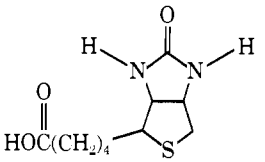
Model Studies on the Mechanism of Biotin Dependent Carboxylations[1]

Harold Kohn

Contribution from the Department of Chemistry, University of Houston, Houston, Texas 77004. *Received October 9, 1975*

Abstract: The syntheses of both *N*-carbomethoxy-2-phenacylthioimidazoline (**8**) and its *N*-methyl salt, *N*-methyl-*N*-carbomethoxy-2-phenacylthioimidazolinium fluoroborate (**5**), are described. Both substrates can be considered potential models for carboxy-biotin complexes. The reactivity of each of these substrates toward base has been evaluated. Treatment of **8** with 1 equiv of either sodium methoxide or sodium hydride yielded the bicyclic 2-benzoyl-3-hydroxy-5,6-dihydroimidazo[2,1-*b*]thiazole (**12**). Formation of **12** is believed to occur through an intramolecular cyclization reaction. When the *N*-methyl salt, **5**, is reacted under analogous conditions (1 equiv of sodium methoxide or potassium *tert*-butoxide), the carbomethoxy group is transferred, giving the neutral thioimidazoline, methyl 2-(1′-methyl-2′-imidazoline-2′-thiyl)-2-benzoylacetate (**13**). **13** can also be prepared by direct alkylation of *N*-methylimidazolidinethione (**16**) with methyl 2-bromo-2-benzoylacetate (**15**). Successful transfer of the carbomethoxy group in **5** and **8** appears to be at least partially dependent upon the presence or absence of a positive charge on the imidazoline ring. These results indicate that carbon dioxide transfer to an acceptor molecule in biotin dependent reactions should be facilitated by prior protonation of the biotin-CO_2 intermediate. Two mechanisms are discussed for biotin dependent carboxylases which are compatible with these results. One mechanism places the carboxy-biotin intermediate and the acceptor molecule in close proximity to one another; protonation of the intermediate then occurs prior to carbon dioxide transfer. Alternatively, a second pathway might exist for carboxylases, one in which the mobile biotin prosthetic group serves as a template for the carbon dioxide source and the acceptor molecule. In this hypothesis, the carbon dioxide group is transferred by way of an intramolecular mechanism.

Biotin (**1**) plays a pivotal role in numerous enzymatic carbon dioxide fixation reactions.[2] For example, it is an essential cofactor in the conversion of acetyl-CoA to malonyl-CoA,[2,3] propionyl-CoA to methylmalonyl-CoA,[2,4] β-methylcrotonyl-CoA to β-methylglutaconyl-CoA,[2,5] and the transcarboxylation of methylmalonyl-CoA and pyruvate to generate propionyl-CoA and oxaloacetate, respectively.[2,6]



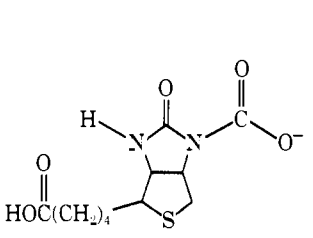
1

The chemical mechanisms of these carboxylations are not well understood at the molecular level. However, efforts aimed at the elucidation of the key role played by this coenzyme have led to the general acceptance of the following two-step reaction pathway for biotin dependent carboxylases.[2]

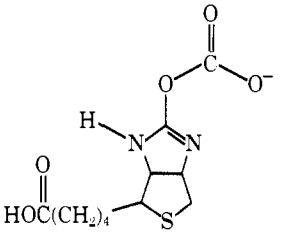
$$\text{enzyme-biotin} + HCO_3^- + \text{ATP} \xrightleftharpoons{M^{++}} \text{enzyme·biotin-}CO_2 + \text{ADP} + P_i \quad (1)$$

$$\text{enzyme·biotin·}CO_2 + \text{acceptor} \rightleftharpoons \text{acceptor·}CO_2 + \text{enzyme-biotin} \quad (2)$$

Each of the above reactions is believed to occur at a different site on the enzyme, with the mobile biotin prosthetic group transporting the active CO_2 from one site to the other.[2,6] Both 1′-*N*-carboxybiotin (**2**)[7] and its imino-anhydride isomer (**3**)[8]



2



3

have been suggested for the structure of the enzyme-biotin-CO_2 intermediate. An analogous mechanism has also been proposed for biotin-dependent transcarboxylases.[6,9]

Considerable controversy still exists concerning the mechanism of reactions 1 and 2, and the precise nature of the enzyme-biotin-CO_2 intermediate.[2] Model reactions directed toward the elucidation of the conditions required for the facile yet selective transfer[10-13] of carbon dioxide required in these transformations have been performed.[8,14-18] All attempts, however, to transfer a carbon dioxide moiety from known carboxylated-biotin derivatives to suitable model acceptors (carboxylation at carbon) have been uniformly disappointing. In all cases studied, carbon dioxide transfer to an incipient carbanion does not occur.

