Model Studies on the Mechanism of Biotin-Dependent Carboxylations. 2. Site of Protonation vs. CO_2 Transfer

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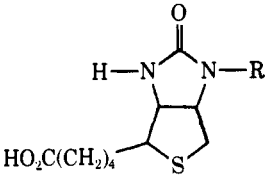
Abstract: Three irreversibly acidified model compounds (**6–8**) of *N'*-carboxybiotin (**2**) have been prepared to assess the importance of prior protonation of the biotin ring system on the CO_2-transfer potential of the *N'*-carboxy group. Substrates **6** and **7** can be considered model compounds of *N'*-carboxybiotin (**2**) in which protonation has occurred at the ureido carbonyl oxygen atom. Conversely, compound **8** was synthesized to evaluate the CO_2-transfer potential of the *N'*-carboxy group, if protonation occurred at the *N'*-nitrogen atom. The reactivity of each substrate with nucleophiles has been evaluated. Of these three compounds, only **8** led to efficient transfer to the carbomethoxy group upon treatment with nitrogen-containing nucleophiles (morpholine, cyclohexylamine, and diisopropylamine). With smaller nucleophiles (i.e., water, methanol) reaction was centered at the ring C-2 position. Correspondingly, treatment of compound **6** with nucleophiles (i.e., alcohols, amines) led to products which can be explained in terms of two competing reactions. One pathway involves initial attack of the nucleophile at the C-2 position of the imidazolinium cation (an $A_{AC}2$ process) to give a tetrahedral intermediate which then undergoes bond cleavage in either of two directions. The competing pathway observed was an irreversible S_N2 displacement reaction (an $A_{AL}2$ process) at the methylene position of the O-alkyl side chain. Factors are presented which account for the overall product distribution obtained from these reactions. Finally, the products obtained from the treatment of compound **7** with nucleophiles (i.e., alcohols, amines) could be accounted for solely by reactions which occurred at the C-2 position of the ring (an $A_{AC}2$ process). The corresponding S_N2 pathway is not a viable route for this substrate. The significance of these results to the mechanism of action of biotin is discussed.

The coenzyme biotin (**1**) plays an indispensable role in numerous naturally occurring carboxylation reactions.[3] Detailed



1, R = H
2, R = CO_2^-

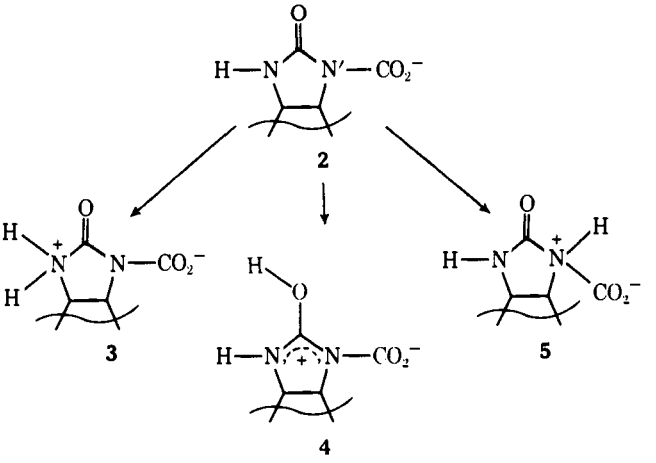
studies on the requirements needed for these transformations have led to the general acceptance of the following two-step reaction pathway for carboxylases:[3]

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

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

Strong support for the intermediacy of a species of general structure **2**[4] as the active carboxy donor has been provided by Lane and co-workers.[5] This result is noteworthy in light of previous model studies, which have shown that the susceptibility of the *N'*-carboxy group to nucleophilic attack is very small.[6-12] Accordingly, considerable effort has been expended on elucidating the pathway for the transfer of the carbon dioxide moiety to the biological acceptor molecule (reaction 2).[13a,14]

It has been suggested by us,[13a,b] as well as by others,[7b,15,16] that the CO_2-transfer step should be assisted by prior protonation of the biotin ring system by an acid on the enzyme surface. Protonation can theoretically occur at any one of three distinctly different basic sites: the two urea nitrogens and the urea carbonyl oxygen. Of these positions, protonation at either the ring carbonyl oxygen atom or the N' position should facilitate the second partial reaction (eq 2). If protonation occurs at the former site (**2** → **4**) in the enzymatic process, CO_2 transfer to an incipient carbanion or urea molecule should occur rapidly with the release of a neutral biotin molecule. This position has been demonstrated to be the thermodynamically most basic site on the imidazolidone ring in biotin (**1**).[17] On the other hand, protonation at the N' position (**2** → **5**) leads to a species in which there is a positive charge localized adjacent to the *N'*-carboxy group, thereby further activating this group toward nucleophilic attack. Additional consideration of this weakly basic site is prompted by the observation of Mildvan and co-workers[16] on the proximity and spatial arrangement of propionyl-CoA and pyruvate on the biotin-dependent enzyme, transcarboxylase. Information garnered from electron paramagnetic resonance and nuclear magnetic studies led to the suggestion that enzymatic protonation of the N' position of carboxybiotin (**2**) may precede the transfer of the carbon dioxide moiety to pyruvate.



Significantly, the effect of protonation of the imidazolidone ring on the CO_2-transfer potential of the *N'*-carboxy moiety has not been previously examined. In this paper we wish to report the syntheses of model compounds for both the O-protonated (**4**) and the N'-protonated (**5**) intermediates, as well as results which provide preliminary information concerning the chemical reactivity of each species.

Selection of Models. Three "irreversibly" acidified compounds were prepared to assess the CO_2-transfer potential of the *N'*-carboxy group. *N*-Carbomethoxy-*N'*-methyl-2-ethoxyimidazolinium fluoroborate (**6**) and *N*-carbomethoxy-*N'*-methyl-2-(2',6'-dimethylphenoxy)imidazolinium fluo-

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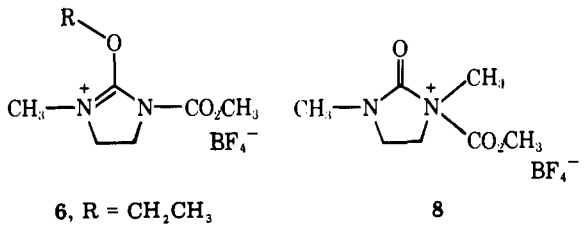
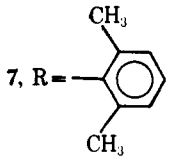


6, R = CH_2CH_3 8



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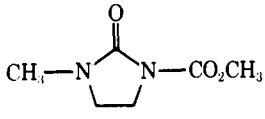
roborate (**7**) modeled the O-protonated species, while *N*-carbomethoxy-*N,N'*-dimethyl-2-oxoimidazolinium fluoroborate (**8**) mimicked the N'-protonated intermediate.

Synthesis of 6–8. Model compound **6** was readily prepared in 48% yield by the addition of 1 equiv of triethyloxonium



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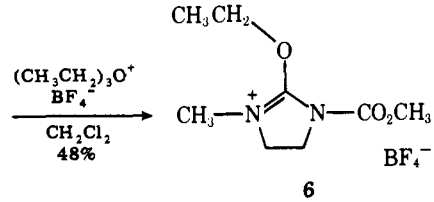
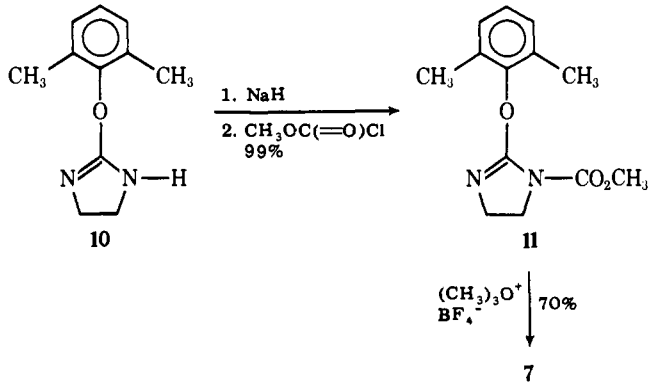


6

fluoroborate[18] to a dichloromethane solution of *N*-methyl-*N'*-carbomethoxyimidazolidone (**9**).[19]

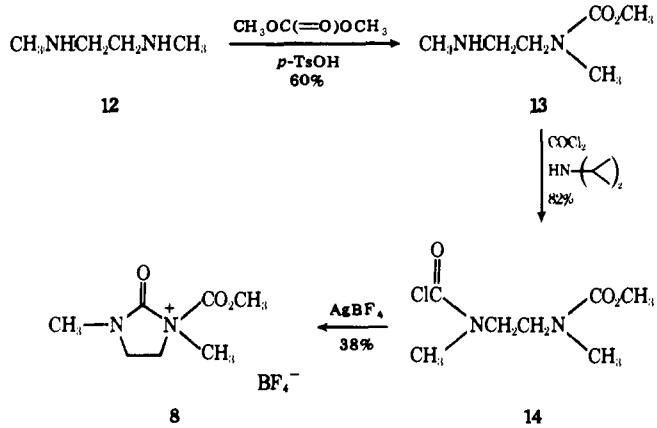
The synthetic strategy adopted for the preparation of **7** differed considerably from the previous approach. Noteworthy, the C-2 substituent was introduced at an initial stage of the synthesis. This was accomplished using the procedure of Trani and Bellasio for the preparation of 2',6'-dimethylphenoxyimidazoline (**10**) from 2-chloro-2-imidazoline sulfate.[20] Subsequent conversion of **10** to the corresponding amide anion



10 11 7

with NaH, followed by the addition of methyl chloroformate, gave *N*-carbomethoxy(2',6'-dimethylphenoxy)imidazoline (**11**). N-Methylation of **11** with 1.1 equiv of trimethyloxonium fluoroborate[21] gave the desired model compound **7**.

