α-Hydroxy Phosphinyl-Based Inhibitors of Human Renin

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Received April 13, 1995®

The design and application of α-hydroxy phosphonates, a new class of transition state analogs, toward the discovery of novel and potent inhibitors of the aspartyl protease renin is described. Tripeptidic α-hydroxy diethyl phosphonate **3**, the first example in this series, was found to be a good inhibitor of human renin (IC_{50} = 29 nM), and preliminary studies led to the choice of α-hydroxy dimethyl phosphonate **15** (IC_{50} = 16 nM) as a base-line compound for further structure–activity relationship study. Corresponding phosphinate (**28–30**) and phosphine oxide (**23** and **24**) analogs of **15** were prepared to assess the steric and electronic requirements around the phosphorus center. Evaluation of these analogs suggested that the presence of at least one alkoxy group on phosphorus was a critical requirement for good activity. Inhibitors with leucine at P_2 possessed better *in vitro* activity than the corresponding P_2 histidine analogs (**15**, IC_{50} = 16 nM vs **37**, IC_{50} = 220 nM; **33**, IC_{50} = 8.5 nM vs **40**, IC_{50} = 41 nM). Compound **34** (IC_{50} = 31 nM), the P_3 aminocaproic analog of **15**, showed complete and long-lasting inhibition of plasma renin activity while eliciting a 10–15 mmHg drop in mean arterial pressure when administered intravenously at 1 μmol/kg in conscious, sodium-depleted, cynomolgus monkeys. In summary, the α-hydroxy phosphonates represent a promising and structurally novel class of transition state analog inhibitors of human renin.

Introduction

Intervention of the renin angiotensin system (RAS) continues to be an attractive strategy for the treatment of hypertension and congestive heart failure.[1] The three most popular areas of research have been (1) inhibition of the aspartyl protease renin, the enzyme that specifically catalyzes the conversion of angiotensinogen to angiotensin I (AI),[2] the first and rate-limiting step of RAS, (2) inhibition of the metalloprotease angiotensin-converting enzyme (ACE) which hydrolyzes inactive AI to the biologically active octapeptide angiotensin II (AII),[3] a potent vasoconstrictor and aldosterone secretion stimulant, and (3) antagonists of the end product AII.[4] In this field, we and others have focused attention toward the discovery of inhibitors of human renin as novel antihypertensive agents.[5] Since angiotensinogen is the only known natural substrate for renin, inhibitors of this enzyme should be highly specific agents potentially free of side effects associated with ACE inhibitors.[6]

We recently reported an application of the concept of activated ketones to the design of novel and potent transition state analog inhibitors of renin.[7] Among several types of ketones that were prepared, the α-keto ester **1** wa found to be a potent inhibitor of human renin (IC_{50} = 15 nM). In the course of this work, it was realized that the corresponding α-hydroxy ester **2**, the penultimate synthetic precursor to **1**, was also a potent renin inhibitor (IC_{50} = 5.3 nM). This suggested to us that the α-hydroxy ester moiety was in itself serving as a good transition state mimic, a finding that was disclosed by other groups as well.[8] It was further envisioned that replacement of the ester group in **2** by its bioisostere, a phosphonate group, should preserve all the binding interactions present in a carboxylic ester group and thus result in good binding.[9] This directed us to propose the α-hydroxy phosphonate group as a transition state mimic and led to the preparation of α-hydroxy phosphonate **3**, which was found to display good inhibitory potency against renin (IC_{50} = 29 nM).[10]

Encouraged by this finding, we undertook a systematic investigation of this novel series of inhibitors and designed analogs aimed at evaluating a broad range of interactions encompassing the enzyme subsites S_3–S_1'. In this paper, the design, synthesis, and *in vitro* structure–activity relationship (SAR) data along with *in vivo* activity of some of the more promising analogs of the α-hydroxy phosphinyl class of renin inhibitors are reported.

Chemistry

Preparation of the Lead Compound 3. Reaction of amino aldehyde **4**[11] with diethyl phosphite in the presence of potassium fluoride as base gave a diastereomeric mixture of α-hydroxy diethyl phosphonates **5** (4:1) in good yields (77%). Deprotection of **5** with anhydrous HCl/EtOAc (89%) followed by coupling of the resulting amine hydrochloride with dipeptide Boc-Phe-Leu-OH (**6**) using DCC/HOBt provided the tripeptidic hydroxy phosphonates as a separable mixture of two diastereomers, **3** (73%) and **7** (14%). The fast moving (silica gel TLC) diastereomer **3** was found to be active against human renin in our *in vitro* assay (*vide infra*) and constituted a new lead in our program.

Preparation of the dimethyl phosphonate analog **13** was undertaken to assess the critical binding features operative at the transition state-mimicking region of the molecule. Thus, reaction of **4** with dimethyl phosphite employing KF/DMF gave essentially a single diastereomer, **9** (>12:1), as determined by ^{31}P NMR.[12] The major isomer **9** was presumed to bear the desired stereochemistry, since the same conditions had previously led to the more active diastereomer as major

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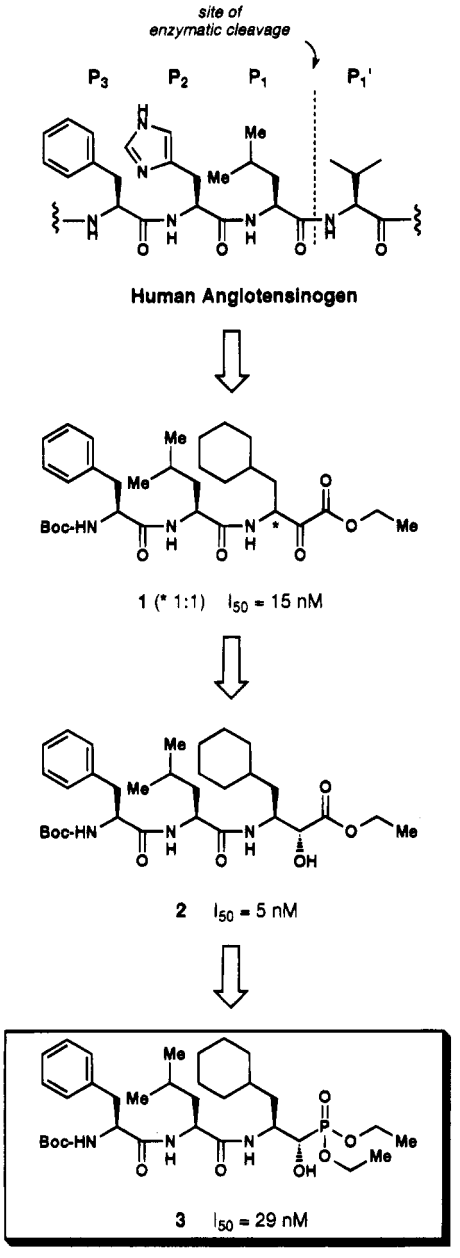
® Abstract published in *Advance ACS Abstracts*, October 1, 1995.

0022-2623/95/1838-4557$09.00/0

Scheme 1. Design of α-Hydroxy Phosphonate-Based Renin Inhibitors



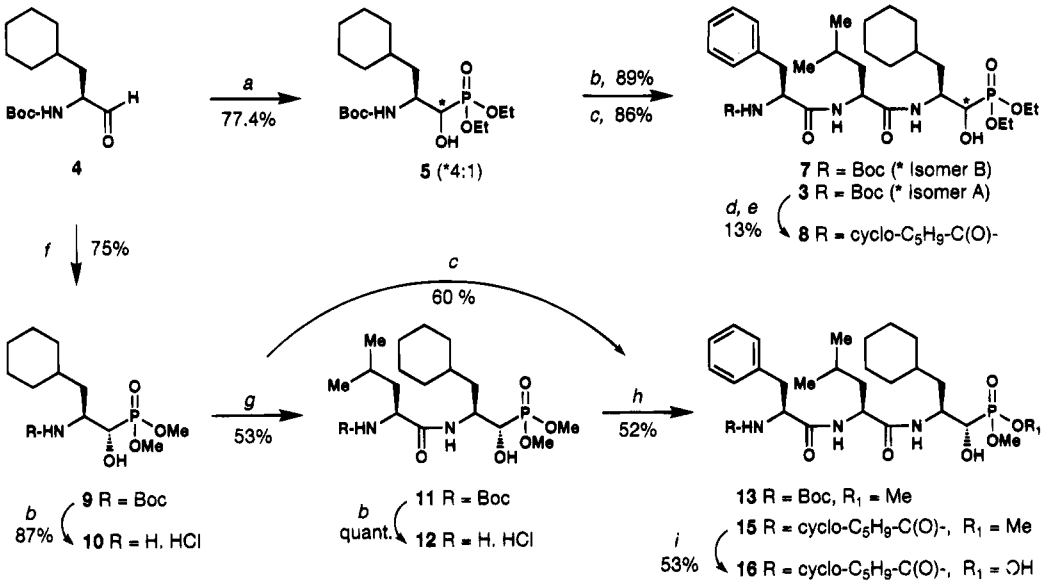
(isomers **3** vs **7** = 4:1) in the diethyl series. Not surprisingly, tripeptide **13** derived from coupling of the major diastereomer **9** with Boc-Phe-Leu-OH (**6**) was found to possess very good inhibitory potency (IC_{50} = 10 nM). Deprotection of **9** deserves some comments. Under strongly acidic conditions (methanol or dioxane saturated with anhydrous HCl), substantial monodealkylation of the dimethyl phosphonate group of **9** was observed. By monitoring the progress of deprotection by ^{31}P NMR under several conditions, it was realized that monodealkylation could be minimized by employing lower temperatures and avoiding prolonged reaction times (1.5 N anhydrous HCl/EtOAc, 0 °C, 2 h). The amine **10** also gradually monodealkylated upon storage, and it was found best to utilize it immediately in the subsequent coupling reactions. Analogs wherein the Boc group is replaced by a cyclopentyl amide group were also prepared. For the diethyl phosphonate series, removal of the Boc group from **3** followed by coupling of the resulting amine with cyclopentanecarboxylic acid gave **8** in an unoptimized yield of 13% yield. The cyclopentyl analog of dimethyl phosphonate **13** was prepared in a sequential fashion by first coupling **10** with Boc-Leu-OH to obtain **11**, which was deprotected to the hydrochloride salt **12**. Coupling of **12** with *cyclo*-C_5H_9CO-Phe-OH (**14**) yielded the desired analog **15**. Upon treatment with anhydrous trimethylamine in acetone at 80 °C in a sealed tube, **15** could be cleanly monodealkylated to provide the monoacid analog **16**.

Determination of Stereochemistry. The condensation reaction of dimethyl phosphite with aldehyde **4** was studied with a variety of different solvents and bases (see table in Scheme 3), and maximum diastereoselection for the desired isomer (**9**:**17**, >12:1) was achieved by employing KF/DMF; in contrast, almost equimolar ratios of the diastereomers was formed upon reaction with DBU in DMF, as determined by ^{31}P NMR (**9** = 25.28 ppm, **17** = 24.66 ppm with respect to H_3PO_4 = 0 ppm as the external reference). For absolute stereochemical assignment, **9** and **17** were converted to the corresponding oxazolidinones **18** and **19**, respectively, by deprotection of the individual isomers with HCl/EtOAc followed by cyclization with carbonyldiimidazole (CDI).

The ring protons (H_1 and H_2) of oxazolidinone **19** appeared downfield and exhibited a larger coupling constant (4.75 ppm, J = 8.4 Hz) when compared to the isomeric oxazolidinone **18** (4.30 ppm, J = 6.3 Hz), suggesting *cis* stereochemistry for the former.[13] Additionally, a 10% NOE was observed for the H_2 proton with **19**, indicative of its *cis* stereochemistry. The ^{13}C NMR data reinforced this finding, as evidenced by the upfield shift of the C-1 carbon atom in **19**. This is based on the steric compression effect according to which the sterically perturbed carbon atoms of *syn* rings are expected to appear at higher field than similar carbons that are not crowded.[14] This translates to the hydroxyl-bearing C-1 carbon of the major diastereomer **9** as having the *S* (*anti*, *threo*) stereochemistry, the one that is preferred for biological activity in the statine[15] and α-hydroxy ester[8] classes of renin inhibitors. The synthetic ease of preparation of the *anti* diastereomer **9** in comparison to the unseparable mixture obtained with the diethyl phosphonate **5**, coupled with 3-fold better activity of the final compound **13** in the dimethyl series when compared to the initial diethyl lead **3** (*vide infra*), led to the choice of dimethyl analog **13** as our base-line compound for further investigation.

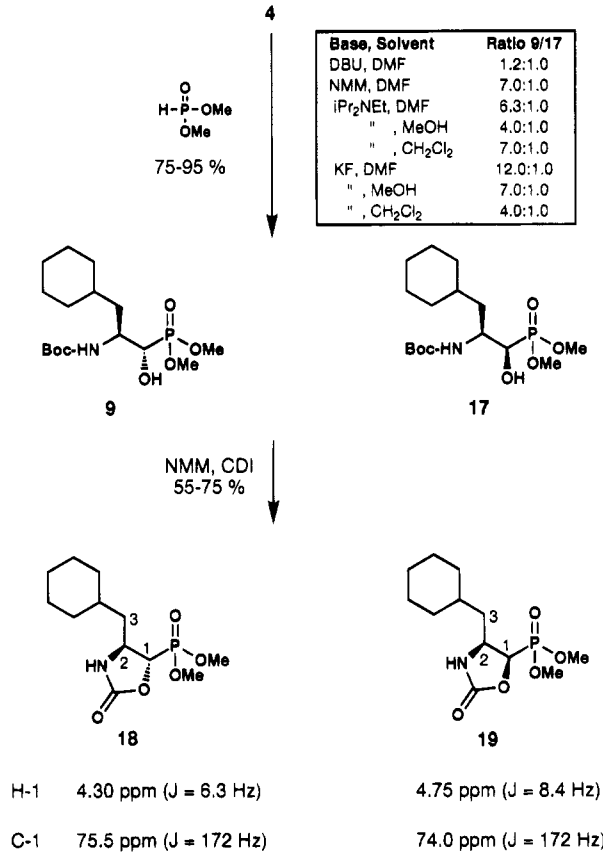
P_1' Modifications: Preparation of Phosphine Oxide and Phosphinate Analogs. Initially, we attempted the synthesis of phosphine oxide intermediate **21** by condensation of diethyl phosphine oxide ($HP(O)(Et)_2$) with aldehyde **4**. $HP(O)(Et)_2$ was prepared by treatment of diethyl phosphonate with 3.0 equiv of ethylmagnesium bromide as described in the literature.[16] However, treatment of $HP(O)(Et)_2$ with nBuLi followed by reaction of the lithioanion with aldehyde **4** gave the desired adduct **21** in only modest yields (20%). Alternatively, when the bromomagnesium phosphine oxide intermediate generated *in situ* was trapped directly with aldehyde **4**, the desired product **21** was obtained in high yield (87%) as a 1:1 mixture of diastereomers. Deprotection of **21** followed by coupling with dipeptide acid **22** using water soluble ethyl[(dimethylamino)propyl]carbodiimide hydrochloride (EDC) as the

Scheme 2. Preparation of Base-Line Compounds[a]



[a] Reagents: (a) KF, $HP(O)(OEt)_2$; (b) anhydrous HCl/EtOAc; (c) Boc-Phe-Leu-OH (**6**), DCC, HOBt, iPr_2NEt; (d) anhydrous HCl/AcOH; (e) $C_5H_9CO_2H$ **6**, DCC, HOBt, iPr_2NEt; (f) KF, $HP(O)(OMe)_2$; (g) Boc-Leu-OH **6**, EDC, HOBt, Et_3N; (h) *cyclo*-C_5H_9-C(O)-Phe-OH (**14**), EDC, HOBt, Et_3N; (i) Me_3N, acetone, 80 °C, 16 h.

