Highly Potent, Orally Active Diester Macrocyclic Human Renin Inhibitors†,[1]

Ann E. Weber,*,‡ Mark G. Steiner,‡ Philip A. Krieter,§ Adria E. Colletti,§ James R. Tata,‡ Thomas A. Halgren,⊥ Richard G. Ball,∥ John J. Doyle,# Terry W. Schorn,# Ralph A. Stearns,§ Randall R. Miller,§ Peter K. S. Siegl,# William J. Greenlee,‡ and Arthur A. Patchett‡

Departments of Exploratory Chemistry, Animal and Exploratory Drug Metabolism, Biophysical Chemistry, and Molecular Systems, Merck Research Laboratories, Rahway, New Jersey 07065, and Department of Pharmacology, Merck Research Laboratories, West Point, Pennsylvania 19486

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Replacing one amide bond in macrocyclic renin inhibitors of the general structure **1** and **2** with an ester linkage gave glutamate-derived inhibitors **3** and serine-derived inhibitors **4**. While this oxygen-for-nitrogen exchange had little effect on potency in the glutamate series, potency was dramatically increased in the serine series. In this series, the 14-membered ring compounds proved to be more potent than the corresponding 13-membered ring derivatives. Substitution of the ring at the position corresponding to P_2' generally increased potency. The absolute configuration at this center was shown to be *R* for the 4-morpholinomethyl derivative (**4o**), both by asymmetric synthesis and X-ray crystallography. Replacing the "Boc-Phe" moiety of inhibitor **4o** with a variety of substituents led to subnanomolar inhibitors, one of which (the "3(*S*)-quinuclidinyl-Phe" derivative **33**) lowered blood pressure 20 mmHg and completely inhibited plasma renin activity for 6 h in sodium-depleted rhesus monkeys. This compound proved to have limited bioavailability (1% in rats) due to cleavage of the serine ester bond and rapid hepatic extraction.

Introduction

The aspartyl protease renin catalyzes the first step in the renin–angiotensin system (RAS), which plays a key role in the regulation of blood pressure and the maintenance of volume homeostasis. Pharmacological intervention in 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. Unlike ACE, renin has a remarkably narrow substrate specificity with angiotensinogen as its only known naturally occurring substrate. This specificity suggests that inhibitors of renin may have pharmocological advantages over ACE inhibitors.[3]

While considerable progress has been made in the synthesis of highly potent inhibitors of renin for the treatment of hypertension, current inhibitors suffer from limited oral absorption and short duration of action.[4] The oral bioavailability of cyclosporin,[5] a cyclic undecapeptide, suggests that macrocyclic renin inhibitors could show enhanced oral absorption.[6,7] Recently we reported the design and sythesis of a series of unique P_2-P_1' linked, macrocyclic renin inhibitors which incorporate a transition-state isostere within a 13- or 14-membered ring.[8] The two most active compounds in this family were 13-membered ring glutamine-derived inhibitor **1** (R = H) and 14-membered ring diaminopropionic acid-derived

† Dedicated to Professor Ralph Hirschmann on the occasion of his 70th birthday.

‡ Department of Exploratory Chemistry, Rahway facility.

§ Department of Animal and Exploratory Drug Metabolism, Rahway facility.

⊥ Department of Molecular Systems, Rahway facility.

∥ Department of Biophysical Chemistry, Rahway facility.

Department of Pharmacology, West Point facility.

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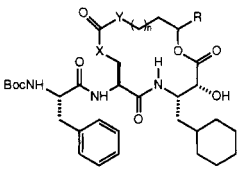
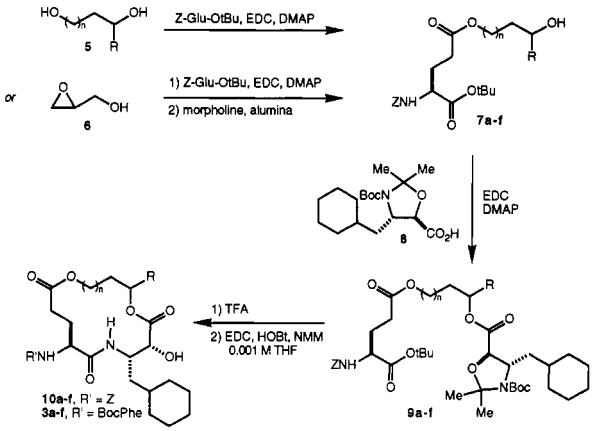


Figure 1. General structure of P_2-P_1' linked macrocyclic human renin inhibitors.

Scheme I. Synthesis of Glutamate-Derived Macrocycles

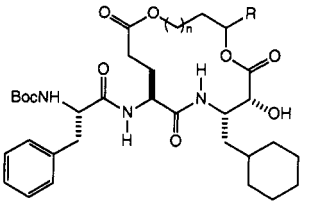


inhibitor **2** (R = H) (Figure 1; IC_{50} = 0.61 and 0.59 μM, respectively). Substitution at "R" corresponding to P_2' greatly increases activity.[9] For example, inhibitor **1** (R = iBu) is 150 times more potent than the parent compound. In an effort to further decrease the peptide character of these macrocycles and increase bioavailability through the elimination of amide bonds in these inhibitors, the side chain amide bond NH (Figure 1, X or Y) was replaced with an ester linkage to give diester macrocycles **3** and **4** (Figure 1). Like inhibitors **1** and **2**, diesters **3** and **4**, which are derived from glutamic acid and serine, respectively, incorporate the "norACHPA" transition-state isostere[10,11] within the macrocyclic ring. Herein we report the synthesis and biological activity of this diester family of macrocyclic renin inhibitors.

Results and Discussion

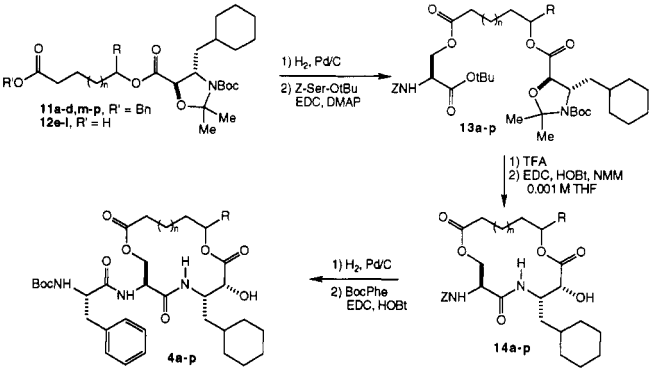
Chemistry. The synthesis of glutamate-derived macrocycles of the general formula **3** is shown in Scheme I. The appropriate diol **5** was coupled to *N*-carbobenzoxy glutamic acid α-*tert*-butyl ester (Z-Glu-OtBu) using 3-ethyl-1-[3-(dimethylamino)propyl]carbodiimide (EDC) and 4-(dimethylamino)pyridine (DMAP) to give alcohol **7**. In the case of R = 4-morpholinomethyl, coupling of Z-Glu-OtBu and epoxy alcohol **6** followed by epoxide opening with morpholine gave alcohol **7**. Alcohol **7** was coupled with Boc-norACHPA acetonide (**8**)[8] using EDC/DMAP to give macrocycle precursor **9**. At this stage, diastereomers (R ≠ H) were separated by silica gel chromatography and the individual isomers carried on through the synthesis. Treatment of compound **9** with trifluoroacetic acid (TFA) followed by macrocyclization of the resultant amino acid employing EDC and hydroxybenzotriazole (HOBt) under high-dilution conditions gave the corresponding macrocycle **10**. Removal of the carbobenzoxy protecting group followed by coupling to BocPhe gave inhibitors **3a–f** (see Table I).

Table I. Human Plasma Renin Activity of Glutamate-Derived Macrocycles **3a–f**



compd (diast 1,2)	*n*	R	IC_{50} (nM) diastereomer 1	diastereomer 2
3a	0	H	840	
3b	1	H	1800	
3c,d	0	nBu	600	200
3e,f	0	$CH_2N(CH_2CH_2)_2O$	17	20000

Scheme II. Synthesis of Serine-Derived Macrocycles



Scheme II illustrates the synthesis of serine-derived macrocycles **4**. Key intermediate acid **12** (or benzyl ester **11**[12] following treatment with H_2 and Pd/C) was coupled to *N*-carbobenzoxy serine *tert*-butyl ester (Z-Ser-OtBu) with EDC/DMAP to give macrocycle precursor **13**. The synthesis of inhibitors **4a–p** (see Table II) was completed following the protocol described above for the synthesis of inhibitors **3a–f**.

Because ring closure to form the macrocyclic amide bond in these serine-derived macrocycles was a low-yield reaction, an alternate route to these derivatives was developed which involved a Keck macrolactonization ring closure.[13] This approach is illustrated in Scheme III for the synthesis of inhibitors **4o,q,r**. Deprotection of key intermediate **11** with TFA followed by coupling of the resultant amine to *N*-*tert*-butyloxycarbonyl serine *O*-ben-

(8) Weber, A. E.; Halgren, T. A.; Doyle, J. J.; Lynch, R. J.; Siegl, P. K. S.; Parsons, W. H.; Greenlee, W. J.; Patchett, A. A. Design and Synthesis of P_2-P_1'-Linked Macrocyclic Human Renin Inhibitors. *J. Med. Chem.* **1991**, *34*, 2692–2701.

(9) (a) Weber, A. E.; Steiner, M. G.; Yang, L.; Dhanoa, D. S.; Tata, J. R.; Halgren, T. A.; Siegl, P. K. S.; Parsons, W. H.; Greenlee, W. J.; Patchett, A. A. Highly Potent, Orally Active, P_2-P_1'-Linked Macrocyclic Human Renin Inhibitors. In *Peptides. Chemistry and Biology. Proceedings of the Twelfth American Peptide Symposium*; Smith, J. A., Rivier, J. E., Eds.; ESCOM: Leiden, 1992; pp 749–751. (b) Dhanoa, D. S.; Parsons, W. H.; Greenlee, W. J.; Patchett, A. A. The Synthesis of Potent Macrocyclic Renin Inhibitors. *Tetrahedron Lett.* **1992**, *33*, 1725–1728.

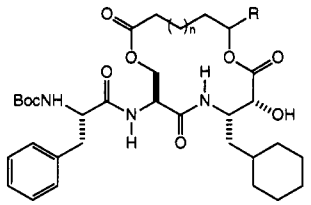
(10) Iizuka, K.; Kamijo, T.; Harada, H.; Akahane, K.; Kubota, T.; Umeyama, H.; Kiso, Y. Design and Synthesis of an Orally Potent Human Renin Inhibitor Containing a Novel Amino Acid, Cyclohexylnorstatine. *J. Chem. Soc., Chem. Commun.* **1989**, 1678–1680.

(11) NorACHPA refers to (2*R*,3*S*)-3-amino-4-cyclohexyl-2-hydroxybutanoic acid.

(12) See ref 8 for the synthesis of compound **11** with R = H.

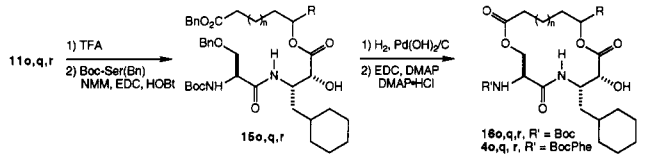
(13) Boden, E. P.; Keck, G. E. Proton-Transfer Steps in Steglich Esterification: A Very Practical New Method to Macrolactonization. *J. Org. Chem.* **1985**, *50*, 2394–2395.

Table II. Human Plasma Renin Inhibition by Serine-Derived Macrocycles **4a–v**

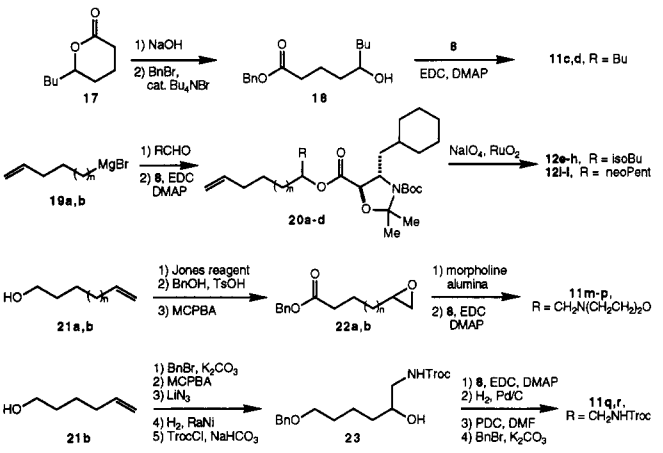


compd (diast 1,2)	*n*	R	IC_{50} (nM) diastereomer 1	diastereomer 2
4a	0	H	58	
4b	1	H	3.4	
4c,d	1	nBu	22	180
4e,f	0	iBu	3.9	6100
4g,h	1	iBu	2.6	330
4i,j	0	neopentyl	580	>20000
4k,l	1	neopentyl	16	1200
4m,n	0	$CH_2N(CH_2CH_2)_2O$	7.6	6400
4o,p	1	$CH_2N(CH_2CH_2)_2O$	0.8	170
4q,r	1	$CH_2NHCO_2CH_2CCl_3$	1.3	180
4s,t	1	CH_2NMe_2	3.9	86
4u,v	1	CH_2NEt_2	7.0	140

Scheme III. Alternate Synthesis of Serine-Derived Macrocycles



Scheme IV. Synthesis of Macrocycle Intermediates 11 and 12



zyl ether (Boc-Ser(Bn)) provided macrocycle precursor **15**. Following hydrogenation, the resultant hydroxy acid was cyclized by slowly adding it to a solution of EDC, DMAP, and DMAP hydrochloride in refluxing chloroform to give macrocycle **16** in typically 70–85% yield. Treatment with TFA followed by coupling to Boc-Phe gave the desired inhibitors **4o,q,r**.

Key intermediates **11** and **12** were synthesized in a variety of different ways as shown in Scheme IV. For esters **11c,d** (R = Bu), the commercially available lactone **17** was hydrolyzed and then esterified to give hydroxy ester **18**. This compound was coupled to Boc-norACHPA acetonide to provide intermediate **11c,d**. For acids **12e–h** and **12i–l** (R = iBu and neopentyl, respectively), Grignard reagent **19** was treated with the appropriate aldehyde and the resultant alcohol was coupled to norACHPA **8** to

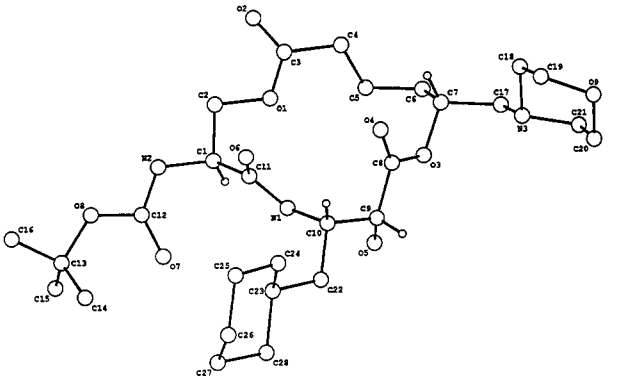
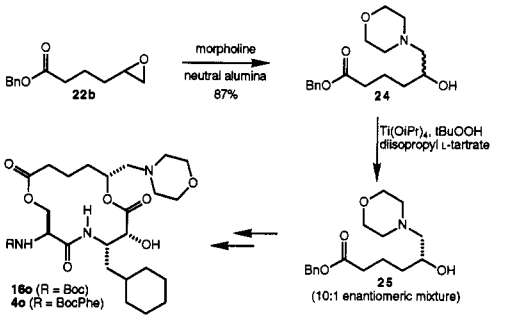


Figure 2. X-ray crystal structure of macrocycle **16o**. Hydrogens have been omitted except at stereogenic centers.

Scheme V. Asymmetric Synthesis of Macrocyclic Renin Inhibitor **4o**



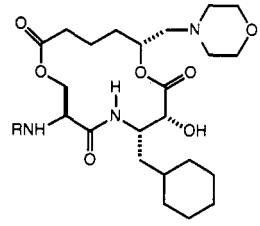
provide alkene **20**. Ruthenium oxidation of the olefin provided the corresponding acids **12e–l**. In the R = 4-morpholinomethyl case (esters **11m–p**), the appropriate alcohol **21** was oxidized to the corresponding acid and esterified. The resultant ester was treated with *m*-chloroperbenzoic acid (MCPBA) to give epoxide **22**. Alumina-catalyzed epoxide opening followed by coupling to norACHPA derivative **8** provided intermediates **11m–p**. Alternatively, alcohol **21b** was protected as its benzyl ether and treated with MCBPA followed by epoxide opening with lithium azide. The resultant azide was reduced and the corresponding amine protected as its *N*-[(trichloroethoxy)carbonyl] (TROC) derivative to give alcohol **23**. Alcohol **23** was coupled to Boc-norACHPA acetonide **8**. The benzyl ether was removed and the alcohol was oxidized to the corresponding acid to give, after esterification, ester **11q,r**. Following conversion of this compound to macrocycles **4q,r**, the TROC group was removed and the corresponding amine was subjected to reductive alkylation with formaldehyde and acetaldehyde to give inhibitors **4s,t** and **4u,v**, respectively.

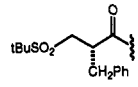
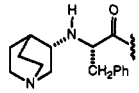
For inhibitor **4o** (R = 4-morpholinomethyl, *n* = 1), the absolute configuration at the morpholinomethyl center was determined by asymmetric synthesis as illustrated in Scheme V. Epoxide **22b** (see Scheme IV) was converted to amino alcohol **24** as described above. Derivative **24** was subjected to Sharpless kinetic resolution,[14] with L-tartrate giving (*R*)-amino alcohol **25** as a 10:1 mixture of enantiomers. This compound was converted to inhibitor **4o**. The stereochemical assignment was confirmed by X-ray crystallography of the *N*-Boc derivative of inhibitor **4o**, macrocycle **16o** (Figure 2).

In Vitro Renin Inhibition. Macrocyclic compounds **3a–f** and **4a–v** were evaluated in vitro for human plasma

(14) Miyano, S.; Lu, L. D.-L.; Viti, S. M.; Sharpless, K. B. Kinetic Resolution of Racemic β-Hydroxy Amines by Enantioselective *N*-Oxide Formation. *J. Org. Chem.* **1983**, *48*, 3608–3611.

Table III. Human Plasma Renin Inhibition by Morpholinomethyl Macrocycle Derivatives



compd	R	IC_{50} (nM)
14o	$PhCH_2OCO$	230
26	$PhOCH_2CO$	61
27	PhCH=CHCO	94
28	indol-2-yl-CO	11
29	$tBuAcNHCH_2CH_2OCO$-Phe	0.23
30	Boc-D-Pro-Phe	0.60
31	TFA·D-Pro-Phe	1.7
32		0.20
33		1.3
34	$BocNHC(CH_3)_2CH_2CO$-Phe	0.5
35	$TFA·H_2NC(CH_3)_2CH_2CO$-Phe	1.1
36	$O(CH_2CH_2)_2NCH_2CH_2OCO$-Phe	0.41
37	$Me(OCH_2CH_2)_3OCO$-Phe	0.50

renin inhibition at pH 7.4 according to the protocol of Boger and co-workers.[15] The results are summarized in Tables I and II. In the glutamate-derived series (Table I), 13-membered ring compound **3a** proved to be more active than 14-membered ring compound **3b**, and nearly equipotent with glutamine-derived macrocycle **1** (R = H, IC_{50} = 0.61 μM). As in the glutamine-derived series, substitution at R, corresponding to P_2', dramatically increased potency. For example, macrocycle **3e** (R = 4-morpholinomethyl) is a 17 nM inhibitor (Table I).

The serine-derived, diester macrocycles **4a** and **4b** proved to be much more potent than the corresponding amide derivatives (Table II). Fourteen-membered ring compound **4b** was more active with an IC_{50} of 3.4 nM. As before, in the substituted derivatives (R ≠ H), one diastereomer proved to be much more active. Substitution at P_2' in the 13-membered ring series generally increased activity (Table II), except in the case of R = neopentyl (inhibitor **4i**). In the 14-membered ring series, substitution at this position had less of an effect on activity, but did give one subnanomolar inhibitor, **4o** (R = (*R*)-4-morpholinomethyl). More importantly, substitution at this position also allowed the introduction of water-solubilizing groups such as morpholino (**4o**) and dialkylamino (**4s** and **4u**) which might improve oral bioavailability.

The "Boc-Phe" component of inhibitor **4o** was replaced with a variety of substituents, some of which are illustrated in Table III. Lower molecular weight derivatives such as phenoxyacetyl (**26**) and indolyl (**28**) gave fairly active inhibitors. Many substituents led to highly potent, subnanomolar derivatives (e.g., compounds **29**, **30**, **32**, **34**, **36**, **37**). Polar, water-solubilizing groups were also incorporated at this position without loss of activity (e.g., compounds **31**–**33**, **35**–**37**).

