Kinetic Evidence for Intramolecular General-Acid Catalysis in the Hydrolysis of a Prostacyclin Model

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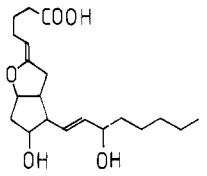
(*E*)-6,9-Epoxynon-5-enoic acid has been synthesized, and the kinetics of its hydrolysis reaction have been studied. The pH profile is very similar to that of (*Z*)-6,9-epoxynon-5-enoic acid, a model of prostacyclin. This suggests that intramolecular general-acid catalysis is responsible for the extra hydrolytic lability of the carboxylate form of the compound. Results from solvent isotope effect measurements using the *Z* isomer support this.

The interesting biological properties[1] of prostacyclin (1) coupled with the extreme hydrolytic lability of the compound have caused a lot of activity in finding analogues of prostacyclin that are more stable but still retain the biological activity.[2]



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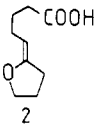
A central issue in this matter has been understanding the reasons behind the hydrolytic lability of prostacyclin, and kinetic investigations have therefore been carried out on prostacyclin and its methyl ester.[3] The kinetic data for prostacyclin could be interpreted in terms of Scheme I.

On the basis of solvent isotope effect data, it has been suggested that the extra hydrolytic lability ($k'_{H^+}/k_{H^+} = 99$) observed for prostacyclin is the result of intramolecular general-acid catalysis by the carboxylic acid group.[3b]

In our study[4] of a model compound (**2**) of prostacyclin we have obtained results very similar to those of prostacyclin, suggesting that **2** is a very good model of prostacyclin from the chemical point of view. However, the model does not have the physiological properties of prostacyclin.[4a]



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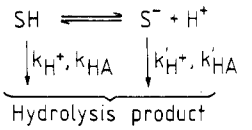
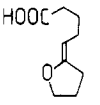
In the present work we report the results from an investigation of the model compound **3** and its methyl ester.

Scheme I



The results support the conclusions drawn from the investigations on prostacyclin.[3b]



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Experimental Section

The ^{1}H NMR spectrum was recorded on a Bruker WH 270 instrument, and chemical shifts are given in ppm downfield from Me_4Si. Mass spectra were obtained with a Finnigan 1020 mass spectrometer and a ZAB-HF mass spectrometer.

Synthetic Procedures. (*E*)-6,9-Epoxynon-5-enoic Acid Methyl Ester. (*E*)-6,9-Epoxynon-5-enoic acid methyl ester was prepared according to ref 4b. After semipreparative HPLC[4b] (high-performance liquid chromatography) the *Z* and *E* isomers were obtained in an 8:1 ratio.

Analysis data for the *E* isomer: ^{1}H NMR (C_6D_6) δ 1.35–1.47 (m, 2 H), 1.61–1.73 (m, 2 H), 1.87–1.98 (m, 2 H), 2.10–2.14 (m, 4 H), 3.37 (s, 3 H), 3.63–3.68 (t, 2 H, J = 7 Hz), 4.85–4.93 (m, 1 H); MS m/e (relative intensity) 53 (11.2), 55 (100), 59 (10.6), 67 (10.9), 68 (10.4), 69 (21.2), 81 (11.5), 82 (12.1), 84 (34.1), 97 (87.9), 110 (32.9), 111 (19.7), 184 (18.0) (all peaks smaller than 10% of the base peak are omitted); HRMS calcd for $C_{10}H_{16}O_3$ 184.1098, found 184.1091.

(*E*)-6,9-Epoxynon-5-enoic Acid Sodium Salt. Saponification of (*E*)-6,9-epoxynon-5-enoic acid methyl ester gave (*E*)-6,9-epoxynon-5-enoic acid sodium salt under the conditions given in ref 4b.

(*Z*)-6,9-Epoxynon-5-enoic Acid Methyl Ester. (*Z*)-6,9-Epoxynon-5-enoic acid methyl ester and the corresponding sodium salt were prepared according to ref 4b.

Kinetic Procedure. The buffer solutions were prepared from commercially obtained buffer components, all of the A.R. grade.

