Fluoroalkenes as Peptide Isosteres: Ground State Analog Inhibitors of Thermolysin

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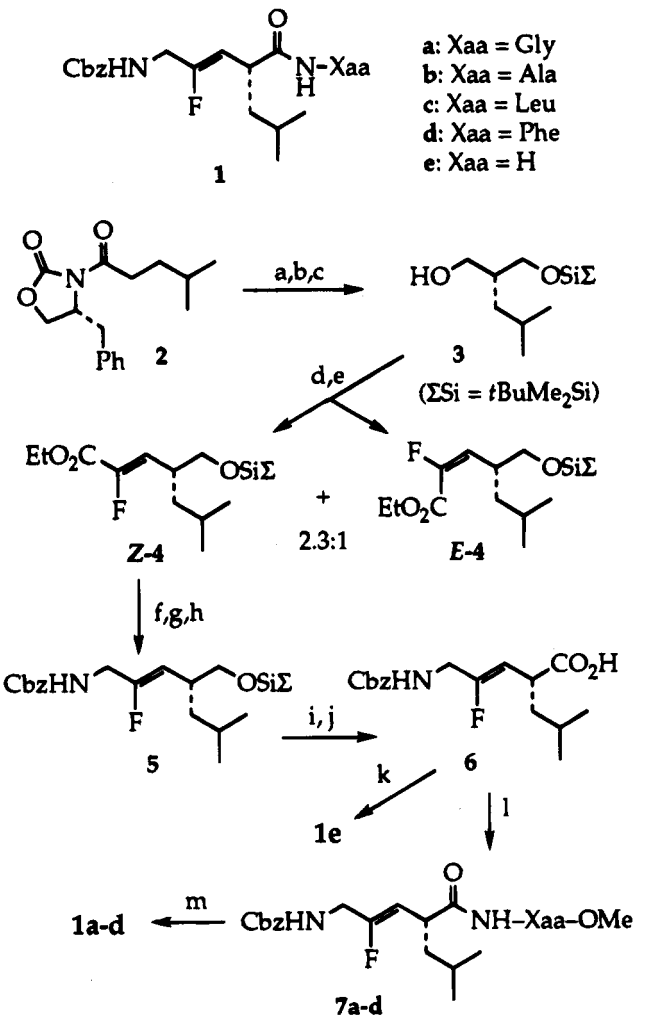
Tripeptide analogs of the form Cbz-GlyΨ[(*Z*)-CF=CH]LeuXaa (**1**, Xaa = Gly, Ala, Leu, Phe, and NH_2) were synthesized to assess the ability of the fluoroalkene moiety to mimic a peptide linkage. These compounds are modest inhibitors of the zinc endopeptidase thermolysin (0.19 mM < K_i < 1.8 mM); the K_i values correlate strongly with the K_m values, but not K_m/k_{cat}, for hydrolysis of the corresponding peptides. The correlation indicates that these inhibitors bind as ground state analogs and represent the first direct assessment of the fluoroalkene unit as a peptide surrogate.

The *trans*-fluoroalkene moiety has been regarded as an isosteric replacement for the peptide linkage on the basis of its planar geometry and direction of polarization. Theoretical studies[1,2] and synthetic routes[3,4] for these analogs have been described, but there has been limited assessment of their behavior as peptidomimetics. In this report, we describe the synthesis of a series of fluoroalkene tripeptide analogs **1a-e** and an evaluation of the way they bind as inhibitors of the zinc peptidase thermolysin.

The GlyΨ[(*Z*)-CF=CH]Leu[5] derivatives were attractive targets for comparison to other inhibitors of thermolysin, specifically for the opportunity to demonstrate a novel structure–activity relationship for these compounds. We have previously evaluated the transition state mimicry of phosphorus-containing peptide analogs by comparing the K_i values of these inhibitors with the K_m/k_{cat} values of the corresponding substrates.[6,7] With the fluoroalkenes, we compare the K_i values of the fluoroalkene analogs with the substrate K_m values.[8] Such a correlation is indicative of *ground state analogy* on the part of the inhibitors, since K_m has been shown to equal K_d for simple tripeptide substrates of thermolysin.[9]

The (*Z*)-fluoroalkene analog of Cbz-Gly-Leu was synthesized and coupled to the C-terminal residues as shown in Scheme 1.[3] The chiral enolate[10,11] of the (4-methylpentanoyl)oxazolidinone, **2**, is condensed with trioxane to give the hydroxymethyl derivative; silylation and reduction then afford the protected diol **3**. After oxidation

Scheme 1



[a] Key: (a) $TiCl_4$, trioxane, iPr_2NEt (85%); (b) imidazole, SSiCl, DMF (99%); (c) $LiBH_4$, ether (76%); (d) PCC, CH_2Cl_2; (e) NaH, $(CO_2Et)_2$, FCH_2CO_2Et (53%); (f) NH_3, MeOH (89%); (g) $LiAlH_4$, ether; (h) CbzCl, Et_3N (67%); (i) TBAF (99%); (j) Jones (91%); (k) NH_3, EDC, HOBt (70%); (l) Xaa-OMe, EDC, HOBt, iPr_2NEt (87–90%); (m) LiOH (quant).

to the aldehyde, condensation with diethyl fluorooxaloacetate, formed *in situ* from diethyl oxalate and ethyl fluoroacetate,[12] provides the fluoroacrylate **4** as a 2.3:1 mixture of *Z*:*E* isomers. The isomers can be separated

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Table 1. Inhibition of Thermolysin by Fluoroalkene Peptide Analogs 1

			substrate[a]	
compd	Xaa	inhibitor K_i (mM)	K_m (mM)	K_m/k_{cat} (μM·s)
1a	Gly	1.80	10.8	165
1b	Ala	1.48	10.6	13.6
1c	Leu	0.32	2.6	7.0
1d	Phe	0.186	2.4	20
1e	NH_2	ND[b]		

[a] 25 °C, pH 7.0; substrate data from ref 8. [b] Not determined because of solubility.

by chromatography and are readily distinguished by their hydrogen–fluorine coupling constants: *Z*: 33.8 Hz; *E*: 22.5 Hz. Amidation of the *Z*-isomer, reduction to the allylic amine, and protection with carbobenzoxy chloride give the allylic carbamate **5**. The reduction process produces a small amount of the defluorinated alkene (ca. 3–6% yield), which can be removed on chromatographic purification. The silyl ether is deprotected, the homoallylic alcohol is oxidized to the acid **6**, and the variable residue is introduced by coupling with the corresponding amino acid esters, to give **7a-d**, or with ammonia, to give **1e**. From the coupling reactions with leucine and phenylalanine, giving **7c** and **7d**, small amounts of diastereomeric products are isolated (<2% yield in both cases). For the leucine derivative, this product ($[\alpha]^{23}_D$ = +44.8°) was shown to be the D,L-isomer (i.e., epimerized at the allylic position) by comparison to authentic L,D-material ($[\alpha]^{23}_D$ = −49.6°) produced on coupling **6** with D-leucine methyl ester. Finally, the inhibitors **1a-d** are formed on hydrolysis of the ester precursors with lithium hydroxide and purified by ion exchange chromatography.

