Although the formation of enol acetates from attack of acyl halides on enolate anions is not uncommon in organic chemistry,[5] reaction B is remarkable in several respects: (i) acetyl chloride is the only source of —(C=O)— in this system; (ii) the acetyl derivative **3** is *not* a precursor to **2**, since treatment of **3** or its deprotonated anion 3^- with acetyl chloride fails to generate **2**; and (iii) there is no evidence of the existence of **3** in solution under our experimental conditions.[6] Moreover, to our knowledge this type of reaction is unprecedented in boron cluster chemistry. For example, the electrophilic attack of acetyl chloride or benzoyl chloride on *nido*-$C_2B_9H_{12}^-$ carborane ions (which are similar to 1^- in having an open C_2B_3 face) generates in each case the expected *B*-acyl derivative.[7] Similarly, the interaction of the 1-lithio derivative of the icosahedral 1,2- or 1,7-$C_2B_{10}H_{12}$ carboranes with acetyl chloride proceeds normally to give the respective 1-acetyl carborane.[8]

Reactions of 1^- with a few other acyl halides have been examined and thus far have not shown behavior of the type seen in reaction B,[9] although studies are continuing. Since the acetyl derivative **3** is not a precursor to **2**, as noted above, a different pathway is involved. Pending the outcome of studies currently under way, we believe it likely that the initial step in reaction B is the attack of an acetyl moiety at the nucleophilic B–B edge of 1^- to form an acetyl-bridged intermediate **4** containing a B–C–B three-center bond (Scheme II). Such a mechanism would correspond to that proposed earlier for the regiospecific B-alkylation[1a] of **1**, its iron–arene and ruthenium–arene counterparts,[1a] and the *nido*-$R_2C_2B_4H_5^-$ carboranes.[10] Electron withdrawal from the bridging acetyl unit in **4** could be expected to polarize the carboxyl region, favoring the enolate species whose C–O^- group may then undergo O-acetylation and rearrangement to give **2**. At least 50% of the neutral **2** starting material is recovered, attributed to the interaction of 1^- with protons generated in the formation of the vinyl acetate.

The isolated B(5)-acetyl derivative **3** is itself a remarkable species, manifesting unexpected metal-coordinating properties which have opened a new synthetic route to multidecker sandwich complexes, as described in the accompanying paper,[11] and also is a potentially important precursor to linked metallacarborane oligomers and polymers via acyl polymerization processes.

Acknowledgment. The support of the National Science Foundation (Grant No. CHE 87-21657) and the U.S. Army Research Office is gratefully acknowledged. We thank Professor Frank Carey for enlightening discussions.

Supplementary Material Available: ORTEP diagram of **2** and tables of positional and thermal parameters, bond lengths, and bond angles for **2** (6 pages); listing of observed and calculated structure factors for **2** (26 pages). Ordering information is given on any current masthead page.

(4) A 0.275-g sample of **2** was dissolved in 50 mL of methanol, 42 mg of NaOH was added, and the solution was heated at 50 °C for 1 h, which produced a color change to pale green. Following addition of excess NH_4Cl, the solution was evaporated, the residue taken up in CH_2Cl_2, the solution filtered, and the filtrate evaporated. The residue was chromatographed on a silica column in 50:50 hexane/CH_2Cl_2, producing several light yellow bands, which were **2** and minor cleavage products, and a major band, which was dark yellow **3**, obtained on evaporation of the solution as a crystalline solid, 0.204 g, 83% yield: ^{1}H NMR (δ, ppm, $CDCl_3$) 2.17 (s, $COCH_3$), 2.05 (m, CH_2), 1.86 (m, CH_2), 1.66 (s, C_5Me_5), 1.07 (t, CH_3); ^{13}C NMR (δ, ppm, $CDCl_3$) 113 (br s, C_2B_3 ring), 93.1 (s, C_5 ring), 37.2 (q, $COCH_3$), 23.0 (t, CH_2), 17.5 (q, CH_3), 9.9 (q, C_5Me_5); ^{11}B NMR (δ, ppm relative to $BF_3{\cdot}OEt_2$, hexane) 7.8 (s, B(5)), 2.8 (d, 130 Hz, B(4,6)); IR (neat, cm^{-1}) 2961 (vs), 2928 (s), 2912 (sh), 2866 (s), 2520 (s), 1886 (m br), 1851 (m br), 1654 (vs), 1610 (m), 1454 (s), 1446 (s), 1428 (m), 1383 (s), 1340 (m), 1199 (s), 1029 (s), 944 (m), 846 (m), 773 (s); exact mass calcd for $^{59}Co^{16}O^{12}C_{18}{}^{11}B_3{}^{11}H_{32}{}^+$ 356.2064, found 356.2057.

