achirotopic points in such a model. With the definition of this class, all possibilities for $(pro)^p$-chirality in E^3 are exhausted.

A desymmetrization lattice for achiral objects is displayed in Figure 3. As shown by transformations such as $D_{2d} \rightarrow C_2$ and $C_{nh} \rightarrow C_n$, and as also illustrated by the examples in Figure 2, desymmetrization need not occur in a stepwise manner, i.e., from $(pro)^p$-chiral to $(pro)^{p-1}$-chiral: replacement of any $(pro)^p$-chirotopic point will necessarily yield a $(pro)^p$-chiral object. Thus, all $(pro)^3$-chiral objects except those with spherical (K_h) symmetry may be rendered $(pro)^1$-chiral (C_s) by replacement of a point that lies on a mirror plane but off an axis, and all achiral objects except those with K_h, $D_{\infty h}$, or $C_{\infty v}$ symmetry may be rendered asymmetric by replacement of a point in a general position.[82,83]

We close this discussion on a historical note. According to our scheme, desymmetrization of an object with T_d symmetry yields an object that can belong to only one of four subsymmetries (C_{3v}, C_{2v}, C_s, or C_1). Van 't Hoff, on the basis of a very different approach to desymmetrization, arrived at the same conclusion for the subsymmetries of substituted methanes.[19]

Acknowledgment. We are deeply grateful to numerous members of the stereochemical community for stimulating discussions and correspondence over a period of years. We also thank the National Science Foundation (CHE-8009670) for support of this work.

(82) Spherical symmetry (K_h) provides a unique model for desymmetrization, in that all points outside the center are $(pro)^2$-chirotopic. Replacement of such a point yields a $(pro)^2$-chiral object ($C_{\infty v}$). In the latter, all points outside of the rotation axis are $(pro)^1$-chirotopic, and replacement of such a point yield a $(pro)^1$-chiral object (C_s). In turn, all points in the last object outside the mirror plane are chirotopic, and replacement of such a point yields a chiral object (C_1). It is thus seen that in a spherical object, stepwise desymmetrization is unavoidable, and that $p_{max} = 3$ in E^3.

(83) If replacements are restricted to ligands on a permutation frame, it may not be possible to desymmetrize the model in other than a stepwise manner. Such is the case, for example, in $(pro)^3$-chiral CH_4 (T_d) and $(pro)^3$-chiral PF_5 (D_{3h}). However, this constraint, which is imposed by giving primacy to constitution over symmetry, is lifted under our treatment of $(pro)^p$-chirality. For example, addition of H^+ to CH_4 yields $(pro)^1$-chiral CH_5^+ (C_s)[84] directly, without the intervention of a $(pro)^2$-chiral intermediate.

(84) According to ab initio calculations, the ground-state symmetry of CH_5^+ is $C_s[C_s(CH_3), C_1(H_2)]$ in Pople's framework group notation as modified by Flurry.[8] See: Raghavachari, K.; Whiteside, R. A., Pople, J. A., Schleyer, P. v. R. *J. Am. Chem. Soc.* **1981**, *103*, 5649.

Study of Proline Peptide Bond Conformation and Ring Dynamics in Crystalline Cyclic Peptides Using ^{13}C MAS NMR

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Contribution from the Mineralized Tissue Research Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20205, Chemistry Department, Illinois Institute of Technology, Chicago, Illinois 60616, and Center for Materials Research, National Bureau of Standards, Washington, DC 20234. *Received September 26, 1983*

Abstract: We have studied three cyclic peptides, *cyclo*(Val-Pro-Gly)$_2$, *cyclo*(Phe-Pro-D-Ala)$_2$, and *cyclo*(Gly-Pro-D-Ala)$_2$, in the crystalline powder form by using ^{13}C MAS NMR. A comparison of chemical shift differences ($\Delta\delta_{B\gamma}$) between the β- and γ-carbons of the proline ring suggests that the Val–Pro and Phe–Pro peptide bonds are cis and that the Gly–Pro bonds are trans. These results for crystalline samples agree with those obtained in solution and are verified by crystal structures of *cyclo*(Phe-Pro-D-Ala)$_2$ and *cyclo*(Gly-Pro-D-Ala)$_2$. Solid-state relaxation data show that the disorder reported at one proline ring in the crystal structure of the latter peptide results from ring motion. A ring correlation time of 1.2×10^{-11} s is obtained when the relaxation data are analyzed by using the two-site exchange model suggested by the crystal structure.

