Non-peptide Renin Inhibitors Containing 2-(((3-Phenylpropyl)phosphoryl)oxy)alkanoic Acid Moieties as P_2–P_3 Replacements

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A series of novel renin inhibitors containing 2-(((3-phenylpropyl)phosphoryl)oxy)alkanoic acid moieties as P_2–P_3 surrogates are presented. The P_2–P_3 mimetics were obtained from (ω-phenylalkyl)-phosphinic acids **1a–c** and 2-hydroxyalkanoic acid benzyl esters **2a–f** by *N,N'*-dicyclohexylcarbodiimide-mediated coupling and subsequent oxidation with sodium metaperjodate. Ester cleavage of these derivatives and coupling with P_1–P_1' transition-state mimetics **I–VII** provided highly selective compounds with inhibitory potencies in the lower nanomolar range. Small renin inhibitors, such as analogues **8c** and **8h** with molecular weights of 539 and 537, respectively, could be prepared. These compounds exhibited IC_{50} values of about 20 nM against human plasma renin. Compound **7i** was examined in vivo for its hypotensive effect. In salt-depleted cynomolgus monkeys, **7i** inhibited plasma renin activity almost completely and lowered blood pressure after oral administration of a dose of 30 mg/kg.

Introduction

The renin–angiotensin system is a complex enzymatic-hormonal system controlling electrolyte homeostasis, fluid volume, and arterial blood pressure by the production of the potent vasopressor and aldosteronogenic octapeptide angiotensin II.[1] The great success of angiotensin-converting enzyme inhibitors in the treatment of hypertension and congestive heart failure[2] provided the impetus to look for alternate approaches to interfering with the renin–angiotensin system by inhibition of renin[3] and antagonism of angiotensin II at the receptor level.[4] Renin is the enzyme that catalyzes the first and rate-limiting step in the production of angiotensin II. Since angiotensinogen is the only substrate for renin, inhibition at this step of the renin–angiotensin system may provide some clinical advantages over ACE inhibition. Therefore, the search for orally active renin inhibitors continues to represent a challenging target for medicinal chemists. Although many potent and selective human renin inhibitors have been developed,[5] oral activity has been poor. Poor absorption, first-pass metabolism, and proteolytic instability appear to be the major problems with many of these inhibitors due to their partly peptidic character, size, and lipophilicity.[6] Therefore, we initiated a synthesis program to overcome these limitations by designing non-peptidic inhibitors with a reduced molecular size. We and other groups[7] speculated that an inhibitor with a surrogate for the P_2–P_3 dipeptide would reduce the peptidic character of these compounds and could lead to inhibitors with enhanced bioavailability. For the P_2–P_3[8] mimetic we chose a phosphonate moiety **A**, a substructure found in the ACE inhibitor SQ 29,852.[9] P_1–P_1' mimetics **I–VII** (Figure 1) were incorporated as transition-state analogues.

A general structure of the resulting non-peptidic renin inhibitor is shown by formula **B**. Modifications of R and R' and variations of *n* were examined. Initial results of these studies on in vitro and in vivo activity are now reported.

Chemistry

The syntheses of the 2-(((ω-phenylalkyl)phosphoryl)-oxy)alkanoic acids and the preparation of a representative example of a non-peptide renin inhibitor are outlined in Schemes 1–3.

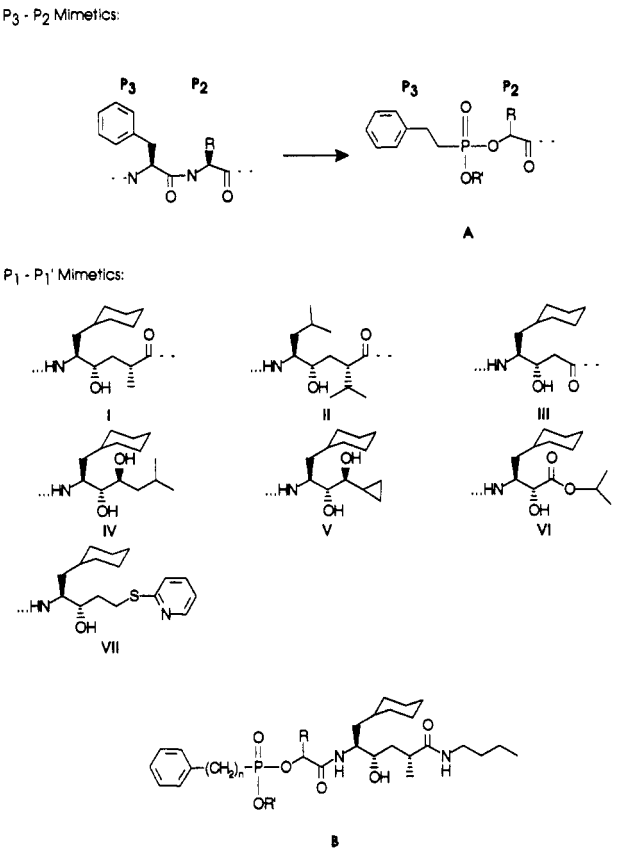

Figure 1. Structures of the P_2–P_3 mimetic **A**, the P_1–P_1' mimetics **I–VII**, and the general structure of a non-peptide inhibitor **B**.

As shown in Scheme 1, the 2-(((ω-phenylalkyl)hydroxy)-phosphoryl)oxy)alkanoic acids **4a–h** were obtained starting from the phosphinic acids **1a–c**. These phosphinic acids were prepared by hydrophosphorylation of the corresponding terminal olfeins under free-radical conditions as described by Karanewsky.[9a]

Coupling of the phosphinic acids **1a–c** with 2-hydroxyalkanoic acid benzyl esters **2a–f** by *N,N'*-dicyclohexylcarbodiimide (DCC)/(*N,N*-dimethylamino)pyridine (DM-

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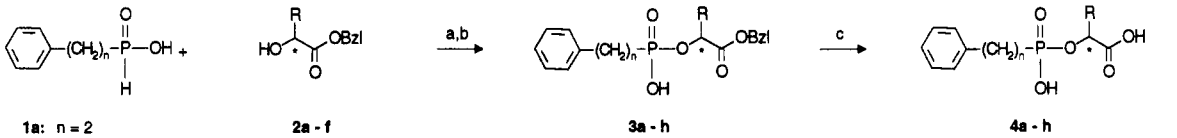
Scheme 1. Synthesis of 2-(((ω-Phenylalkyl)hydroxyphosphoryl)oxy)alkanoic Acids[a]



1a: n = 2
1b: n = 3
1c: n = 4

2a - f 3a - h 4a - h

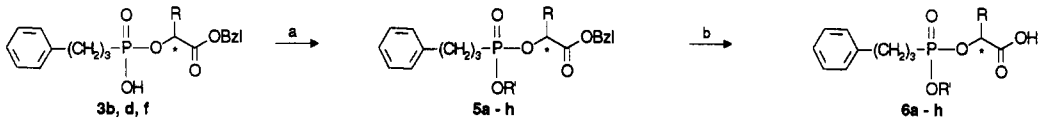
	R	chirality
2a	butyl	S
2b	propyl	S
2c	methyl	S
2d	butyl	R
2e	propyl	R
2f	methyl	R

	n	R	chirality
3a	2	butyl	S
3b	3	butyl	S
3c	4	butyl	S
3d	3	propyl	S
3e	3	methyl	S
3f	3	butyl	R
3g	3	propyl	R
3h	3	methyl	R

	n	R	chiraliry
4a	2	butyl	S
4b	3	butyl	S
4c	4	butyl	S
4d	3	propyl	S
4e	3	methyl	S
4f	3	butyl	R
4g	3	propyl	R
4h	3	methyl	R

[a] (a) DCC, DMAP, THF; (b) $NaJO_4$, dioxane; (c) 1 N LiOH, dioxane.

Scheme 2. Synthesis of 2-(((Alkyloxy)(3-phenylpropyl)phosphoryl)oxy)alkanoic Acids[a]



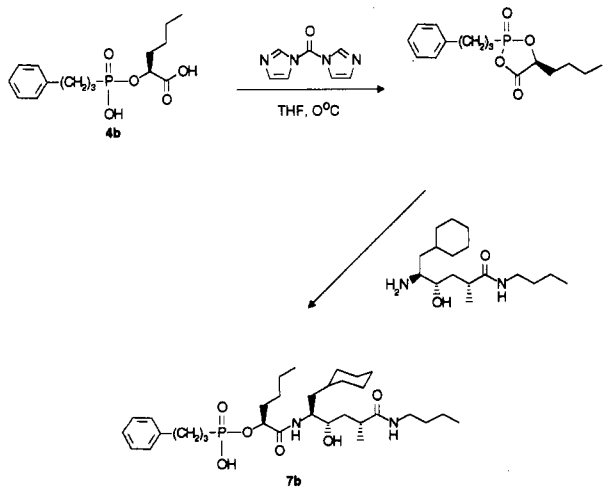
3b, d, f 5a - h 6a - h

	R		R'	chirality
5a	butyl	(less polar)	Et	S
5b	butyl	(more polar)	Et	S
5c	butyl	(less polar)	Et	R
5d	butyl	(more polar)	Et	R
5e	propyl	(less polar)	Et	S
5f	propyl	(more polar)	Et	S
5g	butyl	(less polar)	Bzl	R
5h	butyl	(more polar)	Bzl	R

	R		R'	chiraliry
6a	butyl	(less polar)	Et	S
6b	butyl	(more polar)	Et	S
6c	butyl	(less polar)	Et	R
6d	butyl	(more polar)	Et	R
6e	propyl	(less polar)	Et	S
6f	propyl	(more polar)	Et	S
6g	butyl	(less polar)	Bzl	R
6h	butyl	(more polar)	Bzl	R

[a] (a) EtJ or BzBr, K_2CO_3, DMF; (b) 1 N LiOH, dioxane.

Scheme 3. Synthesis of the Renin Inhibitors



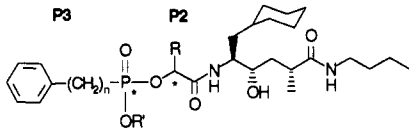
4b 7b

AP) and subsequent oxidation of the resulting phosphonous benzyl esters with sodium metaperiodate in dioxane led to the phosphonic benzyl esters **3a–h**. Saponification of the benzyl esters **3a–h** by lithium hydroxide in dioxane gave the corresponding diacids **4a–h**. The dipeptide mimetics **4a–c** were prepared in an effort to optimize binding of the phenylalkyl moiety to the S_3 subsite of renin. Analogues **4d–h** served as probes to elucidate the influence of the length of the alkyl side chain and its configuration on binding to S_2 subsite of the enzyme.

Scheme 2 illustrates the preparation of the 2-(((alkyloxy)(3-phenylpropyl)phosphoryl)oxy)alkanoic acids **5a–h**. Alkylation of the phosphonic benzyl esters **3b,d,f** with ethyl iodide or benzyl bromide led to diastereomeric mixtures of esters due to the formation of a chiral center at the phosphorus atom. These diastereomers could easily be separated by chromatography on silica gel to yield the pure diastereomers **5a–h**. Saponification of the esters by lithium hydroxide gave the corresponding acids **6a–h**. These analogues were synthesized in order to determine the interaction of the free hydroxyl group and their corresponding ethyl or benzyl esters with the S_4 subsite of renin.