(5) An example of O-acylation in organosilicon chemistry is the reaction of PhCOCl with diethyl (lithio(trimethylsilyl)methyl)phosphonate; the preference for O- over C-acylation was attributed to steric hindrance by the Me_3Si and $P(O)(OEt)_2$ substituents. See: Carey, F. A.; Court, A. S. *J. Org. Chem.* **1972**, *37*, 939. As in most cases, the acylation takes place at an oxygen present in the original substrate, in contrast to the reaction reported here.

(6) The reaction was monitored at room temperature as a function of time, via TLC analysis, with no observable formation of **3** under the conditions employed in the synthesis of **2**.

(7) Brattsev, V. A.; Knyazev, S. P.; Stanko, V. I. *Zh. Obshch. Khim.* **1976**, *46*, 1419.

(8) Zakharkin, L. I.; L'vov, A. I. *Zh. Obshch. Khim.* **1967**, *37*, 1217.

(9) Reactions of 1^- with benzoyl chloride or malonyl chloride yield no identifiable products. Reaction with trimethylacetyl chloride or methyl malonyl chloride forms <5% of the 5-substituted derivative. Trifluoroacetyl chloride yields ~5% of the 4,6-disubstituted metallacarborane.

(10) Davis, J. H., Jr.; Grimes, R. N. *Inorg. Chem.* **1988**, *27*, 4213.

(11) Piepgrass, K. W.; Davis, J. H., Jr.; Sabat, M.; Grimes, R. N. *J. Am. Chem. Soc.*, following paper in this issue.

Electronic Control of Metallacarborane Stacking Reactions. Directed Synthesis of $Cp^*Co(C_2B_3)M(C_2B_3)CoCp^*$ Tetradecker Sandwiches[1]

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Small metallacarboranes of the type $LM(R_2C_2B_3H_5)$ (**1**), in which LM typically is CpCo, $(C_5Me_5)Co$, (arene)Fe, or (arene)Ru, are versatile building block reagents for the preparation of linked and stacked sandwich complexes, including triple-deckers.[1a,b,2] As shown in Scheme I (A), the removal of one or both B–H–B bridge protons to form the mono- or dianion (1^- or 1^{2-}) with subsequent coordination of a transition metal–ligand unit to the open face is a convenient route to homo- and heterobiometallic triple-decker species **2**. An apparently straightforward extension of this approach would be the reaction of 2 equiv of 1^- or 1^{2-} ions with a metal cation to generate tetradecker stacks (**3**) as in Scheme I (B). However, attempts at such reactions in our laboratory have been unsuccessful, and no species of type **3** have been characterized.[3] (A related class of tetradecker complexes, bridged by two C_3B_2 organoboron rings, is well established.[4])

Here we describe an unexpected discovery which has led directly to a solution of the "carborane tetradecker stacking problem" and has opened the way to the utilization of reactions of class B in preparing multidecker sandwiches. More generally, it reflects the sensitivity[1a,b] of the metal-binding capability of the metallacarborane C_2B_3 face to its electron population and charge distribution, a finding with potentially broad synthetic implications. The B(5)-acetyl complex $(C_5Me_5)Co[Et_2C_2B_3H_4$-5-C(O)Me] (**4a**) (compound **3** in the preceding communication[5]), on deprotonation with *n*-butyllithium followed by addition of $NiBr_2$ in THF, afforded a dark brown crystalline air-stable complex, **5a**, in 41% isolated yield.[6] Spectroscopic[6] and crystallographic[7] charac-

[†] Present address: Department of Chemistry, Brandeis University, Waltham, MA 02254.