In recent years high-resolution ^{13}C NMR spectra of powders have been obtained by using cross-polarization and magic-angle sample spinning.[1-3] This technique has been applied to study crystalline peptides where measurement of solid-state and solution chemical shifts permit comparison of peptide conformation in solution and the solid state.[4] In this regard cyclic hexapeptides of the type *cyclo*(Xxx-Pro-D-Yyy)$_2$ or *cyclo*(Xxx-Pro-Gly)$_2$ (where Xxx and Yyy are any other amino acid residues) are particularly attractive because certain aspects of their solution conformation are well-defined by their chemical shifts.[5-10] For instance, the spectrum immediately shows if the peptide conformations have average C_2 symmetry on the NMR time scale. In addition, since the barrier to cis–trans isomerization of a peptide bond (e.g., Xxx–Pro) is about 15–20 kcal/mol,[11] lifetimes of the isomers are large on the NMR time scale and distinct signals are observed for the cis and trans isomers. Therefore, chemical shift measurements have established that these hexapeptides exist in solution in two forms of average C_2 symmetry on the NMR time scale, one with all the peptide bonds as trans[9,10] and the other with two Xxx–Pro bonds as cis.[5-8] The chemical shift difference between the β- and γ-carbon resonances ($\Delta\delta_{\beta\gamma}$) is used to assign the cis and trans isomers. For a cis Xxx-Pro bond $\Delta\delta_{\beta\gamma}$ is ca. 8–12 ppm whereas this difference is smaller, 2–6 ppm, for the trans case.[12]

[†] National Institutes of Health.
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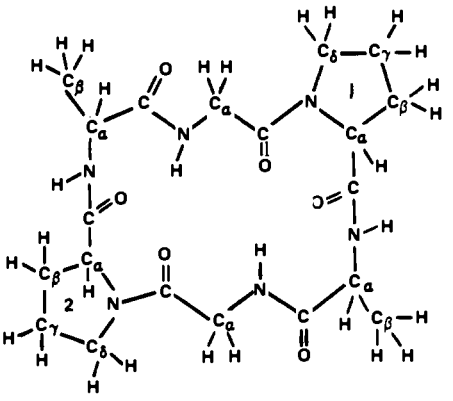
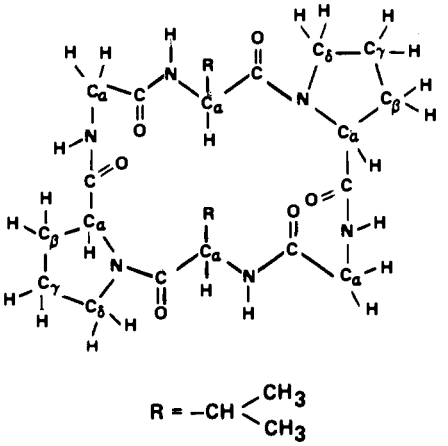
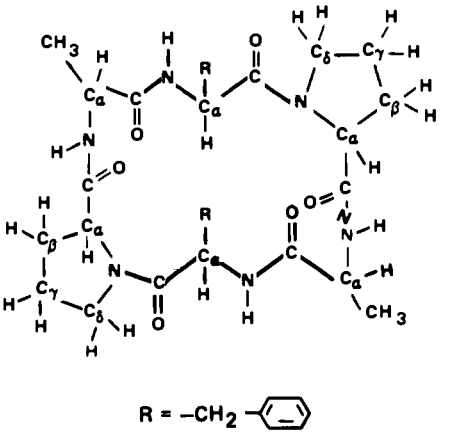


c



R = $-CH(CH_3)_2$

a



R = $-CH_2-C_6H_5$

b

Figure 1. Structure of cyclic peptides: (a) *cyclo*(Val-Pro-Gly)$_2$; (b) *cyclo*(Phe-Pro-D-Ala)$_2$; (c) *cyclo*(Gly-Pro-D-Ala)$_2$. The two prolines in c are labeled 1 and 2 as discussed in the text.

It is of interest to ascertain if these conformational states are preserved in the solid state for a particular peptide.