Because of their limited solubility, the fluoroalkenes **1a-d** were assayed as inhibitors of thermolysin in a buffer solution containing 2.5% DMF (Table 1).[6] We were unable to measure an inhibition constant for the simple amide **1e** because of its low solubility; even in 5% DMF, a saturated solution is only ca. 0.2 mM (the projected K_i value is ca. 3.6 mM). The fluoroalkene tripeptide analogs bind to thermolysin about 1 order of magnitude more tightly than the substrates, as judged by their Michaelis constants.[8] This higher affinity suggests that reduced solvation of the fluoroalkene unit, in comparison to the peptide linkage, is more significant than specific polar interactions in the active site, which would be expected to favor the peptide.

Figure 1 shows the correlation between these K_i values, expressed logarithmically, and the K_m and K_m/k_{cat} values of the corresponding substrates.[8] Alterations at the P_2' position have a similar effect on the affinities of the inhibitors and on the ground state form of the substrates (Figure 1a). In contrast and as expected, there is no relationship between the binding affinities of the inhibitors and the transition state form of the substrates (Figure 1b). The difference between ground state and transition state binding of substrates themselves clearly demonstrates that the influence of the P_2' side chain depends on the geometry and orientation of the scissile linkage. The K_i vs K_m correlation of Figure 1a indicates that the P_2' residue of these inhibitors sits in the thermolysin active site like it does in the substrate Michaelis complex and implies that the fluoroalkene moiety is in turn bound like the peptide linkage. However, the slope of the line in Figure 1a is 1.3 (R = 0.98), which means the binding of inhibitor and substrate are not identical: contributions from the bulky, hydrophobic Leu and Phe side chains are proportionally greater for the inhibitor complexes than for the Michaelis complexes.

Although "ground state analogs" are not expected to be be particularly potent as enzyme inhibitors, they can be useful as substrate models in structural studies where a true substrate would be converted to product. This study demonstrates that (*Z*)-fluoroalkenes have this useful characteristic for the thermolysin system.

Experimental Section[13]

***N*-((*Z*)-(2*R*)-5-(*N*-(Benzyloxycarbonyl)amino)-4-fluoro-2-(2-methylpropyl)3-pentenoyl)-L-leucine Lithium Salt (1c).** To a solution of 203 mg (0.451 mmol) of ester **7c** (see below) in 4 mL of CH_3CN was added 0.496 mL (0.496 mmol) of 1 N aqueous LiOH, and the reaction mixture was stirred for 24 h at room temperature and concentrated in vacuo. After the addition of 15 mL of water to the residue, the solution was lyophilized to afford a white solid. This material was purified by anion exchange chromatography over DEAE Sephadex A-25 (HCO_3^- form) with a step-gradient from H_2O to 0.5 M TBK buffer as an eluant and lyophilized. Cation exchange chromatography (AG50W-X8, Li^+ form) and lyophilization afforded 194 mg (97%) of lithium salt **1c** as a white solid: $[\alpha]^{23}_D$ −48.4° (*c* 0.55, H_2O); IR (KBr) 3313, 2957, 1707, 1603 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 0.83–0.97 (m, 12), 1.34–1.65 (m, 6), 3.50–3.60 (m, 1), 3.87 (d, 2, J = 13.0), 4.18–4.27 (m, 1), 4.88 (dd, 1, J = 9.1, 36.9), 5.17 (s, 2), 7.42–7.56 (m, 5); ^{13}C NMR (D_2O, 100 MHz) δ 21.8, 22.4, 23.1, 23.5, 25.6, 26.3, 41.0, 41.4, 41.9, 54.8, 68.0, 107.2 (d, J = 12), 128.7, 129.4, 129.8, 137.3, 157.9 (d, J = 258), 158.9, 176.7, 180.9; MS (FAB) m/z 443 (MH^+); HRMS (FAB) calcd for $C_{23}H_{33}FN_2O_5Li$ 443.2534, found 443.2533.

The following compounds were prepared in a similar manner.

***N*-((*Z*)-(2*R*)-5-(*N*-(Benzyloxycarbonyl)amino)-4-fluoro-2-(2-methylpropyl)-3-pentenoyl)glycine lithium salt (1a)**: yield 99%; $[\alpha]^{23}_D$ −32.1° (*c* 0.56, H_2O); IR (KBr) 3354, 2957, 1700, 1610 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 0.88 (d, 3, J = 6.2), 0.92 (d, 3, J = 6.2), 1.34–1.62 (m, 3), 3.50–3.60 (m, 1), 3.75 (s, 2), 3.89 (d, 2, J = 12.1), 4.90 (dd, 1, J = 9.7, 36.6), 5.18 (s, 2), 7.42 −7.52 (m, 5); ^{13}C NMR (D_2O, 100 MHz) δ 22.1, 23.2, 26.2, 40.9 (d, J = 3), 41.7 (d, J = 36), 41.9, 44.4, 68.1, 106.5 (d, J = 12), 128.6, 129.4, 129.8, 137.5, 158.0 (d, J = 256), 159.2, 177.4; MS (FAB) m/z 387 (MH^+); HRMS (FAB) calcd for $C_{19}H_{25}FN_2O_5Li$ 387.1908, found 387.1922.

***N*-((*Z*)-(2*R*)-5-(*N*-(Benzyloxycarbonyl)amino)-4-fluoro-2-(2-methylpropyl)-3-pentenoyl)-L-alanine lithium salt (1b)**: yield 99%; $[\alpha]^{23}_D$ −40.2° (*c* 0.56, H_2O); IR (KBr) 3325, 2960, 1692, 1587 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 0.88 (d, 3, J = 6.2), 0.92 (d, 3, J = 6.2), 1.33 (d, 3, J = 7.2), 1.30–1.62 (m, 3), 3.50–3.58 (m, 1), 3.88 (d, 2, J = 12.3), 4.10–4.18 (m, 1), 4.88 (dd, 1, J = 9.6, 36.5), 5.18 (s, 2), 7.40–7.54 (m, 5); ^{13}C NMR (D_2O, 100 MHz) δ 18.5, 22.3, 23.1, 26.2, 40.9 (d, J = 2), 41.7 (d, J = 28), 41.8, 51.9, 68.1, 106.8 (d, J = 12), 128.6, 129.4, 129.8, 137.5, 157.9 (d, J = 260), 159.2, 176.8, 181.0; MS (FAB) m/z 401 (MH^+); HRMS (FAB) calcd for $C_{20}H_{27}FN_2O_5Li$ 401.2064, found 401.2073.