(1) Organotransition-Metal Metallacarboranes. Part 17. For Parts 15 and 16, see: (a) Davis, J. H., Jr.; Attwood, M. E.; Grimes, R. N. *Organometallics* **1990**, *9*, 1171. (b) Attwood, M. E.; Davis, J. H., Jr.; Grimes, R. N. *Organometallics* **1990**, *9*, 1177.

(2) (a) Davis, J. H., Jr.; Sinn, E.; Grimes, R. N. *J. Am. Chem. Soc.* **1989**, *111*, 4776. (b) Davis, J. H., Jr.; Sinn, E.; Grimes, R. N. *J. Am. Chem. Soc.* **1989**, *111*, 4784. (c) Chase, K. J.; Grimes, R. N. *Organometallics* **1989**, *8*, 2492.

(3) A complex of the composition $[CpCo(Me_2C_2B_3H_3)]_2CoH$ having a possible tetradecker structure was partially characterized from ^{11}B and mass spectra: Grimes, R. N. *Coord. Chem. Rev.* **1979**, *28*, 47, ref 95a. Finster, D. A.; Grimes, R. N., unpublished results.

(4) (a) Siebert, W. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 943. (b) Siebert, W. *Pure Appl. Chem.* **1987**, *59*, 947. (c) Several "hybrid" tetradeckers that incorporate both C_2B_3 (carborane) and C_3B_2 (1,3-diborole) bridges have recently been prepared: Fessenbecker, A.; Attwood, M. D.; Grimes, R. N.; Stephan, M.; Pritzkow, H.; Zenneck, U.; Siebert, W. *Inorg. Chem.* **1990**, *29*, 516.

(5) Piepgrass, K. W.; Davis, J. H., Jr.; Sabat, M.; Grimes, R. N. *J. Am. Chem. Soc.*, preceding paper in this issue.

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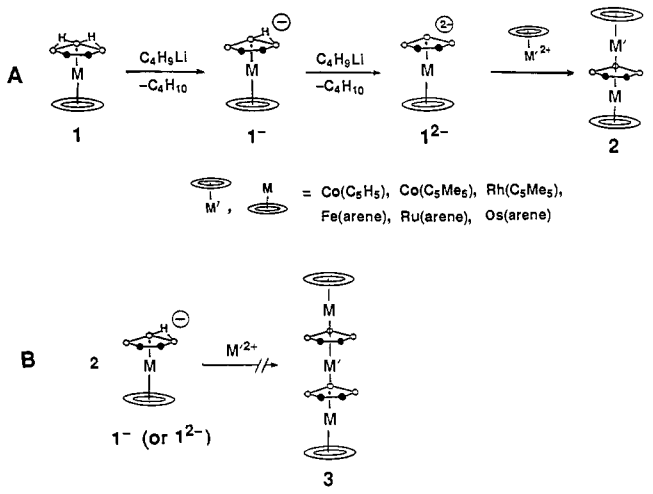
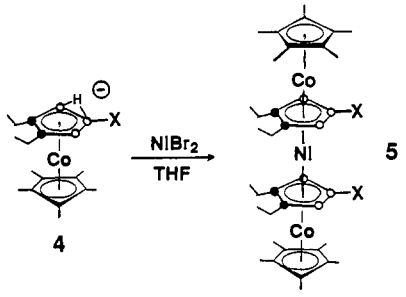
Scheme I[a]



[a](O) BH, (●) CR.

Scheme II[a]



[a](●) C; (O) B, BH; X = C(O)Me, Cl, Br, $CH_2C{\equiv}CMe$.

terization of **5a** revealed it to be tetradecker complex, $\{(C_5Me_5)Co[Et_2C_2B_3H_2$-5-C(O)Me$]\}_2Ni$ (Figure 1). The molecular stack is slightly bent in the middle (Co(1)–Ni–Co(2) angle = 170.6°), but each of the three metal atoms is nearly centered over its neighboring carborane and cyclopentadienyl rings. The complex is a diamagnetic 42-electron system with formal Co(III) and Ni(IV) oxidation states bridged by $Et_2C_2B_3H_3^{4-}$ ring ligands and is unchanged by prolonged exposure to air in solid or solution form.