Molecular Modeling Studies. The computational model of the human renin active site developed at Merck[16] was utilized to gain insight into the mode of binding of the 14-membered ring serine-derived macrocycles to human renin. This model is based on the sequence homology of renin, human pepsin, and several fungal aspartyl proteinases for which crystal structures are available. The morpholinomethyl-substituted macrocycle **16o** (with Boc replaced by acetyl and cyclohexyl replaced by isopropyl) was energetically optimized as bound to the static renin active site using the Merck program OPTIMOL.[17] The minimized conformation (blue) overlayed with the minimized conformation of residues 7–13 of renin's natural substrate angiotensinogen as its scissile bond hydrate (green) is shown in Figure 3. The space accessible to a bound ligand which we classify as predominantly hydrophobic in nature[8] is illustrated in red. The macrocyclic ring occupies the same hydrophobic pocket as angiotensin's P_2 His and P_1' Val side chains. The morpholinomethyl substituent overlays the P_2' Ile side chain. This mode of binding is directly analogous to that calculated for the macrocycle of glutamine-derived inhibitor **1**.[8]

Figure 4 shows in blue the solid-state conformation of macrocycle **16o** obtained by X-ray crystallography. The macrocycle as minimized in the computational model of the human renin active site is illustrated in yellow. The two conformations are strikingly similar. To the extent that the solid-state conformation resembles that found in solution, the macrocycle would be required to undergo minimal torsional changes in binding to the enzyme.

One particularly interesting question arose in connection with the modeling studies. As Figure 1 shows, in macrocycle **4** (n = 1), an ester oxygen is substituted for an amide NH group present in macrocycle **2**. This NH group had been a motivating factor in the design of inhibitors **1** and **2**, as modeling in the static renin active site had suggested that it could donate a hydrogen bond to one of the Asp 226 oxygens (cf. Figure 4 in ref 8). Accordingly, substitution of this NH group by O to give macrocycles **3** and **4** would appear to replace a favorable interaction with renin with an unfavorable one, and hence should lead to a loss in potency. In fact, however, inhibitor **4b** is much more potent (IC_{50} = 3.4 nM for n = 1; see Table II) than are inhibitors **1** and **2** (IC_{50} = 0.61 and 0.59 μM, respectively). We have attempted to find a plausible basis for this surprising reversal in potency, without much success. One possibility is that the two series might bind differently. However, the superposition of the modeled conformations of macrocycles **2** and **4b** shown in Figure 5 suggests that they bind virtually identically. Alternatively, the energy cost entailed in adopting the detailed binding conformation might be substantially higher for **2**, outweighing its more favorable interactions with the renin site. The superpositions shown in Figure 6, however, suggest that the required deformations are minor and are geometrically similar for macrocycles **2** and **4b**. Also, the OPTIMOL

(15) 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. Renin Inhibitors. Synthesis of Subnanomolar, Competitive, Transition-State Analogue Inhibitors Containing A Novel Analogue of Statine. *J. Med. Chem.* **1985**, *28*, 1779–1790.

(16) 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. Renin Inhibitors Containing Conformationally Restricted P_1-P_1' Dipeptide Mimetics. *J. Med. Chem.* **1991**, *34*, 887–900.

(17) The optimizations employed the Merck-developed force-field MM2X as implemented in the molecular-mechanics program OPTIMOL. MM2X shares many force-field parameters with MM2, 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. See ref 8 for a detailed description of the modeling procedures used.

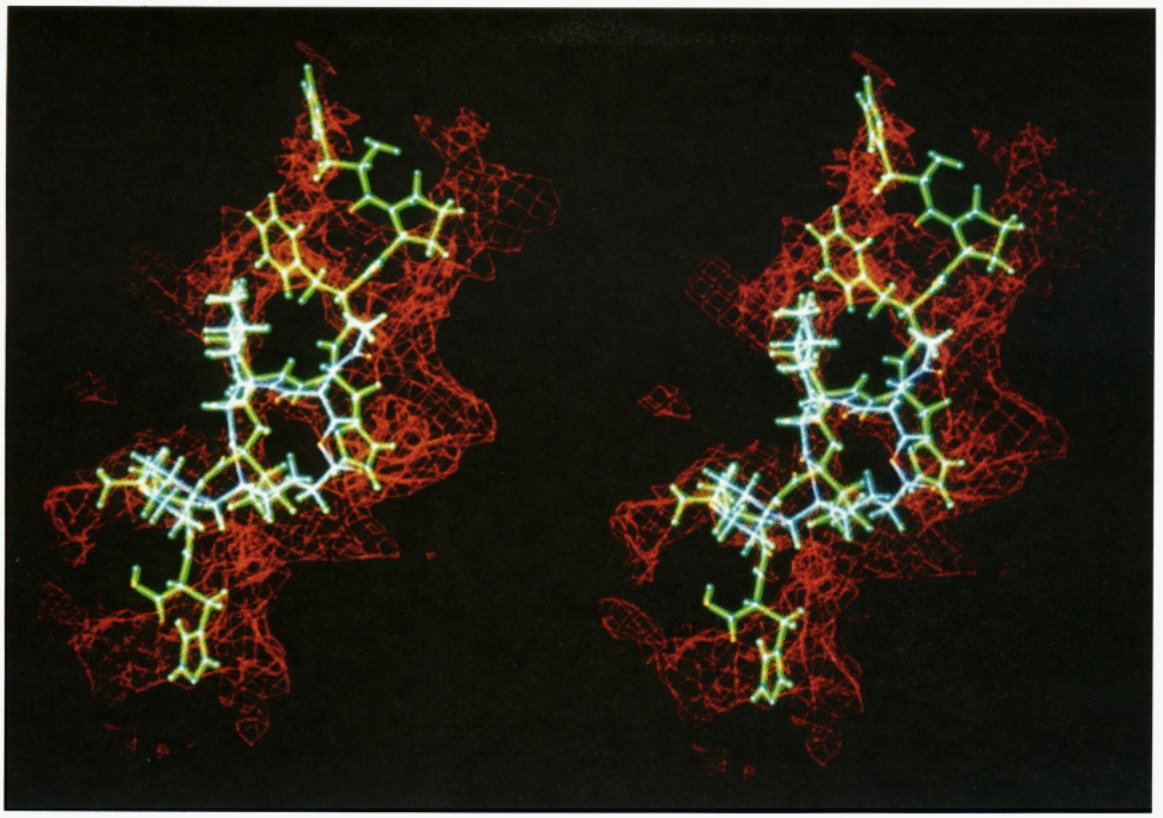


Figure 3. Overlay of the macrocycle of inhibitor **4o** (macrocycle **16o** with the *N*-Boc group modeled as *N*-acetyl and cyclohexyl as isopropyl) in blue and renin's naturally occurring substrate, angiotensinogen, as its scissile bond hydrate in green. Hydrophobic regions in the accessible space available for ligand binding are shown in red.

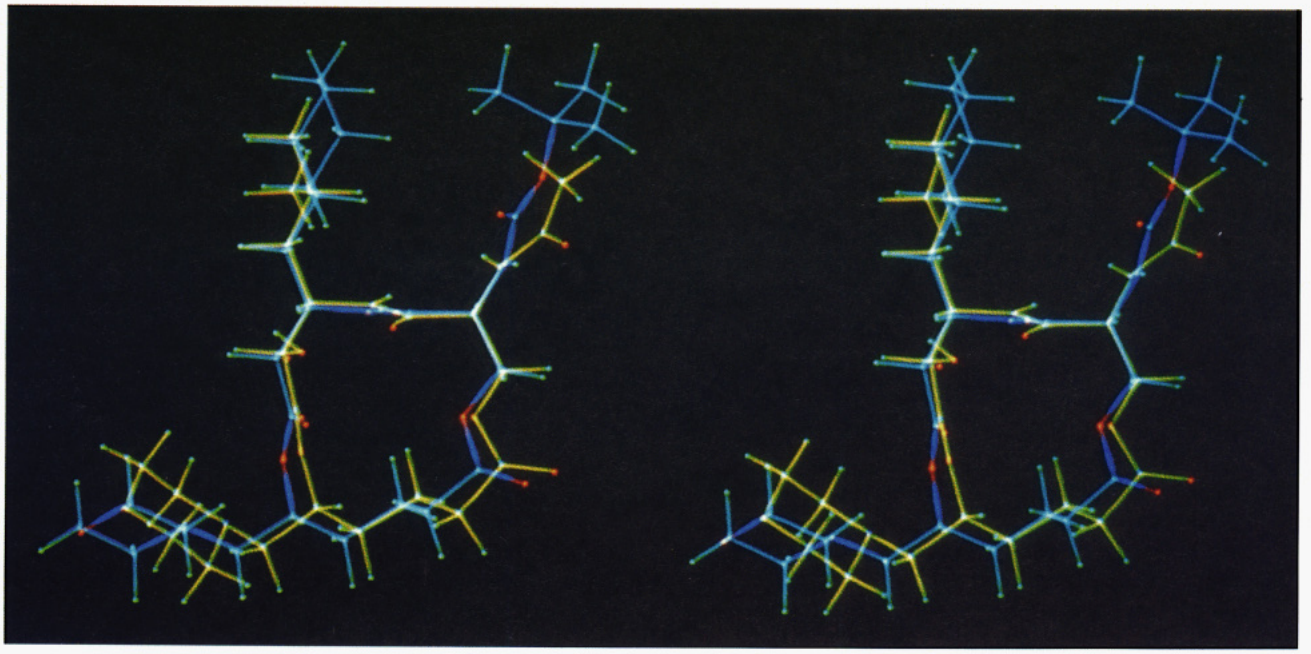


Figure 4. Crystal structure of macrocycle **16o** (blue) and its conformation as minimized in the Merck computational model of the human renin active site (yellow). In the minimized structure, the *N*-Boc group was modeled as *N*-acetyl and cyclohexyl as isopropyl.

energy calculations found the deformation energies arising from changes in bond, angle, torsion, and van der Waals interactions to be about the same, ca. 4.5 kcal/mol, in both cases. A third, related possibility is that conformations approximating the bound conformation might be present in free solution to an appreciably greater extent for inhibitor **4b** than for inhibitor **2**, requiring inhibitor **2** in effect to adopt a different (higher energy) conformation upon binding. A survey of conformations conducted as previously described[8] in fact suggested that the "correct" conformation might occur in free solution to the extent of ca. 3% for **4b**, but only to the extent of about 0.1% for **2**. Such a difference would support a higher potency for inhibitor **4b**. However, it is not certain either that the force field is sufficiently accurate or that modeling studies conducted in the absence of solvent can be relied upon to give a qualitatively correct account. The question of the treatment of solvent also suggests a fourth possibility—that desolvation of the amide group in macrocycle **2** upon binding might outweigh its favorable interaction with the

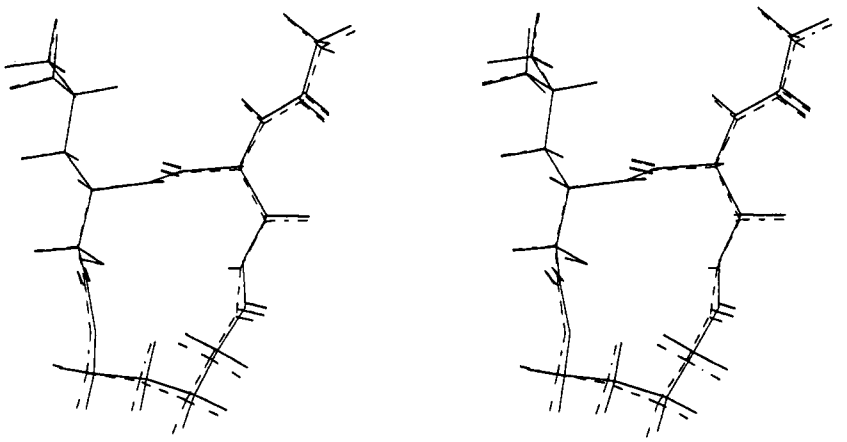


Figure 5. Relaxed stereoscopic view of the superposition of the conformations of macrocycles **2** (dashed) and **4b** as minimized in the human renin active site. The *N*-Boc-Phe group was modeled as *N*-acetyl and cyclohexyl as isopropyl. The -NH- and -O- groups lie along the right-hand edge of the macrocyclic ring. The Asp 38-Asp 226 dyad lies immediately behind the plane of the ring.

renin site. Still another possibility is that the Asp 226 oxygen approached by the serine-derived O in inhibitor **4b** might be protonated, allowing it to donate a hydrogen bond to inhibitor **4b**, thereby substituting a favorable interaction for what would otherwise be an unfavorable one. A number of additional possibilities could also be suggested.

The considerations cited in the previous paragraph illustrate some of the factors which complicate a modeling study of the present kind. The effect examined is not one which can easily be treated through energy minimizations carried out in the absence of explicit solvent using a static, model-built structure for the enzyme active site. The comparison of inhibitors **2** and **4b**, though, is an intriguing one. It would be interesting to see whether the techniques of thermodynamic simulation[18] would be able to account for the observed potencies when a reliable crystallographically-derived structure for human renin becomes available. Such an approach has been found to account reasonably well for the difference in potency of a related pair of inhibitors of the enzyme thermolysin, in which the structural change is also NH → O.[19] Moreover, as here, the former makes a hydrogen bond to the enzyme which the latter lacks. In that case, however, the oxygen-substituted compound is in fact the less potent, as the simple qualitative argument would suggest. Moreover, that case also lacks the extent of conformational variability possessed by macrocycles **2** and **4b**, and is further simplified by the fact that the structure of the enzyme is reliably known. The comparison of **2** and **4b** is therefore intrinsically more difficult, and will constitute a more demanding test for thermodynamic simulation techniques.

In Vivo Activity in Rhesus Monkeys. The in vivo activity of inhibitors **32** and **33** was examined in sodium-depleted rhesus monkeys, by monitoring both blood pressure and PRA.[20] Intravenous administration of inhibitor **32** (0.01 mg/kg)[21] produced an inhibition of PRA and an accompanying drop in blood pressure which persisted for 0.5 h (Figure 7). An oral dose of 15 mg/kg of inhibitor **32** produced a decrease in blood pressure which lasted over 4 h. PRA was inhibited (>80%) over the course of the 6-h experiment.

Intravenous administration (0.05 mg/kg) of inhibitor **33** caused a rapid and complete inhibition of PRA accompanied by a sharp fall in blood pressure (Figure 7). As observed with inhibitor **32**, these effects were short-lived, with blood pressure and PRA returning to normal within 1 and 2 h, respectively. Following an oral dose of 15 mg/kg, macrocycle **33** produced a sustained lowering of mean arterial pressure throughout the 6-h experiment (Figure 7). In addition, plasma renin activity was completely inhibited over the course of the experiment. The improved oral activity for renin inhibitors such as **33** which contain an N-terminal 3(*S*)-quinuclidinyl-Phe has previously been noted.[22] It may be due in part to increased aqueous solubility (>20 mg/mL in 0.1 M aqueous citric acid solution) relative to that of less basic inhibitors such as macrocycle **32**.[22] The incorporation of polar, water-solubilizing groups is an approach which has provided renin inhibitors with increased oral activity.[23]

Bioavailability and Metabolism Studies in Rats. In order to gain a better understanding of the bioavail-

(18) See, for example: *Computer Simulation of Biomolecular Systems*; van Gunsteren, W. F.; Weiner, P. K., Eds.; ESCOM: Leiden, 1989.

(19) Merz, K. M., Jr.; Kollman, P. A. Free Energy Perturbation Simulations of the Inhibition of Thermolysin: Predictions of the Free Energy of Binding of a New Inhibitor. *J. Am. Chem. Soc.* **1989**, *111*, 5649–5658.

(20) See ref 16 for details. Briefly, rhesus monkeys were surgically implanted with chronic arterial catheters with access ports for direct monitoring of mean arterial pressure and repeated collection of blood samples. The monkeys were maintained on a low sodium (1.2 mmol/day) diet for 1 week and then given furosemide (2.5 mg/kg, im) the evening before the experiment. The compound was administered orally as a solution in 0.1 M aqueous citric acid via nasogastric catheter or intravenously as a solution in 0.5% aqueous citric adic/5% dextrose via bolus injection.

(21) Intravenously, inhibitors were tested at doses which were derived by comparing their IC_{50} values to that of the standard renin inhibitor SCRIP (Iva-His-Pro-Phe-His-Sta-Leu-Phe-NH_2). The iv dose of SCRIP required to lower blood pressure by 50% of the maximal response was determined (ED_{50} = 0.039 μM/kg). A projected ED_{50} dose for each inhibitor was calculated using the following formula: ED_{50} (inhibitor) = ED_{50} (SCRIP) × [IC_{50} (inhibitor)/IC_{50} (SCRIP)]. In order to assure initial complete inhibition of endogenous monkey renin after iv administration, a dose of four times the projected ED_{50} dose was utilized.

(22) Greenlee, W. J.; tenBroeke, J.; de Laszlo, S. E.; Chakravarty, P. K.; Camara, V. J.; Fitch, K. J.; Sarnella, C. S.; Patchett, A. A.; Williams, P. D.; Perlow, D. S.; Veber, D. F.; Lynch, R. J.; Doyle, J. J.; Schorn, T. W.; Stourse, J. F.; Siegl, P. K. S. Approaches to Human Renin Inhibitors with Improved Bioavailability. *Peptides: Chemistry, Structure, and Biology. Proceedings of the Eleventh American Peptide Symposium*; Rivier, J. E.; Marshall, G. R., Eds.; ESCOM: Leiden, 1990; pp 411–412.

(23) (a) Rosenberg, S. H.; Woods, K. W.; Sham, H. L.; Kleinert, H. D.; Martin, D. L.; Stein, H.; Cohen, J.; Egan, D. A.; Bopp, B.; Merits, I.; Garren, K. W.; Hoffman, D. J.; Plattner, J. J. Water-Soluble Renin Inhibitors: Design of a Subnanomolar Inhibitor with a Prolonged Duration of Action. *J. Med. Chem.* **1990**, *33*, 1962–1969. (b) Kleinert, H. D.; Martin, D.; Chekal, M. A.; Kadam, J.; Luly, J. R.; Plattner, J. J.; Perun, T. J.; Luther, R. R. Effects of the Renin Inhibitor A-64662 in Monkeys and Rats with Varying Baseline Plasma Renin Activity. *Hypertension* **1988**, *11*, 613–619. (c) Pals, D. T.; Thaisrivongs, S.; Lawson, J. A.; Kati, W. M.; Turner, S. R.; DeGraaf, G. L.; Harris, D. W.; Johnson, G. A. An Orally Active Inhibitor of Renin. *Hypertension* **1986**, *8*, 1105–1112.

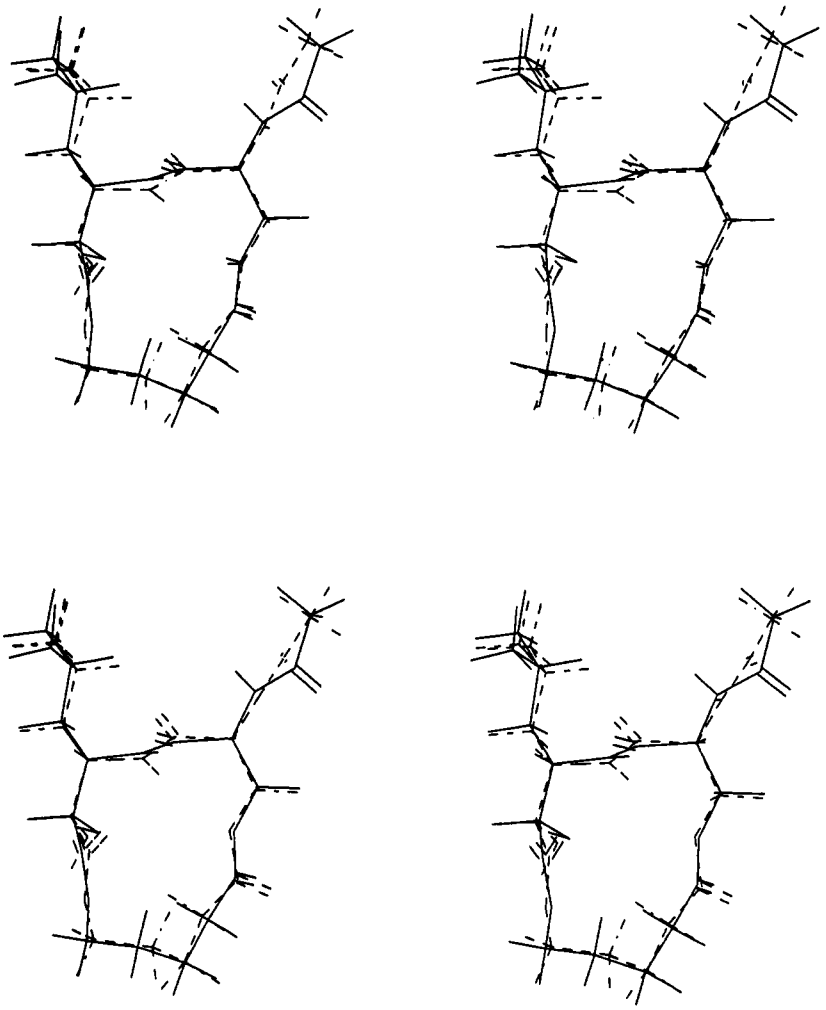


Figure 6. Relaxed stereoscopic views of the superposition of active-site-bound and "free solution" (dashed, minimized with a constant dielectric constant of 50) conformations for inhibitors **2** (top) and **4b** (bottom). The *N*-Boc-Phe group was modeled as *N*-acetyl and cyclohexyl as ispropyl.

ability and in vivo metabolism of inhibitor **33**, a radiolabeled derivative [^{3}H]-**33** was synthesized and studied in rats. As shown in Scheme VI, iodophenylalanine (**38**) was reductively alkylated with 3-quinuclidinone. Following separation of diastereomers using reverse-phase MPLC, the faster-eluting (*S,S*)-diastereomer **39** was coupled to deprotected macrocycle **16o** to give the iodo derivative **40**. Reductive dehalogenation with sodium borotritiide catalyzed by palladium (II) chloride gave [^{3}H]-**33**. In addition, potential ring-opened metabolites of inhibitor **33**, derivatives **41** and **42** (Figure 8), were also synthesized in a straightforward manner. Compounds **41** and **42** themselves do not inhibit human renin (IC_{50} > 20 μM).

