Conformationally Defined Analogs of Prolylamides. *trans*-Prolyl Peptidomimetics

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The *cis* and *trans* conformations of prolylamides are both energetically accessible, in contrast to the peptide bonds of the remaining mammalian amino acids. The synthesis of a rigid, conformationally defined peptidomimetic of the *trans*-prolylamide bond has been developed in this study, and illustrative leucinylproline derivatives (**4**, **10a**, **10b**, and **11**) were assayed for their abilities to inhibit the peptidyl prolyl isomerase activity of recombinant human FK-binding protein 12 (FKBP 12). These *trans*-prolyl peptidomimetics possess a *trans*-substituted alkene in place of the proline peptide bond and were synthesized *via* a six step sequence culminating in the selective addition of isobutylmagnesium bromide to methyl 2(*E*)-(2-oxoethylidene)-1-methylcyclopentanecarboxylate (**9**). Synthesis of the dipeptide analogs was accomplished in six steps with a 20% overall yield. Elaboration of the dipeptide analog gave the Leu-Pro-tyrosyl tripeptide analog in three additional steps. The tripeptide mimic **4** proved to be a potent inhibitor of the prolyl isomerase activity of recombinant hFKBP 12, exhibiting an inhibition constant (K_i) of 8.6 μM; the dipeptidomimetics possessed a modest capacity for isomerase inhibition with inhibition constants ranging from 127 μM for the α-enone analog **11** to 730 and 1390 μM for the allylic alcohol mimetics **10a** and **10b**, respectively.

Proline is the sole imino acid in mammalian proteins. A singular consequence of the secondary amides formed in mammalian peptides by proline is that the energies of the *cis* and *trans* peptidyl conformations (Figure 1) are generally similar ($G_o < \approx 2$ kcal/mol).[1] In contrast, the amide bonds linking amino acids in peptides and proteins are exclusively *trans* unless unusual energetic constraints promote the *cis* conformation, which is ≈5 kcal/mol less stable than the *trans* conformation.[1] The *cis/trans* peptidyl conformations of prolylamides induce strong preferences for certain secondary structural motifs, such as reverse turns and helix-breaking or "kink" behavior in α-helices.[2] Thus, prolylamides play fundamental roles in the structure and function of peptides and proteins, and the structural features unique to prolylamides are often critical in the recognition of protein substrates for their target enzymes and peptide hormones for their receptors.

Although the thermodynamic stabilities of *cis*- and *trans*-prolylamide conformations are generally similar, the rates of *cis-trans*-prolylamide isomerization are typically slow on a "biological" time scale [$E_{act} \approx 17$–21 kcal/mol, however, prolyl amides can be lower].[1,3] Several studies suggest that *cis-trans*-prolyl isomerization may be important contributors to, if not the rate determining steps in, the kinetics of protein folding *in vitro*.[1,3] Nature has addressed this issue through the development of a family of peptidyl prolyl isomerases (PPIases), which catalyze the *cis/trans* isomerization of peptide bonds involving proline (X–Pro bonds). Recently, two members of the PPIase family, which have been collectively termed immunophilins,[4–6] have been shown to be the targets for the potent immunosuppressive agents cyclosporine A,[7] which binds to cyclophilin, and FK506[8] and rapamycin,[9] which both bind to an unrelated immunophilin, FK binding protein 12 (FKBP12). However, immunosuppressive activity is not thought to result from inhibition of PPIase activity ("rotamase model"),[10] but rather results from the drug–immunophilin complex interacting with a third target molecule ("active complex model").[11] The

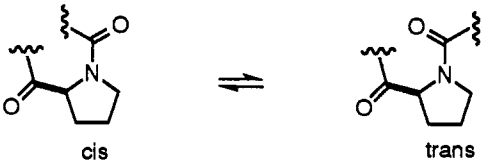

Figure 1. Cis and *trans* conformations of prolylamides.