Preparation of compound **8** required strict adherence to techniques now common for the manipulation of air-sensitive compounds.[22] Starting with *sym*-dimethylethylenediamine (**12**), the corresponding *N*-carbomethoxy-*N,N'*-dimethylethylenediamine (**13**) was prepared by the addition of a catalytic amount of *p*-toluenesulfonic acid to a refluxing benzene solution containing the diamine and dimethyl carbonate.[23] Treatment of this compound with phosgene and *N,N*-diisopropylethylamine in benzene at 50 °C gave *N,N'*-dimethyl-*N*-carbomethoxyethylenediamine carbonyl chloride (**14**). Ring



12 13 8 14

closure with silver fluoroborate in nitromethane in the final step gave **8**. Compound **8** displayed intense peaks in the infrared at 1790 and 1710 cm^{-1}; these bands are characteristic carbonyl absorptions for the *N*-carbomethoxy and urea groups, respectively.[19,24,25] The ^1H NMR showed three singlets at δ 3.14, 3.49, and 4.25, which were assigned to the two *N*-methyl groups and the carbomethoxy methyl group, respectively. The ring ethylene protons gave rise to a complex multiplet between δ 3.68 and 4.87. These chemical shifts are in good agreement with values previously reported for *N*-acylammonium salts.[26]

Product Studies

Compounds **6–8** were each treated with oxygen and nitrogen containing nucleophiles to assess the CO_2-transfer potential of each substrate. The results of these product studies are reported in Tables I–IV. Two numbers for each experiment are listed wherever possible. The first numbers are product yields determined after extensive purification. In most cases the compounds were purified to homogeneity, but in no cases were more than two substrates present in the final mixture. The numbers in parentheses are product ratios determined by ^1H NMR prior to workup. In a number of cases, however, extensive overlapping of peaks in the NMR prevented this analysis.

Model Compound 6. The nucleophiles first reacted with *N*-carbomethoxy-*N'*-methyl-2-ethoxyimidazolinium fluoroborate (**6**) were substrates of general structure ROH (R = H, CH_3, $C(CH_3)_3$) (see Table I). In each case compound **9** was isolated. In the second and third experiments, *N*-methylimidazolidone[27] (**15**) was also obtained. At the conclusion of each of these two experiments, the reaction medium was highly acidic. NMR analysis at this stage indicated only the presence of **9** and a downfield signal. When the solution was neutralized with aqueous bicarbonate, decomposition of **9** to **15** was noted.[28] Significantly, the corresponding ethylated nucleophiles, ethyl methyl ether[29a] and *tert*-butyl ethyl ether,[29b] were detected by GC–MS and ^1H NMR from reactions 2 and 3, respectively. In experiment 2, dimethyl ether[29c] was also observed in the volatile fraction. The identity of these three ethers was confirmed by comparison of their spectra with those from authentic samples.[29,30] In the water reaction, the major product was the open-chain salt, *N*-carboethoxy-*N*-carbomethoxy-*N'*-methylethylenediamine hydrofluoroborate (**16**). In an ef-

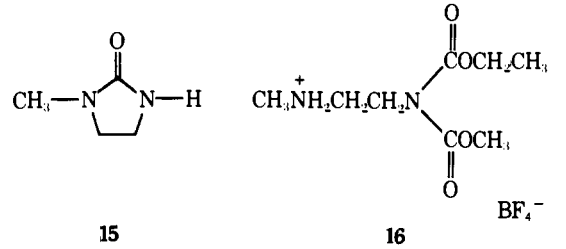


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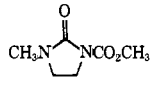
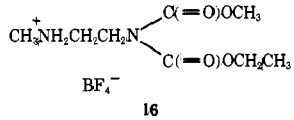
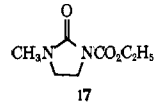
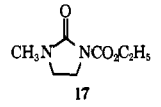
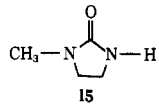
Table I. Reactivity of *N*-Carbomethoxy-*N'*-methyl-2-ethoxyimidazolinium Fluoroborate (**6**) with Nucleophiles

		products[a]				
						
rxn no.	nucleophile (solvent)	**9**	others			
1	H_2O (H_2O)	13 (16)[b]	 **16**	63 (84)		
1A	H_2O (H_2O) (50% ^{18}O)	17.5	**16**	71.5	 **17**	0 (0.2)
2	CH_3OH (CH_2Cl_2)	60[c,d] (100)				
3	*tert*-BuOH (CH_2Cl_2)	54[e,f] (100)				
4	NaOH (H_2O)	66 (68)	**16**	30 (31)	 **17**	0 (1)
5	$NaOCH_3$ (CH_3OH-CH_2Cl_2)	80 (100)[g]				
6	KO-*t*-Bu (CH_2Cl_2)	90 (90)[h]	 **15**	10 (10)		
7	morpholine (CH_2Cl_2)	99 (100)[i]				
8	triethylamine (CH_2Cl_2)	90 (100)[j]				
9	trimethylurea (CH_2Cl_2)	99 (100)[k]				

[a] Purified yields. [b] Numbers in parentheses are product ratios of known compounds determined by ^1H NMR. [c] Neutralization of the remaining reaction residue with aqueous 5% $NaHCO_3$ gave compound **15** in 39% yield. [d] Methyl ethyl ether was detected by GC-MS and ^1H NMR. Dimethyl ether was detected by GC-MS. GC-MS was performed using a 6 ft × 0.25 in. Poropak R column; temperature program, 70-250 °C at 4 °C/min; flow rate, 40 cm^3/min. The retention time for dimethyl ether was 2.8 min; MS *m/e* (rel %) 46 (53), 45 (100), 29 (71). The retention time for methyl ethyl ether was 5.6 min; MS *m/e* (rel %) 60 (33), 59 (12), 45 (100), 43 (6), 31 (14), 29 (31), 28 (6), 27 (12), 15 (9); NMR ($CDCl_3$) δ 1.19 (t, *J* = 7.0 Hz, 3 H), 3.35 (s, 3 H), 3.46 (q, *J* = 7.0 Hz, 2 H). [e] Neutralization of the remaining reaction residue with aqueous 5% $NaHCO_3$ gave compound **15** in 40% yield. [f] *tert*-Butyl ethyl ether was detected by GC-MS and ^1H NMR. GC-MS was performed on column in footnote *d*, under identical conditions: retention time 4.9 min; MS (*m/e*) 56 (58), 55 (25), 41 (100), 39 (69), 29 (17), 28 (30); NMR ($CDCl_3$) δ 1.18 (t, *J* = 7.0 Hz, 3 H), 1.18 (s, 9 H), 3.37 (q, *J* = 7.0 Hz, 2 H). [g] Methyl ethyl ether was detected by ^1H NMR. [h] *tert*-Butyl ethyl ether was detected by ^1H NMR. [i] NMR assay of the product mixture indicated the presence of 4-ethylmorpholine hydrofluoroborate (**18a**) (73%) and morpholine hydrofluoroborate (**18b**) (27%). Further purification gave a 55% yield of the former compound and a 20% yield of the latter. [j] Tetraethylammonium fluoroborate (**19**) was recovered quantitatively. [k] NMR assay indicated only the presence of **9** and *O*-ethyl-1,1,3-trimethylurea hydrofluoroborate (**20**). Further purification gave a 90% yield of the salt.

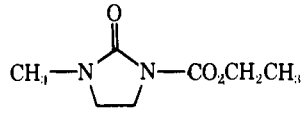
Table II. Percent Oxygen-18 Incorporation Observed from the Treatment of **6** with 50% $H_2{}^{18}O$

compd	^{18}O incorp, %
9	2[a]
16	50[a]
17	50[b]

[a] Accuracy of the measurement is ±0.5%. [b] This compound is present in a very small amount (<0.2%). An accurate quantitative assessment of oxygen-18 incorporation could not be made since the parent peak is less than 3% of that observed for compound **9**. Qualitatively, the *m/e* peaks at 173 and 175 were of equal height.

fort to facilitate the interpretation of experiment 1, the reaction was repeated using 50% oxygen-18 enriched water (experiment 1A). The ratio of the number of equivalents of water to **6** used in the two experiments was 55:1 and 28:1, respectively. The results obtained for these reactions were comparable. Each nonvolatile product (**9**, **17**, and **16**) isolated in experiment 1A was subjected to repetitive-scan mass spectral analysis to determine the extent of oxygen-18 incorporation (Table II).

The inability to detect the desired carbomethoxyl transfer products prompted the examination of **6** with sodium hydroxide, sodium methoxide, and potassium *tert*-butoxide (reactions 4-6). As in the previous two alcohol reactions, compound **9** was the major product. The corresponding ethyl ethers were again detected by ^1H NMR in reactions 5 and 6. Likewise, the open-chain salt **16** was isolated in the hydroxide reaction, although the yield was lower than in the water reaction (experiment 1). Interestingly, NMR analysis of the product mixture indicated a trace amount (ca. 1%) of *N*-methyl-*N'*-carboethoxyimidazolidone[19] (**17**).



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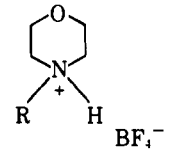
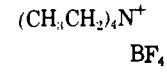
After we completed this study, our attention focused on nitrogen-containing nucleophiles (morpholine, triethylamine, and trimethylurea[31]). The last compound was of special interest since urea is known to undergo carboxylation with the biotin-dependent enzyme ATP:urea amidolyase.[3,32] Compound **9** was the only substrate obtained in reactions 7-9 which could be formally derived from the starting salt **6**. In addition to this compound, the corresponding ethylated nucleophiles, 4-ethylmorpholine hydrofluoroborate (**18a**) and tetraethyl-



18a, R = CH_2CH_3
b, R = H



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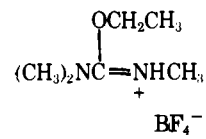

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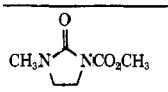
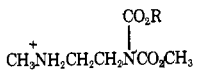
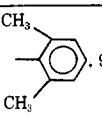
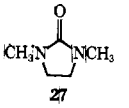
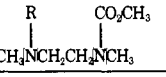
Table III. Reactivity of *N*-Carbomethoxy-*N'*-methyl-2-(2',6'-dimethylphenoxy)imidazolinium Fluoroborate (**7**) with Nucleophiles

rxn no.	nucleophile (solvent)	products[a]	
		 **9**	 BF_4^-
10	H_2O (CH_3CN)	0	**21**, R =  A, 95 (100)[b]
11	CH_3OH (CH_3CN)	45[c]	**22**, R = CH_3, 55[c]
12	CH_3CH_2OH (CH_3CN)	48[c]	**16**, R = CH_2CH_3, 52[c]
13	*t*-BuOH (CH_3CN)	84[c]	**21**, R = A, 16[c]
14	NaOH (CH_3CN)	70[c]	**21**, R = A, 30[c]
15	KO-*t*-Bu (CH_3CN)	92 (100)[b]	0
16	morpholine (CH_3CN)[d]	0	0

[a] Purified yields. [b] Numbers in parentheses are product ratios of known compounds determined by ^1H NMR. [c] Extensive overlapping of peaks in the ^1H NMR prevented the initial product analysis prior to workup. [d] Treatment of **7** with 1 equiv of morpholine in CH_3CN gave a 99% yield of *N*-carbomethoxy-*N'*-methyl-2-morpholinoimidazolinium fluoroborate (**23**).