***N*-((*Z*)-(2*R*)-5-(*N*-(Benzyloxycarbonyl)amino)-4-fluoro-2-(2-methylpropyl)-3-pentenoyl)-L-phenylalanine lithium salt (1d)**: yield 99%; $[\alpha]^{23}_D$ −3.9° (*c* 0.69, H_2O); IR (KBr) 3337, 2956, 1700, 1603 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 0.83 (d, 3,

(13) General. All reactions were carried out under a nitrogen atmosphere unless otherwise noted. Triethylammonium bicarbonate (TBK) buffer was prepared by bubbling CO_2 gas through an aqueous solution of triethylamine until the desired pH (8.5) was obtained. ^1H NMR spectra were obtained in $CDCl_3$ referenced to tetramethylsilane or in D_2O referenced to MeOH as 3.39 ppm. J values are given in Hz. ^{13}C NMR chemical shifts are referenced to $CDCl_3$ (77.0 ppm) or MeOH (49.9 ppm). Thin layer chromatography was performed on silica gel precoated glass plates (250 μm, silica gel 60, E. Merck, Darmstadt). Optical rotations were taken on a Perkin-Elmer 241 polarimeter with a sodium lamp (589 nm, 10-cm path length cell, concentration in g/100mL of the indicated solvent).

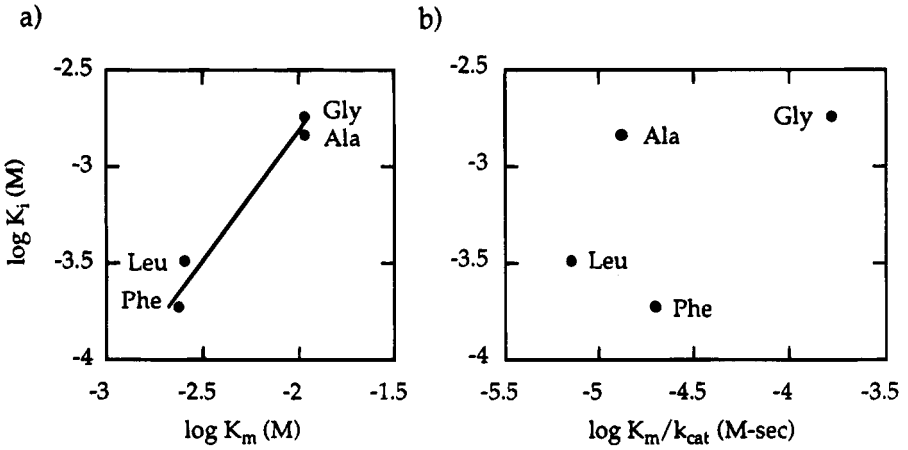

Figure 1. Correlation between inhibition constants for fluoroalkene analogs **1** and kinetic parameters for corresponding thermolysin tripeptide substrates.

J = 6.2), 0.87 (d, 3, J = 6.3), 1.23–1.52 (m, 3), 2.87–2.98 (m, 1), 3.12–3.23 (m, 1), 3.40–3.48 (m, 1), 3.84 (d, 2, J = 13.3), 4.45–4.52 (m, 1), 4.70 (dd, 1H, J = 9.9, 36.2), 5.17 (s, 2), 7.36–7.55 (m, 10); ^{13}C NMR (D_2O, 100 MHz) δ 22.2, 23.3, 26.2, 38.7, 41.0, 41.5, 41.8 (d, J = 33), 56.8, 67.9, 106.9 (d, J = 11), 127.5, 128.7, 129.2, 129.3, 129.6, 130.4, 137.4, 138.6, 157.8 (d, J = 257), 158.7, 176.1, 178.8; MS (FAB) m/z 477 (MH^+); HRMS (FAB) calcd for $C_{26}H_{31}FN_2O_5Li$ 477.2377, found 477.2367.

(*Z*)-(2*R*)-5-(*N*-(Benzyloxycarbonyl)amino)-4-fluoro-2-(2-methylpropyl)-3-pentenamide (1e). To a solution of 180 mg (0.557 mmol) of carboxylic acid **6** in 5 mL of CH_2Cl_2 were added 90.3 mg (0.668 mmol) of HOBT and 2 mL of ammonia (ca. 0.5 N) in CH_2Cl_2 at 0 °C, followed by 128 mg (0.668 mmol) of EDC. After 2 h at 0 °C, to this reaction mixture was added an additional 1 mL of ammonia (ca. 0.5 N) in CH_2Cl_2 and the stirring was continued for 1 h at 0 °C. The reaction mixture was allowed to warm to room temperature and then diluted with 5 mL of 1 N HCl and extracted with 30 mL of EtOAc. The organic layer was washed with $NaHCO_3$, water, and brine and dried over $MgSO_4$. After removal of the solvent in vacuo, the residue was chromatographed on silica gel (3:1 EtOAc/hexane) to afford 125 mg (70%) of amide **1e** as a white solid: R_f 0.39 (3:1 EtOAc/hexane); $[\alpha]^{23}_D$ −50.9° (*c* 0.58, $CHCl_3$); mp 116–118 °C (recrystallized from EtOAc/hexane); IR (KBr) 3331, 2953, 1700, 1653 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (d, 3, J = 6.5), 0.91 (d, 3, J = 6.6), 1.32–1.78 (m, 3), 3.32–3.42 (m, 1), 3.80–3.96 (m, 2), 4.86 (dd, 1, J = 9.8, 35.8), 5.0–5.12 (m, 1), 5.11 (s, 2), 5.24–5.36 (m, 1), 5.70–5.84 (m, 1), 7.26–7.40 (m, 5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.7, 23.0, 25.7, 39.6, 40.9, 41.7 (d, J = 33), 67.1, 106.9 (d, J = 13), 128.1, 128.3, 128.6, 136.2, 156.2, 156.9 (d, J = 258), 175.7; MS (FAB) m/z 323 (MH^+); HRMS (FAB) calcd for $C_{17}H_{24}FN_2O_3$ 323.1771, found 323.1764. Anal. Calcd for $C_{17}H_{23}FN_2O_3$: C, 63.34; H, 7.19; N, 8.69. Found: C, 63.42; H, 7.30; N, 8.68.