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role(s) of unliganded PPIases remains an area of intense investigation. In addition to assisting in the folding of proteins, members of the PPIase family are thought to (1) be important in regulating calcium release receptors;[12] (2) play key roles in protein trafficking between organelles;[13] and (3) aid in protein degradation through catalysis of protein unfolding and prevention of unfolded protein precipitation (associated with heat shock).[14] PPIases may also regulate enzyme activity through isomerization of X–Pro bonds.[15] In efforts directed at probing the structure–function relationships of these enzymes, we sought analogs of proline with conformationally defined character.

Numerous analogs of proline have been prepared by synthetic chemists to probe various mechanistic or structural questions in peptides or to advance the development of peptide-containing drugs.[16b-i] Additionally, small molecule FKBP inhibitors have been synthesized and tested for biological activity.[16a] However, all of these molecules possess prolylamide bonds (or amide bond equivalents) with varying degrees of σ bond character. Although steric constraints can be imposed to favor a single conformational state, these analogs must be considered conformationally "restricted", not "locked". Because of the potent isomerase activities of the PPIases (for example, overcoming $> \approx 4$ kcal/mol between the CsA ground state and the specific *trans*-9,10 conformation in the CP-bound state),[17] we could not be assured that these existing analogs would rigorously remain a single peptidyl conformer upon immunophilin complexation; such conformational ambiguity would obscure our biochemical studies. It was our goal to develop conformationally defined analogs of both the *trans*- and *cis*-prolylamide, which removed the amide bond and therefore the possibility of peptidyl isomerization. This class of prolylamide analogs is depicted in Figure 2. These dipeptidomimetics employ a carbon–carbon double bond to mimic and restrain the prolylamide bond to a single *trans* or *cis* conformation and possess considerable potential for structural diversity. We report here the synthesis of a *trans*-prolylamide peptidomimetic and the biochemical evaluation of representatives incorporating this mimetic substructure in an assay for human FKBP 12 PPIase inhibition.

Figure 2. Prototype structures for the *trans*-prolyl (1) and *cis*-prolyl (2) peptidomimetics.

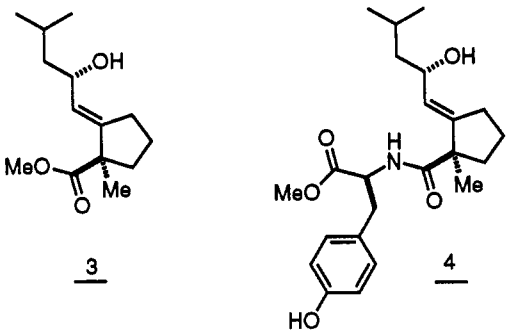

Figure 3. Structures of leucinylproline dipeptidomimetic 3 and Leu-Pro-Tyr tripeptidomimetic 4.

Results and Discussion

Our initial synthetic target was reduced to the leucinylproline peptidomimetic 3 [which corresponds to 1 (A = H; B = isobutyl; R = methyl; X = H; Y = CH(OH)–] (Figure 3). This dipeptidyl residue was incorporated into the Leu-Pro-Tyr tripeptidomimetic 4 (Figure 3) for assay in the inhibition of hFKBP 12 PPIase activity. The tripeptide analog was designed according to the principles outlined by the Schreiber,[6,11,13,14,18] Merck Sharp and Dohme,[8,19] and Wyeth-Ayerst[20,21] groups to rationalize the extremely tight binding of FK506 and rapamycin to FKBP 12 (K_D = 0.4 and 0.2 nM, respectively). Studies by these researchers indicate that the most effective tetrapeptide substrate for the FKBP/PPIase contains the residues X-Leu-Pro-Phe/Tyr,[13,18] although the K_M^{-1} values for PPIase activity are 10^3–10^4 times higher than the K_D values for the immunosuppressive agents.

