ketene–imine cycloaddition reactions in the substituted systems.

The structure of the 2-azetidinone was optimized at the RHF/3-21G, RHF/6-31G*, and RMP2/6-31G* levels; the heat of reaction is calculated to be −38.7, −35.9, and −41.2 kcal/mol, respectively.

In summary, the problem of the mechanism of the ketene–imine cycloaddition reaction has been addressed by ab initio calculations. A very strong dependence of the predicted mechanism on the basis set has been found. At the RHF/3-21G theory level the reaction is a concerted but very asynchronic process, while at RHF/6-31G* and MP2/6-31G* theory levels the reaction is not a concerted cycloaddition, but a two-step process with a zwitterionic intermediate, in agreement with all experimental findings. The electrocyclic conrotatory closure of this intermediate leading to transition structure TS2 is predicted to be the rate-determining step of the process. Further work to study the effect of substituents in the energetic and stereochemistry of the process is in progress.

Registry No. I, 930-21-2; ketene, 463-51-4; formaldimine, 2053-29-4.

Structure-Induced Carbon-13 Chemical Shifts: A Sensitive Measure of Transient Localized Secondary Structure in Peptides
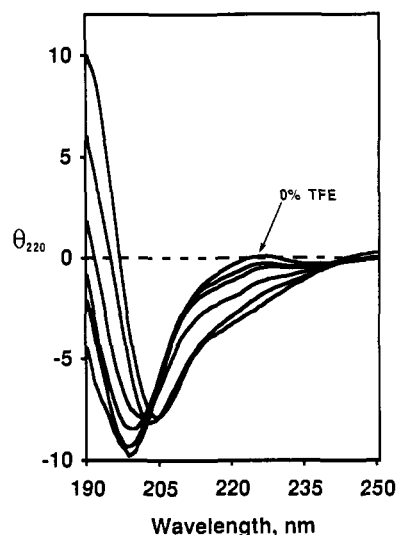
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NMR methods based on nuclear Overhauser effects (NOEs) have become routine tools for determining the secondary and tertiary structure of proteins and peptides in solution.[1] One drawback to these methods, however, is that NOEs require tens to hundreds of milliseconds to manifest themselves, and therefore the existence of multiple conformations in rapid exchange complicates quantitative interpretation of the data. Because of its much faster time scale, circular dichroism (CD) is often more useful in assessing secondary structure[2] in systems such as flexible peptides. It is not possible, however, to distinguish between conformational flexibility and local differences in structure on the basis of CD spectra alone. Recently, Spera and Bax[3] demonstrated that strong correlations exist between the Cα and Cβ chemical shifts and backbone conformation in proteins of well-defined structure. The effect of secondary structure on CαH and NH proton chemical shifts has been reported,[4] but a strict interpretation of these shifts is complicated because, unlike ^{13}C chemical shifts, they are affected by other anisotropic factors such as ring current effects and hydrogen bonding. In this report we demonstrate that the Cα chemical shifts are a sensitive means of identifying domains of polypeptides that have a propensity toward α-helix formation.

Bombesin is a well-characterized 14 amino acid peptide known to exist as a random coil in H$_2$O solution and to adopt a helix between Asn-6 and Met-14 at high concentrations of 2,2,2-trifluoroethanol (TFE)[5] and in phospholipid bilayers.[6] The effect of up to 70% (by volume) of TFE on the CD spectrum of bombesin in aqueous solution is shown in Figure 1. The increase in negative

