Design and Synthesis of P_2-P_1'-Linked Macrocyclic Human Renin Inhibitors[1]

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Using a computer model of the active site of human renin developed at Merck, we designed a series of novel P_2-P_1'-linked, macrocyclic renin inhibitors **3–10**. These unique inhibitors incorporate a transition-state isostere within a 13- or 14-membered ring. The three most active compounds in this family were 13-membered-ring glutamine-derived inhibitor **3**, 14-membered-ring diaminopropionic acid derived inhibitor **6**, and 13-membered-ring diol **9** (IC_{50} 0.61, 0.59, 0.65 μM, respectively). Modification of inhibitor **3** at P_4 led to 56 nM macrocyclic renin inhibitor **39**. This study shows the viability of renin inhibitor designs which incorporate a scissile-bond replacement within a macrocycle.

Introduction

The renin–angiotensin system (RAS) plays a key role in the regulation of blood pressure and the maintenance of volume homeostasis. Pharmacological interruption of the RAS by inhibition of angiotensin-converting enzyme (ACE) has proven to be effective in the treatment of hypertension.[2] Since ACE also catalyzes the cleavage of a variety of endogenous peptides such as bradykinin and substance P, alternate modes of intervention in the RAS are currently being explored. Renin, a member of the aspartyl proteinase family of enzymes, catalyzes the first and rate-limiting step of the RAS. Unlike ACE, renin has a remarkably narrow substrate specificity, with angiotensinogen (Figure 1) as its only known naturally occurring substrate. This specificity suggests that inhibitors of renin may have pharmacological advantages over ACE inhibitors.[3]

While considerable progress has been made in the design and synthesis of highly potent inhibitors of renin, current inhibitors suffer from limited oral absorption and short duration of action.[4] The development of macrocyclic renin inhibitors[5,6] could provide a solution to these problems. Possible advantages of cyclic inhibitors include stabilization of the resulting structures toward proteolytic enzymes[7] and increased binding affinity due to conformational restriction. The oral bioavailability of cyclosporin,[8] a cyclic undecapeptide, suggests that cyclic renin inhibitors could show enhanced oral absorption. As discussed below, second precedent of particular relevance is afforded by K-13 (**2**, Figure 2), a naturally occurring ACE inhibitor of microbial origin which contains a moderately-sized (16-membered) macrocyclic ring.[9]

Examination of the computational model of the human renin active site developed at Merck[10] suggested that the P_2 and P_1' side chains of angiotensinogen could be linked to provide a viable design for macrocyclic renin inhibitors (Figure 1). Unlike previously reported nonpeptidic macrocyclic renin inhibitors,[6] these structures would contain a scissile-bond replacement within the macrocycle itself. Our approach was to develop a macrocyclic framework which could be elaborated by substitution at specific positions, R^1, R^2, and R^3 (Figure 1), to allow for potential hydrophobic interactions with the enzyme in the S_2, S_1'-S_3', and S_2' sites, respectively. The initial goal of this project was to identify a macrocyclic renin inhibitor suitable for further refinement. Our targets were the unsubstituted 13- and 14-membered ring compounds represented by the general structure **1** (Figure 1, $R^1 = R^2 = R^3 = H$), which incorporate either "norACHPA",[11,12] diol,[13] or hydroxy ketone transition-state isosteres. Herein we report the synthesis of eight members of this family of compounds, macrocycles **3–10**, and their renin inhibitory activity.

Results and Discussion

Presynthetic Molecular Modeling Studies. The computational model of the human renin active site developed at Merck was utilized to design cyclic renin in-

(1) Presented in part at the 200th American Chemical Society National Meeting, Washington, DC, August 26–31, 1990.
(2) Wyvratt, M. J.; Patchett, A. A. *Med. Res. Rev.* **1985**, *5*, 364.
(3) Boger, J. In *Annual Reports in Medicinal Chemistry*; Bailey, D. M., Ed., Academic Press: New York, 1985; Vol. 20, p 257.
(4) Recent reviews: (a) Greenlee, W. J. *Med. Res. Rev.* **1990**, *10*, 173. (b) Luther, R. R.; Stein, H. H.; Glassman, H. N.; Kleinert, H. D. *Arzneim.-Forsch./Drug Res.* **1989**, *39*, 1. (c) Greenlee, W. J. *Pharm. Res.* **1987**, *4*, 364. (d) Wood, J. M.; Stanton, J. L.; Hofbauer, K. G. *J. Enzyme Inhib.* **1987**, *1*, 169. (e) Antonaccio, M. J.; Wright, J. J. *Prog. Drug Res.* **1987**, *31*, 161. (f) Kokubu, T.; Hiwada, K. *Drugs Today* **1987**, *23*, 101. (g) Boger, J. *Trends Pharmacol. Sci.* **1987**, *8*, 370.
(5) For cyclic peptide inhibitors of renin, see: (a) Boger, J. In *Aspartic Proteinases and Their Inhibitors*; Kostka, V., Ed.; Walter de Gruyter: Berlin, 1985; p 401. (b) Nakaie, C. R.; Oliviera, M. C. F.; Juliano, L.; Pesquero, J. L.; Paiva, A. C. M. In *Peptides, Structure and Function. Proceedings of the Eight American Peptide Symposium*; Hruby, V. J., Rich, D. H., Eds., Pierce Chemical Co.: Rockford, IL, 1983, p 595. (c) Sawyer, T. K.; Pals, D. T.; Smith, C. W.; Saneii, H. S.; Epps, D. E.; Duchamp, D. J.; Hester, J. B.; TenBrink, R. E.; Staples, D. J.; deVaux, A. E.; Affholter, J. A.; Skala, G. F.; Kati, W. M.; Lawson, J. A.; Schuette, M. R.; Kamdar, B. V.; Emmert, D. E. In *Peptides, Structure and Function. Proceedings of the Ninth American Peptide Symposium*; Deber, C. M.; Hruby, V. J.; Kopple, K. D., Eds., Pierce Chemical Co.: Rockford, IL, 1985; p 729.
(6) For other cyclic renin inhibitors, see: (a) Sham, H. L.; Bolis, G.; Stein, H. H.; Fesik, S. W.; Marcotte, P. A.; Plattner, J. J.; Rempel, C. A.; Greer, J. *J. Med. Chem.* **1988**, *31*, 284. (b) Sham, H. L.; Rempel, C. A.; Stein, H. H.; Cohen, J. *J. Chem. Soc., Chem. Commun.* **1990**, 666.
(7) Ovchinnikov, Y. A.; Ivanov, V. T. *The Proteins*, 3rd ed.; Neurath, H.; Hill, R. L., Eds., Academic Press: New York, 1982; Vol. 5, p 307.
(8) Grevel, J. *Transplant. Proc.* **1986**, *18, Suppl. 5*, 9.
(9) Kase, H.; Kaneko, M.; Yamada, K. *J. Antiobiot.* **1987**, *40*, 450.
(10) 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.; Straus, J. F.; Vlasuk, G. P.; Hoogsteen, K.; Springer, J. P.; Bush, B. L.; Halgren, T. A.; Richards, A. D.; Jay, K.; Veber, D. F. *J. Med. Chem.* **1991**, *34*, 887.
(11) Iizuka, K.; Kamijo, T.; Harada, H.; Akahane, K.; Kubota, T.; Umeyama, H.; Kiso, Y. *J. Chem. Soc., Chem. Commun.* **1989**, 1678.
(12) NorACHPA refers to (2*R*,3*S*)-3-amino-4-cyclohexyl-2-hydroxybutanoic acid.
(13) For other uses of a diol transition-state isostere in renin inhibitors, see: 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. *J. Med. Chem.* **1988**, *31*, 2264 and references therein.

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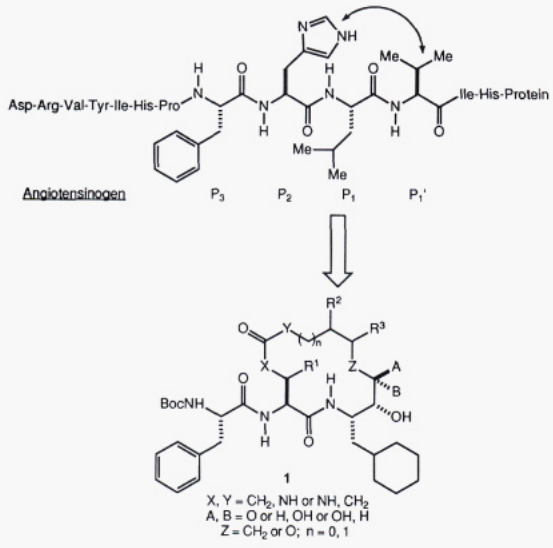


Figure 1. Macrocyclic inhibitor targets.

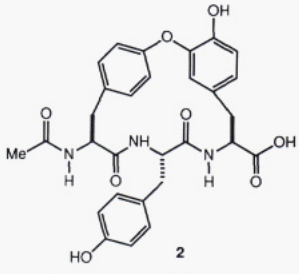


Figure 2. The structure of K-13 (**2**).

hibitors **3–10** as targets for chemical synthesis. This model[10] is based on the sequence homology of renin, human pepsin, and several fungal aspartic proteinases for which crystal structures are available. Examination of the model suggested that the P_2 and P_1' side chains of angiotensinogen occupy a common binding pocket within the enzyme, implying that macrocyclic inhibitors could be developed by linking these elements. After we had explored a number of computational motifs for doing so, we noted with interest the structure of **2** (Figure 2), the naturally occurring ACE inhibitor K-13[9] in which a phenyl-O-phenyl moiety links residues n and $n + 2$. In the context of a renin inhibitor this moiety could be taken to represent bridged P_2 and P_1' side chains, with the phenyl ring nearest the N terminus corresponding to His 9 of angiotensinogen. In our hands, linked aromatic rings provided an unacceptable geometric constraint. However, we did succeed in finding alternative linkages based on the norACHPA replacement for the Leu 10-Val 11 dipeptide of angiotensinogen, which closed the macrocyclic ring in a way which preserved reasonable bond lengths, bond angles, and, in most cases, torsion angles. The calculations on perspective macrocycles used the Merck program OPTIMOL (see Experimental Section) to energetically optimize the macrocycles in isolation and as bound to the static renin active site.

The nature of one such P_2-P_1' linkage is illustrated in Figure 3, which shows an overlay of the macrocyclic portion of inhibitor **3** and angiotensinogen as its scissile bond (Leu 10-Val 11) hydrate, each as energy minimized by OPTIMOL in the static renin active site. The P_3 and P_4 residues of **3** (not shown in Figure 3) and of angiotensinogen adopt closely similar positions, implying that structure–activity relationships observed at P_3 and P_4 in acyclic renin inhibitors may also hold for macrocyclic inhibitors of the present class. As shown in the figure, these N-terminal

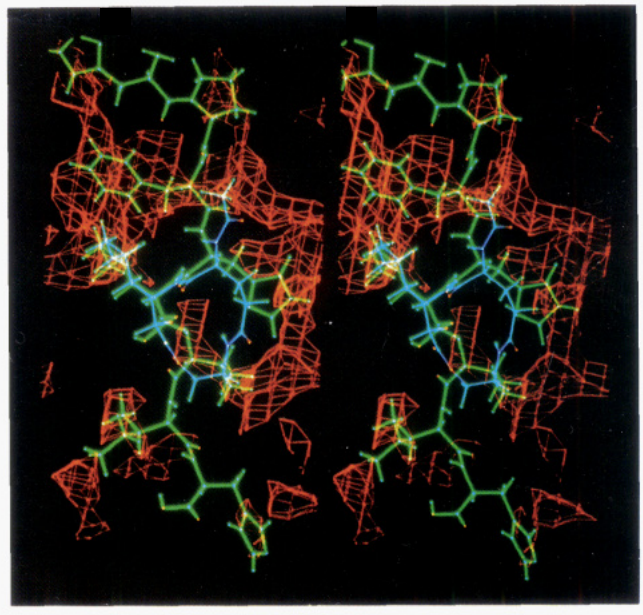


Figure 3. Overlay of the macrocycle of inhibitor **3** (blue) with angiotensinogen as its scissile bond hydrate (green). Hydrophobic regions of the enzyme (see Experimental Section) are mapped in red.

