taldehyde (1.2 g, 4.29 mmol) in dry ether (40 mL) is added. Stirring continues for 2 h at −77 °C. The workup is as for **5e**. Data are in Tables VII and VIII.

(b) Ketones 2e–g. A mixture of the corresponding 2,2-dimesityl-1-arylethanol **5** (0.51 mmol) and PDC (573 mg, 1.53 mmol) in dry DMF (5 mL) was stirred at room temperature for 24 h and then poured into water. The solid obtained was dissolved in ether, the layers were separated, and the organic phase was dried ($MgSO_4$) and evaporated. The ketone was crystallized. Yields, crystallization solvent, and analytical and spectroscopic data are in Tables I and VI.

Equilibration Conditions. (a) Samples of the substrate (15 mg) in hexane (AR) (10 mL) containing trifluoroacetic acid (0.05 mL) were kept at 367.6 K in pressure ampoules. Samples were withdrawn at a predetermined time and washed with an aqueous $NaHCO_3$ solution, the layers were separated, the organic phase was dried and evaporated, the remainder was dissolved in $CDCl_3$, and the enol/ketone ratios were determined by integration. Comparisons were made between the single Mes_2CH proton, the four protons Mes-H singlet and the protons of the α-aryl group (which mostly show a low field doublet of ortho protons) of the ketones on the one hand, and the enolic OH, the two protons signals (one sharp and one broad) of the two Mes-H protons, and the aryl protons of the α-aryl group of the enol on the other. In the methyl region comparison was made between the two sharp *o*-Me and *p*-Me singlets of the ketones and three signals of the enols which are at the lower field side of the methyl region. Comparison of other signals, e.g., the MeO group of **1b/2b** was occasionally made. The average of all the ratios which sometimes includes 15 values is given in Table II. Sources of errors are peak overlap, and peak broadening resulting from coalescence of several *o*-Me and Mes-H signals of the enols due to the ring flip process. Broadening of the OH signal may be due to intermolecular exchange.

(b) Samples of approximately 20 mg (0.04 mmol) of the ketone or the enol were dissolved in 10 mL of hexane (AR) containing 0.01 mL of CF_3COOH in pressure tubes and kept at 367.6 K. After workup as described above, the enol/ketone ratio in the residue was determined by HPLC (Trakor 970 A) with the detector at 254 nm. For analysis of systems **1,2a–f** 10 μm, 250 mm × 4 mm CN-Lichrosorb column was used. The eluants were 9:1 hexane–CH_2Cl_2 (0.7 mL/min) for **1a/2a**, 8:2 hexane–CH_2Cl_2 (0.7 mL/min) for **1b/2b**, hexane (2 mL/min) for **1c/2c**, **1d/2d**, **1e/2e**, and hexane (1 mL/min) for **1f/2f**. A 5 μm, 150 mm/3 mm (Glass cartridge) Diol Lichrosorb column was used for **1g/2g** with hexane as the eluant (0.5 mL/min). The detection absorptions were calibrated by measuring the peak intensities of known enol/ketone ratios.

In spite of the shorter reaction times used for the HPLC analysis small impurity peaks were observed. Since their ε values are unknown, it was impossible to evaluate their percentages.

Acknowledgment. We are indebted to Ilana Cohen and D. Farin for technical assistance, to Dr. S. Cohen for the X-ray diffractions, to Dr. D. Kohn for help with the IR spectra, to Dr. Silvio Biali for discussions, to Prof. W. P. Jencks and Prof. F. Taskinen for correspondence, to Prof. A. Y. Meyer for assisting in the calculations, and to Prof. Y. Apeloig and Dr. M. Karni for the MO calculations. This work was supported by a grant from the United States–Israel Binational Science Foundation (BSF), Jerusalem, to which we are grateful.