Renin inhibitors of Tables 1 and 2 were prepared as shown in Scheme 3 for the synthesis of inhibitor **7b**. The diacid **4b** was converted to the corresponding cyclic mixed anhydride by using carbonyldiimidazole or *N,N'*-dicyclohexylcarbodiimide. Treatment of the cyclic anhydride with (2*R*,4*S*,5*S*)-*N*-butyl-5-amino-6-cyclohexyl-4-hydroxy-

Table 1. Effect of Chain Length Variation in P_3 and Modification in P_2 on Renin Inhibition



7a - 7n

compd	n	R	R'	chirality in P_2	human renin IC_{50} (nM)
7a	2	butyl	H	*S*	46.0
7b	3	butyl	H	*S*	4.8
7c	4	butyl	H	*S*	360.0
7d	3	propyl	H	*S*	3.8
7e	3	methyl	H	*S*	16.0
7f	3	butyl	H	*R*	2.5
7g	3	propyl	H	*R*	4.2
7h	3	methyl	H	*R*	17.5
7i	3	butyl	Et (less polar)	*S*	22.5
7j	3	butyl	Et (more polar)	*S*	350.0
7k	3	butyl	Et (less polar)	*R*	15.0
7l	3	butyl	Et (more polar)	*R*	215.0
7m	3	butyl	Bz (less polar)	*R*	10.5
7n	3	butyl	Bz (more polar)	*R*	45.0

2-methylhexane amide hydrochloride in presence of NEt_3 as base provided inhibitor **7b**.

The following P_1–P_1' transition state mimetics (structures shown in Figure 1) were synthesized according to published reports: Calψ(CHOHCH$_2$)Ala (**I**) and Leuψ-(CHOHCH$_2$)Val (**II**) in analogy to Buhlmayer et al.,[5e] cyclohexylstatine (**III**, ACHPA),[10a] 2(*S*)-amino-1-cyclohexyl-3(*R*),4(*S*)-dihydroxy-6-methylheptane (**IV**, ACDMH),[10b] 2(*S*)-amino-1-cyclohexyl-3(*R*),4(*S*)-dihydroxy-4-cyclopropylbutane (**V**),[10c] 3(*S*)-amino-4-cyclohexyl-2(*R*)-hydroxybutyric acid isopropyl ester (norcyclostatine, **VI**),[10d] 2(*S*)-amino-1-cyclohexyl-3(*S*)-hydroxy-5-(2-pyridylthio)pentane (**VII**).[10e]

Results and Discussion

In Vitro Activity. The structures and in vitro activities of the renin inhibitors are listed in Tables 1 and 2. Human plasma renin inhibition was measured at pH 5.5. Potencies are expressed as IC_{50} values for suppression of angiotensin I formation.

Table 1 shows that inhibitor **7a** with a phenethyl moiety equivalent to the phenylalanine side chain in P_3 (and an *S*-configurated *n*-butyl side chain α to the phosphonate oxygen) has a reasonable activity (IC_{50} = 46 nM). Prolonging the chain length in P_3 by one carbon atom led to compound **7b** displaying a 10-fold increase in inhibitory potency. However, the higher homologue **7c** (n = 4) showed an 80-fold diminution of activity compared to **7b**.

Inhibitor **7b** was chosed as a reference for evaluating the effect of different residues occupying the P_2 position. Truncation of the P_2 side chain by one carbon atom led to derivative **7d** showing by a slight increase in inhibitory potency. A further shortening of the side chain led to the methyl group in P_2 providing inhibitor **7e** which was about 3-fold weaker in activity than compounds **7b**. Our findings that the subsite S_2 can accommodate hydrophobic residues are in good agreement with data previously described,[11] showing that incorporation of L-amino acids with hydrophobic side chains (Nle,Nva) in P_2 led to highly potent renin inhibitors.

Surprisingly, the incorporation of alkyl groups with the opposite configuration α to the phosphonate oxygen

Table 2. Modifications in P_1–P_1'

compd	human renin IC_{50} (nM)	MW
7f	2.5	594
8a	2.8	582
8b	58.0	744
8c	25.0	539
8d	55.0	539
8e	115.0	590
8f (less polar)	17.7	553
8g (more polar)	130.0	553
8h (less polar)	22.5	537
8i (more polar)	155.0	537
8j CGP 38560A	1.60	730

(compounds **7f–h**) were comparable to their *S*-counterparts **7b,d,e** in renin inhibitory potency. This is in contrast to previously described results displaying significantly greater inhibitory potencies for residues derived from the "natural" series (e.g., the L-amino acids and related derivatives).[7b,f,11a,12]

The ethyl and benzyl ester analogues **7i–n** of the corresponding phosphonic acids **7b** and **7f** demonstrated acceptable potency with IC_{50} values between 10 and 300 nM. However, there was a remarkable difference in inhibitory activity between the less and more polar diastereomer.

With the 2(*R*)-(((3-phenylpropyl)phosphoryl)oxy)hexanoic acid moiety optimized for in vitro activity against human plasma renin, we further tried to improve the potency by synthesizing compounds with different transition-state mimetics which are shown in Figure 1. The incorporation of the hydroxyethylene isostere **II** led to inhibitor **8a** which showed a potency comparable to the reference compound **7f**. ACHPA (**III**) in combination with the known P_2'–P_3' C-terminus[7c] provided derivative **8b** accompanied by a 12-fold drop in inhibitory potency. The glycol moiety **IV** and the norcyclohexylstatine **VI** containing inhibitors **8c** and **8d** possessed reasonable IC_{50}

values (25 and 55 nM) and a remarkably low molecular weight of 539 Da. A 77-fold decrease in potency was observed by incorporation of the dipeptide mimetic **VII** into inhibitor **8e**.

Coupling of the ethyl ester derivatives **6e** and **6f** with the glycol transition-state mimetics **IV** and **V** provided the diastereomers **8f, 8g** and **8h, 8i**, respectively. The less polar diastereomers **8f** and **8h** demonstrated an in vitro potency comparable to the glycol-containing inhibitor **8c**.

Modeling Studies

Molecular graphics and model building was used to further clarify the structure–activity relationships for the N-terminal part of this series of inhibitors. For details of the model building, see the Experimental Section.

Variation of the chain length with n = 2–4 methylene groups for the spacer between the phosphonate group and the phenyl ring gave a clear optimum for n = 3 (compound **7b**, Table 1). This observation could not be explained in the context of the hitherto used renin model derived by Blundell and co-workers.[13] Therefore, the X-ray structures of human and mouse renin[14–16] (PDB[17] codes 1RNE, 2REN, 1BBS) which became available only recently were compared to the model (using a method described in ref 18) in order to derive an explanation for the spacer length optimum. While the overall structural similarity of the model and the X-ray structures in the active site is rather good both for the proteins and the inhibitors, it became immediately clear that the loop Pro 111 to Phe 117 was not in good agreement. The position of this loop in the X-ray structure gave clear steric constraints for the S3 site. Therefore, this loop was extracted from the X-ray structure of human renin[14] and incorporated into our model. All subsequent observations are thus derived from a hybrid model of the endothiapepsin-based Blundell model[13] and the loop Pro 111 to Phe 117 from the X-ray structure of human renin.[14] This hybrid model explains convincingly the spacer length optimum of n = 3, compound **7b**. Furthermore, the phenyl rings of the inhibitors of this series can be superimposed without constraint onto the phenyl ring of the inhibitor CGP 38560[5e] (compound **8j**, Table 3) in the X-ray structure (PDB code 1RNE[14]). Figure 2 shows that for this spacer length, there is a possibility for good contact of the terminal phenyl ring of the inhibitor (representing position P_3) to the loop Pro 111 to Phe 117 (taken from the X-ray structure 1RNE), in particular to the hydrophobic residues Pro 111, Phe 112, Leu 114, and Phe 117 (magenta in Figure 2). Thr 12 in the model is also part of the residues bordering this cavity (S3). Shorter spacers would not lead to these mainly hydrophobic contacts; longer chains are not allowed because of steric limitations. The orientation of the phosphate group in compound **7b** (*S* series) is dominated by a hydrogen bond to the amide hydrogen of Ser 219 (Figure 2). For further details of this interaction, see the discussion of compounds **7k** and **7l** (*R* series) below.

The stereopair compounds **7b** and **7f** (see Table 1; the stereocenter is highlighted with an asterisk (*) in the structural formula) differ in their activity only by a factor of 2, i.e., they are similarly potent. This was somewhat surprising as it should be expected that the isomer with the *S* configuration of natural amino acids would be the more active one. This interesting problem was therefore also investigated using model building and computer graphics. The phosphonate group was again oriented to optimize possible hydrogen bonds with main chain atoms of the protein model. The position of the butyl side chain and of the terminal phenyl group were adjusted manually. Figure 3 shows the superposition of compound **7b** (white lines) and **7f** (green lines). The orientation of both the butyl side chains and the terminal phenyl group could be very similar, despite the inversion at the stereocenter. This similarity in the orientation of the butyl side chain despite the different configuration is only possible because of the different orientation of the phosphonate group.

Table 3. Specificity of Renin Inhibitors

compd[a]	IC_{50} (nM)[b] human renin	pepsin	cathespin D
7a	46.0	>10 000	>10 000
7b	4.8	>10 000	>10 000
7c	360.0	>10 000	>10 000
7d	3.8	>10 000	>10 000
7e	16.0	>10 000	>10 000
7f	2.5	>10 000	>10 000
7g	4.2	>10 000	>10 000
7h	17.5	>10 000	>10 000
7i	22.5	>10 000	>10 000
7j	350.0	>10 000	>10 000
7k	15.0	>10 000	2 800
7l	215.0	>10 000	>10 000
7m	10.5	>10 000	2 650
7n	45.0	>10 000	>10 000
8a	2.8	>10 000	2 300
8b	58.0	>10 000	>10 000
8c	25.0	>10 000	>10 000
8d	55.0	>10 000	>10 000
8e	115.0	>10 000	>10 000
8f	17.7	>10 000	2 150
8g	130.0	>10 000	>10 000
8h	20.0	>10 000	4 150
8i	155.0	>10 000	>10 000

[a] See Tables 1 and 2 for structures. Each compound had NMR spectra consistent with structure and expected M + H ion in FAB-MS. [b] IC_{50} values were derived from inhibition experiments in which data points were measured in triplicate. IC_{50} values have an estimated error of ±20%.