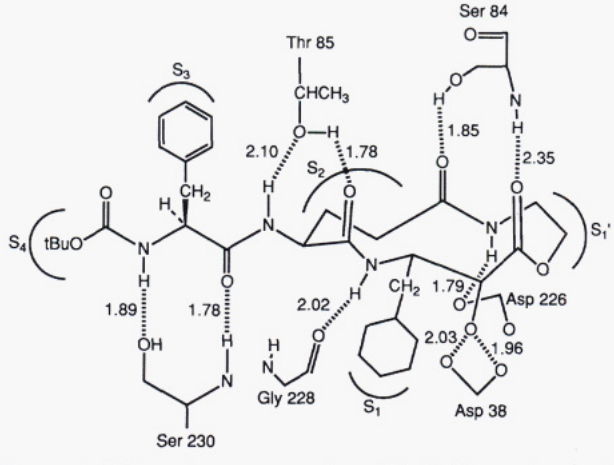
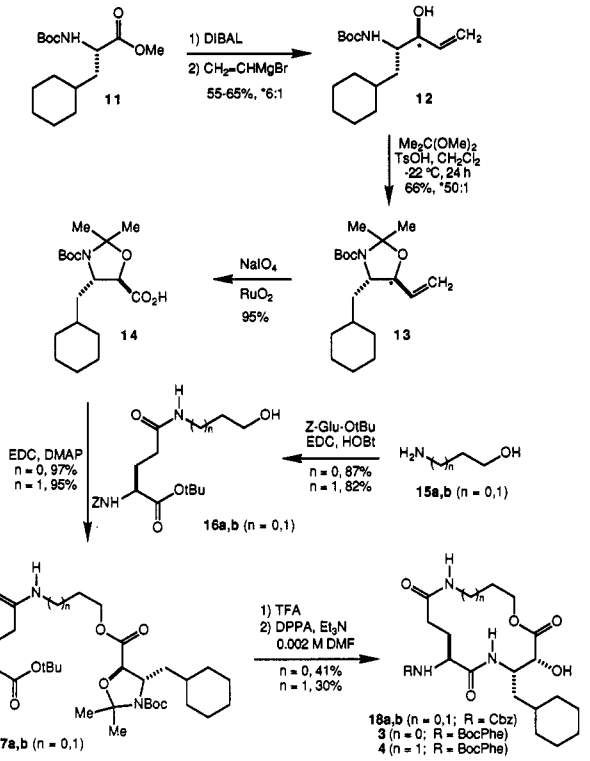


Figure 4. Schematic representation of the hydrogen bond network of macrocycle **3** as energy-optimized in the static human renin model using a dielectric constant of 1 for intermolecular interactions. Hydrogen-bond distances are in angstroms.

residues tend to lie in regions of the space accessible to a bound ligand which we classify as predominantly hydrophobic in nature (see the Experimental Section).

Hydrogen-bonding interactions between the enzyme and the full structure for inhibitor **3** found upon energy minimization in the static renin active site using a gas-phase dielectric constant of 1 are represented schematically in Figure 4. [Hydrogen-bond lengths obtained with a larger dielectric constant of 50 (see Experimental Section) are uniformly longer, usually by 0.1–0.3 Å, but by as much as 0.5 Å (from the Ser 84 hydroxyl), 0.6 Å (to one oxygen of Asp 38) and 1.0 Å (to Gly 228).] In inhibitor **4**, incorporation of the additional methylene group into the P_2-P_1' bridge produces only slight changes in the hydrogen-bond distances. In the computational model, the three macrocyclic carbonyl groups form hydrogen bonds with the renin "flap" residues Thr 85 and Ser 84. Particularly noteworthy in this model is that the glutamine side chain N–H donates a hydrogen bond to one of the active site aspartic acid carboxylate oxygens. The related diaminopropionic acid derived inhibitors **5** and **6** bind in the computational model

Scheme I. Synthesis of Glutamine-Derived Macrocycles **3** and **4**



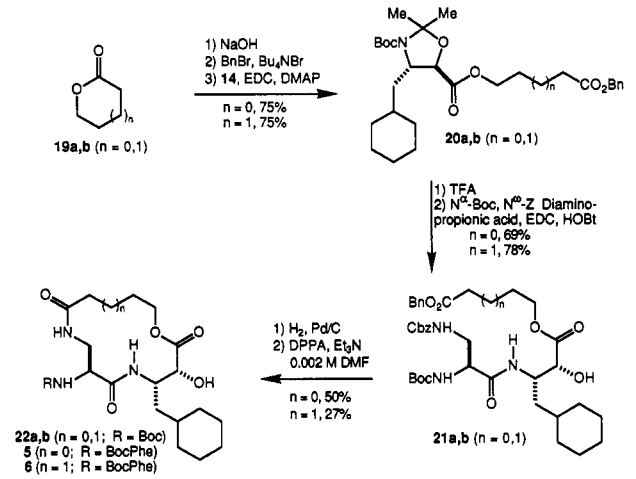
in an analogous fashion with the N–H of the diaminopropionic acid side chain interacting with an active site aspartate. These "reverse amide" structures eliminate an eclipsing interaction between the glutamine β- and γ-carbons in inhibitors **3** and **4**; this eclipsing interaction is required in inhibitors **3** and **4** to allow the glutamine side chain N–H to hydrogen bond to one of the aspartates in the manner shown in Figure 4.

Modeling studies also suggested that replacing the endocyclic ester oxygen with a methylene group would relieve a potentially unfavorable electrostatic interaction between this oxygen and the active site aspartates. This suggestion led to the selection of ketone **8** and diol **9** as synthetic targets. In the computational studies, these inhibitors also bind analogously, with the additional hydroxyl in inhibitor **9** interacting with Ser 84 of the renin flap in the manner shown in Figure 4 for the corresponding carbonyl group of inhibitor **3**. Diol **10** was predicted (and shown) to be the less active diastereomer because its hydroxyl group was not oriented in a manner which would allow for such a hydrogen-bonding interaction.

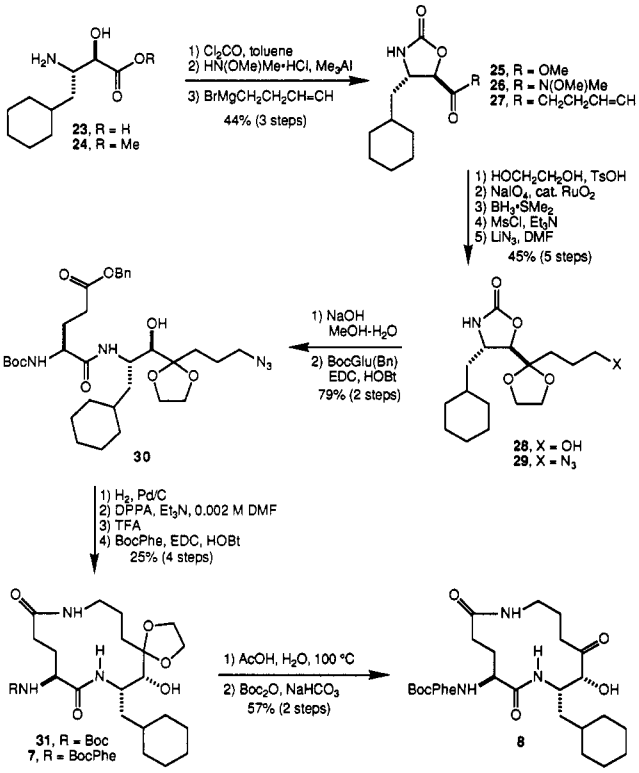
Chemistry. The synthesis of glutamine-derived macrocycles **3** and **4** is illustrated in Scheme I. *N*-Boc-cyclohexylalanine methyl ester (**11**) was reduced with diisobutylaluminum hydride followed by in situ treatment with vinylmagnesium bromide according to the procedure of Abbott workers[14] to give alcohol **12** as a 6:1 mixture at the newly formed asymmetric center. Kinetic resolution occurred during acetonide formation at low temperature to give an enrichment of *trans*-oxazolidine **13**. Typically, this reaction was run at −22 °C for 24 h to provide a 66% yield of oxazolidine **13** as a 50:1 mixture of isomers. Oxidation of the olefin using ruthenium oxide afforded the desired acid **14** in 95% yield. Glutamine derivative **16a** or **16b** was prepared by treatment of the appropriate amino alcohol **15a** or **15b** with *N*-Cbz-glutamic acid benzyl ester employing ethyl(dimethylamino)propylcarbodiimide

(14) Rosenberg, S. H.; Plattner, J. J.; Luly, J. R. Eur. Patent Appl. 0 230 266, 1987.

Scheme II. Synthesis of Diaminopropionic Acid Derived Macrocycles **5** and **6**



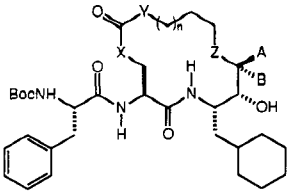
Scheme III. Synthesis of Macrocyclic Ketone **8**



(EDC), and hydroxybenzotriazole (HOBt). The resultant alcohol was then coupled to acid **14** with EDC/4-(dimethylamino)pyridine (DMAP) to provide cyclization precursor **17a** or **17b**. Deprotection followed by macrocyclization with diphenyl phosphorazidate (DPPA) under high-dilution conditions gave the desired macrocycle **18a** or **18b**. The Cbz group was hydrogenolytically removed and the resultant amine was coupled with *N*-Boc-phenylalanine (BocPhe) to provide macrocycle **3** or **4**.

Scheme II outlines the synthesis of diaminopropionic acid derived macrocycles **5** and **6**. The requisite lactone **19a** or **19b** was treated with aqueous sodium hydroxide followed by benzyl bromide. The resultant hydroxy ester was coupled with acid **14** by using EDC/HOBt to provide benzyl ester **20a** or **20b** in 75% overall yield. Following deprotection with trifluoroacetic acid (TFA), the resultant amine was treated with N^{α}-Boc-N^{ω}-Cbz-diaminopropionic acid, EDC, and HOBt to give macrocycle precursor **21a** or **21b**. Hydrogenolysis the benzyl ester and Cbz groups followed by macrocyclization with DPPA afforded the

Table I. Inhibition of Human Plasma Renin at pH 7.4[a]



compound	*n*	X	Y	Z	A	B	IC_{50}, μM
3	0	CH_2	NH	O	=O		0.61
4	1	CH_2	NH	O	=O		7.0
5	0	NH	CH_2	O	=O		9.2
6	1	NH	CH_2	O	=O		0.59
7	0	CH_2	NH	CH_2	$-OCH_2CH_2O-$		>20
8	0	CH_2	NH	CH_2	=O		14
9	0	CH_2	NH	CH_2	H	OH	0.65
10	0	CH_2	NH	CH_2	OH	H	>20

[a] See ref 18 for assay details.

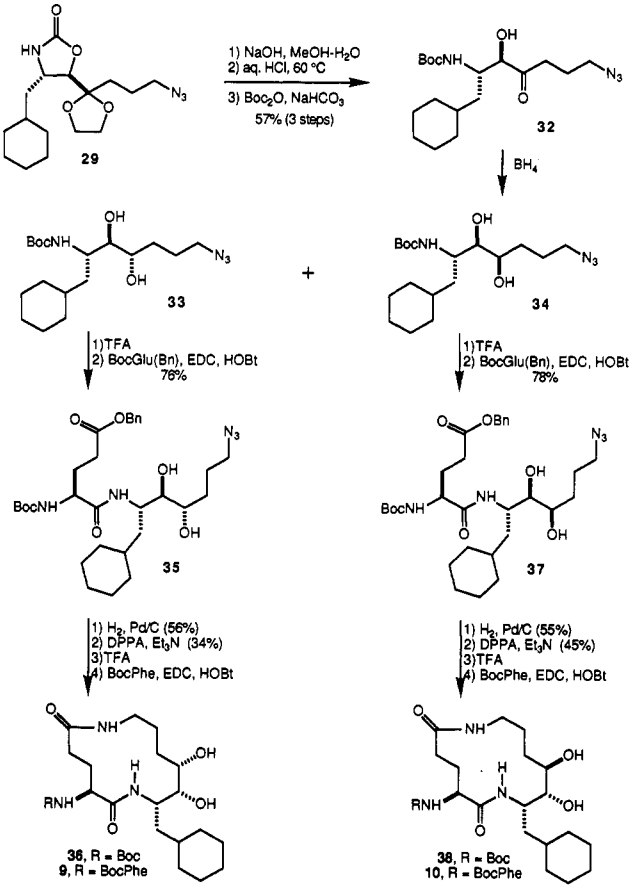
desired macrocycle **22a** and **22b**. The Boc group was removed with TFA and the amine was then coupled to BocPhe to give inhibitors **5** and **6**.

