Structural Evolution and Pharmacology of a Novel Series of Triacid Angiotensin II Receptor Antagonists

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cis-4-(4-Phenoxy)-1-[1-oxo-2(*R*)-[4-[(2-sulfobenzoyl)amino]-1*H*-imidazol-1-yl]octyl]-L-proline derivatives represent a novel class of potent nonpeptide angiotensin II (Ang II) receptor antagonists. These compounds evolved from directed structure–activity relationship (SAR) studies on a lead identified by random screening. Further SAR studies revealed that acidic modification of the 4-phenoxy ring system produced a series of triacid derivatives possessing oral activity in pithed rats. The most potent compound, *cis*-4-[4-(phosphonomethyl)phenoxy]-1-[1-oxo-2(*R*)-[4-[(2-sulfobenzoyl)amino]-1*H*-imidazol-1-yl]octyl]-L-proline (**1e**), inhibited the pressor response to exogenously administered Ang II for periods up to 8 h following oral dosing. The antihypertensive activity of **1e** was evaluated in the Lasix-pretreated conscious spontaneously hypertensive rat (SHR) where it produced a dose-dependent fall in blood pressure following oral dosing lasting >12 h. Antagonists such as **1e** may serve as useful therapeutic agents for the treatment of hypertension as well as for studying the role of Ang II in various disease states.

Introduction

Recent clinical and experimental studies have provided mounting evidence that angiotensin (Ang II)-converting enzyme inhibitors are effective therapy in a wide variety of cardiovascular indications such as heart failure, myocardial hypertrophy, and diabetic nephropathy.[1-4] In view of the diverse pathophysiologic actions of Ang II, interfering with the function of the renin–angiotensin system through other means continues to be an attractive target for new drug development.[5,6] The recent introduction of potent, orally active receptor antagonists of Ang II has added additional impetus to this effort.[7] Thus, there are now a wide variety of antagonists described, most deriving from and/or containing the biphenyl–tetrazole substructure present in losartan (Chart 1).[8-11]

Recently, we described the synthesis and *in vitro* pharmacological evaluation of a novel series of diastereomeric phenoxyproline octanoamides as Ang II (AT_1) receptor antagonists.[12] Studies comparing functional inhibition of AT_1 receptor-mediated responses showed that compound **1b** (Chart 1), possessing the (*R,S,S*) configuration, to be significantly more potent (10–1000-fold) than any of its seven stereoisomers. Compound **1b** evolved from a series of substituted imidazole hexanoic acids (see Chart 2) identified by a random screening effort to uncover potent nonpeptide Ang II receptor antagonists.[13] Although the initial leads from this effort were relatively weak as Ang II receptor antagonists in isolated tissues (Chart 2), they led to the discovery of a potent series of phenoxyproline triacids, exemplified by compounds **1a–g.** In this paper, we report the structural evolution of this novel class of Ang II antagonists, as well as the *in vivo* pharmacology of these agents.

Chemistry

In order to prepare the compounds shown in Tables 1 and 2, we sought a flexible approach that would allow us to readily vary the substitution of the aryloxy ring as well as provide easy access to the desired (*R,S,S*) diastereomer. For this discussion, the synthetic methods used for the preparation of **1a–g** will be described in detail as they are representative of the methods used to explore the SAR of the entire series.[14] Thus, the synthesis of **1a–g** began with the Mitsunobu coupling of phenol derivatives **2a–g** with (2*S*,4*R*)-*N*-Cbz-4-hydroxyproline methyl ester (DEAD, Ph_3P, THF) to give (2*S*,4*S*)-*N*-Cbz-4-phenoxyproline derivatives **3a–g** in 37–83% yield.[15,16] Phenol substrates **2a–c** were prepared by esterification of the commercially available carboxylic acids (MeOH or EtOH, pTsOH, reflux). Tetrazole derivative **2g** was prepared from (4-hydroxyphenyl)acetonitrile, and **2d–f** were prepared as described in Scheme 1.[17-19] Removal of the Cbz protecting groups was accomplished by catalytic hydrogenation (EtOH–EtOAc, 10% Pd/C, 40 psi) to give the requisite intermediate 4-phenoxyproline ester derivatives **4a–g**. The carboxylic acid derivatives (**4a–d**), and the tetrazole **4g**, were converted to the HBr salt form (ethereal–HBr) for characterization.

Construction of the key stereochemically defined 4-nitroimidazole proline octanoamide intermediates **6a–e,g** from **4a–e,g** is shown in Scheme 2. 4-Nitroimidazole was converted to the octanoic acid derivative (±)-**5** by alkylation with ethyl 2-bromooctanoate followed by ester hydrolysis in 90% yield. Nitroimidazole acid (±)-**5** was converted to its acid chloride ($(COCl)_2$, CH_2Cl_2) and reacted with proline ester derivatives **4a–e.** In the cases where the HBr salt was employed, the free base was generated by treatment with diisopropylamine prior to reaction with the acid chloride. In the coupling reaction, diastereomers **6a–e** and **7a–e** were produced as 1:1 mixtures and readily separated by flash chromatography.

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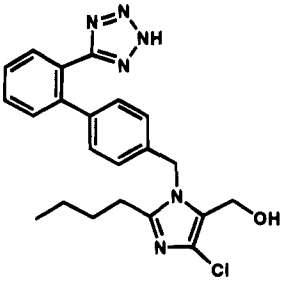
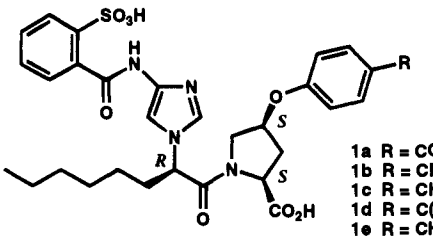
Chart 1

