angle is 140.1° compared to 136.7° for the disolvated analogue, I.[14,24] The average cyclopentadienyl C–Sm bond length in II is 2.79 (1) Å compared to 2.86 (3) Å in I.[14] The difference in these distances is consistent with the reduced ligand crowding in II due to the loss of two THF molecules.[25]

Examination of a packing diagram for II (Figure 2) indicates that the closest approach of one $(C_5Me_5)_2Sm$ molecule to the samarium center of another molecule occurs through the C(11) methyl group.[26] This Sm–C(11)′ distance is 3.22 (1) Å.[27] The smallest calculated separation of a hydrogen on C(11) to the distant samarium is 2.80 Å. In comparison, the longest crystallographically observed agostic metal–hydrogen interaction cited in a recent review is 2.29 Å.[28-30] The closest intermolecular contact between the methyl groups of adjacent molecules (3.34 (1) Å) is, however, significantly less than the sum of the appropriate van der Waals radii (ca. 4.0 Å).[31] We are pursuing the synthesis of closely related compounds in order to determine the importance of this and other factors to the geometry of the molecule.

The high degree of coordinative unsaturation found in $(C_5Me_5)_2Sm$, unprecedented in f-element chemistry, combined with the fact that Sm(II) is the most strongly reducing of the accessible divalent lanthanides,[32] makes decamethylsamarocene a highly reactive species. It should be an ideal molecule for examining the full potential of organosamarium(II) chemistry.

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Supplementary Material Available: Tables of crystal data, bond distances, angles, final fractional coordinates, thermal parameters, and observed and calculated structure factor amplitudes (17 pages). Ordering information is given on any current masthead page.

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(26) The displacement of the methyl groups out of the five-carbon least-squares planes and away from the metal ranges from 0.05 to 0.19 Å in ring 1 and from 0.10 to 0.24 Å in ring 2. The average is 0.13 Å, producing a displacement angle ($\bar{\alpha}$) of 5°. Compare 0.03–0.21 Å for $(C_5Me_5)_2Yb$(THF)(hemitoluene) and 0.13–0.31 Å for $(C_5Me_5)_2Yb(py)_2$.[24] Ring 1 (C(1)–C(5)) exhibits somewhat less thermal motion than ring 2 (C(6)–C(10)) (average isotropic B's for the ring carbons are 3.14 and 4.30 Å^2, respectively). C(11) has the least thermal motion of any of the methyl carbon atoms of ring 1.

(27) For comparison, the tetrameric structure of $[(CH_3C_5H_4)_3Nd]_4$ arises from the interaction of a carbon atom of an η^5-cyclopentadienyl ring in one $(CH_3C_5H_4)_3Nd$ unit with the metal of an adjacent $(CH_3C_5H_4)_3Nd$ unit at a distance of 2.990 (7) –2.978 (7) Å. The average Nd–C (η^5) distance is 2.79 (5) Å: Burns, J. H.; Baldwin, W. H.; Fink, F. H. *Inorg. Chem.* **1974**, *13*, 1916–1920. The Nd–C distances holding $(C_8H_8)_2Nd^-$ to $(C_8H_8)Nd(THF)_2^+$ in a tight ion pair are 2.69 (2), 2.89 (2), and 3.02 (2) Å in comparison to the Nd–C distances in the η^8-C_8H_8 Nd units of 2.68 (1), 2.79 (1), and 2.68 (1) Å: De Kock, C. W.; Ely, S. R.; Hopkins, T. E.; Brault, M. A. *Inorg. Chem.* **1978**, *17*, 625–631. Nd^{3+} is approximately 0.115 Å smaller than Sm^{2+}: Cotton, F. A.; Wilkinson, G. "Advanced Inorganic Chemistry", 4th ed.; Wiley: New York, 1980; pp 982, 1002.

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^{13}C NMR Spectroscopy of "Transition-State Analogue" Complexes of *N*-Acetyl-L-phenylalaninal and α-Chymotrypsin

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Enzymes are predicted to bind transition-state structures more tightly than the ground-state structures and hence a "transition-state analogue", a molecule that resembles the transition state, should have a higher affinity for the enzyme than the substrate or product analogue structures.