The ionic strength varied between 0.05 and 0.10 M (with NaCl or KCl) in the measurements on (*E*)-6,9-epoxynon-5-enoic acid and its methyl ester. We have found that this small difference in ionic strength had very little effect on the reaction rates. All hydrogen ion concentrations were calculated with activity coefficients recommended by Bates.[5]

(1) Moncada, S.; Gryglewski, R.; Bunting, S.; Vane, J. R. *Nature (London)* **1976**, *263*, 663–665.

(2) For a recent review of prostacyclin analogues, see: Nickolson, R. C.; Town, M. H.; Vorbrüggen, H. *Med. Res. Rev.* **1985**, *5*(1), 1–53.

(3) (a) Chiang, Y.; Kresge, A. J.; Cho, M. J. *J. Chem. Soc., Chem. Commun.* **1979**, 129–130. (b) Chiang, Y.; Cho, M. J.; Euser, B. A.; Kresge, A. J. *J. Am. Chem. Soc.* **1986**, *108*, 4192–4196.

(4) (a) Bergman, N.-Å.; Chiang, Y.; Jansson, M.; Kresge, A. J.; Yin, Y. *J. Chem. Soc., Chem. Commun.* **1986**, 1366–1368. (b) Bergman, N.-Å.; Jansson, M.; Chiang, Y.; Kresge, A. J.; Yin, Y. *J. Org. Chem.* **1987**, *52*, 4449–4453.

(5) Bates, R. G. *Determination of pH. Theory and Practice*; Wiley: New York, 1973; p 49.

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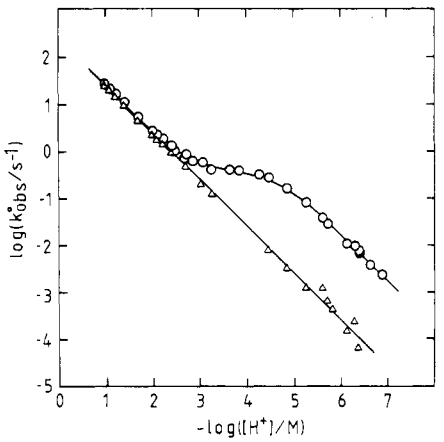


Figure 1. Rate profile for (*E*)-6,9-epoxynon-5-enoic acid (○) and its methyl ester (△) in aqueous solution at 25.0 ± 0.1 °C.

The hydrolysis reaction was followed by monitoring the decrease in absorbance of the vinyl ether group at 220–230 nm. The reaction was followed for at least 2–3 half-lives and showed first-order kinetics.

The kinetic measurements were made with either a Varian Cary 210 spectrophotometer, a Durrum-Gibson stopped-flow spectrophotometer, or a Hi-Tech stopped-flow accessory connected to the Varian 210 spectrophotometer. The buffer solutions and the UV cells were thermostated at 25.0 ± 0.1 °C. The substrate concentration in the cell was ca. 10^{-4} M. The kinetic data conformed to the first-order rate law well, and observed rate constants were evaluated according to ref 4b.

Results

The rates of hydrolysis of (*E*)-6,9-epoxynon-5-enoic acid and its methyl ester were measured in dilute perchloric acid and various buffer solutions. The data are summarized in Tables S1–S4.[6]

At a given pH the observed pseudo-first-order rate constant is given by eq 1 for both the carboxylic acid and

$$k_{obsd} = k^\circ_{obsd} + k^{app}_{HA}[HA] \quad (1)$$

the methyl ester. The buffer-independent part, k°_{obsd}, and the contribution from the buffer acid, k^{app}_{HA}, were evaluated from measurements made at constant buffer ratios but varying buffer concentration.

The hydrolysis of the model compound showed no catalysis by base.

The results from measurements on the methyl ester (Tables S3 and S4) show that the buffer-independent part could be represented by

$$k^\circ_{obsd} = (242 \pm 1)[H^+]$$

i.e., k°_{obsd} is given by $k^\circ_{obsd} = k_{H^+}[H^+]$; $k_{H^+} = 242 \pm 1$ M^{-1} s^{-1}, with no contribution from the solvent.

The hydrolysis of the vinyl ether function of the carboxylic acid shows a more complicated pattern.