We have studied three cyclic hexapeptides, *cyclo*(Val-Pro-Gly)$_2$, *cyclo*(Phe-Pro-D-Ala)$_2$, and *cyclo*(Gly-Pro-D-Ala)$_2$ (Figure 1), in solution and in crystalline powder form by using ^{13}C NMR. One purpose of our study is to compare the conformational characteristics of these peptides in solid state and in solution. Also, since the X-ray structures of *cyclo*(Phe-Pro-D-Ala)$_2$[13] and *cyclo*(Gly-Pro-D-Ala)$_2$[14] are known, we correlate our solid-state NMR results with the crystal structure for these two peptides. In this regard we find that the disorder reported for one proline ring in the *cyclo*(Gly-Pro-D-Ala)$_2$ crystal structure is due to rapid ring motion.

Experimental Section

cyclo(Val-Pro-Gly)$_2$[5,7] and *cyclo*(Phe-Pro-D-Ala)$_2$[13] were prepared by established procedures. *cyclo*(Gly-Pro-D-Ala)$_2$ was synthesized by following the route described in ref 5.

^{13}C spectra for *cyclo*(Val-Pro-Gly)$_2$ and *cyclo*(Phe-Pro-D-Ala)$_2$ in dimethyl sulfoxide solution were recorded on a Nicolet NT-500 spectrometer[15] operating at 125.75 MHz for carbon. The concentration of peptide solutions were 0.009 and 0.008 M, respectively. The 90° pulse width was 20 μs. Free induction decays (16 000 accumulations) were acquired by quadrature phase detection with a 30-kHz spectral window and 16K data points per channel. Pulse delays of 1 s were used between the 90° pulses. The spectrum of *cyclo*(Gly-Pro-D-Ala)$_2$ in a 50:50 mixture of dimethyl sulfoxide/water solution (0.01 M) was obtained at 75.45 MHz for ^{13}C on a Nicolet NT-300 spectrometer.[15] Accumulations (30 000) were collected by quadrature phase detection with a recycling time of 1 s after the 90° pulse (25 μs). A digital resolution of 0.9 Hz was used. Broad-band proton decoupling was employed.

In all the cases chemical shifts were measured with respect to external Me_4Si. An internal deuterium lock was used. Assignment of resonances was based on comparison with model peptides.[5-7]

The solid-state ^{13}C spectra were taken on a Bruker CXP200 spectrometer[15] operating at a magnetic field of 4.7 T (50.3 MHz for ^{13}C nuclei). Carbon signals were generated from the protons by cross-polarization[1] in the presence of magic-angle sample spinning.[2,3] Radio-frequency field strengths of 60 kHz for the carbons and 60 kHz − ν_r for the protons[16] were used, where ν_r, the rotor frequency for magic angle spinning, was set at approximately 3.5 kHz. Cross-polarization times were typically 1 ms, and repetition times were 4–6 s. Proton decoupling radio-frequency levels were the same as those used in cross-polarization. T_1 measurements were carried out by the method of Torchia.[17] T_2 measurements were made by using spectra obtained by Fourier transformation of the second half of ^{13}C spin echoes. These echoes resulted from a single 180° pulse at a time, τ, following the end of cross-polarization. The time τ was set to be an integral number of rotor periods in order to avoid contributions to T_2 from chemical shift anisotropy and unaveraged ^{13}C–^{14}N dipolar interactions. Proton decoupling was main-

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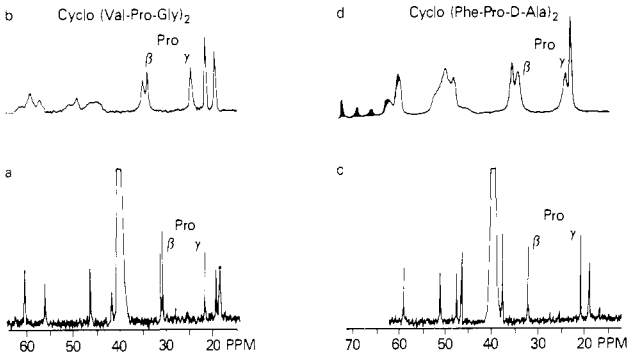

Figure 2. Comparison of ^{13}C NMR spectra of *cyclo*(Val-Pro-Gly)$_2$ and *cyclo*(Phe-Pro-D-Ala)$_2$: (a and c) in dimethyl sulfoxide solution; (b and d) polycrystalline samples; shaded area in d is the region of first side band from the six resolved aromatic resonances of Phe (not shown in the figure).

