded, after recrystallization from hexane, the *tert*-butyl ester in 65% yield: mp 95.5–96.5 °C; ^{1}H NMR (CCl_4) δ 1.55 (s, 9, $C(CH_3)_3$), 3.22 (s, 3, NCH_3), 3.39 (s, 3, NCH_3), 5.86 (s, 1, H-5); IR (KBr) 1666, 1710, 1734 cm^{-1}. Anal. ($C_{11}H_{16}N_2O_4$) C, H, N.

1,3,6-Trimethyluracil. A solution of 6-methyluracil (31.5 g, 0.25 mol) and NaOH (40.0 g, 1.0 mol) in 500 mL of H_2O at 0 °C was treated dimethyl sulfate (95 mL, 1.0 mol) and stirred vigorously at room temperature overnight. The aqueous solution was extracted with $CHCl_3$ (5 × 300 mL), and the combined extracts were dried ($CaSO_4$) and rotary evaporated to dryness. Recrystallization from CH_2Cl_2/cyclohexane afforded 32.0 g (83%) of the product as colorless needles: mp 110–112 °C (lit.[22] mp 114 °C); ^{1}H NMR ($CDCl_3$) δ 2.22 (s, 3, CH_3-6), 3.27 (s, 3, NCH_3), 3.35 (s, 3, NCH_3), 5.51 (s, 1, H-5); IR (KBr) 1621, 1655, 1693 cm^{-1}.

1,3-Dimethylorotaldehyde Diethyl Acetal (6a Diethyl Acetal). This was prepared by a modification of the method used to prepared orotaldehyde[11] and was isolated and characterized as the diethyl acetal. A mixture of 1,3,6-trimethyluracil (2.32 g, 15 mmol) and finely ground selenium dioxide (1.78 g, 16 mmol) in 75 mL of glacial HOAc was heated at 100 °C for 4 h, filtered from the deposited Se while hot, and rotary evaporated to a gum. The residual HOAc was removed in vacuo overnight. The residue was dissolved in 75 mL of $CHCl_3$, filtered, and rotary evaporated to give crude **6a**. A solution of this gum in 75 mL of absolute EtOH was treated with dry HCl for 5 min and heated at reflux under argon overnight. After cooling to room temperature, the solution was filtered and rotary evaporated to afford 0.93 g (26%) of **6a** diethyl acetal as a pale yellow solid. Final purification by sublimation gave the diethyl acetal as a white powder: mp 73.5–74 °C (lit.[23] mp 82 °C); ^{1}H NMR (CCl_4) δ 1.22 (t, J = 7 Hz, 6, $CH(OCH_2CH_3)_2$), 3.18 (s, 3, NCH_3), 3.37 (s, 3, NCH_3), 3.53 (q, J = 7 Hz, 2, CH_2CH_3), 3.57 (q, J = 7 Hz, 2, CH_2CH_3), 5.03 (s, 1, $CH(OCH_2CH_3)_2$), 5.64 (s, 1, H-5); IR (KBr) 1667, 1712 cm^{-1}. Anal. ($C_{11}H_{18}N_2O_4$) C, H, N.

1,3-Dimethylorotaldehyde (6a). A solution of **6a** diethyl acetal (242 mg, 1.0 mmol) in 6 mL of THF was treated with 6 mL of 0.01 M HCl and heated on the steam bath for 3 h. The solution was allowed to cool to room temperature and then rotary evaporated in vacuo. The residue was dried in vacuo at room temperature overnight to give 181 mg (91%) of **6a** hydrate as a white solid. Sublimation (60–70 °C (1.0 mmHg)), followed by removal of trace water as a $CHCl_3$ azeotrope, afforded pure **6a** as a white hygroscopic solid: mp 76–77 °C; ^{1}H NMR ($CDCl_3$) δ 3.32 (s, 3, NCH_3), 3.60 (s, 3, NCH_3), 6.21 (s, 1, H-5), 9.45 (s, 1, CHO); IR (KBr) 1608, 1650, 1704 cm^{-1}. Anal. ($C_7H_8N_2O_3{\cdot}H_2O$) C, H, N. Compound **6a** exists almost exclusively as the hydrate in aqueous solution; ^{1}H NMR (D_2O) δ 3.58 (s, 3, NCH_3), 3.75 (s, 3, NCH_3) 6.20 (s, 1, $CH(OD)_2$), 6.38 (s, 1, H-5).