The superposition of Leu-Pro analog 3 with the analogous dipeptide exhibits strong structural overlap. However, it was critical as we undertook our initial investigations that the prototype structure possess considerable potential variation. An important consideration was the nature of the "R" group in 1, which was important to prolyl structural analogy, but when R = H, it was sensitive to alkene isomerization. Thus, our initial synthetic studies were directed toward prototype structures with R = CH_3, due to their synthetic accessibility. Obviously, the size of the R substituent may be an important feature in

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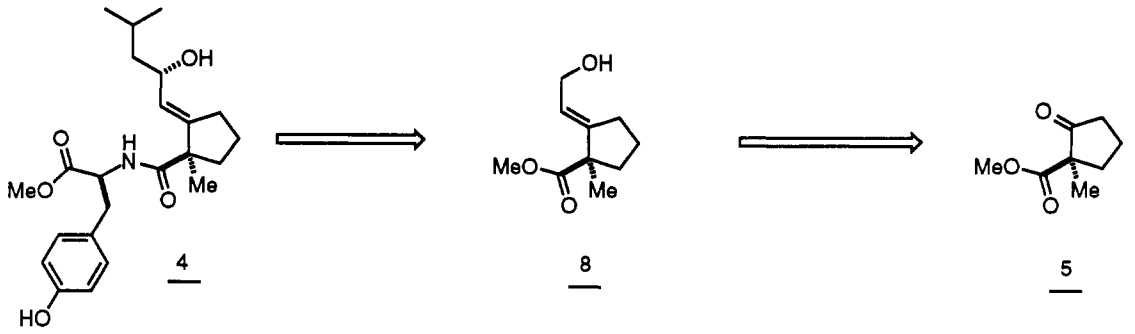

Figure 4. Retrosynthetic analysis of the Leu-Pro-Tyr analog **4**.

recognition of the prolyl peptidomimetic by receptors and enzymes, and ultimately the synthesis may be necessary of compounds with less steric "bulk", such as analogs with R = —C≡CH, —F, and (possibly) H. Our initial studies also employed the five-membered carbocyclic ring (as illustrated in **1**), although the most efficacious ring size for a particular target may well be an alternative size. For example, PPIase binding studies have suggested that the picolinyl ring, relative to the prolyl ring, may promote acquisition of the requisite α-keto amide bound conformation as a consequence of the well defined steric constraints imposed by the six-membered ring.[6,14,21] The nature of the substituent designated as "Y" could be crucial for peptidomimetic recognition. Although as a generic and synthetically facile peptidomimetic structure we selected the hydroxy functionality (Y = —CH(OH)–), selected enzymes or receptors may prefer alternative recognition elements. For example, the α-ketoamide carbonyl of FK506 and rapamycin is proposed to bind as a transition state analog of the "twisted" prolylamide species; such a proposal would suggest that the peptidomimetic **1** with Y = –COCH(OH)– could be a more potent inhibitor. This possibility is discussed below. Finally, the substituent X, although ultimately intended to provide a potential H-bonding acceptor site analogous to the amide carbonyl oxygen (e.g., X = F, OMe), was relegated to H, due to its synthetic accessibility. Retrosynthetic analysis of the peptidomimetic **4** suggested intermediate **8** (Figure 4), whose synthesis from keto ester **5** had been previously reported by Dauben.[22]