Scheme 3. Determination of Stereochemistry

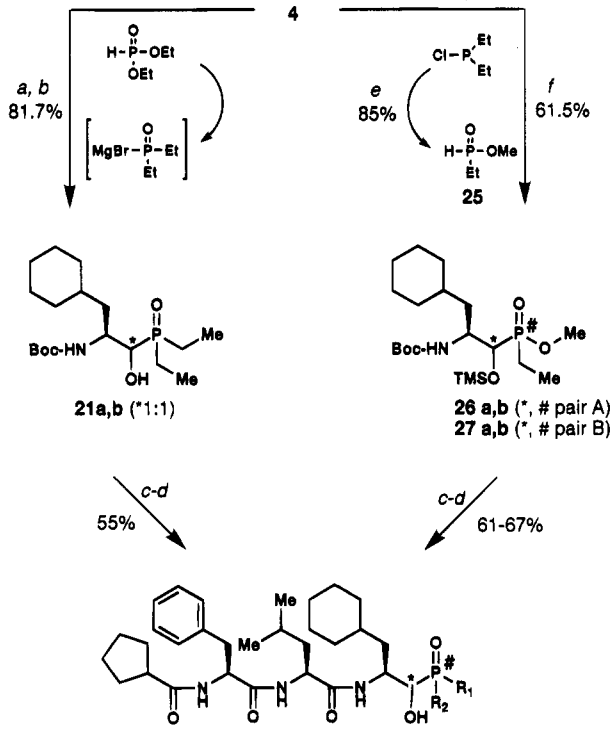


	18	19
H-1	4.30 ppm (J = 6.3 Hz)	4.75 ppm (J = 8.4 Hz)
C-1	75.5 ppm (J = 172 Hz)	74.0 ppm (J = 172 Hz)

coupling reagent yielded the two diastereomers **23** and **24** in 55% overall yield after chromatographic purification.

Preparation of the phosphinate reagent HP(O)(OMe)-(Et) (**25**) required for synthesizing the phosphinate analogs of **15** was initially attempted by treatment of dimethyl chlorophosphite ($(MeO)_2PCl$) with EtMgBr but resulted in poor yields of the desired product. Alternatively, treatment of ethyldichlorophosphine ($EtPCl_2$) with 2 equiv of methanol and 1 equiv of triethylamine

Scheme 4. Preparation of Phosphinates/Phosphine Oxides[a]

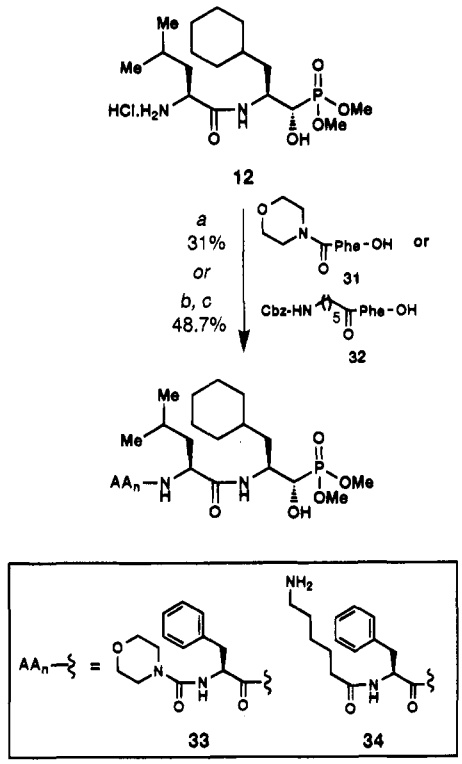


Cpd	R_1	R_2	Isomer (*, #)
23	Et	Et	A
24	Et	Et	B
28	Et	OMe	$A_1 + A_2$
29	Et	OMe	B_1
30	Et	OMe	B_2

[a] Reagents: (a) EtMgBr (3.0), $HP(O)(OEt)_2$ (1.0), THF; (b) **4**, THF; (c) anhydrous HCl/EtOAc; (d) C_5H_9CO-Phe-Leu-OH (**22**), EDC, HOBt, Et_3N; (e) Et_2PCl (1.0), MeOH (2.2), Et_3N (1.0); (f) **4**, iPr_2NEt, TMSCl.

afforded **25** in 85% yield. The reaction of phosphinate **25** with aldehyde **4** generates two new contiguous chiral centers because of the chirality of phosphorus, resulting

Scheme 5. P_3 Modifications in Leucine (P_2) Series[a]



[a] Reagents: (a) **31**, EDC, HOBt, Et_3N; (b) **32**, EDC, HOBt, Et_3N; (c) H_2, 10% Pd/C, 1.0 N HCl, MeOH.

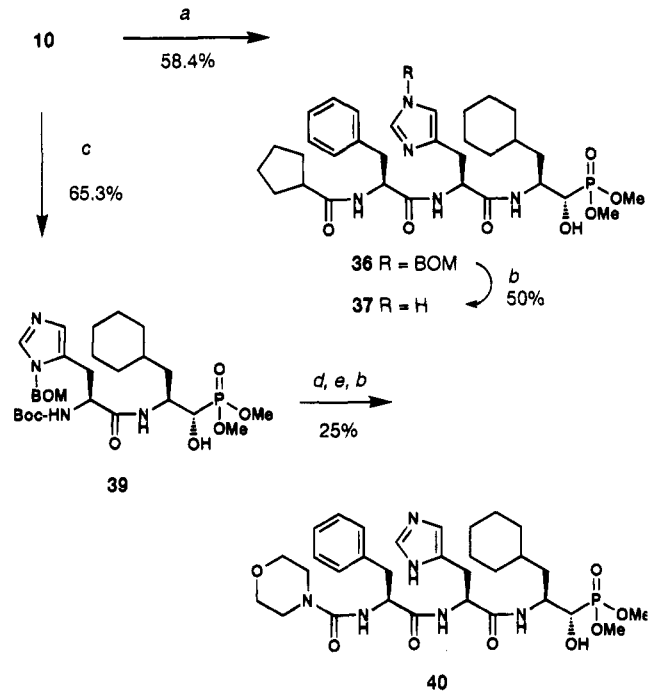
in the formation of four possible diastereomers. Upon condensation of phosphinate **25** with aldehyde **4** in the presence of iPr_2NEt and TMSCl followed by neutral aqueous workup, a mixture of silyl-protected hydroxy phosphinates, **26** and **27**, was obtained in good yields. The two pairs of diastereomers were separated by rapid silica gel chromatography. The individual diastereomeric content of each isomer pair was roughly 1:1 as judged by ^{31}P NMR. The slow-moving isomer pair **27** was deprotected and coupled with acid **22** to afford the diastereomeric tripeptidic phosphinates which were partially separated by flash chromatography (15% **29**, 22% **30**, 30% mixture). Similar deprotection and coupling of the alternate isomer pair **26** afforded an inseparable mixture of diastereomers **28** (isomers A_1 + A_2, 1:1, 61%).

P_3 Modifications in the P_2 Leucine Series. Synthesis of analogs involving modifications of the N-terminal moiety of phenylalanine residue at the P_3 position was undertaken (Scheme 5). The dipeptide amine **12** served as a key synthetic intermediate for this purpose. Thus, the morpholinourea analog **33** was prepared by coupling of dipeptide amine **12** with acid **31**.[17] Similarly, coupling of **12** with the Cbz-protected acid **32**[17] followed by hydrogenolysis of the resulting intermediate afforded the aminocaproic analog **34**.

P_3 Modifications in the P_2 Histidine Series. Preparation of analogs bearing histidine at the P_2 position is summarized in Scheme 6. The dimethyl hydroxy phosphonate intermediate **10** was directly coupled with dipeptide **35**[17] to provide **36**, which after hydrogenolytic cleavage of the (benzyloxy)methyl (BOM) group from the imidazole ring of histidine gave **37**, the P_2 histidine analog of the base-line compound **15**.

A slightly different route was taken for preparing **40**, the N-terminal morpholino analog with P_2 histidine.

Scheme 6. P_3 Modifications in Histidine (P_2) Series[a]



[a] Reagents: (a) C_5H_9CO-Phe-His(BOM)-OH (**35**), EDC, HOBt, Et_3N; (b) H_2, $Pd(OH)_2/C$, MeOH, 1 N HCl; (c) Boc-His(BOM)-OH (**38**), EDC, HOBt, Et_3N; (d) anhydrous HCl/EtOAc; (e) **31**, EDC, HOBt, Et_3N.

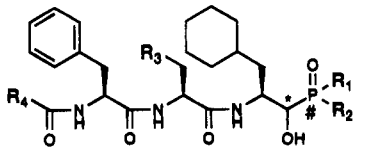
Our intent was to have dipeptide **39**, prepared by reaction of amine **10** with Boc-His(BOM)-OH (**38**),[18] to serve as a common intermediate for various P_3 modifications in the histidine series. However removal of the Boc group from **39** followed by reaction with **31** yielded the coupled product in only 35% yield. It turned out that the dimethyl phosphonate intermediate **39** was itself unstable and decomposed slowly to the corresponding monoester monoacid. Apparently, increased basicity of the imidazole ring in **39**, because of the presence of the BOM group, was responsible for this dealkylation. While this side reaction ruled out the possibility of utilizing **39** as a common precursor for various P_3 modifications, enough material was in hand to complete the synthesis of morpholino analog **40** with histidine at the P_2 site.

Biology

The α-hydroxy phosphonate **3**, in which the diethyl phosphonate moiety serves as a bioisosteric replacement for the ethyl ester group of the α-hydroxy ester **2**, was found to be a good *in vitro* inhibitor of human renin (IC_{50} = 29 nM).[10] This constituted a novel lead in our program and formed the basis for further investigation of this class of compounds as renin inhibitors. Analogous to the statine and norstatine class of renin inhibitors, the hydroxyl-bearing carbon of the active diastereomer **3** bears the *S* (*anti*, *threo*) stereochemistry.[15,8] The >1000-fold difference in activity between **3** and the inactive diastereomer **7** is striking and represents one of the largest degrees of separation in potency between the active and inactive isomers for various classes of compounds designed as renin inhibitors.

Compared to α-hydroxy carbonyls, the α-hydroxy phosphonates involve placement of an sp^3-hybridized phosphorus center possessing two alkoxy side chains

Table 1. Human Renin Inhibitory Activity of Various α-Hydroxy Phosphinyl Renin Inhibitors[a]



Cpd #	R_4	R_3	R_2	R_1	*/#	IC_{50}, nM
3	tBuO-	Me_2CH-	-OEt	-OEt	*A	29 ± 3 (10)
7	tBuO-	Me_2CH-	-OEt	-OEt	*B	41000 ± 1000 (2)
8		Me_2CH-	-OEt	-OEt	*A	38 ± 6 (2)
13	tBuO-	Me_2CH-	-OMe	-OMe	*A	10 ± 1 (3)
15		Me_2CH-	-OMe	-OMe	*A	16 ± 1 (5)
16		Me_2CH-	-OMe	-OH	*A	27,000 (1)
23		Me_2CH-	-Et	-Et	*A	400 ± 32 (3)
24		Me_2CH-	-Et	-Et	*B	5200 ±1200 (2)
28		Me_2CH-	-OMe	-Et	*#A_1 + A_2	210 ± 17 (3)
29		Me_2CH-	-OMe	-Et	*#B_1	44 ± 7 (3)
30		Me_2CH-	-OMe	-Et	*#B_2	2,400 (1)
33		Me_2CH-	-OMe	-OMe	*A	8.5 ± 1.4 (3)
34		Me_2CH-	-OMe	-OMe	*A	31 ± 4 (6)
37			-OMe	-OMe	*A	220 ± 40 (2)
40			-OMe	-OMe	*A	41 ± 14 (3)

[a] See the Experimental Section for a description of the method for determining the IC_{50} values of these inhibitors. Number of determinations is indicated in parentheses.

adjacent to the hydroxyl-bearing C-1 carbon. This results in more steric crowding and may be responsible for making the stereochemical requirements of the adjacent hydroxyl group so stringent, thus leading to the observed activity differences between **3** and **7**. It should be noted that the chemically related but sterically less crowded β-hydroxy phosphonate class of renin inhibitors have been reported to display only a modest preference (2–3-fold) for statine-like *S* stereochemistry for the hydroxyl group.[19]

Choice of a Base-Line Compound. Before embarking on an extensive investigation of the SAR of **3**, a few analogs were synthesized to aid our selection of an appropriate base-line compound. Simple alterations at both the N- and C-terminal ends of **3** were performed for this purpose. Compound **8**, which involved substitution of the acid sensitive *tert*-butoxycarbonyl group in **3** with the stable cyclopentylcarbonyl group, was equiactive (IC_{50} = 38 nM) to the parent compound. The assessment of critical binding features operative at the other end of the molecule was a more intriguing process and commenced with the preparation of dimethyl phosphonate analog **13**. The isosteric replacement of an sp^2-hybridized carbonyl functionality in the α-hydroxy ester **2** with an sp^3-hybridized phosphinyl group in **3** introduces an extra alkoxy group because of the tetravalency of the phosphorus atom. While one of the ethoxy groups in **3** may serve as a P_1' residue, the role of the other extra ethoxy group was largely speculative. It could be engaged in additional favorable binding or undesired steric interaction, or it could be exerting an electronic influence on the adjacent hydroxyl group. The tripeptide dimethyl phosphonate **13** derived from the major diastereomer **8** was found to possess very good inhibitory potency (IC_{50} = 10 nM). On the basis of the equipotency of the isopropyl and methyl ester analogs reported for the α-hydroxy ester series, it was reasonable to expect the P_1'–S_1' binding interaction to be equal for both **3** and **13**.[8] In that event, one possible explanation for the 3-fold improvement of the dimethyl analog **13** over the diethyl analog **3** would be that the second alkoxy group of the diethyl series suffers greater steric hinderance than in the dimethyl case. The synthetic ease of preparation of the *anti* diastereomer **9** in comparison to the unseparable mixture obtained with the diethyl phosphonate **5**, coupled with 3-fold better activity of the final compound **13** when compared to the initial diethyl lead **3**, favored the choice of dimethyl versus diethyl phosphonate as the series of choice for further investigation. With dimethyl phosphonate, replacement of the acid sensitive *tert*-butoxycarbonyl group in **13** by the cyclopentyl group in **15** is well tolerated (IC_{50} = 16 nM). Since the latter may prove advantageous with respect to synthetic manipulations that may be desired in the remainder of the molecule, the dimethyl phosphonate **15** with a cyclopentyl carbonyl at the N-terminal was chosen as the base-line compound for further modifications in the tripeptide inhibitor series.

P_1' Modifications. An understanding of the critical binding features of the hydroxy phosphonate group in these inhibitors was deemed essential at the onset of the project. The monoester monoacid **16** suffered a drastic loss in potency (IC_{50} = 27 μM) compared to the parent dimethyl ester **15**. This suggested that an acidic group is not acceptable at this position in the molecule, possibly due to the unfavorable proximity with the two active site aspartic acid residues. The phosphine oxides **23** and **24** and the phosphinates **28–30** were prepared to further evaluate the role of substituents on the phosphorus center. The steric requirements of these analogs are similar to those of the base-line compound **15**, but they differ markedly in electronic nature. Besides the apparent difference in the electronic nature of alkoxy versus alkyl groups, these substituents also influence the dipolar character of the P=O bond (phosphine oxide > phosphinate > phosphonate). Replacement of both the methoxy side chains on the phosphorus atom of **15** by isosteric ethyl groups results in the relatively inactive phosphine oxides **23** (IC_{50} = 400 nM) and **24** (IC_{50} = 5200 nM), suggesting that one or both of the alkoxy groups in **15** are critically required for good binding. In the phosphinate analogs, one of the methoxy side chains of **15** is replaced by an ethyl group, thereby introducing chirality at the phosphorus center. Among the four diastereomeric phosphinates, isomer B_1 (**29**, IC_{50} = 44 nM) has activity comparable to that of the parent compound **15**. Hence, it can be concluded that only one of the two alkoxy groups in the hydroxy phosphonate series is critical for binding. While isomer B_2 (**30**, IC_{50} = 2400 nM) is >50 fold less active, compound **28** which is a mixture of diastereomers A_1 and A_2 (IC_{50} = 210 nM) is reasonably active. On the basis of the data that were observed for the phosphonate diastereomers **3** and **7**, it may be assumed that only the *trans* statine-like diastereomers having *S* stereochem-

istry at the hydroxyl-bearing carbon center will have good activity in the phosphinate analogs. In that event, the presence of two active diastereomers (**28** and **29**) in the phosphinate series indicates that the chirality on the phosphorus center is not very important with respect to the *in vitro* potency of this class of renin inhibitors.