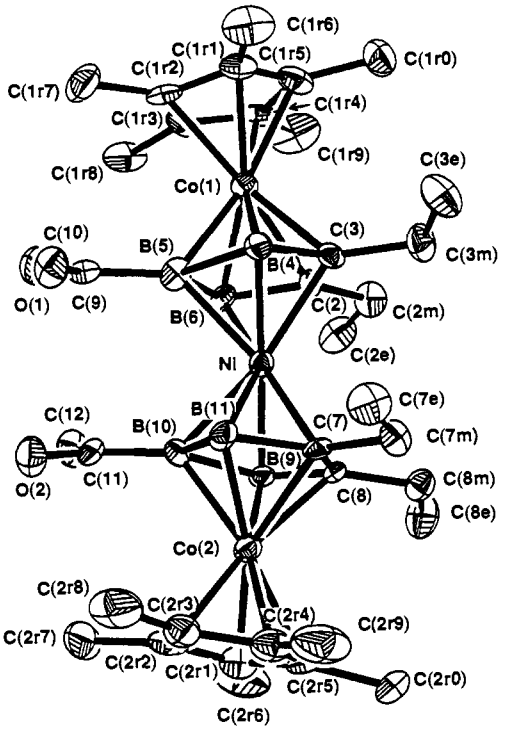


Figure 1. Molecular structure of **5a** (hydrogens omitted). Selected intramolecular distances (Å): Ni(1)–C(2), 2.09 (1); Ni(1)–C(3), 2.16 (1); Ni(1)–B(4), 2.12 (2); Ni(1)–B(5), 2.08 (1); Ni(1)–B(6), 2.09 (1); Ni(1)–C(7), 2.14 (1); Ni(1)–C(8), 2.19 (1); Ni(1)–B(9), 2.15 (2); Ni(1)–B(10), 2.07 (1); Ni(1)–B(11), 2.12 (1); Co(1)–C(2), 2.09 (1); Co(1)–C(3), 2.09 (1); Co(1)–B(4), 2.12 (2); Co(1)–B(5), 2.08 (1); Co(1)–B(6), 2.09 (1); Co(2)–C(7), 2.10 (1); Co(2)–C(8), 2.10 (1); Co(2)–B(9), 2.11 (1); Co(2)–B(10), 2.09 (1); Co(2)–B(11), 2.09 (1); C(2)–C(3), 1.44 (2); C(3)–B(4), 1.54 (2); B(4)–B(5), 1.77 (2); B(5)–B(6), 1.75 (2); B(6)–C(2), 1.59 (1); C(7)–C(8), 1.49 (2); C(8)–B(9), 1.54 (2); B(9)–B(10), 1.79 (2); B(10)–B(11), 1.78 (2); B(11)–C(7), 1.56 (1).

The conversion of **4a** to **5a** is noteworthy in at least two respects: (i) as noted above, other substrates of type **1** (Scheme I) have not exhibited the tetradecker-stacking reaction, and (ii) we have previously observed that alkylation of type **1** complexes at the B(5) position [but not at B(4,6)] renders them apparently inert to transition-metal coordination, blocking formation of even triple-decker sandwiches.[8] Hence the generation of **5a** from **4a** must be associated with electron withdrawal by the B(5)-acetyl substituent. In support of this hypothesis, we find that counterparts of **4a** having other electron-attracting groups at B(5), e.g., X = Cl, Br, or $CH_2C{\equiv}CMe$, also stack with Ni^{2+} to give the corresponding tetradecker species (Scheme II). Conversely, B(5)-X derivatives in which X is less electron withdrawing (e.g., H, alkyl) *do not* generate tetradeckers. Preliminary evidence indicates that these trends extend to other metals; for example, the reaction of **4a** with $CoCl_2$ forms a paramagnetic Co_3 counterpart of **5a**.[9]