(4*R*)-4-Benzyl-3-(4-methyl-1-oxopentyl)-2-oxazolidinone (2). To a solution of 7.00 g (39.5 mmol) of (*R*)-(+)-4-benzyl-2-oxazolidinone in 120 mL of THF was added 24.7 mL (39.5 mmol) of 1.6 N n-BuLi in hexane over 10 min at −78 °C. After 30 min, 6.38 g (47.4 mmol) of 4-methylpentanoyl chloride was added at the same temperature. After being stirred for 30 min at −78 °C, the reaction mixture was allowed to warm to room temperature, and 25 mL of aqueous NH_4Cl and 10 mL of water were added. After removal of the THF in vacuo, the residue was extracted with 100 mL of CH_2Cl_2 and the organic extract was washed with 1 N NaOH, water, and brine and dried over $MgSO_4$. After removal of the solvent in vacuo, the residue was chromatographed on silica gel (1:10 EtOAc/hexane) to afford 10.5 g (96%) of oxazolidinone **2** as a colorless oil: R_f 0.51 (1:4 EtOAc/hexane); $[\alpha]^{23}_D$ −54.7° (*c* 1.27, $CHCl_3$); IR (neat) 2956, 1782, 1698, 1604 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.94 (d, 6, J = 6.4), 1.53–1.70 (m, 3), 2.76 (dd, 1, J = 9.6, 13.4), 2.86–3.03 (m, 2), 3.30 (dd, 1, J = 3.2, 13.4), 4.15–4.27 (m, 2), 4.64–4.72 (m, 1), 7.15–7.45 (m, 5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.3, 27.6, 33.1, 33.5, 37.9, 55.1, 66.1, 127.2, 128.9, 129.4, 135.3, 153.4, 173.6; MS (EI) m/z 275 (M^+). Anal. Calcd for $C_{16}H_{21}NO_3$: C, 69.80; H, 7.69; N, 5.09. Found: C, 69.65; H, 7.80; N, 5.06.

(4*R*)-4-Benzyl-3-((2*S*)-2-(hydroxymethyl)-4-methyl-1-oxopentyl)-2-oxazolidinone. To a solution of 3.26 g (11.9 mmol) of oxazolidinone **2** in 50 mL of dry CH_2Cl_2 was added 1.37 mL (12.5 mmol) of $TiCl_4$ followed by 2.18 mL (12.5 mmol) of diisopropylethylamine at 0 °C. After 1 h, 1.07 g (13.1 mmol) of trioxane and 1.44 mL (13.1 mmol) of $TiCl_4$ were added to this solution and the mixture was stirred for an additional 2.5 h at 0 °C. Fifty mL of aqueous NH_4Cl and 50 mL of water were added, the resultant mixture was extracted with 300 mL of EtOAc, and the organic layer was washed with aqueous $NaHCO_3$, water, and brine and dried over $MgSO_4$. After removal of the solvent in vacuo, the residue was purified by chromatography on silica gel (1:5 EtOAc/hexane) to give 3.09 g (85%) of the aldol adduct as a white solid: R_f 0.32 (1:3 EtOAc/hexane); $[\alpha]^{23}_D$ −54.9° (*c* 1.02, $CHCl_3$); mp 71–72 °C (recrystallized from EtOAc and hexane); IR (KBr) 3526, 2953, 1762, 1690 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.92 (d, 3, J = 6.2), 0.93 (d, 3, J = 6.0), 1.36–1.46 (m, 1), 1.53–1.70 (m, 2), 2.23 (br s, 1, OH), 2.82 (dd, 1, J = 9.5, 13.4), 3.32 (dd, 1, J = 3.4, 13.4), 3.75–3.83 (m, 1), 3.87–3.93 (m, 1), 4.06–4.14 (m, 1), 4.18–4.26 (m, 2), 4.67–4.73 (m, 1), 7.25–7.38 (m, 5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.3, 22.8, 25.9, 37.3, 37.8, 43.6, 55.6, 64.3, 66.1, 127.3, 128.9, 129.4, 135.2, 153.5, 176.1; MS (EI) m/z 305 (M^+). Anal. Calcd for $C_{17}H_{23}NO_4$: C, 66.87; H, 7.59; N, 4.59. Found: C, 66.91; H, 7.83; N, 4.84.

(4*R*)-4-Benzyl-3-((2*S*)-2-(((*tert*-butyldimethylsilyl)oxy)-methyl)-4-methyl-1-oxopentyl)-2-oxazolidinone. To a solution of 825 mg (2.70 mmol) of the aldol adduct in 8 mL of DMF were added 276 mg (4.05 mmol) of imidazole and 529 mg (3.51 mmol) of *tert*-butyldimethylsilyl chloride, and the mixture was stirred for 1 h at room temperature. Eight mL of 1 N HCl was added, the mixture was extracted with 30 mL of EtOAc, and the organic layer was washed with aqueous $NaHCO_3$, water, and brine and dried over $MgSO_4$. After removal of the solvent in vacuo, the residue was chromatographed on silica gel (1:5 EtOAc/hexane) to afford 1.12 g (99%) of the silyl ether as a colorless oil: R_f 0.47 (1:5 EtOAc/hexane); $[\alpha]^{23}_D$ −23.3° (*c* 1.24, $CHCl_3$); IR (neat) 2955, 1783, 1699 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.06 (s, 3), 0.07 (s, 3), 0.89 (s, 9), 0.92 (d, 3, J = 6.6), 0.93 (d, 3, J = 6.3), 1.30–1.37 (m, 1), 1.53–1.70 (m, 2), 2.68 (dd, 1, J = 9.9, 13.4), 3.35 (dd, 1, J = 3.2, 13.4), 3.81 (dd, 1, J = 5.1, 9.5), 3.87 (dd, 1, J = 7.7, 9.5), 4.13–4.25 (m, 3), 4.68–4.75 (m, 1), 7.25–7.40 (m, 5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −5.6, −5.5, 18.2, 22.8, 25.8, 26.1, 37.7, 38.0, 43.7, 55.4, 65.0, 65.8, 127.2, 128.9, 129.4, 135.6, 153.1, 175.5; MS (FAB) m/z 420 (MH^+). Anal. Calcd for $C_{23}H_{37}NO_4Si$: C, 65.83; H, 8.89; N, 3.34. Found C, 65.55; H, 8.90; N, 3.32.