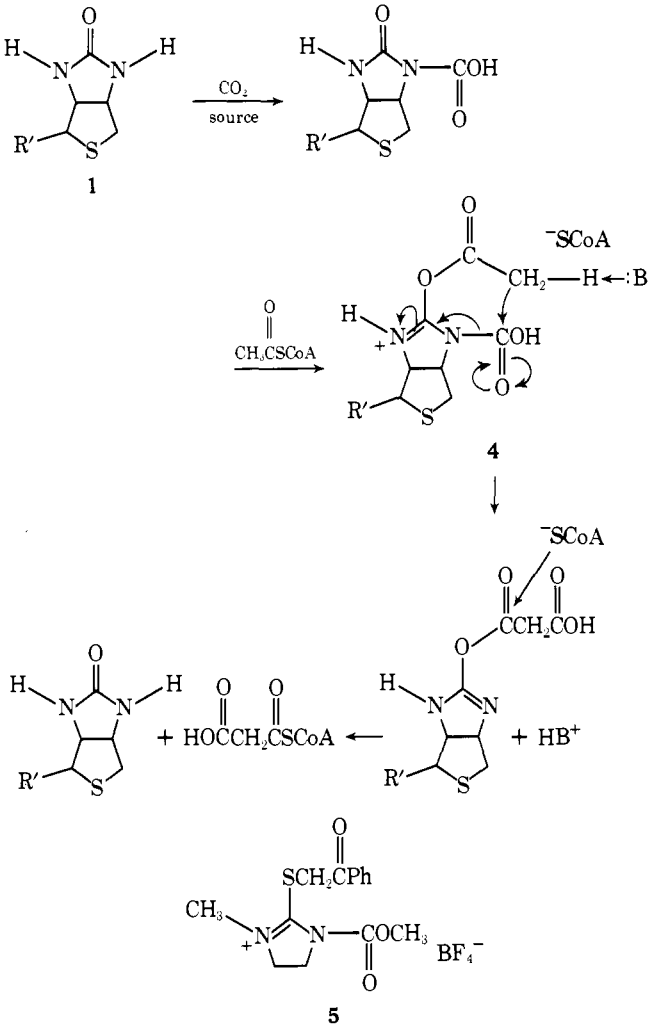
In light of the existing available evidence, it occurred to us that an alternate mechanistic pathway might exist for biotin-dependent reactions, one in which biotin serves as a template for the two reactants, the carbon dioxide source and the acceptor molecule. This hypothesis is depicted in Scheme I using acetyl-CoA carboxylase as an example. We wish to report some tests of this intramolecular mechanism.

Discussion and Results

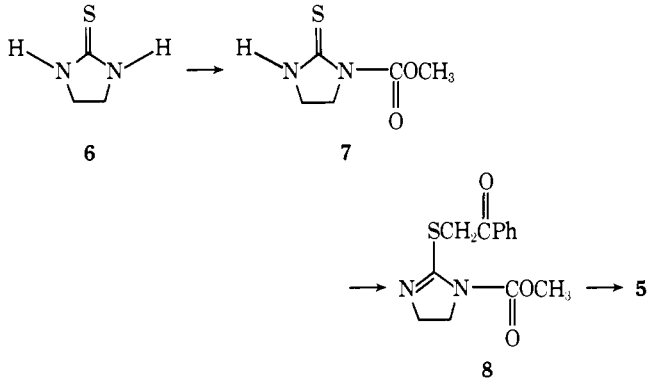
Selection of a Model. The postulated active species in Scheme I is the protonated substrate **4**. A simple test to verify the chemical feasibility of this hypothesis required a chemical model for this intermediate. In devising a suitable substrate to test our mechanism, we considered two structural features to be essential in any working model. First, the acceptor molecule and the carbon dioxide group should be in close proximity to one another, and second, a positive charge should exist on the imidazoline ring system to enhance the reactivity of the carbon dioxide moiety. The model compound that we initially chose to prepare was *N*-methyl-*N*′-carbomethoxy-2-phenacylthioimidazolinium fluoroborate (**5**). Scheme II outlines the synthetic sequence which was utilized for the preparation of **5**.

The acceptor molecule in our model is the covalently attached phenacyl group. The desired carbomethoxyl transfer is envisioned to occur rapidly in this system through an intramolecular pathway. The positive charge on the ring should simultaneously enhance the electrophilicity of the carbome-

Scheme I



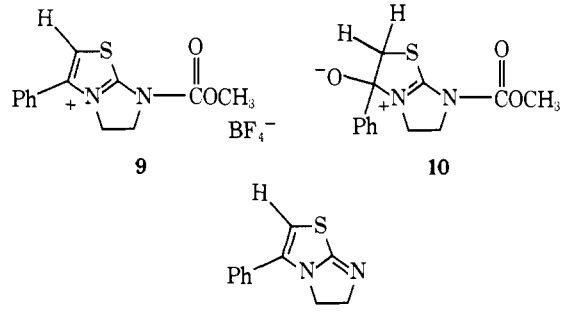
Scheme II



thoxy carbonyl carbon atom and decrease the basicity of the departing imidazoline ring. Comparison of our model **5** with the postulated key intermediate **4** reveals that four important modifications in the molecular framework have been made. The specific nature and purpose of these modifications is as follows: (1) All readily dissociable hydrogen atoms were replaced by methyl groups, in order to evaluate the importance of a positive charge specifically located on the ring. (2) The lower thiophene ring was not included in our model simply because it was felt that its presence or absence should not be a necessary requirement for carbon dioxide transfer.[2,14,15] (3) A sulfur atom was substituted for the oxygen at the 2 position of the starting imidazolidone. This replacement allowed the facile preparation of the neutral precursor to our key intermediate, **8**. (4) An additional methylene unit was inserted at the 2′-thio position in **8**. Without the insulating effect of this saturated carbon atom, the corresponding cyclic isothiourea system undergoes a S → N four-center acyl migration.[8,19]