After intravenous administration of [^{3}H]-**33** (2 mg/kg) to male Sprague–Dawley rats, the plasma concentration of inhibitor **33** declined rapidly (Figure 9a). After 1 h, the plasma concentrations of inhibitor **33** and total equivalents were both approximately 0.1 μg/mL. A shoulder on the leading edge of the inhibitor **33** peak was present in a number of the plasma samples when analyzed by HPLC. Subsequently, it was demonstrated that ring-opened derivative **41** eluted approximately 0.5 min before **33**. It was not possible, therefore, to determine the individual concentrations of the parent and open-chain metabolite. If combined, however, the plasma clearance was 2.1 ± 2.1 L/kg per h and the volume of distribution at steady state was 3.9 ± 3.3 L/kg. Total plasma drug equivalents peaked at 0.4 μg/mL between 1 and 4 h after oral administration of [^{3}H]-**33** (9 mg/kg, Figure 9b). Only two of 36 plasma samples at 1 and 2 h, however, contained compounds **33** and/or **41**. Essentially all of the radioactivity in plasma after oral administration eluted close to the void volume. The bioavailability of inhibitor **33** was approximately 1%. At 24 h, 39 ± 8% and 69 ± 4% of the radioactivity in plasma was volatile after iv and oral administration, respectively, suggesting the formation of tritiated water due to metabolism or tritium exchange.

To determine if the hydrolysis of [^{3}H]-**33** occurred in plasma, the drug was incubated with rat and human plasma at 37 °C and analyzed using a C-18 HPLC column. On this column material, compounds **33** and **42** had the same retention time and **41** eluted before them. After incubation for 1 h with rat plasma, there was considerable degradation to derivative **41**. At 6 and 16 h, only a single peak was apparent; by FAB-MS, this peak was identified as exclusively **42**. In the presence of human plasma, only one peak of radioactivity was observed at all time points. At 16 h, however, the peak was composed of both the parent drug and derivative **42** as determined by FAB-MS. The protonated molecular ions for both compounds were observed in the sample. The plasma, therefore, contains esterases capable of cleaving the diester moiety.

When [^{3}H]-**33** was given iv at 1 mg/kg, almost 20% of the administered dose was in the urine and 47% was in the feces by 48 h (Table IV). In contrast, 3.8% was in the urine and 85% was in the feces in the same time period

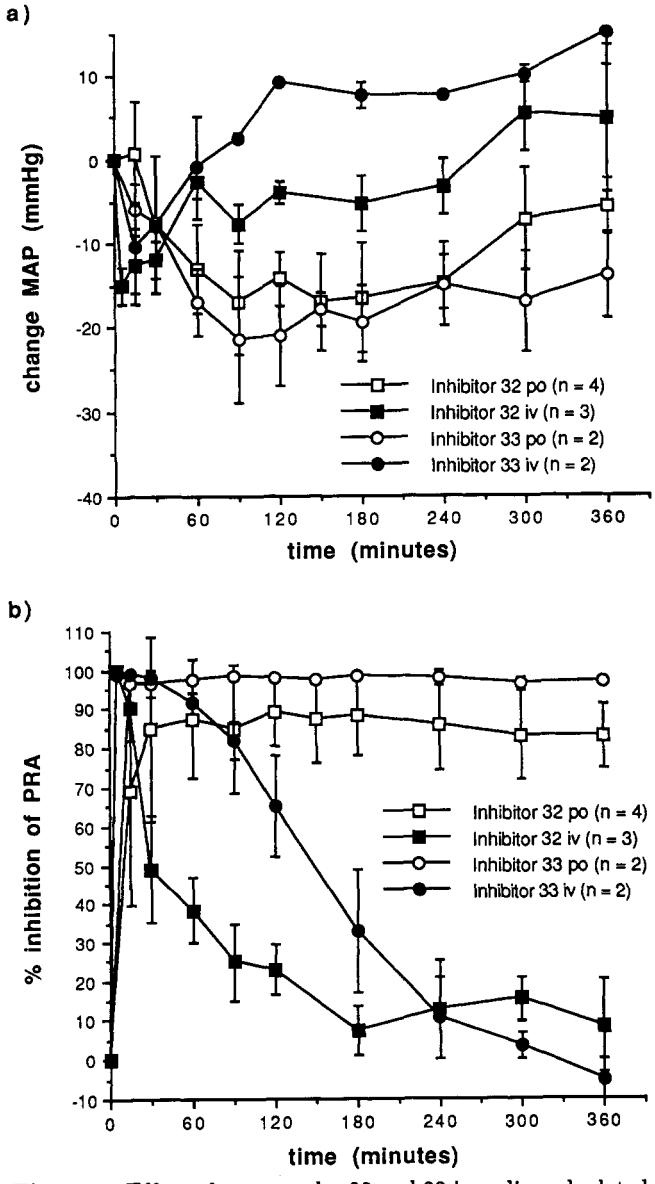
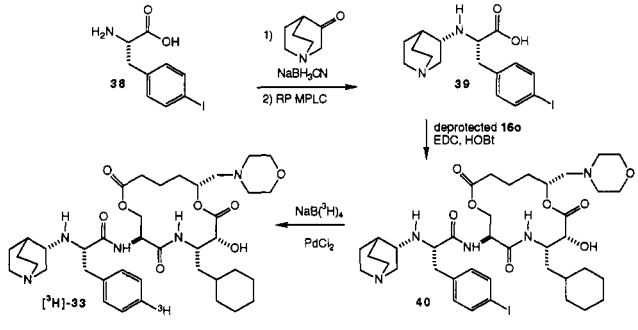


Figure 7. Effect of macrocycles **32** and **33** in sodium-depleted rhesus monkeys on (a) mean arterial pressure and (b) PRA following oral (15 mg/kg) and intravenous (inhibitor **32**, 0.01 mg/kg; inhibitor **33**, 0.05 mg/kg) administration[20,21] (mean ± SD).

Scheme VI. Tritium Incorporation into Inhibitor **33**



when the compound was administered orally at 10 mg/kg. The urinary radioactivity from the intravenously dosed rats was associated with a number of metabolites while that from the orally dosed animals eluted close to the solvent front. When the concentration of acetonitrile in the mobile phase was decreased to 5%, the urinary radioactivity from the orally dosed rats was associated with two peaks, one at 4 min and the other at 9.5 min. The

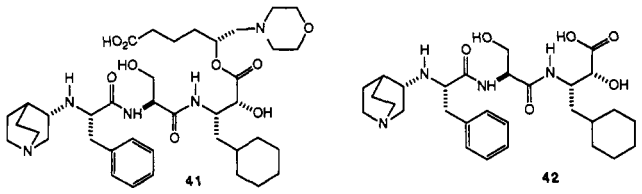


Figure 8. Structures of possible ring-opened metabolites **41** and **42** of inhibitor **33**.

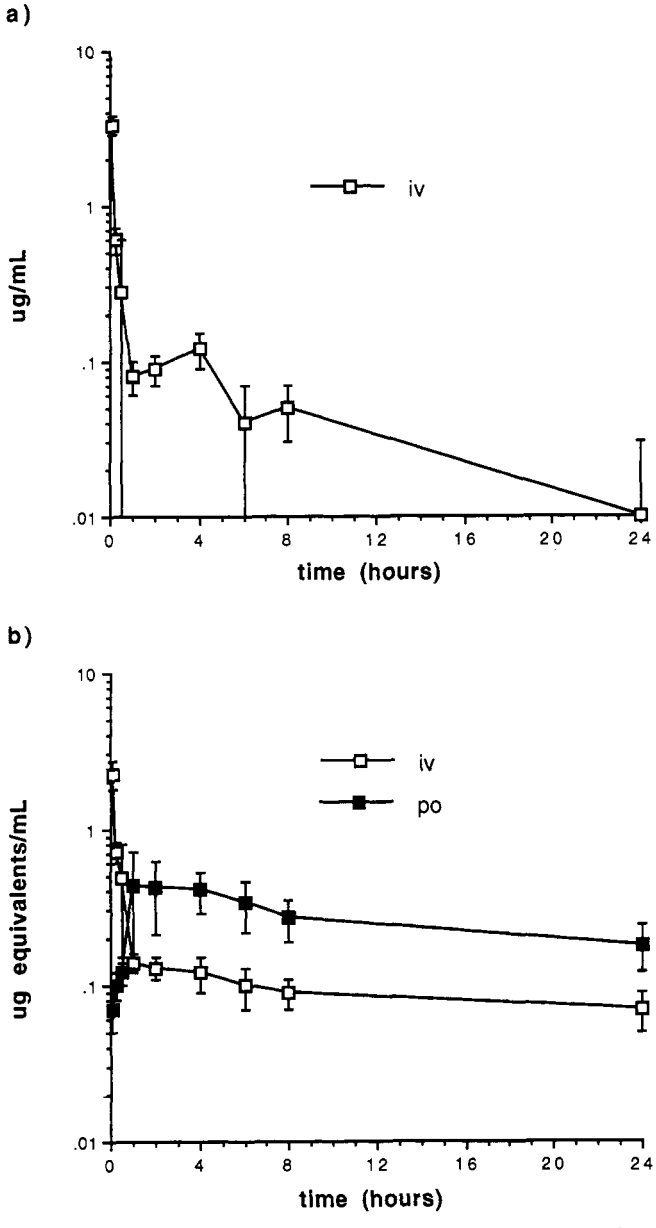


Figure 9. (a) Concentration of **33** after intravenous (2 mg/kg) administration and (b) total drug equivalents in plasma of male Sprague–Dawley rats after oral (9 mg/kg) or intravenous (2 mg/kg) administration of [^{3}H]-**33** (mean ± SD; n = 4).

latter had the same retention time as *N*-3(*S*)-quinuclidinylphenylalanine. Tritiated water eluted at 3.0 min in this system.

After iv administration of [^{3}H]-**33**, 49% of the dose was excreted into bile by 24 h (Table V); nearly three-quarters of the total biliary excretion occurred in the first 30 min. This rapid elimination into the bile indicated extensive hepatic extraction. After id administration, only 4.6% was excreted by 24 h. Bile from the iv and id dosed rats contained a number of radioactive peaks. Two of the peaks had the same retention times as compounds **33** and **41**.

Table IV. Urinary and Fecal Excretion of Radioactivity by Male Sprague–Dawley Rats after Intravenous (1 mg/kg) or Oral (10 mg/kg) Administration of [^{3}H]-33

		% dose excreted	
	time (h)	intravenous[a]	oral[a]
urine	0–24	18.5 ± 3.1	3.1 ± 0.7
	24–48	0.7 ± 0.0	0.7 ± 0.6
	48–72	nd[b]	0.3 ± 0.2
feces	0–24	43.5 ± 9.4	81.7 ± 7.6
	24–48	3.5 ± 0.8	3.3 ± 1.7
	48–72	nd[b]	0.2 ± 0.1
total		66.2 ± 10.2	89.1 ± 7.6

[a] Mean ± SD; n = 3. [b] Not determined.

Table V. Biliary Excretion of Radioactivity by Male Sprague–Dawley Rats after Intravenous (1.0 mg/kg) or Intraduodenal (10 mg/kg) Administration of [^{3}H]-33

	cumulative % dose excreted			cumulative % dose excreted	
time (h)	intravenous[a]	intraduodenal[b]	time (h)	intravenous[a]	intraduodenal[b]
0–0.5	35.7 ± 2.4	2.4	3–4	46.9 ± 4.5	4.2
0.5–1.0	42.7 ± 5.0	3.3	4–6	47.5 ± 4.6	4.3
1.0–1.5	44.6 ± 4.8	3.6	6–8	47.9 ± 4.6	4.4
1.5–2.0	45.5 ± 4.7	3.9	8–24	48.7 ± 4.7	4.7
2–3	46.4 ± 4.6	4.0			

[a] Mean ± SD; n = 3. [b] Average; n = 2.

The peak eluting at 3.0 min from the intraduodenally-dosed rat separated into five peaks when the acetonitrile concentration of the mobile phase was decreased to 5%. One peak had the same retention time as *N*-3(*S*)-quinuclidinylphenylalanine.

Based on the amount of radioactivity in the bile after iv and id administration, approximately 10% of the dose was absorbed. If absorption is calculated using the amount of radioactivity excreted in the urine, 21% was absorbed. These results suggest that inhibitor **33** is poorly absorbed and that there is very efficient first pass extraction by the liver. The presence of parent drug and the open-chain metabolite in the bile but very little of these compounds in the plasma after id administration supports this conclusion.

Conclusions

Replacing one amide bond in macrocycles of the general structure **1** and **2** with an ester linkage gave glutamate-derived renin inhibitors **3** and serine-derived renin inhibitors **4**. While this oxygen for nitrogen exchange had little affect on potency in the glutamate series, potency was dramatically increased in the serine series. In this series, the 14-membered ring compounds proved to be more potent than the corresponding 13-membered ring derivatives. Substitution at "R" corresponding to P_2' generally increased potency. The absolute configuration at this center was shown to be *R* for the R = 4-morpholinomethyl derivative (**4o**), both by asymmetric synthesis and X-ray crystallography. Replacing the "Boc-Phe" moiety of inhibitor **4o** with a variety of substituents led to subnanomolar inhibitors, one of which (inhibitor **33**) lowered blood pressure and completely inhibited plasma renin activity for 6 h in sodium-depleted rhesus monkeys. This compound proved to have limited bioavailability (1% in rats), in part due to the cleavage of the serine ester bond. Replacement of this ester linkage with nonhydrolyzable groups could lead to inhibitors with increased bioavailability. These studies are ongoing and will be reported in due course.

Experimental Section

^{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 using EM Reagents 0.25 mm silica gel 60-F plates, eluting with the indicated solvent system. Chromatography on silica gel was performed using 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 using EM Reagents Lobar silica gel 60 prepacked columns or, where indicated, Sephadex LH-20. Solvents and reagents were used as received.

General Procedure A: 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) were 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 B: 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 hydroxbenzotriazole 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 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 amide.

General Procedure C: 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 D: 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 E: Deprotection Using H_2. A solution of substrate in methanol, ethyl acetate, or dimethylformamide was stirred with 10% Pd/C under 1 atm of hydrogen for several h until the deprotection was judged complete by TLC analysis. The mixture was filtered through Celite and concentrated.

General Procedure F: Macrocyclization Using EDC/HOBt. The substrate was dissolved in tetrahydrofuran to form a 0.001 M solution. The solution was cooled to 0 °C and treated with *N*-methylmorpholine (1.1 equiv), HOBt (4.0 equiv), and EDC (4.0 equiv). The mixture was allowed to warm to room temperature and was stirred for a total of 5–6 days. Solvent 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 G: Macrocyclization Using EDC/DMAP-DMAP·HCl. The substrate was dissolved in tetrahydrofuran to form a 0.1 M solution and added dropwise over 19 h to a refluxing solution of EDC (2.0 equiv), DMAP (1.1 equiv), and DMAP hydrochloride (2.0 equiv) in freshly distilled chloroform (50 mL/mmol substrate). After addition was complete, the solution was cooled, diluted with ethyl acetate, washed sequentially with 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.

Z-Glu(2-hydroxyethyl)-OtBu (7a; R = H, *n* = 0). *N*-Carbobenzoxyglutamic acid α-*tert*-butyl ester dicyclohexylamine salt (Z-Glu-OtBu·DCHA) (215 mg, 0.421 mmol, 1.0 equiv) was coupled to ethylene glycol (261 mg, 4.21 mmol, 10 equiv) according to general procedure A. Purification by flash chromatography (2.5% and 5% methanol/dichloromethane) gave 149 mg (93%) of the title compound: R_f 0.19 (40% ethyl acetate/hexane); MS (FAB) *m/z* 382 (M + 1).

Z-Glu(3-hydroxypropyl)-OtBu (7b; R = H, *n* = 1). Z-Glu-OtBu·DCHA (545 mg, 1.05 mmol, 1.0 equiv) was coupled to 1,3-propanediol (799 mg, 10.5 mmol, 10 equiv) according to general procedure A. Purification by flash chromatography (50% ethyl acetate/hexane) gave 220 mg (53%) of the title compound: R_f 0.23 (50% ethyl acetate/hexane); MS (FAB) *m/z* 396 (M + 1), 340, 296. Anal. ($C_{20}H_{29}NO_{7}\cdot^{1}/_{4}H_2O$) C, H, N.

Z-Glu[(2*S*,2*R*)-2-hydroxyhexyl]-OtBu (7c,d; R = Bu, *n* = 0, diast 1,2). Z-Glu-OtBu·DCHA (1070 mg, 2.00 mmol, 1.0 equiv) was coupled to 1,2-hexanediol (708 mg, 6.00 mmol, 3.0 equiv) according to general procedure A. Purification by MPLC (25% ethyl acetate/hexane) gave 378 mg (43%) of the title compound as a mixture of diastereomers: R_f 0.58 (50% ethyl acetate/hexane); MS (FAB) *m/z* 438 (M + 1), 382, 337. Anal. ($C_{23}H_{35}NO_{7}\cdot^{1}/_{4}H_2O$) C, H, N.

Z-Glu[(2*S*,2*R*)-2-hydroxy-3-(4-morpholino)propyl]-OtBu (7e,f; R = $CH_2N(CH_2CH_2)_2O$, *n* = 0, diast 1,2). Z-Glu-OtBu·DCHA (1720 mg, 3.38 mmol, 1.0 equiv) was coupled to glycidol (0.448 mL, 6.75 mmol, 2.0 equiv) according to general procedure A. Purification by flash chromatography (35% ethyl acetate/hexane) gave 971 mg (73%) of the corresponding glycidyl ester. A 712-mg (1.18 mmol) sample of this derivative was dissolved in ether and stirred overnight with morpholine (0.316 mL, 3.62 mmol, 2.0 equiv) and neutral alumina (1 g). The mixture was filtered and concentrated. Purification by flash chromatography (1.5% and 2.5% methanol/dichloromethane) gave 651 mg (75%) of the title compound as a mixture of diastereomers: MS (FAB) *m/z* 481 (M + 1), 425. Anal. ($C_{24}H_{36}N_2O_{8}\cdot^{1}/_{2}H_2O$) C, H, N.

O^ω^-[2-[[[(4*S*,5*R*)-4-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolidinyl]carbonyl]oxy]ethyl]-N^α^-[(phenylmethoxy)carbonyl]-L-glutamic Acid, 1,1-Dimethylethyl Ester (9a; R = H, *n* = 0). Alcohol **7a** (149 mg, 0.392 mmol, 1.0 equiv) and Boc-norACHPA acetonide **8** (147 mg, 0.431 mmol, 1.1 equiv) were coupled according to general procedure A. Purification by flash chromatography (25% ethyl acetate/hexane) gave a quantitative yield of the title compound: R_f 0.25 (25% ethyl acetate/hexane). Anal. ($C_{37}H_{56}N_2O_{11}$) C, H, N.

O^ω^-[3-[[[(4*S*,5*R*)-4-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolidinyl]carbonyl]oxy]propyl]-N^α^-[(phenylmethoxy)carbonyl]-L-glutamic Acid, 1,1-Dimethylethyl Ester (9b; R = H, *n* = 1). Alcohol **7b** (185 mg, 0.468 mmol, 1.0 equiv) and Boc-norACHPA acetonide **8** (176 mg, 0.515 mmol, 1.1 equiv) were coupled according to general procedure A. Purification by MPLC (25% ethyl acetate/hexane) gave a quantitative yield of the title compound: R_f 0.24 (25% ethyl acetate/hexane); MS (FAB) *m/z* 719 (M + 1), 619. Anal. ($C_{38}H_{58}N_2O_{11}$) C, H, N.

