New Inhibitors of Renin That Contain Novel Phosphostatine Leu-Val Replacements

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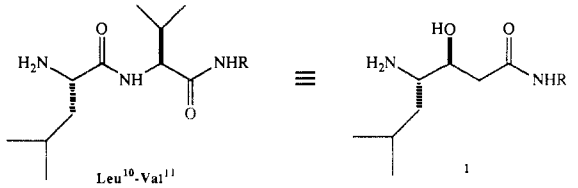
A novel series of renin inhibitors based on the Phe[8]-His[9]-Leu[10]-Val[11] substructure of reinin's natural substrate, angiotensinogen, is reported. These inhibitors retain the Phe[8]-His[9] portion of the native substructure and employ novel phosphostatine Leu[10]-Val[11] replacements (LVRs). The phosphostatine LVRs were prepared by condensing a dialkyl phosphonate ester stabilized anion with either *N*-*t*-Boc-amino aldehydes or *N*-tritylamino aldehydes (derived from the corresponding amino acid). Structure–activity relationships at the Leu[10] side chain revealed that the LVR derived from L-cyclohexylalanine provided a 130-fold boost in potency over the LVR derived from L-leucine. The dialkyl ester moiety was varied and a loss in potency was incurred when the alkyl ester was chain extended or α-branched; dimethyl esters provided optimum potency. The phosphonate moiety was replaced by a half-acid half-ester phosphonate and dimethylphosphinate; both replacements lead to a loss in potency. The more potent inhibitors (IC_{50} = 20–50 nM) were found to be selective inhibitors for renin over porcine pepsin and bovine cathepsin D (little or no inhibition was observed at 10^{-5} M).

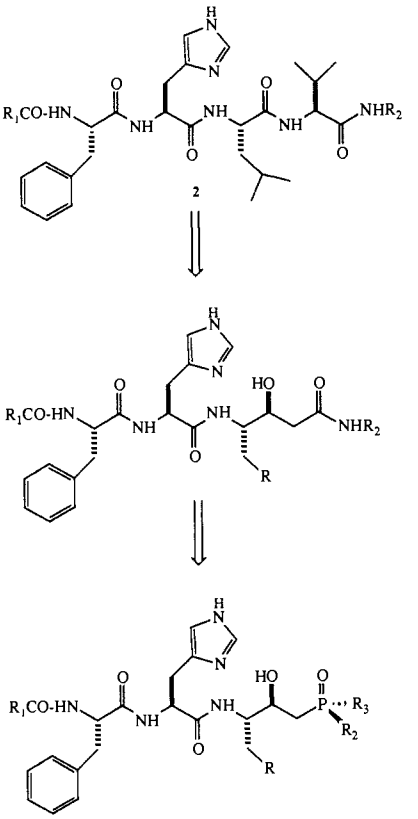
Angiotensin II (AII) is a potent octapeptide pressor agent produced by the renin–angiotensin system (RAS).[1] The RAS is initiated by the renin-catalyzed cleavage of angiotensinogen to the decapeptide angiotensin I, which is subsequently converted by angiotensin-converting enzyme (ACE) to AII. Therapeutically useful antihypertensive responses have been achieved by oral dosing with potent ACE inhibitors.[1b,2] This has served to focus attention on developing an orally active renin inhibitor as a complimentary antihypertensive agent.

Intense efforts along these lines have focused on incorporating proteolytically stabilized Leu[10]-Val[11] replacements (LVRs) at the scissile site of a minimum renin substrate sequence. The discovery that statine (1), an



uncommon, naturally occurring amino acid, served as an excellent LVR[3] was the genesis of several potent renin inhibitors derived from novel, substituted statines such as 2-alkyl-substituted statine,[4] 3-aminostatine,[5] 2,2-difluorostatine,[6] 2,2-difluorostatone,[6] and 3-amino-2,2-difluorostatine.[7]

Chart I



Scheme I

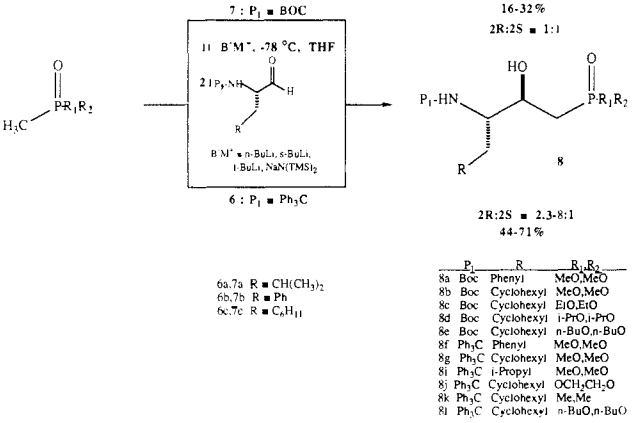


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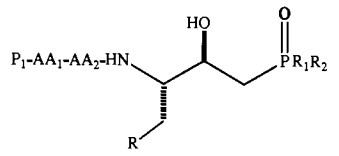
Table I. Inhibitors and in Vitro Activities Obtained at pH 6



no.	P_1	AA_1	AA_2	R	R_1, R_2	renin IC_{50}, nM	% inhibn at 10^{-5} M: porcine pepsin	% inhibn at 10^{-5} M: bovine cathepsin D
12	Boc	Phe	His	$CH(CH_3)_2$	$(OCH_3)_2$	5100	–	
13	Boc	Phe	Ala	C_6H_5	$(OCH_3)_2$	15000	–	
14	Boc	Phe	His	C_6H_5	$(OCH_3)_2$	1500	–	
15	Boc	Phe	His	C_6H_{11}	$(OCH_3)_2$	39	–	
16[a]	Boc	Phe	His	C_6H_5	$(OCH_3)_2$	4000	–	
17[a]	Boc	Phe	His	C_6H_{11}	$(OCH_3)_2$	90	–	
18	Boc	Phe	His	C_6H_{11}	$(OCH_2CH_3)_2$	72	0	0.0
19	Boc	Phe	His	C_6H_{11}	$(OCH(CH_3)_2)_2$	1500	–	
20	Boc	Phe	His	C_6H_{11}	$(O(CH_2)_3CH_3)_2$	400[b]	–	
21	Tba	Phe	His	C_6H_{11}	$(OCH_3)_2$	47	3.0	6.0
22	Etoc	Phe	His	C_6H_{11}	$(OCH_3)_2$	50	4	0.0
23[c]	Boc	Phe	His	C_6H_{11}	$(CH_3)_2$	(24% at 10^{-6} M)[d]	–	
24[c]	Boc	Phe	His	C_6H_{11}	$(CH_3)_2$	(49% at 10^{-6} M)[d]	–	
25	Boc	Phe	Leu	C_6H_{11}	$(OCH_3)_2$	20	21	86.0
26	Boc	Phe	Leu	C_6H_{11}	OH, OCH_3	350	–	
27	Boc	Phe	His	C_6H_{11}	$O(CH_2)_3O$	80	2	0.0

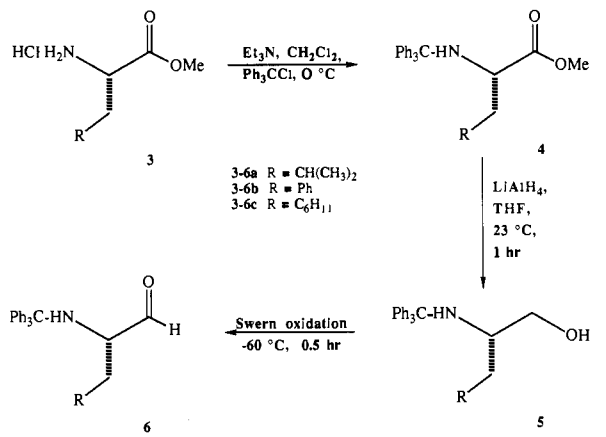
[a] The phosphostatine LVR bears a 2(*S*)-hydroxy group. [b] Tested as a 3:1 mixture of C_2-hydroxy isomers. The major component bears a 2(5)-hydroxy group. [c] Diastereomers; the relative configuration of the 2-hydroxy group in the LVR in unknown. [d] $IC_{50} > 10^{-6}$ M were not determined. The data in parentheses for these compounds are the percent inhibitions measured at the noted concentration.

Previous work from these laboratories established that inhibitors of the general formula **2**[8] represented the minimum renin substrate sequence necessary for potent inhibition of purified human renin. Combining these two concepts lead us to investigate how a phosphonyl diester replacement for the C-terminal carbonyl group of statine would influence the renin inhibitory potency of the resulting compounds (Chart I). Hereinbelow, we describe the structure–activity relationship (SAR) work that lead to the identification of a new and potent series of renin inhibitors which appends an N-protected dipeptide to novel phosphostatine-derived LVRs.

Chemistry

Synthesis: Leu[10]-Val[11] Replacement (LVR). Initially the LVRs were prepared by condensing the phosphonate anion with a *tert*-butyloxycarbonyl (BOC) protected amino aldehyde **7** which provided low yields (16–32%, see Table III) of a 1:1 mixture of isomers at the hydroxyl center. We turned to the *N*-trityl-protected aldehydes, as previously described,[9] and were rewarded with consistently higher yields (44–74%, see Table III) and modest stereoselection varying from 2.3 to 8.0:1 (Scheme I). The *N*-trityl aldehydes **6** were prepared by standard *N*-tritylation of the desired L-amino acid ester hydrochloride salt (**3**), followed by reduction to the corresponding amino alcohol (**5**) with lithium aluminum hydride and subsequent Swern[10] oxidation (Scheme II). The *N*-trityl aldehydes were condensed with the desired dialkyl phosphonate anion by adding the amino aldehydes to a preformed solution of lithium dialkyl methylphosphonate (formed by typically deprotonating the desired phosphonate with *n*-BuLi at −78 °C) in dry tetrahydrofuran at −78 °C. The condensation reaction was quenched at −78 °C, after 30 min, by adding excess saturated aqueous ammonium chloride. In certain cases (**8j** and **8k**), better yields in the condensation were obtained when *tert*-butyllithium or *sec*-butyllithium were employed (see Table III). The unpurified phosphostatine derivatives (**8**) were isolated by an extractive process and purified by column chromatography after determining the ratio of diastereomers by ^1H NMR analysis of the unpurified samples.