Syntheses of 5 and 8. Preparation of the desired *N*-methyl-*N*′-carbomethoxy-2-phenacylthioimidazolinium fluoroborate (**5**) was accomplished in three steps. Starting with commercially available imidazolidinethione (**6**), the corresponding *N*-carbomethoxyimidazolidinethione (**7**) was prepared by the addition of methyl chloroformate to a dichloromethane solution containing the cyclic thiourea and pyridine. Subsequent alkylation of this compound with α-bromoacetophenone in the presence of triethylamine gave the neutral *N*-carbomethoxy-2-phenacylthioimidazoline (**8**) in 68% yield. Alternatively, this substrate could be prepared by direct alkylation of **7** with α-bromoacetophenone in acetone. The neutral compound, **8**, was then liberated from its hydrobromide salt by washing with aqueous 5% sodium bicarbonate. The proton nuclear magnetic resonance spectrum of **8** surprisingly showed a sharp singlet at δ 3.87 for the ethylene unit, rather than the expected A_2B_2 pattern. This observation may be rationalized in terms of exchange processes occurring in solution or an accidental magnetic equivalence of the four-ring protons. The signal remained a sharp singlet in a variety of solvents and at different temperatures. Examination of the ^1H decoupled carbon-13 NMR spectrum, on the other hand, revealed four carbon resonances (40.3, 47.9, 53.6, 54.2 ppm) in the 40–55-ppm region. In the ^1H coupled carbon-13 spectrum, three of these resonances gave rise to triplets (40.3 ($J_{^{13}C-H}$ = 140 Hz), 47.9 ($J_{^{13}C-H}$ = 150 Hz), 54.2 ($J_{^{13}C-H}$ = 145 Hz)), indicating the presence of three methylene carbons. The fourth resonance at 53.6 ppm (a quartet, $J_{^{13}C-H}$ = 145 Hz) was assigned to the methoxy carbon. From the above results it appears that the singlet observed in the proton spectrum can be ascribed to an accidental equivalence of the protons within the ethylene unit. Incidentally we note that from a comparison of the carbon chemical shift positions of **8** to those of other recently synthesized substituted thioimidazolines, the resonances at 47.9 and 54.2 ppm can easily be assigned to the imidazoline ring carbon atoms.[20,21]

Completion of the synthetic sequence required the *N*-alkylation of **8**. Methylation of **8** in nitromethane with 1.5 equiv of trimethyloxonium fluoroborate[22] yielded the desired *N*-methyl-*N*′-carbomethoxy-2-phenacylthioimidazolinium fluoroborate (**5**) in 61% yield along with a 27% yield of the bicyclic *N*-carbomethoxy-3-phenyl-5,6-dihydroimidazo[2,1-*b*]-thiazolium fluoroborate (**9**). The spectral properties and its mode of formation support the structure assigned to **5**. The

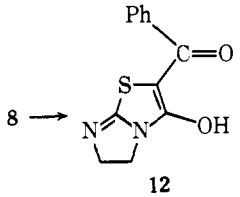


infrared spectrum showed strong carbonyl bands at 1760 and 1680 cm^{-1}. In the NMR the *N*-methyl, carbomethoxy methyl, and phenacyl methylene protons appear as singlets at δ 3.52, 3.84, and 5.02, respectively. The formation of **9** can be envisioned to occur by the initial tautomerism of **8** to give the isomeric *N*-carbomethoxy-3-oxido-3-phenyl-2,3,5,6-tetrahydroimidazo]2,1-*b*]thiazolium (**10**). Subsequent methylation at oxygen followed by rearomatization by loss of methanol would give the bicyclic thiazolium salt, **9**. Analogously, it has

been reported that treatment of *N*-methyl-2-phenacylthioimidazoline with hydrobromic acid gave the corresponding *N*-methyl-3-phenyl-5,6-dihydroimidazo[2,1-*b*]thiazolium bromide.[23] The structure of **9** is supported by its spectral properties. In addition to a single carbonyl stretch at 1710 cm^{-1} in the infrared spectrum, the proton NMR shows a sharp singlet for the lone thiazolium ring proton at 7.27.[24] Further structure proof for **9** is obtained from the treatment of the bicyclic salt, **9,** with 1 equiv of sodium methoxide in methanol. Methanolysis of **9** gave a 72% yield of the known 3-phenyl-5,6-dihydroimidazo[2,1-*b*]thiazole (**11**).[25]

Reactivity of 5 and 8. The thioimidazolinium salt, **5,** and its neutral precursor, **8,** are both active methylene compounds. In both substrates, a base-catalyzed, intramolecular transfer of a carbomethoxy group could occur. We investigated the reactivity of each of these substrates toward base, in order to determine the feasibility of the mechanism outlined in Scheme I and to assess the importance of the positive charge for carbon dioxide transfer.