The synthesis of **4** is depicted in Figure 5. Typical percent yields for each step are shown in parentheses. Keto ester **5** was methylated (95%)[23] and condensed with the anion of *tert*-butyl diethylphosphonoacetate to yield diester (*E*)-**6** (80%).[24] Acidic deprotection of the *tert*-butyl ester gave acid **7** (100%), which was selectively reduced to alcohol **8** (47%) with diborane. Intermediate **8** was oxidized to aldehyde **9** (90%) with the Dess–Martin reagent.[25] Selective addition of isobutylmagnesium bromide to aldehyde **9** gave the pair of diastereomers **10a** and **10b** (60% combined yield), which were enantiomeric at the hydroxyl-bearing carbon. The stereochemistry assigned to **10a** and **10b** is relative and based upon NMR data and molecular modeling, which predicts the more active compound of the pair to have stereochemistry equivalent to that shown in **10a**. To confirm the diastereomeric relationship between **10a** and **10b**, these compounds were separately oxidized with the Dess–Martin reagent to yield enone **11** (88%). Saponification of ester **10a** with lithium hydroxide produced the carboxylic acid **12** (62%).[26] Carboxylic acid **12** was transformed into the corresponding acid fluoride **13** (70%) with cyanuric fluoride.[27] Acid fluoride **13** was reacted with L-tyrosine methyl ester[28] to yield desired tripeptide analog **4** and a diastereomer, which proved inseparable by conventional chromatography (67% combined yield). Attempts to convert compound **10b** into the acyl fluoride derivative were unsuccessful, possibly due to isomerization.

Table I. Inhibition of FKBP Peptidyl Prolyl Isomerase by Prolyl Peptidomimetics

compd	K_i (μM)	compd	K_i (μM)
10a	730	**11**	127
10b	1390	**4**	8.6

Tripeptide analog **4** and several intermediates in its synthesis (**10a**, **10b**, **11**) were assessed for their abilities to inhibit FKBP PPIase activity (Table I).[29,30] This assay has an exceptional range (K_i values from $<\approx 0.1$ to $>\approx 10$ nM), and a competitive mode of inhibition in this assay can be tentatively ascribed to active site binding of the inhibitor.[17,18] A considerable structural diversity in peptide substrates has been shown to competitively inhibit FKBP PPIase activity.[18] However, tripeptidomimetic **4** (K_i = 8.60 μM) is among the most potent tripeptide-like inhibitors yet reported. Even the dipeptide-like precursors, **10a** (K_i = 730 μM), **10b** (K_i = 1.39 mM), and **11** (K_i = 127 μM) exhibit significant levels of inhibition. Interestingly, the α-enone dipeptide analog **11** demonstrated the highest level of inhibition for the three dipeptidomimetics. This result could be a consequence of the ability of FKBP to tightly bind the α-keto amide functionality of FK506 and rapamycin and the structural similarity of **11** with this moiety. The ability of **4** and its precursors to potently inhibit FKBP PPIase activity demonstrates that the simple carbocylic systems, illustrated by **1** and **2**, possess considerable potential in mimicking the prolylamide substructure of polypeptide species. Our studies to develop approaches to enantiomerically enriched dipeptidomimetics and to further define the limitations and expand the potential mechanistic and pharmacologic utility of these agents are currently ongoing.

