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JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Effect of Buffer General Acid−Base Catalysis on the Stereoselectivity of Ester and Thioester H/D Exchange in D_2O

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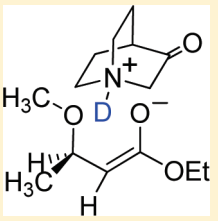
Supporting Information

ABSTRACT: As part of a comprehensive investigation on the stereochemistry of base-catalyzed 1,2-elimination and H/D exchange reactions of carbonyl compounds, we have found that the stereoselectivity of H/D exchange of 3-hydroxybutyryl *N*-acetylcysteamine (**3**) in D_2O is strongly influenced by the presence of buffers. This buffer effect is also operative with a simple acyclic ester, ethyl 3-methoxybutanoate (**7**). Buffers whose general-acid components are cyclic tertiary ammonium ions are particularly effective in changing the stereoselectivity. ^{2}H NMR analysis showed that without buffer, H/D exchange of **3** produces 81−82% of the 2*R**, 3*R** diastereomer of 2-deuterio **3** (the anti product). In the presence of 0.33 M 3-quinuclidinone buffer, only 44% of the 2*R**, 3*R** diastereomer was formed. With ester **7**, the stereoselectivity went from 93−94% in DO^-/D_2O to 60% in the presence of buffer. Phosphate buffer, as well as others, also showed substantial effects. The results are put into the context of what is known about the mechanism of H/D exchange of esters and thioesters, and the relevance of the buffer effect on the mechanism of the enoyl−CoA hydratase reaction is discussed. It is likely that hydrogen bonding in the enolate−buffer acid encounter complex is an important stereochemical determinant in producing a greater amount of the 2*R**, 3*S** diastereomer (the syn product). Studies that involve the protonation of enolate anions in D_2O need to include the buffer general acid in any understanding of the stereoselectivity.



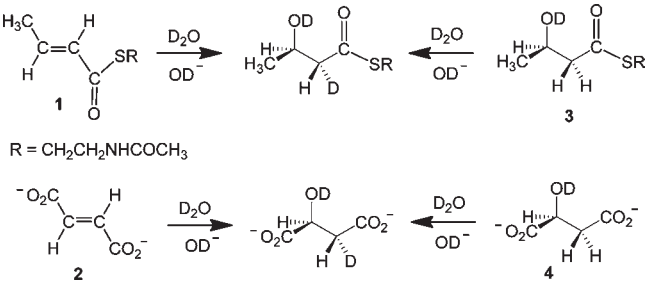
INTRODUCTION

Stereoselectivity is an important criterion in understanding enzymatic catalysis and developing more effective methods for organic synthesis. For example, there are two distinct stereochemical classes of hydratase−dehydratase enzymes. One class includes enoyl−CoA hydratase, which catalyzes the syn addition of water to *S*-crotonyl CoA, an α,β-unsaturated thioester substrate.[1,2] The other class, which includes fumarate hydratase, catalyzes the anti addition of water to conjugated carboxylate substrates. It had been argued that the increased acidity of thioesters could be a determining factor in tilting the stereochemistry toward syn addition−elimination of water in these enzyme-catalyzed reactions.[3]

Study of the stereochemistry of nonenzymatic base-catalyzed addition of D_2O to *S*-crotonyl *N*-acetylcysteamine (**1**) and fumarate (**2**) shown in Scheme 1 suggested that the pK_a difference was not an important stereochemical determinant, but that the syn−anti stereochemistry was the result of the adaptive advantages of separate evolutionary histories.[4] The current view is that enzyme-catalyzed reactions are optimized to take advantage of mechanistic pathways by providing greater stability to key reaction intermediates.[5] Gerlt and Babbit suggest that for the enoyl−CoA hydratase superfamily, divergent evolution with conservation of an oxyanion hole that stabilizes an enolate anion is a key chemical imperative.[6]

We had shown earlier that H/D exchange of 3-hydroxybutyryl *N*-acetylcysteamine (**3**) under base-catalyzed conditions using DO^-/D_2O produced the same stereoselectivity as we observed in the conjugate addition of D_2O to **1**, and that H/D exchange of malate (**4**) gave the same stereoselectivity as we observed in the