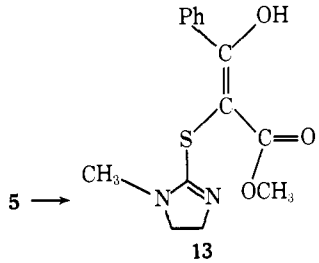
Treatment of **8** in dichloromethane with 1 equiv of 0.5 M sodium methoxide–methanol solution yielded the bicyclic 2-benzoyl-3-hydroxy-5,6-dihydroimidazo[2,1-b]thiazole (**12**) in 71% yield. **12** was also obtained when **8** was treated with 1



12

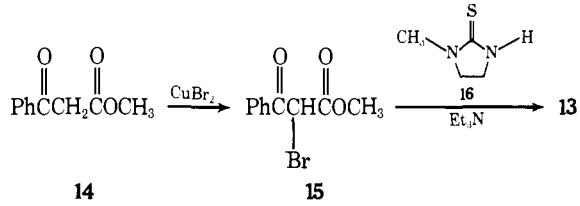
equiv of sodium hydride in benzene. The structure of **12** was supported by its spectral properties. The mass spectrum showed the expected parent peak at *m/e* 246. The presence of the enolic structure rather than the tautomeric β-ketoester was indicated by an infrared band at 1645 cm^{-1}. The proton NMR spectrum was taken in 1 N NaOD–D_2O, since **12** was virtually insoluble in most organic solvents. The NMR spectrum showed the expected A_2B_2 multiplet at δ 3.38–4.32 for the ethylene unit of the imidazoline ring as well as a singlet at δ 7.40 for the aromatic protons. The bicyclic adduct, **12,** undergoes alkaline hydrolysis to give benzoic acid and imidazolidone upon prolonged treatment with aqueous 1 N sodium hydroxide.

When, however, the *N*-methylthioimidazolinium salt (**5**) was treated with 1 equiv of sodium methoxide under the identical conditions used for its neutral precursor, **8,** a 47% yield of the desired methyl 2-(1′-methyl-2′-imidazolin-2′-thiyl)-2-benzoylacetate (**13**) was obtained. In a more meaningful experiment, when potassium *tert*-butoxide was used as the base, the same product was isolated. No ester interchange of the carbomethoxy group by the base was noted. High-resolution mass spectrometry showed a molecular ion at 292.0887 (calcd for $C_{14}H_{16}N_2O_3S$, 292.0881) and fragment ions at *m/e* 233 and 187 corresponding to the loss of a carbomethoxy and a benzoyl unit, respectively. The infrared spectrum showed the expected strong β-keto ester enolic band at 1670 cm^{-1}. The imidazoline ethylene unit protons appeared in the proton NMR as a singlet at δ 3.56, with the *N*-methyl protons and carbomethoxy protons appearing at δ 3.17 and 3.65, respectively.



13

Although the spectral properties obtained for **13** were in agreement with the proposed structure, we felt that an alternate synthesis of this compound would increase our confidence in our structural assignment. Accordingly, starting with methyl benzoylacetate (**14**)[26] the corresponding 2-bromo derivative **15** was prepared by the heterogeneous bromination method

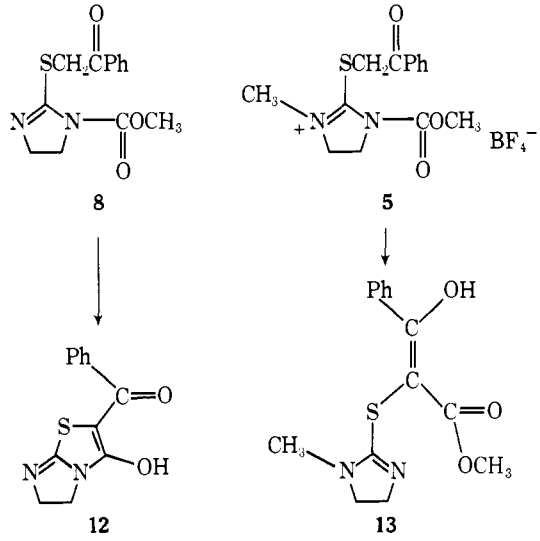


14 **15**

of King and Ostrum.[27] Treatment of this ester with cupric bromide in chloroform–ethyl acetate afforded **15** in 37% distilled yield. Mass spectrometry confirmed a formula of $C_{10}H_9O_3Br$ for **15**. Molecular ions at *m/e* 256 and 258 were obtained in approximately a 1:1 ratio. Surprisingly, the infrared spectrum showed two carbonyl bands at 1760 and 1740 cm^{-1} rather than the corresponding β-keto ester enolic absorption band. The corresponding NMR spectrum exhibited a peak at δ 5.65 for the lone methine hydrogen. Finally, treatment of **15** with *N*-methylimidazolidinethione (**16**)[28] and 2 equiv of triethylamine gave a yellow, crystalline compound whose physical and spectral properties were identical with **13**.

Conclusions

In the conversion of **8** into **5** a methyl group along with a positive charge has been introduced into the imidazoline ring. In the neutral system, **8,** expulsion of methoxide is energetically more favorable than the corresponding release of a thioimidazoline anion, a result very reminiscent of earlier carboxybiotin model studies.[16] This problem does not exist in **5,** where a carbomethoxy group can be transferred to an incipient carbanion upon treatment with base, with the concomitant release of a neutral thioimidazoline, **13**. It appears that successful transfer of the carbomethoxy group in **5** and **8** is at least partially dependent upon the presence or absence of a positive charge on the imidazoline ring.