As was previously found for the *Z* isomer,[4b] the buffer-independent part of the observed rate constant (eq 1) is given by eq 2, which could be derived under the assumption that the reaction takes place according to Scheme I. A nonlinear least-squares fit[7] of eq 2 to the experimental data (Figure 1) gave $K_a = (2.07 \pm 0.14)\ 10^{-5}$ M, $k_{H^+} = 242 \pm 9$ M^{-1} s^{-1}, $k'_{H^+}/k_{H^+} = 73 \pm 3$, and thus $k'_{H^+} = 17700 \pm 1400$ M^{-1} s^{-1}. These parameters are of the same magnitude as the ones obtained for the *Z* isomer and for prostacyclin itself. A comparison is given in Table I.

$$k^\circ_{obsd} = \frac{[H^+]}{1 + [H^+]/K_a} k_{H^+}\left(\frac{[H^+]}{K_a} + \frac{k'_{H^+}}{k_{H^+}}\right) \quad (2)$$

(6) Supplementary material; see paragraph at the end of this paper.
(7) Johnson, K. J. *Numerical Methods in Chemistry*; Marcel Dekker: New York, 1980.

Table I. Reaction Parameters for the Hydrolysis of (*E*)-6,9-Epoxynon-5-enoic Acid, (*Z*)-6,9-Epoxynon-5-enoic Acid, and Prostacyclin in Aqueous Solution at 25 °C

parameter	(*E*)-6,9-epoxynon-5-enoic acid[a]	(*Z*)-6,9-epoxynon-5-enoic acid[a,b]	prostacyclin[a,c]
pK_a[d]	4.87 ± 0.03	4.93 ± 0.02	5.03 ± 0.15
$k_{H^+}/(M^{-1}\ s^{-1})$	242 ± 9	745 ± 17	439 ± 4
$k'_{H^+}/(M^{-1}\ s^{-1})$	17700 ± 1400	61100 ± 2900	43600 ± 900
k_{H^+}(ester)$/(M^{-1}\ s^{-1})$	242 ± 1	697 ± 10	418 ± 5
k'_{H^+}/k_{H^+}	73 ± 3	82 ± 2	99 ± 2

[a] The uncertainties cited are standard deviations derived from statistical analysis of the data; they do not include possible systematic errors. [b] Reference 4b. [c] Reference 3b. [d] Acidity constant at zero ionic strength estimated with activity coefficients recommended by Bates.[5]

Table II. Rate Constants for the Hydrolysis of (*Z*)-6,9-Epoxynon-5-enoic Acid and Its Methyl Ester in D_2O and in H_2O at 25 °C (Ionic Strength = 0.1 M)

catalyst	$k'/(M^{-1}\ s^{-1})$[a]	$k/(M^{-1}\ s^{-1})$[b]
H_3O^+	56400[c]	703
D_3O^+	48900[d]	215
CH_3COOH		1.41[e]
CH_3COOD		0.234[e,f]
$H_2PO_4^-$	0.174[c]	
$D_2PO_4^-$	0.0339[g]	

[a] Rate constant for the carboxylate form of the model compound. [b] Rate constant for the methyl ester. [c] This value is based only on the biphosphate buffer measurements in ref 4b. [d] Evaluated by using eq 3, assuming that the solvent isotope effect on the ionization of the carboxylic acid group of the substrate is the same as for acetic acid.[11] [e] $I = 0.04$ M. [f] $[D^+]$ values were obtained by using a solvent isotope effect on the ionization of acetic acid of $\Delta pK_a = 0.514$.[11] [g] Evaluated by using eq 5, assuming that the solvent isotope effect on the ionization of the carboxylic acid group of the substrate is the same as for acetic acid.[11] $[D^+]$ values were obtained by using a solvent isotope effect on the ionization of biphosphate ion of $\Delta pK_a = 0.535$.[13]

In the present work, the rates of hydrolysis of (*Z*)-6,9-epoxynon-5-enoic acid and its methyl ester were measured also in deuteriated media in order to determine the solvent isotope effect on the reaction. The data are given in Tables S5–S8.[6]