Macrocyclic ketone **8** and its ketal derivative **7** were synthesized as shown in Scheme III. The known acid[11] **23** was converted to its methyl ester **24** by using standard conditions (anhydrous HCl in methanol). Ester **24** was treated with phosgene to provide oxazolidinone **25**. This compound was converted to amide **26** by using the procedure of Weinreb and co-workers.[15] Treatment with 3-butenylmagnesium bromide provided ketone **27**.[16] Following protection of the ketone as a dioxolane derivative, the olefin was oxidized to the corresponding acid with ruthenium oxide. The acid was reduced with borane to provide alcohol **28**. This compound was treated with methylsulfonyl chloride and triethylamine followed by lithium azide to give azide **29**. The oxazolidinone was hydrolyzed with base, and the resultant amine was coupled with *N*-Boc-glutamic acid ω-benzyl ester to provide macrocyclization precursor **30**. Treatment with hydrogen over palladium on carbon followed by cyclization using DPPA gave macrocycle **31**. The Boc protecting group was removed by treatment with TFA and the resultant amine was coupled to BocPhe to afford macrocycle **7**. The ketal was hydrolyzed with hot acetic acid with concommitant removal of the Boc group. Reacylation with di-*tert*-butyl pyrocarbonate gave inhibitor **8**.

The synthesis of diols **9** and **10** is shown in Scheme IV. Oxazolidinine **29** (see Scheme III) was hydrolyzed under basic conditions and the ketal was hydrolyzed with dilute acid. Treatment of the resultant aminoketone with di-*tert*-butyl pyrocarbonate provided ketone **32** in good overall yield. Reduction of this compound with sodium borohydride in methanol at room temperature produced a 4:5 mixture of diols **33** and **34**. In contrast, treatment of ketone **32** with zinc borohydride according to the procedure of Nakata and co-workers[17] gave only diol **33** (>10:1 by 300-MHz ^1H NMR). Since this procedure is known to selectively reduce α-hydroxy ketones via a chelated intermediate, the relative stereochemistry of the two compounds was established as shown.

Diol **33** was treated with TFA and then coupled to *N*-Boc-glutamic acid ω-benzyl ester to give cyclization precursor **35**. This compound was converted to inhibitor **9** by following the same protocol outlined in Scheme III for the conversion of cyclization precursor **30** to inhibitor **7**. Diol **34** was incorporated into inhibitor **10** in an analogous fashion.

Scheme IV. Synthesis of Macrocyclic diols **9** and **10**



In Vitro Renin Inhibition. Compounds **3**–**10** were evaluated in vitro for human plasma renin inhibition at pH 7.4 according to the protocol of Boger and co-workers.[18] The results are summarized in Table I. In the glutamine series, 13-membered-ring compound **3** was 10 times more active than 14-membered-ring compound **4**. The opposite trend was seen in the diaminopropionic acid series. 14-

(15) Levin, J. I.; Turos, E.; Weinreb, S. M. *Synth. Commun.* **1982**, *12*, 989.
(16) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3818.
(17) Nakata, T.; Tanaka, T.; Oishi, T. *Tetrahedron Lett.* **1983**, *24*, 2653.
(18) Boger, J.; Payne, L. S.; Perlow, D. S.; Lohr, N. S.; Poe, M.; Blaine, E. H.; Ulm, E. H.; Schorn, T. W.; LaMont, B. I.; Lin, T.-Y.; Kawai, M.; Rich, D. H.; Veber, D. F. *J. Med. Chem.* **1985**, *28*, 1779.

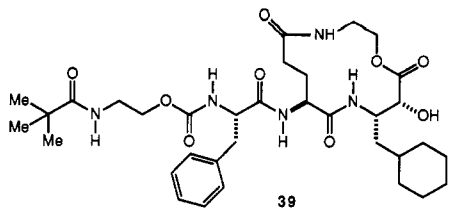


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Figure 5.

membered-ring derivative **6**, nearly equipotent with inhibitor **3**, was 1 order of magnitude more active than 13-membered ring compound **5**. Of the ketal (compound **7**), ketone (compound **8**), and diols (compounds **9** and **10**), only *R,S*-diol **9** showed activity that was comparable to those of inhibitors **3** and **6**.

In an effort to achieve a more potent macrocyclic inhibitor, macrocycle **18a** was deprotected and acylated with *N*-[[2-[(*tert*-butylacetyl)amino]ethoxy]carbonyl]phenylalanine[19] to give glutamine-derived inhibitor **39** (Figure 5), with an IC_{50} of 56 nM.

Molecular Modeling Discussion. While the human renin model enabled us to design novel P_2-P_1'-linked macrocycles, calculations using this model were not able to explain correctly all the trends in renin inhibitory activity found. For example, modeling did not correctly predict which ring size (13 or 14) or which amide placement (glutamine or "reverse amide") would result in the most active inhibitor. In addition, the ester to ketone transformation (inhibitor **3** vs **8**) led to an unexplained 200-fold decrease in activity. These deficiencies might in principle stem from any of a number of sources. For example, our work relied on a static model for human renin[10] which was of computational rather than experimental origin.[20] A second limitation is that we have not been able to completely examine the conformational space available to the isolated and renin-bond inhibitors, and so may have reached conclusions based on inappropriate structures. Moreover, our computational studies on the isolated and renin-bond inhibitors employed a force field model (see the Experimental Section) which does not take into account the very sizeable effects of solvation. The latter point is a crucial one: even when an experimental enzyme structure is employed, interactions which appear favorable in *pseudo-gas-phase* calculations (such as those which underlie the computational models for inhibitor binding presented here) will in reality be unfavorable whenever solvent water stabilizes the inhibitor more strongly than does its interaction with the enzyme.

The calculations did give indications that these inhibitor designs, while reasonable, are not optimal. Thus, each of the proposed macrocyclic ring conformers when optimized in isolation (off the enzyme) was found to lie within 1–3 kcal/mol of the lowest conformer located through conformational searching (see the Experimental Section), such that the estimated conformational population for the proposed bound conformer lay in the range 0.01%–10%. However, the calculations also predicted that the detailed adaptation of the freely optimized conformer upon binding to the renin site would entail an additional energy cost of from 3 to 10 kcal/mol. One measure of the adaptation required can be seen in the optimized hydrogen-bond distances; as noted in the section Presynthetic Molecular Modeling Studies, the distances shown for inhibitor **3** in Figure 4 lengthen, sometimes modestly but by up to 1 Å, when the optimization is carried out with a dielectric constant of 50. Computationally, therefore, these inhibitor designs would appear to suffer to some extent from the availability of lower-energy "free-solution" conformers and from the need to incur moderate strain energy upon binding to the renin site. Probably because of the intrinsic limitations of the modeling approach used here, however, it did not prove possible to acount for the relative inhibitory potency in a consistent fashion on the basis of the calculated conformational populations and adaptation-energy costs.

Conclusions

The computational model of the human renin active site developed at Merck was instrumental in the design of P_2-P_1' linked macrocyclic renin inhibitors. Eight different macrocycles were synthesized and three were shown to be 0.5 μM renin inhibitors: 13-membered-ring glutamine derivative **3**, 14-membered-ring diaminopropionic acid derivative **6**, and 13-membered-ring diol **9**. Modification of the N-terminus of macrocycle **3** led to the synthesis of a more potent macrocyclic renin inhibitor, compound **39** (IC_{50} = 56 nM).

This study showed the viability of renin inhibitor designs which incorporate a scissile-bond isostere with a macrocycle. Further modification of macrocycles **3**, **6**, or **9** by substitution of R^1, R^2, and R^3 (Figure 1) on the macrocyclic ring could allow for potential hydrophobic interactions with the enzyme, leading to more potent inhibitors. These studies are in progress and will be reported in due course.

Experimental Section

Molecular Modeling Procedures. Energy optimizations of inhibitor–renin complexes used a renin binding site model of the type previously described.[10] Macrocyclic portions of compounds **3–10** were constructed by hand modeling and energy optimization within the renin model using an implementation of the Merck modeling program MOLEDIT[21] for PS-300 work stations.[22] This implementation uses graphics protocols derived from the crystallographic modeling program FRODO.[23] The macrocyclic portions used in most optimizations and in conformational searches simplified the full structures of compounds **3–10** by replacing Boc-Phe (P_4-P_3) by acetyl and substituting isopropyl for cyclohexyl at P_1 (as in Leu 10 of angiotensinogen). The optimizations employed the Merck-developed force field MM2X as implemented in the molecular-mechanics program OPTIMOL.[24] MM2X shares many force-field parameters with MM2,[25] and differs from it mainly in the use of partial charges on atoms, instead of bond dipoles, and in the absence of lone-pair centers on nitrogen and oxygen atoms. MM2X (like MM2) by default uses a dielectric constant of 1.5 for intramolecular interactions, but MM2X uses a dielectric constant of 1.0 for intermolecular (e.g., inhibitor–enzyme) interactions. To

(19) In acyclic renin inhibitors, this derivative affords up to a 10-fold increase in potency over the Boc-Phe derivative. Schoen, W. R.; Yager, C. A., unpublished results.

(20) A crystal structure of recombinant human renin at 2.5-Å resolution was published after the completion of this work. Crystallographic coordinates were not, however, reported: Sielecki, A. R.; Hayakawa, K.; Fujinaga, M.; Murphy, M. E. P.; Fraser, M.; Muir, A. K.; Carilli, C. T.; Lewicki, J. A.; Baxter, J. D.; James, M. N. G. *Science* **1989**, *243*, 1346.

(21) (a) Gund, P.; Andose, J. D.; Rhodes, J. B.; Smith, G. M. *Science* **1980**, *208*, 1425. (b) Smith, G. M.; Hangauer, D. G.; Andose, J. D.; Bush, B. L.; Fluder, E. M.; Gund, P.; McIntyre, E. F. *Drug Inf. J.* **1984**, *18*, 167.

(22) Bush, B. L., Merck Sharp and Dohme Research Laboratories, Rahway, NJ, unpublished results.

(23) Jones, T. A. *J. Appl. Crystallogr.* **1978**, *11*, 268.

(24) Halgren, T. A.; Nachbar, R. B.; Bush, B. L.; Smith, G. M.; Fluder, E. M.; Andose, J. D., Merck Sharp and Dohme Research Labs, unpublished results.

(25) (a) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127. (b) Burkert, U.; Allinger, N. L. *Molecular Mechanics*; ACS Monograph 177; American Chemical Society: Washington, DC, 1982.

simulate the effects of solvation in reducing the magnitude of electrostatic interactions, most calculations on isolated inhibitors and on inhibitor-enzyme complexes used a larger constant dielectric constant of 50.