Scheme II



Selection of the *N*-trityl protecting group was crucial to the success of the phosphonate aldol reaction. When *N*-*t*-Boc protection was employed, condensation with lithium dialkyl methylphosphonates **7** resulted in con-

(6) (a) Thaisrivongs, S.; Pals, D. T.; Kati, W. M.; Turner, S. R.; Thomasco, L. M.; Watt, W. *J. Med. Chem.* **1986**, *29*, 2080 and references therein. (b) Fearon, K.; Spaltenstein, A.; Hopkins, P. B.; Gelb, M. H. *J. Med. Chem.* **1987**, *30*, 1617.

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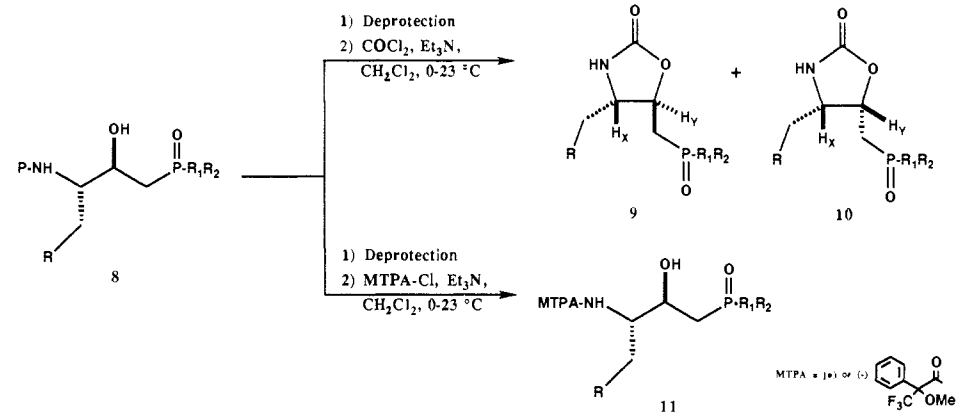
Scheme III



	P	R	R_1,R_2		R	R_1,R_2
8a	Boc	Phenyl	MeO,MeO	9-11a	Phenyl	MeO,MeO
8b	Boc	Cyclohexyl	MeO,MeO	b	Cyclohexyl	MeO,MeO
8c	Boc	Cyclohexyl	EtO,EtO	c	Cyclohexyl	EtO,EtO
8d	Boc	Cyclohexyl	i-PrO,i-PrO	d	Cyclohexyl	i-PrO,i-PrO
8e	Boc	Cyclohexyl	n-BuO,n-BuO	e	Cyclohexyl	n-BuO,n-BuO
8f	Ph_3C	Phenyl	MeO,MeO	f	i-Propyl	MeO,MeO
8g	Ph_3C	Cyclohexyl	MeO,MeO	g	Cyclohexyl	OCH_2CH_2O
8i	Ph_3C	i-Propyl	MeO,MeO	k	Cyclohexyl	Me,Me
8j	Ph_3C	Cyclohexyl	OCH_2CH_2O			
8k	Ph_3C	Cyclohexyl	Me,Me			
8l	Ph_3C	Cyclohexyl	n-BuO,n-BuO			

sistently lower yields (16–32%) and in a 1:1 ratio of the 2-hydroxy diastereomers. Urethane-protected α-amino aldehydes are sensitive to racemization[11a] during silica gel chromatography and storage, even at −30 °C. We have found *N*-tritylcyclohexylalaninal[11b] to be stable to normal-phase silica gel chromatography and to storage as a neat liquid at −19 °C for up to 3 weeks [≤5% racemization as judged by the optical rotation of **6c** immediately after chromatography, $[\alpha]^{24}_D$ +80.6° (c = 2.12, $CHCl_3$), versus after storage at −19 °C for 3 weeks, $[\alpha]^{24}_D$ +76.3° (c = 2.12, $CHCl_3$)].

The C_2-hydroxy isomers were partially separable by chromatography by selecting early and late fractions in the *N*-*t*-Boc-protected series, except for **8e**; only the isomers of **8f** were separable in the *N*-trityl-protected series. The C_2-hydroxy mixture of *N*-trityl-protected LVRs was carried on to the final inhibitors where the isomers were separable (vide supra). The relative stereochemistry between the C_2-hydroxy and C_3-amino centers was determined for all pairs of isomers by ^{1}H NMR techniques on the corresponding oxazolidinones **9** and **10**, which were prepared as described in Scheme III. Despite ample literature precedent[12] indicating that the stereochemical assignments for oxazolidinones of this type can be made based on the magnitude of the vicinal coupling constant between the two centers of interest (i.e. J_{HxHy}), we found that only **9b** and **10b** were consistent with the precedent.[9] The remainder of the oxazolidinones had vicinal coupling constants which differed by ≤2 Hz, making the assignment of relative stereochemistry tenuous at best. Fortunately, we found that a nuclear Overhauser enhancement (NOE) was observed between the phosphonate methylene and side-chain methylene for one of each pair of oxazolidinones (details are given in the Experimental Section). Inspection of Drieding models indicates that only the oxazolidinone having a syn relationship between the two methylenes would show an NOE. This assignment is further strengthened by the fact that the major oxazolidinones in the *N*-trityl series always gave the more potent final inhibitor, and it was shown that the major isomer bore the desired statine-like[13] stereochemistry.[9]

The enantiomeric purity of the condensation adducts was examined by conversion of **8** to the (+)- and (−)-Mosher amides (**11**, Scheme III),[14] which were analyzed by ^{1}H NMR or high-performance liquid chromatography. All phosphostatine LVRs derived from an *N*-*t*-Boc-protected aldehyde were analyzed by ^{1}H NMR (see the Experimental Section for details) and only products having ≥95% ee were utilized to prepare the final inhibitors. High-performance liquid chromatography was utilized to analyze the (+)- and (−)-Mosher amides of **8g** and revealed that no racemization had occurred within our detection limits (±1%, see the Experimental Section). Since we had established earlier that the *N*-tritylamino aldehydes do not racemize under our handling conditions,[11b] we assume by analogy that the other LVRs derived from *N*-tritylamino

(11) (a) Rittle, K. E.; Homnick, C. F.; Ponticello, G. S.; Evans, B. E. *J. Org. Chem.* **1982**, *47*, 3016 and references therein. (b) Control experiments were conducted where the optical rotation of chloroform solutions of the *N*-*t*-Boc- and *N*-trityl-L-cyclohexylalaninal were followed over time in the solvent alone and in the presence of 1.0 equiv of triethylamine hydrochloride or triethylamine. In the case of the *N*-*t*-Boc-L-cyclohexylalaninal (0.078 M), the half-life for the triethylamine (0.078 M) containing solution was ~0.3 **h** and the triethylamine hydrochloride (0.078 M) and solvent-alone solutions gave half-lives approaching 1 day. The *N*-trityl-L-phenylalaninal (0.052 M in aldehyde and in the respective addend) experiments gave no statistically significant change in optical rotation after 19 h. Unpublished results: Dellaria, J. F.; Maki, R. G., Abbott Laboratories.

(12) See footnote 22 in ref 24 of this paper.

(13) "Statine-like" stereochemistry refers to compounds bearing the same relative and absolute stereochemical relationships of statine (4(*S*)-amino-3(*S*)-hydroxy-6-methylheptanoic acid) between the amino- and hydroxy-bearing stereocenters. This circumvents the potential confusion brought on by the *S*/*R* stereochemical convention. [Absolute configurations, "*S*" and "*R*", are as defined by the IUPAC 1974 Recommendations for Section E, Fundemental Stereochemistry: *Pure Appl. Chem.* **1976**, *45*, 13.] For example, the major oxazolidinone **9b** bears "statine-like" stereochemistry but the hydroxyl center is of the *R* configuration.

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Table II. Chemical Characterization of the Inhibitors

no.	HPLC[a] % purity	synth[b] method	R_f	solvent[c] system	formula[d]
12	86	A	0.21	A	$C_{29}H_{46}N_5O_8P\cdot H_2O$
13	94	A	0.38	B	$C_{29}H_{42}N_3O_8P\cdot 0.5\ H_2O$
14	75[f]	A	0.38	C	$C_{32}H_{44}N_5O_8P\cdot H_2O$
15	70[f]	A	0.46	C	$C_{32}H_{50}N_5O_8P\cdot H_2O$[h]
16	79[f]	A	0.32	C	$C_{32}H_{44}N_5O_8P\cdot 1.25\ H_2O$
17	84	A	0.43	C	$C_{32}H_{50}N_5O_6P\cdot H_2O$[h]
18	69[f]	A	0.37	C	$C_{34}H_{54}N_6O_8P\cdot 0.5\ H_2O$
19	76[f]	A	0.37	C	$C_{36}H_{58}N_5O_8P\cdot 1.25\ H_2O$
20	98[g]	B	0.40	C	$C_{38}H_{62}N_5O_8P\cdot H_2O$
21	95	B	0.29	C	$C_{33}H_{52}N_5O_7P\cdot H_2O$
22	92	B	0.27	C	$C_{30}H_{46}N_5O_8P\cdot H_2O$
23[e]	89[f]	A	0.19	C	$C_{32}H_{50}N_5O_6P$[i]
24[e]	82[f]	A	0.12	C	$C_{32}H_{50}N_5O_6P$[i]
25	94	A	0.60	C	$C_{32}H_{54}N_3O_8P$
26			0.18	D	$C_{31}H_{52}N_3O_8P$[i]
27	74[f]	A	0.36	C	$C_{33}H_{50}N_5O_8P\cdot H_2O$

[a] See the Experimental Section for details. [b] Method A: dipeptide coupling to the LVR. Method B: sequential couplings. [c] Solvent systems: A, 5% MeOH/$CHCl_3$; B, 10% MeOH/$CHCl_3$; C, 15% MeOH/$CHCl_3$; D, 25% MeOH/$CHCl_3$. [d] Elemental analyses within ±0.4% of the calculated values for C, H, N were obtained for these compounds unless noted otherwise. [e] Diastereomers; absolute configuration of the 2-hydroxy group in the phosphinylstatine LVR is unknown. [f] Major impurity is the D-His-containing peptide; ≥95% of the compound is accounted for by the total of the L- and D-His peptides (see ref 16). [g] A 3:1 mixture of C_2-hydroxy isomers. [h] Analytical analysis obtained on a mixture of 2(*S*)- and 2(*R*)-hydroxy diastereomers. [i] Exact mass determination within ±4 ppm were obtained for these compounds.

aldehydes have not suffered from racemization at the C_3-amino center.