Equation 2 was used to evaluate the hydronium ion rate constant (k'_{H^+}) for hydrolysis of the ionized form of the substrate. In $H_2PO_4^-$ ($D_2PO_4^-$) buffers $[H^+]/K_a \ll k'_{H^+}/k_{H^+}$, and eq 2 could be simplified to eq 3. This equation

$$k^\circ_{obsd}\frac{[H^+] + K_a}{K_a} = k'_{H^+}[H^+] \quad (3)$$

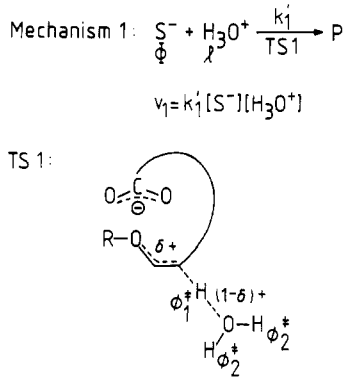
was used to evaluate both $k'_{H^+} = 56400 \pm 500$ M^{-1} s^{-1} and $k'_{D^+} = 48900 \pm 400$ M^{-1} s^{-1} (Table II). The observed isotope effect is given by $k'_{H^+}/k'_{D^+} = 1.15 \pm 0.02$.

The isotope effect on the general-acid catalytic coefficients in biphosphate buffers was also evaluated. In that case eq 4 was used in a simplified form (eq 5):

$$\frac{\Delta k_{obsd}}{\Delta[H_2PO_4^-]}([H^+] + K_a) = \frac{k_{H_3PO_4}}{K_1}[H^+]^2 + \left(k_{H_2PO_4^-} + \frac{k'_{H_3PO_4}K_a}{K_1}\right)[H^+] + k'_{H_2PO_4^-}K_a \quad (4)$$

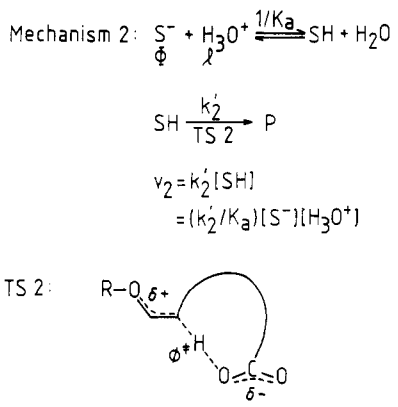
K_1 is the ionization constant of H_3PO_4, and K_a is the

Scheme II



$$v_1 = k'_1[S^-][H_3O^+]$$

Scheme III



$$v_2 = k'_2[SH] = (k'_2/K_a)[S^-][H_3O^+]$$

acidity constant of the model compound. Reaction through both unionized and ionized forms of the substrate as well as possible catalysis by H_3PO_4[4b] have been taken into account. At high pH the first term in eq 4 could be neglected and we obtain eq 5, from which $k'_{H_2PO_4^-}$ and $k'_{D_2PO_4^-}$ (from the corresponding expression for D_2O) given in Table II were obtained.

$$\frac{\Delta k_{obsd}}{\Delta[H_2PO_4^-]}([H^+] + K_a) = \left(k_{H_2PO_4^-} + \frac{k'_{H_3PO_4}}{K_1}K_a\right)[H^+] + k'_{H_2PO_4^-}K_a \quad (5)$$

Discussion

The rate profile (Figure 1) and the hydrolysis rate constants (Table I) obtained for (*E*)-6,9-epoxynon-5-enoic acid (**3**) are very similar to the ones obtained for the *Z* isomer[4b] and for the prostacyclin itself.[3]

Both k_{H^+} and k'_{H^+} are smaller for the *E* isomer than for the *Z* isomer by a factor of ~3, which is quite normal for vinyl ethers.[8] This finding of a retained accelerating effect of the carboxylate group in the *E* isomer can be used as a mechanistic tool in differentiating between the two possible mechanisms (Schemes II and III) for the hydrolysis that have been advanced.[3,4] According to these, the carboxylate group acts as an electrostatic catalyst (Scheme II) or it provides the possibility of an intramolecular general-acid catalysis (Scheme III).

Inspection of molecular models reveals that the developing positive charge in the rate-determining transition state of the hydrolysis of **2** is more accessible for the carboxylate group than in the case of **3** due to the constraint of the methylene chain and the remaining double-bond character. This means that electrostatic stabilization (Scheme II) would be more pronounced for **2** than for **3**. Proton transfer in the intramolecular general-acid catalysis (Scheme III), on the other hand, could be expected to be equally facile for **2** and **3** as the protonation takes place in a plane perpendicular to the plane defined by the double bond and the oxygen.