8 **5**

12 **13**

These preliminary studies clearly demonstrate that carbon dioxide transfer should be facilitated in carboxy-biotin intermediates by the prior protonation of the imidazolidone ring. The results reported herein can be viewed favorably in terms of the intramolecular mechanism set forth in Scheme I for biotin-dependent carboxylations. Alternatively, these results can be interpreted in terms of the traditionally accepted bi-

molecular mechanism for carbon dioxide transfer to the acceptor molecule. It is well known that enzymatic processes proceed within an enzyme-substrate complex, where the reactants are carefully ordered. For this reason many mechanistic studies of known enzymatic bimolecular reactions have been performed on simple models in which both reactants are covalently attached to the same molecule.[8,29,30] In this regard, our experimental results can also be construed in terms of a bimolecular mechanism. One mechanism places the carboxy-biotin intermediate and the acceptor molecule in close proximity to one another; protonation of the intermediate then occurs prior to carbon dioxide transfer. In both mechanisms, however, the biotin prosthetic group is envisioned to transport the active CO_2 group from one binding site to the other.[2,6,9]

At the present time, we are trying to independently evaluate the importance of protonated carboxy-biotin intermediates for carbon dioxide transfer, by way of additional model compounds which do not have the acceptor molecule covalently attached to the ring. We are also in the process of preparing more refined model compounds which better reflect the key protonated intermediate, **4**, to further test the mechanism outlined in Scheme I.

Experimental Section

General. Melting points (mp) were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra (ir) were run on a Perkin-Elmer Model 700 and 237B spectrometer and calibrated against the 1601-cm^{-1} band of polystyrene. Proton nuclear magnetic resonance (NMR) spectra were recorded on a Varian Associates Model T-60 instrument. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were determined at the Baylor College of Medicine, on a Varian Associates Model XL100-15 spectrometer, equipped with a Nicolet Technology Corporation TT-100 data system through the courtesy of Dr. Roger Knapp. Chemical shifts are expressed in parts per million relative to Me_4Si, and coupling constants (J values) in hertz (Hz). Spin multiplicities are indicated by the symbols: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass spectral (MS) data were obtained at an ionizing voltage of 70 eV on a Hitachi Perkin-Elmer Model RMU-6H mass spectrometer. High resolution mass spectra were performed by Dr. James Hudson at the Department of Chemistry, Rice University on a CEC21-110B double focusing magnetic sector spectrometer at 70 eV. Exact masses were determined by peak matching. Elemental analyses were obtained at Spang Microanalytical Laboratories, Ann Arbor, Mich.

The solvents and reactants were of the best commercial grade available and were used without further purification unless noted. When dry solvents were required, dichloromethane was distilled from phosphorus pentoxide, dimethoxyethane was distilled from lithium aluminum hydride, and anhydrous ether was stored over sodium metal. All reactions were run under nitrogen, and all glassware were dried before use.

Preparation of *N*-Carbomethoxyimidazolidinethione (*7*). To a mixture of **6** (22.5 g, 220 mmol) and pyridine (18.2 g, 230 mmol) in dichloromethane (500 ml), methyl chloroformate (17.1 ml, 220 mmol) was added dropwise. The solution was gently refluxed overnight and then washed with water (2 × 100 ml). The organic layer was dried over sodium sulfate and then concentrated in vacuo. Purification of **7** was accomplished by reprecipitation from chloroform–hexanes: yield 11.13 g (32%); mp 156–158 °C; ir (KBr) 1750, 1675 cm^{-1}; NMR ($CDCl_3$) δ 3.37–4.32 (m, 4 H), 3.82 (s, 3 H), 7.78 (broad s, 1 H); MS *m/e* (rel %) 160 (60), 128 (46), 102 (61), 60 (100).

Anal. Calcd for $C_5H_8N_2O_2S$: C, 37.49; H, 5.03; N, 17.49. Found: C, 37.57; H, 4.95; N, 17.45.

Preparation of *N*-Carbomethoxy-2-phenacylthioimidazoline (*8*) with Triethylamine. To a stirred dichloromethane solution (250 ml) containing **7** (6.40 g, 40 mmol) and triethylamine (7.12 g, 80 mmol), 9.56 g of α-bromoacetophenone (48 mmol) was added all at once. After stirring for 72 h at room temperature, the solution was consecutively washed with aqueous 5% sodium bicarbonate (2 × 100 ml) and water (100 ml). The dichloromethane solution was then dried over sodium sulfate and evaporated in vacuo. Purification of **8** was accomplished by reprecipitation from chloroform–hexanes: yield 7.57 g (68%); mp 153.5–155.5 °C; ir (KBr) 1715, 1670, 1580 cm^{-1}; NMR ($CDCl_3$) δ 3.78 (s, 3 H), 3.87 (s, 4 H), 4.54 (s, 2 H), 7.31–8.12 (m, 5 H); ^{13}C NMR ($CDCl_3$) (ppm) 40.3 (t, $J_{^{13}C-H}$ = 140 Hz), 47.9 (t, $J_{^{13}C-H}$ = 150 Hz), 53.6 (q, $J_{^{13}C-H}$ = 145 Hz), 54.2 (t, $J_{^{13}C-H}$ = 145 Hz), 128.7, 128.9, 133.8, 136.4, 152.4. The remaining quaternary carbons were not detected under the experimental conditions used. MS *m/e* (rel %) 278 (14), 134 (47), 105 (100), 77 (75).

Anal. Calcd for $C_{13}H_{14}N_2O_3S$: C, 56.10; H, 5.07; N, 10.07. Found: C, 56.12; H, 5.04; N, 10.10.

