1,2,3-Trisubstituted Cyclopropanes as Conformationally Restricted Peptide Isosteres: Application to the Design and Synthesis of Novel Renin Inhibitors

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The 1,2,3-trisubstituted cyclopropanes **6** and **7** are the first members of a novel class of isosteric replacements for peptide linkages that are more generally represented by the dipeptide mimics **2** and **3**. These unique peptide surrogates are specifically designed to lock a section of a peptide backbone in an extended β-strand conformation (ϕ-angle restriction) while simultaneously enforcing one of two specifically defined orientations for the amino acid side chain (χ_1-angle restriction). Methods were first developed for the stereoselective, asymmetric synthesis of the trisubstituted cyclopropanes **15a–d**, **18a–d**, **22a–d**, and **23a–d** (Scheme II), by an efficient approach featuring the $Rh_2(S\text{-MEPY})_4$ (**11**) and $Rh_2(R\text{-MEPY})_4$ (**20**) catalyzed cyclization of the allylic diazoacetates **10a–d** to give the optically active lactones **12a–d** and **21a–d**, respectively, in up to ≥94% enantiomeric excess. Nucleophilic opening of the lactone ring of **12a–d** gave the corresponding morpholine amides **14a–d**. By exploiting tactics that allowed for selective epimerization of one of the two functionalized side chains on the cyclopropane nucleus, **14a–d** were transformed into the two series of diastereoisomeric morpholine amide carboxylic acids **15a–d** and **18a–d**. Epimerization of the morpholine amide group on **14a–d** followed by Jones oxidation of the intermediate alcohols gave **15a–d**. Alternatively, initial oxidation of the primary alcohol groups in **14a–d** followed by selective, base-catalyzed inversion α to the aldehyde function and then Jones oxidation gave the diastereomeric dicarboxylic acid derivatives **18a–d**. In a similar fashion, the enantiomeric lactones **21a–d** were converted into the two corresponding enantiomeric series of dicarboxylic acid derivatives **22a–d** and **23a–d**. Inhibitors of aspartic proteinases, of which renin is a typical example, are known to bind to the enzyme active site cleft in an extended conformation. Thus, in order to evaluate the efficacy of 1,2,3-trisubstituted cyclopropanes as rigid replacements of β-strand secondary structure in pseudopeptidic ligands, **15a–d**, **18a–d**, **22a–d**, and **23a–d** were incorporated at the P_3 subsite of the potential renin inhibitors **24a–h** and **25a–h** by coupling with the tripeptide replacement **8**. A significant number of these substances inhibited renin at nanomolar concentrations. On the basis of this preliminary test, 1,2,3-trisubstituted cyclopropanes do appear to constitute a viable new class of peptide mimics. Since the stereochemistry at each carbon on the cyclopropane ring may be altered, these novel replacements may also function as stereochemical probes to establish the conformation of pseudopeptide ligands bound to their macromolecular targets.

Introduction

Small peptide ligands exhibit a number of important and diverse biological roles by functioning as hormones, inhibitors, neurotransmitters, growth promoters, and immunomodulators. Following the initial binding of the oligopeptide to its receptor, transduction of the information provided by the resulting bimolecular complex into the cell induces the appropriate response. One of the major challenges in contemporary bioorganic chemistry involves understanding the molecular basis for this transfer of biological information. The first step toward realizing this objective is the development of a detailed picture of the dynamic interactions that occur between the peptide ligand and its receptor(s). However, since the structures of most receptors are unknown, elucidation of the biologically active conformation(s) of the relevant peptide ligands has been pursued to gain preliminary insights into the topographical requirements for interactions within the host–guest complex.

Although small linear oligopeptides adopt well-defined three-dimensional structures upon binding to their respective receptors or enzyme binding sites, they are typically flexible in solution. They appear to interconvert rapidly between a number of conformations, some of which may possess turn or helical motifs, that differ little in energy. Knowledge of the preferred solution conformation(s) of an oligopeptide thus cannot be reliably extrapolated to providing insights regarding the conformation of that ligand bound to its respective host.[2] In the absence of three-dimensional structural data for the ligand–receptor complex itself, insights regarding the biologically active conformation of oligopeptide ligands have been best obtained by introducing conformational restraints at selected sites on the peptide backbone; the resultant effects upon binding and biological activity of the resulting pseudopeptide are evaluated. Conformational constraints have been imposed locally through the agency of modified (i.e., α- or N-alkylated, α,β-dehydro, β,β-disubstituted, etc.) amino acids and globally either by forming rings (i.e., cyclic disulfides and covalent side chain-to-side chain or side chain-to-backbone cyclizations) or by incorporating subunits that are stabilized by amphiphilic helical structures, intramolecular hydrogen bonding, or salt bridges.[3] Another strategy to reduce conformational mobility of oligopeptides involves the rational design and synthesis of rigid, cyclic scaffolds to mimic and replace peptide secondary structural elements (i.e., α-helix, β- and γ-turns, etc.).[4] More recently efforts have been directed to the design of peptide surrogates that position the side chains to enhance binding to the receptor.[5]

Most rigid replacements for backbone fragments of ol-

(1) (a) University of Texas. (b) Abbott Laboratories, Cardiovascular Research Division.

(2) For some excellent examples of the extensive structural changes that may occur upon binding of biologically active ligands to their receptors, see: (a) Weber, C.; Wider, G.; von Freyberg, B.; Traber, R.; Braun, W.; Widmer, H.; Wüthrich, K. The NMR Structure of Cyclosporin A Bound to Cyclophilin in Aqueous Solution. *Biochemistry* **1991**, *30*, 6563–6573. (b) Fesik, S. W.; Gampe, R. T., Jr.; Eaton, H. L.; Gemmecker, G.; Olejniczak, E. T.; Neri, P.; Holzman, T. F.; Egan, D. A.; Edalji, R.; Simmer, R.; Helfrich, R.; Hochlowski, J.; Jackson, M. NMR Studies of [U-^{13}C]Cyclosporin A Bound to Cyclophilin: Bound Conformation and Portions of Cyclosporin Involved in BInding. *Biochemistry* **1991**, *30*, 6574–6582. (c) Van Duyne, G. D.; Standaert, R. F.; Karplus, P. A.; Schreiber, S. L.; Clardy, J. Atomic Structure of FKBP-FK506, an Immunophilin-Immunosuppressant Complex. *Science* **1991**, *252*, 839–842.

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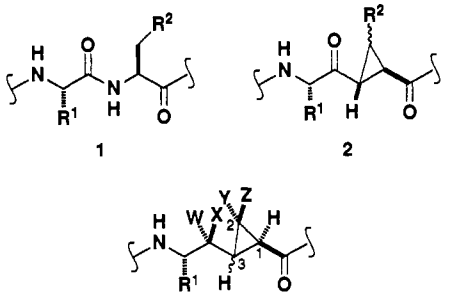
igopeptides are designed to imitate a turn; there are virtually no surrogates that enforce an extended (β-strand) conformation. In order to address this notable deficiency, we undertook a series of molecular modeling studies to identify a novel isosteric replacement for the natural dipeptide segment **1** that would enforce an extended conformation along the peptide backbone while simultaneously orienting the amino acid side chains in a predetermined fashion. On the basis of these studies, we concluded that 1,2,3-trisubstituted cyclopropanes **2** would constitute such a mimic.[6,7] It should be noted that this replacement is conceptually different from the well-known 1-aminocyclopropanecarboxylic acids.[8] Operationally, **2** is derived from **1** by replacing the amide nitrogen with a carbon and forming a single bond between this atom and C(β) on the amino acid side chain. Further generalization of this replacement leads to the formulation of the trisubstituted cyclopropanes **3** as potential surrogates for dipeptide subunits.

The trisubstituted cyclopropane **3** is an unconventional peptide mimic since its geometry and functionality differ from the usual amide bond replacements; its attributes thus merit brief discussion. One design feature of the isosteric replacement **3** is that it endows the peptide



1 **2**

3: W, X = H, OH, O
Y, Z = H, R^2

R^1, R^2 = H, alkyl, aryl, hetaryl

backbone with structural rigidity closely approximating a β-strand by locking the ϕ-angle. Moreover, the R^2 group of the amino acid side chain is rigidly fixed in one of two specifically defined orientations relative to the backbone that approximate χ_1-angles of −60°, gauche(−), and +60°, gauche(+). These structural features may be easily seen upon inspection of the superimpositions depicted in Figure 1. The ability to restrict conformational space available

(3) For reviews of peptide mimics, see: (a) Farmer, P. S. Bridging the Gap between Bioactive Peptides and Nonpeptides: Some Perspectives in Designs. In *Drug Design*; Ariëns, E. J., Ed.; Academic Press Inc.: New York, 1980; Vol. X, pp 119–143. (b) Hruby, V. J. Conformational Restrictions of Biologically Active Peptides via Amino Acid Side Chain Groups. *Life Sciences* **1982**, *31*, 189–199. (c) Spatola, A. F. Peptide Backbone Modifications: A Structure-Activity Analysis of Peptides Containing Amide Bond Surrogates. Conformational Constraints, and Related Backbone Replacements. In *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*; Weinstein, B., Ed; Marcel Dekker, 1983; Vol. 7, pp 267–357. (d) Toniolo, C. Conformationally Restricted Peptides Through Short-Range Cyclizations. *Int. J. Peptide Protein Res.* **1990**, *35*, 287–300. (e) Hruby, B. J.; Al-Obeidi, F.; Kazmierski, W. Emerging Approaches in the Molecular Design of Receptor-Selective Peptide Ligands: Conformational, Topographical and Dynamic Considerations. *Biochem. J.* **1990**, *268*, 249–262.