Scheme 1

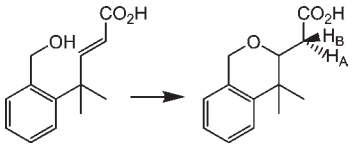


conjugate addition of D_2O to **2**.[4] To extend our earlier study, we chose to ascertain the effect of pH on the stereoselectivity of H/D exchange of **3** in D_2O, using a number of different buffer systems to approach physiological pH levels. We were surprised to discover that the stereoselectivity of H/D exchange of **3** changed depending on which buffer was used. In other words, general-acid catalysis in D_2O produced a change in the stereoselectivity of H/D exchange. This phenomenon is well-known in aprotic organic solvents where proton donors having stringent stereochemical requirements have been used for enantioselective organic synthesis.[7] The diastereoselectivity of intermolecular enolate protonation in organic solvents can also be reversed when intramolecular proton donors compete effectively.[8] However, a change in the stereoselectivity of enolate protonation

Received: January 1, 2011
Published: March 08, 2011

   dx.doi.org/10.1021/ja200014c | *J. Am. Chem. Soc.* 2011, 133, 5124–5128

Scheme 2



5 6

produced by a buffer has been observed only once before in aqueous solution.[9]

When Amyes and Kirby studied the base-catalyzed intramolecular cyclization of the α,β-unsaturated carboxylate **5**, they found that the use of KOD in D_2O gave diastereomer A of **6** (H_B = D) almost exclusively, but in the presence of increasing concentrations of quinuclidine buffers in D_2O, increasing amounts of diastereomer B of **6** (H_A = D) were formed as shown in Scheme 2. They calculated that for the cyclization of the dianion of **5** the water-catalyzed reaction gave ≥90% anti addition, and the reaction involving the conjugate acid of quinuclidine gave >90% syn addition to the conjugated double bond. The authors concluded that more results were needed before they could say that this unprecedented change in stereoselectivity in aqueous solution reflects the fundamental chemistry of the intermediate enolate anion or the special steric requirements of one particular system.[9]

Before presenting our results on the stereoselectivity of H/D exchange of thioester **3** and ethyl 3-methoxybutanoate (**7**), it is important to review briefly what is known about the pathways for the base-catalyzed H/D exchange of thioesters and esters. The results of Amyes and Richard provide our best understanding of the kinetics of proton exchange for simple thioesters in D_2O.[10] Plotting the values of their observed rate constants for deuterium exchange of the α-protons of ethyl thioacetate against buffer concentration in 3-quinuclidinone buffers at pD 7.7−9.3 led to the rate law shown in eq 1, where k_0 equals the rate constant for H/D exchange by the deuteroxide ion.

$$k_{obsd} = k_0 + k_B[\text{buffer}] \qquad k_0 = k_{DO}[DO^-] \tag{1}$$

This kinetic study showed that general-base catalysis produced a free, diffusionally equilibrated, enolate−anion intermediate as shown in Scheme 3.

The rate constants calculated from their data were combined to estimate the pK_a of ethyl thioacetate (20.4−21.5) and k_{BH} (1.7×10^8 to 2×10^9 M^{-1} s^{-1}), whereas the rate constant for diffusional equilibration of the enolate was estimated to be approximately 1.6×10^{10} s^{-1}.[10] The magnitude of k_{HD}^+ for the reaction of the thioester enolate with protonated 3-quinuclidinone showed that there was a chemical barrier to the transfer of a deuteron within the ion pair $BD^+ \cdot {}^-CH_2COSEt$. The lifetime of the enolate−buffer acid intimate ion pair was estimated to be from 10^{-9} to 10^{-10} s with respect to proton transfer to give $B \cdot CH_3COSEt$.

The mechanism of the H/D exchange of ethyl acetate in D_2O has also been carefully studied by Amyes and Richard.[11] Determination of the rate constants for H/D exchange in the presence of 3-substituted quinuclidine buffers made possible the calculation of the pK_a of ethyl acetate (25.6). The mechanism for the H/D exchange of ethyl acetate is summarized in Scheme 3. Proton transfer from ethyl acetate to the deuteroxide ion ($k_{DO}[DO^-]$) or a buffer base ($k_B[B]$) leads to the intimate ion−dipole pair $DOH \cdot {}^-CH_2CO_2Et$ or ion pair $BH^+ \cdot {}^-CH_2CO_2Et$, respectively. The data of Amyes and Richard show that deuterium exchange into ethyl acetate proceeds through the free, diffusionally equilibrated, ester enolate anion.[11] Although diffusional equilibration of the ester enolate is faster than its reaction with solvent ($k_{DOD} \leq k_{HOH} = 5 \times 10^8$ s^{-1}), the reaction rate for transfer of a deuteron from a tertiary ammonium ion buffer to the enolate of ethyl acetate is thought to be limited by the formation of the encounter complex ($k'_{enc}[BD^+]$) rather than the subsequent proton transfer step.