Preparation of *N*-Carbomethoxy-2-phenacylthioimidazoline (*8*) without Triethylamine. To a stirred acetone solution (200 ml) containing α-bromoacetophenone (4.00 g, 20 mmol), an acetone solution (400 ml) containing **7** (3.20 g, 20 mmol) was added dropwise. During the addition a white flocculent solid precipitated. After the addition was complete, the mixture was stirred for 1 h and filtered. The hydrobromide salt was collected and then added to an Erlenmeyer flask containing dichloromethane (200 ml). Liberation of **8** was accomplished by the addition of an aqueous 5% sodium bicarbonate (75 ml) and then washing the dichloromethane layer successively with aqueous 5% sodium bicarbonate (75 ml) and water (75 ml). The organic layer was dried over sodium sulfate and evaporated in vacuo. Reprecipitation of **8** from chloroform–hexanes gave 2.71 g (49%), mp 151–153 °C.

Preparation of *N*-Methyl-*N'*-carbomethoxy-2-phenacylthioimidazolinium Fluoroborate (*5*) and *N*-carbomethoxy-3-phenyl-5,6-dihydroimidazo[2,1-*b*]thiazolium Fluoroborate (*9*). Trimethyloxonium fluoroborate[22] (2.67 g, 18 mmol) in 20 ml of freshly distilled nitromethane was added dropwise to a stirred slurry containing 3.15 g of **8** (12 mmol) in 10 ml of nitromethane. The light red solution was allowed to stir at room temperature overnight and the products (4.32 g) were isolated by precipitation with ether. Purification was accomplished by trituration of the residue with freshly distilled dimethoxyethane (2 × 15 ml) and then selectively extracting **5** from the remaining residue with chloroform. Evaporation of the chloroform layer in vacuo gave the fluoroborate salt, **5**. Reprecipitation of the white solid with ether from a 1:1 nitromethane–dichloromethane solution gave the purified product: yield 2.80 g (61%); mp 132–134.5 °C; ir ($CHCl_3$) 1760, 1680, 1065-1010 cm^{-1}; NMR (CD_3CN) δ 3.52 (s, 3 H), 3.84 (s, 3 H), 4.07–4.24 (m, 4 H), 5.02 (s, 2 H), 7.28–8.17 (m, 5 H).

Anal. Calcd for $C_{14}H_{17}N_2O_3SBF_4$: C, 44.23; H, 4.51; N, 7.37. Found: C, 44.20; H, 4.36; N, 7.40.

The remaining chloroform insoluble, white solid, **9**, was purified by reprecipitation with ether from a 1:1 nitromethane–dichloromethane solution: yield 1.11 g (27%); mp 219–221 °C; ir (KBr) 1710, 1535, 1150–1025 cm^{-1}; NMR (CD_3CN) δ 3.95 (s, 3 H), 4.64 (s, 4 H), 7.27 (s, 1 H), 7.36 (s, 5 H).

Anal. Calcd for $C_{13}H_{13}N_2O_2SBF_4$: C, 44.85; H, 3.76; N, 8.05. Found: C, 44.94; H, 3.80; N, 8.08.

Preparation of 3-Phenyl-5,6-dihydroimidazo[2,1-*b*]thiazole (*11*). **9** (0.70 g, 2 mmol) was dissolved in 4 ml (2 mmol) of 0.5 M sodium methoxide in methanol and the solution allowed to stand at room temperature overnight. The solution was then adjusted to pH 9 by the gradual addition of aqueous 1 N hydrochloric acid, and then concentrated in vacuo. The residue was dissolved in water (20 ml) and then extracted with dichloromethane (2 × 20 ml). The combined organic layer extracts were washed with water (20 ml), dried over sodium sulfate, and evaporated in vacuo to give 0.29 g (72% yield) of **11**: mp 110–113.5 °C (lit.[25] mp 112–113 °C); ir (KBr) 1600 cm^{-1}; NMR ($CDCl_3$) δ 3.52–4.40 (m, 4 H), 5.64 (s, 1 H), 7.37 (s, 5 H); MS *m/e* (rel %) 202 (100), 201 (69), 147 (12), 142 (22), 105 (95), 102 (47), 100 (44), 99 (50), 77 (18).

Preparation of 2-Benzoyl-3-hydroxy-5,6-dihydroimidazo[2,1-*b*]-thiazole (*12*) from Sodium Methoxide. To a stirred dichloromethane solution (30 ml) containing **8** (1.16 g, 4 mmol), 8 ml of 0.5 M sodium methoxide in methanol (4 mmol) was added. The solution was allowed to stir at room temperature overnight during which time a copious precipitate formed. The solid was collected and then successively triturated with water and acetone. Further purification was accomplished by reprecipitation of the product with aqueous 5% hydrochloric acid from a 1 N aqueous sodium hydroxide solution: yield 0.70 g (71%); mp 237–239.5 °C; ir (KBr) 1645, 1555 cm^{-1}; NMR (1 N NaOD–D_2O) δ 3.38–4.32 (m, 4 H), 4.75 (s, HOD), 7.40 (s, 5 H); MS *m/e* (rel %) 246 (26), 168 (16), 105 (81), 77 (100).

Anal. Calcd for $C_{12}H_{10}N_2O_2S$: C, 58.52; H, 4.09; N, 11.38. Found: C, 58.50; H, 4.12; N, 11.38.