Reactions of 6a with Bisulfite. (A). 1,2-Addition Product. Sodium bisulfite (26 mg, 0.25 mmol) was added to a solution of **6a** (42 mg, 0.25 mmol) in D_2O (0.5 mL) in a NMR tube: ^{1}H NMR (D_2O) δ 3.66 (s, 3, NCH_3), 3.90 (s, 3, NCH_3), 6.06 (s, 1, $CH(OD)SO_3Na$), 6.50 (s, 1, H-5).

(B). Bis-Bisulfite Product 7a. A solution of **6a** (42 mg, 0.25 mmol), sodium bisulfite (156 mg, 1.5 mmol), and sodium sulfite (47 mg, 0.38 mmol) in H_2O (0.5 mL) under Ar was heated on a steam bath for 4 h, cooled, and rotary evaporated to dryness in vacuo. The residue was dried over P_2O_5 in vacuo overnight and dissolved in 0.5 mL of D_2O. The NMR spectrum of the product mixture indicated a 95% yield of **7a**: ^{1}H NMR (D_2O) δ 3.47 (s, 3, NCH_3), 3.50 (s, 3, NCH_3), 4.79 (m, 1, H-6), 4.98 ($CH(OD)SO_3Na$), 5.18 (d, J = 2 Hz, 1 H-5). When the reaction was run in D_2O, the NMR signal at 4.79 ppm disappeared, and the signal at 5.18 ppm collapsed to a singlet.

6-Acetyl-1,3-dimethyluracil (6b). A suspension of 1,3-dimethylorotic acid (11.6 g, 63 mmol) in 150 mL of dry C_6H_6 under argon was treated with 100 g of $SOCl_2$ and 10 drops of anhydrous DMF and then heated at reflux under argon overnight. The solution was cooled to room temperature and rotary evaporated to a yellow gum. The last traces of solvent were removed in vacuo, and the residue was dissolved in 250 mL of anhydrous ether under argon. The ethereal solution was treated dropwise with freshly distilled thiophenol (6.7 mL, 65 mmol), heated at reflux under argon for 4.5 h, cooled to room temperature, filtered, and rotary evaporated to an oil. A solution of this oil in 200 mL of acetonitrile was filtered, concentrated to 60 mL on a steam bath, and cooled to afford 7.31 g (42%) of 1,3-dimethyl-6-(carbothiophenoxy)uracil: mp 132–133 °C; ^{1}H NMR ($CDCl_3$) δ 3.30 (s, 3, NCH_3), 3.33 (s, 3, NCH_3), 6.17 (s, 1, H-5), 7.38 (m, 5, C_6H_5); IR (KBr) 1666, 1712 cm^{-1}. Anal. ($C_{13}H_{12}N_2O_3S$) C, H, N, S.

A suspension of CuI (7.43 g, 39 mmol) in 500 mL of anhydrous THF at 0 °C under argon was treated dropwise with CH_3Li (71 mL of a 1.04 M solution in ether, 74 mmol). The resulting $(CH_3)_2CuLi$ solution was cooled to −78 °C, treated dropwise with a solution of 1,3-dimethyl-6-(carbothiophenoxy)uracil (6.74 g, 24.4 mmol) in 200 mL of anhydrous THF, and stirred for 2 h at −78 °C. The reaction mixture was quenched with 500 mL of saturated aqueous NH_4Cl while cold, allowed to warm to room temperature, and extracted with EtOAc (4 × 600 mL). The combined organic extracts were dried ($CaSO_4$) and rotary evaporated to dryness. Recrystallization of the residue from ether afforded 3.28 g (74%) of **6b** as colorless crystals: mp 86–87 °C; ^{1}H NMR ($CDCl_3$) δ 2.47 (s, 3, $COCH_3$), 3.30 (s, 3, NCH_3), 3.34 (s, 3, NCH_3), 5.96 (s, 1, H-5); IR (KBr) 1654, 1702, 1715 cm^{-1}. Anal. ($C_8H_{10}N_2O_3$) C, H, N.