Synthesis: Final Inhibitors. Final inhibitors were prepared from *N*-*t*-Boc and *N*-trityl-protected LVRs by either a single coupling with an intact dipeptide or sequentially with the appropriately protected amino acid derivatives. Deprotection of the *N*-*t*-Boc group was achieved by stirring of the desired LVR with excess acid (4.8 M HCl in dioxane, 23 °C, 2 h), concentrating in vacuo, and drying under high vacuum. The resulting salts were used without further purification. The *N*-trityl group was removed either by exposure to excess acid (4.8 M HCl in dioxane, 23 °C, 0.5 h) and processing as in the *N*-*t*-Boc case or by hydrogenolysis in ethyl acetate in the presence of excess acid (4.8 M HCl in dioxane, 10% Pd/C, 23 °C, 0.5–3 h) and isolation of the corresponding amine hydrochloride salt by extracting the salts into water and lyophilization[15] (the latter process gave better results in subsequent coupling reactions and became the method of choice). Standard peptide-coupling conditions were utilized to couple Boc-Phe-Ala, Boc-Phe-Leu, and Boc-His to the desired LVR. Inhibitors **21** and **22** were prepared by sequential couplings. When couplings were carried out between the dipeptide Boc-Phe-His and the deprotected LVR, significant amounts of racemization were encountered (15–30%) at the histidine site; unfortunately, we were unable to identify conditions to remove the racemates by preparative chromatographic methods or by recrystallization. The mixtures were utilized for testing since other work from these laboratories have demonstrated that the substitution of L-His with D-His results in at least a 350-fold loss in inhibitory potency.[16] The *N*-trityl-derived LVRs were utilized as a mixture at the C_2-hydroxyl group; the hydroxy isomers were separable at the full inhibitor stage with the exception of inhibitor **20**, which was tested as a mixture.

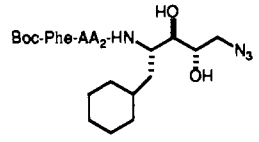
Biological Results and Discussion

In Vitro Enzyme Inhibition. The inhibitory potencies and chemical data for the new compounds are summarized in Tables I and II. The renin IC_{50} data was obtained with use of purified human renal renin at pH 6. Our first point of attack in the SAR of the phosphostatine inhibitors was at the Leu[10] position in the LVR. As first shown by Boger[17] and co-workers, and subsequently utilized by others,[18] optimization at the Leu[10] position can lead to large increases in the binding potency. Compounds **12**, **14**, and **15** revealed that as the LVR side chain is varied from isopropyl to phenyl a modest 3-fold increase in potency is obtained, while the exchange of the phenyl moiety for a cyclohexyl group leads to a nearly 40-fold increase for an overall 130-fold increase in binding potency in going from **12** to **15**. Other work from these laboratories[19] indicated that the cyclohexyl-derived LVR would provide the optimum binding potency at the Leu[10] position; therefore, all subsequent SAR work was carried out in that series.

The influence of the stereochemistry at the 2-hydroxyl position in the LVR on the binding potency was investigated by inhibitors **16** and **17**, which bear the 2(*S*)-hydroxy stereochemistry (non-statine-like). In each case (**14** versus **16** and **15** versus **17**), inverting the 2-hydroxy stereochemistry resulted in a small drop in potency (2.6- and 2.3-fold losses, respectively). This result is interesting, as the inversion of the statine-like stereochemistry at the C_2-hydroxyl center usually leads to a 25–1000-fold loss of potency[20] in the resulting inhibitor. The fact that the potency of the phosphostatine-derived inhibitors are nearly insensitive to the 2-hydroxy stereochemistry may imply that these compounds are binding to renin in an alternate

(15) This method provided salts which were free of trityl sideproducts encountered when the deprotection was carried out in excess acid. Hydrogenolysis under neutral conditions was very slow, giving ~50% mass recovery after 2 days. The combination deprotection by exposure to acid and hydrogenolysis gave optimum purity and conversion.

(16) Inhibitors i and ii have IC_{50}s of 0.7 and 250 nM when AA_2 = L-His and D-His, respectively. Unpublished results: Rosenberg, S., Abbott Laboratories.



i = L-His
ii = D-his

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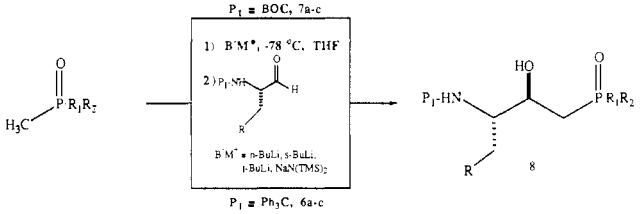
(20) The 3-aminostatine renin inhibitors also demonstrated a small dependence on the stereochemistry at the "statine-like" hydroxyl position. Please refer to ref 5.

Table III. Data for Synthesis of the Phosphostatine Leu-Val Replacements



no.	P_1	R	R_1, R_2	scale,[a] mmol	% yield	R_f	solvent[b] system	isomer[c] ratio 2R:2S	deprotonation base
8a	Boc	phenyl	MeO, MeO	20	29	0.09	A	1:1	*n*-BuLi
8b	Boc	cyclohexyl	MeO, MeO	11.1	32	0.56	B	1:1	*n*-BuLi
8c	Boc	cyclohexyl	EtO, EtO	10.5	17	0.18	A	1:1	*n*-BuLi
8d	Boc	cyclohexyl	*i*-PrO, *i*-PrO	10.9	23	0.33	A	1:1	*n*-BuLi
8e	Boc	cyclohexyl	*n*-BuO, *n*-BuO	6.95	16	0.17	C	1:1	*n*-BuLi
8f	Ph_3C	phenyl	MeO, MeO	0.782	17	0.23 minor	A	3.0:1	*n*-BuLi
8f	Ph_3C	phenyl	MeO, MeO	0.782	51	0.18 major	A	3.0:1	*n*-BuLi
8g	Ph_3C	cyclohexyl	MeO, MeO	18.6	59	0.31	A	2.3:1	*n*-BuLi
8i	Ph_3C	isopropyl	MeO, MeO	4.12	71	0.20	A	2.4:1	*n*-BuLi
8j	Ph_3C	cyclohexyl	$-O(CH_2)_3O-$	0.513	44	0.12	A	3.0:1	*t*-BuLi
8k	Ph_3C	cyclohexyl	Me, Me	4.07	74	0.20	D	8.0:1	*t*-BuLi
8l	Ph_3C	cyclohexyl	*n*-BuO, *n*-BuO	3.25	48	0.45	E	3.0:1	*s*-BuLi

[a] The scale reported is based on 1.0 equiv of the aldehyde (8) and 1.2 equiv of the phosphorous-stabilized anion. [b] Solvents: A, 50% ethyl acetate/chloroform; B, 10% methanol/chloroform; C, 20% ethyl acetate/chloroform; D, 5% methanol/chloroform; E, 25% ethyl acetate/chloroform. [c] Ratio determined by integration of C_2-hydroxy methine protons in 300-MHz ^1H NMR of the unpurified reaction mixture.

fashion. Evaluation of this possibility by molecular modeling techniques has not yet yielded a satisfactory explanation.

Inhibitors **15**, and **18–20** were prepared to investigate steric factors in the diester portion of the LVR. Chain extending the dialkyl esters from methyl (**15**) to ethyl (**18**) and *n*-butyl (**20**) results in a sequential loss in potency from 15 to 50 and 400 nM, respectively. This trend reveals that the space surrounding the phosphonyl group cannot accommodate esters larger than dimethyl. Compound **19**, the diisopropyl ester, barely inhibits the enzyme and demonstrates that an alkyl branch α to the ester oxygen is less acceptable than extending the alkyl chain (compare **19** vs **20**).

Compounds **21** and **22** were prepared to provide less acid-labile *N*-protected inhibitors.[21] Analogue **21** is a direct isostere of **15** and was very similar in binding potency. In contrast, **22** provides a less lipophilic *N*-protecting group and again maintains binding potency. Comparison of **13** versus **14**, and **25** versus **15** reveals that an Ala[9] replacement, for His,[9] reduces binding potency whereas the corresponding Leu[9] replacement provides a small increase in binding potency. These trends are consistent with previously reported results for similar analogues at the His[9] position.[18d]

At this juncture we wished to investigate whether other types of functionality in the phosphorus ligands would be tolerated. The phosphinylstatine compounds **23** and **24** were prepared to probe the role of the ester oxygens. This seemingly modest change lead to a profound loss of binding potency. It is not clear whether this represents an electronic and/or steric influence. The cyclic diester (**27**) was prepared to further explore the size requirements at this site. The inhibitory potency was essentially maintained in comparison to that of the diethyl ester derivative (**18**), an open chain analogue of **27**. These results indicate that there is no apparent advantage to cyclic ester derivatives. Finally, the half-acid half-ester compound **26** was prepared by selective monodemethylation[22] of **25** (judged to be a 2:1 diastereomeric mixture at phosphorus); this leads to a 36-fold loss in potency. These results clearly indicate that good inhibitory potency, in the phosphostatine derived series, is intimately dependent on the pendant ligands of the phosphonate moiety.

The specificity of selected inhibitors toward renin over bovine cathepsin D and porcine pepsin, two related aspartic proteinases, is summarized in Table I. In all cases excellent selectivity in favor of renin was observed. Thus, phosphostatine-derived compounds are potent inhibitors of renin and exhibit excellent enzyme specificity over other related aspartic proteinases.

Conclusions

We have delineated the design and synthesis of a unique series of renin inhibitors which mimic renin's minimum substrate sequence Phe[8]-His[9]-Leu[10]-Val[11] by appending an *N*-protected dipeptide (Phe[8]-His[9]) to novel phosphostatine-derived LVRs. The most potent inhibitors (20–50 nM) were obtained by exchanging a cyclohexyl group for the isopropyl group found at Leu[10] in the natural substrate. The binding potency of the final inhibitors was found to be exquisitely sensitive to the phosphorus ligands; the dimethyl ester derivatives provided the most potent inhibitors. The binding potency of the "statine-like" 2-(*R*)-hydroxy phosphostatines were only ~2 times more potent than the 2(*S*)-hydroxy series. This uncharacteristically small difference in binding potency between the 2-hydroxy diastereomers may be indicative of a unique mode of binding in comparison to most renin inhibitors. Finally, the most potent inhibitors were found to be highly selective for renin over bovine cathepsin D and porcine pepsin, two related aspartic proteinases.