The compounds discussed so far all had a hydrogen in position R′ (see structural formula). Substitution of an ethyl group for hydrogen in that position led to compounds with a chiral phosphonate atom. A pair of compounds, **7k** and **7l**, was chosen for further modeling studies. The less polar compound of the pair had a 14-fold higher activity. Modeling of this pair (Figure 4a,b) showed that they both could form two hydrogen bonds involving the oxygens as acceptors of the amide hydrogen of Ser 219. This function as a hydrogen bond acceptor of one of the terminal phosphonate oxygens is completely analogous to the hydrogen bond acceptor function observed in earlier modeling studies of P_3–P_2 amide bond isosters[19a,b] which were reviewed recently.[20] This shows that the phosphonate moiety is a functional replacement for the amide bond in this context, independent of the stereochemistry defined by *R* (Table 1), see also Figure 2, 3, and 4a for details of the interactions of *S* and *R* series compounds. The marked difference in the activities of compounds **7k** and **7l** could result from some steric hindrance between the ethyl chain of compound **7l** and the protein backbone, especially the carbonyl oxygen of Gly 217 (see Figure 4b). There are no particularly favorable contacts between the ethyl side chain and the protein in **7k** (Figure 4a), but there seems to be no steric hindrance. The closest distance between the non-hydrogen atoms of Thr 12 and compound **7k** is 4.8 Å. It should be noted that, using the results from this modeling study, one could make a *de facto* assignment for the

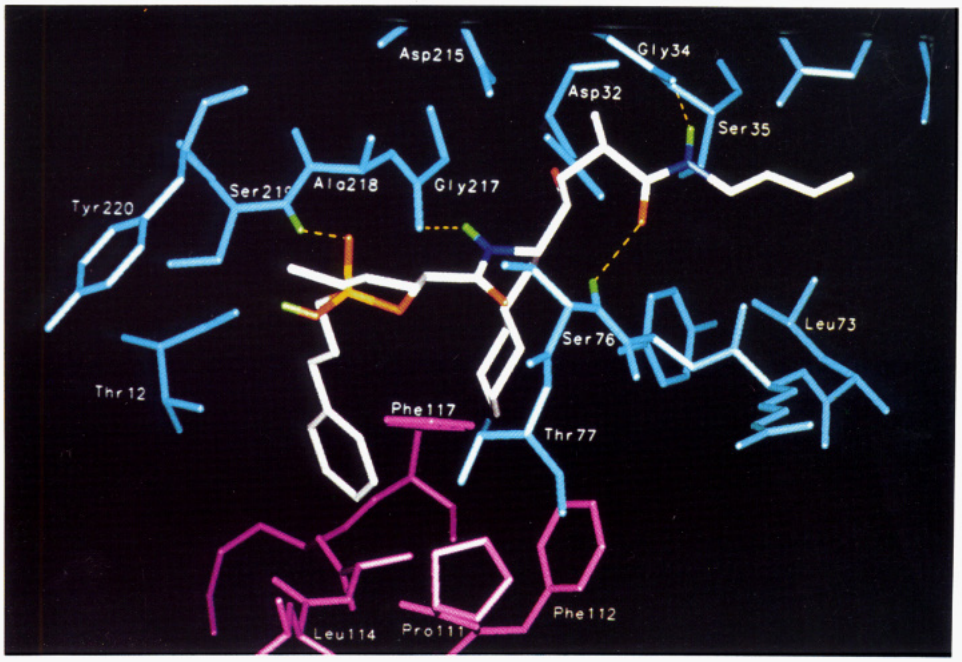


Figure 2. Compound **7b** (white; see also Table 1) in the active site of the renin model (cyan). Only those hydrogen atoms (green) involved in hydrogen bonds (yellow dashed lines) or bound to one of the phosphonate oxygens are displayed. The loop which limits pocket S3 (magenta) was taken from the X-ray structure.[14] The phenyl ring of **7b** (optimal spacer length of 3) makes contacts to the hydrophobic sidechains of Pro 111, Leu 114, and Phe 117.

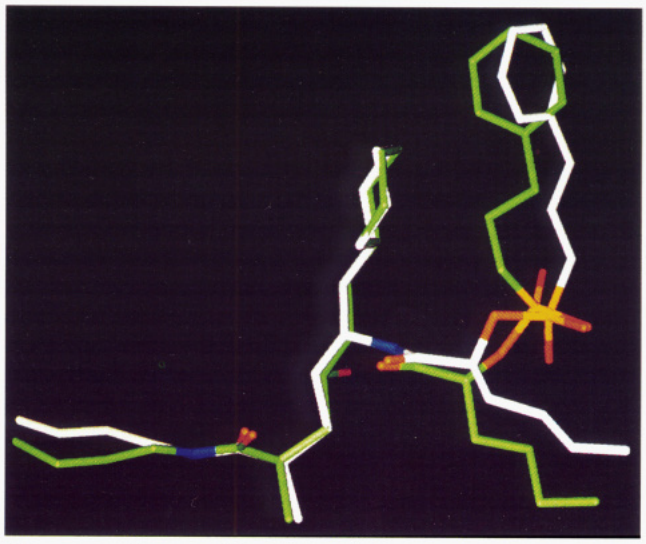


Figure 3. Compounds **7b** (*S* configuration, white) and **7f** (*R* configuration, green) superposed in their presumed receptor-bound conformation. Despite their different stereochemistry, both the phenyl rings in the highly constrained S3 site and to a lesser extent the butyl side chains in the less constrained S2 site occupy similar volumes in space. This could explain the similar activity of the stereoisomers.

stereochemistry of the phosphonate group for compounds **7k** (Figure 4a) and **7l** (Figure 4b).

A somewhat similar situation can be found for compounds **7m** and **7n** containing R′ = benzyl. The relatively good activity of inhibitor **7m** might be a consequence of the possible interaction of the benzyl moiety with Tyr 220 of binding site S4 (see Figure 4c). This edge-to-face interaction is similar to a preferred Phe–Phe interaction found in many protein structures.[21]

Enzyme Selectivity. The renin inhibitors listed in Tables 1 and 2 were assayed for their ability to inhibit other aspartic proteinases. None of these compounds showed significant inhibition of porcine pepsin (Table 3) at a concentration of 10 μM. Compound **7k**, **7m**, **8a**, **8f**, and **8h** did inhibit bovine cathepsin D albeit with very weak activity. These results demonstrate the high specificity of the inhibitors for renin in comparison to related proteinases.

In Vivo Activity. In order to evaluate effects in vivo on plasma renin activity (PRA), blood pressure, and heart rate, selected compounds were administered orally to conscious, sodium-depleted cynomolgus monkeys. Figure 5 shows the results obtained with inhibitor **7i** at an oral dose of 30 mg/kg. This dose produced a maximal hypotensive response of 15 mmHg lasting for about 180 min. PRA was suppressed by 97%, 99%, and 96% at 30, 60, and 180 min, respectivley.

Conclusion

A new series of novel renin inhibitors containing 2-(((3-phenylpropyl)phosphoryl)oxy)alkanoic acid moieties as replacements for P_2–P_3 sites was developed. Our studies resulted in highly selective compounds with inhibitory potencies in the lower nanomolar range. Small renin inhibitors, such as compounds **8c** and **8h** with low molecular weight (539 and 537, respectively), could be prepared and exhibited IC_{50} values of about 20 nM against human plasma renin. Preliminary in vivo studies with compound **7i** demonstrated moderate hypotensive effects after oral administration. Further studies are currently underway to improve the potency of the small molecular weight renin inhibitors and to evaluate their potential in vivo.

Experimental Section

Melting points were determined with a Mettler FP 62 melting point apparatus and are uncorrected. Specific rotations were measured with a Perkin-Elmer 241 MC polarimeter. IR, NMR, and mass spectra are in agreement with the structures cited and were recorded on a Bruker 85 IFS 48 IR spectrophotometer, a Bruker AC 200, WM 250 or AM 500 (TMS as internal standard), and a Vacuum Generator VG 70-70 or 70-250 at 70 eV,

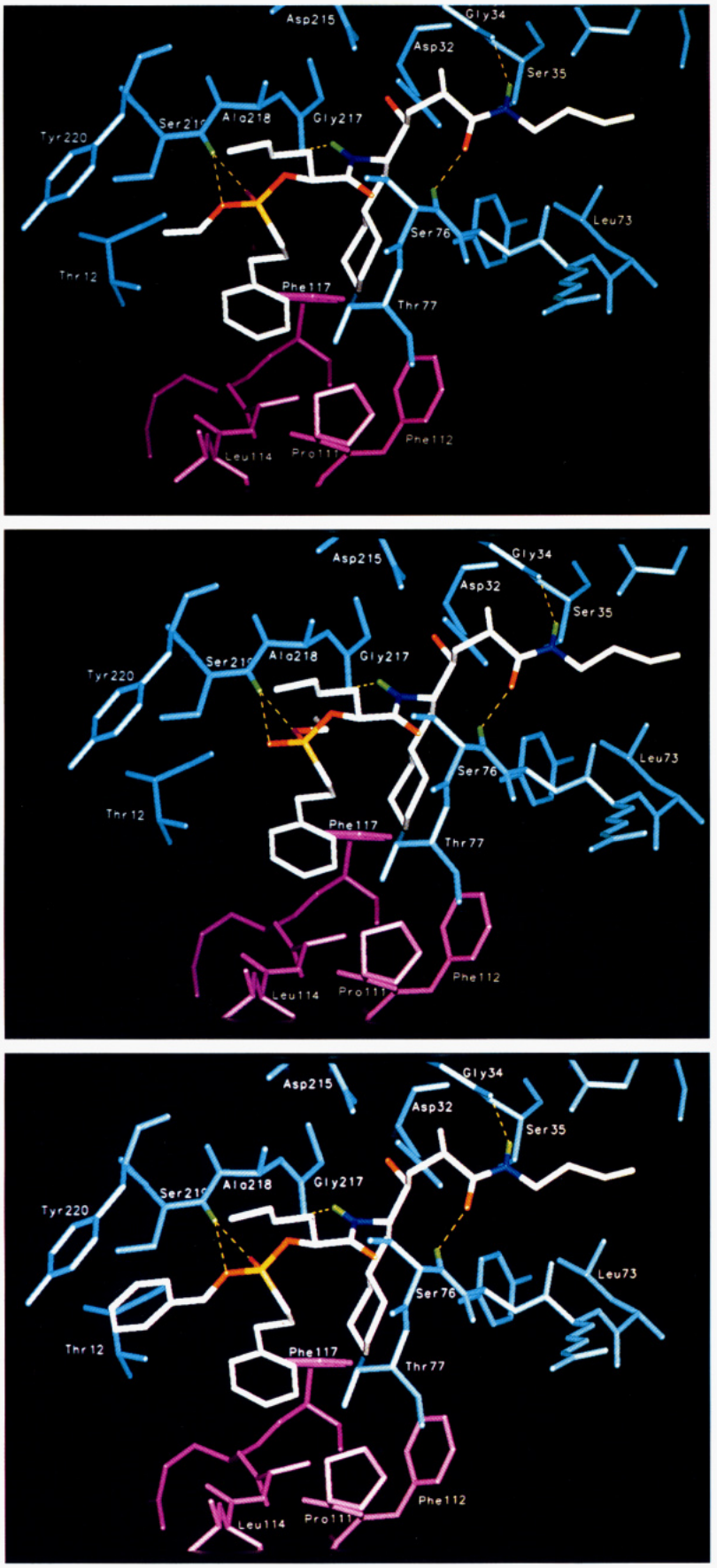


Figure 4. (a) Less polar, more active compound **7k** of the **7k**/**7l** pair in the active site of the renin model. There is ample space for the ethyl group attached to one of the two phosphonate oxygens. (b) More polar, less active compound **7l** of the **7k**/**7l** pair in the active site of the renin model. There is some steric overlap between the ethyl group attached to the other one of the two phosphonate oxygens and the protein backbone (especially the carbonyl oxygen of Gly 217). (c) Compound **7m** in the active site of the renin model. In this configuration, the phenyl group attached to one of the two phosphonate oxygens makes good edge-to-face contact to Tyr 220, which could explain the better activity of compound **7m** relative to **7n**.