Reactions of 6b with Bisulfite. A solution of **6b** (46 mg, 0.25 mmol), sodium bisulfite (156 mg, 1.5 mmol), and sodium sulfite (47 mg, 0.38 mmol) in 0.5 mL of H_2O under argon was stirred for 1 h at room temperature and treated with 2 mL of EtOH, and the solvents were removed by rotary evaporation in vacuo. The residue was dried over P_2O_5 in vacuo overnight and dissolved in 0.5 mL of D_2O. The NMR spectrum of the product mixture indicated a >95% yield of **7b**: ^{1}H NMR (D_2O) δ 2.66 (s, ~3 (exchanges), CCH_3), 3.43 (m, 6, 2(NCH_3)), 4.73 (m, 1, H-6), 5.20 (m, 1, H-5). When the reaction was run in D_2O, the NMR spectrum of the product had no signal at 4.73 ppm, and the signal at 5.20 ppm collapsed to a singlet. All attempts to isolate **7b** in pure form were unsuccessful.

(±)-*trans*-Methyl 1,3-5-Trimethyl-5,6-dihydroorotate (9a). A solution of **8a** (175 mg, 0.88 mmol) in 5 mL of dry THF at −78 °C under argon was treated dropwise with L-Seletride (930 μL of a 1.07 M solution in THF, 0.995 mmol). The resulting pale yellow solution was stirred for 10 min at −78 °C and then was treated with methyl iodide (250 μL, 4.0 mmol). The reaction mixture was stirred for 5 min at −78 °C, allowed to warm to room temperature, and poured onto a rapidly stirred mixture of 20 mL of saturated aqueous NH_4Cl and 20 mL of $CHCl_3$. The layers were separated, and the aqueous phase was extracted with $CHCl_3$ (4 × 20 mL). The combined organic extracts were washed with brine, dried ($CaSO_4$), and rotary evaporated to a pale yellow oil. Preparative thin-layer chromatography (250 μm, 20 cm × 20 cm silica gel plate, 1:1:2 CH_3CN:EtOAc:CCl_4 as eluent) of this material afforded 61 mg (32%) of **9a** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.36 (d, J = 7 Hz, 3, CH_3-5), 3.03 (s, 3, NCH_3), 3.03 (dq, J = 7 Hz, 1.5 Hz, 1, H-5), 3.07 (s, 3, NCH_3), 3.67 (d, J = 1.5 Hz, 1, H-6), 3.70 (s, 3, CO_2CH_3); IR (KBr) 1670, 1713, 1742 cm^{-1}. Anal. ($C_9H_{14}N_2O_4$) C, H, N.

1,3,5-Trimethylorotic Acid. A solution of 5-methylorotic acid (10.2 g, 60 mmol) and NaOH (12.0 g, 300 mmol) in 250 mL of H_2O was cooled to 5 °C and treated with dimethyl sulfate (23 mL, 240 mmol). The mixture was stirred rapidly at 5 °C overnight, acidified with 30 mL of concentrated HCl, and extracted with 3 × 200 mL of EtOAc. The combined organic extracts were dried ($CaSO_4$) and rotary evaporated to afford 8.2 g (69%) of 1,3,5-trimethylorotic acid. Recrystallization from ether/pentane afforded the product as white crystals: mp 203–205 °C (lit.[24] mp 212 °C); ^{1}H NMR (Me_2SO-d_6) δ 1.81 (s, 3, CH_3-5), 3.17 (s, 3, NCH_3), 3.23 (s, 3, NCH_3); IR (KBr) 1625, 1650, 1687, 1741, 3420 cm^{-1}. Anal. ($C_8H_{10}N_2O_4{\cdot}H_2O$) C, H. N.