Registry No. 1a, 106359-74-4; **1b**, 106359-75-5; **1c**, 106359-76-6; **1d**, 77787-80-5; **1e**, 106359-77-7; **1f**, 106359-78-8; **1g**, 106359-79-9; **2a**, 106359-80-2; **2b**, 106359-81-3; **2c**, 106359-82-4; **2d**, 77787-78-1; **2e**, 106359-83-5; **2f**, 106359-84-6; **2g**, 106359-85-7; **5e**, 106359-86-8; **5f**, 106359-87-9; **5a**, 106359-88-0; $Mes_2C{=}C{=}O$, 87871-33-8; *p*-$MeOC_6H_4Br$, 104-92-7; *p*-MeC_6H_4Br, 106-38-7; *m*-ClC_6H_4Br, 108-37-2; *p*-$F_3CC_6H_4Br$, 402-43-7; C_6H_5Br, 108-86-1; *p*-$PhOC_6H_4Br$, 101-55-3; 1,3,5-tribromobenzene, 626-39-1; *m*-dichlorobenzene, 541-73-1; dimesitylacetic acid, 5740-42-1; dimesitylacetaldehyde, 94203-58-4.

Supplementary Material Available: Tables S1–S16 giving the crystallographic data for compounds **2b**, **2d**, **2g**, and **1d**, Figures S1, S2, S3, and S5 giving the stereoviews of these compounds, and Figure S4 giving the ORTEP drawings of **2g** (16 pages). Ordering information is given on any current masthead page.

Exchange Rates of the Amide Protons of *d*-Biotin Sulfoxide Stereoisomers†

Terry L. Fox,*‡ Peter A. Tipton,§ W. W. Cleland,§ and Albert S. Mildvan*‡

Contribution from the Department of Biological Chemistry, The Johns Hopkins University, School of Medicine, Baltimore, Maryland 21205, and the Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706. *Received October 14, 1986*

Abstract: The effects of pH on the exchange rates of the amide NH protons of *d*-biotin *d*-sulfoxide and of *d*-biotin *l*-sulfoxide with water have been measured by the transfer of saturation method. The NH exchange rates of both sulfoxides show typical first-order dependences on $[H^+]$, presumably due to the inability of the sulfoxide sulfur or oxygen to form a transannular bond to the protonated carbonyl group of the amide. A comparison of the second-order exchange rate constants of *d*-biotin *d*-sulfoxide with those of biotin indicates that base catalysis is fivefold faster and acid catalysis is 60-fold slower in the *d*-sulfoxide. The *l*-sulfoxide shows 1.2–1.8-fold inhibition of base catalysis and somewhat greater inhibition of acid catalysis (fivefold) when compared with biotin. These effects are qualitatively consistent with the differing orientations of the $S^+–O^-$ dipole in the *d*- and the *l*-sulfoxides such that the partially positive sulfoxide sulfur stabilizes the anionic intermediate in base catalysis and destabilizes the cationic intermediate in acid catalysis, while the partially negative sulfoxide oxygen exerts opposite effects. A quantitative estimate of the electrostatic effects on the energy levels of the intermediates indicates that such effects, while in the right direction, are insufficient in magnitude to explain the observed differences in the relative exchange rates of the *d*- and *l*-sulfoxides of biotin, unless a significantly lower effective dielectric constant exists at the reaction center. Alternatively, entropic effects must be invoked to explain the relative rates.

In a previous paper[1] Fry et al. reported the unusual kinetics of the exchange of the 1′-NH proton of *d*-biotin, and its methyl ester, with water protons. Unlike all other amide NH protons, including the 3′-NH of *d*-biotin and its methyl ester, the exchange of the 1′-NH proton showed a second-order dependence on $[H^+]^2$ (Figure 1A). This unprecedented kinetic behavior required the presence of sulfur in biotin, since typical first-order dependences on $[H^+]$ were observed with O-heterobiotin, desthiobiotin, and imidazolidone. The second-order term in $[H^+]^2$ was ascribed to the formation of a doubly protonated form of biotin, facilitated

† Supported by National Institutes of Health Grants AM 28616 (A.S.M.) and GM 18938 (W.W.C.).

‡ The John Hopkins University.

§ University of Wisconsin.

(1) Fry, D. C.; Fox, T. L.; Lane, M. D.; Mildvan, A. S. *J. Am. Chem. Soc.* **1985**, *107*, 7659. **Note Added In Proof**: Recent measurements of the acid-catalyzed exchange of the NH protons of *d*-biotin and its methyl ester at the lower temperature of 10 °C (rather than 25 °C), where the decreased rates can be measured more accurately, reveal a kinetic order in $[H^+]$ at the 1′-NH of 1.23 ± 0.07, and 1.30 ± 0.06 (rather than 2.0), and a kinetic order in $[H^+]$ at the 3′-NH of 1.04 ± 0.05 and 0.96 ± 0.12, as originally found (E. H. Serpersu, T. Fox, D. C. Fry, M. D. Lane, and A. S. Mildvan, to be published).