(4) For some examples, see: (a) Freidinger, R. M.; Veber, D. F.; Perlow, D. S.; Brooks, J. R.; Saperstein, R. Bioactive Conformation of Luteinizing Hormone-Releasing Hormone: Evidence from a Conformationally Constrained Analog. *Science* **1980**, *210*, 656–658. (b) Freidinger, R. M.; Perlow, D. S.; Veber, D. F. Protected Lactam-Bridged Dipeptides for Use as Conformational Constraints in Peptides. *J. Org. Chem.* **1982**, *47*, 104–109. (c) Kemp, D. S.; McNamara, P. Amino Acid Derivatives That Stabilize Secondary Structures of Polypeptides II. The Most Stable Conformation of Peptides Containing 3-Amino-2-piperidone-6-carboxylic Acid (Acp). *Tetrahedron Lett.* **1982**, *23*, 3761–3164. (d) Krstenansky, J. L.; Baranowski, R. L.; Currie, B. L. A New Approach to Conformationally Restricted Peptide Analogs: Rigid β-Bends. 1. Enkephalin as an Example. *Biochem. Biophys. Res. Commun.* **1982**, *109*, 1368–1374. (e) Thorsett, E. D.; Harris, E. E.; Aster, S.; Peterson, E. R.; Taub, D.; Patchett, A. A. Dipeptide Mimics. Conformationally Restricted Inhibitors of Angiotensin-Converting Enzyme. *Biochem. Biophys. Res. Commun.* **1983**, *111*, 166–171. (f) Kemp, D. S.; McNamara, P. E. Conformationally Restricted Cyclic Nonapeptides Derived from L-Cysteine and LL-3-Amino-2-piperidone-6-carboxylic Acid (LL-Acp), a Potent β-Turn-Inducing Dipeptide Analogue. *J. Org. Chem.* **1985**, *50*, 5834–5838. (g) Nagai, U.; Sato, K. Synthesis of a Bicyclic Dipeptide with the Shape of β-Turn Central Part. *Tetrahedron Lett.* **1985**, *26*, 647–650. (h) Feigel, M. 2,8-Dimethyl-4-(carboxymethyl)-6-(aminomethyl)phenoxathiin S-Dioxide: An Organic Substitute for the β-Turn in Peptides? *J. Am. Chem. Soc.* **1986**, *108*, 181–182. (i) Zydowsky, T. H.; Dellaria, J. F., Jr.; Nellans, H. N. Efficient and Versatile Synthesis of Dipeptide Isosteres Containing γ- or δ- Lactams. *J. Org. Chem.* **1988**, *53*, 5607–5616. (j) Thaisrivongs, S.; Pals, D. T.; Turner, S. T.; Kroll, L. T. Conformationally Constrained Renin Inhibitory Peptides: γ-Lactam-Bridged Dipeptide Isostere as Conformational Restriction. *J. Med. Chem.* **1988**, *31*, 1369–1376. (k) Yu, K.-L.; Rajakumar, G.; Srivastava, L. K.; Mishra, R. K.; Johnson, R. L. Dopamine Receptor Modulation by Conformationally Constrained Analogues of Pro-Leu-Gly-NH_2. *J. Med. Chem.* **1988**, *31*, 1430–1436. (l) Kemp, D. S.; Curran, T. P. (2S,5S,8S,11S)-1-Acetyl-1,4-diaza-3-keto-5-carboxy-10-thia-tricyclo[2.8.$0^{4,8}$]-tridecane, 1 The preferred Conformation of 1 (1 = αTemp-OH) and its Peptide Conjugates αTemp-L-$(Ala)_n$-OR (n=1 to 4) and α-Temp-L-Ala-L-Phe-L-lys(ϵBoc)-1-Lys(ϵ-Boc)-NHMe Studies of Templates for α-Helix Formation. *Tetrahedron Lett.* **1988**, *29*, 4935–4938. (m) Kemp, D. S.; Stites, W. E. A Convenient Preparation of Derivatives of 3(*S*)-Amino-10(*R*)-carboxy-1,6-diaza-cyclodeca-2,7-dione the Dilactam of L-α,γ-Diaminobutyric Acid and D-Glutamic Acid: A β-Turn Template. *Tetrahedron Lett.* **1988**, *29*, 5057–5060. (n) Kahn, M.; Wilke, S.; Chen, B.; Fujita, K. Nonpeptide Mimetics of β-turns: A facile Oxidative Intramolecular Cycloaddition of an Azodicarbonyl System. *J. Am. Chem. Soc.* **1988**, *110*, 1638–1639. (o) Kemp, D. S.; Carter, J. S. Amino Acid Derivatives that Stabilize Secondary Structures of Polypeptides. 4. Practical Synthesis of 4-Alkylamino-3-cyano-6-azabicyclo[3.2.1]oct-3-enes (Ben Derivatives) as γ-Turn Templates. *J. Org. Chem.* **1989**, *54*, 109–115. (p) Wolf, J.-P.; Rapoport, H. Conformationally Constrained Peptides. Chirospecific Synthesis of 4-Alkyl-Substituted γ-Lactam-Bridged Dipeptides from L-Aspartic Acid. *J. Org. Chem.* **1989**, *54*, 3164–3173. (q) Kahn, M.; Bertenshaw, S. The Incorporation of β-Turn Prosthetic Units into Merrifield Solid Phase Peptide Synthesis. *Tetrahedron Lett.* **1989**, *30*, 2317–2320. (r) Garvey, D. S.; May, P. D.; Nadzan, A. M. 3,4-Disubstituted γ-Lactam Rings as Conformationally Constained Mimics of Peptide Derivatives Containing Aspartic Acid or Norleucine. *J. Org. Chem.* **1990**, *55*, 936–940. (s) Ernest, I.; Kalvoda, J.; Rihs, G.; Mutter, M. Three Novel Mimics for the Construction of Sterically Constrained Protein Turn Models. *Tetrahedron Lett.* **1990**, *31*, 4011–4014. (t) Ede, N. J.; Rae, I. D.; Hearn, M. T. W. Synthesis of a New Protected Lactam-Bridged Dipeptide. *Tetrahedron Lett.* **1990**, *31*, 6071–6074. (u) Holladay, M. W.; Nadzan, A. M. Synthesis of α-Benzyl γ-Lactam, α-Benzyl δ-Lactam, and α-Benzylproline Derivatives as Conformationally Restricted Analogues of Phenylalaninamide. *J. Org. Chem.* **1991**, *56*, 3900–3905. (v) Hinds, M. G.; Welsh, J. H.; Brennand, D. M.; Fisher, J.; Glennie, M. J.; Richards, N. G. J.; Turner, D. L.; Robinson, J. A. Synthesis, Conformational Properties, and Antibody Recognition of Peptides Containing β-Turn Mimetics Based on α-Alkylproline Derivatives. *J. Med. Chem.* **1991**, *34*, 1777–1789.

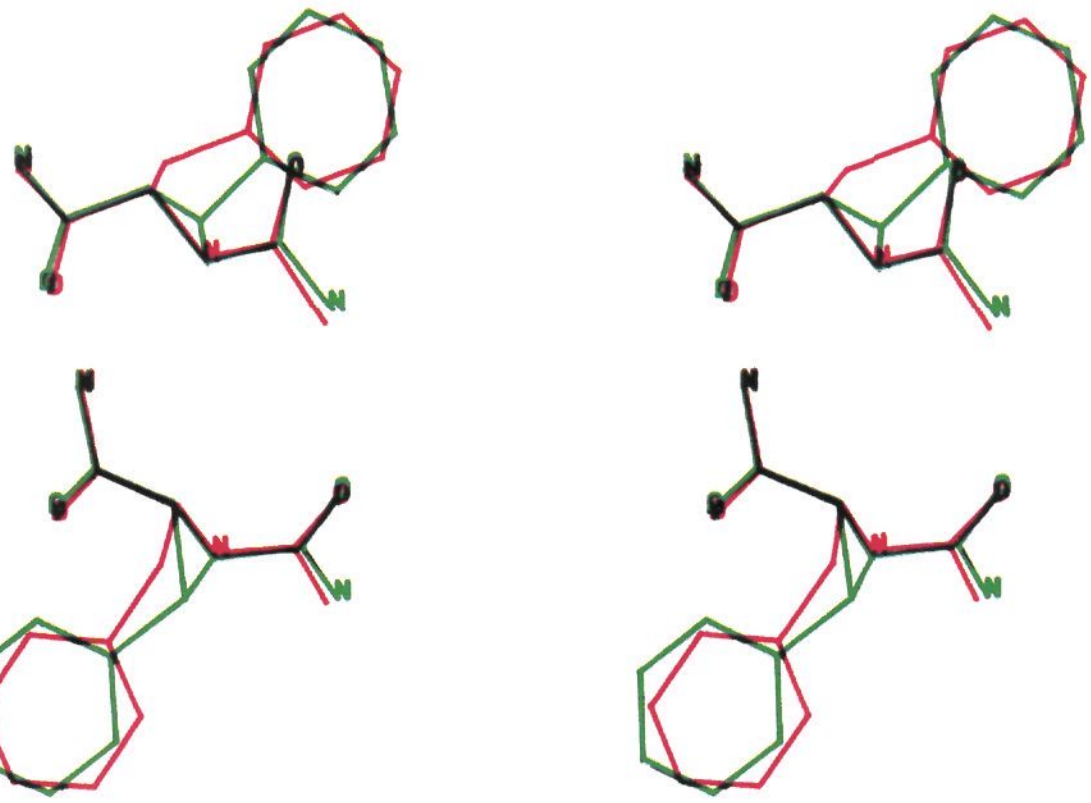


Figure 1. Stereoviews of a partial phenylalanine dipeptide (red) in a β-strand conformation superimposed on a partial cyclopropylphenylalanine derived dipeptide (green) in which the phenylalanine side chain is fixed in a gauche(−) orientation (a, top) and a gauche(+) orientation (b, bottom).

to the side chain Y or Z is significant since these appendages provide crucial sites for recognition, binding, and consequent transduction. The absolute stereochemistry depicted at C(1) of the cyclopropane in **3** correlates with the *S*-configuration of the natural amino acids; inversion at this center would provide the corresponding replacement for a D-amino acid. Inversion of the stereochemistry at C(3) of the cyclopropane in **3** places the backbone chains in a cis-orientation, and this modification would induce a turn in the backbone. Thus, interactions of the pseudopeptide with the targeted receptor may be optimized by controlling the stereochemistry at each of the ring carbons of the cyclopropane. Variable degrees of lipophilicity and hydrogen-bonding capability can be achieved by incorporating functionally as: W, X = O (H-bond acceptor); W or X = OH, X or W = H (directional H-bond donor–acceptor); and W = X = H. Finally, removal of one amide linkage, which suffers the potential disadvantage from the loss of a hydrogen-bond donor function, renders the isostere unsusceptible to enzymatic hydrolysis at that position; the steric bulk of the substituted cyclopropane ring should also increase the enzymatic stability of the adjacent (C-terminal) amide bond.

The design of a new peptide mimic is only the first step in its development, and the evaluation of its efficacy in a well-defined biological system is essential to establish its viability as a structural replacement. In the present context, the aspartic class of proteinases,[9] which possesses a characteristic Asp-Thr-Gly active site triad, seemed to constitute an ideal proving ground. The X-ray structures of a number of complexes of inhibitors bound to the active sites of different aspartic proteinases have been determined, and *in each case the inhibitor binds to the active site cleft of the respective enzyme in an extended, β-strand conformation.*[10] This observation suggested the design of novel inhibitors of aspartic proteinases that incorporated 1,2,3-trisubstituted cyclopropanes related to **3** to enforce the same local β-strand conformation on the backbone that is characteristic of the biologically active (bound) conformation of the known inhibitors. All other factors being equal, the reduced loss of entropy caused by the mimic's inflexible structure would be anticipated to result in more favorable binding energies and more potent enzyme inhibitors.

Although any aspartic proteinase could have been selected as the biological testing ground, we selected renin

(5) (a) Kahn, M.; Devens, B. The Design and Synthesis of a Nonpeptide Mimic of an Immunosuppressing Peptide. *Tetrahedron Lett.* **1986**, *27*, 4841–4844. (b) Flynn, G. A.; Giroux, E. L.; Dage, R. C. An Acyl-Iminium Ion Cyclization Route to a Novel Conformationally Restricted Dipeptide Mimic: Applications to Angiotensin-Converting Enzyme Inhibition. *J. Am. Chem. Soc.* **1987**, *109*, 7914–7915. (c) Kahn, M.; Chen, B.; Zieske, P. The Design and Synthesis of a Nonpeptide Mimic of Erabutoxin. *Heterocycles* **1987**, *25*, 29–31. (d) Olson, G. L.; Voss, M. W.; Hill, D. W.; Kahn, M.; Madison, V. S.; Cook, C. M. Design and Synthesis of a Protein β-Turn Mimetic. *J. Am. Chem. Soc.* **1990**, *112*, 323–333. (e) Kazmierski, W. M.; Yamamura, H. I.; Hruby, V. J. Topographic Design of Peptide Neurotransmitters and Hormones on Stable Backbone Templates: Relation of Conformation and Dynamics to Bioactivity. *J. Am. Chem. Soc.* **1991**, *113*, 2275. (f) Shimamoto, K.; Ishida, M.; Shinozaki, H.; Ohfune, Y. Synthesis of Four Diastereomeric L-2-(Carboxycyclopropyl)glycines. Conformationally Constrained 1-Glutamate Analogues. *J. Org. Chem.* **1991**, *56*, 4167–4176.

(6) Martin, S. F.; Austin, R. E.; Oalmann, C. J. Stereoselective Synthesis of 1,2,3-Trisubstituted Cyclopropanes as Novel Dipeptide Isosteres. *Tetrahedron Lett.* **1990**, *31*, 4731–4734.

(7) For a related example, see: Melnick, M. J.; Bisaha, S. N.; Gammill, R. B. Conformationally Restricted P_1-P_1' Transition State Analogues. Synthesis of 1(*R*), 3(*R*) [1(*S*), 2(*S*)] 3-[3-Cyclohexyl-2-[(Boc)amino]-1-hydroxypropyl]-2,2-dimethylcyclopropane Carboxylic Acids. *Tetrahedron Lett.* **1990**, *31*, 961–964.

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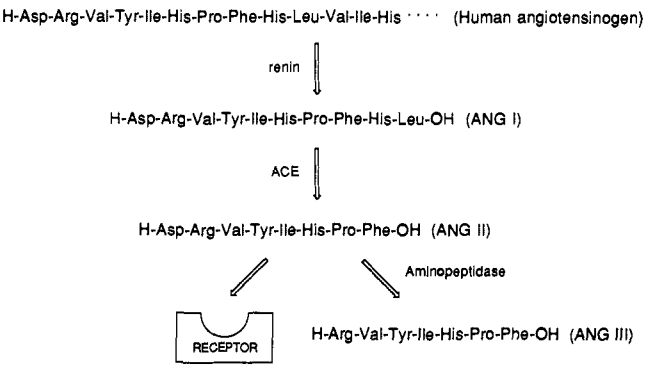
(9) For some leading references, see: (a) *Aspartic Proteinases and Their Inhibitors*, Kostka, V., Ed.; de Gruyter: Berlin, 1985. (b) Jupp, R. A.; Dunn, B. M.; Jacobs, J. W.; Vlasuk, G.; Arcuri, K. E.; Veber, D. F.; Perlow, D. S.; Payne, L. S.; Boger, J.; de Laszlo, S.; Chakravarty, P. K.; Ten Broeke, J.; Hangauer, D. G.; Ondeyka, D.; Greenlee, W. J.; Kay, J. The Selectivity of Statine-Based Inhibitors Against Various Human Aspartic Proteinases. *Biochem. J.* **1990**, *265*, 871–878.