An independent measure of the electron-withdrawing influence of the B(5)-X functional groups in complexes of type **4** is afforded by the ^1H B–H–B signal, which is sensitive to the nature of X. A clear trend is evident in these values, which are at lowest field (most deshielded) for X = Cl (δ −4.00), Br (−4.23), and acetyl (−5.16) and at higher field for less electron attracting groups (e.g., −5.37 for X = Et). Thus far, we find excellent correlation between the downfield B–H–B shift and the metal-stacking properties of these derivatives, even to the extent that the tetradecker complex isolated in highest yield (65%) was obtained from the B(5)-chloro species. At this point, it is not yet clear how the withdrawal of electron density from the C_2B_3 face facilitates metal coordination,

(6) A 0.192-g (0.54 mmol) sample of **4a** in dry THF at 0 °C was treated with 1 equiv of *tert*-butyllithium in hexane. The red solution was stirred for 15 min, and 60 mg (0.27 mmol) of $NiBr_2$ was added in vacuo, following which the solution was stirred for 4 h at room temperature. The dark brown solution was opened to the air and solvent stripped off, and the residue was taken up in dichloromethane and passed through a short silica column, which gave one band consisting of recovered **4a** (61 mg). The column was then stripped with methanol, the solution evaporated, and the dark brown residue chromatographed on a 45-cm silica column in 50:50 CH_2Cl_2/CH_3CN. A minor yellow band, unidentified, eluted first, followed by a major dark brown band which was evaporated and dissolved in 50:50 CH_2Cl_2/hexane, and solvent was removed to give dark brown crystals of **5a** (58 mg, 41% based on **4a** consumed): ^1H NMR (δ, ppm, $CDCl_3$) 2.44 (s, $COCH_3$), 2.30 (m, CH_2), 1.40 (s, C_5Me_5), 1.38 (t, CH_3); ^{13}C NMR (δ, ppm, $CDCl_3$, proton decoupled) 103.0 (C_2B_3 ring), 90.0 (C_5 ring), 37.9 (acetyl CH_3), 24.5 (CH_2), 15.2 (CH_3), 9.5 (C_5Me_5); ^{11}B NMR (β, ppm relative to $BF_3{\cdot}OEt_2$, 50:50 CH_2Cl_2/hexane, B–H coupling not resolved) 65.6 (1B), 11.8 (2B); IR (neat, cm^{-1}) 2965 (s), 2903 (s), 2872 (s), 2511 (s), 2493 (s), 2358 (m), 2327 (m), 1616 (vs), 1380 (s), 1334 (s), 1274 (m), 1241 (w), 1201 (w), 1020 (s), 907 (m), 809 (vs), 780 (m), 732 (s); electron-impact mass spectrum, cutoff at m/z 770 corresponding to parent ion envelope, base peak at m/z 766, intensity pattern consistent with calculated spectrum based on natural isotope abundances for $NiCo_2O_2C_{36}B_6H_{60}^+$; exact mass calcd for $^{60}Ni^{59}Co_2{}^{16}O_2{}^{12}C_{36}{}^{11}B_6{}^1H_{60}^+$ 768.3124, found 768.3143.

(7) Crystal data: space group $P\bar{1}$ (No. 2); $Z = 2$; $a = 10.487$ (3) Å, $b = 13.033$ (3) Å, $c = 17.537$ (6) Å, $\alpha = 106.51$ (2)°, $\beta = 97.71$ (3)°, $\gamma = 108.82$ (2)°; $V = 2107$ (3) Å^3; crystal size 0.20 × 0.20 × 0.05 mm; μ(Mo Kα) = 1.364 mm^{-1}. Data collection parameters: 2θ range 3.5–45.0°; $R = 0.070$ for 3494 reflections having $F_o > 2.0\sigma(F_o)$. The structure was solved by using the Siemens SHELXTL PLUS package using direct methods. A full report on the structure determination will be given elsewhere.