The acetyl-capped macrocyclic rings of inhibitors such as 3 and 6 were constructed from energetically docked acyclic precursors which possessed P_2 and P_1' "stubs" by incrementally "growing" on and energy optimizing one or more additional carbon or heteroatoms until it became possible to visualize a ring closure which could span the open segment with reasonable bond lengths and angles. Other putative inhibitors were generated by simple, localized replacement. To determine whether the renin-bound conformations for the macrocyclic inhibitors were chemically reasonable, extensive (but, given the dimensionality of the conformational space, incomplete) computational searches for low-energy conformations were carried out by generating 200–1000 trial conformations with a distance geometry[26] algorithm implemented in MOLEDIT.[27] The distance-geometry structures were then optimized with OPTIMOL (usually at a constant dielectric constant of 50) and were classified into families having macrocyclic rings of similar shape with ANAL_CONF.[28]

Qualitative judgements of inhibitor binding were aided by specificity maps for the human renin model generated with an "active-site mapping" protocol developed at Merck.[29] The active-site mapping procedure operates in a manner analogous to Goodford's GRID algorithm[30] by constructing a grid of points in the space accessible to a ligand in the region of the enzyme active site. In the Merck approach, "hydrophobic" and "hydrophilic" regions are defined in a way which considers both spatial proximity and suitability for occupancy by solvent water. A putative van der Waals (vdW) energy and the electrostatic field magnitude (calculated in a distance-dependent dielectric formulation) are computed for a probe centered at each such grid point by considering interactions with all atoms of the enzyme site. A measure of "hydrophilicity" is then constructed by adding a (negative) "electric-field reward" term to the vdW term. *Hydrophilic* regions are then those within which the sum of the two terms is sufficiently negative and are revealed graphically by contouring (using FRODO[23]) the "hydrophilic map" at a prescribed negative threshold. Conversely, the quantity representing "hydrophobicity" is constructed by adding a (positive) "electric-field penalty" term to the vdW term, and *hydrophobic* regions are taken to be those for which the negative (favorable) vdW term is not too strongly degraded by the positive electric-field term. Qualitatively, therefore, hydrophobic regions are those which lie in suitably close contact with the surface of the enzyme site but for which the water-dipole orienting electric field produced by the enzyme site is sufficiently small. Hydrophobic regions too, are revealed by contouring the "hydrophobic map" at a suitable negative energy threshold. For the human renin model, the hydrophobic regions defined in this way are illustrated in red in Figure 3.

Chemistry Procedures. ^{1}H NMR were recorded on a Varian XL-300 spectrometer (300 MHz). FAB mass spectra were obtained on a MAT 731 spectrometer at 8 keV. Analytical thin layer chromatography (TLC) was performed with EM Reagents 0.25-mm silica gel 60-F plates, eluting with the indicated solvent system. Chromatography on silica gel was performed with a forced flow of the indicated solvent system (flash chromatography) on EM Reagents silica gel 60 (230–400 mesh). Medium-pressure liquid chromatography (MPLC) was carried out with EM Reagents Lobar silica gel 60 prepacked columns (column size indicated). Solvents and reagents were used as received.

General Procedure A: Amide Formation Using EDC/HOBt. A solution of the appropriate acid (1–2 equiv) and amine (1–3 equiv) [or ammonium salt and 1.2 equiv of *N*-methylmorpholine (NMM) or triethylamine (TEA)] in dichloromethane (0.05–0.2 M) was cooled to 0 °C and treated with hydroxybenzotriazole monohydrate (HOBt, 1–2 equiv) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC, 1–2 equiv). The solution was stirred overnight with gradual warming to room temperature. The mixture was applied directly to a silica gel column or an aqueous workup was performed as follows. The solution was diluted with ethyl acetate, washed sequentially with 1 N aqueous sodium bisfulate, water, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride, dried over anhydrous magnesium sulfate, and concentrated. Purification by silica gel and/or Sephadex LH-20 gel chromatography provided the amide.

General Procedure B: Esterification Using EDC/DMAP. A solution of the appropriate acid and alcohol (0.95–1.2 equiv) in dichloromethane (0.1–0.33 M) was cooled to 0 °C, and 4-(dimethylamino)pyridine (DMAP, 0.05–0.1 equiv) and EDC (1.5–3 equiv) was added. The mixture was stirred at 0 °C for 2–16 h, until the reaction was judged complete by TLC analysis. The solution was then diluted with ethyl acetate, washed sequentially with 1 N aqueous sodium bisulfate, water, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride, dried over anhydrous magnesium sulfate, and concentrated. Purification by silica gel chromatography provided the desired ester in good yield.

General Procedure C: Macrocyclization Using DPPA. The substrate was dissolved in dimethylformamide (DMF) to form a 0.002 M solution. The solution was cooled to 0 °C and treated with diphenyl phosphorazidate (2.0 equiv) and triethylamine (2.2 equiv). After the reaction mixture was stirred at 0 °C for several hours, 7.5 °C for 3 days, and room temperature for 16 h, the DMF was removed in vacuo. The residue was dissolved in ethyl acetate, washed sequentially with 1 N aqueous sodium bisulfate, water, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride, dried over anhydrous magnesium sulfate, and concentrated. Purification by silica gel and/or Sephadex LH-20 gel chromatography provided the macrocycles.

General Procedure D: Deprotection Using TFA. A solution of the substrate in 1:1 dichloromethane/trifluoroacetic acid (TFA) was stirred at room temperature until the reaction was judged complete by TLC analysis (0.5–6 h). The solution was concentrated and trace amounts of acid were removed azeotropically with tetrahydrofuran and toluene. The resultant oil was dried over P_2O_5/KOH under vacuum for several hours prior to use.

General Procedure E: Deprotection Using TFA/DMS. A solution of substrate in 4:1 TFA/dimethyl sulfide (DMS) was stirred at room temperature for 6–8 h or overnight. The solution was concentrated and trace amounts of acid were removed azeotropically with methanol and toluene. The resultant oil was dried over P_2O_5/KOH under vacuum for several hours prior to use.

General Procedure F: Deprotection Using H_2. A solution of substrate in the indicated solvent was stirred with 10% Pd/C under 1 atm of hydrogen for several hours until the deprotection was judged complete by TLC analysis. The mixture was filtered through Celite and concentrated.

(4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyl-5-vinyloxazolidine (13). A solution of 34.6 g (122 mmol, 1.0 equiv) of alcohol **12**[31] (6:1 *S*/*R* mixture at C-3) and 1.16 g (6.10 mmol, 0.05 equiv) of *p*-toluenesulfonic acid monohydrate in 530 mL of dichloromethane was cooled to −78 °C and 63.5 g (75.0 mL, 61.0 mmol, 5 equiv) of dimethoxypropane was added. The reaction mixture was stirred at −22 °C overnight and then quenched by the addition of 1.23 g (1.70 mL, 12.2 mmol, 0.1 equiv) of triethylamine. The solution was washed sequentially with 250-mL portions of saturated aqueous sodium bicarbonate solution and 1 N aqueous sodium bisulfate solution, dried over anhydrous magnesium sulfate, and concentrated to give 43 g of an oil. Purification by silica gel chromatography (Waters Prep 500, 4% ethyl acetate/hexane) gave 25.9 g (66% yield, >97% diastereomeric purity by 300-MHz ^{1}H NMR) of the title compound as an oil: R_f 0.25 (5% ethyl acetate/hexane); NMR ($CDCl_3$)

(26) (a) Crippen, G. M.; Havel, T. F. *Acta Crystallogr. A.* **1987**, *34*, 282. (b) Crippen, G. M. *Distance Geometry and Conformational Calculations*; John Wiley & Sons: New York, 1981.
(27) The MOLEDIT implementation of distance geometry (Andose, J. D., Merck Sharp and Dohme Research Labs, unpublished results) is based on subroutine EMBEDD written by G. M. Crippen.
(28) Halgren, T. A. Unpublished results.
(29) Halgren, T. A.; Bush, B. L., unpublished results.
(30) Goodford, P. *J. Med. Chem.* **1985**, *28*, 849.
(31) For the synthesis of this compound see ref 14.

δ 5.95 (ddd, 1 H, J = 7.1, 10.3, 17.1 Hz), 5.33 (d, 1 H, J = 17.1 Hz), 5.23 (d, 1 H, J = 10.3 Hz), 4.26 (dd, 1 H, J = 3.5, 7.1 Hz), 3.81 (br s, 1 H), 1.98–0.85 (m, 19 H), 1.47 (s, 9 H); MS (FAB) m/e 378 (M + 1 + matrix (dithiothreitol) – Boc). Anal. ($C_{19}H_{33}NO_3$) C, H, N.

(4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyloxazolidine-5-carboxylic Acid (14). To a solution of 25.9 g (80.1 mmol, 1.0 equiv) of oxazolidine **13** in 1500 mL of acetone at room temperature was added in four portions over 3 h a solution of 102.8 g (480 mmol, 6.0 equiv) of sodium periodate and 1.07 g (4.01 mmol, 0.05 equiv) of 50% ruthenium dioxide on carbon in 1500 mL of water. After the final addition, the reaction was judged complete by TLC analysis and excess reagent was quenched by the addition of 14 mL of isopropyl alcohol. The resultant mixture was filtered through Celite and concentrated. The aqueous residue was diluted with 2 L of 1:1 1 N aqueous sodium bisulfate and 1 N aqueous sodium bisulfite and extracted with four 750-mL portions of dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate and decolorized with activated charcoal. Concentration gave 25.9 g (95%) of a slightly green solid. An analytical sample was prepared by recrystallization from ethyl acetate/hexane: R_f 0.30 (10% MeOH/CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 4.38 (s, 1 H), 4.35 (br s, 1 H), 1.93 (br d, J = 12 Hz), 1.80–0.85 (m, 12 H), 1.66 (s, 3 H), 1.58 (s, 3 H), 1.48 (s, 9 H); MS (FAB) m/e 342 (M + 1), 286, 242. Anal. ($C_{18}H_{31}NO_5$) C, H, N.

Z-Glu(2-hydroxyethyl)-OtBu (16a). Ethanolamine (362 mg, 5.92 mmol, 3.0 equiv) and Z-Glu·DCHA (1060 mg, 1.97 mmol, 1.0 equiv) were coupled according to general procedure A. Purification (MPLC, Lobar C, 80:5:0.5 chloroform/methanol/ammonium hydroxide) gave 650 mg (87%) of the title compound: R_f 0.21 (80:5:0.5 chloroform/methanol/ammonium hydroxide); NMR ($CDCl_3$) δ 7.4–7.2 (m, 5 H), 6.39 (br s, 1 H), 5.60 (br s, 1 H), 5.10 (s, 2 H), 4.22 (br m, 1 H), 3.78–3.63 (m, 2 H), 3.54–3.47 (br m, 1 H), 3.30–3.21 (br m, 1 H), 2.34–2.09 (m, 6 H), 1.83 (m, 1 H), 1.46 (s, 9 H); MS (FAB) m/e 381 (M + 1), 325, 281. Anal. ($C_{19}H_{28}N_2O_6$) C, H, N.

Z-Gln(3-hydroxypropyl)-OtBu (16b). Propanolamine (445 mg, 5.92 mmol, 3.0 equiv) and Z-Glu·DCHA (1060 mg, 1.97 mmol, 1.0 equiv) were coupled according to general procedure A. Purification (MPLC, Lobar C, 80:5:0.5 chloroform/methanol/ammonium hydroxide) gave 640 mg (82%) of the title compound: R_f 0.25 (80:5:0.5 chloroform/methanol/ammonium hydroxide); NMR ($CDCl_3$) δ 7.40–7.32 (m, 5 H), 6.54 (br m, 1 H), 5.57 (br m, 1 H), 5.11 (s, 2 H), 3.63 (t, J = 5.7 Hz, 2 H), 3.59–3.32 (m, 3 H), 2.31–2.15 (m, 4 H), 1.98–1.83 (m, 5 H), 1.70–1.53 (m, 2 H), 1.46 (s, 9 H); MS (FAB) m/e 395 (M + 1), 339, 295. Anal. ($C_{20}H_{30}N_2O_6$) C, H, N.

N^ω-[2-[[[(4*S*,5*R*)-4-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolindyl]carbonyl]oxy]ethyl]-N^α-[(phenylmethoxy)carbonyl]-L-glutamine 1,1-Dimethylethyl Ester (17a). Esterification of acid **14** (477 mg, 1.34 mmol, 1.0 equiv) and alcohol **16a** (531 mg, 1.34 mmol, 1.0 equiv) according to general procedure B gave, after flash chromatography (50% ethyl acetate/hexane), 953 mg (97%) of the title compound: R_f 0.24 (50% ethyl acetate/hexane); NMR ($CDCl_3$) δ 7.33–7.26 (m, 5 H), 6.37 (br t, 1 H), 5.60 (br d, 1 H), 5.07 (s, 2 H), 4.34 (br s, 1 H), 4.34–4.15 (m, 4 H), 3.50 (br m, 2 H), 2.24–2.13 (m, 3 H), 1.91–0.90 (m, 14 H), 1.58 (s, 3 H), 1.54 (s, 3 H), 1.44 (s, 9 H); MS (FAB) m/e 704 (M + 1), 604, 548. Anal. ($C_{37}H_{57}N_3O_{10}$) C, H, N.