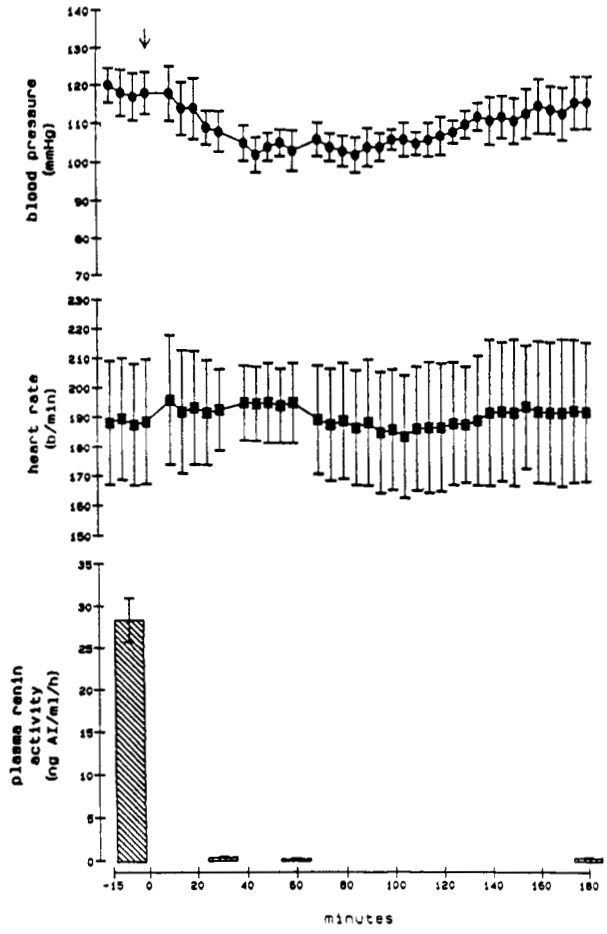


Figure 5. Effects of a 30 mg/kg oral dose of inhibitor **7i** in salt-depleted cynomolgus monkeys. Results are shown as mean ± SEM of three animals. Blood pressure = systolic arterial blood pressure, AI = angiotensin I.

respectively. Microanalyses were obtained with a Perkin-Elmer 240B CHN analyzer. Thin-layer chromatography (TLC) was carried out on precoated gel F_{254} plates with a layer thickness of 0.25 mm from E. Merck, Darmstadt. Visualization was done with UV and I_2. Analytical HPLC data were obtained by using an E. Merck-Hitachi instrument (L-6200 pump, L-4250 detector, D-2500 integrator) equipped with a Lichrospher 60 RP-select B reverse-phase column (250 × 4 mm, E. Merck) at ambient temperature with CH_3CN and NaH_2PO_4 buffer (0.05 m, pH 6) as eluents, and a detection at 210 nm.

General Procedure for 2-Hydroxyalkanoic Acid Benzyl Esters 2a–f. (2*R*)-2-Hydroxyhexanoic Acid Benzyl Ester (2d). To an ice-cooled solution of D-norleucine (25 g, 191 mmol) in 1 N H_2SO_4 (500 mL) was added dropwise a solution of $NaNO_2$ (21.1 g, 306 mmol) in water (100 mL). The mixture was stirred for an additional 2 h at 0 °C and then left to stand overnight. The reaction solution was concentrated in vacuo and extracted with EtOAc. The combined extracts were dried (Na_2SO_4) and evaporated.

To the solution of the crude hydroxy acid (23.7 g, 94%) in DMF (200 mL) was added K_2CO_3 (27.2 g, 197 mmol) and benzyl bromide (33.4 g, 195 mmol). After being stirred for 14 h at room temperature, the reaction mixture was poured into water. The resulting mixture was extracted with EtOAc, and the combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated. The residue was distilled to give 30.2 g (75.9%) of **2d** as colorless liquid: bp 119–21 °C (0.3 Torr); $[\alpha]^{20}_D$ +22.3° (*c* 1.25/MeOH); HPLC 98.8%; TLC R_f 0.42 (hexane, 15% methyl *tert*-butyl ether (MTBE)); ^{1}H NMR (DMSO-d_6) δ 7.37 (s, 5H), 5.34 (d, *J* = 7.5 Hz, 1H), 5.13 (s, 2H), 4.05 (q, *J* = 7.5 Hz, 1H), 1.62 (m, 2H), 1.25 (m, 4H), 0.87 (tr, *J* = 7.2 Hz 3H); FAB MS *m/e* 223 (M^+ + H). Anal. ($C_{13}H_{18}O_3$) C, H.

(2*R*)-2-Hydroxypentanoic acid benzyl ester (2e) was obtained from D-norvaline (25 g, 213 mmol) as described for **2d** as a colorless oil (32.3 g, 82.5%): bp 116–118 °C (0.5 Torr); $[\alpha]^{20}_D$ +16.6° (*c* 0.93/MeOH); HPLC 98.3%; TLF R_f 0.52 (hexane, 20% MTBE); ^{1}H NMR (DMSO-d_6) δ 7.38 (s, 5H), 5.35 (d, *J* = 7.2 Hz, 1H), 5.12 (s, 2H), 4.04 (q, *J* = 7.2 Hz, 1H), 1.65 (m, 2H), 1.35 (m, 2H), 0.88 (tr, *J* = 7.5 Hz, 3H); FAB MS *m/e* 209 (M^+ + H). Anal. ($C_{12}H_{16}O_3$) C, H.

General Procedure for 2-(((ω-Phenylalkyl)hydroxyphosphoryl)oxy)alkanoic Acid Benzyl Esters 3a–h. (2*S*)-2-(((3-Phenylpropyl)hydroxyphosphoryl)oxy)hexanoic Acid Benzyl Ester (3b). To a solution of (2*S*)-2-hydroxyhexanoic acid benzyl ester (**2a**) (5.9 g, 32.03 mmol) and (3-phenylpropyl)phinic acid **1b** (4.5 g, 20.28 mmol) in dry THF (75 mL) at 0 °C under nitrogen was added *N*,*N*′-dicyclohexylcarbodiimide (4.18 g, 20.28 mmol) and 4-(dimethylamino)pyridine (0.95 g, 7.78 mmol). After being stirred for 2 h at 0 °C, the reaction mixture was filtered. The filtrate was diluted with EtOAc (250 mL) and washed with 5% $KHSO_4$ and saturated $NaHCO_3$, dried (Na_2SO_4), and evaporated to dryness. The resulting crude phosphenic ester (7.7 g) was dissolved in dioxane (90 mL) and treated with $NaIO_4$ (4.49 g, 21 mmol) in water (50 mL) and the mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with 2% $KHSO_4$ (300 mL) and extracted with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated to give 7.5 g of a pale yellow liquid. For purification the phosphonic acid was taken up with Et_2O (50 mL) and treated with a solution of admantanamine (2.79 g, 18.42 mmol) in Et_2O (100 mL). The precipitate was filtered off and air-dried, yielding admantanamine salt (8.87 g, 84%): mp 127–8 °C; HPLC 98.6%. The admantanamine salt was dissolved in EtOAc (200 mL) and washed three times with 1 N HCl and brine, dried (Na_2SO_4), and evaporated to give pure **3b** (6.2 g, 84%) as a colorless oil: TLC R_f 0.60 (CH_2Cl_2 75%, MeOH 23%, H_2O 2%); $[\alpha]^{20}_D$ −19.2° (*c* 0.72/MeOH); HPLC 98.4%; ^{1}H NMR (DMSO-d_6) δ 7.38 (s, 5H), 7.23 (m, 5H), 5.15 (s, 2H), 4.73 (m, 1H), 2.61 (tr, *J* = 7.5 Hz, 2H), 1.8–1.5 (m, 6H), 1.27 (m, 4H), 0.91 (tr, *J* = 6.3 H z, 3H); FAB MS *m/e* 405 (M^+ + H). Anal. ($C_{22}H_{29}O_5P$) C, H, P.

(2*S*)-2-(((Phenylethyl)hydroxyphosphoryl)oxy)-hexanoic acid benzyl ester (3a) was obtained from **2a** and phosphenic acid **1a** as described for **3b** as a colorless oil: TLC R_f 0.58 (CH_2Cl_2 82%, MeOH 17%, H_2O 1%); $[\alpha]^{20}_D$ −19.6° (*c* 0.5/MeOH); HPLC 98.7%; ^{1}H NMR (DMSO-d_6) δ 7.18 (m, 5H), 7.23 (m, 5H), 5.21 (s, 2H), 4.85 (m, 1 H), 2.75 (m, 2H), 1.8–1.6 (m, 4H), 1.38 (m, 4H), 0.95 (tr, *J* = 6.5 Hz, 3H); FAB MS *m/e* 391 (M^+ + H). Anal. ($C_{21}H_{27}O_5P$) C, H, P.

(2*S*)-2-(((4-Phenylbutyl)hydroxyphosphoryl)oxy)-hexanoic acid benzyl ester (3c) was obtained from **2a** and phosphenic acid **1c** as described for **3b** as a colorless oil: TLC R_f 0.45 (CH_2Cl_2 85%, MeOH 14%, H_2O 1%); $[\alpha]^{20}_D$ −23.9° (*c* 0.96/MeOH); HPLC 97.8%; ^{1}H NMR (DMSO-d_6) δ 7.39 (s, 5H), 7.23 (m, 5H), 5.16 (s, 2H), 4.72 (m, 1 H), 2.55 (tr, *J* = 7.5 Hz, 2H), 1.85–1.50 (m, 6H), 1.29 (m, 6H), 0.93 (tr, *J* = 6.2 Hz, 3H); FAB MS *m/e* 419 (M^+ + H). Anal. ($C_{23}H_{31}O_5P$) C, H, P.

(2*S*)-2-(((3-Phenylpropyl)hydroxyphosphoryl)oxy)-pentanoic acid benzyl ester (3d) was obtained from **2b** and phosphenic acid **1b** as described for **3b** as a colorless oil: TLC R_f 0.59 (CH_2Cl_2 75%, MeOH 23%, H_2O 2%); $[\alpha]^{20}_D$ −22.0° (*c* 1.03/MeOH); HPLC 97.9%; ^{1}H NMR (DMSO-d_6) δ 7.38 (s, 5H), 7.23 (m, 5H), 5.15 (s, 2H), 4.72 (m, 1H), 2.62 (tr, *J* = 7.5 Hz, 2H), 1.85–1.50 (m, 6H), 1.27 (m, 2H), 0.89 (tr, *J* = 6.5 Hz, 3H); FAB MS *m/e* 391 (M^+ + H). Anal. ($C_{21}H_{27}O_5P$) C, H, P.