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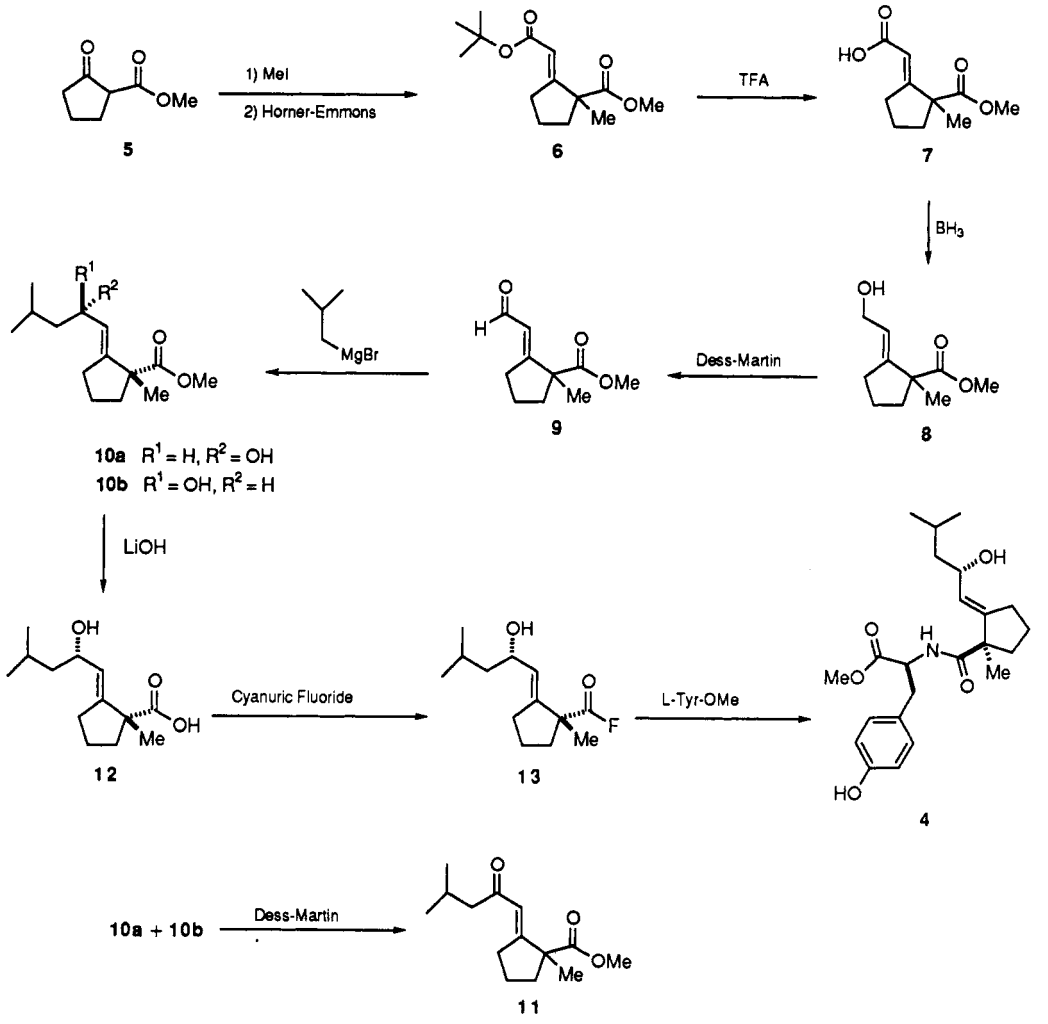

Figure 5.

Experimental Section

General Experimental Section. Solvents were purified prior to use when deemed necessary: tetrahydrofuran (THF) was distilled from sodium metal; acetone was distilled from potassium carbonate; methylene chloride and pyridine were distilled from calcium hydride. All reactions were run in flame dried flasks under an atmosphere of argon, except those reactions where water was present. Analytical thin-layer chromatography was performed utilizing Baker hard-surfaced glass plates of 0.25-mm thickness with a 254-nm fluorescent indicator. Column chromatography was carried out on silica gel (Fisher 230–425 mesh). Chemicals and reagents were procured from commercial sources (Aldrich Chemical Co., Inc., or Lancaster Chemical Co.) and used as received unless otherwise indicated. For the biochemical assay of peptidyl prolyl isomerase (PPIase) inhibition, chymotrypsin was purchased from Sigma Chemical Co. and *N*-succinyl-Ala-Leu-Pro-Phe-*p*-nitroanilide was obtained from Bachem Bioscience. Trifluoroethanol (TFE) was dried over calcium sulfate and vacuum filtered through sodium bicarbonate. Lithium chloride was dried overnight in a vacuum oven at 40°C.