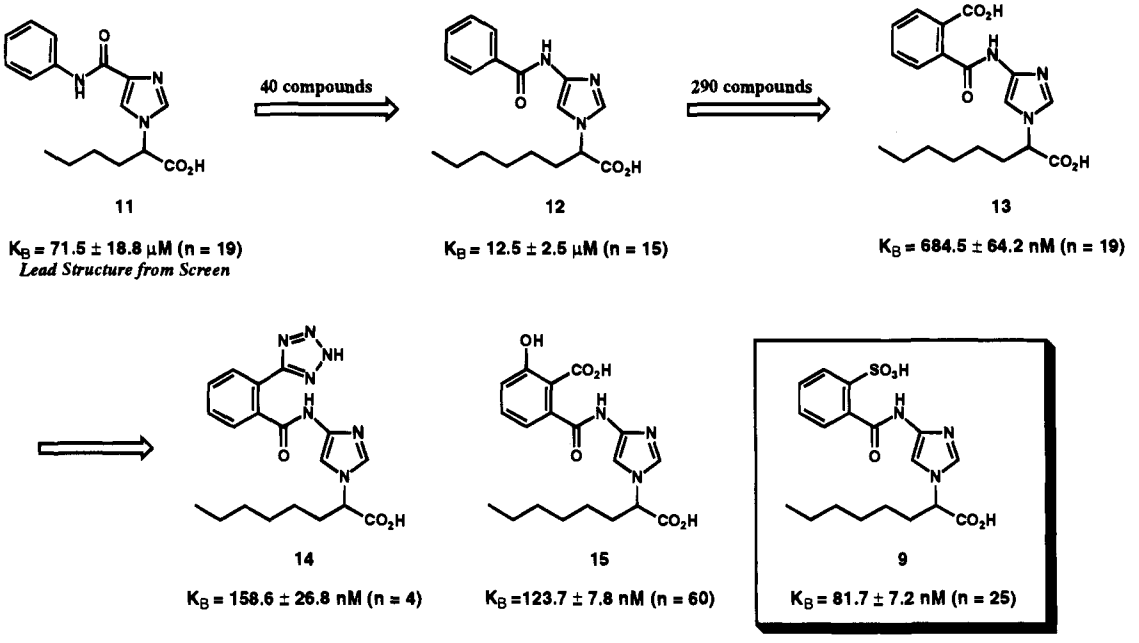


Losartan



1a R = CO_2H
1b R = CH_2CO_2H
1c R = $CH_2CH_2CO_2H$
1d R = $C(CH_3)_2CO_2H$
1e R = $CH_2PO_3H_2$
1f R = $CH_2CH_2PO_3H_2$
1g R = CH_2CN_4H

Chart 2



11 $K_B = 71.5 \pm 18.8$ μM (n = 19)
Lead Structure from Screen

12 $K_B = 12.5 \pm 2.5$ μM (n = 15)

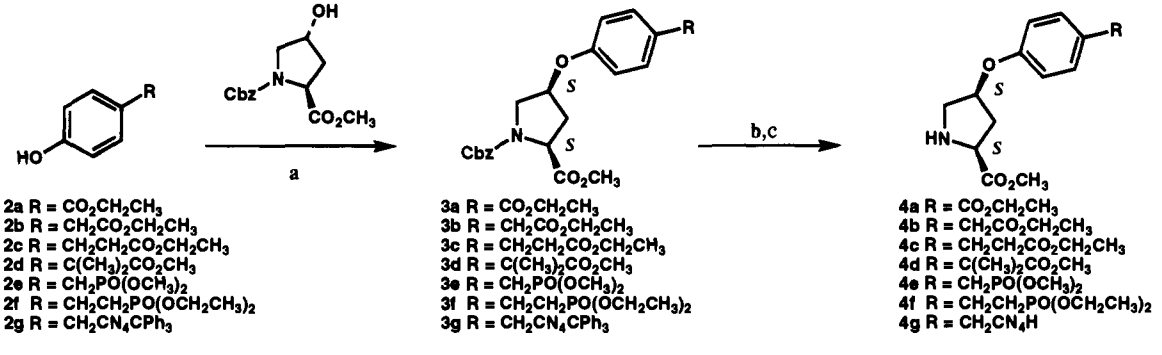
13 $K_B = 684.5 \pm 64.2$ nM (n = 19)

14 $K_B = 158.6 \pm 26.8$ nM (n = 4)

15 $K_B = 123.7 \pm 7.8$ nM (n = 60)

9 $K_B = 81.7 \pm 7.2$ nM (n = 25)

Scheme 1



2a R = $CO_2CH_2CH_3$
2b R = $CH_2CO_2CH_2CH_3$
2c R = $CH_2CH_2CO_2CH_2CH_3$
2d R = $C(CH_3)_2CO_2CH_3$
2e R = $CH_2PO(OCH_3)_2$
2f R = $CH_2CH_2PO(OCH_2CH_3)_2$
2g R = $CH_2CN_4CPh_3$

3a R = $CO_2CH_2CH_3$
3b R = $CH_2CO_2CH_2CH_3$
3c R = $CH_2CH_2CO_2CH_2CH_3$
3d R = $C(CH_3)_2CO_2CH_3$
3e R = $CH_2PO(OCH_3)_2$
3f R = $CH_2CH_2PO(OCH_2CH_3)_2$
3g R = $CH_2CN_4CPh_3$

4a R = $CO_2CH_2CH_3$
4b R = $CH_2CO_2CH_2CH_3$
4c R = $CH_2CH_2CO_2CH_2CH_3$
4d R = $C(CH_3)_2CO_2CH_3$
4e R = $CH_2PO(OCH_3)_2$
4f R = $CH_2CH_2PO(OCH_2CH_3)_2$
4g R = CH_2CN_4H

Preparation of 2d-f

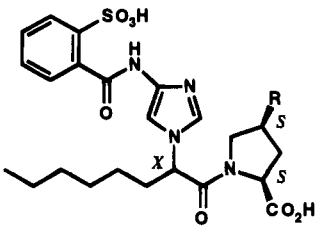
4-methoxyphenylacetic acid —d,e,f→ **2d**
4-benzyloxybenzylchloride —g,b→ **2e**
4-benzyloxybenzaldehyde —h,b→ **2f**

a Conditions: (a) DEAD, Ph_3P, THF, room temperature; (b) H_2 (40 psi), 10% Pd/C, EtOH; (c) ethereal HBr, 25 °C (**4a-d, g**). (d) LDA, MeI; (e) pyridine hydrochloride, 150 °C; (f) MeOH, pTsOH(cat.), reflux; (g) $(MeO)_2POH$, NaH, THF; (h) $((EtO)_2PO)_2CH_2$, NaH, DMF.

In subsequent work, it was found that the *R*-enantiomer of **5** could be readily obtained by selective crystallization of the (−)-cinchonidine salt (Scheme 2). The enantiomeric excess of the resolved acid was found to be >99% after a single recrystallization from EtOH–H_2O. Enantiomeric excess was determined by chiral HPLC analysis of the methyl ester (CH_2N_2, Et_2O). Using the resolution protocol, **6g** was prepared stereoselectively by the DCC coupling of **4g** to (*R*)-**5**.