Table I. Comparison of the Differences in Proline β- and γ-Carbon Chemical Shifts (in ppm) for the Three Peptides in Solution and in the Solid State

peptides	$\Delta\delta_{\beta\gamma}$ (solution)	$\Delta\delta_{\beta\gamma}$ (solid state)	Xxx-Pro conformational state
cyclo(Val-Pro-Gly)$_2$	9.05	9.7	cis
cyclo(Phe-Pro-D-Ala)$_2$	11.34	10.05	cis
cyclo(Gly-Pro-D-Ala)$_2$	3.2	3.5, 6.0	trans

tained from cross-polarization through observation.

The rotor was deuterated poly(methyl methacrylate). This material shows weak resonances due to cross-polarization from residual protons. The sharpest of these resonances, namely that of the tetrahedral carbon, occurs at 45.14 ppm.[18] This resonance, if observed, was used as a calibration for chemical shifts; otherwise chemical shift referencing was made by substitution. The absolute values of chemical shifts are thus accurate to ±1 ppm; the difference values of the chemical shift are accurate to ±10% of the line width or approximately 0.1 ppm for the proline resonances discussed in this paper.

Results and Discussion

***cyclo*(Val-Pro-Gly)$_2$ and *cyclo*(Phe-Pro-D-Ala)$_2$.** Solution ^{13}C NMR spectra of *cyclo*(Val-Pro-Gly)$_2$ and *cyclo*(Phe-Pro-D-Ala)$_2$ (Figure 2a,c) show that each peptide has an average C_2 symmetric conformation on the NMR time scale. In addition, the observed values of $\Delta\delta_{\beta\gamma}$ (Table I) strongly suggest that the Xxx–Pro peptide bonds are cis in each case. These conclusions are in agreement with those obtained previously.[5-7]

The magic-angle spinning spectra of the crystalline peptides are also shown in Figure 2. The single resonances characterizing each carbon in the 10–40-ppm region suggest that the peptides have C_2 symmetric conformation in the solid state. Moreover, solid-state $\Delta\delta_{\beta\gamma}$ values for *cyclo*(Val-Pro-Gly)$_2$ and *cyclo*(Phe-Pro-D-Ala)$_2$ (Table I) agree closely with the corresponding values in solution. This observation indicates that the Xxx–Pro peptide bonds are cis in both peptides in the solid state. These conclusions are confirmed for *cyclo*(Phe-Pro-D-Ala)$_2$ by its recently reported crystal structure.[13] The crystal structure of *cyclo*(Val-Pro-Gly)$_2$ has not been solved.

It has been argued[19] that one must proceed with some caution in interpreting solid-state ^{13}C chemical shifts in terms of conformations since chemical shift deviations of 1–2 ppm have been observed in the normal alkanes. The alkanes examined have the same conformation but different crystal lattices. Although it was argued in that paper that magnetic susceptibility anisotropy was not the principal explanation for these shifts, the argument given there underestimated the contribution from other than next nearest neighbors. Support for the important influence of these more distant neighbors is the observation that resonance differences

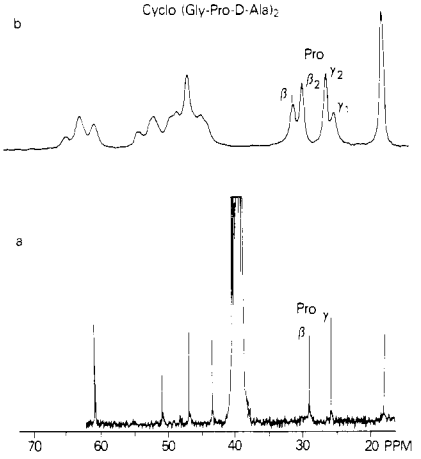

Figure 3. ^{13}C NMR spectra of *cyclo*(Gly-Pro-D-Ala)$_2$: (a) in dimethyl sulfoxide/water mixture; (b) polycrystalline sample. The β and γ resonances of Pro-1 and Pro-2 are referred to as β_1, γ_1 and β_2, γ_2, respectively.