(2*S*)-2-(((3-Phenylpropyl)hydroxyphosphoryl)oxy)-propanoic acid benzyl ester (3e) was obtained from **2c** and phosphenic acid **1b** as described for **3b** as a colorless oil: TLC R_f 0.40 (MTBE 75%, MeOH 23%, H_2O 2%): $[\alpha]^{20}_D$ −15.3° (*c* 0.97/MeOH); HPLC 98.5%; ^{1}H NMR (DMSO-d_6) δ 7.38 (s, 5H), 7.24 (m, 5H), 5.15 (s, 2H), 4.85 (m, 1H), 2.62 (tr, *J* = 7.3 Hz, 2H), 1.85–1.50 (m, 4H), 1.42 (d, *J* = 7.5 Hz, 3H); FAB MS *m/e* 363 (M^+ + H). Anal. ($C_{19}H_{23}O_5P$) C, H, P.

(2*R*)-2-(((3-Phenylpropyl)hydroxyphosphoryl)oxy)-hexanoic acid benzyl ester (3f) was obtained from **2d** and phosphenic acid **1b** as described for **3b** as a colorless oil: TLC R_f 0.44 (CH_2Cl_2 75%, MeOH 23%, H_2O 2%); $[\alpha]^{20}_D$ +25.3° (*c* 0.95/MeOH); HPLC 98.6% ^{1}H NMR (DMSO-d_6) δ 7.38 (s, 5H), 7.24 (m, 5H), 5.15 (s, 2H), 4.60 (m, 1H), 2.65 (tr, *J* = 7.5 Hz, 2H), 1.80–1.55 (m, 6H), 1.45 (m, 4H), 0.93 (tr, *J* = 7.2 Hz, 3H); FAB MS *m/e* 405 (M^+ + H). Anal. ($C_{22}H_{29}O_5P$) C, H, P.

(2*R*)-2-(((3-Phenylpropyl)hydroxyphosphoryl)oxy)-pentanoic acid benzyl ester (3g) was obtained from **2e** and phosphenic acid **1b** as described for **3b** as a colorless oil: TLC R_f 0.50 (CH_2Cl_2 70%, MeOH 28%, H_2O 2%); $[\alpha]^{20}_D$ +25.1° (*c* 0.95/MeOH); HPLC 99.7%; ^1H NMR (DMSO-d_6) δ 7.42 (s, 5H), 7.25 (m, 5H), 5.18 (s, 2H), 4.26 (m, 1H), 2.65 (tr, J = 7.5 Hz, 2H), 1.9–1.5 (m, 6H), 1.34 (m, 2H), 0.89 (tr, J = 7.3 Hz, 3H); FAB MS *m/e* 391 (M^+ + H). Anal. ($C_{21}H_{27}O_5P$) C, H, P.

(2*R*)-2-(((3-Phenylpropyl)hydroxyphosphoryl)oxy)-propanoic acid benzyl ester (3h) was obtained from **2f** and phosphenic acid **1b** as described for **3b** as a colorless oil: TLC R_f 0.40 (MTBE 75%, MeOH 23%, H_2O 2%): $[\alpha]^{20}_D$ +15.6° (*c* 1.02/MeOH); HPLC 99.2%; ^1H NMR (DMSO-d_6) δ 7.38 (s, 5H), 7.23 (m, 5H), 5.12 (s, 2H), 4.82 (m, 1H), 2.63 (tr, J = 7.5 Hz, 2H), 1.85–1.50 (m, 4H), 1.41 (d, J = 7.5 Hz, 3H); FAB MS *m/e* 363 (M^+ + H). Anal. ($C_{19}H_{23}O_5P$) C, H, P.

(2*S*)-2-(((2-Phenylethyl)hydroxyphosphoryl)oxy)-hexanoic Acid (4a). Benzyl ester **3a** (5.5 g, 14.1 mmol) was dissolved in dioxane (70 mL) and treated with 1 N LiOH (35.6 mL). After being stirred for 1 h at room temperature, the reaction mixture was poured into 1 N HCl solution (100 mL) and extracted with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated to dryness to give a colorless oil (4.15 g, 98%): TLC R_f 0.74 (CH_2Cl_2 83%, MeOH 16%, H_2O 1%); $[\alpha]^{20}_D$ −14.5° (*c* 0.96/MeOH); HPLC 97%; ^1H NMR (DMSO-d_6) δ 7.24 (m, 5H), 4.58 (m, 1H), 2.78 (m, 2H), 1.8–1.6 (m, 4H), 0.97 (tr, J = 6.5 Hz, 3H); FAB MS *m/e* 301 (M^+ + H). Anal. ($C_{14}H_{21}O_5P$) C, H, P.

(2*S*)-2-(((3-Phenylpropyl)hydroxyphosphoryl)oxy)-hexanoic Acid (4b). This acid was prepared in 95% yield from benzyl ester **3b** as described for acid **4a**: TLC R_f = 0.16 (CH_2Cl_2 60%, MeOH 38%, H_2O 2%); $[\alpha]^{20}_D$ −13.0° (*c* 1.03/MeOH); HPLC 97.8%; ^1H NMR (DMSO-d_6) δ 7.23 (m, 5H), 4.62 (m, 1H), 2.72 (tr, J = 7.2 Hz, 2H), 1.8–1.5 (m, 6H), 1.33 (m, 4H), 0.93 (tr, J = 7.3 Hz, 3H); FAB MS *m/e* 315 (M^+ + H). Anal. ($C_{15}H_{23}O_5P$) C, H, P.

(2*S*)-2-(((4-Phenylbutyl)hydroxyphosphoryl)oxy)-hexanoic Acid (4c). This acid was prepared in 93% yield from benzyl ester **3c** as described for acid **4a**: TLC R_f = 0.18 (CH_2Cl_2 70%, MeOH 30%, H_2O 3%); $[\alpha]^{20}_D$ −12.5° (*c* 1.05/MeOH); HPLC 98.3%; ^1H NMR (DMSO-d_6) δ 7.25 (m, 5H), 4.63 (m, 1H), 2.73 (tr, J = 7.5 Hz, 2H), 1.83–1.50 (m, 6H), 1.35 (m, 6H), 0.95 (tr, J = 7.5 Hz, 3H); FAB MS *m/e* 329 (M^+ + H). Anal. ($C_{16}H_{25}O_5P$) C, H, P.

(2*S*)-2-(((3-Phenylpropyl)hydroxyphosphoryl)oxy)-pentanoic Acid (4d). This acid was prepared in 96% yield from benyl ester **3d** as described for acid **4a**: TLC, R_f = 0.28 (CH_2Cl_2 60%, MeOH 38%, H_2O 2%); $[\alpha]^{20}_D$ −15.0° (*c* 0.99/MeOH); HPLC 96.7%; ^1H NMR (DMSO-d_6) δ 7.25 (m, 5H), 4.62 (m, 1H), 2.63 (tr, J = 7.5 Hz, 2H), 1.85–1.50 (m, 6H), 1.43 (m, 2H), 0.91 (tr, J = 7.3 Hz, 3H); FAB MS *m/e* 301 (M^+ + H). Anal. ($C_{14}H_{21}O_5P$) C, H, P.

(2*S*)-2-(((3-Phenylpropyl)hydroxyphosphoryl)oxy)-propanoic acid (4e). This acid was prepared in 97% yield from benzyl ester **3e** as described for acid **4a**: TLC R_f 0.16 (CH_2Cl_2 70%, MeOH 30%, H_2O 3%); $[\alpha]^{20}_D$ −10.8° (*c* 1.04/MeOH); HPLC 99.6%; ^1H NMR (DMSO-d_6) δ 7.23 (m, 5H), 4.65 (m, 1H), 2.65 (tr, J = 7.3 Hz, 2H), 1.85–1.50 (m, 4H), 1.39 (d, J = 7.5 Hz, 3H); FAB MS *m/e* 273 (M^+ + H). Anal. ($C_{12}H_{17}O_5P$) C, H, P.

(2*R*)-2-(((3-Phenylpropyl)hydroxyphosphoryl)oxy)-hexanoic Acid (4f). This acid was prepared by 93% yield from benzyl ester **3f** as described for acid **4a**: TLC R_f 0.21 (CH_2Cl_2 60%, MeOH 38%, H_2O 2%); $[\alpha]^{20}_D$ +12.8° (*c* 1.06/MeOH); HPLC 97.5%; ^1H NMR (DMSO-d_6) δ 7.25 (m, 5H), 4.57 (m, 1H), 2.65 (tr, J = 7.5 Hz, 2H), 1.90–1.50 (m, 6H), 1.28 (m, 4H), 0.91 (tr, J = 6.5 Hz, 3H); FAB MS *m/e* 315 (M^+ + H). Anal. ($C_{15}H_{23}O_5P$) C, H, P.

(2*R*)-2-(((3-Phenylpropyl)hydroxyphosphoryl)oxy)-pentanoic Acid (4g). This acid was prepared in 96% yield from benzyl ester **3g** as described for acid **4a**: TLC R_f 0.20 (CH_2Cl_2 60%, MeOH 38%, H_2O 2%); $[\alpha]^{20}_D$ +16.5 (*c* 1.05/MeOH); HPLC 98.2%; ^1H NMR (DMSO-d_6) δ 7.26 (m, 5H), 4.63 (m, 1H), 2.63 (tr, J = 7.5 Hz, 2H), 1.95–1.50 (m, 6H), 1.33 (m, 2H), 0.92 (tr, J = 7.5 Hz, 3H); FAB MS *m/e* 301 (M^+ + H). Anal. ($C_{14}H_{21}O_5P$) C, H, P.

(2*R*)-2-(((3-Phenylpropyl)hydroxyphosphoryl)oxy)-propanoic Acid (4h). This acid was prepared in 96% yield from benzyl ester **3h** as described for acid **4a**: TLC R_f 0.16 (CH_2Cl_2 70%, MeOH 30%, H_2O 3%); $[\alpha]^{20}_D$ +11.2° (*c* 1.01/MeOH); HPLC 98.3%; ^1H NMR (DMSO-d_6) δ 7.24 (m, 5H), 4.63 (m, 1H), 2.63 (tr, J = 7.5 Hz, 2H), 1.85–1.55 (m, 4H), 1.38 (d, J = 7.5 Hz, 3H); FAB MS *m/e* 273 (M^+ + H). Anal. ($C_{12}H_{17}O_5P$) C, H, P.

(2*S*)-2-(((-Ethyloxy)(3-phenylpropyl)phosphoryl)oxy)-hexanoic Acid Benzyl Esters (5a + 5b). To a solution of benzyl ester **3b** (8.2 g, 21 mmol) in 30 mL of dry DMF was added K_2CO_3 (3.19 g, 23.1 mmol) and EtJ (5.36 g, 32.3 mmol). After stirring for 14 h at room temperature, the reaction mixture was poured into water (400 mL) and extracted with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated to dryness. The diastereomers were separated by chromatography on silica gel using hexane/MTBE (40:60) as eluent. The benzyl ester **5a** (less polar isomer) was obtained as pale yellow oil (3.1 g, 35%): TLC R_f 0.50 (hexane/MTBE, 30:70); $[\alpha]^{20}_D$ −33.5° (*c* 0.95/MeOH); HPLC 98.3%; ^1H NMR (DMSO-d_6) δ 7.38 (s, 5H), 7.23 (m, 5H), 5.18 (s, 2H), 4.77 (m, 1H), 3.95 (m, 2H), 2.62 (m, 2H) 1.77 (m, 6H), 1.41 (m, 4H), 1.15 (tr, J = 7.5 Hz, 3H), 0.86 (tr, J = 7.3 Hz, 3H); FAB MS *m/e* 433 (M^+ + H). Anal. ($C_{24}H_{33}O_5P$) C, H, P.