The absolute stereochemical assignment of **6a–e,g** was established as (*R,S,S*) based on the X-ray crystallographic analysis of a closely related compound, **7h**

Table 1. Structure, Physical Properties, and *in Vitro* Antagonism of Ang II by **16**–**40**



compd	R	stereochemistry (X,S,S)	mp, °C	formula[a]	analyses	in vitro $K_B \pm$ SE (nM)[e]
16	H	(*R*,*S*)	190–195	$C_{23}H_{30}N_4O_7S$	C, H, N	50.9 ± 12.7 (5)
17	H	(*S*,*S*)	163–170	$C_{23}H_{30}N_4O_7S$	C, H, N	461 (1)
18	OH	(*R*,*S*,*S*)	198–205	$C_{23}H_{30}N_4O_8S$	C, H, N	25.8 (1)
19	OPh	(*R*,*S*,*S*)	180–190	$C_{29}H_{34}N_4O_8S$	C, H, N	0.80 ± 0.12 (20)
20	OPh	(*S*,*S*,*S*)	>200 dec	$C_{29}H_{34}N_4O_8S{\cdot}1.2HCl$	C, H, N	3.2 ± 1.9 (3)
21	OPh(4-Me)	(*R*,*S*,*S*)	155–165	$C_{30}H_{36}N_4O_8S{\cdot}0.5H_2O$	C, H, N	1.09± 0.5 (7)
22	OPh(4-iPr)	(*R*,*S*,*S*)	175–185	$C_{32}H_{40}N_4O_8S$	C, H, N	25.1 (1)
23	OPh(4-tBu)	(*R*,*S*,*S*)	162–170	$C_{33}H_{42}N_4O_8S$	C, H; N[b]	125.9 (1)
24	OPh(4-F)	(*R*,*S*,*S*)	160–175 dec	$C_{29}H_{33}FN_4O_8S{\cdot}0.5H_2O$	C, H; N[c]	3.0 ± 1.7 (3)
25	OPh(4-CF_3)	(*R*,*S*,*S*)	155–162 dec	$C_{30}H_{33}F_3N_4O_8S{\cdot}1.5H_2O$	C, H, N	34.7 (1)
26	OPh(4-Ph)	(*R*,*S*,*S*)	154–165 dec	$C_{35}H_{38}N_4O_8S$	C, H, N	10.0 (1)
27	OPh(4-OMe)	(*R*,*S*,*S*)	145–155	$C_{30}H_{36}N_4O_9S{\cdot}0.5H_2O$	C, H, N	0.44 ± 0.12 (11)
28	OPh(3-OMe)	(*R*,*S*,*S*)	148–155	$C_{30}H_{36}N_4O_9S$	C, H, N	11.6 (2)
29	OPh(2-OMe)	(*R*,*S*,*S*)	150–162	$C_{30}H_{36}N_4O_9S{\cdot}1.0H_2O$	C, H, N	>30 (2)
30	OPh(4-OiPr)	(*R*,*S*,*S*)	138–145	$C_{32}H_{40}N_4O_9S$	C, H, N	4.1 ± 2.9 (3)
31	OPh(4-OtBu)	(*R*,*S*,*S*)	170–175	$C_{33}H_{40}N_4O_9S$	C, H, N	5.2 ± 1.4 (4)
32	OPh(3,4-OCH_2O-)	(*R*,*S*,*S*)	169–175	$C_{30}H_{34}N_4O_{10}S{\cdot}0.5HCl$	C, H, N	0.92 ± 0.4 (4)
33	OPh(3,4-di-OMe)	(*R*,*S*,*S*)	152–162	$C_{31}H_{36}N_4O_{10}S{\cdot}0.5H_2O$	C, H, N	15.8 (1)
34	O(2-naphthyl)	(*R*,*S*,*S*)	170–180 dec	$C_{33}H_{36}N_4O_8S{\cdot}1.0H_2O$	C, H, N	4.1 ± 1.2 (7)
35	O(1-naphthyl)	(*R*,*S*,*S*)	170–190 dec	$C_{33}H_{36}N_4O_8S{\cdot}1.0H_2O$	C, H, N	4043.4 (2)
36	O(5-benzofuran)	(*R*,*S*,*S*)	170–180 dec	$C_{31}H_{34}N_4O_9S{\cdot}1.2HCl$	C, H, N	0.63 ± 0.16 (6)
37	O(5-isoquinoline)	(*R*,*S*,*S*)	185–190 dec	$C_{32}H_{35}N_5O_8S{\cdot}1.0HCl$	C, H, N	3.3 ± 1.6 (3)
38	O(5-thianaphthene)	(*R*,*S*,*S*)	168–172 dec	$C_{31}H_{34}N_4O_8S_2{\cdot}3.5HCl$	C, H, N	3.6 (2)
39	O(3-pyridyl)	(*R*,*S*,*S*)	180–183 dec	$C_{28}H_{33}N_5O_8S{\cdot}3.0HCl$	C, H; N[d]	148 (1)
40	O(5-isoxazole)	(*R*,*S*,*S*)	225–230 dec	$C_{26}H_{31}N_5O_9S{\cdot}0.75HCl$	C, H, N	94.6 (2)

[a] All compounds had C, H, and N microanalysis within ±0.4% theoretical value unless otherwise noted. [b] N: calcd, 8.50; found, 7.73. [c] N: calcd, 8.90; found, 8.42. [d] N: calcd, 9.90; found, 10.49. [e] Numbers in parentheses represent the number of individual experiments, with each experiment representing the average of at least four tissues.

Table 2. Physical Properties and *in Vitro* and *in Vivo* Antagonism of Ang II by **1a**–**g** and **41**–**42**