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Figure 1. CD spectra of bombesin as a function of TFE concentration. The spectrum of bombesin in H$_2$O (0% TFE) is indicated. Successive traces (decreasing Θ_{220}) are 10%, 15%, 20%, 30%, and 70% TFE (v/v). The averaged spectra (three independent acquisitions) were recorded on a 0.6 mg/mL sample in a 0.1-cm cell. The results were subtracted from the spectrum of a solvent blank and smoothed with a third-order polynomial.
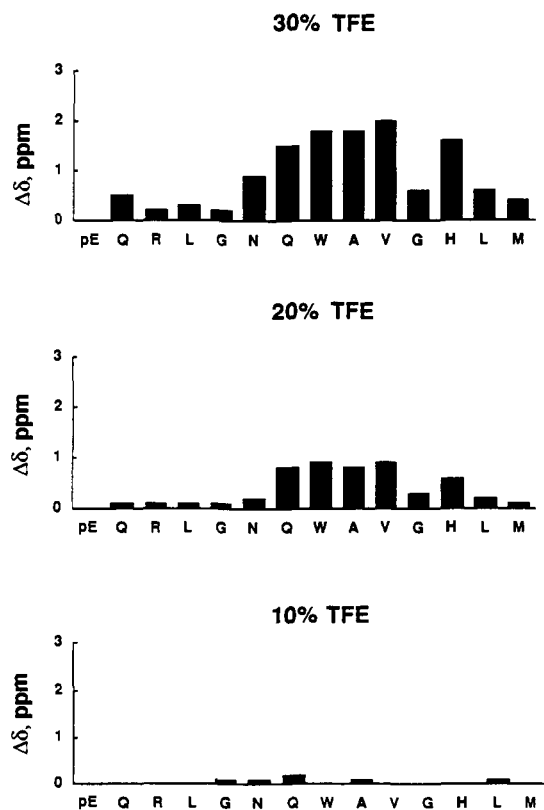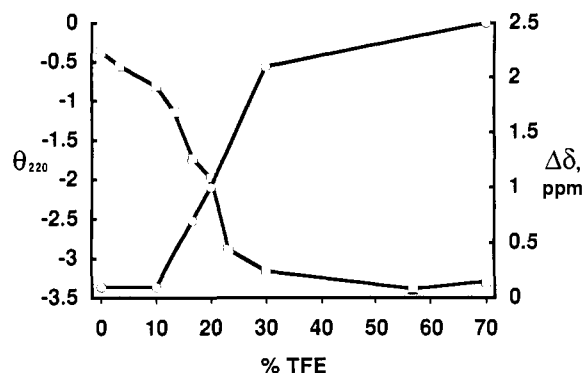

Figure 2. Secondary chemical shifts, $\Delta\delta$ (ppm), of the Cα carbons in bombesin as a function of added TFE. The heights of the bars indicate the change in chemical shift ($\Delta\delta = \delta_{obsd} - \delta_{^2H_2O}$) for each of the C$\alpha$ carbons relative to the chemical shift measured in 100% ^{2}H$_2$O solution, where bombesin is known to exist as a random coil (see text). The NMR measurements were made on 5 mg of bombesin dissolved in H$_2$O or ^{2}H$_2$O mixed with varying amounts of TFE (ca. 0.5 mL total volume) at pH $\sim$3.1, 298 K. Proton assignments were made on the basis of sequentially related NOEs, and heteronuclear multiple quantum coherence (HMQC) spectroscopy was then used to correlate carbon resonances to the assigned protons. HMQC experiments were recorded at natural abundance of ^{13}C in ^{2}H$_2$O and increasing amounts of TFE in ^{2}H$_2$O.

ellipticity, Θ, at 220 nm as a function of TFE concentration is indicative of increasing helical content. These results, however,

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Figure 3. Plot of mean residue ellipticity at 220 nm, Θ_{220}, and secondary shift of the Val-10 Cα, $\Delta\delta$ (ppm), as a function of 2,2,2-trifluoroethanol (TFE) concentration in volume percent.

do not provide site-specific secondary structure information. In contrast, the effect of similar amounts of added TFE on the Cα chemical shifts is more revealing. Figure 2 shows the sequence-specific deviation of the Cα chemical shifts from their random coil values[7] (secondary shifts) at 10%, 20%, and 30% TFE. These histograms clearly indicate that the region from residue 6 to residue 12 is the most affected as the TFE concentration is increased from 10 to 30%. Residues 1–5 show little secondary shift. Large secondary shifts are observed starting at residue 6 and continuing through Val-10, with a somewhat smaller effect at Asn-6. The large secondary shifts are lost at Gly-11 but return at His-12. The last two residues exhibit only small effects. Analysis of these data suggests the formation of a helix between Asn-6 and His-12 which is interrupted by Gly-11.[8] While residues 13 and 14 may also be included in the helix, the smaller induced shifts suggest that these residues are at least partially unordered, possibly as a result of fraying at the peptide's C terminus. A fraying mechanism may also explain the smaller secondary shift observed for Asn-6. This description of the structure of bombesin is similar to that proposed by Carver and Collins[5] on the basis of NOEs and $^3J_{NH,H\alpha}$ measured in 70% TFE.