1,3,5-Trimethylorotoyl Chloride. A suspension of 1,3,5-trimethylorotic acid (4.5 g, 23 mmol) in 100 mL of anhydrous benzene was treated with thionyl chloride (5.0 mL, 68 mmol) and 4 drops of anhydrous DMF. The reaction mixture was heated at reflux under argon overnight. The solution was then allowed to cool to room temperature, the solvents were removed by rotary evaporation, and the residue was purified by Kugelrohr distillation (bp 110–115 °C at 3–5 mmHg) to afford 4.47 g (91%) of the acid chloride as a thick colorless oil. Recrystallization from pentane afforded the product as white prisms: mp 54–56 °C; ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 3, CH_3-5), 2.25 (s, 3, NCH_3), 2.35 (s, 3, NCH_3); IR (KBr) 1650, 1669, 1773 cm^{-1}. Anal. ($C_8H_9ClN_2O_3$) C, H, Cl, N.

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Methyl 1,3,5-Trimethylorotate (8b). A solution of 1,3,5-trimethylorotoyl chloride (1.5 g, 7.0 mmol) in 40 mL of absolute methanol was heated at reflux under argon overnight. The solvent was removed by rotary evaporation to afford 1.45 g (99%) of **8b**. Recrystallization from hexane afforded **8b** as white needles: mp 125–126 °C; ^{1}H NMR ($CDCl_3$) δ 1.78 (s, 3, CH_3-5), 3.28 (s, 3, NCH_3), 3.33 (s, 3, NCH_3), 3.94 (s, 3, CO_2CH_3); IR (KBr) 1670, 1700, 1744 cm^{-1}. Anal. ($C_9H_{12}N_2O_4$) C, H, N.

(±)-1,3-Dimethyl-*r*-5-chloro-5-methyl-*trans*-6-carbomethoxy-5,6-dihydrouracil (9b). A solution of **8b** (1.27 g, 6.0 mmol) in 30 mL of anhydrous THF under argon at 0 °C was treated dropwise with L-Selectride (5.7 mL of 1.07 M solution in THF, 6.1 mmol), was stirred exactly 5 min at 0 °C, and then was transferred through an ice-cold Flex-needle with positive argon pressure into a stirred saturated solution of Cl_2 in 45 mL of anhydrous THF under argon at −78 °C. The reaction mixture was stirred for 10 min at −78 °C, 10 min a 0 °C, and finally 10 min at room temperature before being poured into a rapidly stirred mixture of 120 mL of saturated aqueous NH_4Cl and 120 mL of $CHCl_3$. The layers were separated, and the aqueous phase was extracted with 4 × 120 mL of $CHCl_3$. The combined organic extracts were washed once with brine, dried ($CaSO_4$), and rotary evaporated to a pale yellow oil. Column chromatography (silica gel, 400 g; 1:1:8 CH_3CN:EtOAc:CCl_4 as eluent) of this material afforded 415 mg (27%) of the desired compound as a colorless oil. Recrystallization from pentane gave **9b** as colorless crystals: mp 67.5–69 °C; ^{1}H NMR ($CDCl_3$) δ 1.77 (s, 3, CH_3-5), 3.07 (s, 3, NCH_3), 3.22 (s, 3, NCH_3), 3.73 (s, 3, CO_2CH_3), 3.98 (s, 1, H-6); IR (KBr) 1676, 1690, 1724, 1737 cm^{-1}. Anal. ($C_9H_{13}ClN_2O_4$) C, H, Cl, N.