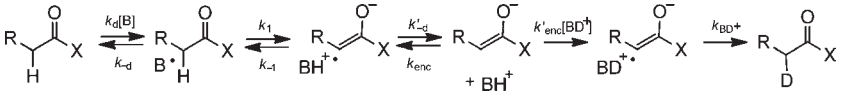
EXPERIMENTAL SECTION

Substrates and Buffers. 3-Hydroxybutyryl *N*-acetylcysteamine **3** and ethyl 3-methoxybutanoate **7** were synthesized by standard methods.[4,12] **3** was purified by silica gel MPLC using 97:3 EtOAc/MeOH. 400 MHz ^{1}H NMR showed 97% purity; ^{1}H NMR (D_2O, δ) 4.08 (m, 1H, CHOH), 3.20 (t, 2H, CH_2N), 2.89 (m, 2H, CH_2S), 2.61 (d, 2H, CH_2CO), 1.78 (s, 3H), 1.05 (d, 3H). Impurities were small amounts of 3-hydroxybutyrate, *N*-acetylcysteamine, and MeOH. Buffer components and their pK_a values were from standard sources. Buffer components were dissolved in D_2O, the solutions were lyophilized, and then redissolved and lyophilized again before redissolving in D_2O (99.9 atom % D) and bringing the pD to the desired value with 11.2 M KOD. The conversion to pD used the equation pD = pH meter reading + 0.4.[13]

H/D Exchange Reactions. H/D exchange reactions on **3** were carried out using 0.5 M solutions in 2.0 mL of stirred 0.05−0.60 M buffer/D_2O solution (N_2) in a 23 °C water bath. Ionic strength was held constant at 0.6 M using $KClO_4$. To avoid dideuteration, the H/D exchange was stopped by the addition of 5% DCl at 4−15% exchange of one C-2 proton. A small amount of hydrolysis occurred under these reaction conditions, and careful additions of 11 M KOD solution were necessary to maintain a constant pD. Extent of H/D exchange was followed using the C-3/C-2 NMR integration ratio in D_2O (HOD = 4.63 ppm). Reaction mixtures of **3** were lyophilized to remove D_2O, back exchanged with 2 mL of H_2O, and again lyophilized. Next, 4:1 C_6H_6/MeOH was added, and the solution was centrifuged to remove buffer salts before NMR analyses. Diastereoselectivity was determined by 61 MHz ^{2}H NMR. Samples that produced asymmetric ^{2}H NMR peak shapes were purified by silica gel MPLC before NMR analysis; all results reported were obtained by multiple integrations of well-formed NMR peaks. The (2*R**, 3*R**) diastereotopic deuteron at C-2 of **3** was at δ 2.43 ppm, and the (2*R**, 3*S**) deuteron was at δ 2.60 ppm (C_6D_6 = 7.15 ppm).[4]

The H/D exchange reactions of **7** were carried out using 0.2 M solutions at 70 °C in 10 mL of 0.33−0.45 M quinuclidinone buffers. The pD was maintained by careful additions of 11 M KOD solution. The H/D exchange was stopped at 5−25% of one proton at C-2, and the reaction mixture was extracted with Et_2O; the solutions were then extracted with 0.1 M HCl followed by 5% $NaHCO_3$ until neutral, dried, and the solvent was evaporated before C_6H_6 was added. Diastereoselectivity was determined by 61 MHz ^{2}H NMR. All results reported were obtained by multiple integrations on well-formed NMR peaks. The (2*R**, 3*R**) diastereotopic deuteron of 2-deuterio **7** was at δ 2.20 ppm, and the (2*R**, 3*S**) deuteron was at δ 2.50 ppm (C_6D_6 = 7.15 ppm). The H/D exchange of **7** was also studied at room temperature using 0.5 M KOD/D_2O (3−13% exchange of one proton).