The benzyl ester **5b** (more polar isomer) was obtained as pale yellow oil (3.46 g, 40%): TLC R_f 0.45 (hexane/MTBE, 30:70); $[\alpha]^{20}_D$ −22.2° (*c* 1.07/MeOH); HPLC 98.8%; ^1H NMR (DMSO-d_6) δ 7.41 (s, 5H), 7.20 (m, 5H), 5.17 (s, 2H), 4.83 (m, 1H), 3.91 (m, 2H), 2.58 (m, 2H), 1.79 (m, 6H), 1.43 (m, 4H), 1.23 (tr, J = 7.5 Hz, 3H), 0.85 (tr, J = 7.3 Hz, 3H); FAB MS *m/e* 433 (M^+ + H). Anal. ($C_{24}H_{33}O_5P$) C, H, P.

(2*R*)-2-(((Ethyloxy)(3-phenylpropyl)phosphoryl)oxy)-hexanoic Acid Benzyl Esters (5c + 5d). These benzyl esters were prepared from **3f** as described for **5a + 5b**. Benzyl ester **5c** (less polar isomer) was obtained as a slightly yellow oil in 32% yield: TLC R_f 0.50 (hexane/MTBE, 30:70); $[\alpha]^{20}_D$ +33.8° (*c* 0.98/MeOH); HPLC 97.9%; ^1H NMR (DMSO-d_6) δ 7.38 (s, 5H), 7.22 (m, 5H), 5.17 (s, 2H), 4.77 (m, 1H), 3.92 (m, 2H), 2.63 (tr, J = 7.2 Hz, 2H), 1.75 (m, 6H), 1.39 (m, 4H), 1.15 (tr, J = 7.5 Hz, 3H), 0.86 (tr, J = 7.5 Hz, 3H); FAB MS *m/e* 433 (M^+ + H). Anal. ($C_{24}H_{33}O_5P$) C, H, P.

Benzyl ester **5d** (more polar isomer) was obtained as a yellow oil in 46% yield: TLC R_f 0.43 (hexane/MTBE, 30:70); $[\alpha]^{20}_D$ +21.6° (*c* 1.07/MeOH); HPLC 98.9%; ^1H NMR (DMSO-d_6) δ 7.42 (s, 5H), 7.23 (m, 5H), 5.17 (s, 1H), 4.83 (m, 1H), 3.89 (m, 2H), 2.61 (tr, J = 7.3 Hz, 2H), 1.77 (m, 6H), 1.43 (m, 4H), 1.23 (tr, J = 7.5 Hz, 3H), 0.85 (tr, J = 7.5 Hz, 3H); FAB MS *m/e* 433 (M^+ + H). Anal. ($C_{24}H_{33}O_5P$) C, H, P.

(2*S*)-2-(((Ethyloxy)(3-phenylpropyl)phosphoryl)oxy)-pentanoic Acid Benzyl Esters (5e + 5f). These benzyl esters were prepared from **3d** as described for **5a + 5b**. Benzyl ester **5e** (less polar isomer) was obtained as a pale yellow oil in 27.8% yield: TLC R_f = 0.40 (hexane/MTBE, 40:60); $[\alpha]^{20}_D$ −33.0° (*c* 1.0/EtOH); HPLC 99.3%; ^1H NMR (DMSO-d_6) δ 7.38 (s, 5H), 7.23 (m, 5H), 5.17 (s, 2H), 4.77 (m, 1H), 3.95 (m, 2H), 2.67 (tr, J = 7.2 Hz, 2H), 1.75 (m, 6H), 1.33 (m, 2H), 1.15 (tr, J = 7.5 Hz, 3H), 0.88 (tr, J = 7.5 Hz, 3H); FAB MS *m/e* 419 (M^+ + H). Anal. ($C_{23}H_{31}O_5P$) C, H, P.

Benzyl ester **5f** (more polar isomer) was obtained as yellow oil in 42% yield: TLC R_f 0.35 (hexane/MTBE, 40:60); $[\alpha]^{20}_D$ −17.5° (*c* 0.99/EtOH); HPLC 98.3%; ^1H NMR (DMSO-d_6) δ 7.42 (s, 5H), 7.25 (m, 5H), 5.19 (s, 2H), 4.86 (m, 1H), 4.0 (m, 2H), 2.61 (tr, J = 7.2 Hz, 2H), 1.55 (m, 6H), 1.41 (m, 2H), 1.23 (tr, J = 7.5 Hz, 3H), 0.87 (tr, J = 7.5 Hz, 3H); FAB MS *m/e* 419 (M^+ + H). Anal. ($C_{23}H_{31}O_5P$) C, H, P.

(2*R*)-2-(((Benzyloxy)(3-phenylpropyl)phosphoryl)oxy)-hexanoic Acid Benzyl Esters (5g + 5h). These benzyl esters were prepared from **3f** as described for **5a + 5b** by using benzyl bromide as alkylating agent. Benzyl ester **5g** was obtained as pale yellow oil in 26.5% yield: TLC R_f 0.49 (hexane/MTBE, 50:50); $[\alpha]^{20}_D$ +20.3° (*c* 1.17/MeOH); HPLC 98.2%; ^1H NMR (DMSO-d_6) δ 7.39 (s, 5H), 7.28 (m, 5H), 7.23 (m, 5H) 5.19 (s, 2H), 5.17 (s, 2H), 4.80 (m, 1H), 2.62 (tr, J = 7.3 Hz, 2H), 1.71 (m, 6H), 1.42 (m, 4H), 0.85 (tr, J = 7.5 Hz, 3H); FAB MS *m/e* 495 (M^+ + H). Anal. ($C_{29}H_{35}O_5P$) C, H, P.

Benzyl ester **5h** (more polar isomer) was obtained as a slightly yellow oil in 46% yield. TLC R_f 0.42 (hexane/MTBE, 50:50);

$[\alpha]^{20}_D$ +23.0° (c 1.05/MeOH); HPLC 99.1%; ^{1}H NMR (DMSO-d_6) δ 7.38 (s, 5H), 7.31 (m, 5H), 7.25 (m, 5H), 5.23 (s, 2H), 5.17 (s, 2H), 4.85 (m, 1H), 2.63 (tr, J = 7.2 Hz, 2H), 1.68 (m, 6H), 1.41 (m, 4H), 0.92 (tr, J = 7.5 Hz, 3H); FAB MS m/e 495 (M^+ + H). Anal. ($C_{29}H_{35}O_5P$) C, H, P.

(2S)-2-(((Ethyloxy)(3-phenylpropyl)phosphoryl)oxy)-hexanoic Acid (6a). The benzyl ester **5a** (6.4 g, 14.8 mmol) was dissolved in EtOH (100 mL) and treated with 5% Pd/C (3 g) and stirred under hydrogen atmosphere (1 bar) for 6 h. The mixture was filtered through Celite, and the filtrate was evaporated to dryness. The crude product was chromatographed on silica gel using CH_2Cl_2/MeOH/H_2O (70%/28%/2%) as eluent. There was obtained acid **6a** (4.15 g, 81.5%) as a pale yellow oil: TLC R_f 0.65 (CH_2Cl_2 70%, MeOH 28%, H_2O 2%); $[\alpha]^{20}_D$ −12.1° (c 0.55/MeOH); HPLC 97.8%; ^{1}H NMR (DMSO-d_6) δ 7.27 (m, 5H), 4.65 (m, 1H), 3.97 (m, 2H), 2.65 (tr, J = 7.3 Hz, 2H), 1.8–1.5 (m, 6H), 1.43 (m, 4H), 1.23 (tr, J = 7.5 Hz, 3H), 0.91 (tr, J = 7.5 Hz, 3H); FAB MS m/e 343 (M^+ + H). Anal. ($C_{17}H_{27}O_5P$) C, H, P.

(2S)-2-(((Ethyloxy)(3-phenylpropyl)phosphoryl)oxy)-hexanoic Acid (6b). This acid was prepared in 86% yield from benzyl ester **5b** as described for acid **5a**: TLC R_f 0.62 (CH_2Cl_2 70%, MeOH 28%, H_2O 2%); $[\alpha]^{20}_D$ −19.5° (c 0.85/MeOH); HPLC 97.8%; ^{1}H NMR (DMSO-d_6) δ 7.25 (m, 5H), 4.63 (m, 1H), 4.05 (m, 2H), 2.61 (tr, J = 7.3 Hz, 2H), 1.85–1.5 (m, 6H), 1.43 (m, 4H), 1.23 (tr, J = 7.5 Hz, 3H), 0.91 (tr, J = 7.5 Hz, 3 H); FAB MS m/e 343 (M^+ + H). Anal. ($C_{17}H_{27}O_5P$) C, H, P.

(2R)-2-((Ethyloxy)(3-phenylpropyl)phosphoryl)oxy)hexanoic Acid (6c). This acid was prepared in 83% yield from benzyl ester **5c** as described for **6a**: TLC R_f 0.58 (CH_2Cl_2 75%, MeOH 23%, H_2O 2%); $[\alpha]^{20}_D$ +18.9° (c 0.83/MeOH); HPLC 98.2%; ^{1}H NMR (DMSO-d_6) δ 7.23 (m, 5H), 4.65 (m, 1H), 3.95 (m, 2H), 2.63 (tr, J = 7.5 Hz, 2H), 1.78 (m, 6H), 1.33 (m, 4H), 1.22 (tr, J = 7.3 Hz, 3H), 0.85 (tr, J = 7.5 Hz, 3H); FAB MS m/e 343 (M^+ + H). Anal. ($C_{17}H_{27}O_5P$) C, H, P.

(2R)-2-(((Ethyloxy)(3-phenylpropyl)phosphoryl)oxy)-hexanoic Acid (6d). This acid was prepared in 87% yield from benzyl ester **5d** as described for acid **6a**: TLC R_f 0.55 (CH_2Cl_2 75%, MeOH 23%, H_2O 2%); $[\alpha]^{20}_D$ +13.1° (c 0.35/MeOH); HPLC 98.2%; ^{1}H NMR (DMSO-d_6) δ 7.25 (m, 5H), 4.62 (m, 1H), 3.92 (m, 2H), 2.62 (tr, J = 7.5 Hz, 2H), 1.75 (m, 6H), 1.43 (m, 4H), 1.20 (tr, J = 7.3 Hz, 3H), 0.92 (tr, J = 7.5 Hz, 3H); FAB MS m/e 343 (M^+ + H). Anal. ($C_{17}H_{27}O_5P$) C, H, P.