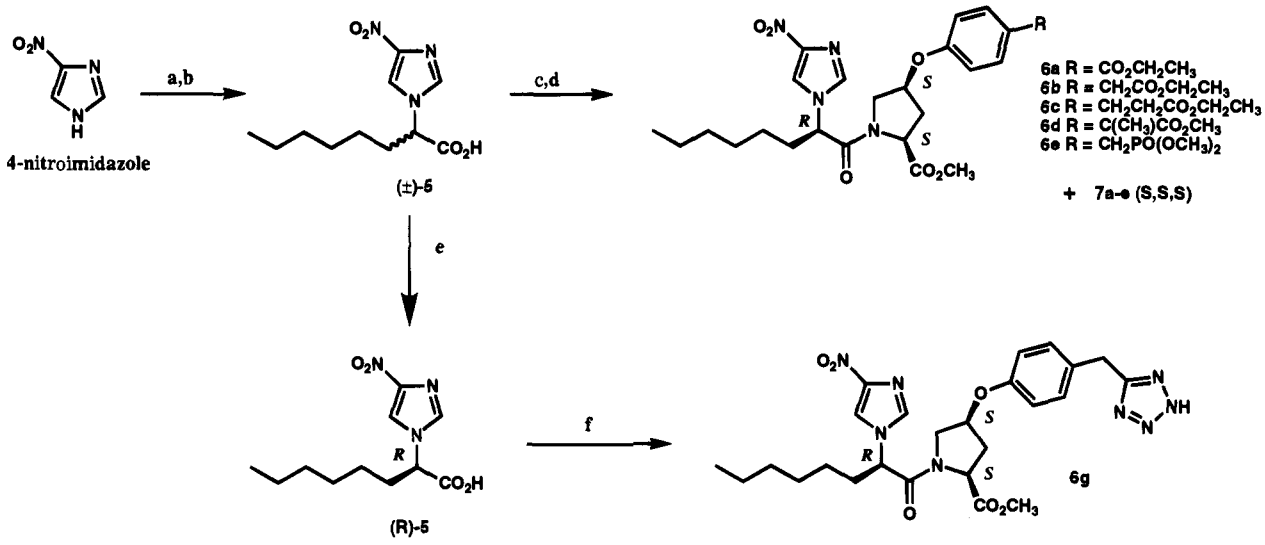
compd	mp, °C	formula[a]	analyses	in vitro $K_b \pm$ SE (nM)[b]	in vivo $K_b \pm$ SE (mg/kg, po)[b,c]
1a	185–195	$C_{30}H_{34}N_4O_{10}S$	C, H, N	1.1 ± 0.3 (5)	NA (3)
1b	160–175	$C_{31}H_{36}N_4O_{10}S$	C, H, N	0.27 ± 0.05 (17)	2.8 ± 0.2 (16)
1c	140–148	$C_{32}H_{38}N_4O_{10}S$	C, H, N	0.6 ± 0.3 (3)	NA (3)
1d	182–187	$C_{33}H_{40}N_4O_{10}S{\cdot}0.6HCl$	C, H, N	0.6 ± 0.3 (3)	6.5 ± 0.3 (3)
1e	190 dec	$C_{30}H_{37}N_4O_{11}PS$	C, H, N	0.9 ± 0.5 (8)	1.8 ± 0.4 (16)
1f	230–234	$C_{31}H_{39}N_4O_{11}PS{\cdot}1.5HCl$	C, H, N	0.4 ± 0.4 (3)	8.3 ± 1.0 (4)
1g	165–172	$C_{31}H_{36}N_8O_8S$	C, H, N	0.6 ± 0.5 (3)	NA (3)
41	166–171	$C_{31}H_{36}N_4O_{10}S$	C, H, N	158, 501 (2)	NA (2)
42	153–167	$C_{31}H_{36}N_4O_{10}S$	C, H, N	6.3, 15.8 (2)	10.6 ± 5.0 (4)

[a] All compounds had C, H, and N microanalysis within ±0.4% theoretical value. [b] Numbers in parentheses represent the number of individual experiments. [c] NA = not active (no shift in the dose response curve noted at this dose and time, i.e. >10 mg/kg).

(Figure 1, precursor to **20** (Table 1)). This compound differs only in the lack of substitution at the *para* position of the 4-(aryloxy)proline group (i.e., R = H). Isomers **6h** and **7h** possess ^{1}H NMR spectral characteristics and silica gel mobility profiles that are directly analogous to those observed for the *para*-substituted derivatives **6a**–**e**,**g** and **7a**–**e**, as well as the intermediates leading to **21**–**40**.[20] The ^{1}H NMR spectra of the (*R*,*S*,*S*) isomers in DMSO-d_6 produce two sets of singlets (doubling due to amide rotamers) at 8.45 and 8.43 ppm, and at 7.99 and 7.95 ppm, respectively, that are the resonances of the two imidazole protons. For the (*S*,*S*,*S*) isomers, the analogous signals are observed at 8.43 and 8.30 ppm and at 7.94 and 7.78 ppm. These spectral characteristics are independent of the substitution on the proline phenoxy ring and are remarkably consistent from compound to compound, differing only within ± 0.02 ppm. Consequently, the ^{1}H NMR spectra provided a simple means of confirming stereochemical assignment as well as assessing diastereomeric purity of the isomers. Additionally, in all solvent systems investigated, the (*R*,*S*,*S*) isomers had greater R_f values on silica gel than the corresponding (*S*,*S*,*S*) isomers.

The elaboration of **6a**–**e**,**g** to **1a**–**e**,**g** is shown in Scheme 3. Catalytic reduction of **6a**–**e**,**g** (EtOAc-EtOH, 10% Pd/C, 40 psi) generated the 4-aminoimidazole intermediate. In our hands, it was not possible to isolate or store the 4-aminoimidazole due to problems of stability. Hence, this material was reacted immediately with sulfobenzoic anhydride (THF or CH_3CN, 25 °C). After 1 h, trituration of the crude reaction mixture provided sulfonic acid derivatives **8a**–**e**,**g** in

Scheme 2



[a] Conditions: (a) NaH, ethyl 2-bromooctanoate, DMF, 25 °C; (b) NaOH, THF; (c) $(COCl)_2$, CH_2Cl_2, DMF(cat.); (d) **4a**–e, TEA, CH_2Cl_2; (e) (−)-cinchonidine, EtOH-H_2O; (f) DCC, HOBT, **4g**, DMF.