N^ω-[3-[[[(4*S*,5*R*)-4-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolindyl]carbonyl]oxy]propyl]-N^α-[(phenylmethoxy)carbonyl]-L-glutamine 1,1-Dimethylethyl Ester (17b). Esterification of acid **14** (466 mg, 1.31 mmol, 1.0 equiv) and alcohol **16b** (516 mg, 1.31 mmol, 1.0 equiv) according to general procedure B gave, after flash chromatography (50% ethyl acetate/hexane), 889 mg (95%) of the title compound: R_f 0.23 (50% ethyl acetate/hexane); NMR ($CDCl_3$) δ 7.35–7.26 (m, 5 H), 6.32 (br t, 1 H), 5.58 (br d, 1 H), 5.09 (s, 2 H), 4.34 (br s, 1 H), 4.34–4.15 (m, 4 H), 3.30 (br dd, 2 H), 2.27–2.14 (m, 3 H), 1.88–0.90 (m, 16 H), 1.59 (s, 3 H), 1.56 (s, 3 H), 1.44 (s, 9 H); MS (FAB) m/e 718 (M + 1), 618, 562. Anal. ($C_{38}H_{59}N_3O_{10}$) C, H, N.

(8*S*,11*S*,12*R*)-11-(Cyclohexylmethyl)-12-hydroxy-8-[[(phenylmethoxy)carbonyl]amino]-5,9,13-trioxo-1-oxa-4,10-diazacyclotridecane (18a). Macrocyclization precursor **17a** (944 mg) was deprotected according to general procedure D. The resultant amino acid was cyclized according to general procedure C. Purification by flash chromatography (5% methanol/dichloromethane) followed by MPLC (2.5% and 5% methanol/dichloromethane) provided 271 mg (41%) of the title compound: R_f 0.31 (10% methanol/dichloromethane); NMR (CD_3OD) δ 7.35–7.27 (m, 5 H), 5.08 (s, 2 H), 4.52 (ddd, 1 H), 4.37 (br t, 1 H), 4.23 (d, J = 2.1 Hz, 1 H), 4.21–4.14 (m, 2 H0, 3.57 (ddd, 1 H), 3.29–3.23 (m, 1 H), 2.43–2.33 (m, 1 H), 2.26–2.12 (m, 2 H), 1.91–0.85 (m, 14 H); MS (FAB) m/e 490 (M + 1). Anal. ($C_{25}H_{35}N_3O_7 \cdot 0.5H_2O$) C, H, N.

(3*R*,4*S*,7*S*)-4-(Cyclohexylmethyl)-3-hydroxy-7-[[(phenylmethoxy)carbonyl]amino]-2,6,10-thioxo-1-oxa-5,11-diazacyclotetradecane (18b). Macrocyclization precursor **17b** (883 mg) was deprotected according to general procedure D. The resultant aminoa cid was cyclized according to general procedure C. Purification by flash chromatography (5% methanol/dichloromethane) followed by MPLC (5% methanol/dichloromethane) provided 193 mg (30%) of the title compound: R_f 0.16 (10% methanol/dichloromethane); NMR (CD_3OD) δ 7.41–7.29 (m, 5 H), 5.11 (d, J = 12.5 Hz, 1 H), 5.06 (d, J = 12.5 Hz, 1 H), 4.38–4.07 (m, 5 H), 3.86 (ddd, 1 H), 2.89 (ddd, 1 H), 2.41–0.75 (m, 19 H); MS (FAB) m/e 504 (M + 1). Anal. ($C_{26}H_{37}N_3O_7 \cdot 0.5H_2O$) C, H, N.

(8*S*,11*S*,12*R*)-11-(Cyclohexylmethyl)-8-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-12-hydroxy-5,9,13-trioxo-1-oxa-4,10-diazacyclotridecane (3). Macrocycle **18a** (7.2 mg) was deprotected according to general procedure E and coupled to BocPhe (5.9 mg, 1.5 equiv) according to general procedure A. Purification by flash chromatography (5% methanol/dichloromethane) gave 8.3 mg (93%) of the title compound: R_f 0.78 (10% methanol/dichloromethane); NMR ($CD_3OD/CDCl_3$) δ 7.44–7.14 (m, 5 H), 4.68 (ddd, 1 H), 4.32 (m, 3 H), 4.20 (d, J = 1.8 Hz, 1 H), 4.03 (br m, 1 H), 3.57 (ddd, 1 H), 3.24 (ddd, 1 H), 3.06 (dd, J = 5.0, 13.7 Hz, 1 H), 2.84 (dd, J = 8.4, 13.7 Hz, 1 H), 2.31–2.08 (m, 3 H), 1.82–0.80 (m, 14 H), 1.33 (s, 9 H); MS (FAB) m/e 603 (M + 1). Anal. ($C_{31}H_{46}N_4O_8 \cdot 0.85H_2O$) C, H, N.

(3*R*,4*S*,7*S*)-4-(Cyclohexylmethyl)-7-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-3-hydroxy-2,6,10-trioxo-1-oxa-5,11-diazacyclotridecane (4). Macrocycle **18b** (33 mg) was deprotected according to general procedure F in methanol and coupled to BocPhe (34 mg, 2.0 equiv) according to general procedure A. Purification by flash chromatography (5 and 10% methanol/dichloromethane) gave 18 mg (90%) of the title compound: R_f 0.69 (10% methanol/dichloromethane); NMR ($CD_3OD/CDCl_3$) δ 7.27–7.18 (m, 5 H), 4.42–4.16 (m, 6 H), 3.87 (ddd, 1 H), 3.05 (dd, J = 4.5, 13.9 Hz, 1 H), 2.89 (ddd, 1 H), 2.75 (dd, J = 10.0, 13.9 Hz, 1 H), 2.43–0.78 (m, 19 H), 1.34 (s, 9 H); MS (FAB) m/e 617 (M + 1). Anal. ($C_{32}H_{48}N_4O_8 \cdot H_2O$) C, H, N.

4-Oxo-4-(phenylmethoxy)butyl (4*S*,5*R*)-4-(Cyclohexylmethyl)-2,2-dimethyl-3-[(1,1-dimethylethoxy)carbonyl]-5-oxazolidinecarboxylate (20a). A mixture of 5.10 g (59.2 mmol) of γ-butyrolactone and 2.37 g (59.2 mmol) of sodium hydroxide in 59 mL of water was heated at 70 °C overnight. The clear solution was then cooled and concentrated. The resultant white solid was suspended in toluene and concentrated to remove trace amounts of water. This solid was suspended in 60 mL of acetone along with 955 mg (2.96 mmol, 0.05 equiv) of tetrabutylammonium bromide and 12.2 g (8.46 mL, 71.1 mmol, 1.2 equiv) of benzyl bromide and heated at reflux for 24 h. The reaction mixture was cooled and concentrated. The residue was partitioned between 750 mL of ethyl acetate and 250 mL of 1 N aqueous sodium bisulfate. The organic phase was washed with 250-mL portions of saturated aqueous sodium bicarbonate and saturated aqueous sodium chloride, dried over anhydrous magnesium sulfate, and concentrated. Purification by MPLC (Lobar C column, 40% ethyl acetate/hexane) provided 8.94 g (78%) of benzyl 4-hydroxybutanoate [R_f 0.21 (40% ethyl acetate/hexane)]. This compound (935 mg, 4.82 mmol, 1.2 equiv) and acid **14** (1370 mg, 4.01 mmol, 1.0 equiv) were coupled according to general procedure B. Purification of MPLC (Lobar C column, 20% ethyl acetate/hexane) gave 1100 mg (96%) of the title compound as an oil: NMR ($CDCl_3$) δ 7.39–7.32 (m, 5 H), 5.12 (s, 2 H), 4.32–4.18 (m, 4 H), 2.46 (t, J = 7.4 Hz, 2 H), 2.02 (quint, J = 6.8 Hz, 2 H), 1.89 (br d, J = 13 Hz, 1 H), 1.83–0.94 (m, 12 H), 1.67 (s, 3 H), 1.63 (s, 3

H), 1.60 (s, 9 H); MS (FAB) *m/e* 518 (M + 1), 418. Anal. ($C_{29}H_{43}NO_7$) C, H, N.

5-Oxo-5-(phenylmethoxy)pentyl (4*S*,5*R*)-4-(Cyclohexylmethyl)-2,2-dimethyl-3-[(1,1-dimethylethoxy)carbonyl]-5-oxazolidinecarboxylate (20b). Benzyl 5-hydroxypentanoate was prepared according to procedure above for the preparation of benzyl 4-hydroxybutyrate. A 208 mg (0.998 mmol, 1.1 equiv) sample was coupled to acid **14** (302 mg, 0.884 mmol, 1.0 equiv) according to general procedure B. Purification by MPLC (Lobar B-column, 15% ethyl acetate/hexane) gave 467 mg (99%) of the title compound as an oil: R_f 0.25 (15% ethyl acetate/hexane); NMR ($CDCl_3$) δ 7.37–7.26 (m, 5 H), 5.11 (s, 2 H), 4.32 (s, 1 H), 4.3–4.2 (br s, 1 H), 4.16 (br m, 2 H), 2.40 (br t, J = 7.0 Hz, 2 H), 1.90 (br d, J = 11.3 Hz, 1 H), 1.83–0.85 (m, 16 H), 1.61 (s, 3 H), 1.59 (s, 1.5 H), 1.56 (s, 1.5 H), 1.47 (s, 9 H); MS (FAB) *m/e* 532 (M + 1), 432. Anal. ($C_{30}H_{45}NO_7$) C, H, N.

4-Oxo-4-(phenylmethoxy)butyl (2*R*,3*S*)-4-Cyclohexyl-2-hydroxy-3-[[(2*S*)-2-[[(1,1-dimethylethoxy)carbonyl]-amino]-3-[[(phenylmethoxy)carbonyl]amino]propanoyl]-amino]butanoate (21a). Compound **20a** (166 mg, 0.320 mmol, 1.0 equiv) was deprotected according to general procedure D and coupled to N^{α}-Boc-N^{ω}-Z-diaminopropionic acid (108 mg, 0.320 mmol, 1.0 equiv) according to general procedure A. Purification by MPLC (Lobar B column, 35%, 40%, 50% ethyl acetate/hexane) gave 155 mg (69%) of the title compound: R_f 0.58 (5% methanol/dichloromethane); NMR ($CDCl_3$) δ 7.37–7.25 (m, 10 H), 6.77 (br m, 1 H), 5.78 (br m, 2 H), 5.11 (s, 4 H), 4.38 (m, 1 H), 4.25–4.05 (m, 4 H), 3.49 (m, 2 H), 2.46 (br t, 2 H), 1.97 (quint, 2 H), 1.80–0.78 (m, 13 H), 1.42 (s, 9 H); MS (FAB) *m/e* 613, 461, 275.

5-Oxo-5-(phenylmethoxy)pentyl (2*R*,3*S*)-4-Cyclohexyl-2-hydroxy-3-[[(2*S*)-2-[[(1,1-dimethylethoxy)carbonyl]-amino]-3-[[(phenylmethoxy)carbonyl]amino]propanoyl]-amino]butanoate (21b). Compound **20b** (100 mg, 0.189 mmol, 1.0 equiv) was deprotected according to general procedure D and coupled to N^{α}-Boc-N^{ω}-Z-diaminopropionic acid (80.2 mg, 0.237 mmol, 1.25 equiv) according to general procedure A. Purification by MPLC (Lobar B column, 40% ethyl acetate/hexane) gave 105 mg (78%) of the title compound: R_f 0.47 (50% ethyl acetate/hexane); NMR ($CDCl_3$) δ 7.39 (m, 10 H), 6.5 (br s, 1 H), 5.62 (br s, 1 H), 5.48 (t, 1 H), 5.13 (m, 4 H), 4.38 (m, 1 H), 4.20–4.06 (m, 4 H), 3.51–3.45 (m, 2 H), 2.38 (br t, 2 H), 1.80–0.79 (m, 26 H); MS (FAB) *m/e* 712 (M + 1), 612.