Comparison of the CD and the secondary shift results (Figure 3) indicates the cooperative formation of a helix with an inflection near 20% TFE and which is nearly complete at 50% TFE. This is readily explained by a two-state coil-to-helix equilibrium mediated by TFE concentration. At the highest TFE concentrations, the magnitude of the largest secondary shifts (2.0–2.5 ppm) is consistent with reported values for stable helices in proteins,[3] indicating that the equilibrium is far to the right and that a stable helix is formed.

Using bombesin as an example, we have shown that the Cα chemical shifts are very sensitive to helical secondary structure in small peptides as previously observed in proteins.[3] There is good agreement between the relative magnitudes of the secondary Cα shifts and the Θ_{220} determined from the CD measurements. This implies that an estimate of the population of the helical conformer can be extracted from the magnitudes of the Cα secondary chemical shifts. Furthermore, only the residues involved in helix formation are affected. Thus, unlike CD measurements, the ^{13}C secondary shifts are indicative of both the localization and the relative amounts of helical content. In systems which contain other types of secondary structures, such as β-sheet, coexisting with helical and random coil forms, two different possibilities arise. In the situation where β-sheet and α-helical structures are in

equilibrium, the secondary shifts would be additive. Since the Cα secondary shifts tend to be opposite in sign for β-sheets than for α-helices,[3] this could result in a cancellation of the observed secondary shifts. On the other hand, if β-sheet and helical structures existed in distinct domains, these regions should be clearly defined by this method. Although not enough is known about the origin of the secondary shift effects to allow detailed quantification of helical content or the extent of coil–helix equilibrium, the method described here is clearly useful for monitoring transient helix formation in dynamic systems such as small peptides.

Ring Fusion and Polycyclic Ring Constructions via Half-Open Titanocenes

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The construction of organic ring systems is of great importance. Such species are especially common in natural products.[3] While many synthetic routes are available for both small (3–6) and large (>10) ring systems,[4] approaches to intermediate-sized rings encounter serious problems as a result of entropic difficulties and severe transannular strain.[5] We report here on the coupling reactions of a (pentadienyl)titanium compound with acetylenes, which lead to fused and polycyclic systems that incorporate such medium-sized rings.[6] These reactions tolerate somewhat surprising functionalization and offer some potential for actual synthetic applications. Furthermore, subsequent carbon–carbon skeletal rearrangements can be promoted, and directed, by appropriate ligand substituents.

The reaction of Ti(C$_5$H$_5$)(2,4-C$_7$H$_{11}$)(PEt$_3$)[7] (C$_7$H$_{11}$ = dimethylpentadienyl) with C$_6$H$_5$C$_2$SiMe$_3$ leads to the incorporation of 2 equiv of alkyne and the formation of a (bicyclo[4.2.1]nonadienyl)titanium complex (I, Scheme I, Figure 1a) in 64% yield. Attempts to limit the incorporation of alkyne, or to observe intermediates, were unsuccessful. Previous results suggest, however, that the first acetylene (C(6), C(7)) should couple to the pentadienyl ends (C(1,5)), leading to a seven-membered ring which rapidly undergoes further reactions of notably selective regiochemistry.[7,8] The product (I) may be considered to be a 16-electron complex by virtue of an apparent "agostic" interaction

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(7) In this study, the random coil chemical shifts for the Cα carbons were taken from the observed values for bombesin dissolved in 100% ^2H_2O solution, where bombesin is known to exist as a random coil (see refs 5 and 6). A similar analysis of the data using random coil shift values previously reported (Howarth, O. W.; Lilley, D. M. J. *Prog. NMR Spectrosc.* **1978**, *12*, 1–40) gave similar results.

(8) The smaller secondary shift of Gly-11 may be due to disruption of the helix, since glycine is a known "helix-breaker"; e.g., see Presta, L. G.; Rose, G. D. *Science* **1988**, *240*, 1632–1641. Another possibility is that glycine may have different secondary structure induced shifts than other amino acids.

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