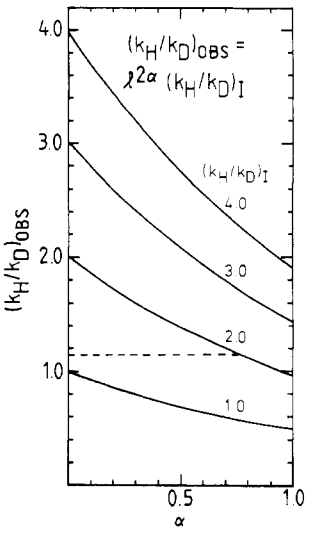


Figure 2. Plot of the observed solvent isotope effect ($(k_H/k_D)_{obsd}$) for the hydronium ion catalyzed hydrolysis of the carboxylate form of the model compound vs the isotopic Brønsted exponent (α) for different values of the primary component of the isotope effect. Broken line indicates magnitude of experimentally determined k_H/k_D.

From the experimental results, where we find very little difference in k'_{H^+}/k_{H^+} between the *Z* and *E* isomers, we conclude that Scheme III most properly represents the mechanism for the hydrolysis reaction.

For prostacyclin no such measurements have been made but the same conclusion has been reached from solvent isotope effect data.[3b]

Corresponding isotope effect measurements have been performed for the *Z* isomer of the model, and the results are summarized in Table II.

The isotope effects ($k_{H_3O^+}/k_{D_3O^+} = 3.3$, $k_{CH_3COOH}/k_{CH_3COOD} = 6.0$) for the methyl ester are quite normal for vinyl ether hydrolysis[9] and are comparable to the corresponding values (2.99, 5.03) for prostacyclin.[3b] $k'_{H_2PO_4^-}/k'_{D_2PO_4^-} = 5.13$ is also of the magnitude expected.

As in the case of prostacyclin the isotope effect on the hydronium ion hydrolysis reaction of the carboxylate form is rather weak, $k'_{H^+}/k'_{D^+} = 1.15$.

In order to investigate the compatibility of this value with either Scheme II or Scheme III, we have used fractionation factor theory.[10]

On the basis of Scheme II the predicted isotope effect is given by[10]

$$(k_H/k_D)_{obsd} = \frac{l^3\Phi}{\phi^\ddagger_1\phi^{\ddagger 2}_2} = \frac{l^2}{\phi^{\ddagger 2}_2}\frac{l\Phi}{\phi^\ddagger_1} \quad (6)$$

Assuming that $\phi^\ddagger_2 = l^{1-\alpha}$, where α is the isotopic Brønsted exponent,[10] eq 6 can be rewritten as in eq 7. $(k_H/k_D)_I$

$$(k_H/k_D)_{obsd} = l^{2\alpha}(k_H/k_D)_I \quad (7)$$

(8) Salomaa, P.; Nissi, P. *Acta Chem. Scand.* **1967**, *21*, 1386–1389. Okuyama, T.; Fueno, T. *J. Org. Chem.* **1974**, *39*, 3156–3158.

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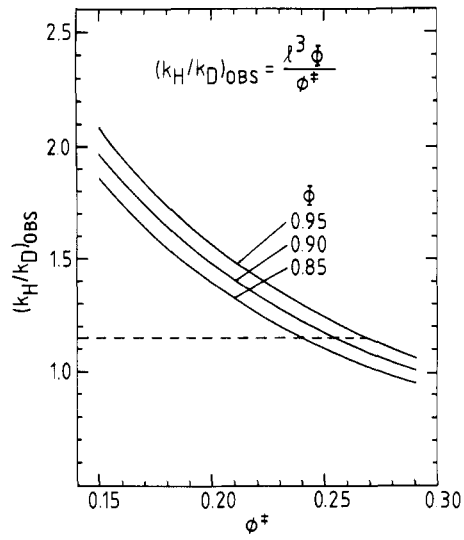

Figure 3. Plot of the observed solvent isotope effect ($(k_H/k_D)_{obsd}$) for the hydronium ion catalyzed hydrolysis of the carboxylate form of the model compound vs the fractionation factor for the proton "in flight" ($\phi^\ddagger$; see Scheme III) for some values of the fractionation factor (Φ) for the carboxylate form of the model compound. Broken line indicates magnitude of experimentally determined k_H/k_D.

corresponds to the primary component of the observed solvent isotope effect.