(3*R*,4*S*,7*S*)-4-(Cyclohexylmethyl)-7-[[(1,1-dimethylethoxy)carbonyl]amino]-3-hydroxy-2,6,10-trioxo-1-oxa-5,9-diazacyclotridecane (22a). Compound **21a** (154 mg, 0.221 mmol) was deprotected according to general procedure F in methanol. The resultant amino acid was cyclized according to general procedure C. Purification by flash chromatography (5% methanol/dichloromethane) gave 51 mg (50%) of the title compound: R_f 0.21 (5% methanol/dichloromethane); NMR (CD_3OD) δ 4.86–4.05 (m, 5 H), 3.51 (dd, 1 H), 3.3 (m, 1 H), 2.40 (ddd, 1 H), 2.27 (ddd, 1 H), 2.14–1.96 (m, 2 H), 1.80–1.56 (br m, 5 H), 1.48–0.82 (m, 8 H), 1.45 (s, 9 H); MS (FAB) *m/e* 456 (M + 1), 356.

(3*R*,4*S*,7*S*)-4-(Cyclohexylmethyl)-7-[[(1,1-dimethylethoxy)carbonyl]amino]-3-hydroxy-2,6,10-trioxo-1-oxa-5,9-diazacyclotetradecane (22b). Compound **21b** (105 mg, 0.147 mmol) was deprotected according to general procedure F in methanol. The resultant amino acid was cyclized according to general procedure C. Purification by flash chromatography (5% methanol/dichloromethane) gave 19 mg (27%) of the title compound: R_f 0.67 (5% methanol/dichloromethane); NMR (CD_3OD) δ 4.23–4.05 (m, 5 H), 3.55 (dd, 1 H), 3.25 (dd, 1 H), 2.34–2.19 (m, 2 H), 1.94–0.76 (m, 26 H); MS (FAB) *m/e* 470 (M + 1), 414, 370.

(3*R*,4*S*,7*S*)-4-(Cyclohexylmethyl)-7-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-3-hydroxy-2,6,10-trioxo-1-oxa-5,9-diazacyclotridecane (5). Macrocycle **22a** (51 mg) was deprotected according to general procedure D and coupled to BocPhe (60 mg, 2.0 equiv) according to general procedure A. Purification by flash chromatography (5% methanol/dichloromethane) gave 61 mg (90%) of the title compound: R_f 0.50 (10% methanol/dichloromethane); NMR (CD_3OD) δ 7.29–7.17 (m, 5 H), 4.52 (dd, J = 3.6, 9.6 Hz, 1 H), 4.39–4.31 (m, 2 H), 4.25 (d, J = 1.8 Hz, 1 H), 4.17–4.02 (m, 2 H), 3.62 (dd, J = 9.9, 13.1 Hz, 1 H), 3.3 (m, 1 H), 3.12 (dd, J = 5.0, 13.9 Hz, 1 H), 2.83 (dd, J = 9.5, 13.9 Hz, 1 H), 2.40 (ddd, 1 H), 2.26 (ddd, 1 H), 2.04 (m, 2 H), 1.79–1.67 (br m, 5 H), 1.57–0.80 (m, 8 H), 1.37 (s, 9 H); MS (FAB) *m/e* 603 (M + 1), 503. Anal. ($C_{31}H_{46}N_4O_8$) C, H, N.

(3*R*,4*S*,7*S*)-4-Cyclohexylmethyl-7-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-3-hydroxy-2,6,10-trioxo-1-oxa-5,9-diazacyclotetradecane (6). Macrocycle **22b** (19 mg) was deprotected according to general procedure D and coupled to BocPhe (21 mg, 2.0 equiv) according to general procedure A. Purification by flash chromatography (2.5 and 5% methanol/dichloromethane) gave 21 mg (83%) of the title compound: R_f 0.43 (5% methanol/dichloromethane); NMR (CD_3OD) δ 7.29–7.18 (m, 5 H), 4.54 (dd, 1 H), 4.36–4.23 (m, 4 H), 4.12 (dd, 1 H), 3.58 (dd, 1 H), 3.37 (dd, 1 H), 3.09 (dd, 1 H), 2.80 (dd, 1 H), 2.35–2.21 (m, 2 H), 1.92–0.80 (m, 26 H); MS (FAB) *m/e* 617 (M + 1), 517. Anal. ($C_{32}H_{48}N_4O_8 \cdot 0.5H_2O$) C, H, N.

Methyl (4*S*,5*R*)-4-(Cyclohexylmethyl)-2-oxo-5-oxazolidinecarboxylate (25). To a solution of 2.58 g (12.0 mmol) of amino ester **24** in 60 mL of toluene at 0 °C was added 2.67 g (3.67 mL, 26.4 mmol, 2.2 equiv) of triethylamine followed by 6.21 mL (12.0 mmol, 1.0 equiv) of phosgene solution (1.93 M in toluene) dropwise over 10 min. The reaction was stirred an addition 30 min, then diluted with ethyl acetate, washed with 2 portions of 1 N aqueous sodium bisulfate solution, dried over anhydrous magnesium sulfate, and concentrated. Purification by MPLC (Lobar C column, 40% ethyl acetate/hexane) provided 2.17 g (75%) of the title compound: R_f 0.48 (50% ethyl acetate/hexane); NMR ($CDCl_3$) δ 6.56 (br s, 1 H), 4.58 (d, 1 H), 3.96 (q, 1 H), 3.82 (s, 3 H), 1.75–0.88 (m, 13 H); MS (FAB) *m/e* 242 (M + 1). Anal. ($C_{12}H_{19}NO_4$) C, H, N.

(4*S*,5*R*)-4-(Cyclohexylmethyl)-*N*-methyoxy-*N*-methyl-2-oxo-5-oxazolidinecarboxamide (26). To a solution of 2.84 g (11.8 mmol) of ester **25** in toluene at 0 °C was added 38.7 mL (25.9 mL) of a 0.67 M solution of Weinreb's reagent[15] in toluene. The reaction was quenched after 1.5 h by the addition of 1 N aqueous hydrochloric acid. Ethyl acetate was added, and the layers were separated. The aqueous phase was extracted several times with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate and concentrated. Purification by MPLC (Lobar C column, 75% ethyl acetate/hexane) gave 2.56 g (80%) of the title compound: R_f 0.33 (75% ethyl acetate/hexane); NMR ($CDCl_3$) δ 6.22 (br s, 1 H), 4.87 (d, J = 4.4 Hz, 1 H), 4.09 (br m, 1 H), 3.76 (s, 3 H), 3.23 (s, 3 H), 1.73–0.85 (m, 13 H); MS (FAB) *m/e* 271 (M + 1). Anal. ($C_{13}H_{22}N_2O_4$) C, H, N.

(4*S*,5*R*)-4-(Cyclohexylmethyl)-5-(1-oxo-4-pentenyl)-2-oxazolidinone (27). To a solution of 2.56 g (9.48 mmol) of amide **26** in 50 mL of anhydrous THF was added a 0 °C solution of 4-butenylmagnesium bromide formed from 6.40 g (4.81 mL, 47.4 mmol, 5.0 equiv) of 4-butenyl bromide and 1.15 g (47.4 mmol, 5.0 equiv) of magnesium turnings in 50 mL of THF. The reaction mixture was stirred at 0 °C for 1 h and then quenched by the addition of saturated aqueous ammonium chloride solution. Volatiles were removed in vacuo, and the resultant residue was partitioned between dichloromethane and 1 N aqueous hydrochloric acid. The aqueous phase was extracted twice with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate and concentrated. Purification by MPLC (Lobar C column, 30% ethyl acetate/hexane) gave 2.07 g (82%) of the title compound: R_f 0.31 (30% ethyl acetate/hexane); NMR ($CDCl_3$) δ 6.35 (br s, 1 H), 5.86–5.73 (dddd, 1 H), 5.08–4.98 (m, 2 H), 4.39 (d, J = 5.0 Hz, 1 H), 3.91 (q, J = 6 Hz, 1 H), 2.83–2.71 (sym m, 2 H), 2.35 (q, J = 7 Hz, 2 H), 1.72–0.87 (m, 13 H); MS (FAB) *m/e* 420 (M + 1 + dithiothreitol matrix). Anal. ($C_{15}H_{23}NO_3$) C, H, N.

(4*S*,5*R*)-4-(Cyclohexylmethyl)-5-[2-(3-hydroxypropyl)-1,3-dioxolan-2-yl]-2-oxazolidinone (28). A two-phase solution of 2.07 g (7.79 mmol) of ketone **27** and 74 mg (0.39 mmol, 0.05 equiv) of *p*-toluenesulfonic acid monohydrate in 78 mL of toluene and 17 mL of ethylene glycol was heated at reflux with removal of water with a Dean–Stark trap. After 24 h, the mixture was cooled, diluted with 300 mL of ethyl acetate, washed with 150-mL portions of saturated aqueous sodium bicarbonate and saturated aqueous sodium chloride solutions, dried over anhydrous magnesium sulfate, and concentrated. Purification by MPLC (Lobar C column, 40% ethyl acetate/hexane) gave 2.28 g (95%) of the corresponding ketal as an oil which crystallized: R_f 0.14 (30% ethyl acetate/hexane); MS (FAB) *m/e* 464 (M + 1 + dithiothreitol matrix). Anal. ($C_{17}H_{27}NO_4$) C, H, N.

To a solution of 1.17 g (3.77 mmol) of the ketal in 150 mL of acetone was added a solution of 6.03 g (28.2 mmol, 7.5 equiv) of sodium periodate and 150 mg of 51% ruthenium dioxide on carbon in 150 mL of water in three equal portions 1–2 h apart. After the last addition, the mixture was stirred an additional 30 min, then quenched with 2-propanol, filtered through Celite, and concentrated. The residue was partitioned between dichloromethane and 1:1 1 N aqueous sodium bisulfite/1 N aqueous sodium bisulfate. The aqueous phase was washed with dichloromethane, and the combined organic phases were dried over anhydrous magnesium sulfate and concentrated to give 1.21 g (98%) of the corresponding acid as a foam: R_f 0.15 (7.5% methanol/dichloromethane); NMR ($CDCl_3$) δ 12 (br s, 1 H), 6.65 (s, 1 H), 4.15–4.00 (m, 5 H), 3.81 (pent, J = 4.5 Hz, 1 H), 2.49–2.37 (m, 2 H), 2.13–1.92 (m, 2 H), 1.76–1.68 (m, 5 H), 1.60–1.22 (m, 6 H), 1.00–0.85 (m, 1 H).

To a solution of 1.20 g (3.67 mmol) of the acid in 18 mL of THF at 0 °C was added 4.58 mL (9.16 mmol, 2.5 equiv) of borane–methyl sulfide (2.0 M in THF). The reaction mixture was stirred at room temperature for 3 h, then quenched with methanol and concentrated. The residue was dissolved in ethyl acetate, washed with saturated aqueous sodium bicarbonate, dried over anhydrous magnesium sulfate, and concentrated. Purification by flash chromatography (ethyl acetate) gave 1.09 g (95%) of the title compound as a clear oil: R_f 0.39 (7.5% methanol/dichloromethane); NMR ($CDCl_3$) δ 5.60 (s, 1 H), 4.17–4.00 (m, 4 H), 3.79 (sym m, 1 H), 3.66 (br m, 2 H), 1.85–0.87 (m, 17 H); MS (FAB) m/e 314 (M + 1), 252. Anal. ($C_{16}H_{27}NO_5 \cdot {}^1/_4H_2O$) C, H, N.