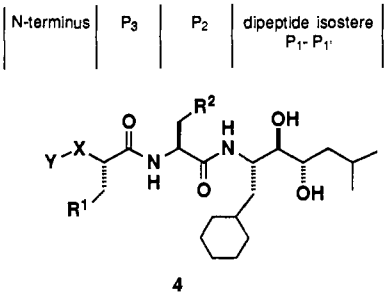
as the target enzyme for these initial investigations. Renin cleaves the N-terminal region of angiotensinogen in the rate-determining (committed) step of the renin–angiotensin–aldosterone system, which controls one of the primary mechanisms involved in the regulation of blood pressure and fluid balance, to produce the decapeptide angiotensin I (ANG I) (Scheme I).[11] Angiotensin-converting enzyme (ACE) then removes the C-terminal dipeptidyl fragment of ANG I to produce the potent effector hormone angiotensin II (ANG II), which may either bind to its receptor(s) or be processed by an aminopeptidase to liberate the heptapeptide angiotensin III (ANG III). Suppression of ANG II production by inhibition of renin

Scheme I



has received considerable attention by many research groups,[12] especially in the pharmaceutical industry, since selective inhibitors of this enzyme, whose only known role is to cleave angiotensinogen, might be effective antihypertensive drugs.[13]

In our search for a series of renin inhibitors that would serve as a reference point for the present studies, we were particularly attracted to those that possess the generic structure **4** in which a glycol replacement serves as the transition-state analogue for the scissile Leu–Val amide bond.[14-17] These inhibitors are comprised of four structural segments that include a P_1–$P_{1'}$ dipeptide isostere, a P_2 amino acid, a P_3 amino acid, and a N-terminal substituent. Extensive structure–activity relationship (SAR) studies in each substructure ultimately lead to the identification of the series of dipeptide glycol renin inhibitors represented by **4** (Y = (dialkylamino)carbonyl or (heterocycloamino)carbonyl; X = CH_2; R^1 = C_6H_5; R^2 = thiazol-4-yl); some members of this class of renin inhibitors exhibited excellent efficacy and intraduodenal bioavailability.[16]



4

Given the broad scope of these SAR studies, it occurred to us that evaluation of the biological profiles of a series of pseudopeptides represented by **5**, which incorporate cyclopropane-derived replacements for the P_3 and N-terminal subunits of **4**, would provide an excellent preliminary test of the viability of 1,2,3-trisubstituted cyclopropanes as peptide mimics. The synthesis of compounds **5** would involve initial preparation of diastereomeric cyclopropanes

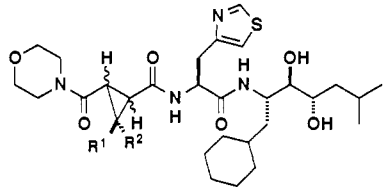
(10) For endothiapepsin: (a) Foundling, S. I.; Cooper, J.; Watson, F. E.; Cleasby, A.; Pearl, L. H.; Sibanda, B. L.; Hemmings, A.; Wood, S. P.; Blundell, T. L.; Valler, M. J.; Norey, C. G.; Kay, J.; Boger, J.; Dunn, B. M.; Leckie, B. J.; Jones, D. M.; Atrash, B.; Hallett, A.; Szelke, M. High Resolution X-ray Analyses of Renin Inhibitor-Aspartic Proteinase Complexes. *Nature* **1987**, *327*, 349–352. (b) Blundell, T. L.; Cooper, J.; Foundling, S. I.; Jones, D. M.; Atrash, B.; Szelke, M. On the Rational Design of Renin Inhibitors: X-ray Studies of Aspartic Proteinases Complexed with Transition-State Analogues. *Biochemistry* **1987**, *26*, 5585–5590. (c) Cooper, J. B.; Foundling, S. I.; Blundell, T. L.; Boger, J.; Jupp, R. A.; Kay, J. X-ray Studies of Aspartic Proteinase-Statine Inhibitor Complexes. *Biochemistry* **1989**, *28*, 8596–8603. (d) Suguna, K.; Padlan, E. A.; Smith, C. W.; Carlson, W. D.; Davies, D. R. Binding of a Reduced Peptide Inhibitor to the Aspartic Proteinase from *Rhizopus Chinensis*: Implications for a Mechanism of Action. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7009–7013. For penicillopepsin: (e) James, M. N. G.; Sielecki, A. R., Aspartic Proteinases and Their Catalytic Pathway. *Biological Macromolecules and Assemblies*; Jurnak, F. A., McPherson, A., Eds.; Wiley: New York, 1987, Vol. 3, pp 413–482. For porcine pepsin: (f) Abad-Zapatero, C.; Rydel, T. J.; Neidhart, D.; Luly, J.; Ericson, J. W. Inhibitor Binding Induces Structural Changes in Porcine Pepsin. *Proceedings of the Sonoma Aspartic Proteinase Meeting*, September 1990; Plenum Press: New York, in press. For HIV-1 protease: (g) Miller, M.; Schneider, J.; Sathyanarayana, B. K.; Toth, M. V.; Marshall, G. R.; Clawson, L; Selk, L.; Kent, S. B. H.; Wlodawer, A. Structure of Complex of Synthetic HIV-1 Protease with a Substrate-Based Inhibitor at 2.3 Å Resolution. *Science* **1989**, *246*, 1149–1152. (h) Miller, M.; Swain, A. L.; Jaskólski, M.; Sathyanarayana, B. K.; Marshall, G. R.; Rich, D. H.; Kent, S. B. H.; Wlodawer, A. X-ray Analysis of HIV-1 Protease and Its Complexes with Inhibitors. *Retroviral Proteases: Control of Maturation and Morphogenesis*; Pearl, L., Ed; Macmillan Press: New York, 1990; pp 93–106. (i) Erickson, J.; Neidhart, D. J.; VanDrie, J.; Kempf, D. J.; Wang, X. C.; Norbeck, D. W.; Plattner, J. J.; Rittenhouse, J. W.; Turon, M.; Wideburg, N.; Kohlbrenner, W. E.; Simmer, R.; Helfrich, R.; Paul, D. A.; Knigge, M. Design, Activity, and 2.8 Å Crystal Structure of a C_2 Symmetric Inhibitor Complexed to HIV-1 Protease. *Science* **1990**, *249*, 527–533. (j) Swain, A. L.; Miller, M. M.; Green, J.; Rich, D. H.; Schneider, J.; Kent, S. B. H.; Wlodawer, A. X-ray Crystallographic Structure of a Complex Between a Synthetic Protease of Human Immunodeficiency Virus 1 and a Substrate-Based Hydroxyethylamine Inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 8805–8809. (k) Jaskólski, M.; Tomasselli, A. G.; Sawyer, T. K.; Staples, D. G.; Heinrikson, R. L.; Schneider, J.; Kent, S. B. H.; Wlodawer, A. Structure at 2.5-Å Resolution of Chemically Synthesized Human Immunodeficiency Virus Type 1 Protease Complexed with a Hydroxyethylene-Based Inhibitor. *Biochemistry* **1991**, *30*, 1600–1609. For renin: (l) Watenpaugh, K. D.; Einspahr, H. M.; Finzel, B. C.; Clancy, L. L.; Mulichak, A. M.; Holland, D. R.; Muchmore, S. W.; Poorman, R. A.; Hui, J. O.; Heinrikson, R. L.; Murakami, K.; Shoda, A.; Maggiora, L. L.; Sawer, T. K. *Pept. Chem. Biol., Proc. 12th Am. Pept. Symp* **1991**, (June 16–21).

(11) (a) Peach, M. J. Renin-Angiotensin System: Biochemistry and Mechanisms of Action. *Physiol. Rev.* **1977**, *57*, 313. (b) Ondetti, M. A.; Cushman, D. W. Enzymes of the Renin-Angiotensin System and Their Inhibitors. *Ann. Rev. Biochem.* **1982**, *51*, 283–308.

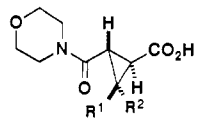
(12) For recent reviews describing inhibitors of renin and their use as antihypertensive agents, see: (a) Kleinert, H. D. Renin Inhibitors: Discovery and Development, An Overview and Perspective. *Am. J. Hypertension* **1989**, *2*, 800–808. (b) Ocain, T. D.; Abou-Gharbia, M. Renin-Angiotensin System (RAS) Dependent Antihypertensive Agents: I. Renin Inhibitors. *Drugs of the Future* **1991**, *16*, 37–51. (c) Kleinert, H. D.; Baker, W. R.; Stein, H. H. Renin Inhibitors. *Adv. Pharm.* **1991**, *22*, 207–250.

(13) (a) Ondetti, M. A.; Cushman, D. W. Inhibition of the Renin-angiotensin System. A New Approach to the Therapy of Hypertension. *J. Med. Chem.* **1981**, *24*, 355–361. (b) Thaisrivongs, S. Renin Inhibitors: A Promising New Class of Antihypertensive Agents. *Drug News Perspect.* **1988**, *1*, 11–16.

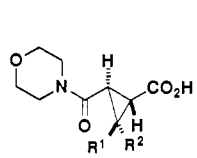
of the general structures **6** and **7**, which represent slightly truncated variants of the dipeptide surrogates **2** and **3**; **6** and **7** would then be coupled with the known P_2–$P_{1'}$ replacement **8**. Introduction of the conformationally restricted replacements **6** and **7** into renin inhibitors **5** could offer some important insights regarding the topographical requirements involved in binding of inhibitors to the active site cleft of renin. For example, a series of rigid replacements **6** and **7** could be utilized to define the biologically active conformation at the N-terminal and P_3 positions of pseudopeptide ligands bound to the active-site cleft of renin; they could also be employed as stereochemical probes to map the S_3 subsite of renin. We now report initial investigations in this area confirming our original hypothesis that 1,2,3-trisubstituted cyclopropanes related to **2** and **3** may be utilized as novel and effective, conformationally restricted peptide mimics.



5: R_1, R_2 = H, alkyl, aryl, Bn



6



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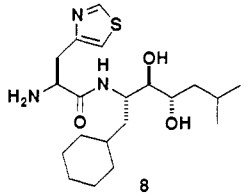
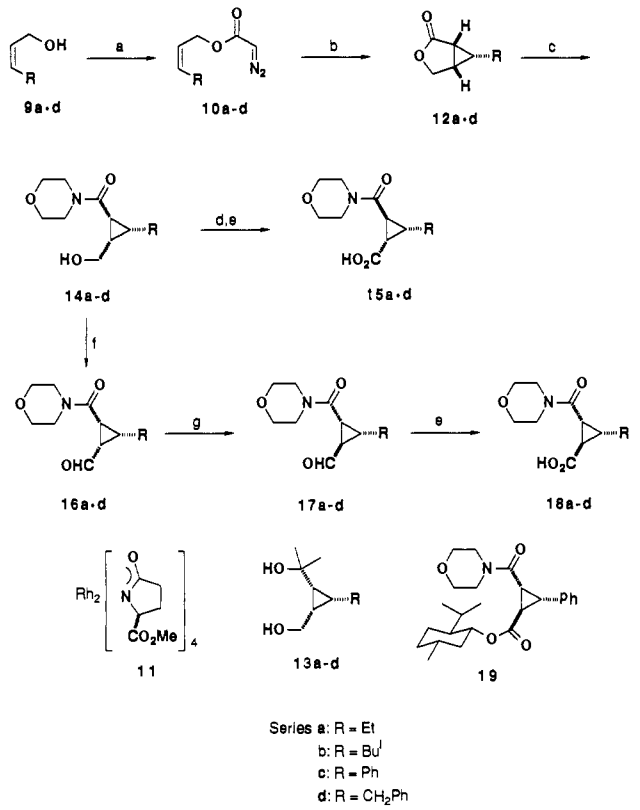


8

Results

Stereoselective Synthesis of Trisubstituted Cyclopropanes. A survey of the literature revealed that there existed a paucity of methods for the stereoselective synthesis of diastereomeric 1,2,3-trisubstituted cyclopropanes of the general types **6** and **7**, and it was necessary to devise

Scheme II[a]



Series a: R = Et
b: R = Bu^t
c: R = Ph
d: R = CH_2Ph

[a] (a) $TsNHN{=}CHCOCl$, $Me_2NC_6H_5$; Et_3N. (b) $Rh_2(5S\text{-MEPY})_4$ (**11**); (c) $O(CH_2CH_2)_2NH$, $AlMe_3$. (d) LiHMDS. (e) Jones' reagent. (f) PCC. (g) K_2CO_3, MeOH.

efficient, reliable procedures for their preparation. After exploring a number of routes, we discovered that the general plan outlined in Scheme II provided an expeditious solution to the problem. The requisite cis allylic alcohols **9a–d** were prepared via the corresponding acetylenes utilizing standard literature procedures.[18] The alcohols **9a–d** were then transformed into the corresponding allylic diazoacetates **10a–d** in 85–93% yield by reaction of **9a–d** with the *p*-toluenesulfonyl hydrazone of glyoxylic acid chloride[19,20] in the presence of *N,N*-dimethylaniline followed by triethylamine. Highly enantioselective intramolecular cyclopropanation of the allylic diazoacetates **10a–d** was achieved upon their exposure to the chiral rhodium catalyst $Rh_2(5S\text{-MEPY})_4$ (**11**) in refluxing methylene chloride to furnish the cyclopropyl lactones **12a–d**.[21] In order to ascertain the levels of asymmetric induction obtained in the cyclizations of **10a–d**, the adducts **12a–d** were converted into the corresponding diols

(14) (a) Luly, J. R.; BaMaung, N.; Soderquist, J.; Fung, A. K. L.; Stein, H.; Kleinert, H. D.; Marcotte, P. A.; Egan, D. A.; Bopp, B.; Merits, I.; Bolis, G.; Greer, J.; Perun, T. J.; Plattner, J. J. Renin Inhibitors. Dipeptide Analogues of Angiotensinogen Utilizing a Dihydroxyethylene Transition-State Mimic at the Scissle Bond to Impart Greater Inhibitory Potency. *J. Med. Chem.* **1988**, *31*, 2264–2276. (b) Plattner, J. J.; Marcotte, P. A.; Kleinert, H. D.; Stein, H. H.; Greer, J.; Bolis, G.; Fung, A. K. L.; Bopp, B. A.; Luly, J. R.; Sham, H. L.; Kempf, D. J.; Rosenberg, S. H.; Dellaria, J. F.; De, B.; Merits, I.; Perun, T. J. Renin Inhibitors. Dipeptide Analogues of Angiotensinogen Utilizing a Structurally Modified Phenylalanine Residue to Impart Proteolytic Stability. *J. Med. Chem.* **1988**, *31*, 2277–2288.