Table II. T_1 and T_2 Values for β- and γ-Carbons of the Two Proline Residues (1 and 2) in Crystalline *cyclo*(Gly-Pro-D-Ala)$_2$

	proline 1		proline 2	
	β(31.1 ppm)	γ(25.1 ppm)	β(29.7 ppm)	γ(26.2 ppm)
T_1, s	~50 ± 10	~40 ± 8	3.0 ± 0.5	3.5 ± 0.5
T_2, ms	9 ± 2	7 ± 2	15 ± 3	17 ± 3
fwhh,[a] Hz	42	55	35	30

[a] Observed full width at half-height.

along the chain showed much less variation as a function of crystal lattice than did the individual chemical shifts. The use of chemical shift differences, such as $\Delta\delta_{\beta\gamma}$, should reduce the influence of magnetic susceptibility shifts due to more distant neighbors in the crystal lattice. Therefore, one should be more confident in correlating such chemical shift differences with conformation, particularly when there are no strong local sources of magnetic susceptibility anisotropy such as aromatic rings. The good correspondence between solid-state and solution values of $\Delta\delta_{\beta\gamma}$ is probably helped considerably by the weak nonbonding interactions of methylene groups. Moreover, the absence of directly bonded ^{14}N nuclei also simplifies interpretation. It is seen in Figures 2 and 3 that the solid-state spectra of the peptides in the 45–65-ppm region due to a α-carbons is complex. These carbons are directly bonded to ^{14}N, and the ^{14}N–^{13}C dipolar coupling is not completely averaged out by magic-angle sample spinning.[20,21]

***cyclo*(Gly-Pro-D-Ala)$_2$.** The ^{13}C spectrum of *cyclo*(Gly-Pro-D-Ala)$_2$ (Figure 3a) shows that this peptide, like the two peptides just discussed, has an average C_2 symmetric conformation in solution.[6,7] However, the Xxx–Pro peptide bonds are trans in this case as is evident from the measured value of $\Delta\delta_{\beta\gamma}$, 3.2 ppm, in solution (Table I). The magic-angle spinning spectrum of polycrystalline *cyclo*(Gly-Pro-D-Ala)$_2$ (Figure 3b) is particularly interesting since two signals of approximately equal integrated intensity are observed for the proline β- and γ-carbons. In addition to their different chemical shifts these signals have different line widths. One pair of C_β, C_γ signals, designated Pro-1, has line widths of 42–55 Hz and $\Delta\delta_{\beta\gamma}$ = 6.0 ppm while the other pair of C_β, C_γ signals, designated Pro-2, has line widths of 30–35 Hz and $\Delta\delta_{\beta\gamma}$ = 3.5 ppm. These results indicate that the Xxx–Pro peptide bonds are trans (as is found in solution) but do not establish the symmetry of the conformation; either a single asymmetric conformation or two different C_2 symmetric conformations are consistent with the NMR data in the solid state. X-ray diffraction

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shows that crystalline *cyclo*(Gly-Pro-D-Ala)$_2$ assumes a single asymmetric conformation with trans Xxx–Pro bonds and that one of the proline rings is significantly disordered.[14]

The proline C_β and C_γ spin–lattice (T_1) relaxation times (Table II) provide strong evidence that the disorder observed in the X-ray study is due to motion of one of the proline rings in the peptide. Table II shows that the Pro-2 T_1 values are an order of magnitude shorter than the corresponding Pro-1 T_1 values. This result indicates that the Pro-2 ring is considerably more flexible than the Pro-1 ring in that the spectral densities in the range of the Larmor frequency are much larger for the Pro-2 C_β and C_γ carbons than for Pro-1.

An estimate of the correlation time for the Pro-2 ring motion is made as follows: First, in accordance with the X-ray data[14] we assume a model in which the proline γ-carbon jumps between two equally populated sites. Second, the difference in angle (2θ) between C_γ–H bonds in the two sites is calculated from the coordinates of the Pro-2 C_γ and H_γ atoms.[14] This calculated value of 2θ is 60°. Finally, since the proline γ-carbon is bonded to two protons and since aliphatic carbons have chemical shift anisotropies generally less than 60 ppm, it is reasonable to assume[22] that the ^1H–^{13}C dipolar mechanism is responsible for relaxing the Pro-2 C_γ spin. We have used the approximate expression for the magic-angle spinning T_1[23] along with the measured value of T_1 of 3.5 s (Table II) to get the two possible values of the correlation time τ_c, 1.2×10^{-11} and 2.6×10^{-7} s. The motion of the Pro-2 ring also causes a substantial reorientation of the C–H bonds at the adjoining C_β carbon. This motion produces comparable carbon T_1's at both C_γ and C_β (Table II); thus there is little doubt that the assignments of the C_β and C_γ resonances at the two proline sites are correct.