(2*R*)-2-(((*tert*-Butyldimethylsilyl)oxy)methyl)-4-methylpentanol (3). To a solution of 1.01 g (2.41 mmol) of

oxazolidinone silyl ether was added 52.5 mg (2.41 mmol) of $LiBH_4$ at 0 °C. The mixture was stirred for 30 min at room temperature, and then 12 mL of 1 N HCl was added. The resultant mixture was extracted with 30 mL of EtOAc, and the organic layer was washed with aqueous $NaHCO_3$, water, and brine. After removal of the solvent in vacuo, the residue was chromatographed on silica gel (1:10 EtOAc/hexane) to give 453 mg (76% yield) of alcohol **3** as a colorless oil: R_f 0.46 (1:5 EtOAc/hexane); $[\alpha]^{23}_D$ +10.3° (*c* 1.02, $CHCl_3$); IR (neat) 3600–3100, 2955 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.08 (s, 6H), 0.895 (d, 6H, J = 6.4), 0.903 (s, 9), 0.95–1.13 (m, 2), 1.55–1.67 (m, 1), 1.78–1.88 (m, 1), 3.56 (dd, 1, J = 7.9, 9.8), 3.60 (dd, 1, J = 7.5, 10.7), 3.72 (dd, 1, J = 3.3, 10.7), 3.80 (dd, 1, J = 4.0, 9.8); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −5.7, −5.6, 18.1, 22.77, 22.81, 25.4, 25.8, 36.9, 39.6, 67.1, 67.7; MS (FAB) *m*/*z* 247 (MH^+). Anal. Calcd for $C_{13}H_{30}O_2Si$: C, 63.35; H, 12.27. Found: C, 63.19; H, 12.06.

Ethyl (*Z*)-(4*R*)-4-(((*tert*-Butyldimethylsilyl)oxy)methyl)-2-fluoro-6-methyl-2-heptenoate and the *E*-Isomer ((*Z*)-4 and (*E*)-4). To a solution of 131 mg (0.531 mmol) of alcohol **3** in 2 mL of CH_2Cl_2 was added 286 mg (1.33 mmol) of PCC, and the mixture was stirred for 3 h at room temperature. The chromium reagent was removed with silica gel chromatography (CH_2Cl_2 as an eluant) to give 106 mg of the aldehyde as a colorless oil: R_f 0.62 (1:8 EtOAc/hexane); IR (neat) 2957, 1729 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.05 (s, 6 H), 0.87 (s, 9 H), 0.90 (d, 3, J = 6.2), 0.91 (d, 3, J = 5.8), 1.24 −1.35 (m, 1), 1.52–1.70 (m, 2), 2.46–2.54 (m, 1), 3.77–3.86 (m, 2), 9.69 (d, 1, J = 2.8). This material was used in the subsequent reaction without further characterization.

To a suspension of 51.0 mg (1.27 mmol) of NaH in 2.5 mL of THF were added 0.180 mL (1.33 mmol) of diethyl oxalate, 5 μL of EtOH, and 5 μL (0.05 mmol) of ethyl fluoroacetate. The mixture was stirred for 15 min at 45 °C, 0.123 mL (1.27 mmol) of ethyl fluoroacetate was added, and the mixture was stirred for an additional 1.5 h at 55 °C. After addition of 106 mg of the aldehyde (0.434 mmol) in 1 mL of THF, the reaction mixture was refluxed for 16 h. The reaction mixture was diluted with 5 mL of aqueous NH_4Cl and 5 mL of water and extracted with 40 mL of EtOAc. The organic layer was washed with water and brine and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on silica gel (1:20 ether/hexane) to give 93.8 mg of a mixture of the *Z*- and *E*-esters (*Z*)-**4** and (*E*)-**4**. This mixture was separated by preparative TLC (2 mm) to afford 64.6 mg (37% yield from alcohol **3**) of *Z* olefin (*Z*)-**4** and 27.6 mg (16% yield) of *E* olefin (*E*)-**4** as colorless oils.

(*Z*)-4: R_f 0.41 (1:20 ether/hexane); $[\alpha]^{23}_D$ −29.8° (*c* 1.07, $CHCl_3$); IR (neat) 2956, 1741, 1678 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.03 (s, 6), 0.876 (d, 3, J = 6.2), 0.880 (s, 9), 0.90 (d, 3, J = 6.6), 1.22–1.40 (m, 2), 1.33 (t, 3, J = 7.2), 1.46–1.60 (m, 1), 2.85–2.95 (m, 1), 3.52 (dd, 1, J = 5.9, 9.8), 3.57 (dd, 1, J = 5.5, 9.8), 4.22–4.34 (m, 2), 5.97 (dd, 1, J = 10.5, 33.8); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −5.5, 14.1, 18.2, 21.8, 23.5, 25.77, 25.80, 36.4, 40.1, 61.5, 65.8, 122.7 (d, J = 11), 148.3 (d, J = 255), 160.9 (d, J = 37); MS (FAB) *m*/*z* 333 (MH^+). Anal. Calcd for $C_{17}H_{33}FO_3Si$: C, 61.40; H, 10.00. Found: C, 61.38; H, 9.83.

(*E*)-4: R_f 0.51 (1:20 ether/hexane); IR (neat) 2955, 1732, 1665 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.022 (s, 3), 0.025 (s, 3), 0.871(d, 3, J = 6.5), 0.874 (s, 9), 0.91 (d, 3, J = 6.6), 1.19 (ddd, 1, J = 5.5, 9.0, 13.5), 1.30–1.40 (m, 1), 1.34 (t, 3, J = 7.2), 1.47–1.60 (m, 1), 3.37–3.48 (m, 1), 3.51 (ddd, 1, J = 1.1, 5.6, 9.7), 3.57 (dd, 1, J = 5.0, 9.7), 4.23–4.36 (m, 2), 5.75 (dd, 1, J = 10.9, 22.5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ -5.5, 14.1, 18.2, 22.2, 23.3, 25.7, 25.8, 36.5 (d, J = 5), 40.8, 61.3, 66.1 (d, J = 3), 125.8 (d, J = 17), 147.3 (d, J = 251), 161.0 (d, J = 37); MS (FAB) *m*/*z* 333 (MH^+).