Scheme 3



dx.doi.org/10.1021/ja200014c |J. Am. Chem. Soc. 2011, 133, 5124–5128

Table 1. Dependence of H/D Exchange Stereoselectivity of 3 on Buffer Concentration[a]

buffer	pK_a	pD	% H/D exchange	reaction time (h)	% 2*R**, 3*R** product[b]
none		12−13	2−15	0.5	81−82[c]
3-quinuclidinone	7.5	8.6	11−13	4−5	44[d]
0.17 M		8.4			48
HEPES	7.5	8.2	7−11	30	39
0.05 M				141	48
Tris	8.2	8.0	20	24	76
phosphate	7.2	8.0	4−9	24−30	60
0.05 M				105−130	70
0.60 M				22	57
4-hydroxybenzenesulfonate	8.5	9.2	5−23	30	86
0.10 M				30	84
0.60 M				5	86

[a] 0.33 M total buffer concentration unless otherwise stated. [b] All values were reproducible to ±2%. [c] Same result with 0.33 M KCl added. [d] Same result at 70 °C (7 min).

RESULTS AND DISCUSSION

Table 1 shows the results of the H/D exchange of thioester **3** using a variety of buffer systems at 0.33 M total buffer concentration at pD 8.0−9.2. After our initial experiments using quinuclidinone buffers, four other buffers were also studied to test the generality of their influence on the stereoselectivity. The general-acid component of two buffers was a tertiary ammonium cation: 3-quinuclidinone and sodium 4-(2-hydroxyethyl)-1-piperazineethanesulfonate (HEPES). The general-acid component of another buffer was anionic, potassium dihydrogen phosphate, and the last was a neutral phenolic acid, potassium 4-hydroxybenzenesulfonate.

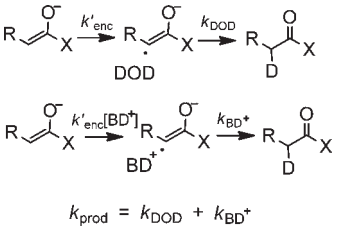
Although pK_a values are listed in Table 1, the more relevant values in D_2O would be pK_{BD}. In the case of 3-quinuclidinone, pK_{BD} is 8.3, 0.8 units higher that the pK_a.[10] On the basis of a limited data set, Glasoe and Long estimated that pK_{BD} values are approximately 0.5 units higher than the pK_a values in water.[13]

Transfer of the deuteron to a free, diffusionally equilibrated, enolate−anion intermediate in D_2O has two competing pathways: one where the solvent D_2O is the acid that transfers the deuteron and another where a buffer general acid does, shown by Scheme 4.

These competing pathways are mirrored in the effect that the total buffer concentration has on the stereoselectivity. When no buffer is present, D_2O is the sole deuterium transfer agent, which produces 81−82% of the 2*R**, 3*R** diastereomer of 3-hydroxy-2-deuteriobutyryl *N*-acetylcysteamine **3**. With greater buffer concentrations and lower pD, the concentration of the intimate ion pair $BD^+ \cdot {}^-RCHCOX$ becomes greater and the stereochemistry changes systematically toward the inherent BD^+ stereoselectivity, although from the data in Table 1 it is not possible to ascertain the BD^+ stereoselectivity. The observed percentages of the 2*R**, 3*R** diastereomer of **3** always reflect a combination of the reactions of both pathways shown in Scheme 4.

The collection of buffers in Table 1 represents a range of different general acids. Perhaps it is not surprising that the most dramatic stereochemical changes are brought about by using 3-quinuclidinone and HEPES buffers, due to their greater steric demands at the cyclic tertiary ammonium site where the labile deuterium is attached. In addition, these two buffer general acids are stronger acids than the conjugate acid of tris-(hydroxymethyl)aminomethane and are presumably stronger hydrogen-bond donors. Although we are unable to assign absolute

Scheme 4



stereoselectivities for 3-quinuclidinone and HEPES, the data in Table 1 clearly show that the general-acid components of these tertiary ammonium buffers lead preferentially to the 2*R**, 3*S** diastereomer of α-deuterated **3**, the opposite stereoselectivity from that shown by deuteration with D_2O. On the other hand, the phenol group of 4-hydroxybenzenesulfonate is actually more stereoselective than D_2O for the 2*R**, 3*R** diastereomer, whereas the dihydrogen phosphate anion produces an intermediate stereoselectivity. It is worth noting that the presence of additional salt and an elevation of the temperature have no effect on the stereochemistry. Better future computational methodologies may provide insight into the subtle stereochemical factors at play in the short-lived encounter complexes that lead to deuterated **3**.[14]