Preparation of 2-Benzoyl-3-hydroxy-5,6-dihydroimidazo[2,1-*b*]thiazole (*12*) from Sodium Hydride. Sodium hydride (50% mineral oil dispersion) (0.46 g, 9.6 mmol) was washed successively three times with 10 ml of dry benzene and then an additional 50 ml of benzene was added to the reaction vessel. **8** (2.22 g, 8 mmol) was added to the stirred sodium hydride slurry. The mixture was heated at 40 °C overnight, and then 4 ml of water was cautiously added. The basic solution was neutralized with aqueous 5% hydrochloric acid, and the precipitate was collected and triturated with water and acetone. The solid was further purified by reprecipitation with aqueous 5% hydrochloric acid from an aqueous 1 N sodium hydroxide solution containing the compound: yield 1.48 g (75%); mp 237.5–239.5 °C.

Basic Hydrolysis of 2-Benzoyl-3-hydroxy-5,6-dihydroimidazo-[2,1-*b*]thiazole (*12*). **12** (0.15 g, 0.6 mmol) was dissolved in 2 ml (2 mmol) of aqueous 1 N sodium hydroxide, and the solution was allowed to stand at room temperature for 1 month. An NMR of the basic solution after 1 month showed that hydrolysis was complete. Acidification of the solution with aqueous 5% hydrochloric acid to approximately pH 3 resulted in the precipitation of a white solid. The solid was filtered, collected, and dried (0.04 g). The material melted at 120–122.5 °C and was identified as benzoic acid (54% yield).[31] The pH of the remaining aqueous solution was then raised to approximately pH 8 with aqueous 1 N sodium hydroxide, then evaporated to dryness in vacuo. The residue was triturated with dichloromethane (2 × 25 ml), and then the organic layer was concentrated in vacuo to give a white solid (0.041 g) (79% yield). The solid melted at 129–131 °C and was identified as 2-imidazolidone.[31]

Preparation of Methyl 2-(1′-Methyl-2′-imidazoline-2′-thiyl)-2-benzoylacetate (*13*) from Sodium Methoxide. To a stirred dichloromethane solution (25 ml) containing 1.05 g of **5** (2.8 mmol), 5.5 ml of 0.5 M sodium methoxide in methanol (2.8 mmol) was added. With the addition of base the solution turned yellow and a small amount of a white granular solid precipitated. The reaction mixture was allowed to stir for 20 min at room temperature and filtered, and the organic layer was evaporated in vacuo. The reaction product was then taken up in 7 ml of chloroform and quickly reprecipitated with 7 ml of hexanes. The yellow precipitate was collected to give 0.38 g of **13** (47%): mp 146–148 °C; ir (KBr) 1670, 1625, 1560 cm^{-1}; NMR ($CDCl_3$) δ 3.17 (s, 3 H), 3.56 (s, 4 H), 3.65 (s, 3 H), 7.25–8.12 (m, 5 H), 8.80 (broad s, 1 H); MS *m/e* (rel %) 292 (15), 233 (5), 187 (37), 105 (100), 77 (74).

Anal. Calcd for $C_{14}H_{16}N_2O_3S$ (292.0881): C, 57.51; H, 5.52; N, 9.58. Found (292.0887): C, 57.49; H, 5.68; N, 9.55.

Preparation of Methyl 2-(1′-Methyl-2′-imidazoline-2′-thiyl)-2-benzoylacetate (*13*) from Potassium *tert*-Butoxide. Potassium *tert*-butoxide (0.17 g, 1.5 mmol) in 10 ml freshly distilled *tert*-butyl alcohol was added dropwise to a stirred solution of **5** (0.57 g, 1.5 mmol) in 20 ml of distilled dichloromethane. The yellow mixture was stirred for 10 min at room temperature. The mixture was filtered and the filtrate evaporated in vacuo. The residue was taken up in chloroform and **13** reprecipitated with ether: yield 0.14 g (32%); mp 147–148 °C.

Preparation of Methyl 2-Bromo-2-benzoylacetate (*15*). Methyl 2-bromo-2-benzoylacetate (**15**) was prepared according to the method of King and Ostrum[27] by the gradual addition of **14**[26] (4.48 g, 25 mmol) in hot chloroform (53 ml) to a stirred mixture of finely granulated cupric bromide (22.32 g, 100 mmol) in refluxing ethyl acetate. The mixture was allowed to reflux for 2 h and then filtered. The light green organic layer was treated with Norit, filtered, and then concentrated in vacuo. Distillation of the remaining oil at 112–114 °C (0.2 mm) gave 2.40 g (37% yield) of **15**: ir (neat) 1760, 1740, 1680 cm^{-1}; NMR ($CDCl_3$) δ 3.76 (s, 3 H), 5.65 (s, 1 H), 7.22–8.08 (m, 5 H); MS *m/e* (rel %) 258 (0.8), 256 (0.8), 227 (0.5), 225 (0.5), 177 (8), 105 (100), 77 (43). Mol wt 255.9744 (calcd for $C_{10}H_9BrO_3$, 255.9733).

Preparation of Methyl 2-(1′-Methyl-2′-imidazolin-2′-thiyl)-2-benzoylacetate (*13*) from (*15*). To a stirred dichloromethane solution (25 ml) containing *N*-methylimidazolidinethione (**16**)[28] (0.46 g, 4 mmol) and triethylamine (0.81 g, 8 mmol), **15** (1.13 g, 4.4 mmol) was added all at once. The solution was allowed to stir for 60 h at room temperature and then consecutively washed with aqueous 5% sodium bicarbonate (2 × 10 ml) and water (10 ml). The organic layer was dried over sodium sulfate and evaporated in vacuo. Further purification was accomplished by reprecipitation from chloroform–hexanes to give 0.40 g (34% yield) of **13**: mp 147–148 °C.