0002-7863/87/1509-2127$01.50/0

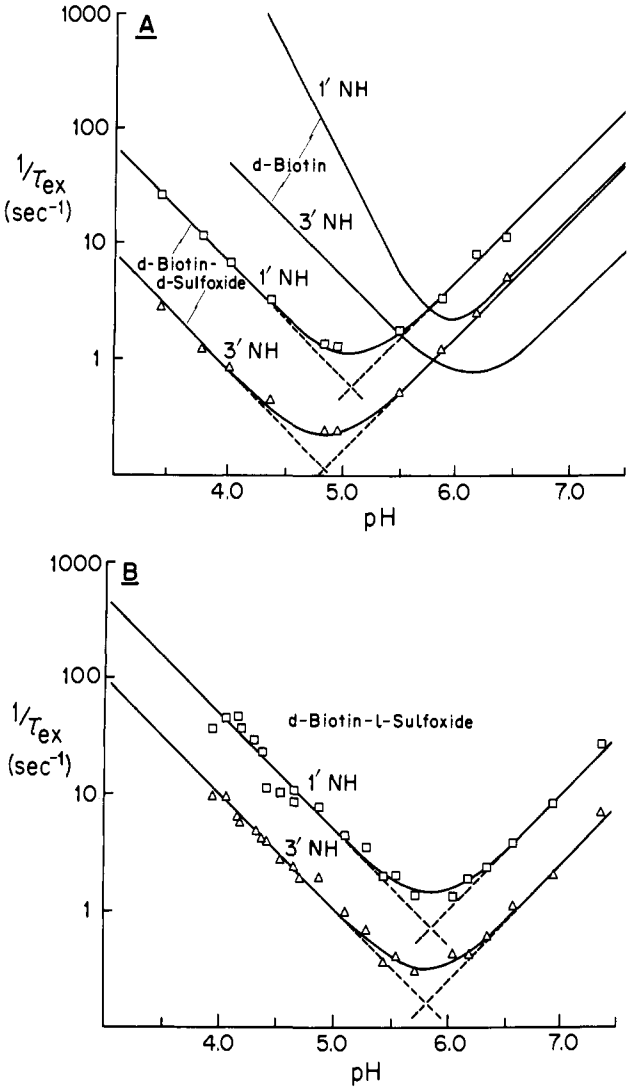

Figure 1. Exchange rates of amide NH proton with water as a function of pH for *d*-biotin *d*-sulfoxide (A) and *d*-biotin *l*-sulfoxide (B). Also shown for comparison are the previously reported exchange rates of *d*-biotin[1] (A). Solutions contained 4.0 mM *d*- or *l*-sulfoxide, 0.1 M KCl, and 10% ^2H_2O. pH was adjusted by the addition of μL amounts of dilute HCl and NaOH. The set of pseudo-first-order exchange rate measurements for each NH proton was fit by a curve representing the sum of two linear functions, corresponding to acid and base catalysis, which are shown as dashed lines. The exchange rates $1/\tau_{ex}$ were fit to the equation $1/\tau_{ex} = k_{OH^-}[OH^-] + k_{H^+}[H^+]$ where k_{OH^-} and k_{H^+} are the second-order rate constants for base- and acid-catalyzed exchange, respectively (Table I). For *d*-biotin[1] and its methyl ester[1] an additional term $k'_{H^+}[H^+]^2$ was needed to fit the exchange rates of 1′-NH, where k'_{H^+} is a third-order rate constant for acid-catalyzed exchange. Temperature, 25 °C.

by the transannular bonding of sulfur to the protonated carbonyl carbon (Figure 2A). We now show that both the *d*- and *l*-stereoisomers of *d*-biotin sulfoxide, in which transannular interaction with sulfur is blocked, show simple exchange kinetics, first order in $[H^+]$.

Experimental Section

The biotin sulfoxide stereoisomers were prepared and characterized as described by Melville.[2,3] The melting points and proton NMR spectra in ^2H_2O indicated negligible cross contamination (<2%) of the stereoisomers. The amide NH proton resonances of the sulfoxides in H_2O containing 10% ^2H_2O and 100 mM KCl were assigned as follows: *d*-biotin *d*-sulfoxide, 1′-NH, 6.78 ppm; 3′-NH, 6.91 ppm; *d*-biotin *l*-sulfoxide, 1′-NH, 6.68 ppm; 3′-NH, 6.82 ppm. These assignments are based on those of *d*-biotin as determined by comparison with the 3′-N methyl derivative.[1]

(2) Melville, D. B. *J. Biol. Chem.* **1954**, *108*, 495.
(3) Berg J. M.; Holm, R. H. *J. Am. Chem. Soc.* **1985**, *107*, 925.