Synthetic Methods and Procedures. Methyl (*E*)-2-(2-Oxoethylidene)-1-methylcyclopentanecarboxylate (9). Methyl 2-(hydroxyethylidene)-1-methylcyclopentanecarboxylate **8** (1.00 g; 5.43 mmol), prepared by the method of Daubin,[22] was dissolved in freshly distilled CH_2Cl_2 (43 mL) and treated with the Dess–Martin reagent (2.55 g; 6.00 mmol) at rt. The reaction was stirred for 20 min and then added to a solution of $Na_2S_2O_3$ (6.4 g; 41.0 mmol) dissolved in 250 mL of saturated $NaHCO_3$. Ether (250 mL) was introduced to this mixture, and the layers were stirred until the aqueous and organic layers were clear (approximately 0.5 h). The layers were separated, and the ether layer was further extracted with a saturated $NaHCO_3$ solution (200 mL), followed by a saturated NaCl solution (brine). The ether layer was dried with $MgSO_4$, filtered, and stripped of solvent. The crude oil was purified by flash chromatography (10:1 CH_2Cl_2/ether) to yield **9** as an oil (90%, 0.890 g). IR (neat) 2955, 2753, 2356, 1730, 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.88 (d, 1 H), 6.01 (dt, 1 H), 3.68 (s, 3 H), 2.95 (dm, 2 H), 2.37 (m, 1 H), 1.90 (dm, 2 H), 1.65 (m, 1 H), 1.38 (s, 3 H); MS *m/e* (rel intensity) 182 (M^+, 2), 150 (80), 123 (100), 107 (20), 93 (70), 81 (80).

Methyl (*E*)-2-(2-Hydroxy-2-isobutylethylidene)-1-methylcyclopentanecarboxylate (10a, 10b). Aldehyde **9** (0.830 g; 4.60 mmol) was dissolved in THF (64 mL) at rt and isobutylmagnesium bromide (2.3 mL of a 2.0 M solution; 4.60 mmol) was added dropwise over 10 min. After addition of the Grignard reagent was complete, the reaction was allowed to stir for an additional 30 min at 0 °C. The reaction was quenched with 50 mL of an aqueous solution containing acetic acid (2 mL). The organic and aqueous layers were separated, and the aqueous layer was further extracted with ether (50 mL). The organic layers were combined, extracted with brine, dried with $MgSO_4$, filtered, and stripped of solvent. The resulting oil was purified by flash chromatography (15:1 CH_2Cl_2/ether) to yield 0.662 g (2.76 mmol) of **10a** and **10b** (60% combined), as an approximate 1 (**10a**) to 2 (**10b**) ratio. **10a:** IR (neat) 3487, 2954, 2341, 1718 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.30 (dt, 1 H), 4.30 (dd, 1 H), 3.62 (s, 3 H), 2.46 (m, 1H), 2.32 (m, 4H), 1.32 (s, 3 H), 1.26 (m, 1 H), 0.88 (d, 6 H); MS *m/e* (rel intensity) 240 (M^+, 1), 223 (35), 180 (20), 163 (35) 151 (30), 123 (100), 95 (50), 85 (75); high-resolution mass spectrum requires 240.3418, measured 240.3422. **10b:** IR (neat) 3396, 2954, 2347, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.30 (dt, 1 H), 4.30 (dd, 1 H), 3.64 (s, 3 H), 2.46 (m, 1 H), 2.36 (dt, 1 H), 2.28 (m, 1 H), 1.80 (m, 1 H), 1.68 (m, 2 H), 1.50 (m, 2 H), 1.27 (s, 3 H), 1.22 (m, 1 H), 0.88 (dd, 6 H); MS *m/e* (rel intensity) 240 (M^+,

0.5), 223 (80), 163 (30), 151 (25), 123 (30), 107 (20), 95 (70), 85 (100), 79 (45); high-resolution mass spectrum requires 240.3418, measured 240.3420.