Experimental Section

Proton magnetic resonance spectra were obtained on a Nicolet QE-300 (300 MHz) spectrometer. Chemical shifts are reported as δ values (ppm) relative to Me_4Si as the internal standard and were obtained in the noted solvent. Mass spectra were obtained with Hewlett-Packard HP5985 (CI, EI), Varian CH7 (EI), and

(21) This was in anticipation of potential *N*-deprotection of the *t*-Boc group in the stomach upon oral administration.

(22) (a) Daub, G. W.; Van Tamelen, E. E. *J. Am. Chem. Soc.* **1977**, *99*, 3526. (b) Takeuchi, Y.; Demachi, Y.; Yoshii, E. *J. Am. Chem. Soc.* **1979**, *14*, 1231.

Dratos MS50 (FAB, HRMS) spectrometers. Elemental analysis and the above determinations were performed by the Analytical Research Department at Abbott Laboratories, Abbott Park and North Chicago, IL.

Thin-layer chromatography (TLC) was carried out using E. Merck precoated silica gel F-254 plates (thickness, 0.25 mm). Chromatographic purification was carried out by either medium-pressure liquid chromatography (MPLC) employing columns packed with EM Silica gel 60 (40–63 μm) at 30–50 psi or by forced-air chromatography employing the previously described silica gel at 5–10 psi of air pressure. Final inhibitor samples were chromatographed on a 50 × 4.6 mm Spherisorb ODS2 (3-μm particles) column (Regis Chemical Co.) using acetonitrile/methanol/0.01 M tetramethylammonium perchlorate in 0.1% trifluoroacetic acid (V/V) mobile phase (50:5:45 by volume) at a flow rate of 1.0 mL/min (Spectroflow 400 isocratic pump (Kratos, Ramsey, NJ)) with UV detection at 205 nm, 0.001 AUFS (Spectroflow 783 variable-wavelength detector (Kratos)). Peak-area response was collected on a DS650 chromatographic data system (Kratos).

Protected amino acids were purchased from Bachem (Torrance, CA). Diethyl-, diisopropyl-, and trimethylphosphine oxide were purchased from Alfa Products, Morton Thiokol Inc., and used as received. Cyclic diester **7e**, 2-methyl-2-oxo-1,3-dioxa-2-phosphacyclohexane, and di-*n*-butyl methylphosphonate were prepared according to the method of McKay.[23] Tetrahydrofuran was distilled from sodium/benzophenone ketyl, and dichloromethane was distilled from P_2O_5. All other solvents and reagents were reagent grade and used without further purification.

Methyl *N*-Tritylcyclohexylalaninate (4c, R = Cyclohexyl). A 1-L flask was charged with methyl cyclohexylalaninate hydrochloride salt[24] (66.67 g, 301 mmol) and 450 mL of dry dichloromethane. After cooling of the suspension in an ice bath, the triethylamine was added neat in a dropwise fashion from a side-arm pressure-equalized addition funnel under a stream of N_2. The cooling bath was removed after addition of the base was complete, and the reaction warmed to room temperature when the trityl chloride (85.6 g, 301 mmol) was added portionwise from a solid addition funnel. The reaction was stirred overnight (18 h) at room temperature and poured into H_2O (400 mL). The organic layer was drawn off and the aqueous layer was back-extracted with dichloromethane (2×, 200 mL). The combined organic extracts were washed with H_2O (1×, 200 mL), saturated aqueous ammonium chloride (1×, 250 mL), and brine (1×, 400 mL); dried over Na_2SO_4; filtered; and concentrated in vacuo to provide unpurified **4c** (118.16 g, 92%) as a golden oil, which was carried on without further purification. **4c**: ^{1}H NMR ($CDCl_3$, TMS) δ ca. 7.49 (m, 6 H), 7.13–7.30 (m, 9 H), 3.37 (d, d, J = 8.5, 6.3 Hz, 1 H), 3.12 (s, 3 H), 2.6 (br s, 1 H), 1.45–1.78 (m, 9 H), 1.12–1.37 (m, 4 H), 0.75–0.98 (m, 2 H); mass spectrum (FAB) $(M + H)^+$ = 428.

Methyl *N*-Tritylleucinate (4a, R = Isopropyl). The title compound **4a** was prepared as previously described for **4c** from methyl leucinate hydrochloride salt. **4a**: ^{1}H NMR ($CDCl_3$) δ ca. 7.5 (m, 6 H), 7.13–7.33 (m, 9 H), 3.33 (d, d, J = 8.5, 5.5 Hz, 1 H), 3.14 (s, 3 H), 2.6 (br s, 1 H), 1.53–1.67 (m, 3 H), 0.88 (d, J = 9 Hz, 3 H), 0.86 (d, J = 9 Hz, 1 H); mass spectrum (CI) $(M + H)^+$ = 388.

Methyl *N*-Tritylphenylalaninate (4b, R = Phenyl). The title compound **4b** was prepared as previously described for **4c** from methyl phenylalaninate hydrochloride salt. **4b**: ^{1}H NMR ($CDCl_3$) δ ca. 7.5 (m, 6 H), 7.10–7.47 (m, 14 H), 3.56 (t, J = 6.5 Hz, 1 H), 3.04 (s, 3 H), 2.97 (*A*BX, J = 13.5, 6.5 Hz, 1 H), 2.92 (A*B*X, J = 13.5, 6.5 Hz, 1 H), 2.63 (br s, 1 H); mass spectrum (FAB) $(M + H)^+$ = 422.

***N*-Tritylcyclohexylalaninol (5c, R = Cyclohexyl).** A 250-mL round-bottom flask was charged with lithium aluminum hydride (0.665 g, 70.11 mmol hydride equiv) and THF (25 mL) and fit with an addition funnel charged with **4c** (9.5 g, 22.2 mmol) in THF (65 mL). The solution, of **4c** in THF, was added to the rapidly stirred reaction mixture over 10 min in a dropwise fashion and the resulting solution was stirred for 1 h at room temperature. The reaction was quenched by adding sequentially H_2O (1.2 mL), freshly prepared 15% aqueous NaOH (1.2 mL), and H_2O (3.6 mL). The resulting mixture was stirred for 1 h at room temperature and filtered through Celite, and the filter cake was rinsed thoroughly with dichloromethane. The combined filtrates were dried over Na_2SO_4, filtered, and concentrated in vacuo to provide unpurified **5c** as a yellow glass. Purification by MPLC (500 g of silica gel, 10% ethyl acetate/hexanes) provided **5c** as a thick, colorless oil. **5c**: ^{1}H NMR ($CDCl_3$) δ 7.45–7.55 (m, 6 H), 7.15–7.33 (m, 9 H), 3.18 (*A*B, J = 11.7, 3.0 Hz, 1 H), 2.98 (A*B*, J = 11.7, 3.7 Hz, 1 H), 2.68 (br d, q, J = 9.6, 3.3 Hz, 1 H), 2.04 (br s, 1 H), 1.55 (br d, J = 7.5 Hz, 3 H), 1.27–1.42 (m, 4 H), 0.93–1.14 (m, 4 H), 0.45–0.77 (m, 3 H); mass spectrum (EI) M^+ = 399. Anal. ($C_{28}H_{33}NO \cdot 0.25H_2O$) C, H, N.

***N*-Tritylleucinol (5a, R = Isopropyl).** The title compound **5a** was prepared in analogy to **5c** from **4a**. **5a**: ^{1}H NMR ($CDCl_3$) δ ca. 7.54 (m, 6 H), 3.22 (*A*BX, J = 10.5, 2.5 Hz, 1 H), 3.05 (A*B*X, J = 10.5, 3.5 Hz, 1 H), 2.65 (d, q, J = 2.5, 2.5, 2.5, 9.5 Hz, 1 H), 1.7–2.3 (br hump, 1 H), 1.58 (br s, 1 H), 1.3–1.45 (m, 2 H), 0.67 (d, J = 6.0 Hz, 3 H), 0.55 (d, J = 6.0 Hz, 3 H); mass spectrum (FAB) $(M + H)^+$ = 360. Anal. ($C_{15}H_{29}NO \cdot H_2O$) C, H, N.

***N*-Tritylphenylalaninol (5b, R = Phenyl).** The title compound **5b** was prepared in analogy to **5c** from **4b**. **5b**: ^{1}H NMR ($CDCl_3$) δ 7.55 (m, 6 H), 7.10–7.35 (m, 12 H), 6.93 (m, 2 H), 3.12 (*A*BX, J = 11.5, 3.0 Hz, 1 H), 2.93 (A*B*X, J = 11.5, 4.0 Hz, 1 H), 2.81 (d, d, d, d, J = 9.0, 4.5, 4.0, 3.0 Hz, 1 H), 2.50 (ABX, J = 13.0, 9.0 Hz, 1 H), 2.27 (ABX, J = 13.0, 4.5 Hz, 1 H), 1.9 (br s, 1 H); mass spectrum (FAB) $(M + H)^+$ = 394. Anal. ($C_{28}H_{27}NO \cdot 0.75H_2O$) C, H, N.

***N*-Tritylcyclohexylalaninal (6c, R = Cyclohexyl).** A 500-mL round-bottom flask was charged with a magnetic stir bar, freshly dried dichloromethane (50 mL), and oxalyl chloride (2.43 mL, 27.9 mmol) and cooled to −60 °C (CO_2(s)/$CHCl_3$) under a stream of N_2. To the cooled reaction solution was added dimethyl sulfoxide (2.88 mL, 37.2 mmol) in dichloromethane (50 mL) over 10 min. The resulting mixture was stirred for 10 min at −60 °C before adding alcohol **5c** (7.42 g, 18.6 mmol) as a solution in dichloromethane (80 mL). Triethylamine (10.4 mL, 74.4 mmol) was added 10 min later in dichloromethane (20 mL). The reaction was quenched 30 min later at −60 °C by adding 20% aqueous $KHSO_4$. The quenched reaction mixture was diluted with hexanes (300 mL) and the organic layer drawn off. The aqueous layer was extracted with ethyl ether (300 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (2×), H_2O (2×), and brine (2×); dried over $MgSO_4$; filtered; and concentrated in vacuo to provide unpurified **6c** (7.39 g, 18.59 mmol, 100% yield) as a sticky, cream foam. The unpurified aldehyde was normally carried on without further purification. A small portion of **6c** (0.5 g) was purified by forced-air chromatography (7 g of silica gel, 5% ethyl acetate/hexanes). **6c**: ^{1}H NMR ($CDCl_3$) δ 8.97 (d, J = 3.0 Hz, 1 H), 7.47–7.53 (m, 6 H), 7.15–7.33 (m, 9 H), 3.33 (br m, 1 H), 2.59 (br s, 1 H), 1.5–1.75 (m, 5 H), 1.1–1.45 (m, 6 H), 0.73–0.87 (m, 1 H).