(±)-1,3-Dimethyl-*r*-5-(methylthio)-5-methyl-*trans*-6-carbomethoxy-5,6-dihydrouracil (9c). A solution of **8b** (850 mg, 4.0 mmol) in 20 mL of anhydrous THF under argon at 0 °C was treated dropwise with L-Selectride (3.9 mL of 1.07 M solution in THF, 4.2 mmol) was stirred exactly 5 min at 0 °C, and then was transferred through an ice-cold Flex-needle with positive argon pressure into a stirred solution of methanesulfenyl chloride (40 mmol) in 30mL of anhydrous THF under argon at −78 °C, prepared by treating dimethyl disulfide (1.8 mL, 20 mmol) with sulfuryl chloride[25] (1.6 mL, 20 mmol) at −23 °C. The reaction mixture was stirred for 10 min at −78 °C, 10 min at 0 °C, and 10 min at room temperature before pouring into a rapidly stirred mixture of 80 mL of saturated aqueous NH_4Cl and 80 mL of $CHCl_3$. The layers were separated, and the aqueous phase was extracted with 4 × 80 mL of $CHCl_3$. The combined organic extracts were washed once with brine, dried ($CaSO_4$), and rotary evaporated to a yellow oil. Column chromatography (silica gel, 150 g; 1:1:2 CH_3CN:EtOAc:CCl_4 as eluent) of this material afforded 221 mg (21%) of the desired compound as a pale yellow oil. Recrystallization from pentane gave **9c** as colorless crystals: mp 59.5–60.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 3, CH_3-5), 2.08 (s, 3, SCH_3), 3.01 (s, 3, NCH_3, 3.17 (s, 3, NCH_3), 3.72 (s, 3, CO_2CH_3), 3.83 (s, 1, H-6); IR (KBr) 1669, 1705, 1741 cm^{-1}. Anal. ($C_{10}H_{16}N_2O_4S$) C, H, N, S.

(±)-1,3-Dimethyl-*r*-5-(methylthio)-5-methyl-*trans*-6-carboxy-5,6-dihydrouracil (15). A solution of **9c** (52 mg, 0.2 mmol) in 2 mL of 1.0 N HCl was heated on a steam bath for 1 h, allowed to cool to room temperature, and then rotary evaporated in vacuo to a clear oil. Residual HCl was removed by adding 5 mL of H_2O and rotary evaporating in vacuo three times. The resulting clear oil was dried in a P_2O_5 vacuum desiccator overnight to afford 50 mg (quantitative) of **15** as a clear oil which could not be recrystallized: ^{1}H NMR (D_2O) δ 1.74 (s, 3, CH_3-5), 2.42 (s, 3, SCH_3), 3.33 (s, 3, NCH_3), 3.44 (s, 3, NCH_3), 4.32 (s, 1, H-6); IR (KBr) 1652, 1667, 1710, 1742 cm^{-1}.

A solution of the oil (14 mg, 0.06 mmol) in 12 mL of anhydrous ether was treated dropwise with a solution of dicyclohexylamine (12 μL, 0.06 mmol) in 5 mL of anhydrous ether, the solvent was removed by rotary evaporation, and the residue (23 mg, 88%) was recrystallized from pentane to afford the dicyclohexylammonium salt as white crystals: mp 156–157 °C; ^{1}H NMR ($CDCl_3$) δ 1.21 and 1.77 (m, br, 22, cyclohexyl), 1.52 (s, 3, CH_3-5), 2.03 (s, 3, SCH_3), 2.85 (m, br, 2, $^+NH_2$), 2.97 (s, 3, NCH_3), 3.02 (s, 3, NCH_3), 3.52 (s, 1, H-6); IR (KBr) 1639, 1665, 1700, 2935, 3435 cm^{-1}. Anal. ($C_{21}H_{37}N_3O_4S$) C, H, N, S.

Methylation of 9c (16b). A solution of **9c** (9 mg, 0.035 mmol) in 0.1 mL of freshly distilled methyl iodide under argon was treated, all at once, with silver tetrafluoroborate (14 mg, 0.07 mmol). The reaction mixture was diluted to ca. 1 mL with CH_2Cl_2, and filtered under argon through glass wool, and the precipitate was washed with 1 mL of anhydrous acetone. The combined organic solutions were rotary evaporated under argon to dryness, and the residue was redissolved in 0.5 mL of anhydrous acetone. Slow addition of 3 mL of anhydrous ether effected separation of 11 mg (88%) of **16b** as hygroscopic white crystals: mp 179–180 °C; ^{1}H NMR (acetone-d_6) δ 1.86 (s, 3, CH_3-5), 3.07 (s, 6, $S(CH_3)_2$), 3.22 (s, 3, NCH_3), 3.28 (s, 3, NCH_3), 3.83 (s, 3, CO_2CH_3), 4.84 (s, 1, H-6); IR (KBr) 1685, 1720, 1739 cm^{-1}. Anal. ($C_{11}H_{19}N_2O_4SBF_4$) C, H, N, S.