In Figure 2 $(k_H/k_D)_{obsd}$ has been plotted vs α for different values of $(k_H/k_D)_I$. It is easily seen that the only $(k_H/k_D)_I$ value compatible with $(k_H/k_D)_{obsd} = 1.15$ is $(k_H/k_D)_I = 2$. This value is quite different from the primary component, $(k_H/k_D)_I = 4.6$, which can be estimated for the hydrolysis of a vinyl ether of this reactivity from a correlation of such isotope effects for a group of these reactions.[9]

A corresponding analysis of Scheme III gives eq 8.

$$(k_H/k_D)_{obsd} = \frac{l^3\Phi}{\phi^\ddagger} \quad (8)$$

In Figure 3 $(k_H/k_D)_{obsd}$ has been plotted vs $\phi^\ddagger$ for some values of Φ. The exact value of Φ is not known but a good approximation would be 0.90, corresponding to that of the acetate ion.[11] The fractionation factor obtained for the proton "in flight" is $\phi^\ddagger = 0.26$; on this basis from the observed values $k'_{H^+}/k'_{D^+} = 1.15$. No exact experimental value can be determined but an estimate could be obtained from the solvent isotope effect on the acetic acid protonation of the methyl ester. Such an analysis gives $\phi^\ddagger = 0.16$.

The outcome of these analyses thus favors the intramolecular general-acid catalysis mechanism of Scheme III.

To investigate the importance of possible electrostatic catalysis further, we have also determined k'_{HA}/k_{HA} (Scheme I) for a neutral buffer acid (CH_3COOH, $pK_a = 4.75$) and for a positively charged buffer acid ($CH_3ONH_3^+$, $pK_a = 4.72$[12]).

The ratio k'_{HA}/k_{HA} was obtained from a plot of $k_{HA}^{app}([H^+] + K_a)$ vs $[H^+]$ (see ref 4b, eq 12). The slope gives k'_{HA}, and k_{HA} is obtained from the intercept.

For acetic acid buffers $k'_{HA}/k_{HA} = 2.1 \pm 0.2$ (Z isomer) and 1.7 ± 0.4 (E isomer). The corresponding ratio obtained from methoxyamine buffer data (Table S9)[6] was $k'_{HA}/k_{HA} = 2.1 \pm 0.6$ (Z isomer).

Due to the rather weak general-acid catalysis, the ratios are not very well determined, but they clearly show that both neutral and positively charged catalysts (of the same pK_a) give about the same rate acceleration.

If electrostatic stabilization by the carboxylate group of the substrate played an important role, then the ratio k'_{HA}/k_{HA} would be expected to be as large (ca. 10^2) for the positively charged catalyst $CH_3ONH_3^+$ as for the H_3O^+ ion. For a neutral catalyst such as CH_3CO_2H, on the other hand, electrostatic destabilization would be expected to occur, as proton transfer from such a species would make it negatively charged and that would lead to an unfavorable interaction with the negative substrate carboxylate ion. Quite different electrostatic effects would therefore be expected for the two different catalyst types, contrary to the experimental results. These results would therefore seem to rule out any significant electrostatic effect of the substrate carboxylate group on the stability of the reaction's transition state.

Conclusion

The results obtained for the E isomer of the model compound suggest that the extra hydrolytic lability showed by the carboxylate form of the compound is due to intramolecular general-acid catalysis. This is supported by isotope effect data and the results from an investigation of two buffer acids of different charge but of about the same pK_a. The results reported in the present investigation thus provide further support to the ideas put forward concerning the role of the carboxylate group in the hydrolysis of prostacyclin.[3]

Acknowledgment. We are grateful to the Swedish Natural Science Research Council and the Natural Sciences and Engineering Research Council of Canada for financial support of this work.

Registry No. 1, 35121-78-9; 2, 108428-26-8; 2 methyl ester, 108428-27-9; 3, 114274-33-8; 3 methyl ester, 114274-34-9.

Supplementary Material Available: Rate data (Tables S1–S9) (20 pages). Ordering information is given on any current masthead page.

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(12) Fox, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 1436–1449.

(13) Gary, R.; Bates, R. G.; Robinson, R. A. *J. Phys. Chem.* **1964**, *68*, 3806–3809.