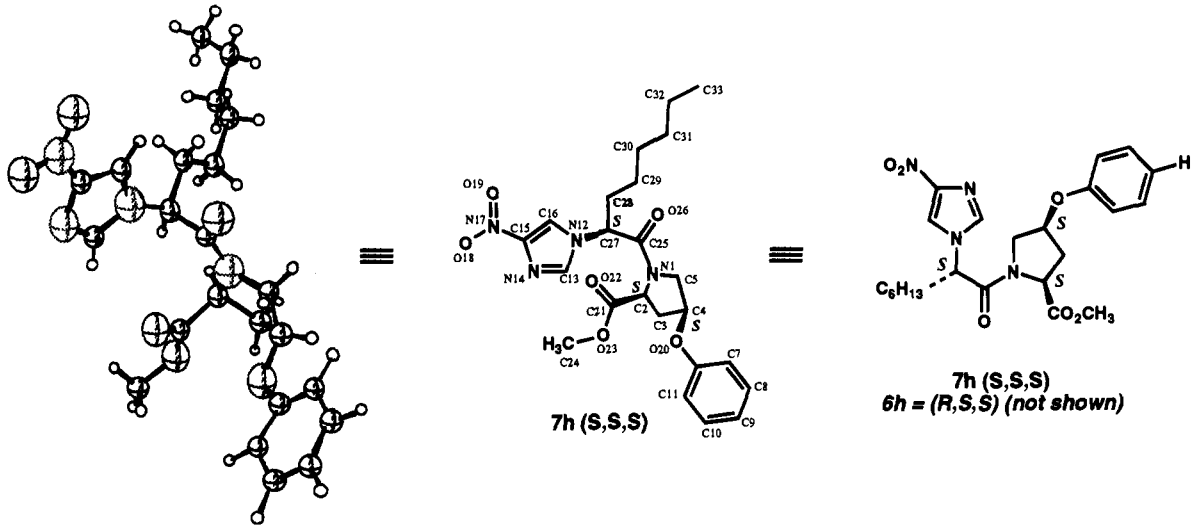
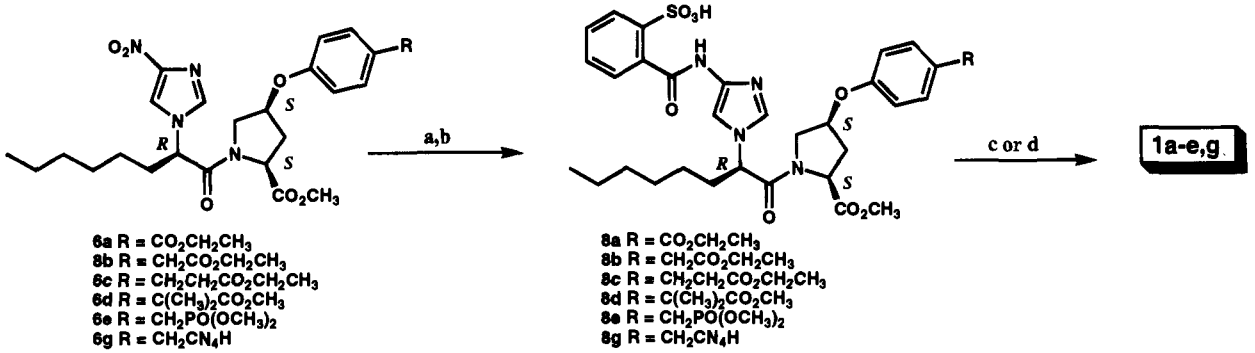


Figure 1. ORTEP drawing of the crystal structure of **7h** (*S,S,S*).

Scheme 3

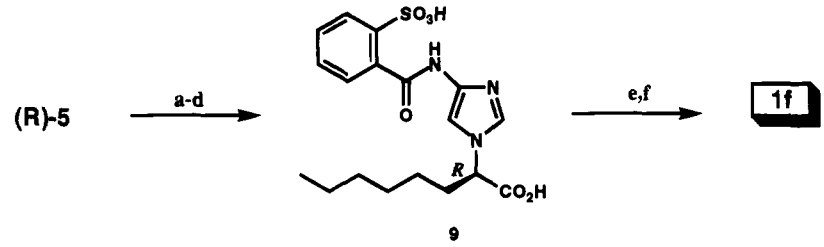


[a] Conditions: (a) H_2 (40 psi), 10% Pd/C, EtOH; (b) sulfobenzoic anhydride, THF; (c) NaOH (**8a-d, g**); (d) TMS–Br, CH_2Cl_2 then NaOH (**8e**).

good yield. In general, it was not necessary to rigorously purify the esters prior to hydrolysis. The conversion of **8a–d,g** to **1a–d,g** was accomplished by treatment with 1 N NaOH, followed by acidification to pH = 1.5 with 5 N HCl. The triacids were isolated either by direct filtration of the aqueous solution or by extraction into EtOAc–EtOH, followed by concentration and trituration of the crude product from CH_3CN/Et_2O. For **8e**, TMS–Br cleavage of the dimethyl phosphonate in CH_2Cl_2 at 0 °C followed by basic workup provided **1e** as a white solid after acidification and isolation using the extraction–trituration protocol.[21]

For the preparation of ethylenephosphonic acid **1f**, we took advantage of the resolution chemistry described

Scheme 4



[a] Conditions: MeOH, pTsOH(cat.), reflux; (b) H2 (40 psl), 10% Pd/C, EtOH; (c) sulfobenzoic anhydride, THF; (d) NaOH; (e) DCC, HOBT, **4g**, DMF; (f) TMS–Br, CH_2Cl_2 then NaOH.

earlier. As detailed in Scheme 4, esterification of (*R*)-**5** followed by catalytic reduction, acylation with sulfobenzoic anhydride, and careful alkaline hydrolysis provided enantiomerically enriched acid **9** in 36% overall yield. Although no attempts were made to determine the enantiomeric purity of this material, we demonstrated that esterification of (*R*)-**5** (MeOH, cat. pTsOH) followed by hydrolysis with 2.0 equiv of NaOH resulted in no measurable loss of ee. Hence, DCC coupling, of **9** to **4f** gave diester **8f**, isolated in 30% yield by flash chromatography (SiO_2, 10% MeOH/$CHCl_3$). ^1H NMR analysis showed this material to be a single diastereomer. Finally, treatment with TMS–Br in CH_2Cl_2 followed by hydrolytic workup (NaOH) provided **1f** in 26% yield. The low yield is representative of the difficulty encountered in isolating these polar species from the aqueous media. However, no exhaustive attempts were made to optimize isolation procedures.

Results and Discussion

Our efforts toward the discovery of nonpeptide Ang II antagonists grew out of a large volume receptor-based screening assay using the rat adrenal glomerulosa preparation.[22] After examining numerous compounds, imidazolehexanoic acid **11** emerged as a lead structure from which extensive SAR studies were begun. This compound proved to be a weak, competitive antagonist of Ang II in isolated rabbit aorta, possessing a K_B of 71.5 μM. As shown in Chart 2, early structural modifications revealed that reversal of the amide linkage from the imidazole to the benzoyl ring and extension of the aliphatic side chain from four to six carbons led to a slight increase in activity (**12**). More significant increases in activity were not realized until acidic substitutions were introduced at the *ortho* position of the benzoyl ring (**13**). Examination of acid isosteres showed that the sulfonic acid derivative **9** was the most potent with a K_B of 81.7 nM.[23] The discovery of **9** coincided with the early publications of the losartan structure and SAR by the DuPont group. It was apparent to us that **9** shared some structural and SAR similarity to the DuPont series, especially with regard to overall dimension, the need for acidic modification of the benzoyl group at the *ortho* position, and the requirement of a lipophilic chain. However, our compounds were significantly less potent *in vitro* than either losartan itself or its carboxylic acid metabolite EXP3174.[24] For comparison, losartan yielded a K_B of 6.3 nM under the same conditions.[25]