Methyl (*E*)-2-(2-Oxo-2-isobutylethylidene)-1-methylcyclopentanecarboxylate (11). Alcohol **10a** (0.053 g; 0.220 mmol) was dissolved in anhydrous CH_2Cl_2 (1.8 mL) and treated with the Dess–Martin reagent (0.104 g; 0.245 mmol) at rt. After 20 min, the reaction was poured into a saturated $NaHCO_3$ solution (20 mL) containing $Na_2S_2O_3$ (0.316 g; 2.00 mmol). The reaction was diluted with ether (20 mL), and the layers were stirred until they became clear. The layers were separated, and the organic layer was extracted with saturated $NaHCO_3$ solution (15 mL), followed by saturated NaCl solution. The combined organic layers were dried with $MgSO_4$, filtered, and stripped of solvent. The crude oil was purified by flash chromatography (15:1 CH_2Cl_2/ether) to give pure **11** in 88% yield (0.046 g, 0.193 mmol). IR ($CHCl_3$) 2956, 2402, 1731, 1689, 1618 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.19 (t, 1 H), 3.68 (s, 3 H), 2.92 (m, 2 H), 2.32 (m, 3 H), 2.12 (m, 1 H), 1.86 (m, 1 H), 1.78 (m, 1 H), 1.58 (m, 1 H), 1.35 (s, 3 H), 0.92 (d, 6 H); MS *m/e* (rel intensity) 238 (M^+, 2), 206 (25), 178 (70), 163 (17), 153 (50), 136 (13), 121 (82), 93 (100), 85 (65), 77 (52); high-resolution mass spectrum requires 238.3260, measured 238.3263.

(*E*)-2-(2-Hydroxy-2-isobutylethylidene)-1-methylcyclopentanecarboxylic Acid (12). Ester **10a** (0.404 g; 1.68 mmol) was dissolved in a solution of THF (4.0 mL), water (4.0 mL), and methanol (2.0 mL) at rt. LiOH·H_2O (0.707 g; 16.9 mmol) was added and the reaction stirred for 10 h. The reaction was then treated with a saturated aqueous solution of citric acid (10 mL) and extracted with ethyl acetate (20 mL). The organic layer was separated, dried with $MgSO_4$, filtered, and stripped of solvent. Flash chromatography of the resulting oil (1:1 $CHCl_3$/methanol) gave the pure acid **12** as an colorless oil in 62% yield (0.240 g, 0.106 mmol): IR ($CHCl_3$) 3369, 2957, 2361, 1701 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.4 (dt, 1 H), 4.35 (q, 1 H), 2.51 (m, 1 H), 2.35 (m, 2 H), 1.85 (m, 1 H), 1.62 (m, 4 H), 1.35 (s, 3 H), 1.28 (m, 1 H), 0.91 (d, 6 H); MS *m/e* (rel intensity) 226 (M^+, 20), 208 (100), 179 (15), 163 (55), 123 (40), 83 (45).

(*E*)-2-(2-Hydroxy-2-isobutylethylidene)-1-methylcyclopentanecarboxylic Acid Fluoride (13). Carboxylic acid **12** (51.2 mg; 0.226 mmol) was dissolved in dry acetonitrile (0.5 mL) at 0 °C, and then anhydrous pyridine (20 μL) and cyanuric fluoride (12.0 mg; 0.089 mmol) were sequentially added. The reaction was stirred for 70 min, and then poured into ice–water (10 mL) and extracted with ether (20 mL). The ether layer was dried with anhydrous Na_2SO_4 and stripped of solvent, and the crude product was subjected to flash chromatography (1:1 ethyl acetate/hexanes) to give pure **13** as a waxy solid oil in 70% yield (36.0 mg, 0.158 mmol): IR ($CHCl_3$) 3383, 2958, 2362, 1627 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.43 (d, 1 H), 4.34 (q, 1 H), 2.54, (m, 1 H), 2.41 (m, 1 H), 2.31 (m, 1 H), 1.88 (m, 1 H), 1.78 (m, 1 H), 1.68 (m, 2 H), 1.50 (m, 1 H), 1.14 (s, 3 H), 1.30 (m, 1 H), 0.91 (dd, 6 H); MS *m/e* (rel intensity) 228 (M^+, 0.1), 211 (10), 183 (35), 163 (30), 151 (20), 143 (10), 123 (100), 105 (20), 95 (30).