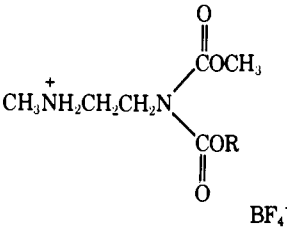
Table IV. Reactivity of *N*-Carbomethoxy-*N,N'*-dimethyl-2-oxoimidazolinium Fluoroborate (**8**) with Nucleophiles

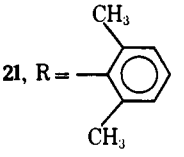
rxn no.	nucleophile (solvent)	products[a]	
		 **27**	
17	H_2O (CD_3NO_2)	0	**25**, R = $HHBF_4$, 68 (100)[b]
18	CH_3OH (CH_3NO_2)	0	**25**, R = $HHBF_4$, 4 (9)[b] **26**, R = CO_2CH_3, 43 (91)[b]
19	*t*-BuOH[c] (CH_3NO_2)	2 (5)[b]	**25**, R = $HHBF_4$, 47 (95)[b]
20	$NaOCH_3$ (CH_3OH-CH_3NO_2)	65 (74)[b]	**26**, R = CO_2CH_3, 23 (26)[b]
21	KO*t*-Bu[d] (CH_3NO_2)	39 (44)[b]	**13**, R = H, 26 (26)[b]
22	morpholine (CD_3NO_2)	65 (100)[b,e]	0
23	cyclohexylamine (CD_3NO_2)	49 (100)[b,f]	0
24	diisopropylamine[g] (CH_3NO_2)	48 (100)[b]	0

[a] Purified yields. [b] Numbers in parentheses are product ratios of known compounds determined by ^1H NMR. [c] Some unidentified material was present; the quantity was estimated at ca. 4% of the total reaction mixture. [d] Some unidentified material was present; the quantity was estimated at ca. 30% of the total reaction mixture. [e] *N*-Carbomethoxymorpholine (**28**) was isolated in 34% yield (based on total morpholine added); NMR estimated yield was 50%. Morpholine hydrofluoroborate was isolated in 39% yield; NMR estimated yield was 50%. [f] *N*-Carbomethoxycyclohexylamine (**29**) was isolated in 19% yield (based on total cyclohexylamine added); NMR estimated yield was 30%. Cyclohexylamine hydrofluoroborate was isolated in 11% yield; NMR estimated yield was 50%. [g] Some unidentified material was present; the quantity was estimated at ca. 7% of the total product mixture. Methyl diisopropylcarbamate (**30**) was isolated in 5% yield (based on total diisopropylamine added). Diisopropylamine hydrofluoroborate was isolated in 82% yield.

ammonium fluoroborate[33] (**19**), were isolated from experiments 7 and 8, respectively. A 20% yield of the hydrofluoroborate salt of morpholine (**18b**) was obtained in reaction 7. Noteworthy, trimethylurea did not react at nitrogen but exclusively at the carbonyl oxygen to give *O*-ethyl-1,1,3-trimethylurea hydrofluoroborate (**20**). Both **18a** and **20** could be independently prepared by the addition of triethyloxonium fluoroborate[18] to a dichloromethane solution of morpholine and trimethylurea, respectively (synthetic details are deposited as microfilm; see supplementary material paragraph at end of paper).

Model Compound 7. An approach analogous to the previous study was used to evaluate the CO_2-transfer potential of *N*-carbomethoxy-*N'*-methyl-2-(2',6'-dimethylphenoxy)imidazolinium fluoroborate (**7**) (Table III). This model compound, unlike the previous substrate **6**, was relatively stable to atmospheric moisture. The first set of nucleophiles examined was of general structure ROH (R = H, CH_3, CH_2CH_3, $C(CH_3)_3$). In reactions 11–13, significant product formation was noted only when an excess of alcohol was present. Even under these conditions, reaction of **7** with 55 equiv of *tert*-butyl alcohol (reaction 13) was complete only after ca. 20 h (NMR analysis). With the exception of the water reaction (reaction 10), compound **9** was isolated in each experiment. The other products obtained from these four reactions were the open-chain salts **21, 22, 16,** and **21,** respectively. The percentage of these compounds decreased as the size of the nucleophile increased. Finally, the exclusive formation of **21** in the water reaction argues that the origin of **9** in experiments 11–13 cannot be





22, R = CH_3
16, R = CH_2CH_3

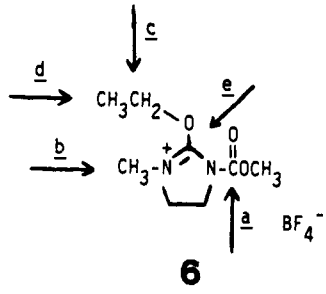
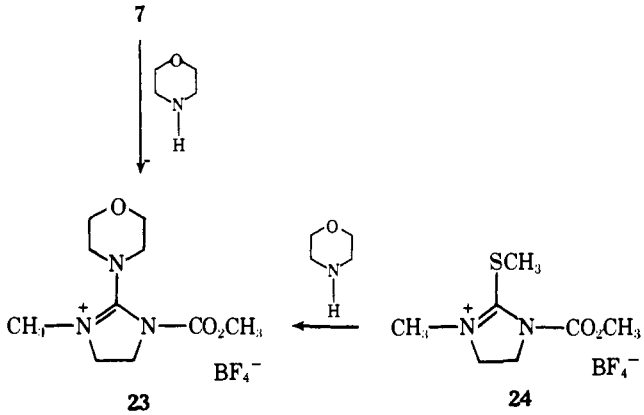


Figure 1.

attributed to trace amounts of water present in the reaction medium.

Treatment of **7** with 1 equiv of NaOH (reaction 14) gave a mixture of two products, the open-chain salt **21** and imidazolidone **9**. The latter compound was the only product observed when **7** was treated with potassium *tert*-butoxide (reaction 15).

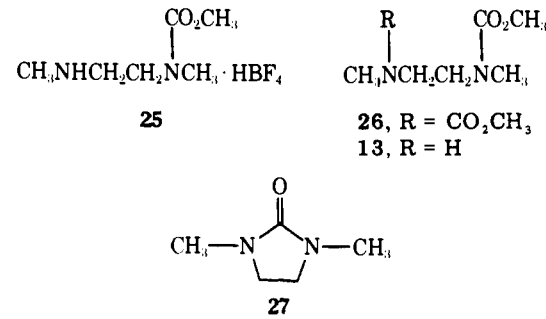
The last nucleophile chosen for study with **7** was the secondary amine, morpholine (experiment 16). The corresponding *N*-carbomethoxy-*N'*-methyl-2-morpholinoimidazolinium fluoroborate salt (**23**) was isolated in quantitative yield. Compound **23** was independently prepared in 50% yield by the treatment of *N*-carbomethoxy-*N'*-methyl-2-methyl-



thioimidazolinium fluoroborate (**24**) with an equivalent of morpholine in nitromethane.

Model Compound 8. The third model compound examined in this study was *N*-carbomethoxy-*N,N'*-dimethyl-2-oxoimidazolinium fluoroborate (**8**) (Table IV). The extreme ease with which this compound reacted with atmospheric moisture precluded weighing it prior to use. Instead, compound **8** was dissolved in a known volume of nitromethane (no appreciable change in volume accompanied dissolution), and then aliquots of the solution were syringed into reaction vessels containing the desired nucleophiles. Inherent in this method are several sources of error which noticeably reduced the accuracy of the final results. These experimental limitations, however, did not affect our confidence in the identification of the products, nor the initial product ratios determined by ^{1}H NMR.

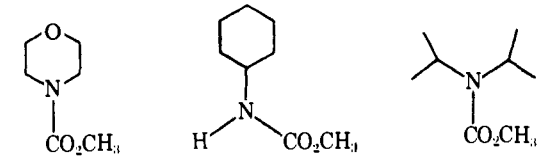
The major compounds observed from the reaction of **8** with nucleophiles of general structure ROH (R = H, CH_3, $C(CH_3)_3$) were ring-opened products. *N*-Carbomethoxy-*N,N'*-dimethylethylenediamine hydrofluoroborate (**25**) was the sole compound detected when **8** was added to a nitromethane solution containing 1 equiv of water (experiment 17). The corresponding neutral carbamate, *N,N'*-dicarbomethoxy-*N,N'*-dimethylethylenediamine (**26**), was isolated in the methanol reaction (reaction 18) along with a small amount of salt **25**. Finally, compound **25** was the major product observed in the *tert*-butyl alcohol reaction (experiment 19). A small amount (2%) of *N,N'*-dimethylimidazolidone[34] (**27**) was also



isolated. GC–MS and NMR analysis of the volatile fraction from reactions 18 and 19 did not reveal the presence of any carbonates or ethers.

The reactivity of compound **8** with sodium methoxide and potassium *tert*-butoxide was next examined. The respective formation of compounds **26** and **13** in reactions 20 and 21 paralleled the results observed in the previous alcohol reactions. It is noteworthy that significant quantities of *N,N*-dimethylimidazolidone[34] (**27**) were recovered in these experiments. Careful examination of the volatile fraction (GC–MS and NMR) did not detect the presence of any carbonates or ethers.