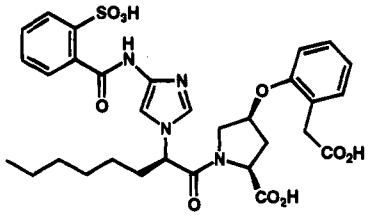
In exploration of the SAR centered around the carboxylic acid of **9**, we found that substitution with L-proline produced a set of diastereomeric derivatives (**16** and **17**, Table 1) with markedly different *in vitro* activity. The (*R,S*) isomer **16** gave a K_B of 50.9 nM, while the (*S,S*) isomer **17** gave a K_B of 461 nM. This result indicated that there is a definite stereochemical preference of the hexyl side chain for interaction with the AT_1 receptor. Additionally, the modest increase in activity gained by introduction of the proline into **9** revealed that repositioning of the carboxyl group as part of the proline system was beneficial to activity. Hence, the proline ring system of **16** provided an attractive template from which to expand our SAR studies.

Due to the availability of L-(*2S,4R*)-4-hydroxyproline, we chose to explore modification of the proline ring by chemical transformations derived from the 4-hydroxyl group. While the *cis*-4-hydroxyproline derivative **18** was weakly active, a large increase in *in vitro* potency (150-fold) was achieved with the introduction of a 4-phenoxy group *cis* to the carboxylic acid (**19**, Table 1). Additionally, while all of our previous compounds were competitive antagonists of Ang II in the rabbit aorta, the more potent phenoxyproline derivative **19** was a noncompetitive antagonist. This is consistent with other studies that demonstrate nonsurmountable antagonism of Ang II with potent diacidic compounds, such as CV-11194, EXP3174, and GR 117289.[11,24,26] As was observed for the simple proline case, there was a large separation in activity between the (*R,S,S*) and (*S,S,S*) isomers.[27] Consequently, all subsequent SAR studies were conducted with derivatives possessing the (*R,S,S*) absolute stereochemistry.

The large increase in *in vitro* potency achieved by the introduction of the phenoxyproline side chain into **9** elevated this series to a comparable level of potency with other known nonpeptide Ang II antagonists. To more fully characterize the interaction of the phenoxy group with the receptor, we investigated structural modifications of the aryl ring as summarized in Table 1. It was possible to substitute the aryl ring at the *para* position with small alkyl groups and alkyl ethers and maintain good potency (**21** and **27**). However, activity dropped off sharply when large sterically demanding groups were introduced (**22**, **23**). Additionally, there appeared to be a regiochemical preference for *para* substitution; the *ortho* and *meta* methoxy derivatives **29** and **28** were significantly less potent. Poly acyclic substitution was not well-tolerated as exemplified by the 3,4-dimethoxy derivative **33**; however, the less sterically demanding 3,4-methelenedioxy compound **32** retains excellent potency. Finally, activity was reduced by direct *para* substitution with strongly electron withdrawing groups such as F and CF_3 (**24** and **25**, respectively).

In addition to substituted aryl derivatives, we explored various bicyclic aromatic systems, also shown in

Chart 3



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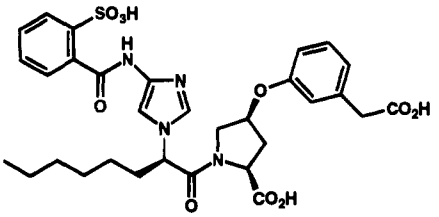

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Table 1. The 5-substituted benzofuran derivative **36** was equipotent with **19**; however, activity was reduced with the more sterically demanding 2-naphthalene (**34**), thianaphthene (**38**), and isoquinoline (**37**). Interestingly, activity was markedly decreased with the 1-naphthyl derivative **35**. This result was consistent with the reduction in potency observed with *ortho* and *meta* substitution of the aryloxy ring as discussed earlier. As a final point, attempts to replace the phenoxy group with other oxygen linked heterocycles such as 3-pyridyl **39** and 5-isoxazole **40** resulted in a substantial loss of potency.

Despite the tremendous increase in *in vitro* potency achieved with the introduction of the phenoxyproline moiety, the more potent compounds in Table 1 showed no activity against the pressor response to Ang II in pithed rats following oral administration at doses up to 30 mg/kg.[28] Modification of the phenoxyproline series to produce analogs with a long duration of action following oral administration in the pithed rat model was subsequently achieved by appending an acidic function at the *para* position of the aryl ring. This yielded a series of triacid derivatives **1a**–**g** (Chart 1), the *in vitro* and *in vivo* data for which are summarized in Table 2. All compounds showed high potency *in vitro* as nonsurmountable Ang II antagonists, with K_B's ranging from 0.3 to 1.1 nM calculated using noncompetitive kinetic models (see Methods).

Also presented in Table 2 are the *ortho* and *meta* acetic acid derivatives **41** and **42**, respectively (Chart 3). The poor *in vitro* activity for these compounds is consistent with a regiochemical preference for substitution of the aryloxy ring at the *para* position.

All compounds in Table 2 were studied for oral activity as antagonists of the pressor response to Ang II in pithed rats. To compare potency of the compounds, we determined an *in vivo* K_B using noncompetitive modeling techniques.[28] Of all the compounds in Table 2 tested at an oral dose of 10 mg/kg, only **1b**, **1d**–**f**, and **42** showed a measurable shift in the dose response curve to exogenously administered Ang II 4 h postdosing. This observation is intriguing in that only minor structural differences distinguish these compounds. A requirement for good oral activity in the carboxylic and phosphonic acid series appears to be a methylene spacer

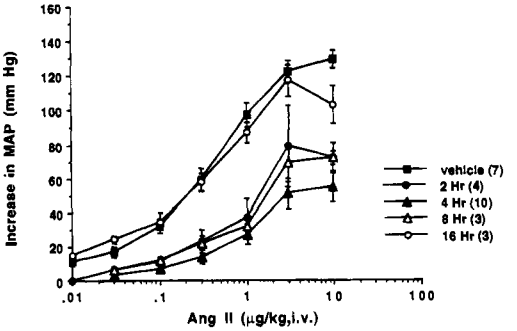

Figure 2. Time course of **1e** antagonism of exogenous Ang II in pithed rats. Compound **1e** (10 mg/kg) or vehicle (0.1 mL of 0.1 N NaOH diluted to ~2 mL with distilled H_2O) was administered to normotensive Sprague–Dawley rats by gavage. At the times indicated, rats were pithed and the mean pressor response to various iv doses of Ang II determined. Values are the mean ± SE of the number of experiments indicated in parentheses. MAP = mean arterial pressure.