***N*-Tritylleucinal (6a, R = Isopropyl).** The title compound was prepared in analogy to **6c** from **5a** and carried on without purification. **6a**: ^{1}H NMR ($CDCl_3$) δ 8.95 (d, J = 3.2 Hz, 1 H), 7.47–7.55 (m, 6 H), 7.17–7.32 (m, 9 H), 3.29 (d, t, J = 3.2, 6.9, 6.9 Hz, 1 H), 1.68 (septet, J = 6.9 Hz, 1 H), 1.36 (t, J = 6.9 Hz, 2 H), 1.28 (br s, 1 H), 0.83 (d, J = 6.9 Hz, 6 H).

***N*-Tritylphenylalaninal (6b, R = Phenyl).** The title compound was prepared in analogy to **6c** from **5b**, and carried on without purification. **6b**: ^{1}H NMR ($CDCl_3$) δ 8.85 (d, J = 2.5 Hz, 1 H), 7.13–7.40 (m, 20 H), 3.57 (d, t, J = 2.5, 6.5, 6.5 Hz, 1 H), 2.78 (*A*BX, J = 13.5, 6.5 Hz, 1 H), 2.74 (A*B*X, J = 13.5, 6.5 Hz, 1 H), 1.57 (br s, 1 H).

General Procedure for the Phosphorus Stabilized Anion Condensation with N-Protected Amino Aldehydes. The following general procedure was used in the preparation of the phosphostatine derivatives unless noted otherwise. An oven-dried flask was charged with a magnetic stir bar, the desired phosphonate or phosphonate derivative (1.1 equiv), and freshly distilled

(23) McKay, A. F.; Braun, R. O.; Vavasour, G. R. *J. Am. Chem. Soc.* **1952**, *74*, 5540. It was necessary to prepare the di-*n*-butyl methylphosphonate, as the commercially available material was found to contain substantial amounts of *n*-butyl *n*-propyl methylphosphonate.

(24) Prepared according to Luly et al.: Luly, J. R.; Dellaria, J. F.; Plattner, J. J.; Soderquist, J. L.; Yi, N. *J. Org. Chem.* **1987**, *52*, 1487.

THF (ca. 0.5 M) and fitted with a septum. The reaction was cooled to −78 °C under a nitrogen atmosphere and the *n*-butyllithium (1.05 equiv, in hexanes 2.3–2.7 M), or the base noted in Table III, was added via a syringe in a dropwise fashion. The resulting solution was stirred at −78 °C for 0.5 h and then the desired *N*-protected amino aldehyde (1.0 equiv) was added via a syringe in THF (ca. 0.5 M). The reaction was stirred for 0.5 h at −78 °C, quenched by adding excess saturated aqueous ammonium chloride, and poured into ethyl acetate. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (2×). The combined organic extracts were washed with saturated aqueous NH_4Cl (1×), H_2O (1×), and brine (2×); dried over $MgSO_4$ or Na_2SO_4; filtered; and concentrated in vacuo to provide the unpurified product. Purification was achieved by MPLC or flash chromatography using a 50:1 loading ratio of silica gel to unpurified product and eluting with the mixed solvent system noted in Table III where the percent of polar solvent was adjusted to give an R_f = 0.15–0.25. In the case of the *N*-*t*-Boc-protected series the 2(*R*)- and 2(*S*)-hydroxy diastereomers of the resulting phosphostatine derivatives were separable except for the di-*n*-butyl phosphostatine derivative. ^1H NMR data is reported for the pure statine-like[13] 2-hydroxy isomers. When the diastereomers were inseparable, resonances corresponding to the minor and major diastereomers are noted accordingly.

(2*R*,3*S*)-Dimethyl [3-(*t*-Boc-amino)-2-hydroxy-4-phenylbutyl]phosphonate (8a): ^1H NMR ($CDCl_3$, TMS) δ 6.98–7.35 (m, 5 H), 4.97 (d, *J* = 9.5 Hz, 1 H), 3.98 (br t, *J* = 9.5 Hz, 1 H), 3.90 (br s, 1 H), 3.76 (d, *J* = 11.4 Hz, 3 H), 3.70 (d, *J* = 12.0 Hz, 3 H), 3.67–3.80 (m, 1 H), 2.92 (d, *J* = 7.0 Hz, 2 H), 2.18 (d, t, *J* = 11.4, 15.3, 15.3 Hz, 1 H), 1.83 (br t, *J* = 17.7 Hz, 1 H), 1.40 (s, 9 H); mass spectrum (EI) M^+ = 373. Anal. ($C_{17}H_{28}NO_6P·0.25H_2O$) C, H, N.

(2*R*,3*S*)-Dimethyl [3-(*t*-Boc-amino)-4-cyclohexyl-2-hydroxybutyl]phosphonate (8b): ^1H NMR ($CDCl_3$) 4.75 (br d, *J* = 9.0 Hz, 1 H), 3.98 (br t, *J* = 9.0 Hz, 1 H), 3.77 (d, J_{HP} = 11.0 Hz, 3 H), 3.75 (d, J_{HP} = 11.0 Hz, 3 H), 3.63 (br d, t, *J* = 9.0, 9.0, 3.3 Hz, 1 H), 1.45 (s, 9 H), 1.07–2.13 (m, 14 H), 0.75–1.05 (m, 2 H); mass spectrum (EI) M^+ = 374. Anal. ($C_{17}H_{34}NO_6P$) C, H, N.

(2*R*,3*S*)-Diethyl [3-(*t*-Boc-amino)-4-cyclohexyl-2-hydroxybutyl]phosphonate (8c): ^1H NMR ($CDCl_3$, TMS) δ 4.73 (br d, *J* = 10 Hz, 1 H), ca. 4.13 (br m, 4 H), (br t, *J* = 10 Hz, 1 H), 3.77 (br s, 1 H), 3.62 (d, t, *J* = 5.1, 10 Hz, 1 H), 1.77–2.08 (m, 4 H), 1.45 (s, 9 H), 0.77–1.75 (m, 11 H), 1.33 (t, *J* = 6.6 Hz, 3 H), 1.36 (t, *J* = 6.6 Hz, 3 H); mass spectrum (EI) M^+ = 407. Anal. ($C_{19}H_{38}NO_6P·0.5H_2O$) C, H, N.

(2*R*,3*S*)-Diisopropyl [3-(*t*-Boc-amino)-4-cyclohexyl-2-hydroxybutyl]phosphonate (8d): ^1H NMR ($CDCl_3$, TMS) δ 4.63–4.81 (m, 3 H), 3.97 (t, *J* = 9.6 Hz, 1 H), ca. 3.8 (br s, 1 H), 3.64 (br m, 1 H), 1.47 (s, 9 H), 1.35 (d, *J* = 7.8 Hz, 6 H), 1.32 (d, *J* = 7.8 Hz, 6 H), 0.75–1.97 (m, 15 H). Anal. ($C_{21}H_{42}NO_6P$) C, H, N.

(2*R*,3*S*)-Di-*n*-butyl [3-(*t*-Boc-amino)-4-cyclohexyl-2-hydroxybutyl]phosphonate (8e): ^1H NMR ($CDCl_3$, TMS) δ 5.32 (br d, *J* = 9.0 Hz, 0.5 H (Boc-N*H*)), 5.07 (d, *J* = 9.0 Hz, 0.5 H (Boc-N*H*)), 3.85–4.18 (m, 6 H), 1.43 (s, 9 H), 0.93 (t, *J* = 7.5 Hz, 6 H), 0.70–1.92 (m, 24 H); mass spectrum (EI) M^+ = 463. Anal. ($C_{23}H_{46}NO_6P·0.25H_2O$) C, H, N.

(2*R*,3*S*)- and (2*S*,3*S*)-Dimethyl [2-hydroxy-4-phenyl-3-(tritylamino)butyl]phosphonate (8f): ^1H NMR ($CDCl_3$) (2*R*,3*S*) δ 7.5–7.57 (m, 6 H), 7.08–7.30 (m, 12 H), 6.78–6.83 (m, 2 H), 3.70 (d, *J* = 11.0 Hz, 3 H), 3.62 (d, *J* = 11.0 Hz, 3 H), 3.47 (br t, *J* = 11.0 Hz, 1 H), 2.72 (br t, *J* = 6.5 Hz, 1 H), 2.58 (*ABX*, *J* = 15, 6.5 Hz, 1 H), 2.43 (A*B*X, *J* = 15, 9.0 Hz, 1 H), 2.17 (d, t, *J* = 15, 10 Hz, 1 H), 1.87 (d, d, d, *J* = 21, 15, 2.5 Hz, 1 H); (2*S*,3*S*) δ 7.43–7.50 (m, 6 H), 7.10–7.30 (m, 12 H), ca. 6.83 (m, 2 H), 3.74 (br t, *J* = 9.5 Hz, 1 H), 3.67 (d, *J* = 12 Hz, 3 H), 3.61 (d, *J* = 12 Hz, 3 H), ca. 2.65 (m, 2 H), 2.36 (q, *J* = 8.8 Hz, 1 H), 2.08 (d, t, *J* = 10, 15, 15 Hz, 1 H), 1.66 (d, d, d, *J* = 20.5, 15.0, 2.5 Hz, 1 H); mass spectrum (FAB) [mixture of (2*R*,3*S*) and (2*S*,3*S*)] $(M + H)^+$ = 516. Anal. ($C_{31}H_{34}NO_4P·H_2O$) C, H, N.