We have had substantial experience in studying the diastereoselectivity of base-catalyzed H/D exchange of β-substituted carbonyl compounds, but except for the influence of aggregation in nonpolar organic solvents, we have always been able to interpret our results using steric and electronic factors pertaining to the substrate.[4,12,15] However, the proton donors that we have studied have been EtOD and D_2O. In retrospect, the results presented in Table 1 for the H/D exchange of **3** with buffer catalysis should not have been a surprise. When an ion pair $BD^+ \cdot {}^-RCHCOX$ transfers a deuteron to carbon, the resulting stereochemistry can depend on both the recognition motif in the enolate−acid encounter complex and the innate stereochemical demands of the enolate anion. This will be true in both aqueous and nonaqueous solvent systems. Of course, the lifetime of the enolate anion will depend on its exact structure and the environment it inhabits. However, even a lifetime of 10^{-9}−10^{-10} s, as has been estimated for deuterium transfer within $BD^+ \cdot {}^-CH_2COSEt$, offers adequate opportunity for different

dx.doi.org/10.1021/ja200014c | J. Am. Chem. Soc. 2011, 133, 5124–5128

stereochemical pathways, depending on the nature of BD^+ and its preferred interactions with the enolate anion. Greater stereoselectivity may result when the activation energy becomes larger for transfer of the deuteron to the α-carbon of the enolate anion.

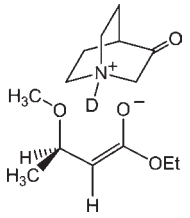
To further ascertain the generality of how a buffer can affect the stereoselectivity of H/D exchange, we have studied the deuteration of a simple acyclic ester, ethyl 3-methoxybutanoate **7** in D_2O. Earlier research had shown that the stereoselectivity of H/D exchange for this ester was higher than that for thioester **3**. The percentage of 2*R**, 3*R** 2-deuterio **7** was 93−94% in D_2O with 0.5 M KOD as the base.[12] Addition of 0.33 M NaCl, KCl, $(CH_3)_4NCl$, or $(CH_3)_4NBr$ had no effect on the observed stereoselectivity in D_2O; 93% of the 2*R**, 3*R** diastereomer of 2-deuterio **7** was produced in every case. However, using 0.33 M 3-quinuclidinone buffer at pD 8.9−9.6 gave 2-deuterio **7** that contained 60 ± 2% of the 2*R**, 3*R** diastereomer (14−25% exchange of one proton). The use of a greater total buffer concentration (0.45 M) at pD 8.2 gave 54 ± 2% of the 2*R**, 3*R** diastereomer. Clearly, the general-acid component of the buffer has a strong influence on the diastereoselectivity of the H/D exchange of **7**.

Even though the transfer of a deuteron to the ester enolate anion of **7** may be limited by the formation of the encounter complex rather than the chemical barrier for deuteron transfer, 3-quinuclidinone buffers produce the same kind of stereochemical outcome for the deuteration of ester **7** as we observed with thioester **3**. This suggests that the formation of the encounter complex of the ester enolate involves a subtle structure recognition motif with the general-acid component of the buffer. However, one must be cautious in making too much of this, in that the ester enolate of 3-methoxybutanoate is likely more stable than the enolate of ethyl acetate. This suggests that there may be a more substantial chemical barrier for deuteration by a tertiary ammonium ion, as also is the case with the enolate from ethyl thioacetate.[10] A greater stability of the ester enolate of **7** is consistent with the faster H/D exchange rate of **7** as compared to that of simpler alkyl esters. An ethoxy group at the 3-position of ethyl butanoate speeds up EtOD/NaOEt catalyzed H/D exchange by ∼80-fold as compared to when the 3-position is substituted with an ethyl group.[15]

The dramatic increase in the amount of 2*R**, 3*S** diastereomer of 2-deuterio **7** in the presence of 3-quinuclidinone buffers, as compared to the stereoselectivity when no buffer is present, is related to the product of syn addition. In the encounter complex shown in Scheme 5, it is not unlikely that hydrogen bonding between the acidic deuteron of the tertiary ammonium ion with the methoxy group at the neighboring stereocenter plays an important role leading to the increased production of the 2*R**, 3*S** diastereomer. Perhaps a three-center hydrogen bond is involved, where the acidic deuteron of the tertiary ammonium ion is bonded both to the methoxy group and to the enolate oxygen. A similar rationale can also account for the stereoselectivity observed in the H/D exchange of thioester **3** in the presence of 3-quinuclidinone buffers. It is more difficult to envision details of solvent hydrogen bonding on the encounter complexes.