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(16) Rosenberg, S. H.; Spina, K. P.; Kleinert, H. D.; Martin, D. L.; Verburg, K. M.; Stein, H. H.; Cohen, J.; Egan, D. A.; Barlow, J. L.; Klinghofer, V.; Hoffman, D. J.; Garren, K. W.; Baker, W. R. Renin Inhibitor: Approaches Toward Oral Activity. *Book of Abstracts*, 201st National Meeting of the American Chemical Society, Division of Medicinal Chemistry, Atlanta, April 1991; American Chemical Society: Washington DC, 1991; Abstract 64.

(17) Rosenberg, S. H.; Kleinert, H. D.; Stein, H. H.; Martin, D. L.; Chekal, M. A.; Cohen, J.; Egan, D. A.; Tricarico, K. A.; Baker, W. R. Design of a Well-Absorbed Renin Inhibitor. *J. Med. Chem.* **1991**, *34*, 469–471.

(18) (a) Vig, O. P.; Jindal, R. T. Syntheses of Acetates of 2(*E*)-Hexenol, 2(*E*)-Octenol & 2(*E*)-Decenol. *Indian J. Chem.* **1983**, *22B*, 919–920. (b) Nakagawa, N.; Mori, K. Synthesis of (3*S*,4*S*)-4-Methyl-3-heptanol and Its (3*S*,4*R*)-Isomer Employing Asymmetric Epoxidation Coupled with Regioselective Cleavage of Epoxides with Trimethylaluminum. *Agric. Biol. Chem.* **1984**, *48*, 2505–2510. (c) Brown, C. A.; Ahuja, V. K. "P-2 Nickel" Catalyst with Ethylenediamine, a Novel System for Highly Stereospecific Reduction of Alkynes to *cis*-Olefins. *J. Chem. Soc., Chem. Commun.* **1973**, 553–554. (d) Hatch, L. F.; Alexander, H. E. Preparation of *cis*- and *trans*-Cinnamyl Chloride. *J. Am. Chem. Soc.* **1950**, *72*, 5643–5645.

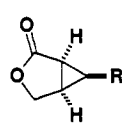
(19) Blankley, C. J.; Sauter, F. J.; House, H. O. Crotyl Diazoacetate (Acetic acid, diazo-, *trans*-2-butenyl ester). *Organic Syntheses*; John Wiley: New York, 1973; Coll. Vol. V, pp 258–263.

(20) Corey, E. J.; Myers, A. G. Efficient Synthesis and Intramolecular Cyclopropanation of Unsaturated Diazoacetic Esters. *Tetrahedron Lett.* **1984**, *23*, 3559–3562.

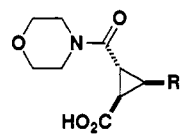
13a–d by treatment with excess methyllithium. Subsequent integration of the enantiotopic peaks in the ^{1}H NMR spectra of 13a–d in C_6D_6 in the presence of Eu(tfc)$_3$[22,23] then provided a measure of the enantiomeric excesses of the lactones 12a–d (12a, ≥94%; 12b, 93%; 12c ≥94%; 12d, ≥94%). The absolute configuration of 12c was determined by a single-crystal X-ray analysis of the (–)-menthyl ester 19, which was prepared by the reaction of 18c (vide infra) with (–)-menthol in the presence of dicyclohexylcarbodiimide and (dimethylamino)pyridine.[24] Since the absolute sense of asymmetric induction in the Rh$_2$(5*S*-MEPY)$_4$-catalyzed intramolecular cyclopropanation of 10c to give 12c is identical to that observed for the Rh$_2$(5*S*-MEPY)$_4$-catalyzed cyclizations of several other allylic alcohols,[21] we assume that the absolute stereochemistry of the cyclopropyl lactones 12a,b,d is as shown; however, this assignment has not been independently verified.

Opening of the lactone rings of 12a–d according to the Weinreb protocol[25] gave morpholine amides 14a–d in 76–90% yield. The two series of diastereoisomeric 1,2,3-trisubstituted cyclopropanes 15a–d and 18a–d, which represent some of the requisite peptide mimics 6 and 7, were then prepared by pathways that featured selective epimerization of one of the two functionalized substituents on the cyclopropane ring.[6] In the event, deprotonation of 14a–d with lithium hexamethyldisilazide (LiHMDS) effected quantitative epimerization of the center α to the morpholinocarbonyl moiety, and subsequent Jones oxidation of the intermediate amide alcohols provided 15a–d in 52–73% (unoptimized) overall yield. Alternatively, oxidation of 14a–d with pyridinium chlorochromate (PCC) gave the aldehydes 16a–d, which were readily epimerized α to the aldehyde function by treatment with methanolic potassium carbonate to furnish 17a–d; Jones' oxidation of 17a–d then delivered 18a–d.

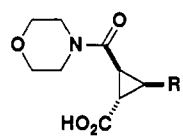
Preparation of the enantiomeric series of 1,2,3-trisubstituted cyclopropanes 22a–d and 23a–d followed in a straightforward fashion from the preceding experiments. Cyclization of the allylic diazoacetates 10a–d in the presence of the chiral rhodium catalyst Rh$_2$(5*R*-MEPY)$_4$ (20) gave the cyclopropyl lactones 21a–d with levels of asymmetric induction that were within experimental error of that observed for the cyclizations of 10a–d catalyzed by 11.[21] The lactones 21a–d were then processed in analogy with those reactions depicted in Scheme II to give 22a–d and 23a–d.



21a-d



22a-d



23a-d

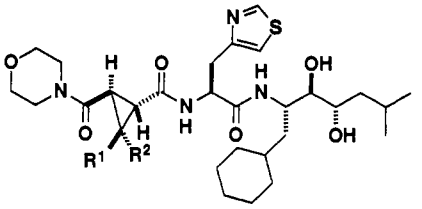
Series a: R = Et
b: R = Bu^t
c: R = Ph
d: R = CH$_2$Ph

(21) Doyle, M. P.; Pieters, R. J.; Martin, S. F.; Austin, R. E.; Oalmann, C. J.; Müller, P. High Enantioselectivity in the Intramolecular Cyclopropanation of Allyl Diazoacetates Using a Novel Rhodium(II) Catalyst. *J. Am. Chem. Soc.* **1991**, *113*, 1423–1424.

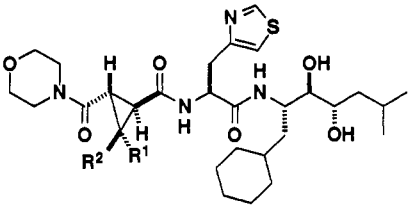
(22) Jakovac, I. J.; Jones, J. B. Determination of Enantiomeric Purity of Chiral Lactones. A General Method Using Nuclear Magnetic Resonance. *J. Org. Chem.* **1979**, *44*, 2165–2168.

(23) The limit of detection of the minor enantiomer in these experiments was ±3%, and consequently an enantiomeric excess of ≥94% is denoted when only one enantiomer was detected.

Synthesis of Pseudopeptides as Potential Renin Inhibitors. With the requisite 1,2,3-trisubstituted cyclopropane carboxylic acids 15a–d, 18a–d, 22a–d, and 23a–d in hand, it simply remained to couple these subunits to the P_2–$P_{1'}$ surrogate 8. Toward this end, the tripeptide replacement 8 was prepared in 86% yield by condensing *N*-(*tert*-butoxycarbonyl)-3-(4-thiazoyl)-L-alanine[26] with (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane[27] using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride in the presence of 1-hydroxybenzotriazole and *N*-methylmorpholine followed by N-terminal deprotection with trifluoroacetic acid. Reaction of 8 with each of the cyclopropane carboxylic acids 15a–d, 18a–d, 22a–d, and 23a–d then delivered the corresponding pseudopeptides 24a–h and 25a–h that represented potential renin inhibitors of the general type 5.



24a-h



25a-h

Rigorous evaluation of the merits of introducing a rigid replacement at the P_3 inhibitor subsite in the pseudopeptides 24a–h and 25a–h required comparison of their biological activities with those of counterparts that do not possess such a conformational constraint. To address this important question, the flexible analogues 29a,b were prepared (Scheme III). In the event, the *tert*-butyl ester protecting group was removed from 26a[14b] and 26b[28] by the action of trifluoroacetic acid; subsequent reaction of mixed anhydrides derived from the intermediate monoacid esters with morpholine then afforded (2*S*)- and (2*R*)-benzyl 2-benzyl-3-(morpholinocarbonyl)propionates 27a[14b] and 27b, respectively. Selective cleavage of the benzyl esters

(24) Lynch, V. M.; Austin, R. E.; Martin, S. F.; George, T. The Determination of the Absolute Configuration of a Novel Dipeptide Isostere. *Acta Crystallogr.* **1991**, *C47*, 1345–1347.

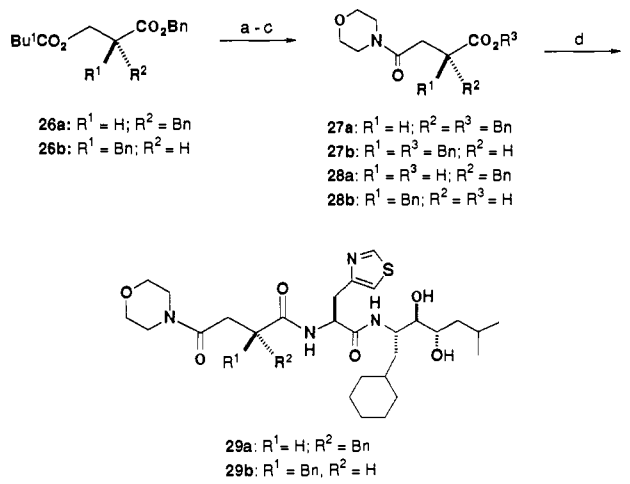
(25) Basha, A.; Lipton, M.; Weinreb, S. M. A Mild, General Method for Conversion of Esters to Amides. *Tetrahedron Lett.* **1977**, 4171–4174.

(26) Hsiao, C. N.; Leanna, M. R.; Bhagavatula, L.; de Lara, E.; Zydowsky, T. M.; Horrom, B. W.; Morton, H. E. Synthesis of *N*-(*tert*-butoxycarbonyl)-3-(4-thiazoyl)-L-alanine. *Synth. Commun.* **1990**, *20*, 3507–3517.

(27) Luly, J. R.; Hsiao, C. N.; BaMaung, N.; Plattner, J. J. A Convenient Stereoselective Synthesis of 1,2,3-Aminodiols from α-Amino Acids. *J. Org. Chem.* **1988**, *53*, 6108–6112.

(28) Benzyl (2*S*)-2-benzyl-3-*tert*-butoxycarbonyl propionate (26b), $[\alpha]^{25}_D$ = –6.25 (MeOH), was prepared by TDC Research, Inc., Blacksburg, VA according to the procedure cited in reference 14b for the preparation of 26a with the modification that (4*R*)-4-(3-benzyl)oxazolidin-2-one was substituted for (4*S*) 4-(3-isopropyl)oxazolidin-2-one.

Scheme III[a]



[a] (a) CF_3CO_2H. (b) Isobutyl chloroformate; morpholine. (c) H_2Pd/C. (d) EDC, HOBT, 8.

of **27a,b** by hydrogenolysis followed by coupling of the resulting acids **28a**[14b] and **28b** to 8 gave the corresponding acyclic pseudopeptide inhibitors **29a**[29] and **29b**.