P_2 and P_3 Modifications. Investigation of the hydroxy phosphonate renin inhibitors was initiated with a leucine residue at the P_2 position, even though the natural substrate angiotensinogen possesses a histidine at P_2. The choice of leucine stemmed from our work in the area of activated ketone-based inhibitors, where histidine was replaced by leucine to avoid synthetic complications that may arise from undesired side reactions between the basic imidazole ring of histidine and the highly electrophilic carbonyl groups.[7] However, unlike activated ketones, the hydroxy phosphonates possess adequate chemical stability and can be expected to be compatible with moderately basic side chain functional groups in the molecule. This culminated in the preparation of **37**, the histidne analog of **15**. To our surprise, **37** was 14-fold less active (IC_{50} = 220 nM) than the leucine-containing base-line compound **15** (IC_{50} = 16 nM). The superiority of P_2 leucine analog **15** in the hydroxy phosphonate series contradicts the trend observed for most classes of renin inhibitors, wherein substitution of the P_2 histidine with an aliphatic side chain possessing amino acids has at best led to retention of activity.

In general, the P_3 group in tripeptide renin inhibitors is a requirement for good activity but appears to offer a great degree of structural flexibility. This has led to the emergence of various N-capping groups as well as replacement of the entire *N*-carbamate moiety of initial lead **3** by various isosteric functional groups as a way of altering the physiochemical characteristics, and thereby oral activity, of these compounds.[5] The morpholino and aminocaproic substitutions were especially attractive since these had shown promising results with imidazole alcohols,[20,21] another class of renin inhibitors studied in our group. In the P_2 leucine series, the morpholino N-terminal modification (**33**, IC_{50} = 8.5 nM) was very well tolerated, while substitution by an aminocaproic moiety (**34**, IC_{50} = 31 nM) suffered a marginal loss in comparison to the parent compound (**15**, IC_{50} = 16 nM). Unlike the previously studied P_2 leucine compounds, the free amino group in **34** confers adequate degree of aqueous solubility, quite often a useful criterion for *in vivo* and oral activity. The superior activity of **33** led to the preparation of a similar morpholine-terminating analog, **40**, in the P_2 histidine series. Compound **40** (IC_{50} = 41 nM) was 5-fold less active than the leucine counterpart **33**, reiterating the trend observed previously with analogs **37** versus **15**.

***In Vivo* Studies.** The real challenge in the area of discovery of renin inhibitors has extended well beyond the arena of *in vitro* activity of small-sized and novel molecules, and major attention in recent years has shifted in the direction of molecules with good oral bioavailability.[5] Several hydroxy phosphonates were evaluated in conscious, sodium-depleted, cynomolgus monkeys for their effects on plasma renin activity (PRA) and mean arterial pressure (MAP), and the data for compound **34**, the aminocaproic analog of **15**, are

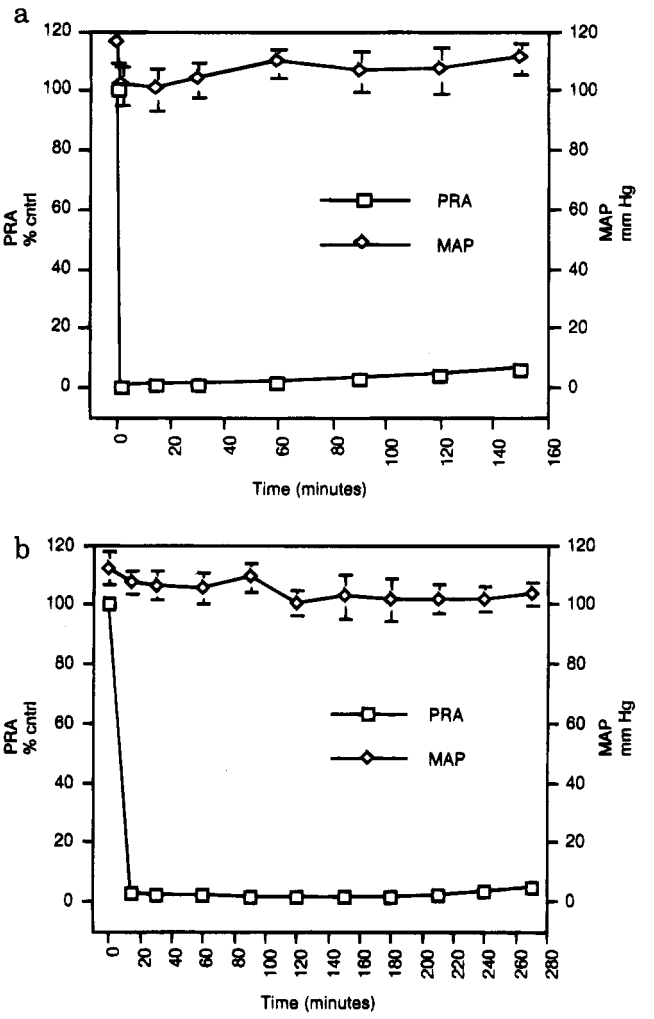


Figure 1. *In vivo* activity of aminocaproic analog **34**. (a) Intravenous time response effect at 1 μmol/kg in sodium-depleted, cynomolgus monkeys. (b) Oral time response effect at 15 μmol/kg of sodium-depleted, cynomolgus monkeys.

discussed briefly. Nearly complete inhibition of PRA was observed upon intravenous administration of **34** at doses as low as 0.01 μmol/kg (data not shown). At 1 μmol/kg, iv, complete and long-lasting PRA inhibition was obtained (Figure 1). This was accompanied by a small (ca. 10−15 mmHg) drop in MAP which returned to normal levels after 60 min. When tested po at 15 μmol/kg, **34** caused nearly complete inhibition of PRA for more than 4 h; however, the effect on blood pressure at this dose was not very pronounced (Figure 1). The absence of significant blood pressure lowering while PRA is strongly inhibited has been observed before with renin inhibitors. Explanations for such discrepancies have included (1) circulating levels of inhibitors being overestimated due to the release of plasma protein-bound renin inhibitor that occurs during the process of measuring PRA[22] and (2) maximal reduction of blood pressure by renin inhibitors possibly requiring their access to less accessible sites such as the vascular interstital space.[23] In our own studies, we have found that blood pressure reduction measured following administration of renin inhibitors to monkeys correlates well with reductions in circulating concentrations of AI (data not shown). However, we have not further investigated the causes of dissociation between PRA and blood pressure reduction in our own studies.

Conclusions

The α-hydroxy phosphonates represent a promising, structurally unique class of transition state analog inhibitors of proteolytic enzymes. We have demonstrated their application and utility in the design of inhibitors of the aspartyl protease renin. Our initial lead was the tripeptidic α-hydroxy diethyl phosphonate **3** (IC_{50} = 29 nM), and preliminary studies identified α-hydroxy dimethyl phosphonate **15** (IC_{50} = 16 nM) as a base-line compound for further SAR study. Synthesis and evaluation of the corresponding phosphinate (**28–30**) and phosphine oxide (**23** and **24**) analogs of **15** highlighted the presence of at least one alkoxy group on the phosphorus center as a requirement for good activity. Surprisingly, inhibitors with leucine at P_2 were found to possess better *in vitro* activity than the corresponding P_2 histidine analogs (**15**, IC_{50} = 16 nM vs **37**, IC_{50} = 220 nM; **33**, IC_{50} = 8.5 nM vs **40**, IC_{50} = 41 nM) for this class of renin inhibitors. Several modifications at the P_3 N-terminal site were aimed at altering the physiochemical properties, and thereby *in vivo* performance, of these inhibitors. Compound **34** (IC_{50} = 31 nM), the P_3 aminocaproic analog of **15**, showed complete and long-lasting inhibition of PRA along with a 10–15 mmHg drop in MAP when administered intravenously at 1 μmol/kg in conscious, sodium-depleted, cynomolgus monkeys.

Experimental Section

General. All reactions were carried out under a positive pressure of dry argon, unless otherwise specified. Tetrahydrofuran (THF) and diethyl ether were distilled from sodium or potassium/benzophenone ketyl prior to use. Acetonitrile, benzene, dichloromethane, diisopropylamine, hexane, methanol, pyridine, and toluene were distilled from calcium hydride prior to use.

TLC was performed using EM Science (E. Merck) 5 × 10 cm plates precoated with silica gel 60 F_{254} (0.25 mm thickness), and the spots were visualized by any of the following: UV, iodine, phosphomolybdic acid (PMA), ceric ammonium sulfate, anisaldehyde, vanillin, or Rydons stain. EM Science silica gel 60 (230–400 mesh ASTM) was used for flash chromatography. A ratio of 25–100:1 silica gel/crude product by weight and a nitrogen pressure of 5–25 psi was normally employed for flash columns. Reverse phase chromatographic purification of final compounds was carried out using CHP20P gel, a 75–150 μm poly(styrene)–divinylbenzene copolymer purchased from Mitsubishi Chemical Industries. Analytical HPLC was performed using two Shimadzu LC-6A pumps with an SCL-6B system controller and a C-R4AX chromatopac and an SPD-6AV UV–vis spectrophotometric detector. HPLC columns were commercially available from either Whatman or YMC Corp.

Melting points were determined on an electrothermal Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on one of the following instruments: JOEL GX-400 operating at 400 (^{1}H) or 100 (^{13}C) MHz, JOEL FX-270 operating at 270 (^{1}H) or 67.8 (^{13}C) MHz, on JOEL FX-60Q operating at 15 MHz (^{13}C). Chemical shifts are reported in δ units in parts per million (ppm) downfield from tetramethylsilane (TMS) and coupling constants (*J*) are in hertz (Hz). ^{31}P NMR spectra were recorded on a JEOL FX-90Q using H_3PO_4 (δ = 0 ppm) as an external reference. IR spectra were recorded on a Mattson Sirius 100 FT-IR spectrophotometer, and the absorption maxima are reported in cm^{-1}. Mass spectra were recorded on a Finnigan MAT TSQ-4600 mass spectrometer (chemical ionization, CI) or a VG-ZAB-2F mass spectrometer (fast atom bombardment, FAB). High-resolution mass spectra (HRMS) were determined using peak-matching techniques versus PEG standards on a VG-ZAB-2F spectrometer. Optical rotations were measured using a Perkin-Elmer model 241 polarimeter and a 10 cm path length optical cell. Microanalysis results were adjusted to obtain the best fit assuming nonstoichiometric hydration.

***In Vitro* Inhibition Studies.** The human kidney renin used for assay of inhibitor potency was a partially purified preparation (no. 216, 2.4 μg of AI/h/mg) generously provided by Dr. E. Haas (Mt. Sinai Medical Center, Cleveland, OH). The source of angiotensinogen substrate in renin incubation mixtures was human plasma (Mercer Regional Blood Center, Trenton, NJ). Incubation mixtures of 0.5 mL were buffered with 0.2 M TES, pH 7.0, and contained 0.10 mM EDTA, 0.10 mM sodium tetrathionate, and 0.04 mM phenylmethanesulfonyl fluoride. Renin concentrations in the mixtures were adjusted to generate AI at rates, constant with time, of 20–80 ng of AI/mL/h. Human plasma was added at concentrations (10–50%) sufficient to provide a final angiotensinogen concentration of 0.5 μM. Inhibition test compounds were dissolved, serially diluted, and added to incubation mixtures in dimethyl sulfoxide (DMSO), with the final DMSO concentration fixed at either 0.5% or 1.0%. Incubations were conducted for 30 min at 37 °C. After the reactions were terminated by reduction of the temperature to 0 °C, AI concentrations were measured by radioimmunoassay. Inhibitor potencies are expressed as IC_{50} values, the interpolated concentrations corresponding to 50% inhibition of renin activity. For most of the compounds, including all of those with IC_{50} concentrations below 2000 nM, the IC_{50} concentrations are mean values determined from 2–10 experiments; standard errors for the mean IC_{50} concentrations ranged from 2% to 34%.

***In Vivo* Studies.** The *in vivo* evaluation of renin inhibitors in conscious, sodium-depleted, cynomolgus monkeys instrumented with indwelling aortic and vena caval catheters has been described before.[21] Compound **34** was dissolved in water for both iv and po dosing. For both studies, the results are expressed as means ±SEM for n = 4 animals. Note that the standard errors for PRA measurements in both the iv and po experiments were generally <1% and thus are not evident on the graphs in Figure 1.

Preparation of (1*S*)-[1-(Cyclohexylmethyl)-2-(diethoxyphosphinyl)-2-hydroxyethyl]carbamic Acid, 1,1-Dimethylethyl Ester (5). A mixture of the aldehyde **4** (2.55 g, 10 mmol), diethyl phosphonate (1.381 g, 10 mmol), and KF (0.581 g, 10 mmol) in 40 mL of CH_2Cl_2 was stirred overnight at room temperature. A TLC check after 14 h revealed incomplete reaction, and hence additional amounts of KF (1.162 g, 20 mmol) and diethyl phosphonate (276 mg, 2 mmol) were added at this stage. After an additional 4 h of stirring, the reaction mixture was filtered through Celite and the filtrate diluted with 60 mL of CH_2Cl_2 and washed sequentially with H_2O (2 × 25 mL), saturated $NaHCO_3$ (2 × 20 mL), and saturated NaCl (1 × 20 mL). Drying (Na_2SO_4) and concentration gave a residue which upon flash chromatographic purification (250 g of silica gel, 2:8:0.1 hexane/EtOAc/AcOH) yielded 3.04 g (77.4%) of pure **5**. TLC: R_f = 0.35 (2:8:0.1 hexane/EtOAc/AcOH). MS: $(M + H)^+$ 394$^+$. $[\alpha]_D = -25.8°$ (c = 3.48, CH_3OH). ^{1}H NMR (60 MHz, $CDCl_3$): 1.47 (s, 9 H), 0.7–2.2 (m, 19 H), 3.7–4.4 (m, 6 H), 5.0–5.4 (m, 2 H). ^{13}C NMR (67.8 MHz, $CDCl_3$): 2 diastereomers (4:1); 16.1 (d, J = 5.7), 25.8, 25.9, 26.1, 26.3, 28.0, 32.7, 33.2, 34.0, 39.6, 37.8 (minor), 48.3, 50.0 (minor), 62.8, 63.1, 63.3, 69.7 (d, J = 160.8), 71.0 (d, J = 156, minor), 78.8, 155.5, 156.1 (minor).