O^ω^-[(2*S*,2*R*)-2-[[[(4*S*,5*R*)-4-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolidinyl]carbonyl]oxy]hexyl]-N^α^-[(phenylmethoxy)carbonyl]-L-glutamic Acid, 1,1-Dimethylethyl Ester (9c,d; R = Bu, *n* = 0, diast 1,2). Alcohol **7c,d** (321 mg, 0.734 mmol, 1.0 equiv) and Boc-norACHPA acetonide **8** (276 mg, 0.808 mmol, 1.1 equiv) were coupled according to general procedure A. Purification by MPLC (20% ethyl acetate/hexane) gave 270 mg (48%) of a faster eluting isomer, **9c**, and 259 mg (46%) of a slower eluting isomer, **9d**. Compound **9c**: R_f 0.21 (15% ethyl acetate/hexane); MS (FAB) *m/z* 661, 605. Anal. ($C_{38}H_{58}N_2O_{11}$) C, H, N. Compound **9d**: R_f 0.17 (15% ethyl acetate/hexane); MS (FAB) *m/z* 661, 605. Anal. ($C_{38}H_{58}N_2O_{11}$) C, H, N.

O^ω^-[(2*S*,2*R*)-2-[[[(4*S*,5*R*)-4-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolidinyl]carbonyl]oxy]-3-(4-morpholino)propyl]-N^α^-[(phenylmethoxy)carbonyl]-L-glutamic Acid, 1,1-Dimethylethyl Ester (9e,f; R = $CH_2N(CH_2CH_2)_2O$, *n* = 0, diast 1,2). Alcohol **7e,f** (652 mg, 1.36 mmol, 1.0 equiv) and Boc-norACHPA acetonide **8** (556 mg, 1.64 mmol, 1.2 equiv) were coupled according to general procedure A. Purification by MPLC (35% and 65% ethyl acetate/hexane) gave 371 mg (34%) of a faster eluting isomer, **9e**, 238 mg (22%) of a slower eluting isomer, **9f**, and 168 mg (15%) of a mixture of the two. Compound **9e**: MS (FAB) *m/z* 804 (M + 1). Anal. ($C_{42}H_{65}N_3O_{12}\cdot 1.15H_2O$) C, H, N. Compound **9f**: MS (FAB) *m/z* 804 (M + 1).

(8*S*,11*S*,12*R*)-11-(Cyclohexylmethyl)-12-hydroxy-8-[[(phenylmethoxy)carbonyl]amino]-5,9,13-trioxo-1,4-dioxa-10-azacyclotridecane (10a; R = H, *n* = 0). Compound **9a** (276 mg, 0.392 mmol) was deprotected according to general procedure C and cyclized according to general procedure F. Purification by flash chromatography (2.5%, 5%, and 7.5% methanol/dichloromethane) gave 113 mg (59%) of the title compound: MS (FAB) *m/z* 447, 357. Anal. ($C_{25}H_{34}N_2O_{8}\cdot^{1}/_{2}H_2O$) C, H, N.

(9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-13-hydroxy-9-[[(phenylmethoxy)carbonyl]amino]-6,10,14-trioxo-1,5-dioxa-11-azacyclotridecane (10b; R = H, *n* = 1). Compound **9b** (317 mg, 0.441 mmol) was deprotected according to general procedure C and cyclized according to general procedure F. Purification by flash chromatography (60% ethyl acetate/hexane) gave 129 mg (58%) of the title compound: R_f 0.08 (50% ethyl acetate/hexane); MS (FAB) *m/z* 505 (M + 1). Anal. ($C_{26}H_{36}N_2O_{8}\cdot^{3}/_{4}H_2O$) C, H, N.

(8*S*,11*S*,12*R*)-2-Butyl-11-(cyclohexylmethyl)-12-hydroxy-8-[[(phenylmethoxy)carbonyl]amino]-5,9,13-trioxo-1,4-dioxa-10-azacyclotridecane (10c; R = Bu, *n* = 0, diast 1). Compound **9c** (240 mg, 0.315 mmol) was deprotected according to general procedure C and cyclized according to general procedure F. Purification by flash chromatography (35% ethyl acetate/hexane) gave 132 mg (75%) of the title compound: R_f 0.17 (35% ethyl acetate/hexane); MS (FAB) *m/z* 547 (M + 1). Anal. ($C_{29}H_{42}N_2O_{8}\cdot^{1}/_{4}H_2O$) C, H, N.

(8*S*,11*S*,12*R*)-2-Butyl-11-(cyclohexylmethyl)-12-hydroxy-8-[[(phenylmethoxy)carbonyl]amino]-5,9,13-trioxo-1,4-dioxa-10-azacyclotridecane (10d; R = Bu, *n* = 0, diast 2). Compound **9d** (249 mg, 0.327 mmol) was deprotected according to general procedure C and cyclized according to general procedure F. Purification by flash chromatography (35% ethyl acetate/hexane) gave 94 mg (53%) of the title compound: R_f 0.17 (35% ethyl acetate/hexane); MS (FAB) *m/z* 547 (M + 1). Anal. ($C_{29}H_{42}N_2O_{8}\cdot^{1}/_{4}H_2O$) C, H, N.

(8*S*,11*S*,12*R*)-11-(Cyclohexylmethyl)-12-hydroxy-2-(4-morpholinomethyl)-8-[[(phenylmethoxy)carbonyl]amino]-5,9,13-trioxo-1,4-dioxa-10-azacyclotridecane (10e; R = $CH_2N(CH_2CH_2)_2O$, *n* = 0, diast 1). Compound **9e** (371 mg, 0.461 mmol) was deprotected according to general procedure C and cyclized according to general procedure F with one modification: in the workup, the acid wash was omitted. Purification by MPLC (LH-20, methanol) gave 87.2 mg (32%) of the title compound: R_f 0.44 (10% methanol/dichloromethane); MS (FAB) *m/z* 590 (M + 1). Anal. ($C_{30}H_{43}N_3O_9$) C, H, N.

(8*S*,11*S*,12*R*)-11-(Cyclohexylmethyl)-12-hydroxy-2-(4-morpholinomethyl)-8-[[(phenylmethoxy)carbonyl]amino]-5,9,13-trioxo-1,4-dioxa-10-azacyclotridecane (10f; R = $CH_2N(CH_2CH_2)_2O$, *n* = 0, diast 2). Compound **9f** (238 mg, 0.296 mmol) was deprotected according to general procedure C and cyclized according to general procedure F with one modification: in the workup, the acid wash was omitted. Purification by flash chromatography (2.5% and 5% methanol/dichloromethane) followed by MPLC (LH-20, methanol) gave 51 mg (29%) of the title compound: R_f 0.56 (10% methanol/dichloromethane); MS (FAB) *m/z* 590 (M + 1). Anal. ($C_{30}H_{43}N_3O_{9}\cdot^{1}/_{2}H_2O$) 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-1,4-dioxa-10-azacyclotridecane (3a; R = H, *n* = 0). Macrocycle **10a** (48.1 mg, 0.981 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (78.0 mg, 0.294 mmol, 3.0 equiv) according to general procedure B. Purification by flash chromatography (75% ethyl acetate/hexane) gave 30.7 mg (52%) of the title compound: ^{1}H NMR (300 MHz, d_6-DMSO) δ 7.88 (d, J = 7.4 Hz, 1 H), 7.70 (d, J = 9.5 Hz, 1 H), 7.32–7.16 (m, 5 H), 7.13 (d, J = 8.8 Hz, 1 H), 5.43 (d, J = 6.5 Hz, 1 H), 4.85 (m, 1 H), 4.38–3.97 (m, 7 H), 2.93 (dd, J = 3.7, 14.0 Hz, 1 H), 2.72 (dd, J = 10.9, 12.3 Hz, 1 H), 2.42 (m, 1 H), 2.28 (dd, J = 7.2, 13.4 Hz, 1 H), 2.09–1.94 (m, 1 H), 1.86–1.77 (m, 2 H), 1.65–1.50 (m, 4 H), 1.36–1.09 (m, 6 H), 1.29 (s, 9 H), 0.91–0.76 (m, 2 H); MS (FAB) m/z 548, 505. Anal. ($C_{31}H_{45}N_3O_9 \cdot {}^1/_2H_2O$) C, H, N.

(9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-13-hydroxy-9-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-6,10,14-trioxo-1,5-dioxa-11-azacyclotetradecane (3b; R = H, *n* = 1). Macrocycle **10b** (58.3 mg, 0.116 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (61.3 mg, 0.231 mmol, 2.0 equiv) according to general procedure B. Purification by flash chromatography (70% ethyl acetate/hexane) followed by MPLC (LH-20, methanol) gave 33.3 mg (47%) of the title compound: R_f 0.23 (70% ethyl acetate/hexane); ^{1}H NMR (300 MHz, CD_3OD) δ 8.11 (d, J = 7.8 Hz, 1 H), 7.74 (d, J = 8.6 Hz, 1 H), 7.29–7.19 (m, 5 H), 6.69 (d, J = 8.0 Hz, 1 H), 4.61–4.19 (m, 7 H), 3.94 (ddd, J = 3.3, 8.2, 11.2 Hz, 1 H), 3.08 (dd, J = 4.2, 13.6 Hz, 1 H), 2.78 (dd, J = 9.6, 13.6 Hz, 1 H), 2.49 (m, 1 H), 2.33–2.12 (m, 2 H), 2.01–1.87 (m, 3 H), 1.80 (br d, J = 12.2 Hz, 1 H), 1.75–0.81 (m, 11 H), 1.35 (s, 9 H); MS (FAB) m/z 618 (M + 1), 562, 518. Anal. ($C_{32}H_{47}N_3O_9$) C, H, N.

(8*S*,11*S*,12*R*)-2-Butyl-11-(cyclohexylmethyl)-12-hydroxy-8-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-5,9,13-trioxo-1,4-dioxa-10-azacyclotridecane (3c; R = Bu, *n* = 0, diast 1). Macrocycle **10c** (37.6 mg, 0.0688 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (37.6 mg, 0.142 mmol, 2.06 equiv) according to general procedure B. Purification by flash chromatography (50% ethyl acetate/hexane) gave 38.5 mg (85%) of the title compound: R_f 0.25 (50% ethyl acetate/hexane); MS (FAB) m/z 660 (M + 1), 604, 560. Anal. ($C_{35}H_{53}N_3O_9$) C, H, N.

(8*S*,11*S*,12*R*)-2-Butyl-11-(cyclohexylmethyl)-12-hydroxy-8-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-5,9,13-trioxo-1,4-dioxa-10-azacyclotridecane (3d; R = Bu, *n* = 0, diast 2). Macrocycle **10d** (30.6 mg, 0.0560 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (30.6 mg, 0.115 mmol, 2.06 equiv) according to general procedure B. Purification by flash chromatography (50% ethyl acetate/hexane) gave 30.6 mg (83%) of the title compound: R_f 0.25 (50% ethyl acetate/hexane); NMR (300 MHz, CD_3OD) δ 7.35–7.17 (m, 5 H), 5.25 (m, 1 H), 4.47–4.25 (m, 5 H), 4.07 (d, J = 11.7 Hz, 1 H), 3.07 (dd, J = 4.5, 13.5 Hz, 1 H), 2.77 (dd, J = 9.3, 13.5 Hz, 1 H), 2.50–2.30 (m, 2 H), 2.10–1.80 (m, 3 H), 1.76–0.87 (m, 21 H), 1.35 (s, 9 H); MS (FAB) m/z 660 (M + 1), 604, 560. Anal. ($C_{35}H_{53}N_3O_9$) C, H, N.

(8*S*,11*S*,12*R*)-11-(Cyclohexylmethyl)-12-hydroxy-2-(4-morpholinomethyl)-8-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-5,9,13-trioxo-1,4-dioxa-10-azacyclotridecane (3e; R = $CH_2N(CH_2CH_2)_2O$, *n* = 0, diast 1). Macrocycle **10e** (65.5 mg, 0.110 mmol, 1.0 equiv) was deprotected according to general procedure D and coupled to Boc-Phe (88.4 mg, 0.333 mmol, 3.0 equiv) according to general procedure B with one modification: in the workup, the acid wash was omitted. Purification by flash chromatography (2.5% and 5% methanol/dichloromethane) gave 22.8 mg (29%) of the title compound: NMR (300 MHz, CD_3OD) δ 7.28–7.20 (m, 5 H), 5.45 (m, 1 H), 4.44–4.27 (m, 5 H), 4.17 (dd, J = 1.9, 11.8 Hz, 1 H), 3.68 (br t, J = 4.2 Hz, 4 H), 3.08 (dd, J = 4.8, 13.7 Hz, 1 H), 2.82–2.71 (m, 2 H), 2.60–2.89 (m, 3 H), 1.75–0.85 (m, 12 H), 1.35 (s, 9 H); MS (FAB) m/z 703 (M + 1). Anal. ($C_{36}H_{54}N_4O_{10} \cdot {}^1/_2H_2O$) C, H, N.

(8*S*,11*S*,12*R*)-11-(Cyclohexylmethyl)-12-hydroxy-2-(4-morpholinomethyl)-8-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-5,9,13-trioxo-1,4-dioxa-10-azacyclotridecane (3f; R = $CH_2N(CH_2CH_2)_2O$, *n* = 0, diast 2). Macrocycle **10f** (20.8 mg, 0.0353 mmol, 1.0 equiv) was deprotected according to general procedure D and coupled to Boc-Phe (28.1 mg, 0.106 mmol, 3.0 equiv) according to general procedure B with one modification: in the workup, the acid wash was omitted. Purification by flash chromatography (2.5% and 5% methanol/dichloromethane) gave 21.1 mg (85%) of the title compound: MS (FAB) m/z 703 (M + 1). Anal. ($C_{36}H_{54}N_4O_{10} \cdot H_2O$) C, H, N.

(1*S*,*R*)-1-Butyl-5-oxo-5-(phenylmethoxy)pentyl (4*S*,5*R*)-4-(Cyclohexylmethyl)-2,2-dimethyl-3-[(1,1-dimethylethoxyl)carbonyl]-5-oxazolidinecarboxylate (11c,d; R = Bu, *n* = 1, diast 1,2). A solution of δ-nonanolactone **17** (2.05 g, 13.1 mmol, 1.0 equiv) and sodium hydroxide (525 mg, 13.1 mmol, 1.0 equiv) in 13 mL of water and 6 mL of THF was heated at 70 °C for 48 h and then concentrated. The resultant waxy solid was suspended in acetone and heated at reflux overnight with benzyl bromide (2.69 g, 15.7 mmol, 1.2 equiv) and tetrabutylammonium bromide (212 mg, 0.656 mmol, 0.05 equiv). The reaction mixture was concentrated and partitioned between ethyl acetate and 1 N aqueous sodium hydrogen sulfate. The organic phase was washed sequentially with 1 N aqueous sodium hydrogen sulfate and saturated sodium chloride, dried over magnesium sulfate, and concentrated. Purification by MPLC (20% ethyl acetate/hexane) gave 1.61 g (6.08 mmol, 46%) of alcohol **18**, which was coupled to Boc-norACHPA acetonide **8** (1.73 g, 5.07 mmol, 0.83 equiv) according to general procedure A. Purification by MPLC (10% ethyl acetate/hexane) gave 2.35 g (66%) of the title compound as a mixture of diastereomers as indicated by ^{1}H NMR: R_f 0.35 (15% ethyl acetate/hexane); MS (FAB) m/z 488. Anal. ($C_{34}H_{53}NO_7$) C, H, N.

(1*S*,*R*)-1-(4-Morpholinomethyl)-4-oxo-4-(phenylmethoxy)butyl (4*S*,5*R*)-4-(Cyclohexylmethyl)-2,2-dimethyl-3-[(1,1-dimethylethoxyl)carbonyl]-5-oxazolidinecarboxylate (11m,n; R = $CH_2N(CH_2CH_2)_2O$, *n* = 0, diast 1,2). Benzyl ester **22a** (*n* = 0, 1.75 g, 8.50 mmol, 1.0 equiv) (available from alcohol **21a** by Jones oxidation followed by Fisher esterification and epoxidation with *m*-chloroperbenzoic acid) was treated with morpholine (1.48 g, 17.0 mmol, 2.0 equiv) and 2 g of neutral alumina in 8.5 mL of ether overnight. The resultant mixture was filtered and concentrated. Purification by flash chromatography (2.5% methanol/dichloromethane) gave 2.11 g (85%) of the corresponding morpholino alcohol. A 590-mg (2.01 mmol, 1.0 equiv) sample was coupled to Boc-norACHPA acetonide **8** (756 mg, 2.21 mmol, 1.1 equiv) according to general procedure A with one modification: in the workup, the acid wash was omitted. Purification by MPLC (30% ethyl acetate/hexane) gave 493 mg (40%) of a faster eluting isomer, **11m**, and 477 mg (38%) of a slower eluting isomer, **11n**. Compound **11m**: R_f 0.35 (35% ethyl acetate/hexane); MS (FAB) m/z 617 (M + 1), 561. Anal. ($C_{34}H_{52}N_2O_8$) C, H, N. Compound **11n**: R_f 0.31 (35% ethyl acetate/hexane); MS (FAB) m/z 617 (M + 1), 561. Anal. ($C_{34}H_{52}N_2O_8$) C, H, N.

(1*S*,*R*)-1-(4-Morpholinomethyl)-5-oxo-5-(phenylmethoxy)pentyl (4*S*,5*R*)-4-(Cyclohexylmethyl)-2,2-dimethyl-3-[(1,1-dimethylethoxyl)carbonyl]-5-oxazolidinecarboxylate (11o,p; R = $CH_2N(CH_2CH_2)_2O$, *n* = 1, diast 1,2). Benzyl ester **22b** (available from alcohol **21b** as described for alcohol **22a**) was treated with morpholine and neutral alumina and a 2.26-g (7.37 mmol) sample of the resultant alcohol was coupled to Boc-norACHPA acetonide **8** according to procedure outlined above in the synthesis of **11m,n**. Purification by MPLC (30% ethyl acetate/hexane) gave 1.65 g (36%) of a faster eluting isomer, **11o**, and 1.66 g (36%) of a slower eluting isomer, **11p**. Diastereomer **11o**: R_f 0.27 (25% ethyl acetate/hexane); MS (FAB) m/z 631 (M + 1). Anal. ($C_{35}H_{54}N_2O_8$) C, H, N. Diastereomer **11p**: R_f 0.21 (25% ethyl acetate/hexane); MS (FAB) m/z 631 (M + 1). Anal. ($C_{35}H_{54}N_2O_8$) C, H, N.

Asymmetric Synthesis of 11o. A solution of 2.8 g (9.0 mmol) of amino alcohol **24** and 2.52 g (10.8 mmol) of L-(+)-diisopropyl tartrate in dichloromethane (20 mL) was cooled to 0 °C and 5.75 mL (19.35 mmol) of titanium isopropoxide was added. The reaction mixture was stirred for 20 min at room temperature and cooled to −22 °C, and then 1.8 mL (5.4 mmol) of *tert*-butyl hydroperoxide was added. After stirring for 2.5 h the reaction mixture was quenched with water (3 mL) and 5 g of sodium fluoride. The mixture was stirred for 3 h and then diluted with ethyl acetate and filtered through Celite. Flash chromatography (silica gel, 1:1 hexane/acetone) gave 391 mg (15%) of **25**.

A 35.1 mg (0.114 mmol) sample of **25** and was coupled to Boc-norACHPA acetonide **8** (58.3 mg, 0.117 mmol, 1.03 equiv)

according to general procedure A with one modification: in the workup, the acid wash was omitted. HPLC analysis (2.6 mm × 25 cm Dynamax silica gel column, hexane/ethyl acetate 60:40, 1.0 mL/min) showed a 10:1 mixture of **11o** (t_R = 3.4 min) and **11p** (t_R = 5.7 min). Flash chromatography (3:1 dichloromethane/ethyl acetate) provided 59.1 mg (82%) of **11o**.