Biological Activities of Renin Inhibitors. The in vitro potencies for the inhibitory candidates **24a–h**, **25a–h**, and **29a,b** against purified human renin (pH 6.0) and human plasma renin (pH 7.4) were then determined according to standard procedures,[14b,30] and the results of these evaluations are summarized in Table I. It is noteworthy that the IC_{50} values against human plasma renin at pH 7.4 were consistently higher than those against purified renin at pH 6.0. This trend in a measured decrease of inhibitor potency against plasma renin relative to purified renin has been observed previously,[14b,31] and although the precise cause of this phenomenon remains unknown, three-dimensional structural variations of renin with pH and/or interactions of the inhibitors with components in plasma may be involved. In order to assess the stability of renin-inhibiting pseudopeptides containing cyclopropane replacements toward degradative enzymes, the stability of **24c** toward bovine pancreatic chymotrypsin was determined using a previously described procedure.[30a] As anticipated, **24c** did not suffer significant proteolysis ($t_{1/2}$ > 300 min) by this enzyme, even after prolonged digestion; the acyclic analogue **29a** was equally stable ($t_{1/2}$ > 300 min).

Table I. In Vitro Potency of Cyclopropane-Derived Pseudopeptide Renin Inhibitors against Purified (pH 6.0) and Plasma Renin (pH 7.4)

			IC_{50}, nM		
compd	R^1	R^2	pH 6.0[a]	pH 7.4[b,c]	formula[f]
24a	Et	H	10	230	[g]
24b	Bu^i	H	2.1	41	$C_{33}H_{54}N_4O_6S\cdot{}^1/_3H_2O$
24c	Ph	H	0.7	20	$C_{35}H_{50}N_4O_6S$
24d	Bn	H	25	>1,000[d]	$C_{36}H_{52}N_4O_6S\cdot{}^4/_5H_2O$
24e	H	Et	16	610	$C_{31}H_{50}N_4O_6S\cdot{}^2/_3H_2O$
24f	H	Bu^i	19	>1,000[e]	[g]
24g	H	Ph	200	nd	[g]
24h	H	Bn	120	nd	$C_{36}H_{52}N_4O_6S\cdot{}^1/_3H_2O$
25a	Et	H	200	nd	$C_{31}H_{50}N_4O_6S\cdot{}^1/_5H_2O$
25b	Bu^i	H	44	nd	$C_{33}H_{54}N_4O_6S$
25c	Ph	H	120	nd	$C_{35}H_{50}N_4O_6S\cdot{}^2/_3H_2O$
25d	Bn	H	10	370	$C_{36}H_{52}N_4O_6S\cdot{}^1/_3H_2O$
25e	H	Et	92	nd	$C_{31}H_{50}N_4O_6S\cdot{}^1/_3H_2O$
25f	H	Bu^i	33	nd	[g]
25g	H	Ph	120	nd	[g]
25h	H	Bn	27	nd	$C_{36}H_{52}N_4O_6S\cdot{}^1/_2H_2O$
29a			0.36	8.3	$C_{35}H_{52}N_4O_6S$
29b			150	nd	$C_{35}H_{52}N_4O_6S\cdot{}^4/_5H_2O$
32			1.1	810	$C_{36}H_{47}N_3O_4S\cdot{}^4/_5H_2O$
33			0.47	54	$C_{36}H_{49}N_3O_4S$

[a] Purified human renin. [b] Human plasma renin. [c] Compounds were selected for IC_{50} determinations against plasma renin if their IC_{50} value in the purified assay, pH 6.0, was 25 nM or less. [d] 30% inhibition at 1×10^{-6} M. [e] 46% inhibition at 1×10^{-6} M. [f] Analyses for C, H, N were correct within ±0.4%. [g] High-resolution mass spectra (±5 ppm) were obtained.

Discussion

The data obtained by the biological evaluation of the pseudopeptides **24a–h** and **25a–h** clearly support our initial hypothesis that their potency as renin inhibitors would be sensitive to the substitution and stereochemical pattern on the cyclopropane ring of the P_3 replacement. The most obvious trends were observed with the series of eight inhibitors **24a–h** in which the stereochemistry at C(1) of the cyclopropane ring mimics L-amino acids. In this family of compounds, more potent inhibition was observed when the side-chain substituent of the cyclopropane, which corresponds to the amino acid side chain, was syn to the N-terminal morpholine amide function; the relative potency varied according to the following: **24a > 24e**; **24b > 24f**; **24c > 24g**; **24d > 24h**. The most active member of this group was **24c** (IC_{50} = 0.7 nM for purified renin) wherein the cyclopropane unit mimicked L-phenylalanine, which is the residue at the P_3 subsite of human angiotensinogen. This result, coupled with the relative activity exhibited by the epimeric analogue **24g** (IC_{50} = 200 nM for purified renin), suggests that the phenyl ring in **24c** is constrained in a favorable position for binding to the S_3 subsite of renin; in **24g** the phenyl substituent does not appear well directed for interaction with the enzyme (vide infra). Perhaps the pseudopeptide ligands **24a** (IC_{50} = 10 nM for purified renin) and **24b** (IC_{50} = 2.1 nM for purified renin), which bear ethyl and isobutyl appendages, are not as potent as **24c** because the aliphatic side chains do not adequately fill the S_3 subsite of renin for optimal van der Waals contacts between the inhibitor and the enzyme. Conversely, the comparatively high IC_{50} (IC_{50} = 25 nM for purified renin) observed for the benzyl analogue **24d** may be interpreted by assuming that the extra methylene unit pushes the phenyl substituent too far into the S_3 site, thereby leading to an unfavorable steric interaction with the enzyme.

The biological activities of the conformationally restricted renin inhibitor **24c** and the flexible analogue **29a**,

(29) Other structure–activity relationships for **29a** and related analogues will be reported in due course.

(30) (a) Sham, H. L.; Stein, H.; Rempel, C. A.; Cohen, J.; Plattner, J. J. Highly Potent and Specific Inhibitors of Human Renin. *FEBS Lett.* **1987**, *220*, 299–301. (b) Bolis, G.; Fung, A. K. L.; Greer, J.; Kleinert, J. D.; Marcotte, P. A.; Perun, T. J.; Plattner, J. J.; Stein, H. H. Renin Inhibitors. Dipeptide Analogues of Angiotensinogen Incorporating Transition-State, Nonpeptidic Replacements at the Scissile Bond. *J. Med. Chem.* **1987**, *30*, 1729–1737.

(31) (a) Bock, M. G.; DiPardo, R. M.; Evans, B. E.; Rittle, K. E.; Boger, J.; Poe, M.; LaMont, B. I.; Lynch, R. J.; Ulm, E. H.; Vlasuk, G. P.; Greenlee, W. J.; Veber, D. F. Renin Inhibitors. Statine-Containing Tetrapeptides with Varied Hydrophobic Carboxy Termini. *J. Med. Chem.* **1987**, *30*, 1853–1857. (b) Williams, P. D.; Perlow, D. S.; Payne, L. S.; Holloway, M. K.; Siegl, P. K. S.; Schorn, T. W.; Lynch, R. J.; Doyle, J. J.; Strouse, J. F.; Vlasuk, G. P.; Hoogsteen, K.; Springer, J. P.; Bush, B. L.; Halgren, T. A.; Richards, A. D.; Kay, J.; Veber, D. F. Renin Inhibitors Containing Conformationally Restricted P_1-$P_{1'}$ Dipeptide Mimics. *J. Med. Chem.* **1991**, *34*, 887–900.

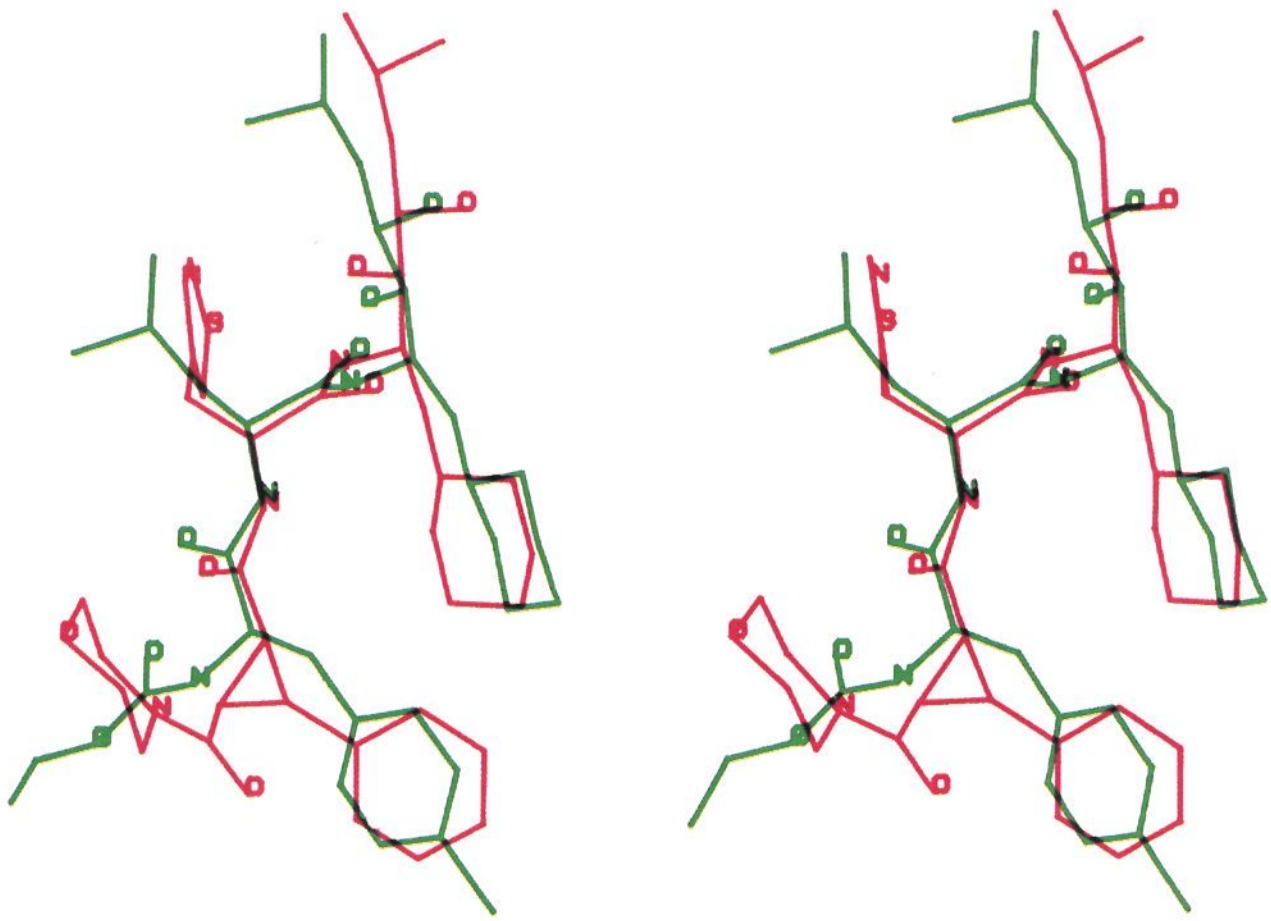


Figure 2. Stereoview of cyclopropane inhibitor **24c** (red), which was minimized in a renin active site model, superimposed on the pseudopeptide renin inhibitor **4** (Y = CO_2Et; X = NH; R^1 = *p*-IC_6H_4; R^2 = $CHMe_2$) (green). The conformation of inhibitor **4** (Y = CO_2Et; X = NH; R^1 = *p*-IC_6H_4; R^2 = $CHMe_2$) is based on X-ray data of the inhibitor and porcine pepsin complex.[10f]

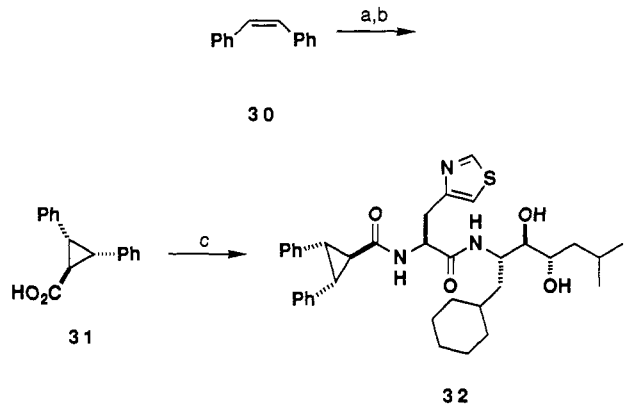
whose structures differ only at the P_3 position, are virtually identical. This observation strongly suggests that there is a close correspondence between the manner in which both **24c** and **29c** bind to the active-site cleft of renin. The phenyl side chain and N-terminal substituent on the cyclopropane ring of **24c** thus appear to be specifically preorganized to interact with the S_3 subsite of renin in a fashion that nicely mimics the spatial arrangements of the respective groups at the P_3 site in the bound (biologically active) conformation of **29a**. Thus, these considerations combined with the design features of the cyclopropane replacements lead to the prediction that the P_3 and N-terminal substituents of **29a** probably bind to renin in an extended (β-strand) mode with the phenyl group residing in the gauche(−) orientation relative to the main chain of the inhibitor.