(2*R*,3*S*)- and (2*S*,3*S*)-Dimethyl [4-cyclohexyl-2-hydroxy-3-(tritylamino)butyl]phosphonate (8g): ^1H NMR ($CDCl_3$) δ 7.45–7.57 (m, 6 H) 7.15–7.30 (m, 9 H), 3.90 (t, d, d, *J* = 9.6, 9.6, 3.3, 3.3, 2.7, 2.7, 1 H), 3.77 (d, *J* = 9 Hz, 3 H, major), 3.73 (d, *J* = 9 Hz, 3 H, major), 3.69 (d, *J* = 11.0 Hz, 3 H, minor), 2.63 (br m, 1 H, major), 2.47 (br t, *J* = 6.3 Hz, 1 H, minor), 2.13 (d, d, d, *J* = 18, 15.0, 2.7 Hz, 1 H, major), 1.93 (d, t, *J* = 11.4, 15.0, 15.0 Hz, 1 H, major), ca. 2.05 (m, 2 H, minor), 0.37–1.7 (m, 13 H); mass spectrum (DCI, NH_3) $(M + H)^+$ = 522, M^+ = 521 (weak); ^{13}C NMR ($CDCl_3$; 125.8 MHz) δ 146.62 (Ar, minor), 146.47 (Ar, major), 128.94 (Ar, major), 128.83 (Ar, minor), 127.69 (Ar, major), 127.64 (Ar, minor), 126.33 (Ar, major), 126.25 (Ar, minor), 70.96 (Ph_3CN, major), 70.68 (Ph_3CN, minor), 67.34 (HCOH, J_{CP} = 5.6 Hz, major), 66.34 (HCOH, J_{CP} = 4.6 Hz, minor), 54.72 ($NCHCH_2$, J_{CP} = 15.7 Hz, minor), 53.71 ($NCCHCH_2$, J_{CP} = 16.7 Hz, major), 52.27–52.49 (br m, $POCH_3$), 39.67 (CH_2, major), 37.93 (CH_2, minor), 33.94, 33.64, 33.60, 33.48, 33.32, 28.18 (PCH_2, J_{CP} = 140.6 Hz, minor), 28.12 (PCH_2, J_{CP} = 139.6 Hz, major), 26.34, 26.19, 26.07. Anal. ($C_{31}H_{40}NO_4P·0.25H_2O$) C, H, N.

(2*R*,3*S*)- and (2*S*,3*S*)-Dimethyl [3-hydroxy-5-methyl-3-(tritylamino)hexyl]phosphonate (8i): ^1H NMR ($CDCl_3$) δ 7.46–7.55 (m, 6 H), 7.13–7.32 (m, 9 H), 3.92 (t, t, *J* = 11, 11, 2.5, 2.5 Hz, 1 H, major) 3.77 (d, *J* = 9 Hz, 3 H, major), 3.73 (d, *J* = 9 Hz, 3 H, major), 3.71 (d, *J* = 11 Hz, 3 H, minor), 3.65 (d, *J* = 11 Hz, 3 H, minor), 2.58 (br septet, *J* = 3 Hz, 1 H, major), 2.44 (br t, *J* = 6 Hz, 1 H, minor), 2.07 (d, d, d, *J* = 19, 15.5, 3.0 Hz, 1 H, major), 1.07 (d, t, *J* = 15, 15, 10.5 Hz, 1 H, major), 1.73 (d, d, d, *J* = 20.5, 15, 3 Hz, 1 H, minor), 1.2–1.35 (m, 2 H), 1.07 (septet, *J* = 6 Hz, 1 H, minor), 0.60–0.80 (m, 1 H), 0.61 (d, *J* = 6 Hz, 3 H, minor), 0.57 (d, *J* = 6 Hz, 3 H, major), 0.47 (d, *J* = 6 Hz, 3 H, minor), 0.44 (d, *J* = 6 Hz, 3 H, major); mass spectrum (CI) $(M + H)^+$ = 482. Anal. ($C_{28}H_{36}NO_4P·0.5H_2O$) C, H, N.

(2*R*,3*S*)- and (2*S*,3*S*)-Dimethyl[4-cyclohexyl-2-hydroxy-3-(tritylamino)butyl]phosphine Oxide (8k). Higher yields were obtained when *tert*-butyllithium was employed in lieu of *n*-butyllithium for the deprotonation: ^1H NMR ($CDCl_3$, TMS) δ 7.45–7.57 (m, 6 H), 7.15–7.35 (m, 9 H), 3.90 (br t, *J* = 9.3 Hz, 1 H), 2.67 (br s, $W_{1/2h}$ = 16.5 Hz, 1 H), 1.75–1.97 (m, 2 H), 1.57 (d, *J* = 13.0 Hz, 3 H), 1.47 (d, *J* = 13.0 Hz, 3 H), 1.26–1.65 (m, 4 H), 0.8–1.15 (m, 7 H), 0.45–0.66 (m, 2 H); ^{13}C NMR (75.48 MHz, $CDCl_3$) δ 146.78 (Ar), 129.22 (Ar), 127.75 (Ar), 126.52 (Ar), 71.16 (Ph_3CN), 68.43 (HCOH, J_{CP} = 4.9 Hz), 54.89 (NCH, J_{CP} = 12.2 Hz), 40.71 (CH_2), 34.54 (CH_2P, J_{CP} = 69.6 Hz), 34.26 ($CH(CH_2)_3$), 33.79 (CH_2), 33.59 (CH_2), 26.48 (CH_2), 26.22 (CH_2), 26.15 (CH_2), 17.7 (CH_3P, J_{CP} = 68.4 Hz), 17.17 (CH_3P, J_{CP} = 68.4 Hz); mass spectrum (EI) M^+ = 489. Anal. ($C_{31}H_{40}NO_2P·H_2O$) C, H, N.

(2′*R*,3′*S*)- and (2′*S*,3′*S*)-2-[4-Cyclohexyl-2-hydroxy-3-(tritylamino)butyl]-1,3-dioxa-2-oxo-2-phosphacyclohexane (8j). Higher yields were obtained when *sec*- or *tert*-butyllithium were employed in lieu of *n*-butyllithium for the deprotonation of the starting phosphonate (39% and 44% vs 4%, respectively): ^1H NMR ($CDCl_3$) δ 7.45–7.57 (m, 6 H), 7.13–7.30 (m, 9 H), 4.42–4.63 (m, 2 H), 4.17–4.35 (m, 2 H), 3.96 (br t, *J* = 10.8 Hz, 1 H, major), 3.79 (br t, *J* = 11.4 Hz, 1 H, minor), 2.64 (br septet, *J* = 2.7 Hz, 1 H, major), 2.47 (br t, *J* = 2.7 Hz, 1 H, minor), 1.92–2.28 (m, 4 H), 1.25–1.73 (m, 6 H), 0.55–1.10 (m, 6 H), 0.40–0.68 (m, 2 H). Anal. ($C_{32}H_{40}NO_4P·H_2O$) C, H, N.

General Procedures for N-Deprotection of the LVRs. Method A. A flask charged with the desired LVR and a stir bar was exposed to a 10-fold excess of HCl in dioxane (4.5 M HCl in dioxane). The reaction was stirred at room temperature for 0.5 h for *N*-trityl-protected LVRs or for 2 h for *N*-*t*-Boc-protected LVRs, concentrated in vacuo, chased (3×) with 3:1 benzene/methanol, and dried under high vacuum. The resulting salts were used without further purification.

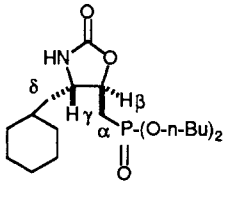
Method B. Under a slow stream of nitrogen, a two-necked flask was charged with a stir bar, 10% Pd/C (1:5 ratio (w:w) of catalyst:substrate), and ethyl acetate (enough to cover the catalyst) and fitted with an inlet for hydrogen and a septum. To this was added sequentially HCl (10 equiv as a 4.8 M solution in dioxane) and the substrate (1.0 equiv) in enough ethyl acetate to give an ~0.25 M solution. After evacuating and flushing with hydrogen three times, the reaction was exposed to 1 atm of hydrogen and stirred at room temperature until TLC indicated the reaction was complete (typically 0.5–3.0 h). The catalyst was removed by filtration through a Celite plug. The filtrate was concentrated in vacuo and partitioned between water and hexanes. The aqueous layer was then lyophilized to provide the corresponding amine hydrochloride salt in nearly quantitative yield (95–98%).

(4*S*,5*R*)- and (4*S*,5*S*)-5-[(Dimethylphosphinyl)-methyl]-4-(phenylmethyl)oxazolidin-2-one (9a and 10a). The

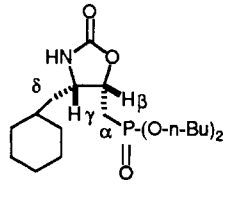
more mobile diastereomer (50.3 mg, 0.13 mmol) of **8a** was deblocked according to method A to provide the corresponding amine hydrochloride salt, which was subsequently suspended in dry dichloromethane (500 μL), treated with triethylamine (56 μL, 0.39 mmol), and cooled to 0 °C. To the resulting cooled suspension was added phosgene (185 μL, 0.20 mmol, 12.5% in toluene) in a single portion. The reaction was stirred for 1 h at 0 °C, the cooling bath was removed, and the reaction mixture was stirred for 1 h at room temperature. The reaction solution was diluted with ethyl acetate and washed sequentially (1×, 10% aqueous HCl; 1×, saturated aqueous $NaHCO_3$; 2×, brine), dried (Na_2SO_4), filtered, and concentrated in vacuo. Purification by forced-air chromatography (FAC) (8 g of silica gel, 5% methanol/chloroform) provided 19.8 mg (51%) of the title compound. (**4*S*,5*R*)-9a**: ^{1}H NMR ($CDCl_3$, TMS) δ 7.18–7.4 (m, 5 H), 5.25 (br s, 1 H, NH), 4.63 (d, d, d, d, J_{HH} = 4.8, 5.5, 7.8, J_{HP} = 8.6 Hz, HCO), 3.95 (d, d, d, J = 4.8, 5.0, 8.8 Hz, HNCH), 3.78 (d, J_{HP} = 12 Hz, $POCH_3$), 3.75 (d, J_{HP} = 12 Hz, $POCH_3$), 3.00 (ABX, J = 5.0, 13.4 Hz, PhCHH), 2.79 (ABX, J = 8.8, 13.4 Hz, PhCHH), 2.27 (ABX_2, J_{HH} = 5.6, 15.2, J_{HP} = 19.3 Hz, OPCHH), 2.13 (ABX_2, J_{HH} = 7.8, 15.2, J_{HP} = 18.5 Hz, OPCHH); mass spectrum (DCI) $(M + H)^+$ = 300, M^+ = 299. Anal. ($C_{13}H_{18}NO_5P$) C, H, N. (**4*S*,5*S*)-10a** was prepared in an identical fashion from the less mobile diastereomer of **8a**. **10a**: ^{1}H NMR ($CDCl_3$, TMS) δ 7.15–7.4 (m, 5 H), 5.04 (d, d, d, d, J = 7.7 Hz, HCO), 4.91 (br s, 1 H, NH), 4.05 (d, d, d, J = 5.8, 7.5, 12 Hz, HNCH), 3.86 (d, J_{HP} = 11.5 Hz, OCH_3), 3.81 (d, J = 11.5 Hz, OCH_3), 3.04 (d, d, J = 3.5, 12.5 Hz, Ph CHH), 2.58 (d, d, J = 11.0, 12.5 Hz, PhCHH), 2.42 (d, d, d, J = 7.7, 15, 20 Hz, HHPO), 2.28 (d, d, d, J = 7.7, 15, 20 Hz, HHPO).

