outside of the cavity in accommodation of the guest.[4]

There is a possibility for an asymmetric reaction to occur in the chiral frameworks of **1-4**. In order to examine this point, we have measured the CD spectrum of **6** in methanol. The sample of **6** was isolated from the reaction mixture of **3**. The CD spectrum exhibits peaks at 230 and 283 nm and a trough at 250 nm with the values of molecular ellipticity, $[\theta]$, of 18800, 5360, and -19300 deg cm^2 dmol^{-1}, respectively, demonstrating that the reaction product is optically active.

Stereochemical control of bimolecular reactions are known to be attained in some crystals.[5] The results shown here demonstrate that similar control can be achieved even in solution by using γ-cyclodextrin template with the advantage of facility in regulating the orientation of the reactive species as desired. Another aspect of this method is that it may assist the study on the physical nature of bimolecular interactions by providing a pair of the species placed with different orientations.

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(4) This type of complexation is suggested from the CD data that **3** decreases CD intensities in the anthracene transition regions upon addition of *l*-borneol. The analysis of the CD variations gave 2000 M^{-1} as the binding constant, so 80% of **3** should exist as the complex at 2 mM of *l*-borneol.

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Isotopically Enhanced Infrared Spectroscopy: A Novel Method for Examining Secondary Structure at Specific Sites in Conformationally Heterogeneous Peptides

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Detailed mapping of secondary structure(s) in flexible peptides and proteins is an important goal of biological chemistry. This knowledge is relevant to the study of protein folding pathways and peptide-macromolecular interactions as diverse as hormone-receptor, antigen-antibody, and substrate-enzyme binding. Vibrational transitions occur very rapidly compared to the time periods needed for interconversion of different secondary structures in peptides. For this reason, Fourier transform infrared (FTIR) spectroscopy has the potential to quantitate populations of peptide conformers in solution[1] and is not subject to the problem of conformational averaging associated with the relatively long time scales of NMR spectroscopy.[2] However, FTIR is subject to an important limitation that also restricts the application of circular dichroism spectroscopy to peptide mapping; i.e., neither can analyze the conformation(s) of amino acids at specific positions. We now report a novel approach in which FTIR, enhanced by site-specific isotopic labels,[3-15] is used to examine secondary structure(s) of amino acid residues at *specific* locations in solutions of conformationally heterogeneous peptides.

The stretching frequencies of peptide backbone carbonyl groups are sensitive indicators of local conformation.[1,16,17] In the data presented below, ^{13}C-labeled carbonyl groups are incorporated into a series of synthetic peptides. Replacing ^{12}C with ^{13}C is expected to reduce the stretching frequency of an isolated carbonyl oscillator by ~37 cm^{-1}.[18] Thus, when the spectra of labeled and natural abundance molecules are compared, the transitions originating at the *labeled sites* can be detected and evaluated.

Peptides and native proteins may have different IR transitions associated with the same class of secondary structure(s).[1] Most experimental work correlating secondary structure(s) with the frequencies of IR transitions has been done for native proteins.[16,17] The isotopic approach described in this report will be a valuable tool for systematically correlating IR absorption bands and secondary structure(s) in relatively unconstrained, dynamic peptide conformers. For illustrative purposes, in this initial demonstration, we have used the secondary structure assignments commonly utilized in the interpretation of spectra of native proteins.[16,17]

Peptide **1** was designed to be water soluble and monomeric and to contain two distinct conformational regions, containing mostly alanine and glycine residues, respectively. In peptide **2**, five alanines are ^{13}C-1 enriched; in peptide **3**, five glycines are similarly labeled (labeled residues are underlined).[19]

peptide **1** AEAE AAAAA EAEWEGE GGGGG EGEG

peptide **2** AEAE <u>AAAAA</u> EAEWEGE GGGGG EGEG

peptide **3** AEAE AAAAA EAEWEGE <u>GGGGG</u> EGEG

The peptide **1** amide I spectrum is shown in Figure 1a. A broad band centered at 1645 cm^{-1} indicates backbone disorder, and a peak at 1621 cm^{-1} indicates a β-strand extended chain conformation.[16,17] A small shoulder at 1687 cm^{-1} is consistent with reverse turn (or possibly β-strand) conformations.[16,17] Peaks at 1564 and 1707 cm^{-1} reflect ionized and protonated carboxylate groups, respectively.[20]

There are prominent differences in the spectra of peptides **1**, **2**, and **3**, due entirely to the presence of ^{13}C in the labeled peptides. In the peptide **2** spectrum, Figure 1b, there is a large decrease in the 1621-cm^{-1} β-strand extended chain peak, and a concurrent increase in area in the ~1575-1595-cm^{-1} region (arrow A in the figure), reflecting an isotopic shift of ~36 cm^{-1}. The reverse turn shoulder at 1687 cm^{-1} is less distinct, with its area shifted into the 1645-cm^{-1} band. These isotopic shifts are easily seen in a *difference spectrum*, obtained by simple algebraic subtraction of the two spectra. The [(peptide **2**) - (peptide **1**)] difference spectrum, Figure 1c, shows distinct negative peaks at 1621 and 1687 cm^{-1} and a positive peak centered at ~1584 cm^{-1}. Therefore it is possible to conclude that the alanines in positions 5-9 of peptide **1** are ordered in a β-strand extended chain conformation with some degree of reverse turn present.