The initial evaluation of the CO_2-transfer potential of compound **8** concluded with the examination of the reactivity of this substrate with nitrogen-containing nucleophiles. Two equivalents of the corresponding amines was used in reactions 22 and 23 to allow for the consumption of the amine by the HBF_4 generated during the reaction. Reactions 22–24 gave *N,N'*-dimethylimidazolidone[34] (**27**), the corresponding carbomethoxylated amines (**28–30,** respectively), and the HBF_4

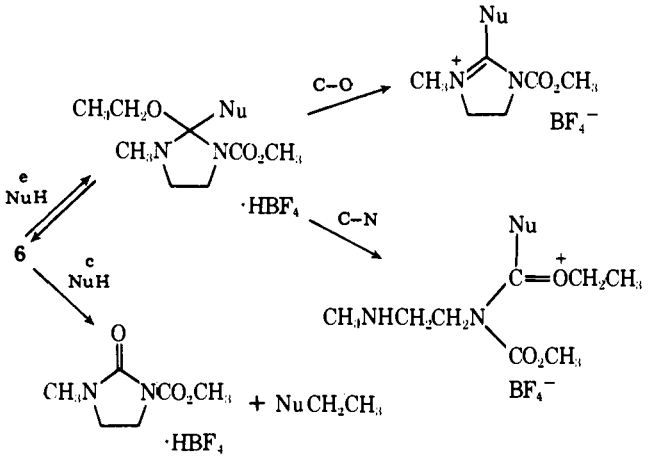


salts of the starting amine. Each carbamate (**28–30**) was identified by comparison with an authentic sample.[35-37] (Please see supplementary material for additional synthetic and spectral information concerning the products generated in experiments 17–24.)

Discussion

Model Compounds 6 and 7. The reactivities of model compounds **6** and **7** can be conveniently considered together. Examination of the molecular framework for compound **6** suggests five potential sites for attack by a nucleophile (Figure 1). The desired pathway is shown by route a. Nucleophilic attack at this point should lead to the formation of a tetrahedral intermediate. Subsequent breakdown of this adduct with *N*-acyl bond cleavage would yield *N*-methyl-*O*-ethylimidazoline[19] and the carbomethoxy-transferred product. Alternatively, the tetrahedral intermediate formed by this pathway could break down by cleavage of the C–OCH_3 bond. Analogy here stems from the work of Knappe and Lynen[10] on the alkaline hydrolysis of *N*-carbomethoxyimidazolidone and *N'*-carbomethoxybiotin. In addition to route a, compound **6** contains two sites activated toward S_N2 displacement. These are depicted by arrows b and c, and would lead to the corresponding methylated and ethylated nucleophiles, respectively. Of these two pathways, route c should be the more likely based on a comparison of the nucleophilicity[38] of the respective leaving group for each process (*N*-carbomethoxy-*O*-ethylimidazoline vs. *N*-methyl-*N'*-carbomethoxyimidazolidone). A fourth pathway (route d) considered for these reactions envisages the abstraction of a proton by a nucleophile from the *O*-ethyl side

Scheme I



chain in **6** to give ethylene and compound **9** via an E2 elimination process. Finally, it was anticipated that alkylation of **9** to give **6** would increase the likelihood of nucleophilic attack taking place at the C-2 position of the imidazolinium ring (arrow e). The resulting tetrahedral intermediate could then undergo ring opening to yield a substituted ethylenediamine or eliminate ethanol to give the appropriately 2-substituted imidazolinium derivative.

Replacement of the ethoxy group in model compound **6** by the 2′,6′-dimethylphenoxy group (**6** → **7**) decreases appreciably the number of potential sites for nucleophilic attack (Figure 2). Pathways c and d outlined for **6** are no longer viable routes in compound **7**. Furthermore, the known steric hindrance of the 2′,6′-dimethylphenoxy group[39] should decrease the ease of nucleophilic attack at the C-2 position of the imidazolinium ring (pathway e).

The most striking result observed for the reaction of nucleophiles with model compounds **6** and **7** (experiments 1–16) was the absence of products emanating from the desired process (carbomethoxy transfer). The compounds obtained from **6** (Table I) can be explained in terms of two competing reactions[40] (Scheme I). One pathway (route e) involves initial attack of the nucleophile at the C-2 position of the imidazolinium cation to give a tetrahedral intermediate. This adduct can then revert back to **6** or undergo bond cleavage in either of the two directions shown. The competing pathway (route c) is an irreversible S_N2 displacement reaction at the methylene position of the *O*-ethyl side chain in **6** to give the ethylated nucleophile and **9**. For small nucleophiles, pathway e (an $A_{AC}2$ process) becomes competitive with the S_N2 displacement reaction (pathway c) (an $A_{AL}2$ process); for larger nucleophiles, however, the S_N2 route is the exclusive pathway.

Although no kinetic data was obtained to substantiate either of these two mechanisms, evidence has been accumulated to support these suggestions. Furthermore, there already exists an impressive amount of information for closely related chemical systems which are in agreement with these findings.[40-47] The principal mode of reaction observed for model compound **6** is the $A_{AL}2$ process (S_N2 displacement).[41] In experiments 2–9, the only product obtained was compound **9**. Significantly, in these reactions the corresponding alkylated nucleophile was either detected or isolated. No evidence for the anticipated tetrahedral intermediate from the $A_{AC}2$ process (path e)[41] or subsequent breakdown products was observed in reactions 3–7. This was particularly apparent in reaction 7 where the stable guanidinium salt **23** should have been formed had the reaction proceeded via route e to give the tetrahedral intermediate followed by expulsion of ethanol. We attribute the predominance of the $A_{AL}2$ pathway over the $A_{AC}2$ route in these reactions to the comparatively unfavorable steric interactions that must be encountered in the transition state for

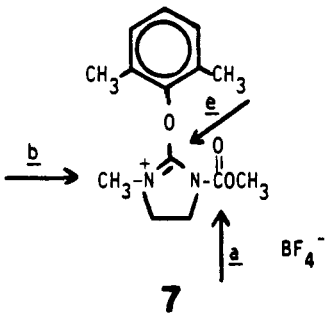
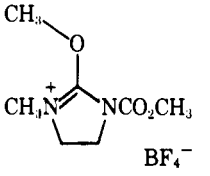


Figure 2.

the latter process as one goes to larger nucleophiles and to the lack of favorable stabilizing dipole-dipole and hydrogen-bonding interactions[41,48] by the solvent in the transition state for this process.

The importance of these steric and solvation effects on the course of the reaction can be ascertained by examining the products observed in the water and methanol reactions (experiments 1 and 2) where both pathways are operative. In the water reaction the open-chain amine salt **16** and **9** are obtained. The isolation of **16** in this reaction strongly implies the prior formation of a tetrahedral intermediate by pathway e. The appearance of compound **9**, on the other hand, does not ensure that pathway c is operable, since this same compound can result from initial formation of the tetrahedral intermediate (path e, Scheme I), followed by expulsion of ethanol or by ring closure of the open-chain amine salt. The use of oxygen-18 enriched water in this reaction provides an easy way to differentiate these two routes (arrows c and e). Mass spectral analysis of the nonvolatile reaction products (see Table II) recovered indicated that 96% of imidazolidone **9** formed occurred through the $A_{AL}2$ process (0% oxygen-18 enrichment) (estimated yield of unlabeled **9**, 16.8%) and that this pathway (route c) accounted for only one-fifth of the total reaction products (fraction based on an 89% recovery of products). Furthermore, of the remaining 4% of **9** isolated (50% oxygen-18 enrichment) (estimated yield of labeled **9**, 0.7%), the major portion of this labeled compound (>0.7) occurred by direct expulsion of ethanol from the tetrahedral intermediate rather than ring opening to **16**, followed by ring closure and loss of ethanol. Evidence bearing on this point emanates from the detection of **17**[19] by mass spectroscopy (50% oxygen-18 incorporation) and ^1H NMR. The upper limit for the overall yield of **17** has been placed at 0.2% by NMR anaylsis. We infer that ring closure of the amine salt to give **17** and methanol should occur at least as readily as closure to give **9** and ethanol. Finally, the major nonvolatile product isolated in this reaction was the open-chain salt **16** (yield of labeled **16**, 71.5%). In agreement with the proposed mechanism for the formation of this compound, mass spectral analysis indicated 50% oxygen-18 incorporation. Additional evidence for the $A_{AC}2$ pathway comes from the methanol reaction. In experiment 2, the only nonvolatile product obtained was **9**. However, examination of the volatile fraction showed not only the corresponding $A_{AL}2$ product, methyl ethyl ether, but also dimethyl ether in an approximate 4:1 ratio. The most likely mechanism for the formation of dimethyl ether involves attack of methanol at the C-2 position of the ring to give the tetrahedral intermediate, followed by expulsion of ethanol to yield **31**.



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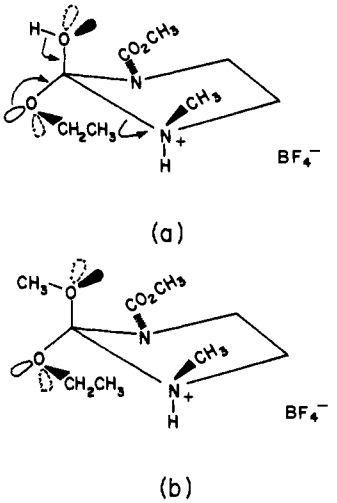
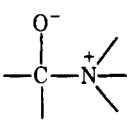


Figure 3. Initial tetrahedral intermediates formed upon addition of nucleophile to compound **6**: (a) H_2O; (b) CH_3OH.

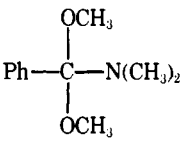
Nucleophilic displacement ($A_{AL}2$ process) at the *O*-methyl site in **31** by the excess methanol present in a subsequent reaction would give **9** and dimethyl ether.