(4*S*,5*R*)- and (4*S*,5*S*)-5-[(Di-*n*-butylphosphinyl)methyl]-4-(cyclohexylmethyl)oxazolidin-2-one (9e and 10e). Following the general procedure for the preparation of oxazolidinones, **8l** (170.2 mg, 0.281 mmol) as a 3:1 mixture of C_2-hydroxy isomers was deprotected by method B, and the resulting amine salts were converted to a 3:1 mixture of **9e** and **10e**. Purification by forced-air chromatography (8 g of silica gel, 50% ethyl acetate/hexanes (fractions 1–30) and then 75% ethyl acetate/hexanes) provided a purified mixture of **9e** and **10e** (80.5 mg, 74% yield) where **9e** was the major component, which was in agreement with an ^{1}H NMR spectrum of the unpurified mixture. Careful selection of early fractions from the chromatographic purification provided a sample of pure **9e**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.45 (d, d, d, d, J = 4.4, 5.5, 8.1, 8.1 Hz, OCH), 4.0–4.12 (m, 4 H, 2[$POCH_2$]), 3.78 (d, d, d, d, J = 0.9, 4.4, 5.5, 10.1 Hz, NCH), 2.27 (d, d, d, J = 5.5, 15.2, 19.3 Hz, PCHH), 2.16 (d, d, d, J = 8.0, 15.2, 18.4 Hz, PCHH), 1.60–1.75 (m, 10 H), 1.47 (d, d, d, J = 2.5, 5.5, 8.3 Hz, $NCHCH_2$), 1.10–1.46 (m, 10 H), 0.94 (d, t; J = 1.5, 7.0, 7.0 Hz, 2[CH_2CH_3]). Anal. ($C_{13}H_{18}NO_5P$) C, H, N.

Determination of Relative Stereochemistry in the Oxazolidinones. All spectra were collected at 500 MHz. The determination of the relative stereochemistry in **9e** and **10e** is given as a typical example. In most cases a pure sample of the more mobile isomer was secured during chromatography by carefully selecting early fractions of the product peak. The procedure can be successfully carried out on a mixture of both isomers; however, having one pure isomer simplifies assigning protons in the 1D ^{1}H NMR spectra.



9e



10e

A 1D ^{1}H NMR spectrum of pure isomer **9e** and a mixture of **9e** and **10e** were collected and H_α, H_β, H_γ, and H_δ were identified with coupling constants and decoupling experiments. Decoupling by irradiation of H_γ was always necessary to locate H_δ. A 2D NOE experiment was then conducted on both the pure sample (**9e**) and the mixture (**9e** and **10e**). The anti isomer **9e** had a small NOE between the two methine proton H_β and H_γ which was approximately $^1/_3$ the magnitude of the NOE observed between the two analogous protons in the syn isomer (**10e**). The syn isomer (**10e**) also always gave an NOE between the H_α methylenes and the H_δ methylenes in contrast to the anti isomer (**9e**), which showed no measurable NOE between the same two methylenes. These results clearly permit the assignment of the relative stereochemistry in the oxazolidinones and hence in the phosphostatine condensation adducts as well.

General Procedure for Mosher Amide Formation. The desired N-protected LVR (1.0 equiv) was deprotected following the previously described procedures. The resulting salts were suspended in dry dichloromethane (0.25 M), cooled to 0 °C, and triethylamine (2.5 equiv) and (+)- or (−)-MTPA-Cl (1.5 equiv) were added via a syringe. The cooling bath was removed; the reaction was stirred for 0.5 h and poured into excess 10% aqueous HCl. The quenched reaction mixture was extracted with ethyl ether (2×). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (1×) and brine (1×), dried over Na_2SO_4, filtered, and concentrated in vacuo. The unpurified material was analyzed for diastereomeric purity by ^{1}H NMR and/or reverse-phase analytical HPLC.

8b-Derived (+)-Mosher Amide [(+)-11b]. Following the general procedure, the more mobile diastereomer of **8b** (28.6 mg, 0.075 mmol) was converted to the (+)-Mosher amide derivative (+)-**11b**. ^{1}H NMR of the unpurified mixture indicated a ≥95:5 ratio of diastereomers by integrating the phosphonate methyl esters at δ 3.75 and 3.65 for the (+)-series. This was consistent with our previous results.[9] Purification by FAC (2 g of silica gel, 50% ethyl acetate/chloroform then 5% methanol/chloroform provided an analytical sample of (+)-**11b** (18.5 mg, 50%) for characterization. (+)-**11b**: ^{1}H NMR ($CDCl_3$, TMS) δ 7.54–7.62 (m, 2 H), 7.37–7.45 (m, 3 H), 6.97 (d, J = 9.5 Hz, HH), 3.96–4.07 (m, 2 H), 3.74 (d, J = 11.5 Hz, 3 H), 3.65 (d, J = 11.5 Hz, 3 H), 3.45 (q, J = 2 Hz, 3 H), 1.57–1.9 (m, 8 H), 1.37–1.48 (m, 1 H), 1.08–1.36 (m, 4 H), 0.75–1.04 (m, 2 H); mass spectrum (EI) M^+ = 495. Anal. ($C_{22}H_{33}NO_6F_3P$) C, H, N.

8b-Derived (−)-Mosher Amide [(−)-11b]. Following the general procedure, the more mobile diastereomer of **8b** (32.6 mg, 0.086 mmol) was converted to the (−)-Mosher amide derivative (−)-**11b**. ^{1}H NMR of the unpurified mixture indicated a ≥95:5 ratio of diastereomers by integrating the phosphonate methyl esters at δ 3.78 and 3.75 for the (−)-series. Purification by FAC (2 g of silica gel, 50% ethyl acetate/chloroform and then 5% methanol/chloroform) provided an analytical sample of (−)-**11b** (14.8 mg, 35%) for characterization. (−)-**11b**: ^{1}H NMR ($CDCl_3$, TMS) δ 7.47–7.55 (m, 2 H), 7.38–7.45 (m, 3 H), 7.15 (d, J = 9.5 Hz, NH), 4.0–4.12 (m, 2 H), 3.78 (d, J = 11.5 Hz, 3 H), 3.75 (d, J = 11.5 Hz, 3 H), 3.40 (q, J = 2 Hz, 3 H), 1.55–2.03 (m, 9 H), 1.33–1.45 (m, 1 H), 1.03–1.23 (m, 3 H), 0.73–1.0 (m, 2 H); mass spectrum (EI) M^+ = 495. Anal. ($C_{22}H_{33}NO_6F_3 \cdot 0.25H_2O$) C, H, N.

8g-Derived (+)-Mosher Amide [(+)-11b]. Following the general procedure, **8g** (21.4 mg, 0.068 mmol) was converted to the corresponding (+)-Mosher amide derivative (+)-**11b** as a 3:1 mixture of isomers at the hydroxyl-bearing carbon. The unpurified mixture of (+)-**11b** (46 mg, 88%) was analyzed by high-performance liquid chromatography (HPLC) using an Applied Biosystems Model 400 pump, a Model 783 variable-wavelength detector, a Waters Model 412 automatic sampler, a 50 × 4.6 mm Spherisorb ODS (3-μm particle) column (Regis Chemical Co.), and a mobile phase consisting of acetonitrile/methanol/0.01 M tetramethylammonium perchlorate in 0.1% trifluoroacetic acid (40/5/55) at a flow rate of 1.5 mL/min. The effluents were monitored at a wavelength of 205 nM. This analysis indicated no racemization within our detection limits (±1%) thus the enantiomeric purity was ≥99%.

8g-Derived (−)-Mosher Amide [(−)-11b]. Following the procedure as described for the (+)-Mosher amide derivative, an identical sample of **8g** (21.4 mg, 0.068 mmol) was converted to (−)-**11b**, analyzed by HPLC without purification, which indicated no detectable racemization (±1%).

General Coupling Method. To a −23 °C (CCl_4/dry ice bath) DMF solution (0.25 M) of the amine·HCl salt (1.0 equiv) was added, in order, the desired N-protected amino acid or N-protected dipeptide (1.0 equiv), 1-hydroxybenzotriazole monohydrate (3.0 equiv), *N*-methylmorpholine (2.0 equiv), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (1.0 equiv). The reaction was stirred for 2 h at −23 °C and for 12–18 h at room

temperature and was quenched by pouring into saturated aqueous $NaHCO_3$. The resulting aqueous mixture was extracted with ethyl acetate (2×). The combined organic layers were washed (1×, brine), dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by normal-phase chromatography was performed using a methanol/chloroform solvent system which gave an R_f between 0.10 and 0.20. When the N-terminus piece contained more than one basic functionality (e.g., H-His-LVR·2HCl), an additional equivalent(s) of *N*-methylmorpholine was (were) added to neutralize all basic moieties.

(2*R*,3*S*)-Dimethyl [3-[[[(*tert*-Butyloxycarbonyl)phenylalaninyl]histidyl]amino]-4-cyclohexyl-2-hydroxybutyl]phosphonate (15). Following method A for N-deprotection, the more mobile isomer of **8b** (85.2 mg, 0.225 mmol) was deblocked to the corresponding hydrochloride salt, which was subsequently coupled to Boc-Phe-His-OH following the general coupling procedure to provide 100.4 mg of unpurified product. Forced-air chromatography (8 g of silica gel, 5% methanol/chloroform for fractions 1–50 and then 10% methanol/chloroform) provided pure title compound (64.4 mg, 43%), which was a 69:31 mixture of His isomers by HPLC. **15**: Partial ^1H NMR ($CDCl_3$) 7.51 (br s, $^1/_3$ H), 7.48 (br s, $^2/_3$ H), 6.83 (br s, $^2/_3$ H), 6.71 (br s, $^1/_3$ H), 5.18 (br d, J = 6 Hz, $^2/_3$ H), 5.62 (br d, J = 6 Hz, $^2/_3$ H), 3.98 and 3.75 (d, J_{HP} = 10.5 Hz, $^1/_3$ OMe), 3.73 (d, J_{HP} = 10.5 Hz, $^2/_3$ OMe), 1.42 (s, $^1/_3$ $OC(CH_3)_3$), 1.38 (s, $^2/_3$ $OC(CH_3)_3$).