In order to gain further structural insights regarding the binding requirements of pseudopeptide inhibitors to aspartic proteinases, the renin inhibitor **4** (Y = CO_2Et; X = NH; R^1 = *p*-IC_6H_4; R^2 = $CHMe_2$) [IC_{50}'s of 2.4 nM (purified renin, pH 6.0) and 300 nM (human plasma renin, pH 7.4)] was crystallized with porcine pepsin, a mammalian aspartic proteinase.[10f] X-ray data of the resulting complex revealed that the inhibitor bound to the enzyme with the backbone in an extended conformation and with the benzyl, isobutyl, and cyclohexylmethyl side chains positioned in a staggered conformation. The phenylalanine at P_3 adopted a conformation in which the NH, α-carbon, and benzylic carbon were coplanar and the phenyl ring eclipsed the ethoxycarbonyl group (i.e., a gauche(−) orientation). These data were combined with other structural information available for aspartic proteinases to develop a working model for the active site of renin.[32] Associated molecular modeling studies of the cyclopropane inhibitor **24c** were then performed in which the pseudopeptide was docked and minimized (DISCOVER) in this active site model for renin. When the three-dimensional structure thus obtained for **24c** was superimposed on the porcine pepsin-bound, acyclic inhibitor **4** (Y = CO_2Et; X = NH; R^1 = *p*-IC_6H_4; R^2 = $CHMe_2$), an excellent fit (rms = 0.59 Å for the backbone and the C(β)'s of the side chains) was obtained as shown in Figure 2. It may be noted that the percent inhibition of porcine pepsin by **4** (Y = CO_2Et; X = NH; R^1 = *p*-IC_6H_4; R^2 = $CHMe_2$) and **24c** at 10^{-5} M were 31 and 7, respectively. In view of these studies coupled with the structural similarities of the active sites of the various aspartic proteinases, it seems likely that **4** (Y = CO_2Et; X = NH; R^1 = *p*-IC_6H_4; R^2 = $CHMe_2$) and **24c** adopt closely related conformations on binding to the active site clefts of renin and porcine pepsin. These data support our hypothesis that 1,2,3-trisubstituted cyclopropane replacements may be exploited as structural probes to establish the biologically active conformation of oligopeptide and pseudopeptide ligands.

The IC_{50}'s of inhibitors **25a–h**, which possess D-amino acid replacements at the P_3 subsite, vary by a factor of approximately 20. Although this result suggests that the nature of the side chain in this series is not overly significant, there is a consistent trend wherein the efficacy of the side chain group follows the order Bn > *i*-Bu > Et $\cong$ Ph. The specific orientation of the side chain also affected inhibitory activity, but the effect was not as dramatic as in the series **24a–h**; the IC_{50}'s were typically within a factor of about 2 for each pair **25a,e**, **25b,f**, **25c,g**, and **25d,h**. With the notable and presently inexplicable exceptions of **25d,g,h**, compounds bearing the D-amino acid replacements at P_3 were less potent than the corresponding L-amino acid mimics. The specificity of the renin S_3 subsite for residues bearing the natural L-configuration is further underscored by comparison of the potency of **29a** and **29b**, which possess an acyclic benzylsuccinimide amino

(32) Hutchins, C.; Greer, J. Comparative Modeling of Proteins in the Design of Novel Renin Inhibitors. *Crit. Rev. Biochem. Mol. Biol.* **1991**, *26*, 77–127.

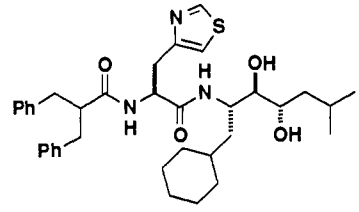
Scheme IV[a]



[a] (a) EtO_2CCHN_2, $Rh_2(OAc)_4$. (b) H_3O^+, Δ. (c) EDC·HCl, HOBT, 8.

acid replacement at the P_3 position. The inhibitor **29b**, which contains a D-benzylsuccinimide amino acid replacement, was over 400 times less active than the corresponding L-analogue **29a**.

Since the optimal orientation of the side chain with the amino terminus of the inhibitors bearing cyclopropanes mimicking L-amino acids was cis, we were intrigued by the possibility that the pseudopeptide **32**, which contains the achiral *cis*-diphenylcyclopropane subunit **31**, might be an effective inhibitor of renin. Consistent with this hypothesis, we found that **32**, which was readily prepared by coupling **31** with **8** (Scheme IV), was a potent inhibitor (IC_{50} = 1.1 nM) of purified renin. Unfortunately, **32** was a weak inhibitor of human renin in plasma (IC_{50} = 810). By comparison, the more flexible acyclic analogue **33**, which was derived from dibenzyl acetic acid, exhibited comparable inhibitory potency against purified renin (IC_{50} = 0.47 nM) but markedly improved potency against human plasma renin (IC_{50} = 54 nM).



33

Conclusions

The 1,2,3-trisubstituted cyclopropanes **6** and **7**, which represent simplified members of a novel class of dipeptide mimics **3**, have been incorporated as combined N-terminal and P_3 replacements in the design of a unique series of renin inhibitors **24a–h** and **25a–h**. These peptide surrogates were designed to enforce localized extended β-strand structure on the peptide backbone (φ-angle restriction) while directing the side chains in one of two specific orientations (χ_1-angle restriction). The viability of trisubstituted cyclopropanes as rigid peptide mimics was convincingly established by the incorporation of the diastereoisomeric cyclopropanes **15a–d**, **18a–d**, **22a–d**, and **23a–d** as the combined N-terminal and P_3 subunit into the renin inhibitors **24a–h** and **25a–h**. These compounds served as effective probes of the topographical preferences of the S_3 subsite of human renin. Since the conformationally constrained inhibitor **24c** and the flexible inhibitor **29a** exhibit virtually identical potencies, the preorganized spatial arrangement of the substituents on the rigid cyclopropane replacement at P_3 in **24c** appears to mimic closely the three-dimensional orientation of these groups in the biologically active conformation of **29a**. More generally it now seems probable that cyclopropane-derived isosteres of natural amino acids may be exploited to help define the biologically active conformation of selected oligopeptide and pseudopeptide ligands and to map the three-dimensional features of their respective receptors. Armed with this knowledge, new insights regarding the complex ligand–receptor interactions that mediate biological response should emerge. Toward these goals, future studies are directed toward the incorporation of 1,2,3-trisubstituted cyclopropanes related to **2**, **3**, **6**, and **7** into novel inhibitors of aspartic and other proteinases as well as into pseudopeptides that are designed as antagonists and agonists of biologically active oligopeptides. The results of these investigations will be reported in due course.

Experimental Section

General. Unless otherwise noted, solvents and reagents were reagent grade and used without purification. Tetrahydrofuran (THF) was distilled from potassium/benzophenone ketyl under nitrogen immediately prior to use. Benzene was distilled from and stored over sodium. Dichloromethane (CH_2Cl_2), triethylamine, *N*,*N*-dimethylaniline, and hexamethyldisilazane were distilled from calcium hydride under nitrogen immediately prior to use. Methanol (MeOH) was distilled from magnesium methoxide and stored over 3-Å molecular sieves. Reactions involving air- or moisture-sensitive reagents or intermediates were performed under an inert atmosphere of nitrogen or argon in glassware that had been oven and/or flame dried. Melting points are uncorrected. Infrared (IR) spectra were recorded either neat on sodium chloride plates or as solutions in $CHCl_3$ as indicated and are reported in wave numbers (cm^{-1}) referenced to the 1601.8 cm^{-1} absorption of a polystyrene film. ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were obtained as solutions in $CDCl_3$ unless otherwise indicated, and chemical shifts are reported in parts per million (ppm, δ) downfield from internal standard Me_4Si (TMS). Coupling constants are reported in hertz (Hz). Spectral splitting patterns are designated as s, singlet; br, broad; d, doublet; t, triplet; q, quartet; m, multiplet; and comp, complex multiplet. Partial ^1H NMR assignments were made for compounds **8**, **24a–h**, **25a–h**, **27b**, **28b**, **29a–b**, **32**, and **33**. Mass spectra were generally obtained using electron ionization except for compounds **24a**, **24c**, **24d**, **24f**, **24g**, **25c**, **25f**, **25h**, **27b**, **28b**, **29a**, **32**, and **33** which were measured using fast atom bombardment (FAB) methods. Elemental analyses were performed by Dr. Franz Scheidl (Hoffmann LaRoche, Inc., Nutley, NJ) and by the Analytical Research Department, Abbott Laboratories. Flash chromatography was performed according to published methods with Merck silica gel 60 (230–400 mesh ASTM).[33] Percent yields are given for compounds that were ≥95% pure as judged by NMR.

(2*S*,3*R*,4*S*)-2-[(L-4-Thiazolylalanyl)amino]-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (8). (2*S*,3*R*,4*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-1-cyclohexyl-3,4-dihydroxy-6-methylheptane[27] (5.05 g, 14.7 mmol) was stirred for 90 min in 4 M HCl in ethanol, whereupon the solvent was evaporated. Ether was added and evaporated three times, and the residue was dried under high vacuum. The residue was then dissolved in DMF (60 mL) containing 1-hydroxybenzotriazole (HOBT) (5.57 g, 41.2 mmol), Boc-L-(4-thiazolyl)alanine[26] (4.00 g, 14.7 mmol), and *N*-methylmorpholine (NMM) (3.40 mL, 30.9 mmol). The mixture was cooled to −23 °C, and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC·HCl) (4.03 g, 21.0 mmol) was added. After stirring for 2 h at −23 °C and 21 h at ambient temperature, the mixture was poured into saturated $NaHCO_3$. The aqueous layer was extracted with EtOAc, and the combined organic layers were washed with water and brine and dried

(33) Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* 1978, *43*, 2923–2925.

(Na_2SO_4). The solvents were then evaporated under reduced pressure to afford a white solid, which was recrystallized from CH_2Cl_2/ether (1:15, v/v) (multiple crops) to afford 6.28 g (86%) of Boc-L-(4-thiazolyl)alanine amide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane as a flaky white solid: mp 159–160 °C; ^{1}H NMR δ 8.78 (d, 1 H), 7.14 (d, 1 H), 6.18 (br d, 2 H), 4.44 (dd, 1 H), 4.27 (m, 1 H), 4.10 (m, 1 H), 3.37 (dd, 1 H), 3.30–3.12 (m, 3 H), 1.89 (m, 1 H), 0.94 (d, 3 H), 0.88 (d, 3 H).

To a solution of the intermediate Boc-protected pseudopeptide (6.27 g, 12.6 mmol) prepared above in CH_2Cl_2 (50 mL) at 0–5 °C was slowly added trifluoroacetic acid (50 mL). The reaction was stirred for 3 h at 0 °C, and the mixture was concentrated in vacuo (40 °C bath) to an oil, which was basified to pH 10–11 with aqueous K_2CO_3. The aqueous layer was extracted with $CHCl_3$, and the combined organic extracts were dried (Na_2SO_4) and concentrated. Recrystallization of the resulting foam from CH_2Cl_2/hexane (1:4, v/v) gave 5.00 g (100%) of **8** as a fluffy white solid: mp 111–112 °C; ^{1}H NMR δ 8.77 (d, 1 H), 7.40 (br d, 1 H), 7.13 (d, 1 H), 4.54 (m, 1 H), 4.25 (m, 1 H), 3.80 (dd, 1 H), 3.33 (dd, 1 H), 3.25–3.12 (m, 3 H), 0.95 (d, 3 H), 0.86 (d, 3 H).

General Procedure for the Transformation of the Alcohols 9a–d into Diazo Esters 10a–d. The *p*-toluenesulfonyl hydrazone of glyoxylic acid chloride[19,20] (1.15 equiv) was added to a solution of the alcohol **9a–d** in dry CH_2Cl_2 (0.20 M) at 0 °C, whereupon *N*,*N*-dimethylaniline (1.10 equiv) was added. After the mixture was stirred at 0 °C for 15 min, Et_3N (5.13 equiv) was added slowly. The resulting dark suspension was stirred for 15 min at 0 °C and then for 30 min at room temperature, whereupon an equal volume of water was added. The reaction mixture was extracted with Et_2O (3 × 1 volume), and the combined organic fractions were dried ($MgSO_4$) and concentrated in vacuo. The crude diazo esters thus obtained were purified by flash chromatography using hexane/EtOAc mixtures to furnish pure **10a–d** as yellow oils in 85–93% yield.

(*Z*)-3-Phenyl-2-propenyl diazoacetate (10c) was obtained as a yellow oil (15:1, hexanes/EtOAc): 85% yield; ^{1}H NMR δ 7.40–7.18 (comp, 5 H), 6.69 (d, J = 11.1 Hz, 1 H), 5.83 (dt, J = 11.1, 6.6 Hz, 1 H), 4.96 (d, J = 6.6 Hz, 2 H), 4.79 (s, 1 H); ^{13}C NMR δ 166.3, 135.9, 133.0, 128.6, 128.3, 127.4, 125.7, 61.6, 46.0; IR (neat) ν 2210, 1690 cm^{-1}; mass spectrum, m/z 202.0747 ($C_{11}H_{10}N_2O_2$ requires 202.0742), 139, 129, 115, 91, 28 (base).