(4*S*,5*R*)-4-(Cyclohexylmethyl)-5-[2-(3-azidopropyl)-1,3-dioxolan-2-yl]-2-oxazolidinone (29). A solution of 1.09 g (3.49 mmol) of alcohol **28**, 424 mg (0.58 mL, 4.19 mmol, 1.2 equiv) of triethylamine, and 440 mg (0.30 mL, 3.84 mmol, 1.1 equiv) of methanesulfonyl chloride in 20 mL of dichloromethane was stirred at 0 °C for 30 min. The reaction mixture was then diluted with 100 mL of dichloromethane, washed with 50-mL portions of 1 N aqueous sodium bisulfate and saturated aqueous sodium bicarbonate, dried over anhydrous sodium sulfate, and concentrated to give a white crystalline solid. This material was dissolved in 5 mL of DMF and stirred with 513 mg (10.5 mmol, 3.0 equiv) of lithium azide at room temperature overnight. The resultant solution was diluted with 50% ethyl acetate/hexane, washed with 200-mL portions of water and saturated aqueous sodium chloride, dried over anhydrous magnesium sulfate, and concentrated to give 1.09 g (92%) of a white solid which was used without further purification: R_f 0.44 (50% ethyl acetate/hexane); NMR ($CDCl_3$) δ 6.65–6.50 (br s, 1 H), 4.12–3.97 (m, 4 H), 3.76 (sym m, 1 H), 3.30 (m, 2 H), 1.86–0.85 (m, 17 H); MS (FAB) m/e 339 (M + 1), 314. Anal. ($C_{16}H_{26}N_4O_4$) C, H, N.

Phenylmethyl (4*S*)-5-[(1*S*,2*R*)-[2-[2-(3-Azidopropyl)-1,3-dioxolan-2-yl]-1-(cyclohexylmethyl)-2-hydroxyethyl]-amino]-4-[[(1,1-dimethylpropyl)carbonyl]amino]-5-oxopentanoate (30). A mixture of 1.09 g (3.21 mmol) of oxazolidinone **29** and 2 g (6.42 mmol, 2 equiv) of barium hydroxide octahydrate in 150 mL of 3:2 dioxane/water was heated at reflux overnight. The cloudy solution was then cooled, filtered, and concentrated. The residue was dissolved in 100 mL of water and washed with three 150-mL portions of dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate and concentrated to give 992 mg of the corresponding amine as a white solid which was used without further purification: R_f 0.21 (80:5:0.5 chloroform/methanol/ammonium hydroxide).

A 93.0 mg (0.298 mmol) sample of the amine was coupled to 121 mg (0.357 mmol, 1.2 equiv) of BocGlu(Bn) according to general procedure A. Purification by MPLC (Lobar B column, 50% ethyl acetate/hexane) provided 149 mg (79%) of the title compound: R_f 0.25 (40% ethyl acetate/hexane); NMR ($CDCl_3$) δ 7.36–7.26 (m, 5 H), 6.34 (d, J = 8.9 Hz, 1 H), 5.27 (d, J = 9 Hz, 1 H), 5.12 (s, 2 H), 4.20 (br q, J = 8 Hz, 1 H), 4.00–3.86 (m, 5 H), 3.53 (br s, 1 H), 3.25 (br t, 2 H), 2.59–2.42 (m, 3 H), 2.21–2.11 (m, 1 H), 1.96–0.83 (m, 18 H), 2.04 (s, 9 H); MS (FAB) m/e 632 (M + 1), 606. Anal. ($C_{32}H_{49}N_5O_8$) C, H, N.

(3*S*,12*R*,13*S*)-13-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-12-hydroxy-2,6,11-trioxo-1,7-diazacyclotridecane Ethylene Glycol Ketal (31). A solution of 136 mg (0.245 mmol) of benzyl ester **30** in 4 mL of methanol was treated with 20 mg of 10% Pd/C under 40 psi of hydrogen overnight. The mixture was then filtered and concentrated. TLC analysis indicated the presence of two products so the mixture was purified by flash chromatography (10%, 30%, 100% methanol/dichloromethane) to give 48 mg of an impurity [R_f 0.83 (1:1:1:1 ethyl acetate/acetic acid/water/butanol)] and 49 mg of the deprotected starting material [R_f 0.54 (1:1:1:1 ethyl acetate/acetic acid/water/butanol); MS (FAB) m/e 516 (M + 1)]. This material was subjected to macrocyclization according to general procedure C to provide, after flash chromatography (2.5%, 5%, and 10% methanol/dichloromethane), 32.5 mg (30% overall yield) of the title compound: R_f 0.39 (7.5% methanol/dichloromethane); NMR (CD_3OD) δ 4.19–4.07 (m, 2 H), 3.99–3.88 (m, 4 H), 3.51 (br t, J = 10 Hz, 1 H), 3.41 (s, 1 H), 2.83 (br m, 1 H), 2.48–2.24 (m, 2 H), 2.05–0.80 (m, 19 H), 1.41 (s, 9 H); MS (FAB) m/e 498 (M + 1), 398. Anal. ($C_{25}H_{43}N_3O_7 \cdot 0.5H_2O$) C, H, N.

(3*S*,12*R*,13*S*)-13-(Cyclohexylmethyl)-3-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-12-hydroxy-2,6,11-trioxo-1,7-diazacyclotridecane Ethylene Glycol Ketal (7). A solution of 32.5 mg (0.0653 mmol) of macrocycle **31** was deprotected according to general procedure D and coupled with 34.7 mg (0.131 mmol, 2.0 equiv) of BocPhe according to general procedure A. Purification by flash chromatography (2.5% and 5% methanol/dichloromethane) gave 30.2 mg (72%) of the title compound: R_f 0.19 (5% methanol/dichloromethane); NMR ($CD_3OD/CDCl_3$) δ 7.28–7.18 (m, 5 H), 4.51 (dd, J = 3.8, 10.0 Hz, 1 H), 4.33 (dd, J = 4.5, 9.3 Hz, 1 H), 4.14 (t, J = 7.0 Hz, 1 H), 4.04–3.88 (m, 3 H0, 4.00 (s, 1 H), 3.54–3.44 (m, 1 H), 3.44 (s, 1 H), 3.06 (dd, J = 4.9, 13.7 Hz, 1 H), 2.89 (m, 1 H), 2.75 (dd, J = 9.7, 13.8 Hz, 1 H), 2.49–2.27 (m, 2 H), 2.18–2.07 (m, 1 H), 1.98–0.81 (m, 18 H), 1.32 (s, 9 H); MS (FAB) m/e 645 (M + 1), 589, 545. Anal. ($C_{34}H_{52}N_4O_8$) C, H, N.

(3*S*,12*R*,13*S*)-13-(Cyclohexylmethyl)-3-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-12-hydroxy-2,6,11-trioxo-1,7-diazacyclotridecane (8). A solution of 25.0 mg (0.0389 mmol) of macrocycle **7** in 3:1 acetic acid/water was heated at 80 °C for 3 h. It was then cooled and concentrated. The resultant ketone was then dissolved in 3:1 THF/water and treated with 12.7 mg (0.0134 mL, 0.05816 mmol, 1.5 equiv) of di-*tert*-butyl dicarbonate and 7.2 mg (0.0853 mmol, 2.2 equiv) of sodium bicarbonate. After the mixture was stirred at room temperature for 1 h, it was partitioned between half-saturated aqueous sodium chloride and dichloromethane. The aqueous phase was washed with several portions of dichloromethane, and the combined organic phases were dried over anhydrous sodium sulfate and concentrated. Purification by flash chromatography (2.5%, 5%, 10% methanol/dichloromethane) gave 13.2 mg (57%) of the title compound: R_f 0.26 (5% methanol/dichloromethane); NMR (CD_3OD) δ 7.30–7.17 (m, 5 H), 4.45–4.29 (m, 3 H), 4.12 (d, J = 2.0 Hz, 1 H), 3.98–3.30 (m, 1 H), 3.28–3.05 (m, 2 H), 2.86–2.72 (m, 3 H), 2.23–2.08 (m, 3 H), 1.89–0.87 (m, 16 H), 1.34 (s, 9 H); MS (FAB) m/e 601 (M + 1), 501. Anal. ($C_{32}H_{48}N_4O_8 \cdot 1.7H_2O$) C, H, N.

(2*S*,3*R*)-7-Azido-1-cyclohexyl-2-[[(1,1-dimethylethoxy)-carbonyl]amino]-3-hydroxy-4-oxoheptane (32). Oxazolidinone **29** was hydrolyzed to the corresponding amine as described above for the synthesis of compound **30**. A solution of 395 mg (1.26 mmol) of this amine in 25 mL of 1 N aqueous hydrochloric acid was heated at 60–65 °C overnight. The solution was then concentrated. The residue was dissolved in 12 mL of 3:1 THF/water, cooled to 0 °C, and treated with 818 mg (3.79 mmol, 3 equiv) of di-*tert*-butyl pyrocarbonate and 319 mg (3.79 mmol, 3.0 equiv) of sodium bicarbonate. After the reaction mixture was stirred at 4 °C overnight, it was diluted with 100 mL of 1 N aqueous sodium bisulfate solution and extracted with dichloromethane (3 × 100 mL). The organic phase was dried over sodium sulfate and concentrated. Purification by flash chromatography (10%, 15%, and 30% ethyl acetate/hexane) gave 267 mg (57%) of the title compound: R_f 0.35 (20% ethyl acetate/hexane); NMR ($CDCl_3$) δ 4.53 (br d, J = 9.9 Hz, 1 H), 4.22 (br sym m, 1 H), 4.08 (br d, J = 3.5 Hz, 1 H), 3.70 (d, J = 3.5 Hz, 1 H), 3.33 (sym m, 2 H), 2.98 (dt, 1 H), 2.51 (dt, 1 H), 1.97–0.88 (m, 15 H), 1.38 (s, 9 H); MS (FAB) m/e 369 (M + 1), 313, 269. Anal. ($C_{18}H_{32}N_4O_4$) C, H, N.

(2*S*,3*R*,4*RS*)-7-Azido-1-cyclohexyl-3,4-dihydroxy-2-[[(1,1-dimethylethoxy)carbonyl]amino]heptane (33, 34). To a 0 °C solution of 205 mg (0.557 mmol) ketone **32** in 5 mL of

methanol was added 21 mg (0.557 mmol) of sodium borohydride. After the reaction stirred for 15 min, it was quenched by the addition of acetic acid and concentrated. The residue was dissolved in methanol and concentrated. This was repeated several times. Purification by flash chromatography (30% and 40% ethyl acetate/hexane) gave 91 mg (44%) of a faster eluting compound, alcohol **33**, and 118 mg (57%) of a slower eluting compound, alcohol **34**. Alcohol **33**: R_f 0.33 (30% ethyl acetate/hexane); NMR ($CDCl_3$) δ 4.60 (d, J = 9.3 Hz, 1 H), 4.46 (d, J = 3.3 Hz, 1 H), 4.03 (dt, J = 4.4, 9.0 Hz, 1 H), 3.39–3.18 (m, 4 H), 1.97–0.84 (m, 17 H), 1.46 (s, 9 H); MS (FAB) m/e 371 (M + 1), 345, 315, 289, 271, 245. Anal. ($C_{18}H_{34}N_4O_4 \cdot 0.30H_2O$) C, H, N. Alcohol **33**: R_f 0.22 (30% ethyl acetate/hexane); NMR ($CDCl_3$) δ 4.63 (d, J = 9.2 Hz, 1 H), 3.73 (br m, 1 H), 3.57 (br m, 1 H), 3.34 (t, J = 6.5 Hz, 2 H), 3.27 (br s, 1 H), 2.97 (br s, 1 H), 2.65 (br s, 1 H), 1.90–0.83 (m, 17 H), 1.44 (s, 9 H); MS (FAB) m/e 371 (M + 1), 345, 315, 289, 271, 245. Anal. ($C_{18}H_{34}N_4O_4$) C, H, N.