Acknowledgment. We would like to thank the National Institutes of Health, The Robert A. Welch Foundation, and The Research Corporation for their support of our work.

References and Notes

(1) Presented at the 170th National Meeting of the American Chemical Society, Chicago, Ill., August, 1975, Abstract Orgn. 11.
(2) (a) J. Moss and M. D. Lane, *Adv. Enzymol. Relat. Areas Mol. Biol.*, **35**, 321 (1971); (b) J. Knappe, *Annu. Rev. Biochem.*, **39**, 757 (1970); (c) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms", Vol. 11, W. A. Benjamin, New York, N.Y., 1966, Chapter 11; see also these references for a review of the earlier literature.
(3) R. B. Guchhait, S. E. Polakis, P. Dimroth, E. Stoll, J. Moss, and M. D. Lane, *J. Biol. Chem.*, **249**, 6633 (1974).
(4) Y. Kaziro, E. Leone, and S. Ochoa, *Proc. Natl. Acad. Sci. U.S.A.*, **46**, 1319 (1960).
(5) J. Knappe, H. G. Schlegel, and F. Lynen, *Biochem. Z.*, **335**, 101 (1961); N. L. Edson, *Biochem. J.*, **29**, 2498 (1935); Y. Ikebe, *J. Biochem.*, **17**, 275 (1933), and references therein.
(6) H. G. Wood, "The Enzymes", Vol. VI, 3rd ed, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1972, p 83; H. G. Wood and M. F. Utter, *Essays Biochem.*, 1, 1 (1965); and S. H. G. Allen, R. W. Kellermeyer, R. L. Stjernholm, and H. G. Wood, *J. Bacteriol.*, **87**, 171 (1964).
(7) J. Knappe, E. Ringelman, and F. Lynen, *Biochem. Z.*, **335**, 168 (1961).
(8) A. F. Hegarty and T. C. Bruice, *J. Am. Chem. Soc.*, **92**, 6561, 6568, 6575 (1970); A. F. Hegarty, R. F. Pratt, T. Giudici, and T. C. Bruice, *ibid.*, **93**, 1428 (1971); R. F. Pratt and T. C. Bruice, *ibid.*, **94**, 2823 (1972); R. F. Pratt and T. C. Bruice, *Biochemistry*, **10**, 3178 (1971); T. C. Bruice and A. F. Hegarty, *Proc. Natl. Acad. Sci. U.S.A.*, **65**, 805 (1970).
(9) D. B. Northrop, *J. Biol. Chem.*, **244**, 5808 (1969).
(10) D. Arigoni, F. Lynen, and J. Retey, *Helv. Chim. Acta*, **49**, 311 (1966).
(11) D. J. Prescott and J. L. Rabinowitz, *J. Biol. Chem.*, **243**, 1551 (1968).
(12) I. A. Rose, *J. Biol. Chem.*, **245**, 6052 (1970).
(13) Y. A. Cheung, C. H. Fung, and C. Walsh, *Biochemistry*, **14**, 2981 (1975).
(14) M. Caplow and M. Yager, *J. Amer. Chem. Soc.*, **89**, 4513 (1967); M. Caplow, *ibid.*, **87**, 5774 (1965).
(15) H. J. Schaeffer and P. S. Bhargava, *J. Pharm. Sci.*, **51**, 1116 (1962); **53**, 137 (1964).
(16) J. Knappe and F. Lynen, *Colloq. Ges. Physiol. Chem.*, 265 (1963).
(17) J. Knappe, *Int. Congr. Biochem., Proc. 6th*, 355 (1965).
(18) Y. Akasaki and A. Ohno, *J. Am. Chem. Soc.*, **96**, 1957 (1974).
(19) H. Kohn and M. J. Cravey, to be published.
(20) L. M. Jackman and T. Jen, *J. Am. Chem. Soc.*, **97**, 2811 (1975).
(21) H. Kohn, M. J. Cravey, and M. R. Willcott, to be published.
(22) H. Meerwein, *Org. Synth.*, **46**, 120 (1966).
(23) R. S. Shadbolt, *J. Chem. Soc. C*, 1669 (1971).
(24) P. Haake, L. P. Bausher, and W. B. Miller, *J. Am. Chem. Soc.*, **91**, 1113 (1969).
(25) C. J. Sharpe, R. S. Shadbolt, A. Ashford, and J. W. Ross, *J. Med. Chem.*, **14**, 977 (1971).
(26) S. M. McElvain and G. R. McKay, *J. Am. Chem. Soc.*, **78**, 6086 (1956).
(27) L. C. King and G. K. Ostrum, *J. Org. Chem.*, **29**, 3459 (1964).
(28) A. F. McKay and M. E. Kreling, *J. Org. Chem.*, **22**, 1581 (1957).
(29) R. Breslow and C. McAllister, *J. Am. Chem. Soc.*, **93**, 7096 (1971).
(30) M. I. Page and W. P. Jencks, *J. Am. Chem. Soc.*, **94**, 8818 (1972).
(31) The experimentally obtained material proved to be identical with commercially available material (Aldrich Co.) with respect to physical and spectral properties.