General Procedure for the Cyclopropanation of the Allyl Diazoacetates 10a–d in the Presence of Rh_2(*S*-MEPY)$_4$ (11) and Rh_2(*R*-MEPY)$_4$ (20). A solution of the diazo ester **10a–d** in dry CH_2Cl_2 (0.010 M) was added via syringe pump to a refluxing solution of the chiral rhodium catalyst **11** or **20** in CH_2Cl_2 (0.01 equiv, 1 × 10^{-4} M) over a period of 12–18 h. The reaction was cooled to room temperature, and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography eluting with hexane/EtOAc to give **12a–d** (using **11**) and **21a–d** (using **20**) in yields ranging from 71 to 87% and enantiomeric excesses ranging from 93 to ≥94%.[21]

[1*R*-(1α,5α,6α)]-6-Phenyl-3-oxabicyclo[3.1.0]hexan-2-one (12c) was obtained as a white solid purified by flash chromatography (hexanes/EtOAc, 2:1). The enantiomeric purity was determined to be ≥94%: 45% yield; mp 114–115 °C; ^{1}H NMR δ 7.36–7.26 (comp, 5 H), 4.36 (dt, J = 9.8, 2.5 Hz, 1 H), 4.05 (d, J = 9.8 Hz, 1 H), 2.78 (t, J = 8.5 Hz, 1 H), 2.60–2.57 (comp, 2 H); ^{13}C NMR δ 174.7, 132.4, 129.4, 128.9, 127.7, 65.7, 26.2, 23.9, 23.5; IR (neat) 1800 cm^{-1}; mass spectrum, m/z 174.0678 ($C_{11}H_{10}O_2$ requires 174.0681), 129, 115.

General Procedure for Determination of the Optical Purity of Chiral Lactones 12a–d. Methyllithium (3 equiv in ether) was slowly added with stirring to a solution of the lactone (1 equiv) in THF (0.10 M) at 0 °C. The reaction was then stirred for 1 h at room temperature and then quenched by addition of an equal volume of water. The resulting mixture was extracted with Et_2O (3 × 2 volumes), and the combined ether extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The enantiomeric purity of the resulting diols **13a–d** was determined without further purification by ^{1}H NMR in C_6D_6 in the presence of Eu(tfc)$_3$ (0.1–0.4 equiv).[22]

(1*R*,2*S*,3*R*)-2-(Hydroxymethyl)-1-(1′-hydroxy-1′-methylethyl)-3-phenylcyclopropane (13c): ^{1}H NMR δ 7.49 (d, J = 7.6 Hz, 2 H), 7.33–7.19 (comp, 3 H), 4.36 (dd, J = 8.3, 11.3 Hz, 1 H), 4.14 (dd, J = 7.6, 11.3 Hz, 1 H), 2.48 (t, J = 9.4 Hz, 1 H), 2.46 (br s, 1 H), 1.89 (br s, 1 H), 1.68–1.56 (comp, 2 H), 1.39 (s, 3 H), 1.33 (s, 3 H); ^{13}C NMR δ 136.9, 130.4, 128.6, 126.4, 71.5, 59.5, 32.1, 31.1, 24.6, 22.0; IR (neat) ν 3350 cm^{-1}; mass spectrum, m/z 206.1281 ($C_{13}H_{18}O_2$ requires 206.1307), 157 (base), 143, 129, 115, 91, 82.

General Procedure for the Opening of Lactones 12a–d To Give Morpholine Amide Alcohols 14a–d. A 2.5 M solution of trimethylaluminum in hexanes (3 equiv) was slowly added to a solution of morpholine (3 equiv) in dry CH_2Cl_2 (0.40 M) at room temperature.[25] After the mixture stirred at room temperature for 20 min, a solution of the lactones **12a–d** in CH_2Cl_2 (0.20 M) was added dropwise. The reaction was heated at 40 °C for 40 h, cooled to 0 °C, and carefully quenched with 1 N HCl (1 volume). The aqueous mixture was extracted with CH_2Cl_2 (3 × 1 volume), and the combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The crude amide alcohols were purified by flash chromatography using a mixture of hexanes and EtOAc as eluent to give pure **14a–d** in 76–90% yield.

[1*R*-(1α,2α,3α)]-2-(Hydroxymethyl)-1-(4-morpholinylcarbonyl)-3-phenylcyclopropane (14c): CH_2Cl_2/MeOH (20:1); 79% yield as a pale yellow solid; mp 101–103 °C; ^{1}H NMR δ 7.30–7.11 (comp, 5 H), 4.25 (dd, J = 4.3, 9.6 Hz, 1 H), 3.91–3.51 (comp, 8 H), 3.11–3.04 (m, 1 H), 2.58 (t, J = 9.4 Hz, 1 H), 2.15 (t, J = 9.4 Hz, 1 H), 1.97–1.89 (m, 1 H), (OH not observed); ^{13}C NMR δ 169.1, 136.0, 128.5, 128.3, 126.7, 66.5, 66.2, 58.6, 46.3, 42.1, 27.2, 25.4, 24.7; IR (neat) 3480, 1650 cm^{-1}; mass spectrum m/z 261.1368 ($C_{15}H_{19}NO_3$ requires 261.1365), 230 (base), 170, 144, 129, 115.

General Procedure for the Epimerization of the Amide Function of 14a–d Followed by Oxidation to Acids 15a–d. To a solution of lithium hexamethyldisilazide (3 equiv) in dry THF (0.15 M) at room temperature was added a solution of the amide alcohol **14a–d** in THF (0.050 M). Upon completion of the reaction (as indicated by TLC), saturated NH_4Cl (2 volumes) was added. The mixture was extracted with CH_2Cl_2 (3 × 2 volumes), and the combined organic fractions were dried ($MgSO_4$) and concentrated under reduced pressure. The crude, epimeric amide thus obtained was dissolved in acetone (0.10 M) at 0 °C, and a solution of 8 N Jones' reagent (0.7 mL/mmol of alcohol) was added. The mixture was stirred for 2 h at 0–5 °C, whereupon the yellow solution was diluted with 1 N HCl (2 volumes) and extracted with CH_2Cl_2 (3 × 2 volumes). The combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure, and the crude acids were purified by flash chromatography using hexane/EtOAc/HOAc (33:66:1) as eluant to give **15a–d** in 52–73% yield.

[1*S*-(1α,2β,3α)]-2-(4-Morpholinylcarbonyl)-3-phenylcyclopropanecarboxylic acid (15c) was obtained as a white solid in 66% yield: mp 165–172 °C; ^{1}H NMR δ 9.80 (br s, 1 H), 7.30–7.20 (comp, 5 H), 3.80–3.50 (comp, 8 H), 3.09 (dd, 1 H, J = 6.5, 10.0 Hz), 2.90 (m, 1 H, J = 4.8, 6.5 Hz), 2.60 (dd, 1 H, J = 4.8, 10.0 Hz); ^{13}C NMR δ 172.4, 168.7, 134.2, 128.6, 128.0, 127.1, 66.4, 45.9, 42.6, 32.6, 29.2, 24.4; IR (neat) 2950, 1730, 1630 cm^{-1}; mass spectrum, m/z 275.1155 ($C_{15}H_{17}NO_4$ requires 275.1158), 230 (base), 115.

General Procedure for Oxidation of Primary Alcohols 14a–d to Aldehydes 16a–d. To a solution of pyridinium chlorochromate (1.5 equiv) in dry CH_2Cl_2 (0.30 M) at room temperature was added a solution of the hydroxy amide **14a–d** in CH_2Cl_2 (0.20 M), and the reaction mixture was stirred for 48 h. After the addition of 2.5 volumes of Et_2O, the dark mixture was filtered through glass wool, and the filtrate concentrated under reduced pressure. The crude aldehydes were purified by flash chromatography using hexane/EtOAc mixtures as eluant to give **16a–d** in 68–85% yield.

[1*R*-(1α,2α,3α)]-1-(4-Morpholinylcarbonyl)-3-phenylcyclopropane-2-carboxaldehyde (16c): hexanes/EtOAc (1:3); 68% yield as a white solid; mp 155–160 °C; ^{1}H NMR δ 9.56 (d, J = 6.3 Hz, 1 H), 7.34–7.23 (comp, 5 H), 3.78–3.49 (comp, 8 H), 3.06 (t, J = 9.2 Hz, 1 H), 2.73 (t, J = 9.2 Hz, 1 H), 2.31–2.23 (m, 1 H); ^{13}C NMR δ 199.5, 166.3, 133.2, 129.3, 128.7, 127.6, 66.8, 66.5, 46.8, 42.5, 32.4, 30.9, 30.4; IR ($CHCl_3$) ν 1720, 1660 cm^{-1}; mass spectrum, m/z 259.1203 ($C_{15}H_{17}NO_3$ requires 259.1208), 230, 145, 117, 115 (base), 91, 70.

General Procedure for the Epimerization of the Aldehyde Function of 16a–d. A solution of the cis-aldehyde amide **16a–d**

in MeOH (0.1 M), which had been degassed with a stream of nitrogen for 30 min, containing K_2CO_3 (4 equiv) was stirred at room temperature for 24 h. The mixture was diluted with saturated NH_4Cl (4 volumes) and extracted with Et_2O (3 × 4 volumes); the combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The crude aldehydes were purified by flash chromatography using hexane/EtOAc mixtures as eluant to give **17a–d** in 80–87% yield.

[1*R*-(1α,2β,3α)]-1-(4-Morpholinylcarbonyl)-3-phenylcyclopropane-2-carboxaldehyde (17c): Hexanes/EtOAc (1:3); 80% yield as a clear oil; ^1H NMR δ 9.93 (d, J = 2.0 Hz, 1 H,), 7.33–7.14 (comp, 5 H), 3.76–3.70 (m, 1 H), 3.60–3.36 (comp, 5 H), 3.22–3.05 (comp, 2 H), 2.99 (dd, J = 5.6, 9.1 Hz, 1 H), 2.81 (dd, J = 5.1, 9.1 Hz, 1 H), 2.77–2.71 (m, 1 H); ^{13}C NMR δ 198.9, 164.6, 134.7, 128.5, 127.5, 127.4, 66.6, 45.8, 42.4, 33.9, 33.6, 33.3; IR ($CHCl_3$) ν 1720, 1640 cm^{-1}; mass spectrum, m/z 259.1213 ($C_{15}H_{17}NO_3$ requires 259.1208), 230 (base), 145, 115, 70.

General Procedure for Jones Oxidation of Aldehydes 17a–d. To an ice cooled solution of the aldehydes **17a–d** in acetone (0.1 M) was added 8 N Jones' reagent (0.5 mL/mmol), and the reaction was stirred for 2 h at 0–5 °C. The mixture was diluted with 1 N HCl (4 volumes) and extracted with CH_2Cl_2 (3 × 4 volumes), and the combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The crude acids were purified by flash chromatography using hexane/EtOAc mixtures as eluant to give **18a–d** in 76–98% yield.

[1*R*-(1α,2β,3β)]-2-(4-Morpholinylcarbonyl)-3-phenylcyclopropanecarboxylic acid (18c): Hexanes/EtOAc/HOAc (33:66:1); 76% yield as a white solid; mp 180 °C dec; ^1H NMR δ 7.60 (s, 1 H), 7.31–7.26 (comp, 3 H), 7.23–7.06 (comp, 2 H), 3.76–3.63 (m, 1 H), 3.59–3.40 (comp, 4 H), 3.23–2.90 (comp, 4 H), 2.79–2.69 (comp, 2 H); ^{13}C NMR δ 176.3, 165.1, 134.6, 128.5, 128.2, 127.4, 66.5, 45.8, 42.4, 32.7, 32.4, 25.4; IR (neat) ν 1730, 1650 cm^{-1}; mass spectrum, m/z 275.1159 ($C_{15}H_{17}NO_4$ requires 275.1158), 230 (base), 115.