Phenylmethyl (4*S*)-5-[((2*S*,3*R*,4*S*)-7-Axido-1-cyclohexyl-3,4-dihydroxyhept-2-yl)amino]-4-[[(1,1-dimethylpropyl)carbonyl]amino]-5-oxopentanoate (35). Diol **33** (83 mg, 0.224 mmol, 1.0 equiv) was deprotected according to general procedure D and coupled with BocGlu(Bn) (113 mg, 0.336 mmol, 1.5 equiv) according to general procedure A. Purification by flash chromatography (40% ethyl acetate/hexane) gave 100 mg (76%) of the title compound: R_f 0.36 (50% ethyl acetate/hexane); NMR ($CDCl_3$) δ 7.40–7.26 (m, 5 H), 6.43 (d, J = 9.2 Hz, 1 H), 5.40 (d, J = 6.8 Hz, 1 H), 5.14 (s, 2 H), 4.31 (dt, J = 4.8, 9.3 Hz, 1 H), 4.12 (dd, J = 7, 7.5 Hz, 1 H), 3.35–3.16 (m, 4 H), 2.61–2.42 (sym m, 2 H), 2.21–0.82 (m, 17 H), 1.44 (s, 9 H); MS (FAB) m/e 612 (M + 23), 590 (M + 1), 564, 534, 490. Anal. ($C_{30}H_{47}N_5O_7$) C, H, N.

(3*S*,11*S*,12*R*,13*S*)-13-(Cyclohexylmethyl)-11,12-dihydroxy-3-[(1,1-dimethylethoxy)carbonyl]-2,6-dioxo-1,7-diazacyclotridecane (36). A solution of 87 mg (0.148 mmol) of benzyl ester **35** in 5 mL of 3:1:1 methanol/THF/acetic acid was treated with 10 mg of 10% Pd/C under 40 psi of hydrogen overnight. The mixture was then filtered and concentrated. Purification by flash chromatography (methanol) gave 39 mg (56%) of deprotected starting material. This material was subjected to macrocyclization according to general procedure C. Purification by flash chromatography (1–10% methanol/dichloromethane) provided 13 mg (34%) of the title compound: R_f 0.64 (10% methanol/dichloromethane); NMR (CD_3OD) δ 4.28–4.14 (m, 2 H), 3.44 (br t, J = 11 Hz, 1 H), 3.33 (m, 1 H), 3.20 (br d, J = 7.3 Hz, 1 H), 3.08 (br d, J = 13.5 Hz, 1 H), 2.44–2.06 (m, 4 H), 1.85–0.82 (m, 17 H), 1.44 (s, 9 H); MS (FAB) m/e 456 (M + 1), 356. Anal. ($C_{23}H_{41}N_3O_6 \cdot 1.25H_2O$) C, H, N.

(3*S*,11*S*,12*R*,13*S*)-13-(Cyclohexylmethyl)-11,12-dihydroxy-3-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-2,6-dioxo-1,7-diazacyclotridecane (9). Macrocycle **36** (11.9 mg, 0.0261 mmol) was deprotected according to general procedure D. The resultant amine was coupled to BocPhe (13.9 mg, 0.0522 mmol, 2.0 equiv) according to general procedure A (with several drops of DMF added to help solubilize the substrate). Purification by flash chromatography (2.5%, 5%, and 7.5% methanol/dichloromethane) gave 11.5 mg (73%) of the title compound: R_f 0.37 (7.5% methanol/dichloromethane); NMR (CD_3OD) δ 7.30–7.18 (m, 5 H), 4.50 (m, 1 H), 4.31 (dd, J = 4.9, 9.4 Hz, 1 H), 4.16 (dd, J = 4.1, 9.6 Hz, 1 H), 3.45 (m, 1 H), 3.19–3.07 (m, 2 H), 2.81 (dd, J = 9.7, 13.3 Hz, 1 H), 2.39–2.12 (m, 4 H), 1.95–0.83 (m, 19 H), 1.36 (s, 9 H); MS (FAB) m/e 603 (M + 1), 503. Anal. ($C_{32}H_{50}N_4O_7 \cdot H_2O$) C, H, N.

Phenylmethyl (4*S*)-5-[((2*S*,3*R*,4*R*)-7-Azido-1-cyclohexyl-3,4-dihydroxyhept-2-yl)amino]-4-[[(1,1-dimethylpropyl)carbonyl]amino]-5-oxopentanoate (37). Diol **34** (164 mg, 0.444 mmol, 1.0 equiv) was deprotected according to general procedure D and coupled with BocGlu(Bn) (299 mg, 0.887 mmol, 2.0 equiv) according to general procedure A. Purification by flash chromatography (50% ethyl acetate/hexane) gave 204 mg (78%) of the title compound: R_f 0.32 (50% ethyl acetate/hexane); NMR ($CDCl_3$) δ 7.39–7.26 (m, 5 H), 6.50 (d, J = 9.9 Hz, 1 H), 5.51 (d, J = 6.9 Hz, 1 H), 5.13 (s, 2 H), 4.09 (br m, 2 H), 3.51 (br m, 1 H), 3.45 (d, J = 5.2 Hz, 1 H), 3.30 (t, J = 6.6 Hz, 2 H), 3.17 (br d, 1 H), 2.56–2.43 (sym m, 2 H), 2.11 (hex, J = 7 Hz, 1 H), 1.95 (hex, J = 7 Hz, 1 H), 1.84–0.80 (m, 17 H), 1.43 (s, 9 H); MS (FAB) m/e 612 (M + 23), 590 (M + 1), 564, 534, 490. Anal. ($C_{30}H_{47}N_5O_7$) C, H, N.

(3*S*,11*R*,12*R*,13*S*)-13-(Cyclohexylmethyl)-11,12-dihydroxy-3-[(1,1-dimethylethoxy)carbonyl]-2,6-dioxo-1,7-diazacyclotridecane (38). A solution of 204 mg (0.345 mmol) of benzyl ester **37** was hydrogenated and purified according to the procedure given for compound **35** to give 85.8 mg (52%) of deprotected starting material. This material was subjected to macrocyclization according to general procedure C. Purification by flash chromatography (2.5%, 5%, 7.5%, and 10% methanol/dichloromethane) provided 37.4 mg (45%) of the title compound: R_f 0.46 (10% methanol/dichloromethane); NMR (CD_3OD) δ 4.28 (dd, J = 4.7, 7.7 Hz, 1 H), 4.11 (br m, 1 H), 3.79 (br t, J = 11 Hz, 1 H), 3.47 (br t, J = 10 Hz, 1 H), 3.27 (br d, J = 7.9 Hz, 1 H), 2.84 (br d, J = 13.8 Hz, 1 H), 2.47 (ddd, 1 H), 2.26 (dt, 1 H), 2.14–0.81 (m, 19 H), 1.44 (s, 9 H). Anal. ($C_{23}H_{41}N_3O_6 \cdot {}^4/_3H_2O$) C, H, N.

(3*S*,11*R*,12*R*,13*S*)-13-(Cyclohexylmethyl)-11,12-dihydroxy-3-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-2,6-dioxo-1,7-diazacyclotridecane (10). Macrocycle **38** (32.2 mg, 0.0.707 mmol) was deprotected according to general procedure D. The resultant amine was coupled to BocPhe (37.5 mg, 0.777 mmol, 2.0 equiv) according to general procedure A (in DMF). Purification by flash chromatography (2.5%, 5%, and 7.5% methanol/dichloromethane) gave 32.6 mg (77%) of the title compound: R_f 0.41 (10% methanol/dichloromethane); NMR (DMSO-d_6) δ 8.27 (d, J = 7.5 Hz, 1 H), 7.38 (d, J = 8.9 Hz, 1 H), 7.35–7.15 (m, 6 H), 6.98 (t, J = 9.5 Hz, 1 H), 4.71 (d, J = 4.0 Hz, 1 H), 4.53 (br m, 1 H), 4.40 (d, J = 5.2 Hz, 1 H), 4.13 (br m, 1 H), 3.93 (br m, 1 H), 3.65 (br m, 1 H), 3.3 (m, 1 H), 3.09 (br d, J = 6.6 Hz, 1 H), 2.87 (dd, J = 3.5, 13 Hz, 1 H), 2.73–2.60 (m, 2 H), 2.44 (q, J = 7 Hz, 1 H), 2.13 (m, 1 H), 1.93–0.70 (m, 17 H), 1.28 (s, 9 H); MS (FAB) m/e 603 (M + 1), 547, 503. Anal. ($C_{32}H_{50}N_4O_7 \cdot 0.25H_2O$) C, H, N.

(8*S*,11*S*,12*R*)-11-(Cyclohexylmethyl)-8-[*N*-[[2-[(2,2-dimethylpropanoyl)amino]ethoxy]carbonyl]-L-phenylalanyl]-12-hydroxy-5,9,13-trioxo-1-oxa-4,10-diazacyclotridecane (39). Macrocycle **18a** (18.9 mg) was deprotected according to general procedure E and coupled to *N*-[[2-[(*tert*-butylacetyl)amino]ethoxy]carbonyl]phenylalanine (16.2 mg, 1.2 equiv) according to general procedure A. Purification by MPLC (Lobar B column, 5% methanol/dichloromethane) gave 20.3 mg (76%) of the title compound: R_f 0.37 (10% methanol/dichloromethane); NMR (CD_3OD) δ 7.34–7.18 (m, 5 H), 4.59 (dd, 1 H), 4.47–4.33 (m, 3 H), 4.25 (d, J = 2.0 Hz, 1 H), 4.18 (ddd, 1 H), 4.07–3.95 (m, 2 H), 3.53 (ddd, 1 H), 3.44–3.33 (m, 3 H), 3.09 (dd, J = 4.7, 13.9 Hz, 1 H), 2.84 (dd, J = 9.4, 13.9 Hz, 1 H), 2.39–2.18 (m, 3 H), 2.05 (s, 2 H), 1.94–0.85 (m, 23 H), 1.00 (s, 9 H); MS (FAB) m/e 689, 688 (M + 1). Anal. ($C_{35}H_{53}N_5O_9 \cdot 2H_2O$) C, H, N.

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Registry No. **3**, 134848-65-0; **4**, 134848-66-1; **5**, 134848-67-2; **6**, 134848-68-3; **7**, 134848-69-4; **8**, 134848-70-7; **9**, 134848-71-8; **10**, 134931-40-1; **11**, 98105-41-0; (3*S*,4*S*)-**12**, 104856-06-6; (3*R*,4*S*)-**12**, 104856-05-5; **13**, 121533-55-9; **14**, 121533-54-8; **15a**, 141-43-5; **15b**, 156-87-6; **16a**, 134848-72-9; **16b**, 134848-90-1; **17a**, 134848-73-0; **17b**, 134848-91-2; **18a**, 134848-74-1; **18b**, 134848-92-3; **19a**, 96-48-0; **19b**, 542-28-9; **20a**, 134879-38-2; **20b**, 134848-93-4; **21a**, 134848-75-2; **21b**, 134848-94-5; **22a**, 134848-76-3; **22b**, 134848-95-6; **24**, 134848-77-4; **25**, 134848-78-5; **26**, 134848-79-6; **27**, 134848-80-9; **27** (ethylene ketal), 134848-97-8; **28**, 134848-81-0; **28** (corresponding acid), 134848-98-9; **29**, 134848-82-1; **30**, 134848-83-2; **31**, 134848-84-3; **32**, 134848-85-4; **33**, 134848-86-5; **34**, 134931-41-2; **35**, 134848-87-6; **36**, 134848-88-7; **37**, 134931-42-3; **38**, 134931-43-4; **39**, 134848-89-8; CH_2=$CHMgBr$, 1826-67-1; $Me_2C(OMe)_2$, 77-76-9; Z-Glu-OtBu, 5891-45-2; Boc-Phe-OH, 13734-34-4; $HO(CH_2)_3CO_2CH_2Ph$, 91970-62-6; $HO(CH_2)_4CO_2CH_2Ph$, 134848-96-7; $PhCH_2Br$, 100-39-0; (*S*)-$ZNHCH_2CH(NHBoc)CO_2H$, 65710-57-8; Cl_2CO, 75-44-5; $HN(OMe)Me \cdot HCl$, 6638-79-5; $BrMgCH_2CH_2CH$=CH_2, 7103-09-5; $HOCH_2CH_2OH$, 107-21-1; Boc-Glu(OBn)-OH, 13574-13-5; $Me_3CCONHCH_2CH_2O_2C$-Phe-OH, 134849-00-6; (*c*-C_6H_{11})CH_2-(*S*)-$CH(NH_2)$-(*R*)-$CH(OH)C(OCH_2CH_2O)(CH_2)_3N_3$, 134848-99-0; renin, 9015-94-5.