(1*S*,*R*)-5-Oxo-5-(phenylmethoxy)-1-[[[(2,2,2-trichloroethoxy)carbonyl]amino]methyl]pentyl (4*S*,5*R*)-4-(Cyclohexylmethyl)-2,2-dimethyl-3-[(1,1-dimethylethoxyl)carbonyl]-5-oxazolidinecarboxylate (11q,r; R = CH_2NH-TROC, *n* = 1, diast 1,2). 5-Hexen-1-ol (**21b**, 3.49 g, 34.8 mmol) in 60 mL of THF was deprotonated with sodium hydride (1.07 g, 35.5 mmol, 1.02 equiv) and treated with benzyl bromide (6.02 g, 35.2 mmol, 1.01 equiv) and tetrabutylammonium iodide (644 mg, 1.74 mmol, 0.05 equiv). After 4 h, 5 g of Florisil was added. The mixture was filtered and concentrated. The resultant benzyl ether was dissolved in 35 mL of dichloromethane and treated with *m*-chloroperbenzoic acid (50–60%, 24.0 g, 69.6 mmol, 2.0 equiv) to give the corresponding epoxide. This derivative was stirred with lithium azide (3.41 g, 69.6 mmol, 2.0 equiv) in 30 mL of DMF overnight. The mixture was diluted with hexane and washed with three portions of water and then brine, dried over magnesium sulfate, and concentrated. Purification by flash chromatography (20% and 25% ethyl acetate/hexane) provided 4.53 g (52% from **21b**) of the corresponding azido alcohol. A 2.08-g (8.34 mmol) portion was reduced with Raney nickel in methanol under an atmosphere of hydrogen. The resultant amine was dissolved in 41 mL of 4:1 THF/water and stirred with sodium bicarbonate (736 mg, 8.76 mmol, 1.05 equiv) and 2,2,2-trichloroethyl chloroformate (1.86 g, 8.76 mmol, 1.05 equiv) overnight. The mixture was concentrated. The residue was dissolved in ethyl acetate, washed with water, 1 N aqueous sodium hydrogen sulfate solution, and brine, dried over magnesium sulfate, and concentrated. Purification by flash chromatography (25%, 35%, and 50% ethyl acetate/hexane) provided 2.89 g (87%) of alcohol **23**. A 2.87-g (7.19 mmol) sample of compound **23** was coupled to Boc-norACHPA acetonide **8** (2.73 g, 7.91 mmol, 1.1 equiv) according to general procedure A. Purification by flash chromatography (15% and 20% ethyl acetate/hexane) provided 4.78 g (92%) of the corresponding ester. This compound was deprotected according to general procedure E. The resultant alcohol was dissolved in 65 mL of DMF and stirred for 2 days with pyridinium dichromate (PDC, 8.63 g, 22.9 mmol, 3.5 equiv). The mixture was concentrated and the residue was dissolved in water and extracted with two portions of ethyl acetate. The combined organic phases were dried over magnesium sulfate and concentrated. The crude acid was treated with benzyl bromide (1.20 g, 7.02 mmol, 1.1 equiv) and potassium carbonate (1.76 g, 12.8 mmol, 2.0 equiv) in refluxing acetone for 6 h. The mixture was concentrated and the residue was dissolved in ethyl acetate, washed with saturated aqueous sodium bicarbonate, water, and brine, dried over magnesium sulfate, and concentrated. Purification by flash chromatography (15%, 20%, and 25% ethyl acetate/hexane) provided 2.13 g (45%) of the title compound: R_f 0.35 (25% ethyl acetate/hexane); MS (FAB) *m/z* 737 (M + 1), 637. Anal. ($C_{34}H_{49}N_2O_9Cl_3$) C, H, N.

(1*S*,*R*)-1-(2-Methylpropyl)-4-oxo-4-hydroxybutyl (4*S*,5*R*)-4-(Cyclohexylmethyl)-2,2-dimethyl-3-[(1,1-dimethylethoxyl)carbonyl]-5-oxazolidinecarboxylate (12e,f; R = iBu, *n* = 0, diast 1,2). To a solution at −78 °C of isovaleraldehyde (2.55 g, 29.59 mmol, 1.0 equiv) in diethyl ether was added 1.0 equiv of 4-butenylmagnesium bromide **19a**. The reaction was stirred at 0 °C overnight and then quenched by the addition of methanol. The solution was washed with 1 N aqueous hydrochloric acid and brine, dried over magnesium sulfate, and concentrated. Purification by MPLC (10% ethyl acetate/hexane) gave 2.86 g (68%) of 2-methyloct-7-en-4-ol. An 800-mg (5.62, 1.0 equiv) sample of this alcohol was coupled to Boc-norACHPA acetonide **8** (2.30 g, 6.75 mmol, 1.2 equiv) according to general procedure A. Purification by flash chromatography (5% ethyl acetate/hexane) gave 2.60 g (5.58 mmol, 99%) of the corresponding olefin **20a** (R = *i*-Bu, *n* = 0, diast 1,2). Anal. ($C_{27}H_{47}NO_5$) C, H, N. This compound was dissolved in acetone and treated portionwise with an aqueous solution of 7.17 g (33.5 mmol, 6 equiv) of sodium periodate and 74 mg (0.558 mmol, 0.1 equiv) of ruthenium dioxide. After the reaction was judged complete by TLC analysis, the mixture was concentrated. The residue was partitioned between dichloromethane and 1:1 1 N aqueous sodium hydrogen sulfite and 1 N aqueous sodium hydrogen sulfate. The aqueous phase was extracted with three portions of dichloromethane. The combined organic phases were dried over magnesium sulfate and decolorized with activated charcoal. Concentration gave 2.39 g (87%) of the title compound which was used without further purification: MS (FAB) *m/z* 484 (M + 1), 384. Anal. ($C_{28}H_{45}NO_7$) C, H, N.

(1*S*,*R*)-1-(2-Methylpropyl)-5-oxo-5-hydroxypentyl (4*S*,5*R*)-4-(Cyclohexylmethyl)-2,2-dimethyl-3-[(1,1-dimethylethoxyl)carbonyl]-5-oxazolidinecarboxylate (12g,h; R = iBu, *n* = 1, diast 1,2). Isovaleraldehyde was treated 4-pentenylmagnesium bromide **19b** and the resultant alcohol was coupled to Boc-norACHPA acetonide **8** according to procedure outlined above in the synthesis of **12e,f**. Purification by flash chromatography (3.5% ethyl acetate/hexane) gave in 50% overall yield the corresponding olefin **20b** (R = iBu, *n* = 1, diast 1,2). Anal. ($C_{28}H_{49}NO_5$) C, H, N. This compound was treated with sodium periodate/ruthenium dioxide as described above for the synthesis of **12e,f** to give a 91% yield of the title compound which was used without further purification: MS (FAB) *m/z* 498 (M + 1), 398. Anal. ($C_{27}H_{47}NO_7$) C, H, N.

(1*S*,*R*)-1-(2,2-Dimethylpropyl)-4-oxo-4-hydroxybutyl (4*S*,5*R*)-4-(Cyclohexylmethyl)-2,2-dimethyl-3-[(1,1-dimethylethoxyl)carbonyl]-5-oxazolidinecarboxylate (12i,j; R = neopentyl, *n* = 0, diast 1,2). *tert*-Butylacetaldehyde was treated 4-butenylmagnesium bromide **19a** and the resultant alcohol was coupled to Boc-norACHPA acetonide **8** according to procedure outlined above in the synthesis of **12e,f**. Purification by MPLC (5% ethyl acetate/hexane) gave the corresponding olefin **20c** (R = neopentyl, *n* = 0, diast 1,2). Anal. ($C_{28}H_{49}NO_5$) C, H, N. This compound was treated with sodium periodate/ruthenium chloride in 2:2:3 carbon tetrachloride/acetonitrile/water and isolated as described above for the synthesis of **12e,f** to give a 98% yield of the title compound which was used without further purification.

(1*S*,*R*)-1-(2,2-Dimethylpropyl)-5-oxo-5-hydroxypentyl (4*S*,5*R*)-4-(Cyclohexylmethyl)-2,2-dimethyl-3-[(1,1-dimethylethoxyl)carbonyl]-5-oxazolidinecarboxylate (12k,l; R = neopentyl, *n* = 1, diast 1,2). *tert*-Butylacetaldehyde was treated 4-pentenylmagnesium bromide **19b** and the resultant alcohol was coupled to Boc-norACHPA acetonide **8** according to procedure outlined above in the synthesis of **12e,f**. Purification by flash chromatography (5% ethyl acetate/hexane) gave the corresponding olefin **20d** (R = neopentyl, *n* = 1, diast 1,2). Anal. ($C_{29}H_{51}NO_5$) C, H, N. This compound was treated with sodium periodate/ruthenium dioxide as described above for the synthesis of **12e,f** to give a 84% yield of the title compound which was used without further purification: MS (FAB) *m/z* 512 (M + 1), 412.

O^{ω}-[4-[[[(4*S*,5*R*)-4-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolidinyl]carbonyl]oxy]butyryl]-N^{ω}-[(phenylmethoxy)carbonyl]-L-serine, 1,1-Dimethylethyl Ester (13a; R = H, *n* = 0). Benzyl ester **11a**[24] (303 mg, 0.586 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled with *N*-carbobenzoxy-L-serine *tert*-butyl ester (Z-Ser-OtBu) (190 mg, 0.644 mmol, 1.1 equiv) according to the general procedure A. Purification by MPLC (20% ethyl acetate/hexane) provided 404 mg (98%) of the title compound: R_f 0.26 (20% ethyl acetate/hexane); MS (FAB) *m/z* 705 (M + 1), 605, 549. Anal. ($C_{37}H_{56}N_2O_{11}$) C, H, N.

O^{ω}-[5-[[[(4*S*,5*R*)-4-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolidinyl]carbonyl]oxy]pentanoyl]-N^{ω}-[(phenylmethoxy)carbonyl]-L-serine, 1,1-Dimethylethyl Ester (13b; R = H, *n* = 1). Benzyl ester **11b** (351 mg, 0.660 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled with Z-Ser-OtBu (214 mg, 0.726 mmol, 1.1 equiv) according to the general procedure A. Purification by MPLC (25% ethyl acetate/hexane) provided 369 mg (78%) of the title compound: R_f 0.32 (25% ethyl acetate/hexane); MS (FAB) *m/z* 719 (M + 1), 619, 563. Anal. ($C_{38}H_{58}N_2O_{11}$) C, H, N.

(24) See ref 8 for the synthesis of this compound.

O^{ω}-[(5S,R)-5-Butyl-5-[[[(4S,5R)-4-(cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolidinyl]carbonyl]oxy]pentanoyl]-N^{α}-[(phenylmethoxy)carbonyl]-L-serine, 1,1-Dimethylethyl Ester (13c,d; R = Bu, n = 1, diast 1,2). Benzyl ester **11c,d** was deprotected according to general procedure E and a 902-mg (1.81 mmol, 1.08 equiv) sample of the resultant acid was coupled with Z-Ser-OtBu (496 mg, 1.68, 1.0 equiv) according to the general procedure A. Purification by MPLC (15% ethyl acetate/hexane) provided 369 mg (28%) of faster eluting isomer **13c**, 445 mg (34%) of slower eluting isomer **13d**, and 274 mg (21%) of a mixture of the two. Diastereomer **13c**: R_f 0.36 (20% ethyl acetate/hexane); MS (FAB) m/z 775 (M + 1), 675, 619. Anal. ($C_{42}H_{66}N_2O_{11}$) C, H, N. Diastereomer **13d**: R_f 0.32 (20% ethyl acetate/hexane); MS (FAB) m/z 775 (M + 1), 675, 619. Anal. ($C_{42}H_{66}N_2O_{11}$) C, H, N.

O^{ω}-[(4S,R)-4-[[[(4S,5R)-4-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolidinyl]carbonyl]oxy]-4-(2-methylpropyl)butyryl]-N^{α}-[(phenylmethoxy)carbonyl]-L-serine, 1,1-Dimethylethyl Ester (13e,f; R = iBu, n = 0, diast 1,2). Acid **12e,f** (2.39 g, 4.95 mmol, 1.0 equiv) was coupled with Z-Ser-OtBu (1.61 g, 5.44 mmol, 1.1 equiv) according to the general procedure A. Purification by flash chromatography (20% ethyl acetate/hexane) provided 3.06 g (81%) of the title compound as a mixture of diastereomers: R_f 0.24 (20% ethyl acetate/hexane); MS (FAB) m/z 761 (M + 1), 661, 605. Anal. ($C_{41}H_{64}N_2O_{11}$) C, H, N.

O^{ω}-[(5S,R)-5-[[[(4S,5R)-4-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolidinyl]carbonyl]oxy]-5-(2-methylpropyl)pentanoyl]-N^{α}-[(phenylmethoxy)carbonyl]-L-serine, 1,1-Dimethylethyl Ester (13g,h; R = iBu, n = 1, diast 1,2). Acid **12g,h** (1.12 g, 2.25 mmol, 1.0 equiv) was coupled with Z-Ser-OtBu (796 mg, 2.70 mmol, 1.2 equiv) according to the general procedure A. Purification by flash chromatography (15% ethyl acetate/hexane) followed by MPLC (15% ethyl acetate/hexane) provided 607 mg (35%) of faster eluting isomer **13g**, 573 mg (33%) of slower eluting isomer **13h**, and 205 mg (12%) of a mixture of the two. Diastereomer **13g**: R_f 0.22 (15% ethyl acetate/hexane); MS (FAB) m/z 675. Anal. ($C_{42}H_{66}N_2O_{11}$) C, H, N. Diastereomer **13h**: R_f 0.18 (15% ethyl acetate/hexane); MS (FAB) m/z 675. Anal. ($C_{42}H_{66}N_2O_{11}$) C, H, N.

O^{ω}-[(4S,R)-4-[[[(4S,5R)-4-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolidinyl]carbonyl]oxy]-4-(2,2-dimethylpropyl)butyryl]-N^{α}-[(phenylmethoxy)carbonyl]-L-serine, 1,1-Dimethylethyl Ester (13i,j; R = neopentyl, n = 0, diast 1,2). Acid **12i,j** (1.51 g, 3.03 mmol, 1.0 equiv) was coupled with Z-Ser-OtBu (985 mg, 3.33 mmol, 1.1 equiv) according to the general procedure A. Purification by MPLC (15% ethyl acetate/hexane) provided 1.88 g (80%) of the title compound as a mixture of diastereomers as indicated by ^{1}H NMR: R_f 0.38 (20% ethyl acetate/hexane); MS (FAB) m/z 776 (M + 1). Anal. ($C_{42}H_{66}N_2O_{11}$) C, H, N.

O^{ω}-[(5S,R)-5-[[[(4S,5R)-4-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolidinyl]carbonyl]oxy]-5-(2,2-dimethylpropyl)pentanoyl]-N^{α}-[(phenylmethoxy)carbonyl]-L-serine, 1,1-Dimethylethyl Ester (13k,l; R = neopentyl, n = 1, diast 1,2). Acid **12k,l** (478 mg, 0.933 mmol, 1.0 equiv) was coupled with Z-Ser-OtBu (331 mg, 1.12 mmol, 1.2 equiv) according to the general procedure A. Purification by MPLC (15% ethyl acetate/hexane) followed by re-MPLC of mixed fractions provided 289 mg (40%) of faster eluting isomer **13k** and 274 mg (38%) of slower eluting isomer **13l**. Diastereomer **13k**: R_f 0.50 (25% ethyl acetate/hexane); MS (FAB) m/z 790 (M + 1), 690. Anal. ($C_{43}H_{68}N_2O_{11}$) C, H, N. Diastereomer **13l**: R_f 0.44 (25% ethyl acetate/hexane); MS (FAB) m/z 790 (M + 1), 690. Anal. ($C_{43}H_{68}N_2O_{11}$) C, H, N.

O^{ω}-[4-[[[(4S,5R)-4-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolidinyl]carbonyl]oxy]-4-(4-morpholinomethyl)butyryl]-N^{α}-[(phenylmethoxy)carbonyl]-L-serine, 1,1-Dimethylethyl Ester (13m; R = $CH_2N(CH_2CH_2)_2O$, n = 0, diast 1). Benzyl ester **11m** (465 mg, 0.754 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled with Z-Ser-OtBu (245 mg, 0.830 mmol, 1.1 equiv) according to the general procedure A with one modification: in the workup, the acid wash was omitted. Purification by MPLC (Sephadex LH-20, methanol) followed by MPLC (35% ethyl acetate/hexane) provided 494 mg (81%) of the title compound: R_f 0.52 (50% ethyl acetate/hexane); MS (FAB) m/z 804 (M + 1). Anal. ($C_{42}H_{65}N_3O_{12}$) C, H, N.

O^{ω}-[4-[[[(4S,5R)-4-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolidinyl]carbonyl]oxy]-4-(4-morpholinomethyl)butyryl]-N^{α}-[(phenylmethoxy)carbonyl]-L-serine, 1,1-Dimethylethyl Ester (13n; R = $CH_2N(CH_2CH_2)_2O$, n = 0, diast 2). Benzyl ester **11n** (443 mg, 0.789 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled with Z-Ser-OtBu (233 mg, 0.789 mmol, 1.1 equiv) according to the general procedure A with one modification: in the workup, the acid wash was omitted. Purification by MPLC (Sephadex LH-20, methanol) followed by MPLC (40% ethyl acetate/hexane) provided 510 mg (88%) of the title compound: R_f 0.46 (50% ethyl acetate/hexane); MS (FAB) m/z 804 (M + 1). Anal. ($C_{42}H_{65}N_3O_{12}$) C, H, N.

O^{ω}-[5-[[[(4S,5R)-4-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolidinyl]carbonyl]oxy]-5-(4-morpholinomethyl)pentanoyl]-N^{α}-[(phenylmethoxy)carbonyl]-L-serine, 1,1-Dimethylethyl Ester (13o; R = $CH_2N(CH_2CH_2)_2O$, n = 1, diast 1). Benzyl ester **11o** (1.65 g, 2.62 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled with Z-Ser-OtBu (782 mg, 2.65 mmol, 1.01 equiv) according to the general procedure A with one modification: in the workup, the acid wash was omitted. Purification by MPLC (Sephadex LH-20, methanol) followed by MPLC (35% ethyl acetate/hexane) provided 1.78 g (83%) of the title compound: MS (FAB) m/z 819 (M + 1), 719. Anal. ($C_{43}H_{67}N_3O_{12}$) C, H, N.

O^{ω}-[5-[[[(4S,5R)-4-(Cyclohexylmethyl)-3-[(1,1-dimethylethoxy)carbonyl]-2,2-dimethyl-5-oxazolidinyl]carbonyl]oxy]-5-(4-morpholinomethyl)pentanoyl]-N^{α}-[(phenylmethoxy)carbonyl]-L-serine, 1,1-Dimethylethyl Ester (13p; R = $CH_2N(CH_2CH_2)_2O$, n = 1, diast 2). Benzyl ester **11p** (295 mg, 0.469 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled with Z-Ser-OtBu (139 mg, 469 mmol, 1.0 equiv) according to the general procedure A with one modification: in the workup, the acid wash was omitted. Purification by MPLC (Sephadex LH-20, methanol) provided 287 g (75%) of the title compound: MS (FAB) m/z 819 (M + 1), 762. Anal. ($C_{43}H_{67}N_3O_{12}$) C, H, N.

(8S,11S,12R)-11-(Cyclohexylmethyl)-12-hydroxy-8-[[(phenylmethoxy)carbonyl]amino]-5,9,13-trioxo-1,6-dioxa-10-azacyclotridecane (14a; R = H, n = 0). Compound **13a** (376 mg, 0.533 mmol) was deprotected according to general procedure C and cyclized according to general procedure F. Purification by flash chromatography (60% ethyl acetate/hexane) provided 138 mg (53%) of the title compound: R_f (60% ethyl acetate/hexane); MS (FAB) m/z 491 (M + 1), 447. Anal. ($C_{25}H_{34}N_2O_8$) C, H, N.

(9S,12S,13R)-12-(Cyclohexylmethyl)-13-hydroxy-9-[[(phenylmethoxy)carbonyl]amino]-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (14b; R = H, n = 1). Compound **13b** (349 mg, 0.485 mmol) was deprotected according to general procedure C and cyclized according to general procedure F. Purification by flash chromatography (50% ethyl acetate/hexane) provided 114 mg (46%) of the title compound: R_f 0.24 (50% ethyl acetate/hexane); MS (FAB) m/z 505 (M + 1). Anal. ($C_{26}H_{36}N_2O_8$) C, H, N.

(9S,12S,13R)-2-Butyl-12-(cyclohexylmethyl)-13-hydroxy-9-[[(phenylmethoxy)carbonyl]amino]-6,10,14-trioxo-1,5-dioxa-11-azacyclotetradecane (14c; R = Bu, n = 1, diast 1). Compound **13c** (325 mg, 0.421 mmol) was deprotected according to general procedure C and cyclized according to general procedure F. Purification by flash chromatography (35% ethyl acetate/hexane) followed by MPLC (Sephadex LH-20, methanol) provided 72.5 mg (31%) of the title compound: R_f 0.54 (50% ethyl acetate/hexane); MS (FAB) m/z 561 (M + 1). Anal. ($C_{30}H_{44}N_2O_8$) C, H, N.

(9S,12S,13R)-2-Butyl-12-(cyclohexylmethyl)-13-hydroxy-9-[[(phenylmethoxy)carbonyl]amino]-6,10,14-trioxo-1,5-dioxa-11-azacyclotetradecane (14d; R = Bu, n = 1, diast 2). Compound **13d** (414 mg, 0.535 mmol) was deprotected according to general procedure C and cyclized according to general procedure F. Purification by MPLC (Sephadex LH-20, methanol) followed by flash chromatography (1% methanol/dichlo-

romethane) provided 70 mg (23%) of the title compound: R_f 0.48 (50% ethyl acetate/hexane); MS (FAB) m/z 561 (M + 1). Anal. ($C_{30}H_{44}N_2O_8$) C, H, N.

(8S,11S,12R)-11-(Cyclohexylmethyl)-12-hydroxy-2-(2-methylpropyl)-8-[[(phenylmethoxy)carbonyl]amino]-5,9,-13-trioxo-1,6-dioxa-10-azacyclotridecane (14e,f; R = iBu, n = 0, diast 1,2). Compound **13e,f** (3.05 g, 4.01 mmol) was deprotected according to general procedure C and cyclized according to general procedure F. Purification by MPLC (20% ethyl acetate/hexane; mixed fractions were chromatographed a second time) provided 114 mg (5%) of faster eluting isomer **13e** and 151 mg (7%) of slower eluting isomer **13f**. Diastereomer **13e**: R_f 0.38 (25% ethyl acetate/hexane); MS (FAB) m/z 547 (M + 1). Anal. ($C_{29}H_{42}N_2O_8$) C, H, N. Diastereomer **13f**: R_f 0.38 (25% ethyl acetate/hexane); MS (FAB) m/z 547 (M + 1). Anal. ($C_{29}H_{42}N_2O_8 \cdot {}^1/_3H_2O$) C, H, N.