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(18) In many molecules the carbonyl stretch can be accurately modeled as a simple harmonic oscillator. The frequency of harmonic motion $\nu = 1/2\pi(\sqrt{k/\mu})$ in which k is the bond force constant and μ is the reduced mass of the bonded atoms [$\mu = (m_1)(m_2)/(m_1 + m_2)$ where m_1 and m_2 represent the masses of the oxygen and carbon].

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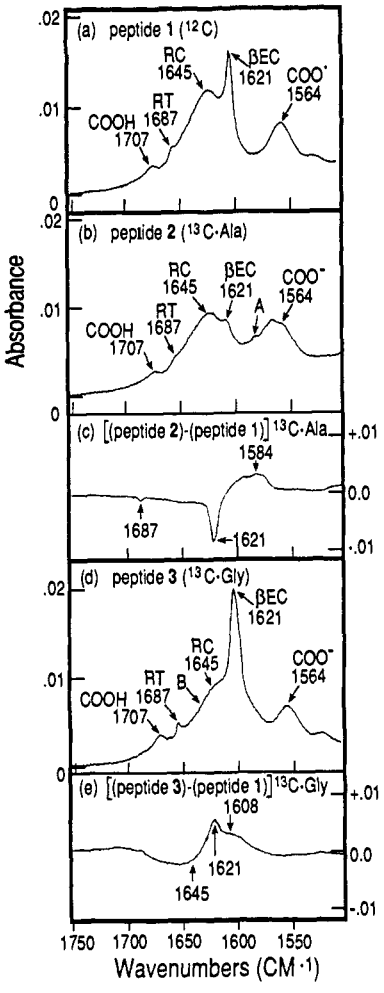

Figure 1. Infrared spectra (at 2-cm^{-1} resolution) of the amide I region for peptides **1**, **2**, and **3** are shown in (a), (b), and (d), respectively. RC represents "random coil"; RT, reverse turn; βEC, β-extended chain. The peptide [**2**-**1**] and [**3**-**1**] difference spectra are shown in (c) and (e), respectively. The 4 mM peptide samples were examined by attenuated total reflectance in phosphate-buffered D_2O (pD = 7.0; 23 °C) containing 1% sodium dodecyl sulfate (SDS) to ensure substantial amounts of random coil peptide. (Measuring small amounts of ordered conformations in the presence of abundant random coil is an important aspect of the current work, since it is difficult to do this with other spectroscopic methods.) Additional details of the experimental conditions are available in the supplementary material.

The peptide **3** spectrum shows a significant decrease in absorption at ~1645 cm^{-1} (disorder), see arrow B in Figure 1d, with a shift of area to lower energy causing a relative increase in the maximum height of the 1621-cm^{-1} peak. This appears as a broad nonsymmetrical negative band at ~1645 cm^{-1} in the difference spectrum, Figure 1e, with a corresponding positive band centered at ~1608 cm^{-1} ($\Delta \approx 37$ cm^{-1}). Therefore, it is apparent that the glycines at positions 17-21 of peptide **1** are predominantly disordered.

These results show that isotopically enhanced FTIR can be used to examine the secondary structure(s) of residues at specific locations in conformationally heterogeneous peptides. With a relatively simple model for bond vibrations, i.e., isolated harmonic motion, it is possible to understand the major isotopic shifts observed in three model peptides. Quantitative analyses of complex structures will be possible when additional details of vibrational transitions are considered. For example, molecular vibrations, rather than being truly harmonic, are actually anharmonic.[21,22] This is especially characteristic of functional groups that participate in hydrogen bonding, such as the amide carbonyl. Other factors that may cause complex isotopic effects are the interactions that can take place between neighboring oscillators during vibrational transitions. In effect, phase-dependent *summation* transition dipoles can exist, causing splitting of the carbonyl absorption bands that ordinarily would be associated with isolated oscillators (transition dipole coupling). The degree of splitting and the relative intensities of the split bands is highly dependent upon the geometry of the interacting transition dipoles.[1,23,24] When these additional factors are considered, the data provided by mathematical deconvolutions[16,17,25-27] of isotopically shifted infrared spectra, together with normal mode calculations and systematic assignments of labeled residues to secondary structures, will allow detailed conformational analyses of peptides and proteins in solution, in the solid state, and at interfacial surfaces.

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Supplementary Material Available: CD spectra of peptide **1** (and analyses of these spectra by the methods of W. C. Johnson and colleagues) (4 pages). Ordering information is given on any current masthead page.

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Stereospecific Synthesis of Cyclobutanol Derivatives Using a 5 Minus 1 Methodology and Platinum(II)

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The literature is replete with strategies and methods for the preparation of three-, five-, and six-membered carbocyclic ring systems. In contrast, four-membered ring construction is uniquely difficult.[1,2] The most common preparative methods for this system are 2 + 2 cycloadditions which are often facilitated by light. However, thermal cyclizations using ketene may be used in the formation of cyclobutanones.[3] A less common methodology, but one of equal validity is that of a 3 + 1 process. This has been amply demonstrated by the ring strain release reactions in which cyclopropane is ring expanded.[4]

In principle, a 5 minus 1 strategy would yield cyclobutanes. Previous results in all carbon-based systems have been reported,

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