Preparation of (1*S*,2*S*)-*N*-[1-(Cyclohexylmethyl)-2-(diethoxyphosphinyl)-2-hydroxyethyl]-*N*^{2}-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-L-leucinamide, Isomers A (3) and B (7). Compound **5** (2.4 g, 6.1 mmol) was dissolved in 10 mL of EtOAc, the solution cooled to 0 °C, and HCl gas bubbled through it for ~15 min, after which a TLC check revealed complete disappearance of starting material. The solution was concentrated and the residue redissolved in 1:1 petroleum ether/EtOAc, and concentrated to give a white solid which was dried overnight *in vacuo* to yield the amine hydrochloride salt (1.786 g, 88.8%). The amine and Boc-Phe-Leu-OH (**6**; 2.04 g, 5.4 mmol) were dissolved in 20 mL of DMF at 0 °C, and HOBt (826 mg, 5.4 mmol) was added to the solution. After 5 min, iPr_2NEt (0.94 mL, 5.4 mmol) was added, and this was followed immediately by addition of DCC (1.112

g, 5.4 mmol). The reaction mixture was stirred at 0 °C for 2 h and overnight at room temperature, after which the precipitated urea was filtered off and the reaction mixture diluted with EtOAc (50 mL) and washed sequentially with H_2O (2 × 25 mL), 10% citric acid (1 × 25 mL), saturated $NaHCO_3$ (2 × 25 mL), and saturated NaCl (1 × 25 mL). Direct concentration without drying afforded 3.585 g of a white solid, which after flash chromatographic purification (60 g of silica gel, 3:3:3:0.05 hexane/Et_2O/EtOAc/AcOH) afforded pure slow-moving diastereomer **3** (1.11 g, 31.5%), pure fast-moving diastereomer **7** (0.116 g, 3.3%), and a mixture of two isomers (2.27 g). Repeated purifications of the latter yielded additional **3** (1.45 g, 41.1%) and **7** (0.363 g, 10.3%).

Characterization of 3. TLC: R_f = 0.45 (90:10:1.0:0.1 $CHCl_3$/MeOH/H_2O/AcOH). MS: $(M + H)^+$ 654^+. Mp: 136–138 °C. $[\alpha]_D$ = −48.0° (*c* = 1.18, CH_3OH). IR (KBr): 1027, 1050, 1170, 1233, 1367, 1499, 1524, 1647, 1689, 2925, 3290, 3310, 3314. ^1H NMR (400 MHz, $CDCl_3$): 0.82 (d, 6H, *J* = 7), 1.27 (t, 6 H, *J* = 7), 1.34 (5, 9 H), 0.95–1.78 (m, 16 H), 2.95–3.15 (m, 2 H), 3.82 (m, 1 H), 4.1 (q, 4 H, *J* = 7), 4.0–4.35 (m, 4 H), 4.90 (d, 1 H, *J* = 6.5), 6.47 (d, 1 H, *J* = 7.5), 6.63 (d, 1 H, *J* = 8.0), 7.10–7.25 (m, 5 H). ^{13}C NMR (67.8 MHz, $CDCl_3$): 16.45 (d, *J* = 3.8), 21.8, 23.0, 24.5, 26.0, 26.1, 26.4, 28.2, 32.7, 33.3, 34.1, 38.0, 38.2, 41.4, 49.1, 52.0, 55.7, 62.6, 62.7, 62.8, 69.5 (d, *J* = 162.8), 80.1, 126.7, 128.4, 129.3, 136.6, 155.5, 171.3, 172.0. Anal. Calcd for $C_{33}H_{56}N_3O_8P$: C, 60.62; H, 8.63; N, 6.43; P, 4.74. Found: C, 60.51; H, 8.53; N, 6.41; P, 4.59.

Characterization of 7. TLC: R_f = 0.55 (90:10:1.0:0.1 $CHCl_3$/MeOH/H_2O/AcOH). MS: $(M + H)^+$ 654^+. Mp: 173–175 °C. $[\alpha]_D$ = +10.0° (*c* = 0.14, CH_3OH). IR (KBr): 66595. ^1H (270 MHz, $CDCl_3$): 0.80 (q, 3 H, *J* = 7), 0.85 (d, 3 H, *J* = 7), 1.37 (q, 6 H, *J* = 7), 1.44 (5, 9 H), 1.03–1.95 (m, 16 H), 2.90–3.18 (m, 2 H), 3.8–3.92 (m, 1 H), 3.94–4.68 (m, 8 H), 5.32 (m, 1 H), 7.03 (d, 1 H, *J* = 6), 7.15–7.35 (m, 5 H), 7.42 (d, 1 H, *J* = 6). ^{13}C NMR (67.8 MHz, $CDCl_3$): 16.4 (d, *J* = 4.0), 21.3, 23.4, 24.2, 26.0, 26.1, 26.5, 28.2, 32.8, 33.5, 33.8, 37.0, 38.2, 39.1, 47.6, 49.8, 55.3, 62.2, 62.4, 70.7 (*J* = 162), 80.9, 126.9, 128.5, 129.1, 136.4, 156.4, 171.5, 171.9. Anal. Calcd for $C_{33}H_{56}N_3O_8P$: C, 60.62; H, 8.63; N, 6.43; P, 4.74. Found: C, 60.44; H, 8.91; N, 6.37; P, 4.42.

Preparation of (1*S*,2*S*)-*N*-[1-(Cyclohexylmethyl)-2-(diethoxyphosphinyl)-2-hydroxyethyl]-*N*²-[*N*-(cyclopentylcarbonyl)-L-phenylalanyl]-L-leucinamide (8). **3** (915.3 mg, 1.4 mmol) was dissolved in a solution of HCl/AcOH (7 mL), reacted for 0.5 h at 25 °C, and concentrated to dryness, yielding 815.9 mg (98.6%) of amine hydrochloride. A portion of this amine (809 mg, 1.37 mmol) was added to a solution of cyclopentanecarboxylic acid (148 μL, 1.37 mmol) in THF (5.5 mL) and cooled to 0 °C. HOBt (209.6 mg, 1.37 mmol), iPr_2NEt (262.5 μL, 1.51 mmol), and DCC (282.6 mg, 1.37 mmol) were added sequentially. After 48 h at 0 °C, the reaction mixture was filtered and concentrated to dryness. The residue was dissolved in EtOAc (40 mL), washed sequentially with 10% citric acid (15 mL), H_2O (25 mL), saturated aqueous $NaHCO_3$ (15 mL), and saturated aqueous NaCl (15 mL), dried, and concentrated, yielding 517 mg of crude compound. Purification by flash chromatography (1:1 hexane/EtOAc, 3:3:3:0.05 hexane/Et_2O/EtOAc/AcOH) followed by crystallization of relatively pure fractions from CH_2Cl_2/iPr_2O afforded pure **8** (117 mg, 13.2%). TLC: R_f = 0.5 (9:1.0:0.1 $CHCl_3$/MeOH/AcOH). MS: $(M + H)^+$ 650^+. Mp: 180–195 °C slow dec. $[\alpha]_D$ = −36.7° (*c* = 0.46, CH_3OH). IR (KBr): 967, 1029, 1229, 1541, 1642, 2925, 3289. ^1H NMR (270 MHz, $CDCl_3$): 0.95 (m, 6 H), 1.05–1.95 (m, 30 H), 2.50 (m, 1 H), 2.97–3.22 (m, 2 H), 3.95 (m, 1 H), 4.05–4.45 (m, 7 H), 4.75–5.0 (m, 2 H), 6.32 (m, 1 H), 6.92 (m, 1 H), 7.05–7.30 (m, 5 H). ^{13}C NMR (67.8 MHz, $CDCl_3$): 16.5, 22.1, 23.0, 24.7, 25.8, 25.9, 26.1, 26.2, 26.5, 29.9, 30.6, 32.8, 33.5, 34.3, 37.6, 38.0, 41.3, 45.4, 49.4, 52.4, 54.0, 62.5, 62.6, 62.8, 62.9, 69.7 (*J* = 162.7), 126.8, 128.4, 129.3, 136.3, 172.0, 171.4, 176.7. Anal. Calcd for $C_{34}H_{56}N_3O_7P$·0.5AcOH·1.0H_2O: C, 60.24; H, 8.67; N, 6.02. Found: C, 60.25; H, 8.27; N, 6.05.

Preparation of [*S*-(*R,*R**)]-[1-(Cyclohexylmethyl)-2-(dimethoxyphosphinyl)-2-hydroxyethyl]carbamic Acid, 1,1-Dimethylethyl Ester (9).** Dimethyl phosphite (Aldrich; 4.383 mL, 47.8 mmol) and anhydrous KF (6.109 g, 105.2 mmol) were added sequentially to a solution of the aldehyde **4** (12.2 g, 47.8 mmol) in 120 mL of DMF. After vigorous stirring for 16 h at room temperature, the reaction mixture was filtered and the filtrate concentrated *in vacuo* to afford 21.92 g of oily residue. Flash chromatographic purification (1 kg of Merck 230–400 mesh silica gel, 19.1 chloroform/methanol) afforded 13.12 g (75.2%) of **9** as a light yellow-colored solid whose diastereomeric ratio was 12.7:1.0 as determined by ^{31}P NMR. TLC: R_f = 0.20 (9:1 $CHCl_3$/MeOH). MS: $(M + H)^+$ 366^+. Mp: 77–83 °C. $[\alpha]_D$ = −38.4° (*c* = 1.14, CH_3OH). IR (KBr): 1042, 1176, 1200, 1531, 1702, 2922, 3330. ^1H NMR (270 MHz, $CDCl_3$): 1.48 (s, 9 H), 0.79–1.97 (m, 13 H), 3.83 (d, 3 H, *J* = 8), 3.87 (d, 3 H, *J* = 7), 3.80–4.15 (m, 2 H), 5.40 (m, 1 H), 5.55 (d, 1 H, *J* = 8). ^{13}C NMR (67.8 MHz, $CDCl_3$): 26.4, 26.5, 26.7, 28.5, 33.0, 33.7, 34.4, 39.5 (*J* = 11.7), 49.2, 53.3 (d, *J* = 5.8), 53.75 (d, *J* = 5.9), 68.95 (*J* = 160.4), 79.4, 156.2; remants of minor isomer, 79.9, 51.0. ^{31}P NMR: 25.28 (major), 24.77 (minor), ratio = 12.70:1.0. Anal. Calcd for $C_{16}H_{32}NO_6P$: C, 52.59; H, 8.83; N, 3.83; P, 8.48. Found: C, 52.78; H, 8.91; N, 3.81; P, 8.72.

Preparation of [*S*-(*R,*R**)]-(2-Amino-3-cyclohexyl-1-hydroxypropyl)phosphonic Acid, Dimethyl Ester, Hydrochloride (10).** **9** (1.82 g, 5 mmol) was treated with 25 mL of 1.42 N anhydrous HCl in EtOAc at 0 °C, and the progress of the reaction was carefully monitored by TLC. It was judged to be complete after 6.5 h at 0 °C. Concentration *in vacuo* followed by trituration first with CH_2Cl_2 and then with Et_2O yielded pure **10** (1.27 g, 86.6%). TLC: R_f = 0.15 (90:20:2.5:1.0 $CHCl_3$/MeOH/H_2O/AcOH). MS: $(M + H)^+$ 266^+. Mp: 60–66 °C. IR (KBr): 1055, 1230, 1448, 1504, 2851, 2913, 2930. ^{13}C NMR (CD_3OD): 26.8, 26.9, 27.3, 33.8, 34.0, 34.4, 38.05 (*J* = 9.5), 51.2, 54.0 (*J* = 7.6), 55.3 (*J* = 7.6), 66.21 (*J* = 166.5). Anal. Calcd for $C_{11}H_{25}ClNO_4P$·0.41H_2O: C, 42.87; H, 8.45; N, 4.55; P, 10.05. Found: C, 42.61; H, 8.21; N, 4.60; P, 9.76.

Preparation of (1*S*,2*S*)-*N*-[1-(Cyclohexylmethyl)-2-(dimethoxyphosphinyl)-2-hydroxyethyl]-*N*²-[(1,1-dimethylethoxy)carbonyl]-L-leucinamide (11). A solution of Boc-L-leucine hydrate (3.18 g, 12.7 mmol) in DMF (25 mL) was added to a solution of **10** (3.50 g, 11.6 mmol) in DMF (25 mL) and cooled to 0 °C. HOBt (1.95 g, 12.7 mmol), Et_3N (3.55 mL, 25.5 mmol), and EDC (2.44 g, 12.7 mmol) were added sequentially. After 16 h at 25 °C, the reaction was quenched with pH 4.01 buffer solution (250 mL, Mallinckrodt "buffer pH 4.01") and the mixture stirred for an additional 10 min. The reaction mixture was then extracted with EtOAc (2 × 200 mL), and the combined organic extracts were washed sequentially with saturated aqueous $NaHCO_3$ (2 × 150 mL) and saturated aqueous NaCl (100 mL), dried, and concentrated. Flash chromatographic purification of the crude product (6.68 g, 400 g of Merck silica gel, 2:8:0.1 hexane/EtOAc/AcOH) gave nearly pure product which was crystallized from hexane/EtOAc to yield pure **11** (2.90 g, 53%). TLC: R_f = 0.20 (2:8:0.1 hexane/EtOAc/AcOH). MS: $(M + H)^+$ 479^+. Mp: 157–159 °C. $[\alpha]_D$ = −62.0° (*c* = 1.37, CH_3OH). IR (KBr): 1026, 1046, 1178, 1248, 1532, 1656, 1704, 2924, 3307. ^1H NMR ($CDCl_3$): 0.95 (d, 3 H, *J* = 7), 0.97 (d, 3 H, *J* = 7), 1.48 (s, 9 H), 0.75–1.83 (m, 16 H), 3.81 (d, 6 H, *J* = 10), 3.9–4.2 (m, 3 H), 4.85–5.05 (m, 2 H), 6.90 (d, 1 H, *J* = 8). ^{13}C NMR ($CDCl_3$): 21.8, 22.9, 24.6, 26.0, 26.1, 26.3, 28.2, 32.6, 33.3, 34.0, 38.0 (*J* = 9.4), 41.5, 49.0, 53.1, 53.2, 53.4, 69.6 (*J* = 162.7), 79.5, 155.4, 173.3. ^{31}P NMR ($CDCl_3$): 24.7. Anal. Calcd for $C_{22}H_{43}N_2O_7P$: C, 55.21; H, 9.06; N, 5.86; P, 6.47. Found: C, 55.34; H, 9.04; N, 5.78; P, 6.28.

Preparation of (1*S*,2*S*)-*N*-[1-(Cyclohexylmethyl)-2-(dimethoxyphosphinyl)-2-hydroxyethyl]-L-leucinamide, Hydrochloride (12). Compound **11** (2.29 g, 4.8 mmol) was dissolved in a solution of anhydrous HCl/EtOAc (25 mL). After 1 h, the reaction mixture was concentrated, yielding 2.2 g of **12** (quantitative yield) which was directly utilized in the next step. TLC: R_f = 0.20 (9:1:0.05 $CHCl_3$/MeOH/NH_4OH). MS: $(M + H)^+$ 379^+. ^1H NMR (CD_3OD): 0.80, 2.07 (m, 22 H), 3.65–4.4 (m, 9 H), 5.9 (m, 1 H), 6.25 (m, 1 H). ^{13}C NMR (CD_3OD): 21.8, 23.4, 25.2, 26.9, 27.0, 27.2, 27.6, 33.7, 34.7, 35.0, 39.9, 41.6, 52.7, 53.1, 53.8, 53.9, 54.2, 54.3, 69.4 (*J* = 166.5). ^{31}P NMR (CD_3OD): 29.22. Anal. Calcd for $C_{17}H_{34}ClN_2O_5P$·

$0.21H_2O$: C, 49.12; H, 8.35; N, 6.74; Cl, 8.29; P, 7.04. Found: C, 49.39; H, 8.58; N, 6.65; Cl, 8.69; P, 7.47.