(*Z*)-(4*R*)-4-(((*tert*-Butyldimethylsilyl)oxy)methyl)-2-fluoro-6-methyl-2-heptenamide. A mixture of 32.6 mg (0.0980 mmol) of ester (*Z*)-**4** and 2 mL of 2 N NH_3 in MeOH was stirred for 40 h at room temperature. The reaction mixture was concentrated in vacuo, and the residue was chromatographed on silica gel (1:2 EtOAc/hexane) to give 26.5 mg (89% yield) of the amide as a white solid: R_f 0.49 (1:2 EtOAc/hexane); $[\alpha]^{23}_D$ −27.4° (*c* 0.76, $CHCl_3$); IR (KBr) 3391, 3210, 2956, 1656 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.027 (s, 3), 0.033 (s, 3), 0.87 (d, 3, J = 6.4), 0.88 (s, 9), 0.89 (d, 3, J = 6.6), 1.18 −1.28 (m, 1), 1.30–1.38 (m, 1), 1.46–1.60 (m, 1), 2.80–2.92 (m, 1), 3.50 (dd, 1, J = 6.4, 9.8), 3.56 (dd, 1, J = 5.7, 9.8), 5.58 (br s, 1), 5.96 (dd, 1, J = 10.6, 36.9), 6.10 (br s, 1); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −5.4, 18.2, 21.7, 23.5, 25.7, 25.8, 36.4, 40.1, 65.9, 119.4 (d, J = 12), 151.0 (d, J = 264), 162.1 (d, J = 32); MS (FAB) *m*/*z* 304 (MH^+). Anal. Calcd for $C_{15}H_{30}FNO_2Si$: C, 59.36; H, 9.96; N, 4.62. Found: C, 59.59; H, 9.83; N, 4.59.

***N*-(Benzyloxycarbonyl)-*N*-((*Z*)-(4*R*)-4-(((*tert*-butyldimethylsilyl)oxy)methyl)-2-fluoro-6-methyl-2-heptenyl)amine (5).** To a suspension of 93.4 mg (2.34 mmol) of $LiAlH_4$ (95%) in 5 mL of ether was added a solution of 284 mg (0.934 mmol) of the amide in 3 mL of ether at 0 °C. The reaction mixture was stirred for 1 h at room temperature and refluxed for 20 min, and then 5 mL of water was added at 0 °C to the reaction mixture. After filtration of the mixture through Celite, the solid was washed with ether (3 × 10 mL), and the combined filtrate was washed with brine and dried over $MgSO_4$. Removal of the solvent gave 256 mg of crude amine, which was used in the next step without further purification. To a solution of this amine in 4 mL of THF were added 0.391 mL (2.80 mmol) of triethylamine and 0.266 mL (1.87 mmol) of benzyl chloroformate at 0 °C. The mixture was stirred for 1 h at 0 °C and then poured into 5 mL of 1 N HCl. The mixture was extracted with 30 mL of EtOAc, washed with aqueous $NaHCO_3$, water, and brine, and dried over $MgSO_4$. After removal of the solvent in vacuo, the residue was chromatographed on silica gel (1:100 CH_3CN/CH_2Cl_2) to afford 265 mg (67% from the amide) of the desired carbobenzoxyamine **5** and 12.4 mg (3.3% from the amide) of defluorinated material as colorless oils.

5: R_f 0.58 (1:100 CH_3CN/CH_2Cl_2); $[\alpha]^{23}_D$ −21.1° (*c* 1.02, $CHCl_3$); IR (neat) 3332, 2956, 2857, 1709 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.02 (s, 6), 0.85 (d, 3, J = 6.5), 0.8762 (d, 3, J = 6.1), 0.8763 (s, 9), 1.06–1.16 (m, 1), 1.23 −1.32 (m, 1), 1.45–1.58 (m, 1), 2.67–2.78 (m, 1), 3.45 (d, 2, J = 5.9), 3.89 (dd, 2, J = 5.9, 14.9), 4.59 (dd, 1, J = 10.2, 37.2), 4.92 (br s, 1), 5.12 (s, 2), 7.30–7.40 (m, 5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −5.4, 18.3, 21.8, 23.6, 25.6, 25.9, 35.4, 40.7, 41.9 (d, J = 33), 66.4, 67.0, 109.9 (d, J = 14), 128.1, 128.2, 128.5, 136.4, 155.7 (d, J = 254), 156.0; MS (FAB) *m*/*z* 424 (MH^+). Anal. Calcd for $C_{23}H_{38}FNO_3Si$: C, 65.21; H, 9.04; N, 3.31. Found: C, 64.86; H, 8.90; N, 3.25.

Desfluoro-**5**: R_f 0.53 (1:100 CH_3CN/CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.02 (s, 6), 0.83 (d, 3, J = 6.5), 0.85–0.90 (m, 12), 1.06–1.16 (m, 1), 1.18–1.28 (m, 1), 1.46–1.58 (m, 1), 2.18 −2.29 (m, 1), 3.44 (d, 2, J = 6.2), 3.72–3.82 (m, 2), 4.66–4.74 (m, 1), 5.11 (s, 2), 5.39 (dd, 1, J = 8.4, 15.5), 5.49 (dt, 1, J = 5.8, 15.5), 7.28–7.38 (m, 5).

***N*-(Benzyloxycarbonyl)-*N*-((*Z*)-(4*R*)-2-fluoro-4-(hydroxymethyl)-6-methyl-2-heptenyl)amine.** To a solution of 2.01 g (4.74 mmol) of silyl ether **5** in 25 mL of THF was added 2.48 g (9.48 mmol) of nBu_4NF at 0 °C. After being stirred for 2 h at room temperature, the reaction mixture was poured into 30 mL of water and extracted with 120 mL of EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. After removal of the solvent in vacuo, the residue was purified by chromatography on silica gel (1:2–2:3 EtOAc/hexane) to give 1.45 g (99%) of the deprotected alcohol as a colorless oil: R_f 0.38 (1:1 EtOAc/hexane); $[\alpha]^{23}_D$ −14.5° (*c* 1.08, $CHCl_3$); IR (neat) 3327, 2954, 1698 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.86 (d, 3, J = 6.6), 0.89 (d, 3, J = 6.6), 1.10–1.24 (m, 2), 1.47–1.59 (m, 1), 2.75–2.87 (m, 1), 3.38 (dd, 1, J = 7.8, 10.5), 3.55 (dd, 1, J = 5.1, 10.5), 3.89 (dd, 2, J = 5.2, 14.2), 4.57 (dd, 1, J = 10.1, 36.9), 5.02–5.16 (m, 3), 7.30–7.40 (m, 5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.7, 23.4, 25.6, 35.6, 40.4, 41.9 (d, J = 33), 66.3, 67.0, 109.3 (d, J = 13), 128.1, 128.2, 128.5, 136.3, 156.2, 156.7 (d, J = 255); MS (FAB) *m*/*z* 310 (MH^+). Anal. Calcd for $C_{17}H_{24}FNO_3$: C, 66.00; H, 7.82; N, 4.53. Found: C, 65.63; H, 7.60; N, 4.60.