It is noteworthy that the yields assessed in experiment 1A for those compounds which formally derive from the breakdown of the tetrahedral intermediate (**16** [71.5%] and **9** [0.5%]) suggest that this adduct partitions approximately 140:1 in favor of carbon–nitrogen bond cleavage vs. rupture of the carbon–ethoxy bond. This number is in good agreement with the ratio observed by previous workers for the hydrolysis of *N,N,O*-trimethylbenzimidatium ion[43] and benzamide.[43,49] In a recent article,[50] McClelland has postulated that the principal factor which governs this breakdown stems from the hydrogen bonding ($>O^+-H\cdots OH_2$) provided by the solvent, water. It was suggested that this bonding sufficiently stabilizes the cations *derived* from the tetrahedral intermediate such that the transition state for bond cleavage resembles the tetrahedral intermediate to a greater extent, thereby leading to preferential breakage of the C–N bond. Two alternative explanations for this result were also mentioned. The first one took into consideration that zwitterionic forms such as **32** might exist for



32

the adduct.[51] This structure should facilitate C–N bond cleavage but was discounted on the basis of the low pH of the reaction solution. A similar objection can probably be raised in our system. The second hypothesis discussed took into consideration the stereoelectronic theory recently proposed by Deslongchamps and co-workers[52] for the selective breakdown of tetrahedral intermediates. This explanation was ruled out on the basis of kinetic experiments which suggested that the lifetime of the intermediates formed in the reactions was great enough to allow conformational equilibration. Application of Deslongchamps' theory to experiment 1 in our study leads to a prediction which is consistent with the experimental results. Dreiding models reveal that the 2-ethoxy group in **6** should adopt the conformation shown in Figure 1 on the basis of steric factors. The resulting tetrahedral intermediate (Figure 3a) should undergo preferential C–N bond cleavage by an orbital-assisted mechanism because the two oxygen atoms each have a lone-pair orbital properly oriented antiperiplanar to the *N*-methyl group. Although this hypothesis provides a satisfactory explanation for the water reaction, it does not explain the apparent absence of C–N bond cleavage from the corresponding tetrahedral intermediate formed in the methanol experiment (Figure 3b). In this adduct, neither the ejection of the *N*-methyl group nor the loss of the *O*-ethyl moiety should be facilitated by an orbital-assisted mechanism. Under these conditions, conformational change by rotation (C–OCH_3 bond rotation) should compete favorably with either loss of a proton and inversion of the *N*-methyl nitrogen or immediate breakdown of the initially formed intermediate.[52] On the other hand, consideration of the presence or absence of hydrogen-bonding interactions from the solvent in the breakdown of the tetrahedral intermediate satisfactorily accounts for the lack of C–N bond cleavage in the methanol experiment. The cations ($>O^+-CH_3$) derived from this intermediate do not benefit from this type of hydrogen bonding. The absence of this stabilizing effect should shift the transition state for bond breakage in experiment 2 closer to the product cation as compared to the corresponding transition state in experiment 1. The effect of this shift is to predict an increased ratio of C–O to C–N bond cleavage in the methanol reaction as compared to the water reaction. A similar trend has been observed for **33** vs. **34**.[50]



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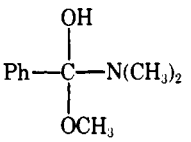

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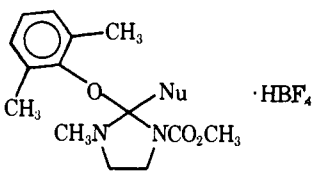
The only site of reaction observed for compound **7** with nucleophiles was the C-2 position of the imidazolinium ring (arrow e). All products listed in Table III can be satisfactorily explained by invoking initial formation of tetrahedral intermediate **35**.[53]

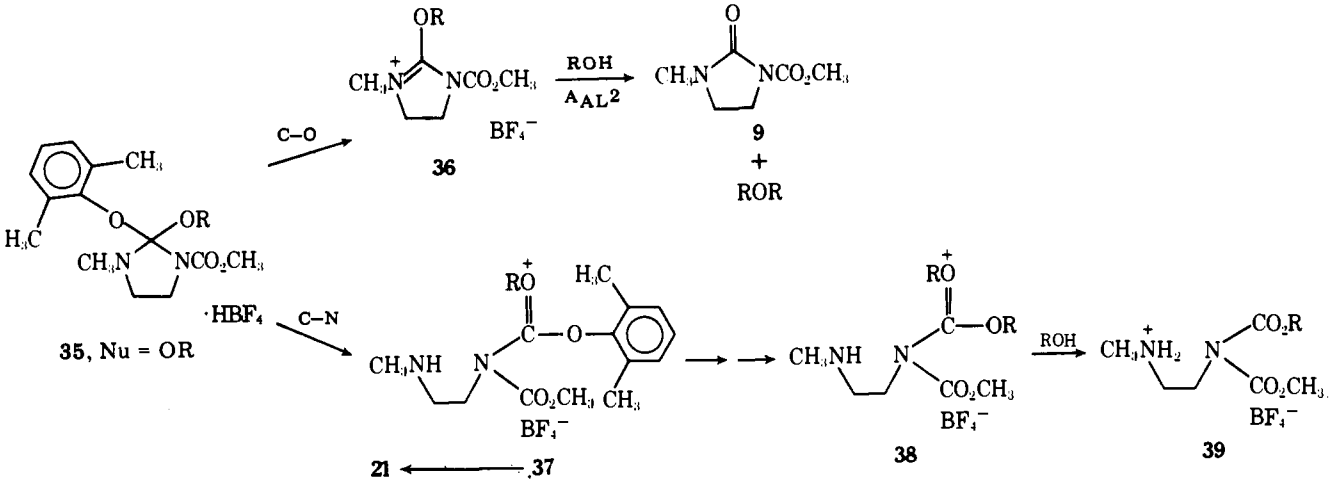


35

The product ratios determined reflect the manner in which this adduct partitions between C–N and C–O bond cleavage. In the water reaction (experiment 10), we observed exclusive C–N bond breakage. This preference may again be due to the stabilization (H bonding) provided by the solvent in the transition state for the breakdown of the tetrahedral intermediate.[50] In reactions 11 → 13 the ratio of C–O to C–N bond cleavage steadily increases. Although the number and accuracy of these experiments do not permit definitive conclusions to be made concerning this trend, two likely contributing factors may be the absence of hydrogen-bonding interactions from the solvent for the cations derived from the tetrahedral intermediates,[50] and the effect of the mixed-solvent systems on the basicity of the *N*-methyl nitrogen atom in the tetrahedral intermediate **35**. The molar concentration of alcohol decreases from experiment 11 → 13 (see Experimental Section). The net effect of this reduction is to decrease the basicity of the *N*-methyl nitrogen atom in the tetrahedral intermediate, thereby permitting C–O bond breakage to become more competitive with C–N bond cleavage.[50] The interesting result in light of the previous model study (compound **6**) is that C–N bond cleavage is observed at all in the alcohol reactions (experiments 11–13). This observation may reflect the lower basicity of the 2,6-dimethylphenoxy group in **35** as compared to the ethoxy group in the corresponding tetrahedral intermediate.

Numerous mechanisms can be drawn for the breakdown of **35** to give the products obtained in experiments 10–13. One set of pathways is depicted in Scheme II. Significantly, the

Scheme II



initial step drawn for the C–O bond cleavage pathway (**35** → **36**) leads to an intermediate which is analogous to our first model compound **6**. Nucleophilic substitution ($A_{AL}2$ process) at the *O*-alkyl group[46] by the excess alcohol present would then give compound **9**. In the case where R = *tert*-butyl, intermediate **36** could ionize directly to give **9** and *tert*-butyl cation.[54] On the other hand, cleavage of the C–N bond in intermediate **35** leads to the open-chain salt **37**. Subsequent ionization of a proton (experiment 10) or a *tert*-butyl group (experiment 13) from **37** would yield compound **21**. Alternatively the open-chain intermediate **37** could undergo addition of a second molecule of alcohol[46] (experiments 11 and 12) to eventually produce the products isolated.[54,55]

Finally, we note the formation of the stable guanidinium salt **23** in quantitative yield from the treatment of **7** with 1 equiv of morpholine (experiment 16). This salt provides conclusive proof for the initial formation of adduct **35**. Apparently in this intermediate C–N bond cleavage does not compete with C–O bond breakage. This result is in accord with previous observations for the hydrolysis of amide acetals in neutral and basic solutions.[50] A similar shift in the C–O to C–N bond cleavage ratio was observed for the sodium hydroxide and potassium *tert*-butoxide reactions (experiments 14 and 15).

Model Compound 8. Specific alkylation of carboxy-biotin model compounds at the N′ position should activate four sites toward nucleophilic attack. These positions are indicated by arrows a → d for *N*-carbomethoxy-*N*,*N*′-dimethyl-2-oxoimidazolinium fluoroborate (**8**) (Figure 4). The desired reaction to give **27** and the carbomethoxy-transferred product is denoted by arrow a. Alternatively, nucleophilic attack can occur at site b. Significantly, this reaction yields the same product (**27**) (after loss of carbon dioxide) anticipated from the first route (path a) along with the methylated nucleophile. Analogy for this pathway stems from the work of Phillips and coworkers on the reaction of tertiary amines with optically active alkyl chloroformates.[56] A third conceivable route (pathway c) is nucleophilic attack at the *N*′-methyl group to yield **9** and the methylated nucleophile. The general synthetic utility of this dealkylation reaction has been investigated by Hobson and McCluskey.[57] Finally, N′-methylation should increase the probability of attack at the C-2 position of the ring (pathway d). Reaction at this site should ultimately give ring-opened products.

In the eight reactions reported for this compound, there is evidence for only two of these pathways (routes a and d). These can be operationally classified by the type of nucleophile employed in the reaction. For oxygen-containing reagents (experiments 17–21), the results are best rationalized in terms of attack occurring at the C-2 position of the ring (route d, Figure 4). When R = H (experiment 17) the decarboxylated salt **25**

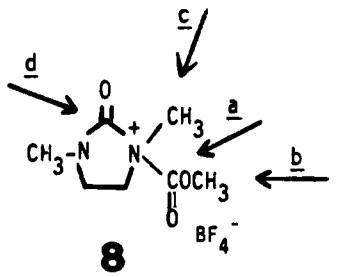


Figure 4.

was obtained rather than compound **41**. In experiment 19, **25** was once again observed to be the major product (before and after workup). This result implies that under the conditions of the reaction, **42** or **40** undergoes rapid ionization of the *tert*-butyl group followed by loss of carbon dioxide to give **25**.[54] Interestingly, a small percentage (2%) of compound **27** was detected (NMR and GC–MS) in this reaction. The products obtained in the two alkoxide reactions (experiments 20 and 21) paralleled the results observed in the methanol and *tert*-butyl alcohol experiments. Noticeably, significant quantities of compound **27** were isolated from these reactions.[58] No evidence (NMR and GC–MS), however, was obtained for the formation of the corresponding carbonates (path a) or ethers (path b).

The other site of reaction noted for model compound **8** with nucleophiles was the carbomethoxy carbonyl group (route a). Reaction at this position with subsequent transfer of the carbomethoxy group to give the corresponding carbamates (**28**–**30**) and **27** was virtually the sole pathway observed for nitrogen-containing nucleophiles (experiments 22–24). A similar result was observed by Paukstelis and Kim in the reaction of *N*-alkoxycarbonyl-*N*,*N*,*N*-trialkylammonium fluoroborates with *N*-methylcyclohexylamine and cyclohexylamine.[26a]

Conclusions

Protonated intermediates are often postulated as key intermediates in enzyme-induced reactions. Model compounds **6**, **7**, and **8** are irreversibly acidified equivalents of two potential intermediates (**4** and **5**). Of these three substrates, only **8** led to transfer of the carbomethoxy group upon treatment with nucleophiles.