Peptide aldehydes related to substrates have proven to be potent inhibitors of serine proteases,[1-4] and it has been suggested that the tighter binding of the aldehydes derives from stabilization of a hemiacetal tetrahedral adduct (resembling the transition state) formed between the enzyme active site serine and the aldehyde carbonyl. In a collaborative experiment, Chen et al.[5] applied proton NMR spectroscopy to the interaction of *N*-benzoyl- and *N*-acetyl-L-phenylalaninals with α-chymotrypsin (Cht) and [195-dehydroalanine]α-chymotrypsin. From line width changes and cross-saturation effects, it was shown that these specific aldehyde transition-state analogues do bind as the hemiacetal to Cht. Proton NMR signals for the hemiacetal structures were never directly observed and were only inferred from the selective cross-saturation experiments.[5,6] However, in a proton and fluorine NMR investigation of *N*-acetyl- and *N*-benzoyl-DL-*p*-fluorophenylalaninal binding to Cht, Gorenstein and Shah[3] provided the first direct observation of signals (fluorine) representing the hemiacetal structure and the noncovalent Michaelis complex.

The wide range and diagnostic power of ^{13}C NMR suggested that structural assignment of the complex could be made for an aldehyde inhibitor binding to Cht, and we now report ^{13}C NMR spectra which allows us to monitor for the first time two interconverting forms for the hemiacetal complex. During the course of our studies a related ^{13}C NMR study of an aldehyde transition-state analogue binding to papain appeared.[7]

The ^{13}C NMR spectrum of the *N*-acetyl-L-phenylalaninal-*1*-^{13}C[8] (prepared from L-phenylalanine-*1*-^{13}C (90% ^{13}C enriched) reveals that a substantial amount (92 ± 2%) of hydrate (δ(C) 91.6 from Me_4Si) is present in Me_2SO-d_6 (20%) together with a small signal (8 ± 2% at 203.6 ppm) for the free aldehyde, which is in agreement with our previous assignments for the ^{19}F NMR spectrum for *N*-acetyl-DL-*p*-fluorophenylalaninal in Me_2SO-d_6 (20%). Addition of the *N*-acetyl-L-phenylalaninal to Cht at 30 °C resulted in the appearance of a new signal at ~94.0 ppm and broadening of the signal at ~203.6 ppm (Figure 1).

The new signal at ~94.0 ppm and the 203.7 ppm signal are assigned to the hemiacetal signal and noncovalent Michaelis complex, respectively (Scheme I), on the basis of the chemical shifts of analogous systems and earlier NMR studies.[3,4,6]

Note that the signals of enzyme-bound complexes are >25 Hz broader than the hydrate signal and also that the noncovalent enzyme-bound complex is broader by about 25 Hz at pH 5 than the free aldehyde signal at 203.6 ppm in the absence of Cht. This broadening of the ^{13}C signals for the enzyme complexes is at-

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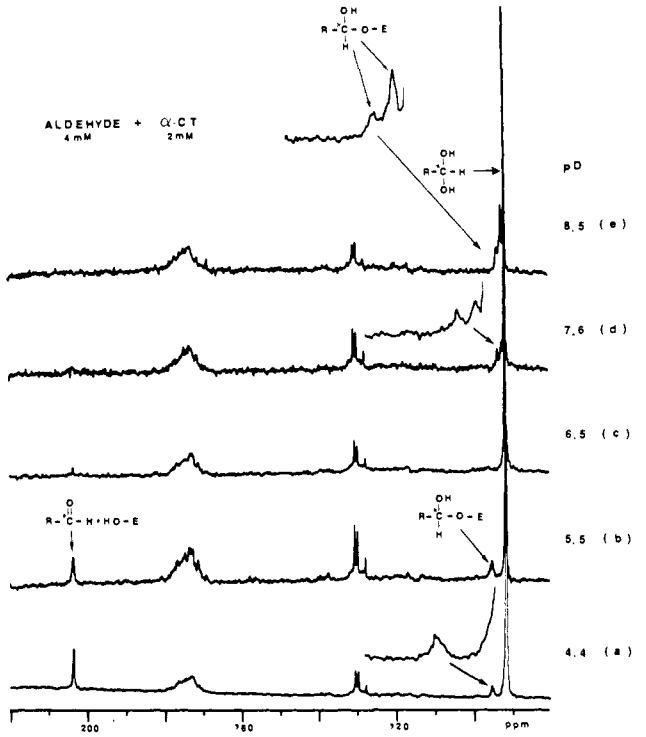

Figure 1. ^{13}C NMR, 50.32 MHz, proton gated decoupled spectra, 8K time domain data points, 7.1-μs pulse width (25 μs = 90° pulse) on an IBM WP-200 SY spectrometer. α-chymotrypsin was dissolved in 0.1 M sodium phosphate buffer solution in 99.9% D_2O containing 1 mM EDTA. To this solution was added the aldehyde dissolved in Me_2SO-d_6 so that the final concentration of Me_2SO was about 20% (v/v). *N*-acetyl-L-phenylalaninal 4 mM; fully active α-chymotrypsin 2mM. pD: (a) 4.4 (inset of hemiacetal, expanded region, 92–104 ppm), (b) 5.5, (c) 6.5, (d) 7.6 (inset of hemiacetal, expanded region, 92–104 ppm), (e) 8.5 (inset of hemiacetal, expanded region, 92–104 ppm).