(9S,12S,13R)-12-(Cyclohexylmethyl)-13-hydroxy-2-(2-methylpropyl)-9-[[(phenylmethoxy)carbonyl]amino]-6,10,-14-trioxo-1,5-dioxa-11-azacyclotetradecane (14g; R = iBu, n = 1, diast 1). Compound **13g** (559 mg, 0.721 mmol) was deprotected according to general procedure C and cyclized according to general procedure F. Purification by flash chromatography (1.75%, 2.5%, and 3.75% methanol/dichloromethane) followed by MPLC (Sephadex LH-20, methanol) and flash chromatography (1.75% and 2.5% methanol/dichloromethane) provided 216 mg (53%) of the title compound: R_f 0.25 (5% methanol/dichloromethane); MS (FAB) m/z 561 (M + 1). Anal. ($C_{30}H_{44}N_2O_8$) C, H, N.

(9S,12S,13R)-12-(Cyclohexylmethyl)-13-hydroxy-2-(2-methylpropyl)-9-[[(phenylmethoxy)carbonyl]amino]-6,10,-14-trioxo-1,5-dioxa-11-azacyclotetradecane (14h; R = iBu, n = 1, diast 2). Compound **13h** (522 mg, 0.674 mmol) was deprotected according to general procedure C and cyclized according to general procedure F. Purification by MPLC (Sephadex LH-20, methanol) followed by flash chromatography (1.75% and 2.5% methanol/dichloromethane) provided 115 mg (30%) of the title compound: MS (FAB) m/z 561 (M + 1). Anal. ($C_{30}H_{44}N_2O_8$) C, H, N.

(8S,11S,12R)-11-(Cyclohexylmethyl)-2-(2,2-dimethylpropyl)-12-hydroxy-8-[[(phenylmethoxy)carbonyl]amino]-5,9,-13-trioxo-1,6-dioxa-10-azacyclotridecane (14i,j; R = neopentyl, n = 0, diast 1,2). Compound **13i,j** (1.83 g, 2.36 mmol) was deprotected according to general procedure C and cyclized according to general procedure F. Purification by MPLC (Sephadex LH-20, methanol) followed by flash chromatography (1% and 2.5% methanol/dichloromethane) and MPLC (20% ethyl acetate/hexane) provided 114 mg (9%) of faster eluting isomer **14i** and 110 mg (8%) of slower eluting isomer **14j**. Diastereomer **14i**: R_f 0.32 (20% ethyl acetate/hexane); MS (FAB) m/z 561 (M + 1). Anal. ($C_{30}H_{44}N_2O_8$) C, H, N. Diastereomer **14j**: R_f 0.16 (20% ethyl acetate/hexane); MS (FAB) m/z 561 (M + 1). Anal. ($C_{30}H_{44}N_2O_8$) C, H, N.

(9S,12S,13R)-12-(Cyclohexylmethyl)-2-(2,2-dimethylpropyl)-13-hydroxy-9-[[(phenylmethoxy)carbonyl]amino]-6,-10,14-trioxo-1,5-dioxa-11-azacyclotetradecane (14k; R = neopentyl, n = 1, diast 1). Compound **13k** (289 mg, 0.375 mmol) was deprotected according to general procedure C and cyclized according to general procedure F. Purification by flash chromatography (2.5% methanol/dichloromethane) followed by MPLC (Sephadex LH-20, methanol) provided 54 mg (25%) of the title compound: R_f 0.26 (20% ethyl acetate/dichloromethane); MS (FAB) 575 (M + 1). Anal. ($C_{31}H_{46}N_2O_8 \cdot {}^1/_4H_2O$) C, H, N.

(9S,12S,13R)-12-(Cyclohexylmethyl)-2-(2,2-dimethylpropyl)-13-hydroxy-9-[[(phenylmethoxy)carbonyl]amino]-6,-10,14-trioxo-1,5-dioxa-11-azacyclotetradecane (14l; R = neopentyl, n = 1, diast 2). Compound **13l** (274 mg, 0.359 mmol) was deprotected according to general procedure C and cyclized according to general procedure F. Purification by flash chromatography (2.5% methanol/dichloromethane) followed by MPLC (Sephadex LH-20, methanol) provided 26.4 mg (12%) of the title compound: R_f 0.37 (20% ethyl acetate/dichloromethane); MS (FAB) m/z 575 (M + 1). Anal. ($C_{31}H_{46}N_2O_8$) C, H, N.

(8S,11S,12R)-11-(Cyclohexylmethyl)-12-hydroxy-2-(4-morpholinomethyl)-8-[[(phenylmethoxy)carbonyl]amino]-5,9,-13-trioxo-1,6-dioxa-10-azacyclotridecane (14m; R = $CH_2N(CH_2CH_2)_2O$, n = 0, diast 1). Compound **13m** (461 mg, 0.574 mmol) was deprotected accoding to general procedure C and cyclized according to general procedure F with one modification: in the workup, the acid wash was omitted. Purification by flash chromatography (2.5% and 5% methanol/dichloromethane) followed by MPLC (Sephadex LH-20, methanol) provided 187 mg (55%) of the title compound: R_f 0.33 (5% methanol/dichloromethane); MS (FAB) m/z 590 (M + 1). Anal. ($C_{30}H_{43}N_3O_9$) C, H, N.

(8S,11S,12R)-11-(Cyclohexylmethyl)-12-hydroxy-2-(4-morpholinomethyl)-8-[[(phenylmethoxy)carbonyl]amino]-5,9,-13-trioxo-1,6-dioxa-10-azacyclotridecane (14n; R = $CH_2N(CH_2CH_2)_2O$, n = 0, diast 2). Compound **13n** (465 mg, 0.579 mmol) was deprotected according to general procedure C and cyclized according to general procedure F with one modification: in the workup, the acid wash was omitted. Purification by flash chromatography (2.5% methanol/dichloromethane) followed by MPLC (Sephadex LH-20, methanol) provided 100 mg (29%) of the title compound: R_f 0.29 (5% methanol/dichloromethane); MS (FAB) 590 (M + 1). Anal. ($C_{30}H_{43}N_3O_9$) C, H, N.

(2R,9S,12S,13R)-12-(Cyclohexylmethyl)-13-hydroxy-2-(4-morpholinomethyl)-9-[[(phenylmethoxy)carbonyl]amino]-6,10,14-trioxo-1,5-dioxa-11-azacyclotetradecane (14o; R = $CH_2N(CH_2CH_2)_2O$, n = 1, diast 1). Compound **13o** (398 mg, 0.486 mmol) was deprotected according to general procedure C and cyclized according to general procedure F with one modification: in the workup, the acid wash was omitted. Purification by MPLC (Sephadex LH-20, methanol) followed by flash chromatography (2.5% methanol/dichloromethane) provided 104 mg (35%) of the title compound: R_f 0.33 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, CD_3OD) δ 7.35–7.27 (m, 5 H), 5.18–5.11 (m, 1 H), 5.11 (s, 2 H), 4.51 (ddd, J = 2.5, 10 Hz, 1 H), 4.42 (dd, J = 4.6, 9.4 Hz, 1 H), 4.23 (d, J = 2.1 Hz, 1 H), 4.23–4.11 (m, 2 H), 3.70–3.60 (m, 4 H), 2.73 (dd, J = 9.2, 13.1 Hz, 1 H), 2.60–2.51 (m, 2 H), 2.44–2.33 (m, 5 H), 1.86–0.80 (m, 17 H); MS (FAB) m/z 604 (M + 1). Anal. ($C_{31}H_{45}N_3O_9$) C, H, N.

(2R,9S,12S,13R)-12-(Cyclohexylmethyl)-13-hydroxy-2-(4-morpholinomethyl)-9-[[(phenylmethoxy)carbonyl]amino]-6,10,14-trioxo-1,5-dioxa-11-azacyclotetradecane (14p; R = $CH_2N(CH_2CH_2)_2O$, n = 1, diast 2). Compound **13p** (278 mg, 0.340 mmol) was deprotected according to general procedure C and cyclized according to general procedure F with one modification: in the workup, the acid wash was omitted. Purification by MPLC (Sephadex LH-20, methanol) followed by flash chromatography (2.5% methanol/dichloromethane) provided 29.6 mg (14%) of the title compound: R_f 0.38 (5% methanol/dichloromethane); MS (FAB) m/z 604 (M + 1).

(1R)-1-(4-Morpholinomethyl)-5-oxo-5-(phenylmethoxy)-pentyl (2R,3S)-4-Cyclohexyl-2-hydroxy-3-[[(2S)-2-[[(1,1-dimethylethoxy)carbonyl]amino]-3-(phenylmethoxy)propanoyl]amino]butanoate (15o; R = $CH_2N(CH_2CH_2)_2O$, n = 1, diast 1). Benzyl ester **11o** (4.00 g, 6.34 mmol) was deprotected according to general procedure C and coupled with *N*-Boc-*O*-benzyl-Ser (Boc-Ser(Bn)) (2.81 g, 9.51 mmol, 1.5 equiv) according to the general procedure B with one modification: in the workup, the acid wash was omitted. Purification MPLC (60% and 100% ethyl acetate/dichloromethane) gave 4.05 g (83%) of the title compound: R_f 0.32 (5% methanol/dichloromethane); MS (FAB) m/z 678 (M + 1). Anal. ($C_{43}H_{67}N_3O_{12} \cdot {}^1/_2H_2O$) C, H, N.

(1S,R)-5-Oxo-5-phenylmethoxy)-1-[[[(2,2,2-trichloroethoxy)carbonyl]amino]methyl]pentyl (2R,3S)-4-Cyclohexyl-2-hydroxy-3-[[(2S)-2-[[(1,1-dimethylethoxy)carbonyl]amino]-3-(phenylmethoxy)propanoyl]amino]butanoate (15q,r; R = CH_2NH-TROC, n = 1, diast 1,2). Benzyl ester **11q,r** (2.03 g, 2.75 mmol, 1.0 equiv) was deprotected according to general procedure C and coupled with Boc-Ser(Bn) (1.22 g, 4.13 mmol, 1.5 equiv) according to the general procedure B with one modification. Purification by flash chromatography (35% ethyl acetate/hexane) followed by MPLC of mixed fractions (35% ethyl acetate/hexane) gave 1.09 g (45%) of a faster eluting isomer, **15q**, and 954 mg (40%) of a slower eluting isomer, **15r**. Diastereomer **15q**: R_f 0.26 (30% ethyl acetate/hexane); MS (FAB) m/z 872 (M + 1), 772. Anal. ($C_{41}H_{53}N_3O_{11}Cl_3$) C, H, N. Diastereomer **15r**: R_f 0.17 (30% ethyl acetate/hexane); MS (FAB) m/z 872 (M + 1), 772. Anal. ($C_{41}H_{53}N_3O_{11}Cl_3 \cdot {}^1/_4H_2O$) C, H, N.

(2R,9S,12S,13R)-12-(Cyclohexylmethyl)-9-[[(1,1-dimethylethoxy)carbonyl]amino]-13-hydroxy-2-(4-morpholinomethyl)-6,10,14-trioxo-1,5-dioxa-11-azacyclotetradecane (16o; R = $CH_2N(CH_2CH_2)_2O$, n = 1, diast 1). Compound **15o** (247 mg, 0.365 mmol) was deprotected according to general procedure E and cyclized according to general procedure G. Purification by flash chromatography (2.5% methanol/dichloromethane) gave 159 mg (76%) of the title compound as a white solid: R_f 0.31 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, CD_3OD) δ 5.16 (m, 1 H), 4.51 (m, 1 H), 4.32 (dd, J = 3.3, 9.7 Hz, 1 H), 4.23 (d, J = 2.0 Hz, 1 H), 4.22–4.06 (m, 2 H), 3.70–3.60 (m, 4 H), 2.73 (dd, J = 9.0, 13.1 Hz, 1 H), 2.60–2.55 (m, 2 H), 2.46–2.35 (m, 5 H), 1.91–0.86 (m, 17 H); MS (FAB) m/z 570 (M + 1), 514, 470. Anal. ($C_{31}H_{45}N_3O_9 \cdot {}^1/_2H_2O$) C, H, N.

(9S,12S,13R)-12-(Cyclohexylmethyl)-9-[[(1,1-dimethylethoxy)carbonyl]amino]-13-hydroxy-2-[[[(2,2,2-trichloroethoxy)carbonyl]amino]methyl]-6,10,14-trioxo-1,5-dioxa-11-azacyclotetradecane (16q; R = CH_2NH-TROC, n = 1, diast 1). Compound **15q** (531 mg, 0.610 mmol) was deprotected according to general procedure E and cyclized according to general procedure G. Purification by flash chromatography (1.25%, 2.5%, and 3.75% methanol/dichloromethane) gave 332 mg (81%) of the title compound: MS (FAB) m/z 674 (M + 1), 574. Anal. ($C_{27}H_{42}N_3O_{10}Cl_3$) C, H, N.

(9S,12S,13R)-12-(Cyclohexylmethyl)-9-[[(1,1-dimethylethoxy)carbonyl]amino]-13-hydroxy-2-[[[(2,2,2-trichloroethoxy)carbonyl]amino]methyl]-6,10,14-trioxo-1,5-dioxa-11-azacyclotetradecane (16r; R = CH_2NH-TROC, n = 1, diast 2). Compound **15r** (489 mg, 0.562 mmol) was deprotected according to general procedure E and cyclized according to general procedure G. Purification by flash chromatography (2.5% methanol/dichloromethane) gave 305 mg (80%) of the title compound: R_f 0.35 (5% methanol/dichloromethane); MS (FAB) m/z 674 (M + 1), 574. Anal. ($C_{27}H_{42}N_3O_{10}Cl_3$) C, H, N.

(8S,11S,12R)-11-(Cyclohexylmethyl)-8-[N-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-12-hydroxy-5,9,13-trioxo-1,6-dioxa-10-azacyclotridecane (4a; R = H, n = 0). Macrocycle **14a** (48.5 mg, 0.0988 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (52.5 mg, 0.198 mmol, 2.0 equiv) according to general procedure B. Purification by flash chromatography (70% ethyl acetate/hexane) gave 43.4 mg (73%) of the title compound as a white solid: R_f 0.48 (75% ethyl acetate/hexane); ^{1}H NMR (300 MHz, CD_3OD) δ 7.27–7.18 (m, 5 H), 4.65 (dd, J = 3.4, 8.0 Hz, 1 H), 4.42–4.22 (m, 4 H), 4.15 (d, J = 2.5 Hz, 1 H), 4.14–4.00 (m, 2 H), 3.13 (dd, J = 5.1, 13.5 Hz, 1 H), 2.83 (dd, J = 9.5, 13.5 Hz, 1 H), 2.59 (ddd, J = 3.0, 10, 15 Hz, 1 H), 2.41 (ddd, J = 3.0, 8.2, 15 Hz, 1 H), 2.18–1.96 (m, 2 H), 1.83–0.85 (m, 13 H), 1.36 (s, 9 H); MS (FAB) m/z 604 (M + 1), 548, 504, 357. Anal. ($C_{31}H_{45}N_3O_9$) C, H, N.

(9S,12S,13R)-12-(Cyclohexylmethyl)-9-[N-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-13-hydroxy-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (4b; R = H, n = 1). Macrocycle **14b** (58.3 mg, 0.116 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (61.3 mg, 0.231 mmol, 2.0 equiv) according to general procedure B. Purification by flash chromatography (70% ethyl acetate/hexane) followed by MPLC (Sephadex LH-20, MeOH) gave 33.3 mg (47%) of the title compound: R_f 0.16 (70% ethyl acetate/hexane); ^{1}H NMR (300 MHz, CD_3OD) δ 7.30–7.18 (m, 5 H), 4.67 (dd, J = 3.9, 7.4 Hz, 1 H), 4.45–4.23 (m, 5 H), 4.15–4.04 (m, 2 H), 3.12 (dd, J = 4.8, 13.8 Hz, 1 H), 2.81 (dd, J = 9.8, 13.8 Hz, 1 H), 2.46–2.32 (m, 2 H), 1.88 (m, 17 H), 1.36 (s, 9 H), 1.04–0.87 (m, 2 H); MS (FAB) m/z 618 (M + 1), 562, 518. Anal. ($C_{32}H_{47}N_3O_9$) C, H, N.

(9S,12S,13R)-2-Butyl-12-(cyclohexylmethyl)-9-[N-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-13-hydroxy-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (4c; R = Bu, n = 1, diast 1). Macrocycle **14c** (25.2 mg, 0.0449 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (23.8 mg, 0.0899 mmol, 2.0 equiv) according to general procedure B. Purification by flash chromatography (1% and 2.5% methanol/dichloromethane) followed by MPLC (Sephadex LH-20, MeOH) gave 14.5 mg (48%) of the title compound: R_f 0.46 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31–7.15 (m, 5 H), 6.88 (br d, J = 4.0 Hz, 1 H), 6.76 (br d, J = 6.9 Hz, 1 H), 5.16 (br d, J = 5.7 Hz, 1 H), 4.88 (br m, 1 H), 4.66 (br m, 1 H), 4.47–4.31 (m, 3 H), 4.23 (s, 1 H), 3.95 (dd, J = 3.5, 10.6 Hz, 1 H), 3.76 (br s, 1 H), 3.06 (br d, J = 5.6 Hz, 2 H), 2.28 (br d, J = 5.0 Hz, 2 H), 1.87–0.86 (m, 26 H), 1.40 (s, 9 H); MS (FAB) m/z 674 (M + 1), 618, 574. Anal. ($C_{36}H_{55}N_3O_9 \cdot {}^3/_2H_2O$) C, H, N.

(9S,12S,13R)-2-Butyl-12-(cyclohexylmethyl)-9-[N-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-13-hydroxy-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (4d; R = Bu, n = 1, diast 2). Macrocycle **14d** (26.1 mg, 0.0465 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (24.7 mg, 0.0931 mmol, 2.0 equiv) according to general procedure B. Purification by flash chromatography (1% and 2.5% methanol/dichloromethane) followed by MPLC (Sephadex LH-20, MeOH) gave 23.4 mg (75%) of the title compound: R_f 0.52 (5% methanol/dichloromethane); MS (FAB) m/z 674 (M + 1), 618, 574. Anal. ($C_{36}H_{55}N_3O_9$) C, H, N.

(8S,11S,12R)-11-(Cyclohexylmethyl)-8-[N-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-12-hydroxy-2-(2-methylpropyl)-5,9,13-trioxo-1,6-dioxa-10-azacyclotridecane (4e; R = iBu, n = 0, diast 1). Macrocycle **14e** (30.7 mg, 0.0562 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (44.7 mg, 0.169 mmol, 3.0 equiv) according to general procedure B. Purification by flash chromatography (1.25%, 2.5%, 3.75%, and 5% methanol/dichloromethane) followed by MPLC (Sephadex LH-20, MeOH) and then flash chromatography (2% of 10:1 methanol/ammonium hydroxide in chloroform) gave 6 mg (16%) of the title compound: R_f 0.33 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.30–7.17 (m, 5 H), 5.05 (m, 1 H), 4.62 (dd, J = 3.8, 8.5 Hz, 1 H), 4.44 (m, 1 H), 4.37–4.25 (m, 2 H), 4.15 (d, J = 2.3 Hz, 1 H), 4.08 (dd, J = 3.5, 10.7 Hz, 1 H), 3.13 (dd, J = 4.8, 13.6 Hz, 1 H), 2.84 (dd, J = 9.6, 13.6 Hz, 1 H), 2.62 (ddd, J = 3.0, 10.5, 13.4 Hz, 1 H), 2.30 (ddd, J = 2.7, 7.5, 10.2 Hz, 1 H), 2.10–0.84 (m, 18 H), 1.36 (s, 9 H), 0.92 (d, J = 6.3 Hz, 3 H), 0.91 (d, J = 6.3 Hz, 3 H); MS (FAB) m/z 660 (M + 1), 604, 560. Anal. ($C_{35}H_{53}N_3O_9$) C, H, N.

(8S,11S,12R)-11-(Cyclohexylmethyl)-8-[N-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-12-hydroxy-2-(2-methylpropyl)-5,9,13-trioxo-1,6-dioxa-10-azacyclotridecane (4f; R = iBu, n = 0, diast 2). Macrocycle **14f** (31.6 mg, 0.0578 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (46.0 mg, 0.173 mmol, 3.0 equiv) according to general procedure B. Purification by flash chromatography (1.25% methanol/dichloromethane) gave 13.9 mg (37%) of the title compound: R_f 0.38 (5% methanol/dichloromethane); MS (FAB) m/z 660 (M + 1), 604, 560. Anal. ($C_{35}H_{53}N_3O_9$) C, H, N.