The results observed for model compounds **6** and **7** argue that if protonation occurs at the ureido oxygen atom prior to CO_2 transfer then the enzyme must also steer the acceptor molecule away from other activated positions on the biotin ring system. Alternatively, the success noted for compound **8** suggests that if N′-protonation does occur, then transfer of the carboxy group to the acceptor molecule can potentially be an

efficient step in the overall catalytic process. These results tend to support the observations of Mildvan and co-workers, which suggested that in transcarboxylase protonation at the N′ position may have preceded CO_2 transfer to pyruvate.[16] A criticism of this latter mechanism, however, is the exceedingly low basicity of the N′ position in comparison to the ureido oxygen atom. The central oxygen atom has previously been demonstrated to be the most basic position in the imidazolidone ring of biotin.[17] Furthermore, Fersht has estimated that the relative pK_as for O- and N-protonation of simple amides is 10^7 in favor of the former site.[60] We anticipate that this difference is even greater in *N*′-carboxybiotin. Although these facts are important mechanistic considerations, they do not necessarily rule out this second stepwise pathway or a comparable concerted mechanism involving general acid catalysis. The enzyme may specifically deliver a proton to the N′ position or N′-protonation may lead to a kinetically active species which is product determining.[61] Analogy comes from the postulation that protonation at the amide nitrogen in peptide substrates occurs in certain proteolytic enzymes,[63] including carboxypeptidase A,[64] even though convincing evidence exists in favor of the O-protonation mechanism for acid-catalyzed amide hydrolysis.[49,62,65] However, if prior N′-protonation is indeed important in biotin catalysis, then the beneficial aspects (i.e., increased CO_2 transfer potential and selectivity) which accompany this protonation must help to offset the energy required to protonate this weakly basic site.

Experimental Section

General. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra (IR) were run on Perkin-Elmer Model 700 and 237B spectrometers and calibrated against the 1601-cm^{-1} band of polystyrene. Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on Varian Associates Model T-60 and EM-390 instruments. Chemical shifts are expressed in parts per million relative to Me_4Si, and coupling constants (J values) are in hertz. Spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), q (quartet), sept (septet), 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 and a Hewlett-Packard 5930 gas chromatograph–mass spectrometer. High-resolution mass spectra were performed by Dr. James Hudson at the Department of Chemistry, Rice University, or Dr. Ronald Grigsby at the Department of Biochemistry and Biophysics, Texas A&M University, on CEC21-110B double focusing magnetic sector spectrometers 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, benzene was distilled from and stored over sodium, dimethylformamide was stored over sodium sulfate and then distilled from calcium hydride, anhydrous ether was distilled from and stored over sodium metal, nitromethane was distilled before use, and 1,2-dimethoxyethane was distilled from lithium aluminum hydride and stored over molecular 4A sieves. Morpholine was dried over potassium hydroxide and distilled from calcium hydride and triethylamine was distilled from calcium hydride. The 50% oxygen-18 enriched water was obtained from Bio-Rad Laboratories. All reactions were run under nitrogen and all glassware was oven or flame dried before use.

Preparation of *N*-Carbomethoxy-*N*′-methyl-2-ethoxyimidazolinium Fluoroborate (6). To a stirred CH_2Cl_2 solution (25 mL) containing *N*-carbomethoxy-*N*′-methylimidazolidone[19] (**9**) (3.16 g, 0.02 mol), a CH_2Cl_2 solution (15 mL) containing triethyloxonium fluoroborate[18] (3.80 g, 0.02 mol) was added dropwise. The solution was refluxed overnight and then cooled in a dry ice–acetone bath, and Et_2O (250 mL) was added causing the separation of an oil. The liquor was quickly decanted and the oil dried in vacuo to yield a solid which by NMR analysis contained only **6**: yield 4.82 g (88%). The sample was further purified by reprecipitation from hot CH_2Cl_2 with Et_2O to yield 2.64 g (48% yield) of the title compound: mp 113–115 °C; IR ($CDCl_3$) 1770, 1730, 1660, 1515, 1070 cm^{-1}; NMR ($CDCl_3$) δ 1.50 (t, J = 7.0 Hz, 3 H), 3.15 (s, 3 H), 3.85 (s, 3 H), 3.65–4.48 (m, 4 H), 4.72 (q, J = 7.0 Hz, 2 H).

Anal. ($C_8H_{15}N_2O_3BF_4$) C, H, N.

Preparation of *N*-Carbomethoxy-(2′,6′-dimethylphenoxy)imidazoline (11). NaH (0.19 g, 0.0039 mol) was washed with DME (3 × 5 mL) and then an additional 20 mL of DME was added. A DME solution (15 mL) of 2′,6′-dimethylphenoxyimidazoline[20] (**10**) (0.57 g, 0.003 mol) was slowly added and the mixture stirred for 2 h. Methyl chloroformate (0.30 mL, 0.0039 mol) was then added quickly, resulting in the immediate precipitation of a white solid and the evolution of heat. The mixture was stirred at room temperature (18 h) and filtered and the filtrate was evaporated in vacuo and then distilled to give 0.74 g (99%) of **11**: bp 90 °C (external temperature, 1.1 mm); IR (neat, NaCl) 2960, 1760, 1715, 1660 cm^{-1}; NMR ($CDCl_3$) δ 2.26 (s, 6 H), 3.40–4.20 (m, 4 H), 3.83 (s, 3 H), 7.01 (s, 3 H); MS *m/e* (rel %) 248 (18), 182 (44), 102 (100), 77 (75); mol wt 248.1167 (calcd for $C_{13}H_{16}N_2O_3$, 248.1161).

Preparation of *N*-Carbomethoxy-*N*′-methyl-2-(2′,6′-dimethylphenoxy)imidazolinium Fluoroborate (7). A CH_2Cl_2 solution (5 mL) of *N*-carbomethoxy-2-(2′,6′-dimethylphenoxy)imidazoline (**11**) (0.72 g, 0.0029 mol) was added to trimethyloxonium fluoroborate[23] (0.47 g, 0.0032 mol), and then the mixture was refluxed for 18 h. The dark red solution was concentrated in vacuo and the residue reprecipitated from a 1:1 CH_2Cl_2–CH_3CN solution with Et_2O to give 0.69 g (70%) of a white crystalline material: mp 110–111 °C; IR (KBr) 2960, 1770, 1665, 1510, 1070 cm^{-1}; NMR ($CDCl_3$) δ 2.37 (s, 6 H), 2.85 (s, 3 H), 3.86 (s, 3 H), 4.13–4.44 (m, 4 H), 7.16 (s, 3 H).

Anal. ($C_{14}H_{19}N_2O_3BF_4$) C, H, N.

Preparation of *N*-Carbomethoxy-*N*,*N*′-dimethylethylenediamine (13). *N*,*N*′-Dimethylethylenediamine (**12**) (8.80 g, 0.10 mol), methyl carbonate (9.90 g, 0.11 mol), and *p*-toluenesulfonic acid (1.92 g, 0.01 mol) were combined in C_6H_6 (150 mL) and the solution was refluxed for 66 h. The solution was dried (Na_2SO_4), concentrated in vacuo to one-third of the original volume, and then Et_2O (400 mL) was added. The milky white solution was refrigerated (4 h) resulting in the precipitation of a white solid. The mixture was filtered, the filtrate concentrated, and then the remaining oil distilled at 60 °C (external temperature, 1 mm) to give 6.74 g (60%) of product: IR (neat, NaCl) 3300, 2940, 1700, 1480 cm^{-1}; NMR ($CDCl_3$) δ 1.19 (br s, 1 H), 2.46 (s, 3 H), 2.60–2.97 (m, 2 H), 2.97 (s, 3 H), 3.40 (t, J = 6.0 Hz, 2 H), 3.74 (s, 3 H); MS *m/e* (rel %) 146 (8), 102 (11), 88 (12), 57 (33), 44 (100).

Anal. ($C_6H_{14}N_2O_2$) C, H, N.

Preparation of *N*-Carbomethoxy-*N*,*N*′-dimethylethylenediamine Carbonyl Chloride (14). A phosgene solution (12.5% in C_6H_6) (70 mL, 0.09 mol) was further diluted by the addition of 100 mL of C_6H_6. A C_6H_6 solution (30 mL) containing *N*-carbomethoxy-*N*,*N*′-dimethylethylenediamine (**13**) (7.30 g, 0.05 mol) and diisopropylethylamine (6.45 g, 0.05 mol) was then rapidly added to the C_6H_6 solution of phosgene. The resulting pale yellow solution was stirred at 50 °C (18 h) and then cooled causing the precipitation of a white solid. Et_2O (50 mL) was added to the mixture resulting in the precipitation of additional material. The mixture was filtered, and the filtrate concentrated in vacuo to yield a yellow oil. Et_2O (300 mL) was added to the oil and the milky white solution refrigerated (4 h) and then filtered. Evaporation in vacuo of the remaining filtrate yielded 8.53 g (82%) of the title compound, a light yellow oil: IR (neat, NaCl) 2955, 1750, 1735 cm^{-1}; NMR ($CDCl_3$) δ 2.91 (s, 3 H), 3.00–3.20 (m, 3 H), 3.33–3.70 (m, 4 H), 3.70 (s, 3 H); MS *m/e* (rel %) 208 (1), 173 (21), 115 (46), 102 (100); mol wt 208.0604 (calcd for $C_7H_{13}ClN_2O_3$, 208.0615).

Preparation of *N*-Carbomethoxy-*N*,*N*′-dimethyl-2-oxoimidazolinium Fluoroborate (8). Owing to the extremely high reactivity of this compound toward water, completely *anhydrous conditions* must be maintained throughout this procedure.[22] The CH_3NO_2 and CH_2Cl_2 were distilled immediately before use; anhydrous Et_2O was dried twice over sodium ribbon. The solvents were all stored under Ar and sealed with septum caps. All glassware was oven-dried and assembled hot, and all liquid transfers were made with predried syringes. The contents of the reaction were continually blanketed with an atmosphere of Ar.