(*Z*)-(2*R*)-5-(*N*-(Benzyloxycarbonyl)amino)-4-fluoro-2-(2-methylpropyl)-3-pentenoic Acid (6). To a solution of 224 mg (0.724 mmol) of the alcohol in 9 mL of acetone was added 0.9 mL of Jones reagent at 0 °C, and the reaction

mixture was stirred for 1 h at room temperature. Isopropyl alcohol (0.4 mL) and 10 mL of water were added, and the mixture was extracted with 40 mL of EtOAc; the organic layer was washed with water and brine and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on silica gel (1:2 EtOAc/hexane–water saturated EtOAc) to give 214 mg (91% yield) of carboxylic acid **6** as a colorless oil: $[\alpha]^{23}_D$ −39.8° (*c* 1.02, $CHCl_3$); IR (neat) 3328, 2958, 1713 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.90 (d, 3, *J* = 6.3), 0.93 (d, 3, *J* = 6.3), 1.34–1.46 (m, 1), 1.50–1.68 (m, 2), 3.57 (dt, 1, *J* = 6.7, 9.5), 3.93 (dd, 2, *J* = 6.1, 13.4), 4.88 (dd, 1, *J* = 9.5, 35.2), 4.96–5.05 (m, 1), 5.13 (s, 2), 7.32–7.42 (m, 5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.8, 22.7, 25.7, 38.8 (d, *J* = 3), 41.47, 41.51 (d, *J* = 32), 67.1, 105.4, (d, *J* = 12), 128.1, 128.2, 128.5, 136.2, 156.1, 156.6 (d, *J* = 259), 179.3; MS (FAB) *m/z* 324 (MH^+). Anal. Calcd for $C_{17}H_{22}FNO_4$: C, 63.14; H, 6.86; N, 4.33. Found: C, 63.53; H, 6.49; N, 4.42.

***N*-((*Z*)-(2*R*)-5-(*N*-(Benzyloxycarbonyl)amino)-4-fluoro-2-(2-methylpropyl)-3-pentenoyl)-L-leucine Methyl Ester (7c).** To a solution of 197 mg (0.609 mmol) of carboxylic acid **6** in 8 mL of CH_2Cl_2 were added 98.8 mg (0.731 mmol) of 1-hydroxybenzotriazole (HOBT) and 133 mg (0.731 mmol) of L-leucine methyl ester hydrochloride at 0 °C, followed by 127 μL (0.731 mmol) of diisopropylethylamine and 140 mg (0.731 mmol) of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC). The reaction mixture was stirred for 1 h at 0 °C and for an additional 1 h at room temperature and then poured into 5 mL of 1 N HCl. The resulting mixture was extracted with 30 mL of EtOAc, and the organic layer was washed with aqueous $NaHCO_3$, water, and brine and dried over $MgSO_4$. After removal of the solvent in vacuo, the residue was chromatographed on silica gel (1:2 EtOAc/hexane) to afford 247 mg (90%) of the desired amide **7c** as a white solid and 5.0 mg (1.8%) of the D,L-diastereomer.

7c: R_f 0.46 (2:3 EtOAc/hexane); $[\alpha]^{23}_D$ −43.2° (*c* 0.67, $CHCl_3$); mp 70–71 °C (recrystallized from ether/hexane); IR (KBr) 3329, 2957, 1751, 1734, 1695, 1648 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.87 (d, 3, *J* = 6.5), 0.88–0.95 (m, 9), 1.33–1.43 (m, 1), 1.48–1.77 (m, 5), 3.30–3.40 (m, 1), 3.72 (s, 3), 3.90 (dd, *J* = 5.9, 13.6), 4.59 (dt, *J* = 5.1, 8.5), 4.89 (dd, *J* = 9.8, 35.8), 4.96–5.04 (m, 1), 5.10 (d, 1, *J* = 12.2), 5.13 (d, 1, *J* = 12.2), 5.95 −6.07 (m, 1), 7.28–7.42 (m, 5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.8, 21.9, 22.7, 22.8, 24.8, 25.6, 40.2, 41.0, 41.3, 41.6 (d, *J* = 33), 50.7, 52.1, 67.0, 107.0 (d, *J* = 12), 128.0, 128.2, 128.5, 136.2, 156.1, 156.6 (d, *J* = 258), 172.8, 173.4; MS (FAB) *m/z* 451 (MH^+). Anal. Calcd for $C_{24}H_{35}FN_2O_5$: C, 63.98; H, 7.83; N, 6.22. Found: C, 63.73; H, 7.77; N, 6.09.

D,L-**7c**: R_f 0.38 (2:3 EtOAc/hexane); $[\alpha]^{23}_D$ +44.8° (*c* 0.250, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.87 (d, 3, *J* = 6.5), 0.91 (d, 3, *J* = 6.5), 0.94 (d, 6, *J* = 5.9), 1.35–1.71 (m, 6), 3.32–3.40 (m, 1), 3.71 (s, 3), 3.86–3.95 (m, 2), 4.59 (dt, 1, *J* = 4.9, 8.5), 4.87 (dd, 1, *J* = 9.9, 35.7), 5.04–5.15 (m, 3), 5.98–6.05 (m, 1), 7.28–7.38 (m, 5).

The following compounds were prepared in a similar manner.

***N*-((*Z*)-(2*R*)-5-(*N*-(Benzyloxycarbonyl)amino)-4-fluoro-2-(2-methylpropyl)-3-pentenoyl)glycine methyl ester (7a)**: yield 87%; R_f 0.30 (1:1 EtOAc/hexane); $[\alpha]^{23}_D$ −52.2° (*c* 0.64, $CHCl_3$); mp 97–98 °C (recrystallized from EtOAc/hexane); IR (KBr) 3317, 2955, 1757, 1695, 1647 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.87 (d, 3, *J* = 6.5), 0.91 (d, 3, *J* = 6.6), 1.36–1.45 (m, 1), 1.47–1.59 (m, 1), 1.63–1.73 (m, 1), 3.34–3.43 (m, 1), 3.74 (s, 3), 3.91 (dd, 2, *J* = 6.0, 13.6), 4.00 (d, 2, *J* = 5.3), 4.89 (dd, 1, *J* = 9.9, 35.7), 5.02–5.12 (m, 1), 5.12 (s, 2), 6.16–6.24 (m, 1), 7.27–7.40 (m, 5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.8, 22.8, 25.6, 40.0, 41.0, 41.1, 41.6 (d, *J* = 33), 52.2, 67.0, 106.7 (d, *J* = 12), 128.0, 128.1, 128.5, 136.2, 156.1, 156.7 (d, *J* = 258), 170.3, 173.3; MS (FAB) *m/z* 395 (M^+). Anal. Calcd for $C_{20}H_{27}FN_2O_5$: C, 60.90; H, 6.90; N, 7.10. Found: C, 61.01; H, 6.88; N, 7.21.