Preparation of (1*S*,2*S*)-*N*-[1-(Cyclohexylmethyl)-2-(dimethoxyphosphinyl)-2-hydroxyethyl]-*N*²-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-L-leucinamide (13). Amine **10** (810 mg, 2.7 mmol) was added to a solution of acid **6** (1.02 g, 2.7 mmol) in THF (12 mL) and cooled to 0 °C. HOBt (412 mg, 2.7 mmol), iPr_2NEt (515 mL, 2.9 mmol), and DCC (555 mg, 2.7 mmol) were added sequentially. After 18 h at 0 °C, the reaction mixture was filtered and concentrated. The residue was dissolved in CH_2Cl_2 (50 mL), washed with 10% citric acid (2 × 30 mL), saturated aqueous $NaHCO_3$ (2 × 30 mL), and saturated aqueous NaCl (30 mL), dried over Na_2SO_4, and concentrated, yielding 1.34 g of crude product. Purification by flash chromatography (107 g of Merck silica gel, 1:1 hexane/ethyl acetate to 30:1 $CHCl_3$/MeOH) afforded pure **5** (1.01 g, 60.1%). A small amount of material was crystallized from hexane/ethyl acetate. TLC: $R_f = 0.26$ (19:1.0:0.05 $CHCl_3$/MeOH/NH_4OH). MS: $(M + H)^+$ 626^+. Mp: 129–130 °C. $[\alpha]_D = -45.3°$ ($c = 0.44$, CH_3OH). IR (KBr): 1049, 1172, 1244, 1523, 1650, 2925, 3302. ^1H NMR ($CDCl_3$): 0.93 (m, 6H), 1.41 (s, 9 H), 0.78–1.98 (m, 16 H), 3.0–3.2 (m, 2 H), 3.79 (1, 6 H, $J = 9$), 3.9–4.5 (m, 4 H), 5.05 (m, 1 H), 7.2–7.4 (m, 5 H). ^{13}C NMR (CD_3OD): 22.0, 23.6, 25.7, 27.2, 27.4, 27.6, 28.6, 33.7, 34.8, 35.3, 38.9, 40.3, 41.9, 49.4, 53.2, 54.2, 54.1, 53.9, 57.2, 69.7 ($J = 166.5$), 80.7, 127.6, 129.4, 130.3, 138.7, 157.6, 173.8, 174.3. ^{31}P NMR (CD_3OD): 24.7. Anal. Calcd for $C_{31}H_{52}N_3O_8P \cdot 0.29H_2O$: C, 59.01; H, 8.40; N, 6.66; P, 4.91. Found: C, 59.01; H, 8.35; N, 6.79; P, 4.69.

Preparation of (1*S*,2*S*)-*N*-[1-(Cyclohexylmethyl)-2-(dimethoxyphosphinyl)-2-hydroxyethyl]-*N*²-[*N*-(cyclopentylcarbonyl)-L-phenylalanyl]-L-leucinamide (15). Amine **12** (1.7 g, 4.1 mmol) was added to a solution of C_5H_9-CO-Phe-OH (**14**;[17] 1.07 g, 4.1 mmol) in DMF (16 mL) and cooled to 0 °C. HOBt (627.3 mg, 4.1 mmol), Et_3N (628.5 mL, 4.5 mmol), and EDC (785.9 mg, 4.1 mmol) were added sequentially. After 18 h at room temperature, pH 4.01 buffer solution (160 mL, Mallinckrodt "buffer pH 4.01") was added and the reaction mixture was stirred for 10 min. The resulting precipitate was filtered, washed sequentially with pH 4.01 buffer solution (3 × 60 mL), water (4 × 60 mL), and saturated aqueous $NaHCO_3$ (4 × 60 mL), and dried *in vacuo*, yielding 3.0 g of crude product. Purification by flash chromatography (200 g of silica gel, 30:1:0.1 $CHCl_3$/MeOH/AcOH to 2:8:0.1 hexane/EtOAc/AcOH) afforded pure **15** (1.27 g, 52%). TLC: $R_f = 0.38$ (19:1.0:0.1 $CHCl_3$/MeOH/AcOH). MS: $(M + H)^+$ 622^+. Mp: 145–147 °C. $[\alpha]_D = -51.7°$ ($c = 0.97$, CH_3OH). IR (KBr): 1034, 1226, 1448, 1540, 1640, 2924, 3297. ^1H NMR (400 MHz, DMSO-d_6): 0.87 (d, 3 H, $J = 7$), 0.90 (d, 3 H, $J = 7$), 0.7–1.85 (m, 24 H), 2.55 (m, 1 H), 2.74 (dd, 1 H, $J = 10, 12$), 3.04 (dd, 1 H, $J = 4.5, 12$), 3.65 (d, 3 H, $J = 10$), 3.75 (d, 3 H, $J = 10$), 3.82 (m, 1 H), 4.15 (m, 1 H), 4.30 (m, 1 H), 4.54 (m, 1 H), 5.82 (m, 1 H), 7.13–7.28 (m, 5 H), 7.40 (d, 1 H, $J = 9$), 7.92 (d, 1 H, $J = 9$), 8.02 (d, 1 H, $J = 8$). ^{13}C NMR (CD_3OD): 22.0, 23.6, 25.7, 26.8, 27.2, 27.3, 27.5, 30.9, 31.6, 33.7, 34.8, 35.2, 38.2, 40.1, 41.8, 46.0, 53.2, 53.8, 53.9, 54.1, 54.2, 55.2, 69.7 ($J = 164.6$), 127.5, 129.2, 130.2, 138.5, 173.7, 178.8. ^{31}P NMR (CD_3OD): 25.0. Anal. Calcd for $C_{32}H_{52}N_3O_7P \cdot 0.6H_2O$: C, 60.76; H, 8.48; N, 6.65; P, 4.90. Found: C, 60.93; H, 8.28; N, 6.62; P, 4.51.

Preparation of (1*S*)-*N*-[1-(Cyclohexylmethyl)-2-hydroxy-2-(hydroxymethoxyphosphinyl)ethyl]-*N*²-[*N*-(cyclopentylcarbonyl)-L-phenylalanyl]-L-leucinamide (16). A solution of **15** (125 mg, 0.2 mmol) in 25 mL of acetone was saturated with trimethylamine and heated for 16 h at 80 °C in a sealed tube. The solvents were removed on a rotary evaporator, and the residue was dissolved in EtOAc (30 mL), washed with 10% HCl (2 × 15 mL), dried (Na_2SO_4), and concentrated to afford 102 mg of crude product. Chromatographic purification (20 g of silica gel, 9:1 chloroform/methanol to 90:10:1:0.1–90:20:2.5:1.0 chloroform/methanol/water/acetic acid) yielded pure **16** (65 mg, 53.5%). TLC: $R_f = 0.41$ (48:20:6:11 EtOAc/pyridine/AcOH/H_2O). MS: $(M - H)^-$ 606^-. Mp: 218–227 °C. IR (KBr): 1055, 1447, 1555, 1643, 2924, 2954, 3301, 3341, 3403, 3412. ^1H NMR (400 MHz, DMSO-d_6 + CD_3CO_2D): 0.85 (d, 3 H, $J = 7$), 0.88 (d, 3 H, $J = 7$), 0.67–1.90 (m, 34 H), 2.57 (m, 1 H), 2.82 (m, 1 H), 3.18 (m, 1 H), 3.42 (d, 3 H, $J = 10$), 4.07 (m, 1 H), 4.26 (m, 1 H), 4.58 (m, 1 H), 7.13–7.29 (m, 5 H), 8.13 (m, 1 H), 8.28 (m, 1 H). ^{13}C NMR (CD_3CO_2D): 22.9, 23.6, 25.8, 26.9, 27.3, 27.5, 30.8, 31.9, 33.2, 35.0, 35.5, 38.3, 40.0, 41.2, 46.1, 53.1, 56.2, 54.0, 71.08 ($J = 161$), 127.7, 129.4, 130.3, 138.6, 174.5, 175.0, 179.7. ^{31}P NMR (CD_3CO_2D): 18.88. Anal. Calcd for $C_{31}H_{50}N_3O_7P \cdot 0.5AcOH \cdot 0.7H_2O$: C, 59.14; H, 8.05; N, 6.47. Found: C, 59.10; H, 8.28; N, 6.46.

Preparation of 18 and 19. *N*-Methylmorpholine (NMM; 0.272 mL, 2.4 mmol) was added to a solution of **9** (603 mg, 2 mmol) in THF (8 mL) at 0 °C. CDI (0.389 mg, 2.4 mmol) was added after 5 min, and the reaction mixture was stirred overnight with gradual warming to room temperature. The next day, the reaction mixture was concentrated and the residue was partitioned between EtOAc (40 mL) and saturated aqueous NH_4Cl (25 mL). The aqueous layer was reextracted with EtOAc (2 × 25 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to afford 510 mg of crude product, which after chromatographic purification (2:8:0.1 hexane/EtOAc/AcOH) yielded **18** (432 mg, 74.2%). MS: $(M + H)^+$ 292^+. Mp: 44–48 °C. ^1H NMR ($CDCl_3$): 0.85–1.8 (m, 13 H), 3.88 (d, 6 H, $J = 10.7$), 4.15 (m, 1 H), 4.33 (d, 1 H, $J = 6.2$), 6.55 (s, 1 H). ^{13}C NMR ($CDCl_3$): 25.7, 25.8, 26.1, 32.4, 33.3, 33.8, 38.3 ($J = 4.7$), 51.2, 53.6 ($J = 7.6$), 54.1 ($J = 7.5$), 75.5 ($J = 172$), 158.5. ^{31}P NMR ($CDCl_3$): 19.1. Anal. Calcd for $C_{12}H_{22}NO_5P \cdot 0.035H_2O$: C, 48.43; H, 7.69; N, 4.71; P, 10.41. Found: C, 48.43; H, 7.68; N, 4.67; P, 10.33.

DBU (1.49 mL, 10 mmol) was added to a solution of aldehyde **4** (1.28 g, 5 mmol) and dimethyl phosphite (0.458 mL, 5 mmol) in DMF (25 mL), and the mixture was stirred overnight at room temperature. The next day, additional DBU (0.35 mL) was added. After 3 h, the reaction mixture was taken up in EtOAc (200 mL) and washed sequentially with 10% citric acid (100 mL) and saturated aqueous NaCl (100 mL). Drying and concentration afforded 1.36 g of crude product, which upon chromatographic purification yielded an inseparable mixture of **9** and **17**. A portion of this mixture (279 mg, 0.763 mmol) was deprotected with anhydrous HCl in EtOAc. The crude product was dissolved in THF (3 mL) and treated with NMM (0.103 mL, 0.92 mmol) and CDI (149 mg, 0.92 mmol) overnight at room temperature. The next day, the reaction mixture was concentrated and the residue was partitioned between EtOAc (25 mL) and saturated aqueous NH_4Cl (20 mL). The aqueous layer was reextracted with EtOAc (2 × 25 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to afford 203 mg of crude product, which after chromatographic purification (2:8:0.1 hexane/EtOAc/AcOH) yielded a 1.4:1.0 mixture of **18** and **19** (122 mg, 55%).

Characterization of 19. MS: $(M + H)^+$ 292^+. ^1H NMR ($CDCl_3$): 0.85–1.8 (m, 13 H), 3.9 (d, 6 H, $J = 10$), 4.05–4.13 (m, 1 H), 4.75 (d, 1 H, $J = 8$), 6.35 (s, 1 H). ^{13}C NMR ($CDCl_3$): 25.9, 26.0, 26.2, 31.6, 34.2, 34.4, 44.0 ($J = 7.5$), 51.8, 53.2 ($J = 7.5$), 54.0 ($J = 7.5$), 74.0 ($J = 172$), 158.2. ^{31}P NMR ($CDCl_3$): 17.1.

Preparation of (1*S*)-[1-(Cyclohexylmethyl)-2-(diethylphosphinyl)-2-hydroxyethyl]carbamic Acid, 1,1-Dimethylethyl Ester (21). A solution of diethyl phosphite (Aldrich; 2.29 g, 16.6 mmol) in THF (8.3 mL) was added dropwise over a period of 10 min to a flask containing a 3 M ethereal solution of ethylmagnesium bromide (16.6 mL, 3 M solution, 49.8 mmol) and 8.3 mL of THF at 0 °C. The reaction mixture was warmed to room temperature and refluxed for 1 h to ensure complete formation of the reagent, after which it was cooled to 0 °C and treated with a 10 mL THF solution of the aldehyde **4** (2.2078 g, 8.3 mmol). The reaction mixture was refluxed for 30 min and cooled to 0 °C and the reaction quenched with aqueous 10% HCl (75 mL). The aqueous solution was extracted with ethyl acetate (3 × 50 mL), and the combined organic extracts were dried (Na_2SO_4) and concentrated to yield 3.191 g of residue. Flash chromatographic purification (150 g of silica gel, 2:8:0.1 hexane/ethyl acetate/acetic acid) afforded pure **21** (2.452 g, 81.7%) as a 1:1.23 mixture of diastereomers as determined by ^{31}P NMR: 55.0, 55.7 (1.23:1.00). TLC: $R_f = 0.15$ (2:8:0.1 hexane/EtOAc/AcOH). MS: $(M + H)^+$ 362^+. Mp: 163–165 °C. ^1H NMR (270 MHz, $CDCl_3$): (two diaster-

eomers) 1.44 (s, 9 H), 0.8–2.0 (m, 23 H), 3.8–4.1 (m, 2 H), 5.41 and 5.56 (d, 1 H, J = 6, 8). ^{13}C NMR: 5.35, 18.1, 18.3, 26.0, 26.2, 26.4, 28.2, 32.1, 32.8, 33.4, 34.1, 38.2 and 40.0, 48.1 and 50.5, 71.2 (J = 68.6), 72.3 (J = 63.3), 79.2 and 79.5, 156.0 and 156.7. Anal. Calcd for $C_{18}H_{36}NO_4P$: C, 59.81; H, 10.04; N, 3.88; P, 8.57. Found: C, 59.54; H, 10.09; N, 3.63; P, 8.46.

Preparation of (1*S*)-*N*-[1-(Cyclohexylmethyl)-2-(diethylphosphinyl)-2-hydroxyethyl]-*N*^{2}-[*N*-(cyclopentylcarbonyl)-L-phenylalanyl]-L-leucinamide, Isomers A (23) and B (24). Phosphine oxide **21** (860 mg, 2.38 mmol) was treated with a solution of anhydrous HCl/EtOAc at 0 °C for 2 h and for 1.5 h at room temperature, after which a TLC check ensured completion of the reaction. Concentration afforded an oily residue which was redissolved in 1:1 hexane/ethyl acetate and concentrated to afford a white solid (741 mg) which was utilized directly for the next reaction. HOBt (364.1 mg, 2.38 mmol), triethylamine (364.9 mL, 2.618 mmol), and EDC (456 mg, 2.38 mmol) were sequentially added to a 10 mL DMF solution of the amine prepared above and acid **22**[7a] at 0 °C. After stirring for 2 h at 0 °C, the reaction mixture was left for overnight stirring at room temperature. The resulting solid mass was treated with 25 mL of pH 4.00 buffer for 5 min and filtered. The precipitates were washed sequentially with buffer solution (2 × 10 mL) and H_2O (10 × 10 mL) and dried *in vacuo* (1.038 g). Flash chromatographic purification (200 g of silica gel, 30:1 $CHCl_3$/MeOH) afforded 395 mg (26.9%) of pure fast-moving isomer **23**, 217 mg (14.8%) of pure slow-moving isomer **24**, and 197 mg (13.4%) of **24** contaminated with small amounts of **23** (55.1% overall yield).