Reaction of 15 with Methyl Iodide (Decarboxylative Elimination). A solution of **15** (13 mg, 0.05 mmol) in 0.1 mL of freshly distilled methyl iodide under argon was treated, all at once, with silver tetrafluoroborate (10.5 mg, 0.05 mmol). The reaction mixture was diluted to ca. 1 mL with CH_2Cl_2 and filtered under argon through glass wool, and the precipitate was washed with 1 mL of anhydrous acetone. The combined filtrates were rotary evaporated to a yellow oil which was purified by preparative thin-layer chromatography (250 μm, 20 cm × 20 cm silica gel plate; 1:1:2 CH_3CN:EtOAc:CCl_4 as eluent) to afford 4.2 mg (55%) of 1,3-dimethylthymine **14**: mp 150–152 °C (lit.[26] mp 151–153 °C); mixed melting point with authentic sample[26] showed no depression; the IR spectrum was identical with that of authentic 1,3-dimethylthymine.

Base Hydrolysis of 9b and 9c. A solution of **9b** (25 mg, 0.1 mmol) in 2.0 mL of H_2O and 0.5 mL of CH_3OH was treated with 1.0 mL of 0.1 M NaOH dropwise over 5 min with stirring. The solution was extracted with $CHCl_3$ (4 × 4 mL), and the combined organic extracts were dried ($CaSO_4$) and rotary evaporated to give 19 mg (89%) of **8b** as an off-white solid. Recrystallization from hexane afforded pure **8b**: mp 125–126 °C; mixed melting point with an authentic sample showed no depression; the IR spectrum was identical with that of the authentic sample.

Substitution of **9c** for **9b** in the above procedure afforded a 75% crude yield of methyl 1,3,5-trimethylorotate; recrystallization from hexane afforded pure **8b**: mp 125–126 °C; mixed melting point and IR spectrum as above.

Acid Hydrolysis of 9b (Decarboxylative Elimination). A solution of **9b** (25 mg, 0.01 mmol) in 2 mL of 1.0 N HCl was heated at reflux for 2 h. The solution was cooled to room temperature and rotary evaporated to dryness in vacuo four times to remove residual HCl. The dry solid (16 mg, quantitative) was recrystallized from ether to afford pure **14**: mp 152.5–153.5 °C; mixed melting point with an authentic sample showed no depression; the IR spectrum was identical with that of authentic 1,3-dimethylthymine.[26]

When **9b** was allowed to stand in 6 N HCl at room temperature, the production of **14** was complete within 24 h as followed by NMR spectroscopy. Direct conversion of **9b** to **14** in refluxing acetone-d_6 saturated with sodium iodide was observed to 40% completion in 40 h (NMR).

Acknowledgment. We are grateful to the National Institutes of Health (Grant CA 21156) and the Research Corp. for financial support.

Registry No. 6a, 83174-90-7; **6a**, diethyl acetal, 83174-91-8; **6b**, 83174-92-9; **7a**, 83174-93-0; **7b**, 83174-94-1; **8a**, 4116-39-6; **8b**, 83174-95-2; **9a**, 83174-96-3; **9b**, 83174-97-4; **9c**, 83174-98-5; **14**, 4401-71-2; **15**, 83174-99-6; **16b**, 83175-01-3; orotidine 5'-phosphate decarboxylase, 9024-62-8; orotic acid, 65-86-1; 1,3-dimethylorotic acid, 4116-38-5; ethyl 1,3-dimethylorotate, 83175-02-4; *tert*-butyl 1,3 dimethylorotate, 83175-03-5; 6-methyluracil, 626-48-2; 1,3,6-trimethyluracil, 13509-52-9; bisulfite, 15181-46-1; 1,3-dimethyl-6-(carbothiophenoxy)uracil, 83175-04-6; L-Selectride, 38721-55-7; 5-methylorotic acid, 3993-73-5; 1,3,5-trimethylorotic acid, 4116-37-4; 1,3,5-trimethylorotoyl chloride, 83175-05-7.

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