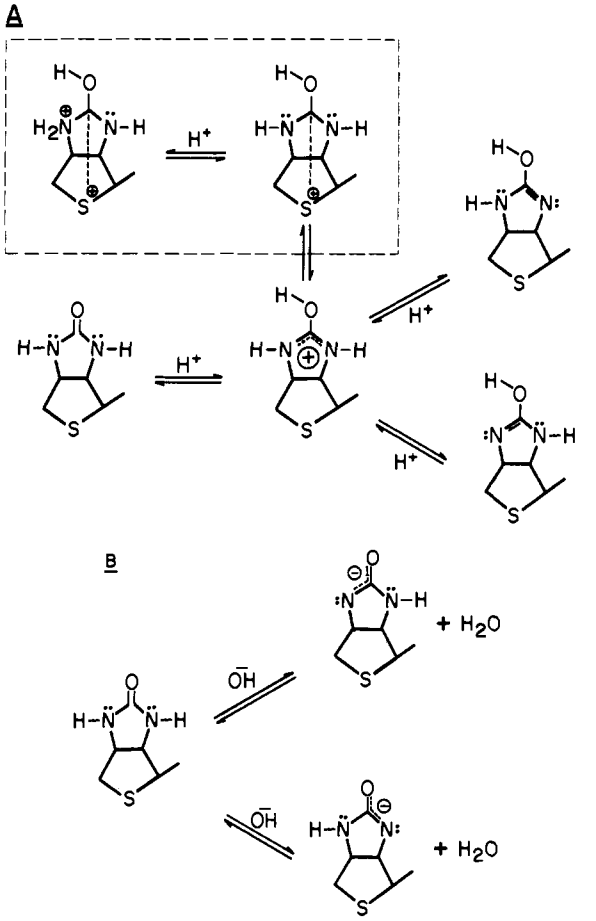

Figure 2. Mechanisms of exchange of the amide NH protons of biotin.[1] (A) Acid-catalyzed exchange. The pathway enclosed in dashed lines is available only for *d*-biotin and its methyl ester but not for *d*-biotin-*d* sulfoxide, *d*-biotin *l*-sulfoxide (present paper), *O*-heterobiotin,[1] desthiobiotin,[1] or imidazolidone.[1] (B) Base-catalyzed exchange.

Table I. Rate Constants for Acid- and Base-Catalyzed Proton Exchange on Biotin Derivatives Used to Fit the Data of Figure 1

compound	proton	k_{H^+} (M^{-1} s^{-1})	k_{OH^-} (M^{-1} s^{-1})
d-biotin *l*-sulfoxide	1′-NH	5.0×10^5	1.0×10^8
	3′-NH	1.0×10^5	2.5×10^7
d-biotin *d*-sulfoxide	1′-NH	6.9×10^4	4.9×10^8
	3′-NH	8.1×10^3	1.6×10^8
d-biotin[a]	1′-NH	*b*	1.8×10^8
	3′-NH	4.9×10^5	2.9×10^7

[a] From ref 1. [b] Second order in $[H^+]$; $k' = 3.8 \times 10^{11}$ M^{-2} s^{-1}.

The exchange rates of these NH protons with water protons were determined as a function of pH by proton NMR at 250 MHz by using the transfer of saturation method, suppressing the strong water signal by the 45°–τ–45°, hard pulse technique as previously described.[1]

Results and Discussion

Figure 1 shows the effects of pH on the pseudo-first-order rate constants for exchange with water of the 1′-NH and 3′-NH protons of *d*-biotin *d*-sulfoxide and *d*-biotin *l*-sulfoxide as well as those previously reported for *d*-biotin.[1] The second-order rate constants for H^+- and OH^--catalyzed exchange used to fit the data of Figure 1 are given in Table I, which also includes the rate constants previously obtained for *d*-biotin for comparison.