(8) (a) Davis, J. H., Jr.; Benvenuto, M. A.; Grimes, R. N., submitted for publication. (b) Davis, J. H., Jr.; Attwood, M. D.; Grimes, R. N., unpublished observations.

(9) Note **Added in Proof**: X-ray data has confirmed the tetradecker structure of this species.

but studies designed to probe this question are in progress.

Acknowledgment. We are grateful to Dr. Charles F. Campana for his assistance with the X-ray data collection and structure determination and to the Siemens Corporation, Madison, WI, for the use of their diffractometer. The support of the National Science Foundation (Grant No. CHE 87-21657) and the U.S. Army Research Office is gratefully acknowledged.

Supplementary Material Available: Tables of crystal structure data, thermal parameters, bond distances and angles, and mean planes for **5a** (14 pages); listing of observed and calculated structure factors for **5a** (19 pages). Ordering information is given on any current masthead page.

Engineering Subtilisin for Reaction in Dimethylformamide[1]

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Enzyme catalysis in organic solvents has emerged as a useful technology in organic synthesis.[2] Nevertheless, problems are often encountered in such environments, particularly in polar organic solvents,[3] mainly due to the intrinsic instability and low catalytic activity of enzymes. We have recently reported the use of site-directed mutagenesis to prepare a mutant subtilisin BPN′ (subtilisin 8350) to improve the enzyme stability and activity in *N*,*N*-dimethylformamide (DMF).[4] We report here another subtilisin variant (8397) which is even more stable than the 8350 variant in DMF.

The 8350 variant contains the following amino acid substitutions which improve stabilizing interactions:[5] Asn 218 Ser (hydrogen bonding), Gly 169 Ala (hydrophobic interaction and conformational restriction), Met 50 Phe (hydrophobic interaction), Tyr 217 Lys (hydrogen bonding), Gln 206 Cys (oxidized to Cys-SH during posttranslational modification, van der Waals interaction) and Asn 76 Asp (Ca^{2+} binding and hydrogen bonding). Each of these mutations was found to have only small and localized effects on the protein structure and also to have an additive effect on the enzyme stability in aqueous media.[5] This variant was about 100 times more stable in aqueous solution and 50 times more stable in DMF than the wild-type enzyme. A marked improvement of stability in DMF was found when the Lys-217 residue of 8350 was changed back to the wild-type Tyr (Figure 1). This new variant 8397 has a half-life of 350 h in DMF and 1600 h in aqueous solution at pH 8.4 and 25 °C, compared to 20 min and 15 h, respectively, for the wild-type enzyme.[4] Since the residue at position 217 is located on the surface, change of the charged Lys back to Tyr may make the enzyme more compatible with

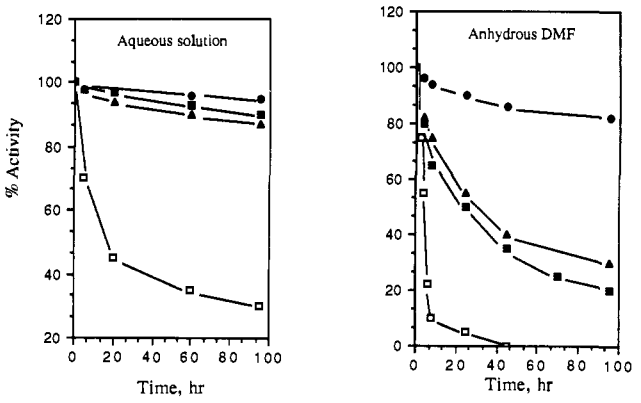

Figure 1. Stability of subtilisin BPN′ and mutants in aqueous solution (0.05 M Tris·HCl, pH 8.4) and in DMF at 25 °C: □, wild-type BPN′; ■, 8350; ●, 8397; ▲, 8399. The rate of inactivation was measured on the basis of the remaining activity as described previously.[4] The inactivation is not simple first order. 8350: Met 50 Phe (hydrophobic), Gly 169 Ala (hydrophobic and configurational entropy), Asn 76 Asp (Ca^{2+} binding and H bonding), Gln 206 Cys (oxidized to Cys-SH, van der Waals), Tyr 217 Lys (H bonding), Asn 218 Ser (H bonding). 8397: The same as 8350 except no change for Tyr 217. 8399: The same as 8350 except no changes for Gly 169 and Tyr 217.