(*E*)-2-(2-Hydroxy-2-isobutylethylidene)-1-methylcyclopentane-(L)-tyrosylcarboxamide (4). Acyl fluoride **13** (36.0 mg, 0.160 mmol) was dissolved in THF (4 mL) at rt and L-tyrosine methyl ester (70 mg, 0.360 mmol) was added as a solid at once. The reaction was stirred until complete (20 min), the solvent was then removed *in vacuo*, and the resulting solid residue was dissolved in CH_2Cl_2 (2 × 10 mL). The organic layer was extracted twice each with an aqueous HCl solution (5% v/v; 2 × 20 mL) and an aqueous $NaHCO_3$ solution (10% w/w; 2 × 20 mL) and then with brine and dried with $MgSO_4$. Flash chromatography of the residue (2:1 ethyl acetate/hexanes) afforded **4** and its inseparable isomer as soft, thin, white fibers of hairlike consistency in 67% combined yield (43.0 mg, 0.107 mmol). IR ($CHCl_3$) 3300, 3020, 2955, 2349, 1745, 1652, 1507 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.90 (t, 4 H), 6.76 (d, 2 H), 6.71 (d, 2 H), 6.48 (d, 1 H), 6.38 (d, 1 H), 5.40 (d, 1 H), 5.28 (d, 1 H), 4.79 (q, 2 H), 4.34 (m, 2 H), 3.74 (m, 8 H), 3.11 (d, 1 H), 3.06 (d, 2 H), 2.98 (d, 1 H), 2.92 (d, 1 H), 2.87 (d, 1 H), 2.38 (m, 3 H), 2.20 (m, 1 H), 1.63 (m, 2 H), 1.40 (m, 2 H), 1.25 (m, 6 H), 0.95 (m, 12 H); MS *m/e* (rel intensity) 403 (M^+, 1), 386 (100), 208 (15), 177 (10), 164 (10), 106 (20), 83 (30).

Procedures for the Assay of Peptidyl Prolyl Isomerase Inhibition. PPIase Assay. The PPIase assay was carried out using a modified assay of Kofron *et al.*,[29] as detailed by Ocain *et al.*[30] and outlined here. The assay was conducted using a Cary 14 spectrophotometer with an IBM interface. Data acquisition was carried out with OLIS (On Line Instrument Systems Inc., Bogart, GA) software. The reaction was run at 0 °C to minimize uncatalyzed isomerization. Stock solutions of substrate were made to 4 mM with 235 mM LiCl in TFE. The assay was carried out as follows: The assay buffer (865 μL of 50 mM HEPES, 100 mM NaCl, pH 8.0) was equilibrated with 10 μL of inhibitor in EtOH and 10 μL of human recombinant FKBP 12 at 0°C for 10 min. Final inhibitor concentrations were typically 10–1000 nM; final PPIase concentrations were typically 6–60 nM. Chymotrypsin (100 μL; 6 mg/mL final) was added, followed by the addition (with stirring) of substrate solution (15 μL; final $[S]_{total}$ = 60 uM; 30–32% *cis*) to a total assay volume of 1 mL. Following a delay of 15 s, data were collected at 390 nm at 1-s intervals for 2 min. Each concentration of inhibitor was run at least two times. Fitting of progress curves to an integrated rate equation that accounts for the background uncatalyzed rate was accomplished with KineTic (Madison, WI) software; each initial velocity data point is derived from 120 individual data points along the progress curve. Enzyme concentrations were determined by active site titration with rapamycin (K_i=0.25 nM) and by absorbance at 280 nm (ϵ = 9.6 mM^{-1} cm^{-1}).

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Supplementary Material Available: ^1H NMR spectra for compounds **4** and **9–13**, ^{13}C NMR spectra of **9-11**, and the curves for compounds **4**, **10a**, **10b**, and **11** depicting the inhibitor concentration *vs* initial velocity of peptidyl prolyl isomerase activity from which the K_i values in Table I were calculated (12 pages). 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.