As found with *d*-biotin, both acid- and base-catalyzed proton exchange of the 1′-NH of the sulfoxides is faster than at the 3′-NH probably due to steric effects at the 3′-N by the side chain. In contrast with exchange at the 1′-NH of *d*-biotin, no evidence for a process second order in $[H^+]^2$ is detected with the sulfoxides, presumably due to the inability of the sulfoxide sulfur to bond to the protonated amide carbonyl group. Although the distance

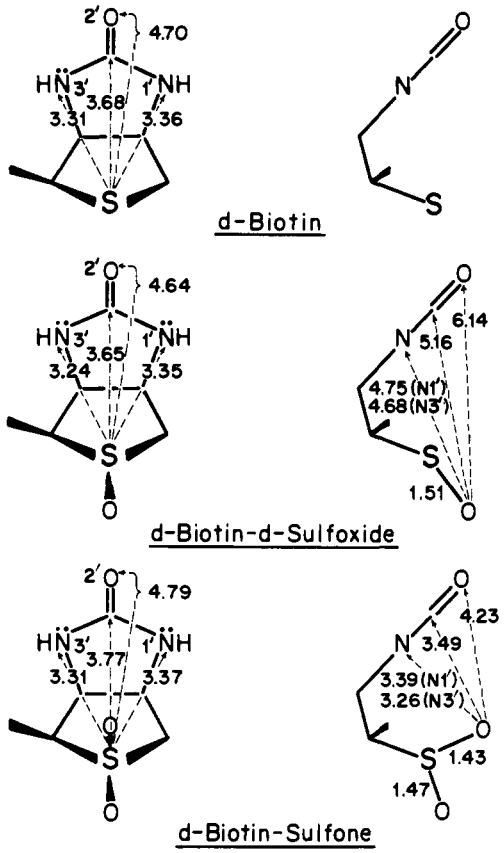

Figure 3. Two views of the structures of *d*-biotin, *d*-biotin *d*-sulfoxide, and *d*-biotin sulfone based on their X-ray structures.[4,5] Distances in Å are calculated from the X-ray coordinates.[4,5]

from the sulfur to the C2′ carbonyl carbon in *d*-biotin *d*-sulfoxide (3.65 Å, Figure 3) as calculated from its X-ray coordinates[4] is very similar to that of *d*-biotin (3.68 Å)[5] and its methyl ester (3.62 Å)[4] and exceeds the sum of the van der Waals radii (3.55 Å) by only 0.1 Å, transannular bonding in the sulfoxide is unlikely, due to the partially positive charge of sulfur.[6-8] The partially negative equatorial sulfoxide oxygen points away from the amide carbonyl group and is 5.16 Å from the C2′ carbon (Figure 3) precluding transannular bonding by the oxygen.

Although no X-ray structure is available for *d*-biotin *l*-sulfoxide, similar effects in this stereoisomer would be expected to prevent transannular bonding. Thus, the sulfoxide sulfur is partially positive, and the axial sulfoxide oxygen, estimated from the X-ray structure of *d*-biotin sulfone[4] to be 3.49 Å from the C2′ carbonyl carbon (Figure 3), is at a distance which exceeds the van der Waals sum by 0.39 Å.

A comparison of the second-order rate constants for *d*-biotin *d*-sulfoxide with those of *d*-biotin itself indicates that base catalysis is fivefold faster and acid catalysis is 60-fold slower in the *d*-sulfoxide (Table I). In the *d*-sulfoxide[4] the axis of the polarized S^+–O^- bond points 121.9° away from that of the C2′ carbonyl group, with the partially cationic sulfur very near N1′ (3.35 Å) and N3′ (3.24 Å) and somewhat further (4.64 Å) from the C2′ carbonyl oxygen of the ureido group (Figure 3). Hence, the anionic imidate intermediate in base catalysis (Figure 2B) would be stabilized, facilitating this process, while the protonated carbonyl intermediate in acid catalysis (Figure 2A) would be destabilized, resulting in inhibition. The fact that acid catalysis is inhibited an order of magnitude more than base catalysis is stimulated suggests that the positive charge of the cationic intermediate in acid catalysis resides largely on N1 and N3 (Figure 2A), while the negative charge of the anionic intermediate in base catalysis is primarily on the carbonyl oxygen (Figure 2B).