DMF, which is a poor solvator, thereby resulting in a large, positive effect on the enzyme stability in the organic solvent. Further change of variant 8397 at position 169 from Ala to the wild-type Gly generated the mutant 8399, which is more stable than the wild-type enzyme but less stable than 8397. It had a half-life of 1000 h in aqueous solution and 43 h in DMF. The 8-fold increase in stability in DMF compared to the 3-fold increase in stability in aqueous solution for the Gly 169 Ala mutation may indicate that the increase in the conformational restriction of the enzyme in DMF (due to the loss of configurational entropy for the unfolded form)[6] seems more significant than the negative hydrophobic effect experienced in DMF vs H_2O. It is noted that the solvent-accessible surface of Gly 169 in aqueous media is only 1% as measured by water probe calculation.[7]

To examine the effect of mutations on catalysis, the kinetic parameters of 8397 and 8399 for the hydrolysis of selected esters, thioester, and amides were determined and compared to those for the wild-type enzyme and 8350 (Table I).[4] It was found that, in general, the mutations have little effect on catalysis, as indicated by the k_{cat}/K_m values. We then investigated the active-site geometry of the mutant enzymes by studying the inhibition kinetics with Boc-Ala-Val-Phe-CF_3, a designed inhibitor that forms an enzyme–inhibitor complex resembling the transition-state complex of the enzyme reaction.[4] As expected, the smaller K_i values[8] for the mutant enzymes interacting with the transition-state-based inhibitor indicate that the mutant enzymes bind to the reaction transition state of the peptide substrate slightly more strongly than the wild-type enzyme, as reflected by the higher k_{cat}/K_m values for the mutant enzymes. In addition to the L-isomer specificity at the P_1 site, we also examined the P_2 specificity of the mutant and the wild-type enzymes in the hydrolysis of a number of N-protected dipeptide esters containing a D- and an L-amino acid residue at the P_2 position.[9] All four enzymes showed a very high

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(1) Supported by NSF (CHE 8806182), NIH (GM 44154), and DOE (DE-AC-03-76SF00098) grants to C.-H.W. at Scripps.

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(7) Richard, E. M. *Annu. Rev. Biophys. Bioeng.* **1977**, *6*, 151.

(8) Slow binding behavior as described previously[4] was observed in each case. The observed rate constant (k_{obsd}) of inhibition was thus determined and plotted vs the inhibitor concentration for K_i ($=k_{off}/k_{on}$) determination. Both k_{on} (=slope × {1 + [S]/K_m}, where [S]/K_m = 0.5 for 8399, 0.4 for 8397, and 0.45 for other enzymes), and k_{off} (intercept) were determined to be the following: wild type, $k_{on} = (1.17 \pm 0.11) \times 10^3$ M^{-1} s^{-1}, $k_{off} = (5.86 \pm 0.91) \times 10^{-3}$ s^{-1}, $K_i = 5.0$ μM; 8350, $k_{on} = (6.48 \pm 0.25) \times 10^2$ M^{-1} s^{-1}, $k_{off} = (7.17 \pm 0.22) \times 10^{-4}$ s^{-1}, $K_i = 1.05$ μM; 8397, $k_{on} = (1.26 \pm 0.03) \times 10^3$ M^{-1} s^{-1}, $k_{off} = (1.32 \pm 0.36) \times 10^{-3}$ s^{-1}, $K_i = 1.1$ μM; 8399, $k_{on} = (6.50 \pm 0.01) \times 10^2$ M^{-1} s^{-1}, $k_{off} = (4.67 \pm 0.06) \times 10^{-4}$ s^{-1}, $K_i = 7.02$ μM.

(9) The peptide esters tested were Boc-L(D)-Ala-Phe-OMe, Boc-L(D)-Phe-Phe-OMe, Boc-L(D)-Ala-Gly-OMe, and Boc-L(D)-Tyr-Gly-OMe.

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