To resolve the ambiguity between the two correlation times just calculated for the Pro-2 C_γ carbon, the expected T_2 values corresponding to these correlation times were also calculated. A τ_c value of 2.6×10^{-7} s corresponds to a T_2 of 3 ms, which is a factor of 5 less than that observed. Although contributions to T_2 from sources other than molecular motion[24] are present in these spectra, and although other slower modes of molecular motion could, in principle, contribute to T_2, neither of these possible contributions to T_2 can explain an observed T_2 *longer* than that expected from the τ_c since it is assumed that this τ_c describes the fastest motion. Thus, we conclude that the Pro-2 ring is undergoing fast reorientation over a 60° range ($2\theta = 60°$) with a correlation time of ca. 1.2×10^{-11} s. At that correlation time, this motion yields a T_2 which contributes less than a 1-Hz broadening to the line width. The fact that T_2 for the Pro-2 C_γ carbon corresponds to a 19-Hz line width is undoubtedly related to the influence of other broadening mechanisms referred to earlier. An analysis of the particular T_2 contributions is beyond the scope of this paper. Suffice it to say that the T_2 and line-width data combined suggest that the contribution to the Pro-1 and Pro-2 C_β and C_γ carbon line width from chemical shift dispersion and/or residual ^{13}C–^{14}N dipolar couplings is at most 0.2 ppm. In other words, the major line-width contributions are those sensed in the T_2 measurements.

Our studies show that $\Delta\delta_{\beta\gamma}$ measurements are reliable indicators of Xxx–Pro peptide bond conformation in solids and thus can be used to compare conformations of peptides in solution and in crystalline powder forms. Furthermore, they demonstrate that the disorder observed in the crystal structure of *cyclo*(Gly-Pro-D-Ala)$_2$ is a consequence of molecular motion of one proline ring in the peptide.

Acknowledgment. We thank Anita Go for synthesizing all the peptides used in the present study.

Registry No. *cyclo*(Val-Pro-Gly)$_2$, 56777-37-8; *cyclo*(Phe-Pro-D-Ala)$_2$, 85761-33-7; *cyclo*(Gly-Pro-D-Ala)$_2$, 69854-33-7.

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Inhibition of Water-Catalyzed Ester Hydrolysis in Hydrophobic Microdomains of Poly(methacrylic acid) Hypercoils

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Abstract: The water-catalyzed hydrolysis of *p*-methoxyphenyl dichloroacetate (**1**) and 2,2-dichloropropionate (**2**) in aqueous solution at 25 °C is strongly retarded by neutral atactic (at) and syndiotactic (st) poly(methacrylic acid) (PMAA), but not by poly(acrylic acid) and poly(*N*-vinylpyrrolidone). The rates and thermodynamic activation parameters are consistent with binding of the substrates to hydrophobic microdomains within the PMAA hypercoil. A conformational transition of PMAA to an extended coil leads to disappearance of the rate inhibition. This transition is induced either by ionization of PMAA or, at constant pH (ca. 3), by addition of urea and can be monitored by potentiometric titrations. Solubility measurements employing the water-insoluble dye Orange OT further established hydrophobic bonding to neutral at-PMAA. Whereas inhibition of the hydrolysis of **1** and **2** in water in the presence of hydrophobic cosolvents or micelles is characterized by initial-state stabilization, it appears that the inhibition by at- and st-PMAA primarily involves destabilization of the transition state. The effect of PMAA may be explained in terms of a lack of water penetration into the hydrophobic microdomains.

Many enzyme-catalyzed hydrolysis reactions occur at hydrophobic active sites,[1] where the access and local concentration of water molecules is restricted and the substrate reactivity modified by hydrophobic interactions.[2] In an attempt to gain insight into these factors with model systems, we have investigated the neutral (i.e., water-catalyzed) hydrolysis of the acyl-activated esters **1** and **2**[4] in the presence of neutral atactic (at) and syndiotactic (st)

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