Characterization of **23**. TLC: R_f = 0.33 (90:10:1.0:0.1 $CHCl_3$/MeOH/H_2O/AcOH). MS: $(M + H)^+$ 618$^+$. Mp: 209–209 °C. $[\alpha]_D$ = −29.8° (c = 0.51, AcOH, 589 nm). IR (KBr): 1070, 1118, 1446, 1531, 1654, 2853, 2924, 2951, 3092, 3113, 3307, 3418. ^{1}H NMR (400 MHz, DMSO-d_6 + CD_3CO_2D): 0.85 (d, 3 H, J = 7), 0.89 (d, 3 H, J = 7), 0.72–1.87 (m, 34 H), 2.55 (m, 1 H), 2.78 (dd, 1 H, J = 9, 12), 3.04 (dd, 1 H, J = 5, 12), 3.84 (m, 1 H), 4.26 (m, 2 H), 4.54 (dd, 1 H, J = 4, 9), 7.13–7.28 (m, 5 H), 7.47 (d, 1 H, J = 7), 7.93 (d, 1 H, J = 8), 8.07 (d, 1 H, J = 8). ^{13}C NMR (CD_3CO_2D): 5.63 (d, J = 9), 5.68 (d, J = 7.5), 18.23 (d, J = 62.4), 18.39 (d, J = 62.4), 21.5, 23.5, 25.4, 26.6, 26.7, 26.9, 27.1, 27.3, 30.8, 31.2, 33.3, 34.6, 34.8, 38.5, 40.3, 41.3, 46.0, 49.0, 53.3, 55.2, 71.0 (J = 75.7), 127.1, 129.3, 130.2, 137.6, 174.4, 174.6, 179.1. ^{31}P NMR (CD_3CO_2D): 65.9. Anal. Calcd for $C_{34}H_{56}N_3O_5P{\cdot}0.2H_2O$: C, 65.72; H, 9.15; N, 6.76; P, 4.98. Found: C, 65.74; H, 8.81; N, 6.81; P, 4.97.

Characterization of **24**. TLC: R_f = 0.30 (90:10:1.0:0.1 $CHCl_3$/MeOH/H_2O/AcOH). MS: $(M - H)^-$ 616$^-$. Mp: 170–175 °C. $[\alpha]_D$ = −32° (c = 0.53, AcOH, 589 nm). IR (KBr): 1141, 1448, 1633, 2924, 3299, 3408, 3455. ^{1}H NMR (400 MHz, DMSO-d_6 + CD_3CO_2D): 0.88 (d, 3 H, J = 7), 0.92 (d, 3 H, J = 7), 0.75–1.9 (m, 34 H), 2.53 (m, 1 H), 2.74 (dd, 1 H, J = 11, 15), 3.01 (dd, 1 H, J = 4, 15), 3.7 (m, 1 H), 4.12 (m, 1 H), 4.27 (m, 1 H), 4.52 (m, 1 H), 5.52 (app t, J = 8), 7.12–7.28 (m, 5 H), 7.72 (d, 1 H, J = 10), 7.90 (d, 1 H, J = 9), 8.05 (d, 1 H, J = 8). ^{13}C NMR (CD_3CO_2D): 5.60, 17.7, 18.3, 18.6, 21.7, 23.3, 25.4, 26.6, 26.8, 27.1, 27.3, 30.7, 31.2, 32.8, 34.8, 35.1, 38.4, 38.6, 41.3, 46.0, 49.7, 53.3, 55.2, 71.43 (d, J = 61.7), 127.7, 129.3, 130.2, 137.6, 174.3, 179.1. ^{31}P NMR (CD_3CO_2D): 66.6. Anal. Calcd for $C_{34}H_{56}N_3O_5P{\cdot}0.2H_2O$: C, 65.72; H, 9.15; N, 6.76; P, 4.98. Found: C, 65.68; H, 8.89; N, 6.68; P, 4.98.

Preparation of (1*S*)-[1-(Cyclohexylmethyl)-2-(ethylmethoxyphosphinyl)-2-[(trimethylsilyl)oxy]ethyl]carbamic Acid, 1,1-Dimethylethyl Ester, Isomer Pairs A (26) and B (27). A 60 mL ethereal solution of methanol (10.82 mL, 267 mmol) and triethylamine (16.92 mL, 121.4 mmol) was added dropwise to a solution of ethyl dichlorophosphine (15.9 g, 121.4 mmol) in 120 mL of ether at 0 °C. (Caution! Reaction is very exothermic during the first half of addition.) After the addition was complete, the resulting slurry was refluxed for 1 h, cooled to 0 °C, and filtered. The precipitated solid was washed with an additional 100 mL of ether. Most of the ethyl ether was removed on a rotary evaporator and the residue vacuum-distilled (18 mmHg) to afford methyl ethyl phosphinate **25** at 105–114 °C (11.2 g, 85.4%). **25** (1.05 mL, 10.4 mmol) was added to a solution of the aldehyde **4** (2.66 g, 10.4 mmol) in THF (40 mL). Diisopropylethylamine (3.62 mL, 20.8 mmol) and trimethysilyl chloride (2.64 mL, 20.4 mmol) were added sequentially, and the reaction mixture was left for overnight stirring at room temperature. After 17 h, the reaction was quenched with H_2O and THF was removed on the rotary evaporator. The residue was dissolved in ethyl acetate (125 mL), washed sequentially with water (2 × 30 mL) and saturated NaCl (1 × 30 mL), dried (Na_2SO_4), and concentrated to give 4.174 g of crude product. Chromatographic purification (200 g of silica gel, 2:1 hexane/ethyl acetate) yielded 678 mg (14.9%) of pure fast-moving diastereomer pair **26**, 1.348 g (30.84%) of pure slow-moving diastereomer pair **27**, and 719 mg (15.8%) of a mixture of **26** and **27** (61.5% overall yield).

Characterization of **26**. TLC: R_f = 0.17 (1:1 hexane/EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): 0.18 (m, H), 1.46 (s, 9 H), 0.78–1.95 (m, 18 H), 3.71 (d, 3 H, J = 10), 3.72 (d, 3 H, J = 10), 3.93–4.10 (m, 2 H), 5.10 and 5.24 (d, 1 H, J = 9, 11). ^{31}P NMR ($CDCl_3$): 57.64, 58.50.

Characterization of **27**. TLC: R_f = 0.13 (1:1 hexane/EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): 0.21 (m, 9 H), 1.45 (s, 9 H), 0.80–1.90 (m, 18 H), 3.72 (d, 3 H, J = 10.2), 3.76 (d, 3 H, J = 10.2), 3.94 (m, 1 H), 4.09 (m, 1 H), 4.75 and 5.00 (d, 1 H, J = 9). ^{31}P NMR ($CDCl_3$): 53.81, 54.27.

Preparation of (1*S*)-*N*-[1-(Cyclohexylmethyl)-2-(ethylmethoxyphosphinyl)-2-hydroxyethyl]-*N*^{2}-[*N*-(cyclopentylcarbonyl)-L-phenylalanyl]-L-leucinamide, Isomer Pair A (28). The fast-moving isomer pair **26** (658 mg, 1.5 mmol) was treated with a solution of anhydrous HCl/EtOAc at room temperature for 2 h, after which a TLC check ensured completion of the reaction. Concentration *in vacuo* afforded a white solid (410 mg, 91.4%) which was direclty utilized for the next reaction. HOBt (210 mg, 1.37 mmol), triethylamine (229 μL, 1.644 mmol), and EDC (263 mg, 1.37 mmol) were sequentially added to a 6 mL DMF solution of the amine hydrochloride (40 mg, 1.37 mmol) and acid **22** (512 mg, 1.37 mmol) at 0 °C. After 2 h at 0 °C and 12 h at room temperature, the reaction mixture was treated with 20 mL of pH 4.01 buffer for 5 min and filtered. The solid was washed sequentially with the buffer solution (4 × 20 mL) and water (5 × 10 mL) and then dried *in vacuo* to afford 658 mg of crude product. Flash chromatographic purification (100 g of silica gel, 30:1:0.05 $CHCl_3$/MeOH/NH_4OH) afforded pure **28** (520 mg, 61.3%) as a mixture of two diastereomers. TLC: R_f = 0.42 (90:10:1.0:0.1 $CHCl_3$/MeOH/H_2O/AcOH). MS: $(M + H)^+$ 620$^+$. Mp: 155–165 °C. IR (KBr): 1047, 1191, 1449, 1542, 1640, 2925, 3289. ^{1}H NMR (270 MHz, $CDCl_3$): 0.90 (d, 3 H, J = 6.0), 0.94 (d, 3 H, J = 6.0), 0.7–2.0 (m, 29 H), 2.58 (m, 1 H), 2.89 (dd, 1 H, J = 9, 12), 3.18 (dd, 1 H, J = 4.5, 12), 3.73 and 3.77 (d, 3 H, J = 10), 3.84–3.96 (m, 1 H), 4.20–.4.44 (m, 2 H), 4.67 (m, 1 H), 7.22 (s, 5 H). ^{31}P NMR ($CDCl_3$): 62.3, 62.4. Anal. Calcd for $C_{33}H_{54}N_3O_6P{\cdot}0.5H_2O$: C, 63.04; H, 8.82; N, 6.68. Found: C, 63.09; H, 8.64; N, 6.56.

Preparation of (1*S*)-*N*-[1-(Cyclohexylmethyl)-2-(ethylmethoxyphosphinyl)-2-hydroxyethyl]-*N*^{2}-[*N*-(cyclopentylcarbonyl)-L-phenylalanyl]-L-leucinamide, Isomers, B$_1$ (29) and B$_2$ (30). The slow-moving isomer pair **27** (500 mg, 1.144 mmol) was treated with a solution of anhydrous HCl/EtOAc at room temperature for 2 h, after which a TLC check ensured completion of the reaction. Concentration followed by trituration with ether afforded a white solid (296 mg, 86.4%) which was directly utilized for the next reaction. HOBt (153 mg, 1.0 mmol), triethylamine (167.3 μL, 1 .2 mmol), and EDC (191.7 mg, 1.0 mmol) were sequentially added to a 6 mL DMF solution of the amine hydrochloride (296 mg, 0.988 mmol) and acid **22** (374.4 mg, 1.0 mmol) at 0 °C. After 2 h at 0 °C and 60 h at room temperature, the reaction mixture was treated with 25 mL of pH 4.01 buffer for 5 min and filtered. The solid was washed sequentially with the buffer solution (2 × 25 mL) and water (5 × 20 mL) and then dried *in vacuo* to afford 495 mg of crude product. Flash chromatographic purification (100 g of silica gel, 40:1:0.05 $CHCl_3$/MeOH/NH_4OH) afforded 90 mg (14.7%) of the fast-moving isomer **29**, 186 mg (30.4%) of a diastereomeric mixture, and 136 mg (22.2%) of the pure slow-moving isomer **30** (67.3% overall yield).

Characterization of **29**. MS: $(M + H)^+$ 620^+. Mp: 191–196 °C. IR (KBr): 1536, 1541, 1640, 2923, 2951, 3301. ^{1}H NMR (400 MHz, DMSO-d_6): 0.83 (d, 3 H, J = 6.2), 0.88 (d, 3 H, J = 8.2), 0.7–1.84 (m, 29 H), 2.55 (m, 1 H), 2.72 (dd, 3.1 H, J = 10.6, 13.9), 3.03 (dd, 1 H, J = 3.66, 13.9), 3.57 (d, 3 H, J = 9.9), 3.70 (m, 1 H), 4.18 (m, 1 H), 4.28 (m, 1 H), 4.53 (m, 1 H), 5.68 (app t, 1 H, J = 7.7, 8.8), 7.13–7.27 (m, 5 H), 7.40 (d, 1 H, J = 9.16), 7.92 (d, 1 H, J = 8.42), 8.05 (d, 1 H, J = 8.43). ^{31}P NMR ($CDCl_3$): 56.3. Anal. Calcd for $C_{33}H_{54}N_3O_6P \cdot 0.5H_2O$: C, 63.04; H, 8.82; N, 6.68. Found: C, 62.92; H, 8.41; N, 6.61.

Characterization of **30**. TLC: R_f = 0.49 (90:10:1.0:0.1 $CHCl_3$/MeOH/H_2O/AcOH). MS: $(M + H)^+$ 620^+. Mp: 170–174 °C. $[\alpha]_D$ = −55.5° (c = 0.38, MeOH). IR (KBr): 1032, 1045, 1185, 1542, 1640 ,2852, 2867, 2924, 2952, 3287, 3392, 3416. ^{1}H NMR (270 MHz, $CDCl_3$): 0.6–2.0 (m, 35 H), 2.57 (m, 1 H), 2.92–3.22 (m, 2 H), 3.77 (d, 3 H, J = 9), 4.02 (m, 1 H), 4.22–4.53 (m, 2 H), 4.85 (m, 1 H), 5.18 (m, 1 H), 6.88 (d, 1 H, J = 8), 7.03–7.32 (m, 5 H), 7.45 (d, 1 H, J = 8), 7.53 (d, 1 H, J = 8). ^{31}P NMR ($CDCl_3$): 55.7. Anal. Calcd for $C_{33}H_{54}N_3O_6P \cdot 0.22H_2O$: C, 63.55; H, 8.80; N, 6.74; P, 4.97. Found: C, 63.54; H, 8.65; N, 6.59; P, 5.06.

Preparation of (1*S*,2*S*)-*N*-[1-(Cyclohexylmethyl)-2-(dimethoxyphosphinyl)-2-hydroxyethyl]-*N*^{2}-[*N*-(4-morpholinylcarbonyl)-L-phenylalanyl]-L-leucinamide (33). Acid **31** (993.5 mg, 3.42 mmol) was added to a solution of amine **12** (1.42 g, 3.42 mmol) in DMF (17 mL) and cooled to 0 °C. HOBt (523.3 mg, 3.42 mmol), Et_3N (0.572 mL, 4.10 mmol), and EDC (655.6 mg, 3.42 mmol) were added sequentially. After 20 h at room temperature, a pH 4.01 buffer solution (75 mL, Mallinckrodt "buffer pH 4.01") was added and the reaction mixture was allowed to stir for 10 min. The aqueous layer was extracted with Et_2O (3 × 75 mL), and the combined organic portions were washed with saturated aqueous $NaHCO_3$ (75 mL), dried (Na_2SO_4), and concentrated, yielding 1.8 g of crude product. Repeated purifications by flash chromatography (126 g of silica gel, 30:1:0.05 $CHCl_3$/MeOH/NH_4OH; 50 g of silica gel, 2:8:0.1 hexane/EtOAc/AcOH; 150 g of silica gel 19.1 $CHCl_3$/MeOH) finally afforded pure **33** (674 mg, 32.1%). TLC: R_f = 0.38 (9:1.0:0.1 $CHCl_3$/MeOH/AcOH). MS: $(M + H)^+$ 639^+. Mp: 99–105 °C. $[\alpha]_D$ = −60.0° (c = 1.07, MeOH). IR (KBr): 1034, 1261, 1447, 1536, 1631, 2923, 3305. ^{1}H NMR (270 MHz, $CDCl_3$): 0.90 (d, 3 H, J = 7), 0.92 (d, 3 H, J = 7), 1.08–1.90 (m, 16 H), 3.00–3.38 (m, 6 H), 3.80 (m, 4 H), 3.77 (d, 3 H, J = 8), 3.82 (d, 3 H, J = 8), 39.6 (m, 1 H), 4.16 (m, H), 4.33–4.56 (m, 2 H), 4.99 (d, 1 H, J = 6), 6.82 (d, 1 H, J = 8), 7.15–7.39 (m, 5 H). ^{13}C NMR ($CDCl_3$): 22.0, 22.9, 24.6, 26.0, 26.3, 32.6, 33.3, 34.1, 37.8, 38.1, 41.3, 43.9, 49.1, 52.1, 52.8, 53.2, 53.3, 55.3, 66.2, 69.08 (J = 160.8), 126.5, 128.2, 129.2, 136.8, 137.0, 157.2, 172.1, 172.2. ^{31}P NMR ($CDCl_3$): 24.77. Anal. Calcd for $C_{31}H_{51}N_4O_8P$: C, 58.29; H, 8.05; N, 8.77; P, 4.85. Found: C, 58.63; H, 8.18; N, 8.82; P, 4.63.