Scheme I

$$RC(O)H \rightleftharpoons RC(OH)_2H$$

$$RC(O)H + Cht \rightleftharpoons RC(O)H\cdot Cht \rightleftharpoons \underset{H}{\overset{OH}{RC}}-O-CH_2-Ser\text{-}195-Cht$$

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tributed to the slower tumbling of the macromolecular complex.[3,9]

The chemical shifts and signal intensities for the two enzyme complex signals are pH dependent (Figure 1). The relative signal intensity of the noncovalent enzyme complex at 203.6 ppm increases at lower pH suggesting more of the inhibitor is noncovalently bound, which in turn explains why the inhibitor binds less tightly at low pH.

Significantly, at higher pH (>7) *two signals for the hemiacetal* have been observed, which are also pH dependent (insert, Figure 1c,d, expanded region of 110–80 ppm of Figure 1).

Kennedy and Schultz[10] and recently Shah and Gorenstein[4] have observed that K_i decreases only ~4-fold from pH 3.0 to 8.0 for the association of *N*-benzoyl-L-phenylalaninal and *N*-acetyl-DL-*p*-fluorophenylalaninal to Cht. This small variation in the binding constant was attributed to the binding of the aldehyde as the neutral hemiacetal throughout this pH range. The His_{57} imidazole likely functions as in the normal enzymatic mechanism to deprotonate Ser_{195} Oγ-H to initially yield the hemiacetal anion. This complex then rapidly picks up a proton to yield the neutral hemiacetal. While the two hemiacetal signals could be assigned to the neutral and anionic hemiacetals, they could also arise from slowly interconverting conformational isomers[7] or from interaction of the hemiacetal with an active site titrable group (such as His_{57}).

Recently it has been claimed that a tetrahedral intermediate (99 ppm) has been detected by ^{13}C NMR with pepsin and a ketone inhibitor.[11] Also a tetrahedral intermediate (98 ppm) has been detected by ^{13}C NMR with trypsin and a chloromethyl ketone specific inhibitor.[12] Very recently Gamcsik et al.[7] have studied the structure of the tetrahedral adduct of papain and an aldehyde by ^{13}C NMR. The results reported herein and taken together with the previous ^{13}C NMR studies indicate that ^{13}C NMR is an excellent probe for the detailed characterization of the protease complexes.

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Carboxylate Heme Complexes as Models for Hemoglobin J Altgeld Gardens (β-F8-His → Asp)

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One of the more intriguing mutant hemoglobins discovered in recent years is Hb J Altgeld Gardens (β-F8-His → Asp) (Hb J AG), where the proximal histidine of the normal β-subunits is replaced by Asp.[1] Surprisingly, this hemoglobin (Hb) is reported to exhibit normal O_2 affinity, only a slightly decreased Hill coefficient, and "normal" absorption spectra of the oxy, met, and cyanmet derivatives. Hb J AG is the only functional Hb known where the F8 residue is other than histidine. Since it is not apparent how the nearly normal functionality of Hb J AG can be reconciled with the mechanisms currently thought to operate in Hb[2] and since samples of this Hb are not presently available, it was thought that a study of carboxylate heme complexes might provide some insight into the molecular mechanism employed by this unusual Hb.

A titration of heme diester with potassium propionate is shown in Figure 1. Tight isosbestic points were not observed but the close approach to isosbesty in the visible region indicates that the visible spectrum of the intermediate complex(es) is similar to that of the final product. Plots of log $[(A - A_0)/(A_\infty - A)]$ vs. log [propionate] (see ref 4) at either 592 or 558 nm were nonlinear with slopes of approximately 1 and 2 during the early and latter stages, respectively, of the titrations. Additional evidence that the final complex is 6-coordinate comes from the close similarity between the spectra of the carboxylate complexes and those of the heme diphenolate complexes[5] and the dihydroxyl complex.[6] Also the acetate complex is probably low spin[7] whereas a 5-co-

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