between the acidic moiety and aryl ring (**1b** and **1e**). The homologous phosphonoethyl compound **1f** was less potent than **1e**, and the corresponding carboxylic acid **1c** was inactive following oral administration. Additionally, geminal substitution of the methylene group (**1d**) resulted in diminished oral activity. Based on the acid isosteres we investigated, good oral activity was particular to the carboxylic and phosphonic acid derivatives, as the methylenetetrazole analog **1g** was devoid of oral activity. Of the orally active compounds, phosphonic acid **1e** was the most potent, with an *in vivo* K_B of 1.8 mg/kg. A time course for inhibition of the pressor response to Ang II for **1e** (Figure 2) demonstrated significant *in vivo* antagonism for at least 8 h following a single oral dose of 10 mg/kg. The inhibition was nonsurmountable *in vivo* as anticipated from the *in vitro* data (Table 2). The improved oral activity observed upon introduction of a third acid function to the phenoxyproline derivative **19** is intriguing. Attempts to replace either of the carboxyl groups of **1b** with simple alkyl esters resulted in a loss of oral activity when evaluated under the same conditions described for **1a**–**g**.

The potential of **1e** as an orally active antihypertensive agent was evaluated further by studying the compound in Lasix-pretreated (10 mg/kg, sc) conscious spontaneously hypertensive rats (SHR). In this model, **1e** was dosed orally, and mean blood pressure and heart rate were determined by an implanted blood pressure transducer and transmitted by radiotelemetry. Figure 3 shows a dose response relationship with a fall in blood pressure seen at doses as low as 1 mg/kg and a maximal effect achieved at 15 mg/kg (30 mmHg reduction from baseline). The antihypertensive effect had a rapid onset (within 1 h following dosing) and lasted for the duration of the monitoring period (>12 h). Heart rate was generally unchanged; however, at the 1 mg/kg dose there was a decrease compared to the vehicle group which reached statistical significance 9 h after dosing (-46 ± 13 b/min, $p = 0.01$).

In a final experiment, we compared the antihypertensive effect of **1e** to other known agents that interfere with the renin–angiotensin pathway. Shown in Figure 4 is the time course of the blood pressure reduction for **1e** and losartan following a single oral dose of 15 μmol/kg in the non-Lasix pretreated SHR. Also, included in

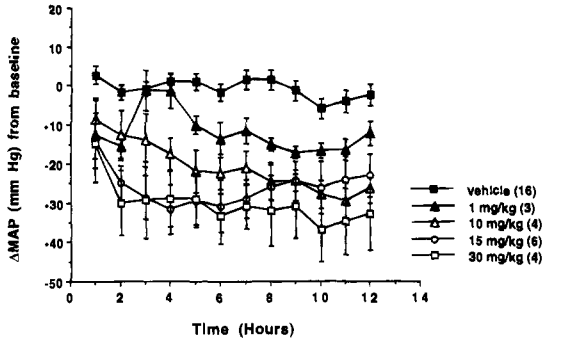

Figure 3. Effect of **1e** on mean pressure of Lasix-pretreated SHR monitored by radio-telemetry. Compound or vehicle was administered by gavage in the doses indicated and mean pressure monitored for 12 h. Mean baseline pressures for the 2 h period before dosing was (mmHg): 147 ± 3, 148 ± 5, 150 ± 5, 143 ± 4, and 149 ± 5 for the vehicle, 1, 10, 15, and 30 mg/kg group, respectively. Mean baseline pressures were not significantly different among groups (ANOVA). In the vehicle group, only the 10 h point was significantly different from baseline (Student's *t*). In the 1 mg/kg group, pressure was significantly decreased from baseline values at 2, 5, 8, 9, 10, and 11 h. All other doses at all time points showed significant decreases from baseline. In general, doses of 10–30 mg/kg yielded significant differences from the vehicle group at most time points.

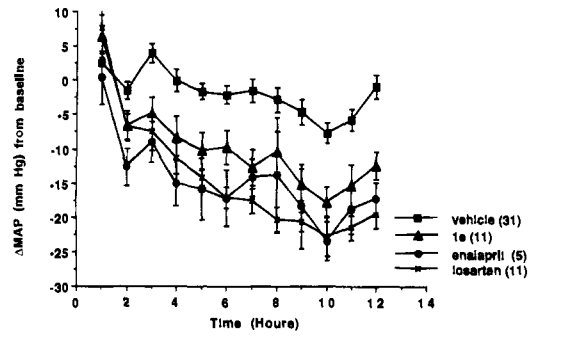

Figure 4. Time course of the effect of losartan (15 μmol/kg, po), enalapril (55 μmol/kg, po), and **1e** (15 μmol/kg, po) on mean blood pressure in conscious SHR monitored by radiotelemetry. Compound or vehicle was administered by gavage in the doses indicated and mean pressure monitored for 12 h. There were no significant differences in the antihypertensive effects of losartan and **1e** or enalapril (ANOVA). Significant effects of all three compounds were evident for at least 12 h after dosing.

this study is the angiotensin converting enzyme inhibitor enalapril, given in a larger dose of 55 μmol/kg (po). All test compounds produced a substantial reduction of blood pressure for >12 h that were not significantly different from one another. In considering this experiment, it is important to note that in rats, losartan is readily transformed into a metabolite (EXP3174) that is intrinsically at least 10-fold more potent than **1e**.[24]

Conclusion

In summary, we have identified a structurally novel class of potent nonpeptide Ang II (AT_1) receptor antagonists with functional *in vitro* K_B values as low as 0.3 nM. Of these compounds, triacids **1b**, **1d**–**1e**, and **42** displayed a significant *in vivo* blockade of the AT_1 receptor in pithed rats following oral dosing. The most potent compound of the series, **1e**, produced a dose-dependent antihypertensive effect in the Lasix-pretreated conscious SHR with a long duration of action (>12 h) when administered orally. Only a few Ang II receptor antagonists structurally diverse from the biphenyl–tetrazole-derived agents have been described. For example, SKB 108566, a 1-(carboxybenzyl)imidazole-5-acrylic acid, was designed based on a putative model of the conformation of Ang II.[29] Thus, the unique structure of **1e** (MW = 694, three ionizable groups at physiologic pH, and three chiral centers) identifies it as a novel entry to the relatively small list of orally effective Ang II (AT_1) antagonists not derived from losartan. Moreover, the pharmacological profile of **1e** suggests its utility for studying the role of Ang II in many pathologic settings.