Silver fluoroborate (2.74 g, 0.014 mol) was added to a two-necked flask, in which one outlet was sealed with a septum cap, and then dried (3 days) in vacuo in the dark.[66] The flask was then fitted to a Schlenk apparatus (80 mL capacity) which was topped with a 250-mL flask

equipped with a two-way valve. The silver salt was dissolved in CH_3NO_2 (20 mL) and then a CH_3NO_2 solution (6 mL) of *N*-carbomethoxy-*N*,*N'*-dimethylethylenediamine carbonyl chloride (**14**) (3.49 g, 0.0168 mol) was added by syringe. The addition of **14** caused the immediate precipitation of a white solid. The reaction mixture was stirred in the dark (2 h). Inversion of the Schlenk apparatus allowed the solution to be filtered into the empty 250-mL flask. Et_2O (40 mL) was added to the filtrate through the Schlenk apparatus, and then the Schlenk apparatus was removed and replaced with a septum cap. The solution under Ar was then cooled in a dry ice–acetone bath causing the separation of an oil. The supernatant was removed by a "pumping out" procedure with the aid of a long stainless steel tube (18 gauge) under Ar pressure. The remaining oil was triturated with Et_2O (2 × 40 mL) and dried in vacuo (3 h) during which time it became a semisolid. This material was then triturated with CH_2Cl_2 (3 × 20 mL) to remove the colored impurities, leaving a pure white crystalline material which was again dried in vacuo to yield 1.39 g (38%) of **8**: mp 84 °C (sealed tube); IR ($CH_3CH_2NO_2$) 1790, 1710, 1680, 1205, 1060 cm^{-1}; NMR (CD_3NO_2) δ 3.14 (s, 3 H), 3.49 (s, 3 H), 3.68–4.87 (m, 4 H), 4.25 (s, 3 H).

Anal. ($C_7H_{13}N_2O_3BF_4$) C, H, N.

Treatment of *N*-Carbomethoxy-*N'*-methyl-2-ethoxyimidazolinium Fluoroborate (6) with Nucleophiles. General Procedure for Neutral Nucleophiles. To a rapidly stirred slurry of compound **6** (0.55 g, 2.0 mmol) and CH_2Cl_2 (7 mL), the neutral nucleophile was added all at once. In reactions 3 and 7–9 the concentrations of nucleophile and substrate were equimolar. In the water reaction (reaction 1), 55 equiv of nucleophile was added per equiv of **6**, while in reaction 2 the ratio was 2:1. A homogeneous solution resulted upon completion of the addition. The solution was stirred at room temperature (18 h), then gently evaporated, taking care to ensure the presence of a small quantity of CH_2Cl_2. NMR analysis at this point indicated the presence of any relatively volatile products, and identified the initial reaction products. The volatile component of the reaction products was then collected by a short-path distillation (room temperature, ~1 mm) and further analyzed by NMR and GC–MS if needed. In the event (reactions 2 and 3) where products more volatile than the solvent were expected, the initial distillate containing solvent and ethers was collected and then examined by ^1H NMR and GC–MS. The pH of the nonvolatile material was adjusted to 8 or 9 with 5% $NaHCO_3$ and it was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated in vacuo. Analysis of the organic soluble material by NMR indicated the presence of compound **9** and sometimes compound **15**. Analysis of the water portion after evaporation often indicated additional organic material. In these cases the residue was triturated with an organic solvent, analyzed by NMR, and further purified.

General Procedure for Basic Nucleophiles. To a rapidly stirred slurry containing compound **6** (0.55 g, 2.0 mmol) and CH_2Cl_2 (10 mL) the desired base (2.0 mmol) was added, effecting immediate solution. In reaction 6, the base was added directly to the reaction solution, while in reactions 4 and 5 1.00 and 1.03 M solutions (titrated against 0.10 N HCl), respectively, of the desired nucleophile were used. The reaction was stirred at room temperature (18 h) and then filtered if necessary. The precipitate was determined to be inorganic in all cases by NMR analysis. The filtrate was gently evaporated in vacuo and the remaining material analyzed by NMR for any relatively volatile components and the initial reaction products. In reactions 5 and 6, the initial distillate was analyzed by ^1H NMR. After drying completely in vacuo, the residue was triturated with warm Et_2O. The Et_2O layer was concentrated to induce crystallization of compound **9**. The filtrate was then evaporated to dryness and was analyzed by NMR to identify any additional products. If an organic salt was present it was generally purified by reprecipitation with Et_2O from a 1:1 solution of CH_3CN and CH_2Cl_2.

Products isolated from reactions 1–9 but not previously described in the Experimental Section are as follows.

***N*-Carboethoxy-*N*-carbomethoxy-*N'*-methylethylenediamine Hydrofluoroborate (16)**: mp 132–133 °C; IR (KBr) 3375, 2950, 1775, 1250, 1075 cm^{-1}; NMR (CD_3CN) δ 1.32 (t, J = 7.0 Hz, 3 H), 2.73 (s, 3 H), 3.27 (t, J = 5.0 Hz, 2 H), 3.81 (s, 3 H), 4.04 (t, J = 5.0 Hz, 2 H), 4.27 (q, J = 7.0 Hz, 2 H), 6.12–6.58 (br s, 2 H); MS *m/e* (rel %) 204 (19), 158 (42), 44 (100).

Anal. ($C_8H_{17}N_2O_4BF_4$) C, H, N.

4-Ethylmorpholine Hydrofluoroborate (18a): IR (neat, NaCl) 3590, 1470, 1090 cm^{-1}; NMR (CD_3CN) δ 1.32 (t, J = 7.0 Hz, 3 H), 2.94–3.55 (m, 6 H), 3.55–4.18 (m, 4 H), 6.42–6.81 (br s, 1 H); MS *m/e* (rel %) 218 (2), 105 (100).

Anal. ($C_6H_{14}NOBF_4$) C, H, N.

Morpholine Hydrofluoroborate (18b): mp 141–143 °C; IR (KBr) 3420, 1105, 1085, 1035 cm^{-1}; NMR (CD_3CN) δ 2.92–3.51 (m, 4 H), 3.97–4.04 (m, 4 H), 5.40–5.78 (br s, 2 H).

Anal. ($C_4H_{10}NOBF_4$) C, H, N.

***O*-Ethyl-1,1,3-trimethylurea Hydrofluoroborate (20)**: IR (neat, NaCl) 3390, 1660, 1540, 1070 cm^{-1}; NMR (CD_3CN) δ 1.42 (t, J = 7.0 Hz, 3 H), 2.76–3.17 (m, 3 H), 3.01 (s, 6 H), 4.40 (q, J = 7.0 Hz, 2 H), 6.02–7.37 (br s, 1 H); MS *m/e* (rel %) 218 (2), 105 (100).

Anal. ($C_6H_{15}N_2OBF_4$) C, H, N.

Mass Spectrometric Analysis (Experiment 1A). Compounds **9**, **16**, and **17** were analyzed for oxygen-18 incorporation after purification using a Hewlett-Packard 5930 mass spectrometer. Multiple scans (>20) were taken for each compound. The same data were collected on unlabeled samples in order to take into account the natural abundance of oxygen-18. The method outlined in ref 54b was used to calculate the percent oxygen-18 incorporation in each sample. The accuracy of the measurement for compounds **9** and **16** is ±0.5%. For compound **17** no quantitative assessment of oxygen-18 could be made. The parent peak for **17** in the mass spectrometer was less than 3% of that observed for 9. Qualitatively, the *m/e* peaks at 173 and 175 were of equal height.

The chemical ionization mode in the mass spectrometer was used to analyze the percent oxygen-18 incorporation in compounds **9** and **17**. For *N*-methyl-*N'*-carbomethoxyimidazolidone (**9**), the ratio of 159 (P + 1):161 (P + 3) peaks was determined, while for *N*-methyl-*N'*-carboethoxyimidazolidone (**17**) the 173 (P + 1):175 (P + 3) ratio was obtained.

The salt, *N*-carboethoxy-*N*-carbomethoxy-*N'*-methylethylenediamine hydrofluoroborate (**16**), did not give a discernible parent peak in the EI (70 eV) mass spectrum. However, a reliable P − HBF_4 (*m/e* at 204) could be observed. The ratio of the peaks at 204:206 was used to analyze the percent incorporation of oxygen-18 in this compound.

Treatment of *N*-Carbomethoxy-*N'*-methyl-2-(2',6'-dimethylphenoxy)imidazolinium Fluoroborate (7) with Nucleophiles. General Procedure for Neutral Nucleophiles. To a stirred CH_3CN solution (15 mL) of compound **7** (0.075 g, 0.02 mmol) was rapidly added the neutral nucleophile. In reactions 10, 11, 12, 13, and 16 the following number of equivalents of nucleophile was added per equivalent of substrate: 280, 125, 85, 55, and 1, respectively. The solution was stirred at least 4 h at room temperature, and then evaporated in vacuo. NMR analysis at this stage provided the initial product ratios. If compound **9** was detected, selective removal of this substrate from the residue was accomplished by trituration with Et_2O. Further purification of **9** was accomplished by recrystallization from Et_2O. Any additional products remaining in the residue were then isolated by reprecipitation of the compound with Et_2O from a 1:1 solution of CH_3CN and CH_2Cl_2.

General Procedure for Basic Nucleophiles. The above procedure was adopted for reactions 14 and 15. In these experiments, the concentrations of nucleophile and substrate were equimolar, and the reaction times 18 h. In reaction 15, the nucleophile was added directly to the reaction solution, while in reaction 14 a 1.02 M solution (titrated against 0.10 N HCl) of NaOH was used.

Products isolated from reactions 10–16 but not previously described in the Experimental Section are as follows.

***N*-Carbo(2',6'-dimethylphenoxy)-*N*-carbomethoxy-*N'*-methylethylenediamine Hydrofluoroborate (21)**: mp 188–190 °C; IR (KBr) 3420, 2960, 1770, 1735, 1145, 1085 cm^{-1}; NMR (CD_3CN) δ 2.20 (s, 6 H), 2.79 (t, J = 5.0 Hz, 3 H), 3.10–3.60 (m, 2 H), 3.93 (s, 3 H), 4.18 (t, J = 5.0 Hz, 2 H), 6.29–7.33 (br s, 2 H), 7.06 (s, 3 H).

Anal. ($C_{14}H_{21}N_2O_4BF_4$) C, H, N.