[1*R*-[1α(1*R,2*R**,3*S**),2β,5α]]-2-(4-Morpholinylcarbonyl)-3-phenylcyclopropanecarboxylic Acid, (–)-Menthol Ester (19).** To a solution of optically pure **18c** (28.8 mg, 0.105 mmol), DCC (33.4 mg, 0.126 mmol), and DMAP (1.30 mg, 0.0105 mmol) in a mixture (1:1) of CH_2Cl_2 and DMF (2 mL) was added (–)-menthol (20.0 mg, 0.126 mmol) at 0 °C. The reaction was warmed to room temperature and stirred for 72 h, whereupon the cloudy mixture was filtered through glass wool and concentrated in vacuo. The crude product was purified by flash chromatography (2:1, hexanes/EtOAc) to give **19** (20.0 mg, 46% yield) as a white solid: mp 124–125 °C; ^1H NMR δ 7.31–7.20 (comp, 3 H), 7.18–7.12 (comp, 2 H), 4.74 (dt, J = 4.0, 10.9 Hz, 1 H), 3.73 (m, 1 H), 3.61–3.44 (comp, 4 H), 3.22–3.05 (comp, 2 H), 3.00 (t, J = 5.0 Hz, 1 H), 2.91–2.79 (comp, 2 H), 2.68 (dd, J = 5.0, 10.2 Hz, 1 H), 1.99–1.90 (comp, 2 H), 1.72–1.66 (comp, 2 H), 1.53–1.37 (comp, 2 H), 1.21 (m, 1 H), 1.12–0.95 (comp, 2 H,), 0.91 (d, J = 7.0 Hz, 3 H), 0.89 (d, J = 6.4 Hz, 3 H), 0.76 (d, 3 H); ^{13}C NMR δ 172.1, 164.9, 134.9, 128.4, 127.4, 127.2, 75.1, 66.6, 46.9, 45.7, 42.1, 40.8, 34.1, 32.3, 31.9, 31.4, 25.9, 25.5, 23.2, 22.0, 20.8, 16.1; IR (CH_2Cl_2) ν 1740, 1660 cm^{-1}; mass spectrum, m/z 413.2574 ($C_{25}H_{35}NO_4$ requires 413.2566), 276, 258, 230 (base), 144, 115.

General Procedure for Coupling Cyclopropane Carboxylic Acids 15a–d, 18a–d, 22a–d, 23a–d, 28a,b, 31, and Dibenzylacetic Acid with the Pseudotripeptide 8. A solution of the appropriate cyclopropane carboxylic acid **15a–d, 18a–d, 22a–d, 23a–d,** and **31** (0.058 mmol), 1-hydroxybenzotriazole (HOBT) (25 mg, 0.186 mmol), and the amine **8** (28 mg, 0.07 mmol) in DMF (0.75 mL) was cooled in a carbon tetrachloride–dry ice bath, and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC·HCl) (13 mg, 0.068 mmol) was added. The solution was allowed to warm to room temperature and stirred for 24 h. The resulting solution was partitioned between EtOAc and brine, and the organic layer was dried ($MgSO_4$) and evaporated to give a thick yellow oil. The crude product was purified by flash chromatography (3% MeOH/CH_2Cl_2) to give the corresponding pseudopeptides **24a–h, 25a–h,** and **32** in 21–82% yield as indicated.

(2*S*,3*R*,4*S*)-2-[[*N*-[[(1*S*,2*S*,3*S*)-3-Phenyl-2-(4-morpholinylcarbonyl)cyclopropyl]carbonyl]-3-(4-thiazolyl)-L-alanyl]amino]-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (24c): 63% yield (24 mg); ^1H NMR δ 8.80 (s, 1 H), 7.92 (d, J = 6.0 Hz, 1 H), 7.33–7.24 (m, 5 H), 7.16 (s, 1 H), 6.39 (d, J = 9.0 Hz, 1 H), 4.75 (q, J = 8.0 Hz, 12.0 Hz, 1 H), 4.26 (m, 1 H), 4.08 (d, J = 4.5 Hz, 1 H), 3.51 (comp, 3 H), 2.96 (m, 1 H), 2.87 (m, 1 H), 2.79–2.65 (m, 2 H), 2.32 (d, J = 7.5 Hz, 1 H), 1.87 (m, 1 H), 0.94 (d, J = 6.0 Hz, 3 H), 0.88 (d, J = 6.0 Hz, 3 H); mass spectrum, m/z 655.3511 ($C_{35}H_{51}N_4O_6S$ requires 655.3529).

Benzyl (2*S*)-2-Benzyl-3-(4-morpholinylcarbonyl)-propionate (27b). A solution of **26b** (1.53 g, 4.32 mmol) in CH_2Cl_2/CF_3CO_2H (1:1 v/v; 20 mL) was stirred at room temperature for 18 h, whereupon an additional amount of CF_3CO_2H (5 mL) was added and stirring continued for 48 h. The solvents and excess CF_3CO_2H were removed in vacuo to provide 0.92 g (71%) of the crude acid as a brown oil: ^1H NMR δ 7.40–7.10 (comp, 10 H), 5.11 (s, 1 H), 3.17 (m, 1 H), 3.07 (dd, J = 6.0, 15.0 Hz, 1 H), 2.82 (d, J = 9.0 Hz, 1 H), 2.77 (dd, J = 3.0, 7.5 Hz, 1 H), 2.71 (d, J = 9.0 Hz, 1 H), 2.46 (dd, J = 4.5, 6.5 Hz, 1 H); mass spectrum, m/z (M + H)$^+$ 299. To a solution of a portion of the crude acid (890 mg, 2.98 mmol) from above in CH_2Cl_2 (10 mL) containing *N*-methylmorpholine (NMM) (0.728 mL, 6.56 mmol) at 0–5 °C was added dropwise isobutyl chloroformate (0.410 mL, 3.13 mmol). The reaction mixture was stirred for 15 min at 0–5 °C, at room temperature for 15 min. After recooling the mixture to 0–5 °C, morpholine (0.394 mL, 4.47 mmol) in CH_2Cl_2 (5 mL) was added, and the reaction mixture was stirred at room temperature overnight. The mixture was then diluted with EtOAc (50 mL), and the resulting mixture was washed sequentially with 1 M HCl, saturated $NaHCO_3$, water, and brine, and dried ($MgSO_4$). Evaporation of the solvents gave a yellow oil, which was purified by flash chromatography (40–50% EtOAc/hexane) to give 0.53 g (48%) of **27b** as an oil: ^1H NMR δ 7.36–7.10 (comp, 10 H), 5.17 (d, J = 12.0 Hz, 1 H), 5.05 (d, J = 12.0 Hz, 1 H), 3.61 (comp, 6 H), 3.39–3.29 (comp, 3 H), 3.06 (dd, J = 6.0, 12.0 Hz, 1 H), 2.80 (dd, J = 9.0, 13.5 Hz, 1 H), 2.71 (dd, J = 9.0, 16.5 Hz, 1 H), 2.32 (dd, J = 4.5, 16.5 Hz, 1 H); mass spectrum, m/z (M + H)$^+$ 368.

(2*S*)-2-Benzyl-3-(4-morpholinylcarbonyl)propionic Acid (28b). A solution of the benzyl ester **27b** (0.5 g, 136 mmol) in MeOH (10 mL) containing 10% Pd/C (250 mg) was hydrogenated at 1 atm for 1.5 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated to give 339 mg (90%) of **28b** as a white foamy solid: ^1H NMR δ 7.35–7.15 (comp, 5 H), 3.62 (comp, 6 H), 3.32–3.16 (comp, 4 H), 2.76 (dt, J = 12.0, 3.0 Hz, 1 H), 2.61 (dd, J = 9.0, 18.0 Hz, 1 H), 2.41 (dd, J = 3.0, 15.0 Hz, 1 H); mass spectrum, m/z (M + H)$^+$ 278.

(2*S*,3*R*,4*S*)-2-[[*N*-[(2*S*)-2-Benzyl-3-(4-morpholinylcarbonyl)propionyl]-3-(4-thiazolyl)-L-alanyl]amino]-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (29a). Prepared according to the general procedure for coupling cyclopropane carboxylic acids using acid **28a**[14b] (188 mg, 0.68 mmol), HOBT (248 mg, 1.8 mmol), EDC·HCl (186 mg, 0.97 mmol), NMM (97 mL, 0.88 mmol), **8** (270 mg, 0.68 mmol): 68% yield (304 mg); mp, 95–112 °C; ^1H NMR ($CDCl_3$) δ 8.77 (d, 1 H), 8.08 (br d, 1 H), 7.42–7.11 (m, 6 H) 6.88 (br d, 1 H), 4.79–4.68 (m, 1 H), 4.35–4.11 (m, 2 H), 3.74–3.42 (m, 8 H), 2.48–2.33 (m, 1 H), 1.98–1.78 (m, 1 H), 0.98–0.83 (2d, 6 H); mass spectrum, m/z (M + H)$^+$ 657.

(1α,2β,3β)-2,3-Diphenylcyclopropanecarboxylic Acid (31). To a solution of *cis*-stilbene (**30**) (1.00 g, 5.56 mmol) and $Rh_2(OAc)_4$ (42 mg, 0.010 mmol) was added ethyl diazoacetate (57 mg, 0.50 mmol) via syringe pump over a period of 8 h. The unreacted stilbene was removed by flash chromatography (20:1; hexanes/EtOAc), and the residue was purified by HPLC (25:1; hexanes/EtOAc) to yield 28 mg (21%) of the *trans*-cyclopropane together with minor amounts (8 mg, 6%) of the all-cis-diastereoisomer. A solution of the cyclopropyl ester (25 mg, 0.094 mmol) in EtOH (3 mL) containing NaOH (38 mg, 0.940 mmol) was heated at reflux for 4 h. The EtOH was evaporated under reduced pressure, and the residue was taken up in H_2O and heated to 80 °C. The H_2O was acidified (2 N HCl), and the product was collected by vacuum filtration and recrystallized from EtOH/H_2O to give **31** as a white solid in 99% yield: mp 153–154 °C; ^1H NMR δ 7.17–7.12 (comp, 6 H), 6.97–6.93 (comp, 4 H), 3.15–3.13 (d, J = 5.2 Hz, 2 H), 2.59–2.55 (t, J = 5.2 Hz, 1 H) (OH's not observed); ^{13}C NMR δ 178.7, 135.1, 128.9, 128.0, 126.6, 33.9, 27.1; IR (CH_2Cl_2) ν 3020, 1700 cm^{-1}; mass spectrum, m/z 238.1011 ($C_{16}H_{14}O_2$ requires 238.0994), 193, 178, 165, 115 (base) 91, 69.

(2*S*,3*R*,4*S*)-2-[[*N*-[(1α,2β,3β)-2,3-Diphenylcyclopropyl)-carbonyl]-3-(4-thiazolyl)-L-alanyl]amino]-1-cyclohexyl-3,4-

dihydroxy-6-methylheptane (32): 82% yield (44 mg); mp 210–214 °C; ^{1}H NMR (DMSO-d_6) δ 9.00 (d, J = 2.0 Hz, 1 H), 8.70 (d, J = 7.5 Hz, 1 H), 7.68 (d, J = 7.5 Hz, 1 H), 7.39 (d, J = 2.0 Hz, 1 H), 7.15–7.05 (comp, 6 H), 6.96 (d, J = 6.0 Hz, 4 H), 4.83 (q, J = 6.0, 12.0 Hz, 1 H), 4.72 (d, J = 6.0 Hz, 1 H), 4.42 (d, J = 4.5 Hz, 1 H), 2.85 (d, J = 4.5 Hz, 1 H), 1.72 (m, 1 H), 0.85 (d, J = 6.0 Hz, 3 H), 0.77 (d, J = 6.0 Hz, 3 H); mass spectrum, m/z $(M + H)^+$ 618. Anal. Calcd for $C_{36}H_{47}N_3O_4S \cdot {}^4/_5H_2O$: C, 68.39; H, 7.75; N, 6.65. Found: C, 68.51; H, 7.66; N, 6.82.

(2*S*,3*R*,4*S*)-2-[[*N*-(Dibenzylacetyl)-3-(4-thiazolyl)-L-alanyl]amino]-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (33). Prepared according to the general procedure for coupling cyclopropane carboxylic acids using dibenzylacetic acid (52 mg, 0.22 mmol), HOBT (82 mg, 0.61 mmol), EDC·HCl (61 mg, 0.32 mmol), NMM (27 mL, 0.25 mmol), **8** (75 mg, 0.19 mmol): 97% yield (114 mg); mp 155–157 °C; ^{1}H NMR ($CDCl_3$) δ 8.61 (d, 1 H), 7.32–7.11 (m, 10 H), 6.69 (br d, 1 H), 6.57 (d, 1 H), 5.70 (br d, 1 H), 4.46–4.36 (m, 1 H), 4.19–4.05 (m, 2 H), 3.24–2.68 (comp, 9 H), 2.43 (br d, 1 H); mass spectrum, m/z $(M + H)^+$ 620.

In Vitro Enzyme Inhibition. Enzyme assays using purified human renin at pH 6.0 and plasma renin at pH 7.4 were performed as previously described.[14b,30] The accuracy of the IC_{50} value determinations is ±25%.

Chymotrypsin Stability Experiments. The stability of compounds **24c** and **29a** toward bovine pancreatic chymotrypsin were determined using a previously described procedure.[30a]

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Supplementary Material Available: Experimental procedures and spectral data for compounds **10a,b,d**, **12a,b,d**, **13a,b,d**, **14a,b,d**, **15a,b,d**, **16a,b,d**, **17a,b,d**, **18a,b,d**, **24a,b,d–h**, **25a–h**, and **29b** (10 pages). Ordering information is given on any current masthead page.