(2S)-2-(((Ethyloxy)(3-phenylpropyl)phosphoryl)oxy)-pentanoic Acid (6e). This acid was prepared in 89% yield from benzyl ester **5e** as described for acid **6a**: TLC R_f 0.35 (CH_2Cl_2 85%, MeOH 15%, H_2O 1%); $[\alpha]^{20}_D$ −11.7° (c 1.04/MeOH); HPLC 98.3%; ^{1}H NMR (DMSO-d_6) δ 7.26 (m, 5H), 4.62 (m, 1H), 4.02 (m, 2H), 2.62 (tr, J = 7.5 Hz, 2H), 1.8–1.5 (m, 6H), 1.43 (m, 2H), 1.22 (tr, J = 7.5 Hz, 3H), 0.93 (tr, J = 7.5 Hz, 3H); FAB MS m/e 329 (M^+ + H). Anal. ($C_{16}H_{25}O_5P$) C, H, P.

(2S)-2-(((Ethyloxy)(3-phenylpropyl)phosphoryl)oxy)-pentanoic Acid (6f). This acid was prepared in 83% yield from benzyl ester **5f** as described for **6a**: TLC R_f 0.33 (CH_2Cl_2 85%, MeOH 15%, H_2O 1%); $[\alpha]^{20}_D$ −24.1° (c 0.99/MeOH); ^{1}H NMR (DMSO-d_6) δ 7.24 (m, 5H), 4.66 (m, 1H), 3.97 (m, 2H), 2.65 (tr, J = 7.5 Hz, 2H), 1.8–1.5 (m, 6H), 1.42 (m, 2H), 1.21 (tr, J = 7.5 Hz, 3H), 0.88 (tr, J = 7.5 Hz, 3H); FAB MS m/e 329 (M^+ + H). Anal. ($C_{16}H_{25}O_5P$) C, H, P.

(2R)-2-(((Benzyloxy)(3-phenylpropyl)phosphoryl)oxy)-hexanoic Acid (6g). This acid was prepared in 88% yield from benzyl ester **5g** as described for acid **6a**: TLC R_f = 0.43 (CH_2Cl_2 85%, MeOH 15%, H_2O 1%); $[\alpha]^{20}_D$ +17.9° (c 1.02/EtOH); HPLC 97.2%; ^{1}H NMR (DMSO-d_6) δ 7.28 (m, 5H), 7.25 (m, 5H), 5.17 (s, 2H), 4.62 (m, 1H), 2.63 (tr, J = 7.5 Hz, 2H), 1.8–1.5 (m, 6H), 1.42 (m, 4H), 0.89 (tr, J = 7.5 Hz, 3H); FAB MS m/e 405 (M^+ + H). Anal. ($C_{22}H_{29}O_5P$) C, H, P.

(2R)-2-(((Benzyloxy)(3-phenylpropyl)phosphoryl)oxy)-hexanoic Acid (6h). This acid was prepared in 92% yield from benzyl ester **5h** as described for acid **6a**: TLC R_f 0.37 (CH_2Cl_2 85%, MeOH 15%, H_2O 1%); $[\alpha]^{20}_D$ +13.4° (c 0.97/EtOH); HPLC 97.2%; ^{1}H NMR (DMSO-d_6) δ 7.27 (m, 5H), 7.25 (m, 5H), 5.15 (s, 2H), 4.66 (m, 1H), 2.65 (tr, J = 7.5 Hz, 2H), 1.8–1.5 (m, 6H), 1.41 (m, 4H), 0.88 (tr, J = 7.5 Hz, 3H): FAB MS m/e 405 (M^+ + H). Anal. ($C_{22}H_{29}O_5P$) C, H, P.

General Coupling Procedure for Inhibitors 7a–n, 8a–i, and 9a–f. (2R,4S,5S)-N-Butyl-6-cyclohexyl-4-hydroxy-2-methyl-5-((2S)-2-((phenethylhydroxyphosphoryl)oxy)-hexanamido)hexanamide (7a). A solution of acid **4a** (600 mg, 2 mmol) in dry THF (20 mL) at 0 °C was treated with 1,1'-carbonyldiimidazole (390 mg, 2.4 mmol) and stirred for 1 h. To this solution were added (2R,4S,5S)-N-butyl-6-cyclohexyl-5-amino-4-hydroxy-2-methylhexanamide hydrochloride (670 mg, 2 mmol) and NEt_3 (670 mg, 6.6 mmol). The mixture was diluted with EtOAc and washed successively with 1 N HCl, saturated $NaHCO_3$ solution, and 5% $KHSO_4$, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on silical gel using CH_2Cl_2/MeOH (9:1) as eluent, yielding 610 mg (42.5%) of **7a** as white solid: mp 81–2 °C; $[\alpha]^{20}_D$ −47.1° (c 0.80/MeOH); HPLC 97.6%; FAB MS m/e 581 (M^+ + H). Anal. ($C_{31}H_{53}N_2O_6P$) C, H, N, P.

(2R,4S,5S)-N-Butyl-6-cyclohexyl-4-hydroxy-2-methyl-5-((2S)-2-(((3-phenylpropyl)hydroxyphosphoryl)oxy)-hexanamido)hexanamide (7b). This title compound was prepared from **4b** as described for **7a** to give a white solid: mp 100–2 °C; $[\alpha]^{20}_D$ −52.0° (c 0.86/MeOH); HPLC 98.9%; FAB MS m/e 595 (M^+ + H). Anal. ($C_{32}H_{55}N_2O_6P$) C, H, N, P.

(2R,4S,5S)-N-Butyl-6-cyclohexyl-4-hydroxy-2-methyl-5-((2S)-2-(((4-phenylbutyl)hydroxyphosphoryl)oxy)-hexanamido)hexanamide (7c). The title compound was prepared from **4c** as described for **7a** to give a white solid: mp 112–4 °C; $[\alpha]^{20}_D$ −50.9° (c 1.03/MeOH); HPLC 97.9%; FAB MS m/e 609 (M^+ + H). Anal. ($C_{33}H_{57}N_2O_6P\cdot 0.3H_2O$) C, H, N, P.

(2R,4S,5S)-N-Butyl-6-cyclohexyl-4-hydroxy-2-methyl-5-((2S)-2-(((3-phenylpropyl)hydroxyphosphoryl)oxy)-pentanamido)hexanamide (7d). The title compound was prepared from **4d** as described for **7a** to give a white solid: mp 178–9 °C; $[\alpha]^{20}_D$ −40.9° (c 0.89/MeOH); HPLC 97.6%; FAB MS m/e 581 (M^+ + H). Anal. ($C_{31}H_{53}N_2O_6P$) C, H, N, P.

(2R,4S,5S)-N-Butyl-6-cyclohexyl-4-hydroxy-2-methyl-5-((2S)-2-(((3-phenylpropyl)hydroxyphosphoryl)oxy)-propanamido)hexanamide (7e). The title compound was prepared from **4e** as described for **7a** to give a white solid: mp 123–5 °C; $[\alpha]^{20}_D$ −22.2° (c 1.03/MeOH); HPLC 96.7%; FAB MS m/e 553 (M^+ + H). Anal. ($C_{29}H_{49}N_2O_6P$) C, H, N, P.

(2R,4S,5S)-N-Butyl-6-cyclohexyl-4-hydroxy-2-methyl-5-((2R)-2-(((3-phenylpropyl)phosphoryl)oxy)hexanamido)-hexanamide (7f). The title compound was prepared from **4f** as described for **7a** to give a white solid: mp 139–42 °C; $[\alpha]^{20}_D$ −10.5° (c 0.87/MeOH); HPLC 96.5%; FAB MS m/e 595 (M^+ + H). Anal. ($C_{32}H_{55}N_2O_6P\cdot H_2O$) C, H, N, P.

(2R,4S,5S)-N-Butyl-6-cyclohexyl-4-hydroxy-2-methyl-5-((2R)-2-(((3-phenylpropyl)phosphoryl)oxy)pentanamido)-hexanamide (7g). The title compound was prepared from **4g** as described for **7a** to give a white solid: mp 153–7 °C; $[\alpha]^{20}_D$ −13.7° (c 0.69/MeOH); HPLC 97.3%; FAB MS m/e 581 (M^+ + H). Anal. ($C_{31}H_{53}N_2O_6P$) C, H, N, P.

(2R,4S,5S)-N-Butyl-6-cyclohexyl-4-hydroxy-2-methyl-5-((2R)-2-(((3-phenylpropyl)phosphoryl)oxy)propanamido)-hexanamide (7h). The title compound was prepared from **4h** as described for **7a** to give a white solid: mp 127–9 °C; $[\alpha]^{20}_D$ −23.9° (c 0.75/MeOH); HPLC 98.3%; FAB MS m/e 553 (M^+ + H). Anal. ($C_{29}H_{49}N_2O_6P\cdot 0.2H_2O$) C, H, N, P.

(2R,4S,5S)-N-Butyl-6-cyclohexyl-4-hydroxy-2-methyl-5-((2S)-2-(((ethyloxy)(3-phenylpropyl)phosphoryl)oxy)hexanamido)hexanamide (7i). The title compound was prepared from **6a** as described for **7a** to give a slightly yellow oil: $[\alpha]^{20}_D$ −55.3° (c 0.90/MeOH); TLC R_f 0.26 (EtOAc/MeOH, 100:1); HPLC 98.6%; FAB MS m/e 623 (M^+ + H). Anal. ($C_{34}H_{59}N_2O_6P$) C, H, N, P.

(2R,4S,5S)-N-Butyl-6-cyclohexyl-4-hydroxy-2-methyl-5-((2S)-2-(((ethyloxy)(3-phenylpropyl)phosphoryl)oxy)hexanamido)hexanamide (7j). The title compound was prepared from **6b** as described for **7a** to give a slightly yellow oil: $[\alpha]^{20}_D$ −31.1° (c 0.85/MeOH); TLC R_f 0.20 (EtOAc/MeOH, 100:1); HPLC 97.9%; FAB MS m/e 623 (M^+ + H). Anal. ($C_{34}H_{59}N_2O_6P$) C, H, N, P.

(2R,4S,5S)-N-Butyl-6-cyclohexyl-4-hydroxy-2-methyl-5-((2R)-2-(((ethyloxy)(3-phenylpropyl)phosphoryl)oxy)hexanamido)hexanamide (7k). The title compound was prepared from **6c** as described for **7a** to give a slightly yellow oil: $[\alpha]^{20}_D$

−18.0° (c 0.49/MeOH); TLC R_f 0.28 (EtOAc/MeOH, 99:1); HPLC 98.6%; FAB MS m/e 623 ($M^+ + H$). Anal. ($C_{34}H_{59}N_2O_6P \cdot 0.5H_2O$) C, H, N, P.

(2*R*,4*S*,5*S*)-*N*-Butyl-6-cyclohexyl-4-hydroxy-2-methyl-5-((2*R*)-2-(((ethyloxy)(3-phenylpropyl)phosphoryl)oxy)hexanamido)hexanamide (7l). The title compound was prepared from **6d** as described for **7a** to give a slightly yellow oil: $[\alpha]^{20}_D$ −25.5° (c 0.90/MeOH); TLC R_f 0.22 (EtOAc/MeOH, 99:1); HPLC 97.9%; FAB MS m/e 623 ($M^+ + H$). Anal. ($C_{34}H_{59}N_2O_6P$) C, H, N, P.