Preparation of (1*S*,2*S*)-*N*^{2}-[*N*-(6-Amino-1-oxohexyl)-L-phenylalanyl]-*N*-[1-(cyclohexylmethyl)-2-(dimethoxyphosphinyl)-2-hydroxyethyl]-L-leucinamide, Hydrochloride (34). Amine **12** (1.24 g, 3 mmol) was added to a solution of acid **32**[17] (1.27 g, 3 mmol) in DMF (15 mL) and cooled to 0 °C. HOBt (459 mg, 3 mmol), Et_3N (501.9 mL, 3.6 mmol), and EDC (575.1 mg, 3 mmol) were added sequentially. After 16 h at 25 °C, pH 4.01 buffer solution (75 mL, Mallinckrodt "buffer pH 4.01") was added and the reaction mixture was stirred for 10 min. The resulting precipitate was filtered, washed sequentially with pH 4.01 buffer (3 × 50 mL), H_2O (3 × 50 mL), and saturated aqueous $NaHCO_3$ (50 mL), and dried (Na_2SO_4), yielding 1.6 g of crude material. Purification by flash chromatography (220 g of silica gel, 19:1:0.05 $CHCl_3$/CH_3OH/NH_4OH; 192 g of silcia gel, 19.1 $CHCl_3$/CH_3OH) afforded the pure Cbz-protected precursor to **34** (1.336g, 58%). TLC: R_f = 0.31 (9:1.0:0.05 $CHCl_3$/MeOH/NH_4OH). ^{1}H NMR (270 MHz, $CDCl_3$): 0.88 (m, 6 H), 1.04–1.84 (m, 22 H), 2.14 (t, 2 H, J = 7), 2.97–3.22 (m, 4 H), 3.77 (d, 3 H, J = 10), 3.83 (d, 3 H, J = 10), 4.0 (m, 1 H), 4.16 (m, 1 H), 4.38 (m, 1 H), 4.73 (m, 1 H), 5.10 (s, 2 H), 6.35 (d, 1 H, J = 6), 6.92 (d, 1 H, J = 8), 7.15–7.40 (m, 10 H). ^{13}C NMR ($CDCl_3$): 22.2, 22.9, 24.6, 25.0, 25.8, 26.0, 26.1, 26.3, 29.4, 32.3, 32.8, 33.8, 35.8, 37.7, 38.0, 40.7, 41.3, 49.2, 52.2, 52.9 (d, J = 5.67), 53.3 (J = 7.6), 53.8, 66.4, 69.0 (J = 168), 126.7, 127.9, 128.6, 129.2, 136.6, 156.4, 171.5, 172.1, 173.2.

A portion of this intermediate (537 g, 0.69 mmol) 10% Pd/C (209 mg; Aldrich) and 1.0 N hydrochloric acid (759 mL, 0.75 mmol) in methanol (7 mL) were stirred under a hydrogen atmosphere for 16 h, after which the reaction mixture was filtered and concentrated. Purification by flash chromatography (81 g of silica gel, 90:10:1:0.1 $CHCl_3$/CH_3OH/H_2O/AcOH) afforded a residue which was dissolved in water containing 980 mL of 1.0 N hydrochloric acid, millipore-filtered, and lyophilized to give pure **34** as a fluffy white solid (390.5 mg, 84%). TLC: R_f = 0.15 (90:20:2.5:1.0 $CHCl_3$/MeOH/H_2O/AcOH). MS: $(M + H)^+$ 639^+. Mp: 155–168 °C. $[\alpha]_D$ = −41.6° (c = 0.44, MeOH). IR (KBr): 1035, 1221, 1539, 1643, 2924, 3291, 3419. ^{1}H NMR (270 MHz, CD_3CO_2D): 0.90 (d, 3 H, J = 7), 0.92 (d, 3 H, J = 7), 1.05–1.93 (m, 22 H), 2.14 (t, 2 H, J = 7), 2.75–2.90 (m, 3 H), 3.17 (dd, 1 H, J = 4, 13), 3.72 (d, 3 H, J = 10), 3.76 (d, 3 H, J = 10), 4.2–4.4 (m, 2 H), 4.66 (m, 1 H), 7.22 (m, 5 H). ^{13}C NMR (67.8 MHz, $CDCl_3$): 21.7, 21.9, 23.6, 25.7, 26.0, 26.6, 27.2, 27.3, 27.5, 28.1, 33.7, 34.8, 35.2, 36.2, 38.7, 40.2, 41.8, 49.9, 53.3, 53.8, 53.9, 54.1, 54.2, 55.8, 69.74 (J = 164), 127.6, 129.3, 130.2, 138.6, 173.8, 175.6, 176.6. ^{31}P NMR (CD_3OD): 29.38. Anal. Calcd for $C_{32}H_{55}N_4O_7P \cdot 1.2HCl \cdot 1.0H_2O$: C, 54.86; H, 8.37; N, 8.00; P, 4.42; Cl, 6.07. Found: C, 54.98; H, 8.75; N, 8.23; P, 4.25; Cl, 6.15.

Preparation of (1*S*,2*S*)-*N*-[1-(Cyclohexylmethyl)-2-(dimethoxyphosphinyl)-2-hydroxyethyl]-*N*^{2}-[*N*-(cyclopentylcarbonyl)-L-phenylalanyl]-3-[(phenylmethoxy)methyl]-L-histidinamide (36). Acid **35** (1.73 g, 3.32 mmol) was added to a solution of amine **10** (1.20 g, 3.98 mmol) in DMF (15 mL) and cooled to 0 °C. HOBt (558.7 mg, 3.65 mmol), Et_3N (696.5 μL, 4.98 mmol), and EDC (699.8 mg, 3.65 mmol) were added sequentially. After 16 h at 25 °C, pH 4.01 buffer solution (80 mL, Mallinckrodt "buffer pH 4.01") was added and the reaction mixture was stirred for an additional 10 min. The aqueous portion was extracted with EtOAc (2 × 75 mL), and the combined organic portions were washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated. Purification of the crude product (2.4 g) by flash chromatography (150 g of Merck silica gel, 19:1:0.1 $CHCl_3$/CH_3OH/AcOH) yielded pure **36** (1.32 g, 58.4%). TLC: R_f = 0.35 (9:1.0:0.1 $CHCl_3$/MeOH/AcOH). MS: $(M + H)^+$ 766^+. $[\alpha]_D$ = −31.1° (c = 0.54, MeOH). ^{1}H NMR (270 MHz, $CDCl_3$): 0.8–1.85 (m, 21 H), 2.45 (m, 1 H), 2.9–3.25 (m, 4 H), 3.75 (d, 3 H, J = 10), 3.80 (d, 3 H, J = 10), 3.9 (m, 1 H), 4.15 (m, 1 H), 4.44 (d, 2 H, J = 6), 4.5 (m, 2 H), 4.8 (m, 1 H), 5.25 (d, 1 H, J = 10), 5.40 (d, 1 H, J = 10), 6.05 (d, 1 H, J = 6), 6.7–6.9 (m, 2 H), 7.1–7.5 (m, 14 H). Anal. Calcd for $C_{40}H_{56}N_5O_8P \cdot 0.56H_2O$: C, 61.91; H, 7.42; N, 9.03; P, 3.99. Found: C, 62.21; H, 7.28; N, 8.80; P, 3.74.

Preparation of (1*S*,2*S*)-*N*-[1-(Cyclohexylmethyl)-2-(dimethoxyphosphinyl)-2-hydroxyethyl]-*N*^{2}-[*N*-(cyclopentylcarbonyl)-L-phenylalanyl]-L-histidinamide (37). A mixture of **36** (1.24 g, 1.6 mmol), 20% palladium hydroxide on carbon (480 mg; Aldrich), and 1.0 N HCl (1.6 mL, 1.6 mmol) in methanol (11 mL) was stirred under hydrogen (balloon) for 20 h, after which it was filtered and concentrated. Purification of the crude (1.17 g) by flash chromatography (80 g of Merck silica gel, 90:10:1:0.5 $CHCl_3$/CH_3OH/H_2O/AcOH) yielded a residue which was dissolved in water containing 1.1 mL of 1 N HCl, millipore-filtered, and lyophilized to give pure **37** (541 mg, 49.6%) as a white fluffy solid. TLC: R_f = 0.31 (90:20:2.5:1.0 $CHCl_3$/MeOH/H_2O/AcOH). MS: $(M + H)^+$ 646^+. Mp: 110–130 °C slow dec. $[\alpha]_D$ = −14.5° (c = 0.85, MeOH). IR (KBr): 1039, 1229, 1446, 1534, 1643, 2923, 3429. ^{1}H NMR (270 MHz, DMSO-d_6): 0.7–1.8 (m, 21 H), 2.55 (m, 1 H), 2.7–3.0 (m, 4 H), 3.65 (d, 3 H, J = 10), 3.75 (d, 3 H, J = 10), 3.88 (m, 1 H), 4.15 (m, 1 H), 4.2 (m, 1 H), 4.48 (m, 1 H), 4.65 (m, 1 H), 6.05 (br s, 1 H), 7.1–7.45 (m, 7 H). ^{31}P NMR (CD_3OD): 28.9. Anal. Calcd for $C_{32}H_{48}N_5O_7P \cdot 1.2HCl \cdot 0.68H_2O$: C, 54.86; H, 7.27; N, 10.00; P, 4.42; Cl, 5.90. Found: C, 55.05; H, 7.28; N, 9.89; P, 4.56; Cl, 5.95.

Preparation of (1*S*,2*S*)-*N*-[1-(Cyclohexylmethyl)-2-(dimethoxyphosphinyl)-2-hydroxyethyl]-*N*^{2}-[(1,1-dimethylethoxy)carbonyl]-3-[(phenylmethoxy)methyl]-L-histidinamide (39). Acid **38** (9.29 g, 23.06 mmol) was added to a solution of **10** (8.34 g, 27.6 mmol) in DMF (115 mL) and cooled

to 0 °C. HOBt (3.87 g, 25.4 mmol), Et_3N (4.82 mL, 34.6 mmol), and EDC (4.86 g, 25.4 mmol) were added sequentially. After 16 h at 25 °C, a pH 4.01 buffer solution (400 mL, Mallinckrodt "buffer pH 4.01") was added and the reaction mixture was stirred for an additional 10 min. The aqueous layer was extracted with EtOAc (2 × 500 mL), and the combined organic extracts were dried (Na_2SO_4) and concentrated. Purification of the crude product by flash chromatography (1400 g of Merck silica gel, 19:1:0.05 $CHCl_3/CH_3OH/NH4OH$) yielded pure **39** (9.37 g, 65.3%). TLC: R_f = 0.19 (90:1.0:0.05 $CHCl_3$/MeOH/NH_4OH). MS: $(M + H)^+$ 623^+. Mp: 80–90 °C. $[\alpha]_D = -32.9°$ (c = 0.54, MeOH). ^1H NMR (270 MHz, $CDCl_3$): 1.38 (s, 9 H), 0.7–1.9 (m, 13 H), 2.98 (dd, 1 H, J = 7, 14), 3.25 (dd, 1 H, J = 5, 14), 3.77 (d, 3 H, J = 10), 3.82 (d, 3 H, J = 10), 3.93 (m, 1 H), 3.95–4.5 (m, 2 H), 4.5 (s, 2 H), 5.33 (s, 2 H), 6.80 (d, 1 H, J = 8), 6.93 (s, 1 H), 7.14–7.43 (m, 5 H), 7.47 (s, 1 H). ^{13}C NMR ($CDCl_3$): 25.9, 26.0, 26.3, 28.1, 32.6, 33.3, 33.9, 38.3, 48.6, 53.2, 53.3, 53.8, 69.1 (J = 162.8), 69.7, 73.2, 79.9, 127.6, 127.9, 128.1, 128.5, 135.9, 136.0, 137.9, 155.3, 171.0. Anal. Calcd for $C_{30}H_{47}N_4O_8P \cdot 0.35H_2O$: C, 57.28; H, 7.64; N, 8.91; P, 4.92. Found: C, 57.28; H, 7.63; N, 8.62; P, 4.61.

Preparation of (1S,2S)-*N*-[1-(Cyclohexylmethyl)-2-(dimethoxyphosphinyl)-2-hydroxyethyl]-*N*²-[*N*-(4-morpholinylcarbonyl)-L-phenylalanyl]-3-[(phenylmethoxy)methyl]-L-histadinamide (40). Compound **39** (7.14 g, 11.48 mmol) was dissolved in a solution of HCl/EtOAc (55 mL, 1.4 N), reacted for 1 h, and concentrated, yielding 7.1 g of amine hydrochloride as a white solid. A portion of this amine (3.4 g, 6 mmol) was added to a solution of **31** (1.45 g, 5 mmol) in DMF (25 mL) and cooled to 0 °C. HOBt (840 mg, 5.5 mmol), Et_3N (1.04 mL, 7.5 mmol), and EDC (1.05 g, 5 mmol) were added sequentially. After 16 h at 25 °C, a pH 4.01 buffer solution (100 mL, Mallinkrodt "buffer pH 4.01") was added and the reaction mixture was stirred for 10 min. The aqueous layer was extracted with EtOAc (3 × 75 mL), and the combined extracts were dried and concentrated, yielding 4.0 g of crude product. Chromatographic purification (300 g of Merck silica gel, 9:1:0.05 $CHCl_3/CH_3OH/NH_4OH$; 100 g of Merck silica gel, 1:1 Hex/EtOAc to 9:1:0.05 $CHCl_3/CH_3OH/NH_4OH$) afforded the pure BOM-protected penultimate precursor of **40** (1.35 g, 34.5%). A mixture of this intermediate (1.22 g, 1.56 mmol), 20% palladium hydroxide on carbon (552 mg; Aldrich), and 1.0 N hydrochloric acid (2.02 mL, 2.02 mmol) in methanol (18 mL) was stirred under hydrogen (balloon) for 16 h, after which time it was filtered over Celite and concentrated *in vacuo*. Purification of the crude product (1.28 g) by flash chromatography (512 g of Merck silica gel, 90:20:2.5:1 $CHCl_3$/MeOH/H_2O/AcOH) yielded a residue which was dissolved in water containing 1.2 mL of 1 N HCl, millipore-filtered, and lyophilized to give **40** (767.3 mg, 71.0%) as a white solid. Traces of impurities were removed from a portion of the material by repurification by preparative HPLC using a fully capped C-18 column (YMC 1–15 ODS, 30 × 500 mm, 15 m spherical, 25.6 mL/min, UV monitoring at 220 nm) and eluting with 70% methanol in water containing 1% trifluoroacetic acid. The appropriate fractions (t_R = 14 min) were concentrated to dryness, dissolved in water (4 mL), millipore-filtered, and lyophilized to give 319 mg of repurified **40**. TLC: R_f = 0.22 (90:20:2.5:1.0 $CHCl_3$/MeOH/H_2O/AcOH). MS: $(M + H)^+$ 663^+. Mp: 85–96 °C slow dec. $[\alpha]_D = -15.6°$ (c = 0.50, MeOH). IR (KBr): 1040, 1262, 1534, 1626, 1650, 2923, 3376. ^1H NMR (DMSO-d_6): 0.7–1.8 (m, 13 H), 2.8–3.55 (m, 12 H), 3.66 (d, 3 H, J = 10), 3.68 (d, 3 H, J = 10), 3.85 (m, 1 H), 4.1–4.3 (m, 2 H), 4.6 (m, 1 H), 6.8 (d, 1 H, J = 8), 7.12–7.35 (m, 7 H), 7.64 (d, 1 H, J = 9), 8.28 (d, 1 H, J = 8), 8.74 (s, 1 H). ^{31}P NMR (CD_3OD): 28.98. Anal. Calcd for $C_{31}H_{47}N_6O_8P \cdot 1.0C_2F_3O_2H \cdot 1.2H_2O$: C, 49.65; H, 6.36; N, 10.53; P, 3.88; F, 7.14. Found: C, 49.30; H, 6.00; N, 11.17; P, 3.69; F, 7.04.