***N*,*N*-Dicarbomethoxy-*N'*-methylethylenediamine Hydrofluoroborate (22)**: mp 162–164 °C; IR (KBr) 3400, 2920, 1785, 1100, 1080, 1030 cm^{-1}; NMR (CD_3CN) δ 2.69 (t, J = 5.0 Hz, 3 H), 2.95–3.47 (m, 2 H), 3.76 (s, 6 H), 3.86–4.10 (t, J = 5.0 Hz, 2 H), 6.54–7.15 (br s, 2 H).

Anal. ($C_7H_{15}N_2O_4BF_4$) C, H, N.

***N*-Carbomethoxy-*N'*-methyl-2-morpholinoimidazolinium Fluoroborate (23)**: mp 119–121 °C; IR (KBr) 1760, 1610, 1110, 1070 cm^{-1}; NMR (CD_3CN) δ 3.20 (s, 3 H), 3.29–4.02 (m, 8 H), 3.51–4.32 (m, 4 H), 3.81 (s, 3 H).

Anal. ($C_{10}H_{18}N_3O_3BF_4$) C, H, N.

Preparation of *N*-Carbomethoxy-*N'*-methyl-2-methylthioimidazolinium Fluoroborate (24). Trimethyloxonium fluoroborate[21] (1.63 g, 0.011 mol) in CH_3NO_2 (12 mL) was rapidly added to a CH_3NO_2 solution (5 mL) of *N*-carbomethoxy-*N'*-methylimidazolidinethione[19] (1.74 g, 0.01 mol). The reaction was slightly exothermic and was stirred at room temperature for 18 h; then Et_2O (~75 mL) was added causing the separation of an oil. After cooling in an ice bath, the solvents were decanted and the oil was dissolved in CH_2Cl_2 (5 mL) and oiled out with Et_2O. This process was repeated (3×), and then the oil was dried in vacuo to give 2.48 g (90%): IR (neat, NaCl) 1755, 1595, 1280, 1215, 1050 cm^{-1}; NMR (CD_3CN) δ 2.74 (s, 3 H), 3.49 (s, 3 H), 3.89 (s, 3 H), 3.95-4.31 (m, 4 H).

Anal. ($C_7H_{13}N_2O_2SBF_4$) C, H, N.

Preparation of *N*-Carbomethoxy-*N'*-methyl-2-morpholinoimidazolinium Fluoroborate (23). Compound **24** (0.55 g, 0.002 mol) was dissolved in CH_3NO_2 (10 mL) and morpholine (0.175 g, 0.002 mol) was quickly added. The solution was stirred at 40 °C (18 h) while N_2 gas was bubbled through the reaction. The reaction mixture was concentrated in vacuo, dissolved in CH_3CN, and cooled in a dry ice-acetone bath. The addition of Et_2O caused the precipitation of 0.29 g (50%) of the desired compound. The product was further purified by reprecipitation from chloroform-hexanes.

Treatment of *N*-Carbomethoxy-*N,N'*-dimethyl-2-oxoimidazolinium Fluoroborate (8) with Nucleophiles. General Procedure for Neutral Nucleophiles. A freshly prepared, weighed batch of compound **8** was dissolved in a known volume of CH_3NO_2 or CD_3NO_2. No noticeable increase in volume accompanied dissolution. Aliquots of this solution were withdrawn by syringe and injected into a reaction vessel containing the premeasured amount of nucleophile. In reactions 17-19 and 24 1 equiv of nucleophile was added per equiv of substrate. In reactions 22 and 23, the ratio of the number of equivalents of nucleophile to substrate was 2:1. The amount of compound **8** varied from 0.69 to 1.16 mmol. The reactions were allowed to stir at room temperature overnight under Ar, and then gently concentrated in vacuo. NMR analysis of the mixture at this point provided the initial product ratios. The mixture was then redissolved in a 1:1 solution of either CH_3NO_2-$CHCl_3$ or CH_3NO_2-CH_2Cl_2 and then any organic salts (compounds **25**, **18b**, cyclohexylamine hydrofluoroborate, and diisopropylamine hydrofluoroborate) precipitated out by the addition of Et_2O. The filtrate was evaporated in vacuo, and the remaining neutral organic compounds were further purified by either recrystallization (compounds **26** and **29**[36]) or distillation (compounds **26**, **27**,[34] **28**,[35] and **30**[37]).

General Procedure for Basic Nucleophiles. Reactions 20 and 21 were performed under conditions identical with those described above for neutral nucleophiles. In both reactions, the precipitate collected after the addition of compound **8** proved to be inorganic by NMR. The filtrate was then carefully concentrated in vacuo, and NMR analysis of the residue at this point provided the initial product ratios. Further purification of the neutral compounds (**26**, **27**,[34] and **13**) formed was accomplished by distillation and/or recrystallization.

Products isolated from reactions 17-24 but not previously described in the Experimental Section are as follows.

***N*-Carbomethoxy-*N,N'*-dimethylethylenediamine Hydrofluoroborate (25):** mp 133-134 °C; IR (KBr) 3430, 1690, 1490, 1205, 1125, 1085 cm^{-1}; NMR (CD_3NO_2) δ 2.95 (s, 3 H), 2.95 (t, J = 6.0 Hz, 3 H), 3.15-3.82 (m, 4 H), 3.70 (s, 3 H), 6.72-7.34 (br s, 2 H); MS *m/e* (rel %) 147 (9), 146 (14), 115 (29), 102 (32), 57 (100).

Anal. ($C_6H_{15}N_2O_2BF_4$) C, H, N.

***N,N'*-Dicarbomethoxy-*N,N'*-dimethylethylenediamine (26):** mp 32-34 °C; IR (KBr) 1685, 1460 cm^{-1}; NMR ($CDCl_3$) δ 2.95 (s, 3 H), 3.40 (s, 2 H), 3.70 (s, 3 H); MS *m/e* (rel %) 204 (1), 173 (2), 115 (76), 102 (100).

Anal. ($C_8H_{16}N_2O_4$) C, H, N.

Cyclohexylamine Hydrofluoroborate: mp 179-181 °C; IR (KBr) 3440, 1125, 1085, 1035 cm^{-1}; NMR (CD_3NO_2) δ 1.94-2.40 (m, 10 H), 3.25-3.67 (m, 1 H), 5.07-5.71 (br s, 3 H).

Anal. ($C_6H_{14}NBF_4$) C, H, N.

Diisopropylamine Hydrofluoroborate: mp 155-156 °C; IR (KBr) 3470, 1125, 1085, 1040 cm^{-1}; NMR (CD_3NO_2) δ 1.40 (d, J = 7.0 Hz, 12 H), 3.40-4.07 (m, 2 H), 5.76-6.07 (br s, 2 H).

Anal. ($C_6H_{16}NBF_4$) C, H, N.

Acknowledgment. We wish to thank the Robert A. Welch Foundation and the National Institutes of Health for support of this research.

Supplementary Material Available: Additional experimental procedures employed for the preparation of all new compounds, as well as physical and spectral properties observed for compounds isolated, are reported (2 pages). Ordering information is given on any current masthead page.

References and Notes

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Reactions of Water-Soluble Metalloporphyrins with the Serum Protein, Hemopexin

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Abstract: Rabbit hemopexin is capable of binding a wide variety of synthetic porphyrins and metalloporphyrins including those of the meso-substituted variety. The protein has a requirement for negatively charged peripheral substituents on the porphyrin suggesting that the binding site(s) have a residual positive charge. The stable porphyrin-protein complexes are 1:1 and involve monomeric porphyrin units regardless of the state of aggregation of the porphyrin in solution. The kinetics of the reactions of tetra(4-sulfonatophenyl)porphinatoferrate(III) ($Fe^{III}TPPS$) with rabbit hemopexin has been studied as a function of pH. The protein is capable of interacting with either monomers or dimers leading to substantial changes in metalloporphyrin absorbance and protein fluorescence. When the bound $Fe^{III}TPPS$ is dimeric, a much slower process ensues in which the intermediate complex loses a monomer unit to form the stable product.

Introduction

The degradation of red blood cells in disease-related hemolytic events leads to the occurrence of extraerythrocytic hemoglobin in the bloodstream. Several pathways exist to clear the serum of this circulating hemoglobin (cf. Figure 1). The plasma protein, haptoglobin, binds $\alpha\beta$ dimers of dissociated hemoglobin molecules and transports them to the liver,[2] but even moderate amounts of hemoglobin in the plasma are sufficient to markedly deplete this transfer protein. Part of the remaining hemoglobin dissociates into its components—heme and globin—with conversion of the iron from the 2+ to the 3+ oxidation state.[2] This resultant heme (defined here to be iron protoporphyrin IX) is then complexed by the serum proteins, hemopexin and albumin. Hemopexin, which is present at about $^1/_{50}$ the concentration of albumin, binds the metalloporphyrin about 10^5 times[3,4] more tightly than does the latter protein and carries it to the parenchymal cells of the liver[5-9] where the heme is degraded.[7] In contrast, the heme-albumin complex continues to circulate[6] until apohemopexin again becomes available for heme transport. The metalloporphyrin is then transferred from one protein to the other in a slow step[10] and is carried to the liver for degradation and excretion as a bile pigment.

The absorption spectrum of the heme-hemopexin complex, unlike that of the heme-albumin complex, displays the characteristics of a low-spin hemoprotein; e.g., there is no absorption band near 620 nm.[3,5,11] The low-spin nature of the complex has been confirmed by Mössbauer, ESR, and magnetic circular dichroism (MCD) spectroscopy.[12,13] The visible region MCD spectrum of the ferriprotoporphyrin IX-hemopexin complex closely resembles those characteristic of cytochrome b_5 and other bisimidazole-coordinated ferriprotoporphyrin IX derivatives.[13] Furthermore, while the hemopexin complex of ferrideuteroporphyrin IX exhibits an MCD spectrum similar to that for ferriprotoporphyrin IX, neither the cobalt nor nickel derivatives display these effects.[13]

Although hemopexin interacts with a wide variety of naturally occurring and synthetic metalloporphyrins, only the binding of iron porphyrins induces major changes in the tertiary structure of the protein as evidenced by circular dichroism spectra.[5,14] These changes in tertiary structure may lead to recognition of the complex by hepatocytes.[5,9] Solvent perturbation studies employing ethylene glycol indicate that the heme chromophore is about 70% exposed to solvent when bound to hemopexin.[15] The model which emerges from these data is one in which heme is tightly bonded near or at the surface of he-

0002-7863/80/1502-3939$01.00/0