(2*R*,4*S*,5*S*)-*N*-Butyl-6-cyclohexyl-4-hydroxy-2-methyl-5-((2*R*)-2-(((benzyloxy)(3-phenylpropyl)phosphoryl)oxy)hexanamido)hexanamide (7m). The title compound was prepared from **6g** as described for **7a** to give an oil: $[\alpha]^{20}_D$ −29.3° (c 0.78/MeOH); TLC R_f 0.49 (EtOAc/MeOH, 100:1), HPLC 98.7%; FAB MS m/e 685 ($M^+ + H$). Anal. ($C_{39}H_{61}N_2O_6P$) C, H, N, P.

(2*R*,4*S*,5*S*)-*N*-Butyl-6-cyclohexyl-4-hydroxy-2-methyl-5-((2*R*)-2-(((benzyloxy)(3-phenylpropyl)phosphoryl)oxy)hexanamido)hexanamide (7n). The title compound was prepared from **6h** as described for **7a** to give a slightly yellow oil: $[\alpha]^{20}_D$ −21.8° (c 0.45/MeOH): TLC R_f 0.42 (EtOAc/MeOH, 100:1); HPLC 98.3%; FAB MS m/e 685 ($MH^+ + H$). Anal. ($C_{39}H_{61}N_2O_6P \cdot 0.8H_2O$) C, H, N, P.

(2*S*,4*S*,5*S*)-*N*-Butyl-4-hydroxy-2-isopropyl-7-methyl-5-((2*R*)-2-(((3-phenylpropyl)hydroxyphosphoryl)oxy)hexanamido)octanamide (8a). The title compound was prepared from **4f** and (2*S*,4*S*,5*S*)-*N*-butyl-5-amino-4-hydroxy-2-isopropyl-7-methyloctanamide as described for the preparation of **7a**: mp 96–7 °C; $[\alpha]^{20}_D$ −13.8° (c 0.87/MeOH); HPLC 98.5%; FAB MS m/e 583 ($M^+ + H$). Anal. ($C_{31}H_{55}N_2O_6P$) C, H, N, P.

((2*R*)-2-(((3-Phenylpropyl)hydroxyphosphoryl)oxy)hexanoyl)-ACHPA-Ile-[*N*-((4-amino-2-methyl-5-pyrimidinyl)methyl)amide] (8b). The title compound was prepared from **4f** and H-ACHPA-Ile-[*N*-((4-amino-2-methyl-5-pyridinyl)-methyl)amide] dihydrochloride as described for the preparation of **7a**: mp 197–8 °C; $[\alpha]^{20}_D$ −37.3° (c 0.95/MeOH); HPLC 97.8%; FAB MS m/e 745 ($M^+ + H$). Anal. ($C_{38}H_{61}N_6O_7P \cdot 0.8H_2O$) C, H, N, P.

(4*S*,5*R*,6*S*)-7-Cyclohexyl-2-methyl-6-((2*R*)-2-(((3-phenylpropyl)hydroxyphosphoryl)oxy)hexanamido)heptane-4,5-diol (8c). The title compound was prepared by using **4f** and (4*S*,5*R*,6*S*)-6-amino-7-cyclohexyl-2-methylheptane-4,5-diol in the general coupling procedure to give a white solid: mp 197–9 °C; $[\alpha]^{20}_D$ −12.0° (c 1.12/MeOH); HPLC 98.5%; FAB MS m/e 540 ($M^+ + H$). Anal. ($C_{29}H_{50}NO_6P$) C, H, N, P.

(2*S*,3*S*)-4-Cyclohexyl-2-hydroxy-3-((2*R*)-2-(((3-phenylpropyl)hydroxyphosphoryl)oxy)hexanamido)butyric Acid Isopropyl Ester (8d). The title compound was prepared by using **4f** and (2*R*,3*S*)-2-amino-4-cyclohexyl-2-hydroxybutyric acid isopropyl ester hydrochloride as described for the synthesis of **7a**: mp 178–81 °C; $[\alpha]^{20}_D$ −15.7° (c 0.78/MeOH); HPLC 97.9%; FAB MS m/e 540 ($M^+ + H$). Anal. ($C_{28}H_{46}NO_7P \cdot 0.75H_2O$) C, H, N, P.

(2*S*,3*S*)-1-Cyclohexyl-2-((2*R*)-2-(((3-phenylpropyl)hydroxyphosphoryl)oxy)hexanamido)-5-(2-pyridylthio)-3-pentanol (8e). The title compound was prepared by using **4f** and (2*S*,3*S*)-2-amino-1-cyclohexyl-5-(2-pyridylthio)-3-pentanol dihydrochloride in the coupling procedure as described for **7a** to give a white solid: mp 168–9 °C; $[\alpha]^{20}_D$ −37.2° (c 0.27/MeOH); HPLC 98.7%; FAB MS m/e 591 ($M^+ + H$). Anal. ($C_{31}H_{47}N_2O_5PS$) C, H, N.

(4*S*,5*R*,6*S*)-7-Cyclohexyl-6-((2*S*)-2-(((ethyloxy)(3-phenylpropyl)phosphoryl)oxy)pentanamido)-2-methylheptane-4,5-diol (8f). The title compound was prepared by using **6e** and (4*S*,5*R*,6*S*)-6-amino-7-cyclohexyl-2-methylheptane-4,5-diol in the general coupling procedure to give a white solid: mp 111–2 °C; $[\alpha]^{20}_D$ −51.9° (c 0.93/MeOH); HPLC 97.2%; FAB MS m/e 554 ($M^+ + H$). Anal. ($C_{30}H_{52}NO_6P$) C, H, N, P.

(4*S*,5*R*,6*S*)-7-Cyclohexyl-6-((2*S*)-2-(((ethyloxy)(3-phenylpropyl)phosphoryl)oxy)pentanamido)-2-methylheptane-4,5-diol (8g). The title compound was prepared by using **6f** and (4*S*,5*R*,6*S*)-6-amino-7-cyclohexyl-2-methylheptane-4,5-diol in the general coupling procedure to give a white solid: mp 103–5 °C; $[\alpha]^{20}_D$ −43.8° (c 0.85/MeOH); HPLC 97.3%; FAB MS m/e 554 ($M^+ + H$). Anal. ($C_{30}H_{52}NO_6P \cdot H_2O$) C, H, N, P.

(2*S*,3*R*,4*S*)-1-Cyclohexyl-4-cyclopropyl-2-((2*S*)-2-(((ethyloxy)(3-phenylpropyl)phosphoryl)oxy)pentanamido)-butane-3,4-diol (8h). The title compound was prepared by using **6e** and (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-4-cyclopropylbutane-3,4-diol hydrochloride in the general coupling procedure as described for **7a** to give an oil: TLC R_f 0.25 (EtOAc/MeOH, 99:1); $[\alpha]^{20}_D$ −47.9° (c 0.43/MeOH, FAB MS m/e 538 ($M^+ + H$). Anal. ($C_{29}H_{48}NO_6P$) C, H, N, P.

(2*S*,3*R*,4*S*)-1-Cyclohexyl-4-cyclopropyl-2-((2*S*)-2-(((ethyloxy)(3-phenylpropyl)phosphoryl)oxy)pentanamido)-butane-3,4-diol (8i). The title compound was prepared by using **6f** and (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-4-cyclopropylbutane-3,4-diol hydrochloride in the general coupling procedure as described for **7a** to give an oil: TLC R_f 0.22 (EtOAc/MeOH, 99:1); $[\alpha]^{20}_D$ −32.7° (c 0.35/MeOH); FAB MS m/e 538 ($M^+ + H$). Anal. ($C_{29}H_{48}NO_6P \cdot 0.3H_2O$) C, H, N, P.

Biological Methods. In Vitro Enzyme Inhibition. The renin IC_{50} values were obtained with human EDTA plasma, utilizing the endogenous renin and angiotensinogen. Test compounds were dissolved in DMSO and diluted so that prior to addition to the assay system the solutions were 10% in DMSO. At least three different concentrations of the inhibitor that bracketed the IC_{50} value were used for determining the IC_{50} value. The final incubation mixture (750 μL) contained the following: plasma, 100 μL; maleate buffer, pH 5.5, 76 mM; EDTA, 7.2 mM; DMSO, 1%; 8-hydroxyquinoline sulfate, 8.3 mM. Samples were incubated at 30 °C for 2 h and then placed on ice; an aliquot was analyzed for angiotensin I by radioimmunoassay utilizing a commercial kit (DuPont NEN Research). The percent inhibition of the reaction was determined and the IC_{50} value was calculated.

The pepsin and cathepsin D IC_{50} values were determined by incubating hemoglobin with 20 units of porcine pepsin at pH 1.8 for 10 min at 35.5 °C and with 100 milliunits of bovine cathepsin D at pH 3.2 for 20 min at 37 °C, respectively. Hemoglobin is degraded by these enzymes to liberate peptides soluble in trichloroacetic acid. The concentration of the peptides was determined by their absorbance at 280 nm. The concentration of the inhibitor that inhibited peptide liberation (= pepsin or cathepsin D activity) by 50% was calculated.

In Vivo Activity. Female cynomolgus monkeys (Macaca fascicularis) weighing 2.5–4 kg were used. The animals were housed under constant temperature and lighting conditions and provided with food consisting of a cereal mixture, barely germ, bread fruit, and vegetables. The animals were treated daily with furosemide, 4 mg/kg im, beginning on the fourth day before an experiment. On the day of the experiment the animals were treated with the final dose of furosemide together with haloperidol, 0.3 mg/kg im, for sedation. About 1.5 h after the last treatment, the monkeys were restrained in a chair and blood pressure (BP) and heart rate (HR) were measured by the tail cuff method (Blood-Pressure-Monitor, TSE, Kronberg) as described by Wood et al.[22] for conscious marmosets. In detail, a pneumatic cuff (18-20 mm/id) and a piezoelectric pressure sensor were positioned on the tail of the monkeys. Systolic blood pressure and HR were measured every 5 min and were allowed to stabilize before drug administration. Following this, test substances were applied orally and BP and HR were measured every 5 min. Blood samples for the measurement of plasma renin activity (PRA) were collected before and after administration of the compounds as indicated. The blood samples were taken by direct puncture of the saphenous vein.

Molecular Models. Homology derived models of human renin based on the homologous aspartic protease penicillopepsin[23] or mainly on high-resolution X-ray structures of endothiapepsin[13] were used for the structure–activity studies. The hybrid model finally used for the derivation of results presented here is the later model derived by Blundell and co-workers,[13] with an exchange of loop Pro 111 to Phe 117 by the loop present in the X-ray structure of human renin (PDB[17] code 1RNE[14]).

The positioning of this loop relative to the rest of the model structure was achieved by a structural alignment of the X-ray structure to the model. The similarity scores derived for each residue pair were used for a weighted superposition of the two structures. Ligand coordinates were not used in the calculation.

The resulting superposition matrix after centering of the molecules on the origin is

0.9112	0.4098	0.0432
−0.4084	0.9121	−0.0367
−0.0545	0.0158	0.9984

Further details of this general method for the structural comparison and superposition of even distantly related protein structures are described in ref 18. The different inhibitors were model built into the protease following as far as possible the orientation and local conformation of the endothiapepsin inhibitors in their complexes. The phosphonate group was oriented to optimize possible hydrogen bonds with main chain atoms of the protein model.

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