Acknowledgment. We wish to thank Ms. Debra Skeens for her help in the preparation of this manuscript.

References

(1) Ferrario, C. M. The Renin-Angiotensin System: Importance in Physiology and Pathology. *J. Cardiovasc. Pharmacol.* **1990**, *15* (Suppl. 3), S1–S5.

(2) (a) Greenlee, W. J. Renin Inhibitors. *Med. Res. Rev.* **1990**, *10*, 173–226. (b) Ocain, T. D.; Abou-Gharbia, M. Renin-Angiotensin System (RAS) dependent Antihypertensives: I. Renin Inhibitors. *Drugs Future* **1991**, *16*, 37–51. (c) Kleinert, H. D.; Baber, W. R.; Stein, H. H. Renin Inhibitors. *Adv. Pharmacol.* **1991**, *22*, 207–250.

(3) (a) Petrillo, E. W., Jr.; Ondetti, M. A. Angiotensin-Converting Enzyme Inhibitors: Medicinal Chemistry and Biological Actions. *Med. Res. Rev.* **1982**, *2*, 1–38. (b) Wyvratt, M. J.; Patchett, A. A. Recent Developments in the Design of Angiotensin-Converting Enzyme Inhibitors. *Med. Res. Rev.* **1985**, *5*, 483–531. (c) Kostis, J. B. Angiotensin-Converting Enzyme Inhibitors. Emerging Differences and New Compounds. *Am. J. Hypertens.* **1989**, *2*, 57–64.

(4) (a) Timmermans, P. B. M. W. M.; Wong, P. C.; Chin, A. T.; Herblin, W. F. Nonpeptide Angiotensin II Receptor Antagonists. *TIPS* **1991**, *12*, 55–62. (b) Steinberg, M. I.; Wiest, S. A.; Palkowitz, A. D. Nonpeptide Angiotensin II Receptor Antagonists. *Cardiovasc. Drug. Rev.* **1993**, *11*, 312–358. (c) Murray, W. V. Angiotensin II Receptor Antagonists: The Next Step in the Evolution of Antihypertensive Therapy. *Chemtracts-Org. Chem.* **1993**, *6*, 263–284. (d) Siegl, P. K. S.; Kivlighn, S. D.; Broten, T. P.; Schaffer, L. W. Pharmacology of angiotensin II receptor antagonists: comparison with renin inhibitors and angiotensin-converting enzyme inhibitors. *Exp. Opin. Invest. Drugs* **1994**, *3*, 925–944.

(5) For examples of recent research, see: (a) Thaisrivongs, S.; Blinn, J. R.; Pals, D. T.; Turner, S. R. Conformationally Constrained Renin Inhibitory Peptides: Cyclic (3-1)-1-(Carboxymethyl)-L-prolyl-L-phenylalanyl-L-histidinamide as a Conformational Restriction at the P_2-P_4 Tripeptide Portion of the Angiotensinogen Template. *J. Med. Chem.* **1991**, *34*, 1276–1282. (b) Fisher, J. F.; Harrison, A. W.; Bundy, G. L.; Wilkinson, K. F.; Rush, B. D.; Ruwart, M. J. Peptide to Glycopeptide: Glycosylated Oligopeptide Renin Inhibitors with Attenuated In Vivo Clearance Properties. *J. Med. Chem.* **1991**, *34*, 3140–3143. (c) Ashton, W. T.; Cantone, C. L.; Tolman, R. L.; Greenlee, W. J.; Lynch, R. J.; Schorn, T. W.; Strouse, J. F.; Siegl, P. K. S. Inhibitors of Human Renin with C-Termini Derived from Amides and Esters of α-Mercaptoalkanoic Acids. *J. Med. Chem.* **1992**, *35*, 2772–2781. (d) Doherty, A. M.; Sircar, I.; Kornberg, B. E.; Quin, J., III; Winters, R. T.; Kaltenbronn, J. S.; Taylor, M. D.; Batley, B. L.; Rapundalo, S. R.; Ryan, M. J.; Painchaud, C. A. Design and Synthesis of Potent, Selective, and Orally Active Fluorine-Containing Renin Inhibitors. *J. Med. Chem.* **1992**, *35*, 2–14. (e) Kleinert, H. D.; Rosenberg, S. H.; Baker, W. R.; Stein, H. H.; Klinghofer, V.; Barlow, J.; Spina, K.; Polakowski, J.; Kovar, P.; Cohen, J.; Dennisen, J. Discovery of a Peptide-Based Renin Inhibitor with Oral Bioavailability and Efficacy. *Science* **1992**, *257*, 1940–1943. (f) Boyd, S. A.; Fung, A. K. L.; Baker, W. R.; Mantei, R. A.; Armiger, Y.; Stein, H. H.; Cohen, J.; Egan, D. A.; Barlow, J.; Klinghofer, V.; Verburg, K. M.; Martin, D. L.; Young, G. A.; Polakowski, J. S.; Hoffman, D. J.; Garren, K. W.; Perun, T. J.; Kleinert, H. D. C-Terminal Modifications of Nonpeptide Renin Inhibitors: Improved Oral Bioavailability via Modification of Physiochemical Properties. *J. Med. Chem.* **1992**, *35*, 1735–1746. (g) Martin, S. F.; Austin, R. E.; Oalmann, C. J.; Baker, W. R.; Condon, S. L.; de Lara, E.; Rosenberg, S. H.; Spina, K. P.; Stein, H. H.; Cohen, J.; Kleinert, H. D. 1,2,3-Trisubstituted Cyclopropanes as Conformationally Restricted Dipeptide Isoteres: Applications to the Design and Synthesis of Novel Renin Inhibitors. *J. Med. Chem.* **1992**, *35*, 1710–1721. (h) Rosenberg, S. H.; Spina, K. P.; Woods, K. W.; Polakowski, J.; Martin, D. L.; Yao, Z.; Stein, H. H.; Cohen, J.; Barlow, J. L.; Egan, D. A.; Tricarico, K. A.; Baker, W. R.; Kleinert, H. D. Studies Directed Toward the Design of Orally Active Renin Inhibitors. 1. Some Factors Influencing the Absorption of Small Peptides. *J. Med. Chem.* **1993**, *36*, 449–459. (i) Rosenberg, S. H.; Spina, K. P.; Condon, S. L.; Polakowski, J.; Martin, D. L.; Yao, Z.; Kovar, P.; Stein, H. H.; Cohen, J.; Barlow, J. L.; Klinghofer, V.; Egan, D. A.; Tricarico, K. A.; Perun, T. J.; Baker, W. R.; Kleinert, H. D. Studies Directed Toward the Design of Orally Active Renin Inhibitors. 2. Development of the Efficacious, Bioavailable Renin Inhibitor (2S)-2-Benzyl-3-[[(1-methylpiperazin-4-yl)sulfonyl]propionyl]-3-thiazol-4-yl-L-alanine. Amide of (2S,3R,4S)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (A-72517). *J. Med. Chem.* **1993**, *36*, 460–467. (j) Hurley, T. R.; Colson, C. E.; Hicks, G.; Ryan, M. J. Orally active water-soluble N,O-acyl transfer products of a β,γ-bishydroxyl amide containing renin inhibitor. *J. Med. Chem.* **1993**, *36*, 1496–1498. (k) Heitsch, H.; Henning, R.; Kleeman, H.; Linz, W.; Nickel, W.; Ruppert, D.; Urbach, H.; Wagner, A. Renin inhibitors containing a pyridyl amino diol derived C-terminus. *J. Med. Chem.* **1993**, *36*, 2788–2800. (l) Lunney, E. A.; Hamilton, H. W.; Hodges, J. C.; Kaltenbronn, J. S.; Repine, J. T.; Badasso, M.; Cooper, J. B.;

Dealwis, C.; Wallace, B. A.; Lowther, W. T.; Dunn, B. M.; Humblet, C. Analyses of ligand binding in five endothiapepsin crystal complexes and their use in the design and evaluation of noven renin inhibitors. *J. Med. Chem.* **1993**, *36*, 3809–3820. (m) Raddatz, P.; Minck, K.; Rippmann, F.; Schmitges, C. Non-Peptide renin inhibitors containing 2-(((3-Phenylpropyl)phosphoryl)oxy)alkanoic acid moieties as P_2-P_3 replacements. *J. Med. Chem.* **1994**, *37*, 486–497.

(6) Garvas, H.; Garvas, I. Angiotensin-Converting Enzyme Inhibitors. Properties and Side Effects. *Hypertension* **1988**, *11* (3, Suppl. 11), 37–41.

(7) (a) Patel, D. V.; Rielly-Gauvin, K.; Ryono, D. E.; Free, C. A.; Smith, S. A.; Petrillo, E. W., Jr. Activated Ketone based Inhibitors of Human Renin. *J. Med. Chem.* **1993**, *36*, 2431–2447. (b) Patel, D. V.; Rielly-Gauvin, K.; Ryono, Denis, E. Peptidic Trifluoromethyl Alcohols and Ketones. A General Synthesis and Application as Renin Inhibitors. *Tetrahedron Lett.* **1988**, *29*, 4665–4668.

(8) (a) Iizuka, K.; Kamijo, T.; Kubota, T.; Akahane, K.; Umeyama, H.; Kiso, Y. New Human Renin Inhibitors Containing an Unnatural Amino Acid, Norstatine. *J. Med. Chem.* **1988**, *31*, 701–704. (b) Iizuka, K.; Kamijo, T.; Harada, H.; Akahane, K.; Kubota, T.; Umeyama, H.; Kiso, Y. Orally Potent Human Renin Inhibitors Derived from Angiotensinogen Transition State: Design, Synthesis, and Mode of Interaction. *J. Med. Chem.* **1990**, *33*, 2707–2714.

(9) Lipinski, C. A. Bioisosterism in Drug Design. In *Annual Reports in Medicinal Chemistry*; Bailey, D. M., Ed.; Academic Press Inc.: New York, 1986; Vol. 21, pp 283–292.

(10) For a preliminary account of this work, see: (a) Patel, D. V.; Rielly-Gauvin, K.; Ryono, D. E. Preparation of Peptidic α-hydroxy Phosphonates: A New Class of Transition State Analog Renin Inhibitors. *Tetrahedron Lett.* **1990**, *31*, 5587–5590. (b) Patel, D. V.; Rielly-Gauvin, K.; Ryono, D. E. Peptidic α-hydroxy Phosphinyls: C-terminal modification methodology. *Tetrahedron Lett.* **1990**, *31*, 5591–5594.

(11) Amino aldehyde **4** was prepared as described in ref 7a. For alternate preparations, see: (a) Bock, M. G.; Dipardo, R. M.; Evans, B. E.; Freidinger, R. M.; Rittle, K. E.; Payne, L. S.; Boger, J.; Whitter, W. L.; LaMont, B. I.; Ulm, E. H.; Blaine, E. H.; Schorn, T. W.; Veber, D. F. Renin Inhibitors Containing Hydrophilic Groups. Tetrapeptides with Enhanced Aqueous Solubility and Nanomolar Potency. *J. Med. Chem.* **1988**, *31*, 1918–1923. (b) Luly, J. R.; Dellaria, J. F.; Plattner, J. J.; Soderquist, J. L.; Yi, N. A Synthesis of Protected Aminoalkyl Epoxides from α-Amino Acids. *J. Org. Chem.* **1987**, *52*, 1487–1492. For a general review on preparation of α-amino aldehydes, see: Optically Active N-Protected α-Amino Aldehydes in Organic Synthesis. *Chem. Rev.* **1989**, *89*, 149–164.

(12) In general, we have found ^{31}P NMR as the most convenient method for determining the isomeric contents and purities of various phosphorus-containing synthetic intermediates and final products.

(13) (a) Rich, D. H.; Sun, E. T. O.; Ulm, E. Synthesis of Analogues of the Carboxyl Protease Inhibitor Pepstatin. Effect of Structure on Inhibition of Pepsin and Renin. *J. Med. Chem.* **1980**, *23*, 27–33. (b) Defour, M.; Jouin, P.; Poncet, J.; Pantalino, A.; Castro, B. Synthesis and Reduction of α-Amino Ketones Derived from Leucine. *J. Chem. Soc., Perkin Trans. I* **1986**, 1895–1899.

(14) Cardillo, G.; Orena, M.; Sandri, S.; Tamasini, C. Synthesis of Oxazolidin-2-ones Using Carbonate Ion on a Polymeric Support. *Tetrahedron* **1985**, *41*, 163–167.

(15) (a) Boger, J.; Payne, L. S.; Perlow, D. S.; Lohr, N. S.; Poe, M.; Blaine, E. H.; Ulm, E. H.; Schorn, T. W.; Lamont, B.; Lin, T. Y.; Kawai, M.; Rich, D. H.; Veber, D. F. Renin Inhibitors. Syntheses of Subnanomolar, Competitive, Transition-State Analogue Inhibitors Containing a Novel Analogue of Statine. *J. Med. Chem.* **1985**, *28*, 1779–1790. (b) Kokubu, T.; Hiwada, K.; Nagae, E.; Murakami, E.; Morisawa, Y.; Yabe, Y.; Koike, H.; Iijima, Y. Statine-Containing Dipeptide and Tripeptide Inhibitors of Human Renin. *Hypertension* **1986**, *8* (Suppl. II), II-1–II-5.

(16) Hays, H. R. The Reaction of Diethyl Phosphonate with Methyl and Ethyl Grignard Reagents. *J. Org. Chem.* **1968**, *33*, 3690.

(17) For preparation of **31**, **32**, and **35**, see ref 20. For a similar preparation of the naphthyl derivative of the phenylalanine analog **31**, see: Harada, H.; Yamaguchi, T.; Iyobe, A.; Tsubaki, A.; Kamijo, T.; Iizuka, K.; Ogira, K.; Kiso, Y. A Practical Synthesis of the (2*R*)-3-(Morpholinocarbonyl)-2-(1-naphthylmethyl)propionyl]-L-histidine Moiety (P_4-P_2) in Renin Inhibitors. *J. Org. Chem.* **1990**, *55*, 1679–1682.

(18) Brown, T.; Jones, J. H. Protection of Histidine Side-chains with Benzyloxmethyl- or Bromobenzyloxymethyl- groups. *J. Chem. Soc., Chem. Commun.* **1981**, 648–649.

(19) Dellaria, J. F., Jr.; Maki, R. G.; Stein, H. H.; Cohen, J.; Whittern, D.; Marsh, K.; Hoffman, D. J.; Plattner, J. J.; Perun, T. J. New Inhibitors of Renin that contain Novel Phosphostatine Leu-Val Replacements. *J. Med. Chem.* **1990**, *33*, 534–542.

(20) Weller, H. N., III; Ryono, D. E. U.S. Patent 5,055,466, October 8, 1991.

(21) DeForrest, J. M.; Waldron, T. L.; Oehl, R. S.; Scalese, R. J.; Free, C. A.; Weller, H. N.; Ryono, D. E. Pharmacology of Novel Imidazole Alcohol Inhibitors of Primate Renin. *J. Hypertens.* **1989**, *7* (Suppl. 2), S15–S19.

(22) Derkx, F. H. M.; Meiracker, A. H.; Fischli, W.; Admiraal, P. J. J.; Man in't Veld, A. J.; Brummelen, P.; Schalekamp, M. A. D. H. Nonparallel Effects of Renin Inhibitor Treatment on Plasma Renin Activity and Angiotensins I and II in Hypertensive Subjects - An Assay-Related Artifact. *Am. J. Hypertens.* **1991**, *4*, 602–609.

(23) Jeunemaitre, X.; Menard, J.; Nussberger, J.; Guyene, T. T.; Brunner, H. R.; Corvol, P. Plasma Angiotensins, Renin, and Blood Pressure during Acute Renin Inhibition by CGP38560A in Hypertensive Patients. *Am. J. Hypertens.* **1989**, *2*, 819–827.

JM9502807