(9S,12S,13R)-12-(Cyclohexylmethyl)-9-[N-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-13-hydroxy-2-(2-methylpropyl)-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (4g; R = iBu, n = 1, diast 1). Macrocycle **14g** (69.6 mg, 0.124 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (98.8 mg, 0.372 mmol, 3.0 equiv) according to general procedure B. Purification by flash chromatography (1.25% methanol/dichloromethane) gave 55.4 mg (66%) of the title compound: R_f 0.29 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.26–7.17 (m, 5 H), 4.94 (br m, 1 H), 4.66 (dd, J = 3.8, 7 Hz, 1 H), 4.47–4.28 (m, 3 H), 4.21 (d, J = 1.9 Hz, 1 H), 3.93 (dd, J = 3.7, 10.6 Hz, 1 H), 3.11 (dd, J = 5.0, 13.8 Hz, 1 H), 2.82 (dd, J = 9.5, 13.8 Hz, 1 H), 2.30 (m, 2 H), 1.89–0.81 (m, 20 H), 1.35 (s, 9 H), 0.90 (t, J = 6.0 Hz, 6 H); MS (FAB) m/z 674 (M + 1), 574. Anal. ($C_{36}H_{55}N_3O_9$) C, H, N.

(9S,12S,13R)-12-(Cyclohexylmethyl)-9-[N-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-13-hydroxy-2-(2-methylpropyl)-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (4h; R = iBu, n = 1, diast 2). Macrocycle **14h** (72.3 mg, 0.129 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (103 mg, 0.387 mmol, 3.0 equiv) according to general procedure B. Purification by flash chromatography (2% methanol/dichloromethane) gave 53.1 mg (61%) of the title compound: R_f 0.20 (2% methanol/dichloromethane); MS (FAB) m/z 674 (M + 1), 574. Anal. ($C_{36}H_{55}N_3O_9$) C, H, N.

(8S,11S,12R)-11-(Cyclohexylmethyl)-8-[N-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-2-(2,2-dimethylpropyl)-12-hydroxy-5,9,13-trioxo-1,6-dioxa-10-azacyclotridecane (4i; R = neopentyl, n = 0, diast 1). Macrocycle **14i** (40.0 mg, 0.0713 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (37.9 mg, 0.142 mmol, 3.0 equiv)

according to general procedure B. Purification by flash chromatography (0–5% methanol/dichloromethane) followed by MPLC (Sephadex LH-20, methanol) and flash chromatography (1%, 2%, and 2.5% methanol/dichloromethane) gave 10.9 mg (23%) of the title compound: R_f 0.36 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.29–7.17 (m, 5 H), 5.16 (m, 1 H), 4.63 (dd, J = 4.8, 6.8 Hz, 1 H), 4.49 (m, 1 H), 4.33 (dd, J = 4.7, 9.2 Hz, 1 H), 4.29–4.15 (m, 2 H), 4.09 (d, J = 2.0 Hz, 1 H), 3.14 (dd, J = 4.5, 13.7 Hz, 1 H), 2.83 (dd, J = 9.4, 13.7 Hz, 1 H), 2.60 (ddd, J = 2.5, 9.7, 13.6 Hz, 1 H), 2.34 (ddd, J = 2.7, 5.3, 15.7 Hz, 1 H), 2.08 (m, 1 H), 1.96–1.82 (m, 2 H), 1.75–0.85 (m, 12 H), 1.36 (s, 9 H), 0.27 (s, 9 H); MS (FAB) m/z 674 (M + 1), 618, 574. Anal. ($C_{36}H_{55}N_3O_9 \cdot {}^1/_4H_2O$) C, H, N.

(8*S*,11*S*,12*R*)-11-(Cyclohexylmethyl)-8-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-2-(2,2-dimethylpropyl)-12-hydroxy-5,9,13-trioxo-1,6-dioxa-10-azacyclotridecane (4j; R = neopentyl, *n* = 0, diast 2). Macrocycle **14j** (39.9 mg, 0.0711 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (37.8 mg, 0.142 mmol, 3.0 equiv) according to general procedure B. Purification by flash chromatography (1.25% and 2.5% methanol/dichloromethane) followed by MPLC (Sephadex LH-20, methanol) gave 7.1 mg (15%) of the title compound: R_f 0.46 (5% methanol/dichloromethane); MS (FAB) m/z 674 (M + 1), 618, 574. Anal. ($C_{36}H_{55}N_3O_9 \cdot {}^1/_2H_2O$) C, H, N.

(9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-9-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-2-(2,2-dimethylpropyl)-13-hydroxy-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (4k; R = neopentyl, *n* = 1, diast 1). Macrocycle **14k** (16.9 mg, 0.0294 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (23.4 mg, 0.0882 mmol, 3.0 equiv) according to general procedure B. Purification by flash chromatography (3% methanol/dichloromethane) followed by MPLC (Sephadex LH-20, methanol) gave 15.9 mg (79%) of the title compound: R_f 0.27 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.28–7.16 (m, 5 H), 5.02 (m, 1 H), 4.66 (dd, J = 3.8, 6.2 Hz, 1 H), 4.48–4.31 (m, 3 H), 4.15 (d, J = 1.9 Hz, 1 H), 3.95 (dd, J = 3.8, 10.8 Hz, 1 H), 3.11 (dd, J = 4.9, 13.7 Hz, 1 H), 2.83 (dd, J = 9.1, 13.7 Hz, 1 H), 2.30 (br t, J = 5.6 Hz, 2 H), 1.86–0.89 (m, 17 H), 1.36 (s, 9 H), 0.89 (s, 9 H); MS (FAB) m/z 688 (M + 1), 588. Anal. ($C_{37}H_{57}N_3O_9 \cdot {}^1/_2H_2O$) C, H, N.

(9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-9-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-2-(2,2-dimethylpropyl)-13-hydroxy-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (4l; R = neopentyl, *n* = 1, diast 2). Macrocycle **14l** (15.1 mg, 0.0263 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (20.9 mg, 0.0788 mmol, 3.0 equiv) according to general procedure B. Purification by flash chromatography (1.7% methanol/dichloromethane) followed by MPLC (Sephadex LH-20, methanol) and flash chromatography (15% ethyl acetate/dichloromethane) gave 8.6 mg (47%) of the title compound: R_f 0.31 (20% ethyl acetate/dichloromethane); MS (FAB) m/z 688 (M + 1), 588. Anal. ($C_{37}H_{57}N_3O_9 \cdot 1.2H_2O$) C, H; N: calcd, 5.92; found, 5.32.

(8*S*,11*S*,12*R*)-11-(Cyclohexylmethyl)-8-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-12-hydroxy-2-(4-morpholinomethyl)-5,9,13-trioxo-1,6-dioxa-10-azacyclotridecane (4m; R = $CH_2N(CH_2CH_2)_2O$, *n* = 0, diast 1). Macrocycle **14m** (25.7 mg, 0.0436 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (23.1 mg, 0.0871 mmol, 2.0 equiv) according to general procedure B with one modification: in the workup, the acid wash was omitted. Purification by flash chromatography (2.5% methanol/dichloromethane) gave 12.5 mg (41%) of the title compound: R_f 0.24 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, CD_3OD) δ 7.32–7.17 (m, 5 H), 5.15 (m, 1 H), 4.66 (dd, J = 3.6, 8.2 Hz, 1 H), 4.34–4.27 (m, 3 H), 4.18 (d, J = 2.3 Hz, 1 H), 4.14–4.06 (m, 1 H), 3.63 (br t, J = 4.0 Hz, 4 H), 3.14 (dd, J = 4.6, 13.7 Hz, 1 H), 2.83 (dd, J = 9.7, 13.7 Hz, 1 H), 2.68–2.57 (m, 4 H), 2.42–2.26 (m, 4 H), 2.12–1.99 (m, 2 H), 1.86 (br d, J = 12.6 Hz, 1 H), 1.68–0.86 (m, 12 H), 1.37 (s, 9 H); MS (FAB) m/z 703 (M + 1), 647, 603. Anal. ($C_{36}H_{54}N_4O_{10} \cdot {}^1/_2H_2O$) C, H, N.

(8*S*,11*S*,12*R*)-11-(Cyclohexylmethyl)-8-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-12-hydroxy-2-(4-morpholinomethyl)-5,9,13-trioxo-1,6-dioxa-10-azacyclotridecane (4n; R = $CH_2N(CH_2CH_2)_2O$, *n* = 0, diast 2). Macrocycle **14n** (21.4 mg, 0.0363 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (19.3 mg, 0.0726 mmol, 2.0 equiv) according to general procedure B with one modification: in the workup, the acid wash was omitted. Purification by flash chromatography (2.5% methanol/dichloromethane) gave 9.1 mg (36%) of the title compound: R_f 0.20 (5% methanol/dichloromethane); MS (FAB) m/z 703 (M + 1), 647, 603. Anal. ($C_{36}H_{54}N_4O_{10}$) C, H, N.

(2*R*,9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-9-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-13-hydroxy-2-(4-morpholinomethyl)-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (4o; R = $CH_2N(CH_2CH_2)_2O$, *n* = 1, diast 1). Macrocycle **14o** (23.3 mg, 0.0386 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (20.5 mg, 0.0772 mmol, 2.0 equiv) according to general procedure B with one modification: in the workup, the acid wash was omitted. Purification by flash chromatography (1%, 2%, 3%, 4%, and 5% methanol/dichloromethane) gave 23.4 mg (84%) of the title compound: R_f 0.29 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.23 (m, 5 H), 6.97 (br m, 1 H), 6.79 (br m, 1 H), 5.10 (br m, 1 H), 4.95 (br m, 1 H), 4.68 (br m, 1 H), 4.45–4.20 (br m, 3 H), 4.29 (s, 1 H), 4.00 (dd, J = 6.8, 10.7 Hz, 1 H), 3.65 (br s, 4 H), 3.16–3.00 (br m, 2 H), 2.68 (br dd, J = 10.7, 12.0 Hz, 1 H), 2.61–2.24 (m, 7 H), 2.04 (br s, 1 H), 1.40 (s, 9 H), 1.86–0.82 (m, 17 H); MS (FAB) m/z 717 (M + 1). Anal. ($C_{37}H_{56}N_4O_{10}$) C, H, N.

(2*S*,9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-9-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-13-hydroxy-2-(4-morpholinomethyl)-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (4p; R = $CH_2N(CH_2CH_2)_2O$, *n* = 1, diast 2). Macrocycle **14p** (24.9 mg, 0.0412 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to Boc-Phe (21.9 mg, 0.0825 mmol, 2.0 equiv) according to general procedure B with one modification: in the workup, the acid wash was omitted. Purification by flash chromatography (2.5%, and 5% methanol/dichloromethane) gave 2.7 mg (9%) of the title compound: R_f 0.30 (5% methanol/dichloromethane); MS (FAB) m/z 717 (M + 1).

(9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-9-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-13-hydroxy-2-[[[(2,2,2-trichloroethoxy)carbonyl]amino]methyl]-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (4q; R = CH_2NH-TROC, *n* = 1, diast 1). Macrocycle **16q** (114 mg, 0.170 mmol, 1.0 equiv) was deprotected according to general procedure C and coupled to Boc-Phe (67.4 mg, 0.254 mmol, 1.5 equiv) according to general procedure B. Purification by flash chromatography (1.25%, and 2.5% methanol/dichloromethane) gave 105 mg (76%) of the title compound: R_f 0.30 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.29–7.19 (m, 5 H), 5.04 (m, 1 H), 4.77 (s, 2 H), 4.63 (dd, J = 4.3, 8.7 Hz, 1 H), 4.50 (m, 1 H), 4.32 (dd, J = 4.8, 9.0 Hz, 1 H), 4.25 (d, J = 1.9 Hz, 1 H), 4.24–4.10 (m, 2 H), 3.37 (d, J = 5.6 Hz, 2 H), 3.10 (dd, J = 4.5, 13.6 Hz, 1 H), 2.81 (dd, J = 9.9, 13.6 Hz, 1 H), 2.38–2.25 (m, 2 H), 1.88–0.91 (m, 17 H), 1.35 (s, 9 H); MS (FAB) m/z 821 (M + 1), 721. Anal. ($C_{36}H_{51}N_4O_{11}Cl_3 \cdot {}^1/_4H_2O$) C, H, N.

(9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-9-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-13-hydroxy-2-[[[(2,2,2-trichloroethoxy)carbonyl]amino]methyl]-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (4r; R = CH_2NH-TROC, *n* = 1, diast 2). Macrocycle **16r** (107 mg, 0.158 mmol, 1.0 equiv) was deprotected according to general procedure C and coupled to Boc-Phe (63.0 mg, 0.238 mmol, 1.5 equiv) according to general procedure B. Purification by flash chromatography (1%, 2%, 3%, 4%, and 5% methanol/dichloromethane) gave 111 mg (85%) of the title compound: R_f 0.33 (5% methanol/dichloromethane); MS (FAB) m/z 821 (M + 1), 721. Anal. ($C_{36}H_{51}N_4O_{11}Cl_3$) C, H, N.

(9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-2-(*N*,*N*-dimethylamino)-9-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-13-hydroxy-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (4s; R = CH_2NMe_2, *n* = 1, diast 1). Macrocycle **4q** (34.5 mg, 0.042 mmol, 1.0 equiv) was dissolved in 1 mL of acetic acid and treated with 250 mg of Zn dust. After 5 h, the mixture was filtered through Celite with DMF. The filtrate was concentrated and then dissolved in 1 mL of 1:1 acetic acid/pyridine. Aqueous

formaldehyde (30%, 12.6 mg, 0.420 mmol, 10 equiv) and sodium cyanoborohydride (7.9 mg, 0.126 mmol, 3.0 equiv) were added. The mixture was stirred overnight and then concentrated. Purification by flash chromatography (2.5%, 5%, and 7.5% of 10:1 methanol/ammonium hydroxide in dichloromethane) followed by more flash chromatography (2.5% to 15% methanol/dichloromethane) gave 14.5 mg (51%) of the title compound: R_f 0.22 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.27–7.20 (m, 5 H), 5.16 (m, 1 H), 4.65 (dd, J = 4.3, 8.8 Hz, 1 H), 4.56 (br ddd, 1 H), 4.33 (m, 1 H), 4.25 (d, J = 1.7 Hz, 1 H), 4.20 (t, J = 9.7 Hz, 1 H), 4.11 (dd, J = 4.0, 10.2 Hz, 1 H), 3.11 (dd, J = 4.6, 13.5 Hz, 1 H), 2.88–2.77 (m, 2 H), 2.35–2.22 (m, 3 H), 2.29 (s, 6 H), 1.89–0.86 (m, 17 H), 1.35 (s, 9 H); MS (FAB) m/z 675 (M + 1), 575. Anal. ($C_{35}H_{54}N_4O_9 \cdot {}^3/_4H_2O$) C, H, N.

(9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-2-(*N*,*N*-dimethylamino)-9-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-13-hydroxy-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (4t; R = CH_2NMe_2, *n* = 1, diast 2). Macrocycle **4r** (29.3 mg, 0.0356 mmol, 1.0 equiv) was treated with Zn dust and the resultant amine was reductively alkylated with formaldehyde as outlined above for the synthesis of compound **4s**. Purification by flash chromatography (2.5%, 5%, 7.5%, and 10% of 10:1 methanol/ammonium hydroxide in dichloromethane) followed by more flash chromatography (2.5% to 20% methanol/dichloromethane) gave 17.0 mg (71%) of the title compound: R_f 0.20 (5% methanol/dichloromethane); MS (FAB) m/z 675 (M + 1), 575. Anal. ($C_{35}H_{54}N_4O_9 \cdot {}^3/_4H_2O$) C, H; N: calcd, 8.14; found, 7.69.

(9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-2-(*N*,*N*-diethylamino)-9-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-13-hydroxy-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (4u; R = CH_2NEt_2, *n* = 1, diast 1). Macrocycle **4q** (28.3 mg, 0.0344 mmol, 1.0 equiv) was treated with Zn dust and the resultant amine was reductively alkylated with acetaldehyde (22.7 mg, 0.516 mmol, 15 equiv) as outlined above for the synthesis of compound **4s**. Purification by flash chromatography (2.5% to 20% of 10:1 methanol/ammonium hydroxide in dichloromethane) followed by more flash chromatography (2.5% to 25% methanol/dichloromethane) gave 5.7 mg (24%) of the title compound: R_f 0.2 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.29–7.19 (m, 5 H), 5.10 (m, 1 H), 4.63 (dd, J = 5.6, 7.6 Hz, 1 H), 4.53 (m, 1 H), 4.32 (dd, J = 5.1, 9.0 Hz, 1 H), 4.23 (d, J = 1.8 Hz, 1 H), 4.22–4.13 (m, 2 H), 3.10 (dd, J = 4.6, 13.3 Hz, 1 H), 2.88–2.30 (m, 9 H), 1.89–0.77 (m, 17 H), 1.35 (s, 9 H), 1.05 (t, J = 7.1 Hz, 6 H); MS (FAB) m/z 703 (M + 1). Anal. ($C_{37}H_{58}N_4O_9 \cdot 2CO_2$) C, H, N.

(9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-2-(*N*,*N*-diethylamino)-9-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-13-hydroxy-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (4v; R = CH_2NEt_2, *n* = 1, diast 2). Macrocycle **4r** (27.5 mg, 0.0334 mmol, 1.0 equiv) was treated with Zn dust and the resultant amine was reductively alkylated with acetaldehyde (22.1 mg, 0.502 mmol, 15 equiv) as outlined above for the synthesis of compound **4s**. After concentrating the reaction mixture, the residue was dissolved in ethyl acetate, washed with saturated aqueous sodium bicarbonate and brine, dried over magnesium sulfate, and concentrated. Purification by flash chromatography (2.5% to 15% methanol/dichloromethane) gave 10.2 mg (43%) of the title compound: MS (FAB) m/z 703 (M + 1), 603. Anal. ($C_{37}H_{58}N_4O_9 \cdot 1.75CO_2$) C, H, N.

(2*R*,9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-13-hydroxy-2-(4-morpholinomethyl)-9-[(phenoxyacetyl)amino]-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (26). Macrocycle **14o** (20.1 mg, 0.0333 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to phenoxyacetic acid (15.2 mg, 0.0999 mmol, 3.0 equiv) according to general procedure B with one modification: in the workup, the acid wash was omitted. Purification by flash chromatography (1.25% and 2.5% methanol/dichloromethane) gave 5.3 mg (26%) of the title compound: R_f 0.29 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.33–7.26 (m, 2 H), 7.01–6.94 (m, 3 H), 5.17 (m, 1 H), 4.76 (dd, J = 5.0, 8.0 Hz, 1 H), 4.62–4.49 (m, 3 H), 4.30–4.19 (m, 3 H), 3.71–3.60 (m, 4 H), 2.74 (dd, J = 9.2, 13.1 Hz, 1 H), 2.60–2.53 (m, 2 H), 2.45–2.32 (m, 5 H), 1.87–0.85 (m, 17 H); MS (FAB) m/z 604 (M + 1). Anal. ($C_{31}H_{45}N_3O_9 \cdot H_2O$) C, H, N.

(2*R*,9*S*,12*S*,13*R*)-9-(*trans*-Cinnamoylamino)-12-(cyclohexylmethyl)-13-hydroxy-2-(4-morpholinomethyl)-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (27). Macrocycle **14o** (20.0 mg, 0.0331 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to *trans*-cinnamic acid (14.7 mg, 0.0994 mmol, 3.0 equiv) according to general procedure B with one modification: in the workup, the acid wash was omitted. Purification by flash chromatography (1.25%, 2.5%, 3.75%, and 5% methanol/dichloromethane) followed by flash chromatography (1% methanol/dichloromethane) gave 3.9 mg (18%) of the title compound: R_f 0.20 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.60 (d, J = 5.8 Hz, 1 H), 7.53 (t, J = 2.7 Hz, 2 H), 7.40–7.33 (m, 3 H), 6.63 (d, J = 5.8 Hz, 1 H), 5.21 (m, 1 H), 4.77 (dd, J = 4.6, 9.9 Hz, 1 H), 4.54 (m, 1 H), 4.29 (t, J = 10.0 Hz, 1 H), 4.23 (d, J = 1.8 Hz, 1 H), 4.20 (dd, J = 4.5, 10.2 Hz, 1 H), 3.67 (m, 4 H), 2.76 (dd, J = 9.2, 13.1 Hz, 1 H), 2.61–2.53 (m, 2 H), 2.45–2.33 (m, 5 H), 1.84–0.81 (m, 17 H); MS (FAB) m/z 754 (M + 1 + dithiothreitol matrix), 600 (M + 1).