(2*S*,3*S*)-Dimethyl [3-[[[(*tert*-Butyloxycarbonyl)phenylalaninyl]histidyl]amino]-4-cyclohexyl-2-hydroxybutyl]phosphonate (17). Following method A for N-deprotection, the less mobile isomer of **8b** (52.9 mg, 0.139 mmol) was converted to the corresponding hydrochloride salt and subsequently coupled to Boc-Phe-His-OH following the general coupling procedures. Purification by forced-air chromatography (8 g of silica gel, 5% methanol/chloroform fractions for 1–50 and then 10% methanol/chloroform) provided the pure title compound (22.9 mg, 25% yield), which was a 84:15 mixture of His isomers by HPLC. **17**: Partial ^1H NMR ($CDCl_3$) δ 7.55 (br s, 1 H), 7.05–7.35 (m, 5 H), 6.87 (br s, 1 H), 3.84 (d, J_{HP} = 12 Hz, 3 H, major), 3.70 (d, J_{HP} = 12 Hz, 3 H, major), 1.38 (s, 9 H, minor), 1.35 (s, 9 H, major).

(2*R*,3*S*)-Dimethyl [3-[[[(*tert*-Butyloxycarbonyl)phenylalaninyl]leucyl]amino]-4-cyclohexyl-2-hydroxybutyl]phosphonate (25). Following method A for N-deprotection, **8g** (152.2 mg, 0.293 mmol) was deprotected and coupled to Boc-Phe-Leu (111.2 mg, 0.294 mmol) according to the general coupling procedure. Purification by forced-air chromatography (16 g of silica gel, 2% methanol/chloroform for fractions 1–70 and then 5% methanol/chloroform) provided pure **25** (187 mg, 77% yield), which was 94% pure by HPLC analysis. **25**: Partial ^1H NMR ($CDCl_3$) δ 7.2–7.35 (m, 5 H), 6.4 (br s, 1 H), 6.23 (br d, J = 6 Hz, 1 H), 4.97 (br s, 1 H), 4.25–4.37 (m, 2 H), 4.0 (m, 2 H), 3.78 (d, J_{HP} = 12 Hz, 3 H), 3.76 (d, J_{HP} = 12 Hz, 3 H), 3.09 (d, J = 6 Hz, 2 H), 1.42 (s, 9 H).

(2*R*,3*S*)-Methyl [3-[[[(*tert*-Butyloxycarbonyl)phenylalaninyl]leucyl]amino]-4-cyclohexyl-2-hydroxybutyl]phosphonate (26). Following the procedure of Van Tamelen,[22] **25** (56 mg, 0.0875 mmol) was treated with triethylamine (48 μL, 0.341 mmol) and thiophenol (19 μL, 0.350 mmol) and stirred overnight at 40 °C. The volatiles were removed in vacuo, and the resulting residue was purified by adsorption on to a 35-g bed of XAD-8 resin (prewashed with 250 mL of 1:1 MeOH/H_2O and 500 mL of H_2O) and eluted with 1500 mL of 25% EtOH/H_2O. This provided 13.3 mg of pure **26** (24%), which appeared to be a 5:1 diastereomeric mixture of monomethyl phosphonate esters by ^1H NMR, and an additional 10.4 mg of product, which contained an impurity. **26**: Partial ^1H NMR (CD_3OD) δ 7.18–7.3 (m, 5 H), 4.45 (br t, J = 7.5 Hz, 1 H), 4.33 (d, d, J = 4.5, 10.5 Hz, 1 H), 3.56 (d, J_{HP} = 10.5 Hz, major, OMe), 3.54 (d, J = 10.5 Hz, minor, OMe), 1.34 (s, major, $OC(CH_3)_3$), 0.93 (overlapping d, J = 7.0 Hz, 6 H).

(2*R*,2*S*)-Methyl [3-[[[(*tert*-Butylacetyl)phenylalaninyl]histidyl]amino]-4-cyclohexyl-2-hydroxybutyl]phosphonate (21). Following method A for N-deprotection, the more mobile isomer of **8b** (195.9 mg, 0.516 mmol) was deprotected and subsequently coupled to Boc-His-OH (134 mg, 0.516 mmol) following the general coupling procedure to provide 260 mg of unpurified title compound. Purification by forced-air chromatography (10 g of silica gel, 4% methanol/chloroform (fractions 1–20) and then 6% methanol/chloroform) provided 159 mg (60% yield) of Boc-His-LVR: Partial ^1H NMR ($CDCl_3$) δ 7.56 (s, 1 H), 6.85 (s, 1 H), 6.6 (br d, J = 9 Hz, 1 H), ~6.17 (br s, 1 H), 4.37 (q, J = 6 Hz, 1 H), 3.92 (br d, J = 9 Hz, 2 H), 3.77 (d, J_{HP} = 11.5 Hz, 3 H), 3.74 (d, J = 11.5 Hz, 3 H), 3.13 (d, d, J = 5, 15 Hz, 1 H), 3.0 (d, d, J = 6.5, 15 Hz, 1 H), 1.45 (s, 1 H). Anal. ($C_{23}H_{41}N_4O_7PO·H_2O$) C, H, N.

The preceding compound (71 mg, 0.137 mmol) was N-deprotected according to method A and subsequently coupled to Tba-Phe-OH (37.2 mg, 0.141 mmol) following the general procedure. Purification by forced-air chromatography (5 g of silica gel, 7% methanol/chloroform) provided 95% pure **21** (34 mg, 37%) as judged by HPLC. **21**: Partial ^1H NMR ($CDCl_3$) δ 7.51 (s, 1 H), 6.85 (s, 1 H), 6.67 (br d, J = 9 Hz, 1 H), 3.75 (d, J_{HP} = 10.5 Hz, 6 H), 0.84 (s, 9 H). Anal. ($C_{33}H_{52}N_5O_7P·H_2O$) C, H, N.

In Vitro Enzyme Inhibition. The renin-inhibition data were obtained using purified human renal renin at pH 6.0 using the previously described method.[18d] Bovine cathepsin D (Sigma) and porcine pepsin (Sigma) activities were assessed by the hydrolysis of hemoglobin at pH 3.1 and 1.9, respectively, at 37 °C, and measurements of the absorbance at 280 nm of the supernatant were made after precipitation with trichloroacetic acid.[18d]

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Registry No. **3a**, 7517-19-3; **3b**, 7524-50-7; **3c**, 17193-39-4; **4a**, 18598-66-8; **4b**, 18598-80-6; **4c**, 123381-13-5; **5a**, 100841-17-6; **5b**, 100841-19-8; **5c**, 123381-14-6; **6a**, 107600-13-5; **6b**, 107591-60-6; **6c**, 112190-46-2; **7b**, 72155-45-4; **7c**, 98105-42-1; (2*R*)-**8a**, 107591-58-2; (2*S*)-**8a**, 107591-59-3; (2*R*)-**8b**, 123381-15-7; BOC-deblocked (2*R*)-**8b**·HCl, 123381-48-6; (2*S*)-**8b**, 123381-16-8; BOC-deblocked (2*S*)-**8b**·HCl, 123381-49-7; (2*R*)-**8c**, 123381-17-9; (2*S*)-**8c**, 123381-18-0; (2*R*)-**8d**, 123381-19-1; (2*S*)-**8d**, 123381-20-4; (2*R*)-**8e**, 123381-21-5; (2*S*)-**8e**, 123381-22-6; (2*R*)-**8f**, 107591-62-8; (2*S*)-**8f**, 107591-63-9; (2*R*)-**8g**, 123381-23-7; (2*S*)-**8g**, 123381-24-8; (2*R*)-**8i**, 123381-25-9; (2*S*)-**8i**, 123381-26-0; (2*R*)-**8j**, 123381-27-1; (2*S*)-**8j**, 123381-28-2; (2*R*)-**8k**, 123381-29-3; (2*S*)-**8k**, 123381-30-6; (2*R*)-**8l**, 123381-31-7; (2*S*)-**8l**, 123381-32-8; **9a**, 107591-64-0; **9e**, 123381-33-9; **10a**, 107591-65-1; **10e**, 123381-34-0; (2*R*)-(+)-**11b**, 123381-35-1; (2*R*)-(−)-**11b**, 123482-51-9; (2*S*)-(+)-**11b**, 123482-56-4; **12**, 123381-36-2; **13**, 123381-37-3; **14**, 123381-38-4; D-**His-14**, 123482-59-7; **15**, 123381-39-5; D-**His-15**, 123482-57-5; **16**, 123482-52-0; D-**His-16**, 123482-60-0; **17**, 123482-53-1; D-**His-17**, 123482-58-6; **18**, 123381-40-8; D-**His-18**, 123482-61-1; **19**, 123381-41-9; D-**His-19**, 123482-62-2; (2*R*)-**20**, 123381-42-0; (2*S*)-**20**, 123482-54-2; **21**, 123381-43-1; **22**, 123381-44-2; (2*R*)-**23**, 123381-45-3; D-**His-(2*R*)-23**, 123482-63-3; (2*S*)-**23**, 123482-55-3; D-**His-(2*S*)-23**, 123482-64-4; **25**, 123381-46-4; **26**, 123381-47-5; **27**, 123484-23-1; D-**His-27**, 123381-51-1; (+)-MTPA-Cl, 20445-33-4; (−)-MTPA-Cl, 39637-99-5; Ph_3CCl, 76-83-5; $MeP(O)(OMe)_2$, 756-79-6; $MeP(O)(OEt)_2$, 683-08-9; $MeP(O)(OPr\text{-}i)_2$, 1445-75-6; $MeP(O)(OBu)_2$, 2404-73-1; $MeP(O)(-OCH_2CH_2CH_2O-)$, 13407-03-9; $Me_3P{=}O$, 676-96-0; BOC-Phe-His-OH, 63648-87-3; BOC-Phe-Leu-OH, 33014-68-5; BOC-His-OH, 17791-52-5; BOC-His-LVR, 123381-50-0; Tba-Phe-OH, 103127-85-1; renin, 9015-94-5.