Recognition that hydrogen bonding may be a stereochemical determinant in an enolate anion−Bronsted acid encounter complex leads one to consider the possibility that hydrogen bonding may also affect the stereoselectivity of H/D exchange in the absence of buffers. To be specific, can a hydrogen bond between a nascent OD^- and a hydroxyl group at C-3 within an enolate anion−water encounter complex stabilize the transition state for

Scheme 5



syn deuteration? We have studied the H/D exchange reactions of two substrates in KOD/D_2O, thioester **3** and ethyl 3-hydroxybutanoate, which touch upon this question. In each case, there was more 2*R**, 3*S** syn deuterated product than with comparable 3-alkoxybutanoate substrates. The H/D exchange in D_2O of thioester **3** in the absence of buffers produces 18−19% of the 2*R**, 3*S** product, whereas H/D exchange of ethyl 3-methoxybutanoate **7** produces only 6−7% of the 2*R**, 3*S** product. With ethyl 3-hydroxybutanoate as the substrate, 15% of the 2*R**, 3*S** deuterated product is formed.[12] Although the internal stabilization of the transition state for *syn* deuteration by an adjacent hydroxyl group, which could hydrogen bond to the developing deuteroxide anion, is consistent with our data, one must keep in mind that the energy differences are small.

Interestingly, this differential internal hydrogen bonding in the enolate anion−solvent encounter complex is more important in ethanol than in water. Whereas the H/D exchange of **7** with NaOEt/EtOD gives 13% of the 2*R**, 3*S** syn product, H/D exchange of ethyl 3-hydroxybutanoate with NaOEt/EtOD produces 41% of 2*R**, 3*S** product.[12] It is not surprising that an internal C-3 hydroxyl group could effectively stabilize a nascent EtO^- in EtOD and that this effect would be greater than with the stabilization of a nascent OD^- in D_2O.

Finally, we wish to point out that the influence of buffer general-acid catalysis on the stereoselectivity of H/D exchange may have implications in understanding the syn addition of water observed in the enoyl−CoA hydratase reaction.[1,2] The enzymatic mechanism by which a molecule of water is added across the α,β-double bond of enoyl-CoA substrates has been thoroughly studied by a variety of experimental methods, including kinetic isotope effects (KIE) and crystallography.[16] Whether the addition of water to the C=C at the enzyme active site is concerted with a single transition state or whether it involves an enolate intermediate in a two-step process has been controversial. The structure of the E−S complex as determined by X-ray diffraction, together with previously determined KIEs, is consistent with either a concerted mechanism or an E1cB stepwise mechanism.[17] One factor that has been cited in favor of a concerted syn addition of H_2O is the observation that the deuteroxide-catalyzed nonenzymatic addition of D_2O to *S*-crotonyl *N*-acetylcysteamine **1** and H/D exchange of thioester **3** produces 81−82% of the 2*R**, 3*R** anti diastereomer of 3-hydroxy-2-deuteriobutyryl *N*-acetylcysteamine.[4]

The catalysis by enoyl−CoA hydratase involves two glutamic acid residues at the active site, which are part of a hydrogen-bonding network with the molecule of water that is added to the C=C.[17] The C-2 deuteron is transferred by a glutamic acid residue acting as a Bronsted general acid. The buffer effect on the stereoselectivity of protonation of an enolate anion with a Bronsted acid that we have described in this Article shows that the syn product is favored. Thus, a carbanion intermediate that is protonated by a general acid in the pathway of the enoyl−CoA

dx.doi.org/10.1021/ja200014c | *J. Am. Chem. Soc.* 2011, 133, 5124–5128

hydratase reaction would seem to be entirely consistent with the overall syn stereochemistry.

CONCLUSION

Future studies of the stereochemistry of the protonation of enolate anions in D_2O in the presence of buffer systems need to include the buffer general acid in any understanding of the stereoselectivity of H/D exchange.

ASSOCIATED CONTENT

Supporting Information. Experimental procedure for the synthesis of **3** and its NMR spectrum, and full citation for ref 15. This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT

We thank the National Science Foundation (NSF Grant CHE-0110700) and the Howard Hughes Medical Institute for their generous support, and acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research. We also thank the 3M Foundation for helping to fund the purchase of a 400 MHz NMR spectrometer and Prof. Thomas Hoye of the University of Minnesota for help with the purification of **3**.

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