(2*R*,9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-13-hydroxy-9-[(2-indolylcarbonyl)amino]-2-(4-morpholinomethyl)-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (28). Macrocycle **16o** (22.3 mg, 0.0391 mmol, 1.0 equiv) was deprotected according to general procedure C and coupled to indole-2-carboxylic acid (9.5 mg, 0.0587 mmol, 1.5 equiv) according to general procedure B with one modification: in the workup, the acid wash was omitted. Purification by flash chromatography (1.75%, 2.5%, 3.75%, and 5% methanol/dichloromethane) gave 15.1 mg (63%) of the title compound: R_f 0.09 (2.5% methanol/dichloromethane); ^{1}H NMR (300 MHz, CD_3OD) δ 8.21 (d, J = 9.6 Hz, 1 H), 7.63 (d, J = 8.2 Hz, 1 H), 7.51 (d, J = 8.3 Hz, 1 H), 7.25–7.20 (m, 2 H), 7.07 (t, J = 7.1 Hz, 1 H), 5.24–5.19 (m, 2 H), 4.68 (m, 1 H), 4.42–4.29 (m, 3 H), 3.65 (m, 4 H), 2.60–2.48 (m, 3 H), 2.42–2.35 (m, 2 H), 2.29–2.19 (m, 3 H), 1.86 (br d, J = 11.5 Hz, 1 H), 1.76–0.78 (m, 16 H); MS (FAB) m/z 613 (M + 1). Anal. ($C_{32}H_{44}N_4O_8 \cdot 0.657H_2O$) C, H; N: calcd, 8.97, found, 7.51.

(2*R*,9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-9-[*N*-[[2-[(2,2-dimethylpropanoyl)amino]ethoxy]carbonyl]-L-phenylalanyl]-13-hydroxy-2-(4-morpholinomethyl)-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (29). Macrocycle **16o** (41.1 mg, 0.0721 mmol, 1.0 equiv) was deprotected according to general procedure C and coupled to *N*-[[2-[(*tert*-butylacetyl)amino]-ethoxy]carbonyl]-Phe (30.3 mg, 0.0866 mmol, 1.2 equiv) according to general procedure B with one modification: in the workup, the acid wash was omitted. Purification by flash chromatography (1.25%, 2.5%, 3.75%, 5%, and 6.25% methanol/dichloromethane) gave 43.6 mg (75%) of the title compound: R_f 0.40 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.29–71.8 (m, 5 H), 5.16 (m, 1 H), 4.64 (dd, J = 4.3, 9.0 Hz, 1 H), 4.52 (ddd, 1 H), 4.39 (dd, J = 4.9, 9.1 Hz, 1 H), 4.25 (d, J = 2.0 Hz, 1 H), 4.21 (dd, J = 4.4, 10.4 Hz, 1 H), 4.14 (dd, J = 9.0, 10.3 Hz, 1 H), 4.06–3.97 (m, 2 H), 3.66 (m, 4 H), 3.70–3.50 (m, 2 H), 3.10 (dd, J = 5.0, 13.9 Hz, 1 H), 2.85 (dd, J = 9.1, 13.9 Hz, 1 H), 2.85 (dd, J = 9.1, 13.9 Hz, 1 H), 2.74 (dd, J = 9.1, 13.1 Hz, 1 H), 2.58–2.55 (m, 2 H), 2.44–2.34 (m, 5 H), 2.05 (s, 2 H), 1.90–0.87 (m, 17 H), 0.99 (s, 9 H); MS (FAB) m/z 801 (M + 1). Anal. ($C_{41}H_{63}N_5O_{11} \cdot {}^1/_4H_2O$) C, H, N.

(2*R*,9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-9-[*N*-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-prolinyl]-L-phenylalanyl]-13-hydroxy-2-(4-morpholinomethyl)-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (30). Macrocycle **16o** (237 mg, 0.417 mmol, 1.0 equiv) was deprotected according to general procedure C and coupled to Boc-D-Pro-Phe (226 mg, 0.625 mmol, 1.5 equiv) according to general procedure B with one modification: in the workup, the acid wash was omitted. Purification by flash chromatography (1.25% to 5% methanol/dichloromethane) provided 261 mg (77%) of the title compound: R_f 0.27 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, CD_3OD) δ 7.28–7.16 (m, 5 H), 5.17 (br m, 1 H), 4.78 (br dd, $^1/_2$ H), 4.68 (br m, $^1/_2$ H), 4.61 (br m, 1 H), 4.51 (br m, 1 H), 4.25 (br s, 2 H), 4.11–4.07 (overlapping d's, 2 H), 3.67 (br m, 4 H), 3.44–3.34 (m, 2 H), 3.22 (br dd, $^1/_2$ H), 3.10 (br dd, $^1/_2$ H), 2.88 (br m, 1 H), 2.75 (dd, J = 9.1, 13 Hz, 1 H), 2.59–2.54 (m, 2 H), 2.45–2.32 (m, 5 H), 2.04–0.87 (m, 30 H); MS (FAB) m/z 814 (M + 1), 714. Anal. ($C_{42}H_{63}N_5O_{11} \cdot {}^1/_5H_2O$) C, H, N.

(2*R*,9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-13-hydroxy-2-(4-

morpholinomethyl)-9-(*N*-L-prolinyl-L-phenylalanyl)-6,10,-14-trioxo-1,7-dioxa-11-azacyclotetradecane, Bis(trifluoroacetate) Salt (31). Macrocycle **30** (17.2 mg) was deprotected according to general procedure C. Purification by MPLC (Sephadex LH-20, methanol) gave 18.1 mg of the title compound: ^{1}H NMR (300 MHz, CD_3OD) δ 8.02 (d, J = 9.4 Hz, 1 H), 7.31–7.20 (m, 5 H), 5.34 (m, 1 H), 4.82 (dd, J = 4.6, 10.5 Hz, 1 H), 4.69–4.60 (m, 2 H), 4.34 (d, J = 1.5 Hz, 1 H), 4.23–4.12 (m, 2 H), 3.84 (br s, 4 H), 3.29–3.17 (m, 3 H), 3.08 (br s, 6 H), 2.84 (dd, J = 10.7, 14.0 Hz, 1 H), 2.45–2.30 (m, 2 H), 2.17 (dt, J = 8.3, 12.9 Hz, 1 H), 1.94–0.89 (m, 20 H); MS (FAB) m/z 714 (M + 1). Anal. ($C_{37}H_{55}N_5O_9 \cdot 2C_2HF_3O_2$) C, H, N.

(2*R*,9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-9-[[(2*R*)-3-[(1,1-dimethylethyl)sulfonyl]-2-(phenylmethyl)propanoyl]amino]-13-hydroxy-2-(4-morpholinomethyl)-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (32). Macrocycle **14o** (212 mg, 0.351 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to (2*R*)-3-(*tert*-butylsulfonyl)-2-(phenylmethyl)-propionic acid (200 mg, 0.702 mmol, 2.0 equiv) according to general procedure B with one modification: the aqueous workup was omitted. The reaction mixture was subjected directly to flash chromatography (2.5% and 5% methanol/dichloromethane). Further purification by MPLC (2% 10:1 methanol/ammonium hydroxide in chloroform) gave 184 mg (71%) of the title compound: R_f 0.26 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.31–7.19 (m, 5 H), 5.16 (br m, 1 H), 4.59 (dd, J = 4.2, 9.7 Hz, 1 H), 4.45 (dt, J = 1.7, 6.2 Hz, 1 H), 4.25 (dd, J = 4.3, 10.3 Hz, 1 H), 4.24 (d, J = 1.8 Hz, 1 H), 4.13 (t, J = 10.0 Hz, 1 H), 3.66 (br s, 4 H), 3.55 (dd, J = 10.2, 13.3 Hz, 1 H), 3.31–3.21 (m, 1 H), 3.05 (dd, J = 6.8, 13.7 Hz, 1 H), 2.92 (dd, J = 2.6, 13.4 Hz, 1 H), 2.80–2.72 (overlapping dd, 2 H), 2.59–2.53 (m, 2 H), 2.49–2.31 (m, 5 H), 1.30 (s, 9 H), 1.88–0.88 (m, 17 H); MS (FAB) m/z 736 (M + 1). Anal. ($C_{37}H_{57}N_3O_{10}S$) C, H, N.

(2*R*,9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-13-hydroxy-2-(4-morpholinomethyl)-9-[*N*-(3*S*)-3-quinuclidinyl-L-phenylalanyl]-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (33). Macrocycle **14o** (350 mg, 0.580 mmol, 1.0 equiv) was deprotected according to general procedure E and coupled to *N*-3(*S*)-quinuclidinylphenylalanine[25] (222 mg, 0.638 mmol, 1.1 equiv) according to general procedure B with two modifications: DCC (*not* EDC) was used and the aqueous workup was omitted. The reaction mixture was concentrated and the residue was submitted directly to flash chromatography (117.5:7.5, 110:15 and 102.5:22.5 chloroform/10:1 methanol–ammonium hydroxide) and purified further by MPLC (160:7.5 chloroform/10:1 methanol–ammonium hydroxide) to give 255 mg (60%) of the title compound: R_f 0.63 (105:20 chloroform/10:1 methanol–ammonium hydroxide); ^{1}H NMR (300 MHz, CD_3OD) δ 7.33–7.20 (m, 5 H), 5.14 (m, 1 H), 4.64 (dd, J = 4.2, 8.7 Hz, 1 H), 4.49 (m, dt, J = 2, 7 Hz, 1 H), 4.25 (d, J = 2.0 Hz, 1 H), 4.20 (dd, J = 4.3, 10.5 Hz, 1 H), 4.07 (dd, J = 8.8, 10.5 Hz, 1 H), 3.66 (br s, 4 H), 3.34 (dd, J = 3.0, 5.3 Hz, 1 H), 3.06 (dd, J = 5.1, 13.5 Hz, 1 H), 2.89 (ddd, J = 2.5, 9.5, 13.4 Hz, 1 H), 2.81–2.52 (m, 10 H), 2.44–2.26 (m, 5 H), 2.14 (ddd, J = 2.0, 4.3, 13.3 Hz, 1 H), 1.89–0.85 (m, 21 H); MS (FAB) m/z 726 (M + 1). Anal. ($C_{39}H_{59}N_5O_{11} \cdot 1.3H_2O$) C, H, N.

(2*R*,9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-9-[*N*-[3-[[(1,1-dimethylethoxy)carbonyl]amino]-3-methylbutanoyl]-L-phenylalanyl]-13-hydroxy-2-(4-morpholinomethyl)-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (34). Macrocycle **16o** (32.9 mg, 0.0578 mmol, 1.0 equiv) was deprotected according to general procedure C and coupled to *N*-[3-(*tert*-butoxcarbonylamino)-3-methylbutanoyl]phenylalanine (31.6 mg, 0.0866 mmol, 1.5 equiv) according to general procedure B with one modification: in the workup, the acid wash was omitted. Purification by flash chromatography (1.25%, 2.5%, 3.75%, and 5% methanol/dichloromethane) gave 42.2 mg (89%) of the title compound: R_f 0.33 (5% methanol/dichloromethane); ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.29–7.17 (m, 5 H), 5.17 (m, 1 H), 4.69–4.61 (m, 2 H), 5.50 (ddd, 1 H), 4.25–4.10 (m, 3 H), 3.66 (m, 4 H), 3.14 (dd, J = 5.2, 14.1 Hz, 1 H), 2.85 (dd, J = 9.4, 13.9 Hz, 1 H), 2.74 (dd, J = 9.1, 13.1 Hz, 1 H), 2.59–2.31 (m, 10 H), 1.90–0.85 (m, 17 H), 1.41 (s, 9 H), 1.21 (s, 3 H), 1.16 (s, 3 H); MS (FAB) m/z 815 (M + 1), 715. Anal. ($C_{42}H_{65}N_5O_{11}$) C, H, N.

(2*R*,9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-9-[*N*-(3-amino-3-methylbutanoyl)-L-phenylalanyl]-13-hydroxy-2-(4-morpholinomethyl)-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane, Bis(trifluoroacetate) Salt (35). Macrocycle **34** (22.7 mg, 0.278 mmol) was deprotected according to general procedure C. Purification by MPLC (Sephadex LH-20, methanol) gave 20.2 mg (77%) of the title compound: ^{1}H NMR (300 MHz, CD_3OD) δ 7.99 (d, J = 9.3 Hz, 1 H), 7.26–7.20 (m, 5 H), 5.33 (m, 1 H), 4.74 (dd, J = 4.7, 10.1 Hz, 1 H), 4.67–4.59 (m, 2 H), 4.33 (d, J = 1.6 Hz, 1 H), 4.24–4.13 (m, 2 H), 3.83 (br s, 4 H), 3.17 (dd, J = 4.8, 14.0 Hz, 1 H), 3.05 (br s, 6 H), 2.85 (dd, J = 10.2, 14.0 Hz, 1 H), 2.51–2.35 (m, 4 H), 1.93–0.90 (m, 17 H), 1.30 (s, 3 H), 1.12 (s, 3 H); MS (FAB) m/z 717 (M + 1). Anal. ($C_{37}H_{57}N_5O_9 \cdot 2C_2HF_3O_2$) C, H, N.

(2*R*,9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-13-hydroxy-9-[*N*-[[2-(4-morpholino)ethoxy]carbonyl]-L-phenylalanyl]-2-(4-morpholinomethyl)-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (36). Macrocycle **16o** (286 mg, 0.502 mmol, 1.0 equiv) was deprotected according to general procedure C and coupled to *N*-[(2-morpholin-4-ylethoxy)carbonyl]phenylalanine (405 mg, 1.25 mmol, 2.5 equiv) according to general procedure B with one modification: the aqueous workup was omitted. The mixture was subjected directly to flash chromatography (5% methanol/dichloromethane and 10% and 20% 10:1 methanol/ammonium hydroxide in chloroform). Further purification by MPLC (Sephadex LH-20, MeOH, and then Lobar B silica gel column, 2% 10:1 methanol/ammonium hydroxide in chloroform) provided 270 mg (70%) of the title compound as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 7.33–7.19 (m, 5 H), 5.17 (br m, 1 H), 4.60 (dd, J = 4.5, 8.7 Hz, 1 H), 4.50 (t, J = 7 Hz, 1 H), 4.40 (dd, J = 5.0, 9.0 Hz, 1 H), 4.24–4.09 (m, 5 H), 3.68 (br m, 8 H), 3.11 (dd, J = 5.0, 14 Hz, 1 H), 2.86 (dd, J = 9.1, 14 Hz, 1 H), 2.76 (dd, J = 9.4, 13 Hz, 1 H), 2.64–2.32 (m, 13 H), 1.90–0.86 (m, 17 H); MS (FAB) m/z 774 (M + 1). Anal. ($C_{39}H_{59}N_5O_{11} \cdot {}^1/_2H_2O$) C, H, N.

(2*R*,9*S*,12*S*,13*R*)-12-(Cyclohexylmethyl)-13-hydroxy-9-[*N*-[(4,7,10-trioxadecanoxy)carbonyl]-L-phenylalanyl]-2-(4-morpholinomethyl)-6,10,14-trioxo-1,7-dioxa-11-azacyclotetradecane (37). Macrocycle **16o** (48.9 mg, 0.0858 mmol, 1.0 equiv) was deprotected according to general procedure C and coupled to *N*-(4,7,10-trioxadecanoxycarbonyl)phenylalanine (61.0 mg, 0.172 mmol, 2.0 equiv) according to general procedure B with one modification: the aqueous workup was omitted. The mixture was subjected directly to flash chromatography (2.5% and 5% methanol/dichloromethane) to provide 63.7 mg (92%) of the title compound: ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.31–7.17 (m, 5 H), 5.16 (m, 1 H), 4.62 (dd, J = 4.4, 9.0 Hz, 1 H), 4.50 (m, 1 H), 4.39 (dd, J = 5.1, 9.0 Hz, 1 H), 4.24 (d, J = 2.0 Hz, 1 H), 4.23–4.05 (m, 4 H), 3.66–3.51 (m, 14 H), 3.53 (s, 3 H), 3.10 (dd, J = 4.9, 13.7 Hz, 1 H), 2.85 (dd, J = 9.1, 13.7 Hz, 1 H), 2.75 (dd, J = 9.2, 13.1 Hz, 1 H), 2.60–2.53 (m, 2 H), 2.44–2.33 (m, 5 H), 1.90–1.48 (m, 11 H), 1.34–1.13 (m, 4 H), 1.03–0.86 (m, 2 H); MS (FAB) m/z 807 (M + 1). Anal. ($C_{40}H_{62}N_4O_{13}$) C, H, N.

Single-Crystal X-ray Diffraction Analysis of Macrocycle 16o. X-ray quality crystals of **16o** were grown by vapor diffusion of ether into a benzene solution of **16o** over a 24-hour period: M_r = 569.70, trigonal, $P3_121$, a = 18.595 (2) Å, c = 18.928 (3) Å, V = 5667 Å^3, Z = 6, D_x = 1.001 g cm^{-1}, monochromatized radiation λ(CuKα) = 1.54184 Å, μ = 0.58 mm^{-1}, F(000) = 1848, T = 153 K. Data were collected by Molecular Structure Corporation on a Rigaku AFC5R diffractometer to a 2θ limit of 100° with 1887 observed, $I \geq 3\sigma(I)$, reflections out of 4887 measured. The structure was solved by direct methods using SHELXS-86[26] and refined using full-matrix least-squares on F. No hydrogen atoms were included in the calculations. The cyclohexyl group on C22 is disordered as are what appears to be two benzene molecules of solvation. The crystal was of poor enough quality that this disorder was impossible to model adequately. Final agreement statistics are R = 0.126, ωR = 0.122, S = 4.27, $(\Delta/\sigma)_{max}$ = 0.01. The weighting scheme is $1/\sigma^2(F)$. The maximum peak height in the final difference Fourier map is 0.6 (1) eÅ^{-3}. Full crystallo-

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graphic details are presented in the supplementary material. All calculations were performed on a Sun Microsystems computer using SDP-Plus software.[27]

Rat Bioavailability Studies. [^{3}H]-**33** was synthesized by the Merck Labeled Compound Synthesis Group, Department of Animal and Exploratory Drug Metabolism; the radiochemical purity was 99.0% by HPLC.

Polyethylene cannulas were implanted in the left femoral vein and artery of male Sprague–Dawley rats (270–350 g, Charles Rivers, Wilmington, DE) 2 days before the experiment.[28] After fasting the second night, they were administered [^{3}H]-**33** either iv at 2 mg/kg or orally at 9.0 mg/kg in phosphate-buffered saline (PBS), pH 7.4, and put into restraining cages. Blood samples (0.4 mL) were withdrawn at intervals from 5 min to 8 h at which time the rats were put into cages and given food and water. The 24-h sample was obtained by exsanguination.

To collect bile, Silastic cannulas were implanted in the bile duct and the duodenum. They were both externalized at the back of the neck and connected. Half of the rats also had a cannula implanted in the femoral vein for iv dosing. The day after surgery, they were dosed either iv at 1 mg/kg or 10 mg/kg intraduodenally (id) with the labeled drug. An infusion of 10 mM sodium taurocholate/5% dextrose (2 mL/kg id) was started and bile was collected in tared vials for 24 h. Urinary and fecal excretion of the drug was determined in rats dosed either iv via the tail vein at 1.0 mg [^{3}H]-**33** or orally at 10 mg/kg.

Aliquots of plasma, bile, urine, and cage washings were mixed with Packard Instagel XF liquid scintillation fluid and counted in a Beckman LS5000TD spectrometer. The amount of nonvolatile radioactivity in urine and plasma was determined by drying an aliquot under a stream of nitrogen before water and scintillation fluid were added. Feces were homogenized with 3 volumes of water and aliquots were combusted in a Packard Model 306 sample oxidizer after drying.

Plasma concentrations of inhibitor **33** were determined using a reverse isotope dilution assay (RIDA). Aliquots of plasma (0.15 or 1 mL) were mixed with 20 μg of nonradioactive **33** in PBS. After the addition of 2 mL of 8 M urea, the solution was brought to pH 3.0 by the addition of saturated $KHSO_4$.[29] It was then passed through a Waters Sep-Pak ODS cartridge and washed sequentially with 5 mL of 0.1% TFA in water and 0.1% TFA in methanol. The methanol extract was dried under a stream of nitrogen and resuspended in 0.2 mL HPLC mobile phase. One hundred microliters was injected onto an analytical Zorbax RX-C8 column equilibrated with a buffer composed of 27 volumes of acetonitrile, 73 volumes of 0.1 M glycine, pH 3.0, and 0.1 volume of TFA. The flow rate was 1.0 mL/min and the effluent was monitored at 210 nm; 20-s fractions were collected and counted. The amount of total drug injected was determined by comparison to known amounts of inhibitor **33**. The concentration of drug in a sample was calculated as the amount of **33** added/[(initial sp act./final sp act.) − 1].

Urine and bile were mixed with an equal volume of the HPLC mobile phase and chromatographed using the same conditions as for the plasma samples.

The stability of [^{3}H]-**33** (10 μg/mL) was determined in rat and human plasma at 37 °C. At 1, 3, 6, and 16 h, aliquots were removed and extracted as outlined above. The extract was analyzed on a Vydac C-18 column (0.46 × 25 cm) using a mobile phase of 27 volumes of acetonitrile, 73 volumes of 20 mM ammonium acetate, and 0.2 volumes of TFA. Under these conditions, inhibitor **33** and **42** coeluted at 11 min while **41** eluted at 10 min. The peaks were collected and analyzed by FAB MS.

The area under the curve (AUC) was calculated by the logarithmic trapezoid method. Plasma clearance was calculated as dose/AUC; the volume of distribution was calculated as dose × AUMC/AUC^2.[30]

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Supplementary Material Available: Details of the crystallography and tables of atomic positional and thermal parameters and bond distances and angles (5 pages). Ordering information is given on any current masthead page.

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