***N*-((*Z*)-(2*R*)-5-(*N*-(Benzyloxycarbonyl)amino)-4-fluoro-2-(2-methylpropyl)-3-pentenoyl)-L-alanine methyl ester (7b)**: yield 89%; R_f 0.41(1:1 EtOAc/hexane); $[\alpha]^{23}_D$ −40.6° (*c* 0.63, $CHCl_3$); mp 114–115 °C (recrystallized from EtOAc/hexane); IR (KBr) 3318, 2954, 1744, 1693, 1642 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.87 (d, 3, *J* = 6.5), 0.91 (d, 3, *J* = 6.5), 1.34–1.43 (m, 1), 1.39 (d, 3, *J* = 7.2), 1.47–1.58 (m, 1), 1.60–1.70 (m, 1), 3.30–3.40 (m, 1), 3.74 (s, 3), 3.91 (dd, 2, *J* = 5.6, 13.8), 4.55 (quintuplet, 1, *J* = 7.2), 4.90 (dd, 1, *J* = 9.8, 35.8), 4.98–5.06 (m, 1), 5.10 (d, 1, *J* = 12.3), 5.12 (d, 1, *J* = 12.3), 6.14–6.24 (m, 1), 7.27–7.40 (m, 5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.1, 21.8, 22.8, 25.6, 40.1, 41.1, 41.6 (d, *J* = 33), 47.9, 52.3, 67.0, 106.8 (d, *J* = 12), 128.0, 128.1, 128.5, 136.2, 156.1, 156.6 (d, *J* = 258), 172.6, 173.4; MS (FAB) *m/z* 409 (MH^+). Anal. Calcd for $C_{21}H_{29}FN_2O_5$: C, 61.75; H, 7.16; N, 6.86. Found: C, 61.86; H, 7.13; N, 6.94.

***N*-((*Z*)-(2*R*)-5-(*N*-(Benzyloxycarbonyl)amino)-4-fluoro-2-(2-methylpropyl)-3-pentenoyl)-L-phenylalanine methyl ester (7d)** (87% yield): R_f 0.40 (2:3 EtOAc/hexane); $[\alpha]^{23}_D$ +6.5° (*c* 0.68, $CHCl_3$); mp 72–74 °C (recrystallized from ether/hexane); IR (KBr) 3337, 2954, 1740, 1695, 1653 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.83 (d, 3, *J* = 6.5), 0.87 (d, 3, *J* = 6.5), 1.28–1.37 (m, 1), 1.43–1.66 (m, 2), 3.05 (dd, 1, *J* = 6.3, 13.9), 3.15 (dd, 1, *J* = 5.7, 13.9), 3.24–3.33 (m, 1), 3.72 (s, 3), 3.88 (dd, 2, *J* = 5.9, 13.6), 4.70–4.88 (m, 2), 4.91–5.00 (m, 1), 5.11 (d, 1, *J* = 12.1), 5.13 (d, 1, *J* = 12.1), 6.02–6.09 (m, 1), 7.07 (d, 2, *J* = 7.1), 7.18–7.38 (m, 8); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.8, 22.8, 25.5, 37.6, 40.1, 40.7, 41.5 (d, *J* = 33), 52.2, 52.8, 67.0, 106.8 (d, *J* = 12), 127.0, 128.05, 128.14, 128.4, 128.5, 129.2, 135.8, 136.1, 156.0, 156.6 (d, *J* = 258), 171.9, 172.5; MS (FAB) *m/z* 485 (MH^+). Anal. Calcd for $C_{27}H_{33}FN_2O_5$: C, 66.93; H, 6.86; N, 5.78. Found: C, 66.68; H, 6.77; N, 5.73.

D,L-**7d** (1.6% yield): R_f 0.33 (2:3 EtOAc/hexane); $[\alpha]^{23}_D$ +63.1° (*c* 0.26, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.83 (d, 3, *J* = 6.5), 0.86 (d, 3, *J* = 6.5), 1.25–1.48 (m, 2), 1.51–1.65 (m, 1), 3.06 (dd, 1, *J* = 6.3, 13.9), 3.15 (dd, 1, *J* = 5.7, 13.9), 3.23–3.33 (m, 1), 3.72 (s, 3), 3.88 (dd, 2, *J* = 5.8, 13.7), 4.74–4.88 (m, 2), 4.92–5.02 (m, 1), 5.11 (s, 2), 6.00–6.06 (m, 1), 7.09 (d, 2, *J* = 6.7), 7.18–7.40 (m, 8); MS (FAB) *m/z* 485 (MH^+).

Enzymology.[14] The standard buffer for all assays was 0.1 M 3-(*N*-morpholino)propanesulfonic acid (MOPS), 2.5 M NaBr, 10 mM $CaCl_2$, and 2.5% (v/v) DMF at pH 7.0. Assays were performed at 25 °C and monitored by absorbance change at 345 nm. Velocities were determined for ≤10% reaction and were reproducible within ±8%. Kinetic parameters for thermolysin hydrolysis of faGLa were determined from the assay carried out with 8.5 nM thermolysin and 0.10–9.0 mM faGLa. Assays for determination of K_i values were carried out with 8.5 nM thermolysin and initiated by the addition of faGLa (2mM). The K_i values for **1a-d** were determined from v_0/v_i versus [I] plots ($v_0/v_i = [I]/K_i\ (1 + [S]/K_m) + 1$) at six or more different concentrations of I. The kinetic plots are reproduced in the supplementary material.

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Supplementary Material Available: Kinetic plots for determination of inhibition constants (1 page). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(14) General. All stock solutions were prepared with doubly distilled water and filtered through Millipore filters (0.45 μm pore size). Buffers were prepared and adjusted to pH at room temperature. Assays were performed on a Uvikon 860 spectrophotometer. A Lauda Model RM 20 circulating constant-temperature bath connected to a water-jacketed sample holder was used for temperature regulation (25 ± 0.2 °C). Inhibitor concentrations were determined by careful dilution of a precisely weighed sample of the inhibitor. Thermolysin was obtained from Calbiochem (3× recrystallized) and used without further purification; enzyme concentration was determined by UV absorbance. The thermolysin substrate furanacryloyl-glycyl-leucinamide (faGLa) was obtained from Sigma and used without further purification; stock substrate concentration was determined by UV absorbance (ϵ_{345} = 766).