A comparison of the rate constants of the *l*-sulfoxide with those of biotin reveals slight inhibition of base catalysis (1.2–1.8 fold) and somewhat greater inhibition of acid catalysis (fivefold). In the *l*-sulfoxide, judging from the X-ray structure of the sulfone,[4] the axis of the polarized S^+–O^- bond is more nearly parallel (29.9°) to that of the C2′ carbonyl group (Figure 3) with the partially negative sulfoxide oxygen only 3.39 Å from N1, 3.26 Å from N3, and 4.23 Å from the C2′ carbonyl oxygen. Hence, in the *l*-sulfoxide, the partially negative sulfoxide oxygen is in a position to attenuate the effects of the partially positive sulfur, as noted with the *d*-sulfoxide, by an order of magnitude.

In the same way, the electrostatic effects of the sulfoxide dipole might be invoked to explain the relative rate constants for the *d*- and *l*-sulfoxides of biotin (Table I) which reveal a 4.90- and 6.4-fold faster base-catalyzed exchange and a 7.2- and 12.3-fold slower acid-catalyzed exchange at N1′ and N3′, respectively, for the *d*-isomer ascompared to the *l*-isomer.

While the orientation and resulting electrostatic effects of the sulfoxide dipole provide a simple and reasonable qualitative explanation of these relative rate constants, a quantitative estimate suggests that electrostatic effects on the energy levels of the intermediates, while correct in direction, are too small in magnitude to fully explain the observed differences in the kinetic barriers, unless the local dielectric constant at the reaction center is less than that of bulk water. From the bond dipole moment (1.0×10^{-29} C·m)[9] and length (1.51 Å)[4] of the S–O bond, the charge on the sulfur is estimated as +0.41 and on the oxygen as −0.41. By using these values and the known distances (Figure 3), we estimate that the electrostatic effects of the sulfoxide group in water would lower the energy level of the anionic intermediate in base catalysis by 0.15 Kcal/mol and would raise the energy level of the cationic intermediate in acid catalysis by a similar amount in the *d*- vs. the *l*-sulfoxide of biotin. Since the observed differences in kinetic barriers between the *d*- and *l*-sulfoxides are −0.9 and −1.1 Kcal/mol for base-catalyzed exchange at N1 and N3 and 1.2 and 1.5 Kcal/mol for acid-catalyzed exchange at these positions, additional effects must be operative.[10] Such effects may be a lower effective dielectric constant (~10) at the reaction center, the selective solvation and neutralization of the negative charge of the sulfoxide oxygen in the *d*-sulfoxide, and/or the orientation of the attacking $^-$OH ion by the sulfoxide dipole. Solvation and orientational effects would be expected to alter the entropy barrier rather than the energy barrier to the exchange reaction and would, therefore, not show up in an electrostatic energy calculation.

Acknowledgment. We are grateful to Jeremy M. Berg, William R. Kirk, Saul Wolfe, and Charles C. Price for substantive advice.

(4) de Titta, G. T.; Parthasarathy, R.; Blessing, R. H.; Stallings, W. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 333.
(5) de Titta, G. T.; Edwards, J. W.; Stallings, W.; Donohue, J. *J. Am. Chem. Soc.* **1976**, *98*, 1920.
(6) Szmant, H. H. In *Organic Sulfur Compounds*; Kharash, N., Ed.; Pergamon: New York, 1962; p 154.
(7) Martin, D; Hanthal H. G. In *Dimethyl Sulphoxide*, Wiley: New York, 1975; p 49.
(8) Price, C. C.; Oae, S. In *Sulfur Bonding*; Ronald Press: New York, 1962; p 129.
(9) Huheey, J. E. In *Inorganic Chemistry*; Harper and Row: New York, 1983; p 163.
(10) We are grateful to Professor Saul Wolfe of Queens University, Kingston, Ontario, Canada, for informing us that somewhat higher net atomic charges on the sulfoxide sulfur (+1.00) and oxygen (−0.68) are obtained by ab initio quantum mechanical calculations on dimethyl sulfoxide by using the 3-21G* basis set[11] which predicts the proper bond angles and molecular dipole moment of this molecule. The use of these charges yields calculated electrostatic effects on kinetic barriers of at most 0.27 Kcal/mol which are also significantly lower than those observed.
(11) Pietro, W. J.; Francl, M. M.; Hehre, W. J.; DeFrees, D. J.; Pople, J. A.; Binkley, J. S. *J. Am. Chem. Soc.* **1982**, *104*, 5039.