Experimental Section

General. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained on a GE QE-300 spectrometer at 300.15 MHz in the solvent indicated. Field desorption (FD) mass spectra were recorded on a VG Analytical ZAB-3F instrument. High-resolution (HR) and fast atom bombardment (FAB) mass spectra were recorded on a VG Analytical ZAB2-SE instrument. Elemental analyses were determined by the Physical Chemistry Department at Lilly Research Laboratories and are within ±0.4% of the theoretical values unless otherwise indicated.

Methyl 4-Hydroxy-α,α-dimethylphenylacetate (2d). To a solution of diisopropylamine (20.2 g, 200 mmol) in 200 mL of anhydrous THF at −5 °C under N_2 was added *n*-BuLi (125 mL, 200 mmol, 1.6 M solution in hexanes) dropwise via syringe. After stirring for 15 min, 4-methoxyphenylacetic acid (8.31 g, 50 mmol) was added in small portions. The mixture was stirred at −5 °C for 30 min and then treated with iodomethane (20.0 mL, 319 mmol). The reaction mixture was allowed to gradually warm to room temperature, stirred for 30 min, and then quenched by pouring into 300 mL of saturated NH_4Cl solution. The aqueous was extracted with Et_2O (3 × 100 mL). The organic was dried (Na_2SO_4) and concentrated *in vacuo* to give 9.50 g (97%) of 4-methoxy-α,α-dimethylphenylacetic acid as a white solid: mp 78–81 °C; ^{1}H NMR (DMSO-d_6) δ 12.20 (bs, 1H), 7.21 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 3.69 (s, 3H), 1.40 (s, 6H); FD MS 194. Anal. ($C_{11}H_{14}O_3$) C, H.

4-Methoxy-α,α-dimethylphenylacetic acid (9.25 g, 47.7 mmol) was combined with pyridine hydrochloride (30.0 5. 260 mmol) and heated to 170–190 °C under a N_2 atmosphere for 5 h. After cooling to room temperature, the solid residue was partitioned between EtOAc and H_2O. The layers were separated, and the organic was extracted several times with H_2O. The organic was then dried (Na_2SO_4) and concentrated *in vacuo* to give 8.10 g (94%) of 4-hydroxy-α,α-dimethylphenylacetic acid as a tan solid: mp 130–133 °C; ^{1}H NMR (DMSO-d_6) δ 12.10 (bs, 1H), 9.24 (bs, 1H), 7.94 (d, *J* = 8.6 Hz, 2H), 6.60 (d, *J* = 8.6 Hz, 2H), 1.37 (s, 6H). Anal. ($C_{10}H_{12}O_3$) C, H.

4-Hydroxy-α,α-dimethylphenylacetic acid (8.0 g, 44.4 mmol) was dissolved in 150 mL of anhydrous MeOH along with 3 mL of concentrated H_2SO_4. The mixture was heated to reflux for 12 h. Upon cooling, the MeOH was removed *in vacuo*. The concentrate was dissolved in Et_2O (200 mL) and washed several times with H_2O. The organic was then dried (Na_2SO_4) and concentrated *in vacuo* to provide 8.05 g (93%) of methyl 4-hydroxy-α,α-dimethylphenylacetate (**2d**) as a white solid: mp 91–94 °C; ^{1}H NMR (DMSO-d_6) δ 9.28 (bs, 1H), 7.05 (d, *J* = 8.5 Hz, 2H), 6.63 (d, *J* = 8.5 Hz, 2H), 3.52 (s, 3H), 1.41 (s, 6H). Anal. ($C_{11}H_{14}O_3$) C, H.

Dimethyl (4-Hydroxybenzyl)phosphonate (2e). To a solution of dimethyl phosphite (22.4 mL, 244 mmol) in 400 mL of anhydrous THF at 0 °C was added NaH (9.3 g, 232 mmol, 60% dispersion in mineral oil) in small portions. (Benzyloxy)benzyl chloride (53.7 g, 232 mmol) was then introduced via canula as a solution in 100 mL of anhydrous THF. The resulting mixture was warmed to room temperature and stirred for 18 h. The solvent was then removed *in vacuo*, and the resulting oil was partitioned between H_2O/Et_2O (300

mL each). The layers were separated, and the aqueous layer was extracted with Et_2O (2 × 200 mL). The organic was combined, dried (Na_2SO_4), and concentrated *in vacuo* to give 78.3 g of a thick oil. Chromatography (SiO_2, 75% EtOAc/25% hexane) provided 36.6 g (52%) of dimethyl (4-hydroxybenzyl)-phosphonate as a solid residue; ^1H NMR ($CDCl_3$) δ 7.43–7.25 (m, 5H), 7.21 (dd, J = 9.0, 3.0 Hz, 2H), 6.91 (d, J = 9.0 Hz, 2H), 5.14 (s, 2H), 3.65 (s, 3H), 3.61 (s, 3H), 3.11 (d, J = 21 Hz); FD MS 306. Anal. ($C_{16}H_{19}O_4P$) C, H.

A solution of dimethyl [4-(benzyloxy)benzyl]phosphonate (19.4 g, 63.0 mmol) in 100 mL of 1% concentrated HCl in EtOH was treated with 840 mg of 5% Pd/C. The mixture was hydrogenated at 40 psi for 30 min. The reaction mixture was then filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to give 13.6 g (100%) of **2e** as a white solid: mp 126–129 °C; ^1H NMR ($CDCl_3$) δ 6.98 (dd, J = 9.0, 3.0 Hz, 2H), 6.67 (d, J = 9.0 Hz, 2H), 3.56 (s, 3H), 3.52 (s, 3H), 2.96 (d, J = 21 Hz, 2H). Anal. ($C_9H_{13}O_4P$) C, H.

Diethyl (4'-hydroxy-2-phenethyl)phosphonate (2f). To a −30 °C solution of tetraethyl methylenediphosphonate (6.22 g, 21.6 mmol) in 30 mL of anhydrous THF under N_2 was added *n*-BuLi (14.9 mL, 23.8 mmol, 1.6 M solution in hexanes) dropwise via syringe. After stirring for 30 min, 4-(benzyloxy)-benzaldehyde (4.58 g, 21.6 mmol) was added as solution in 15 mL of anhydrous THF. The resulting mixture was allowed to warm to room temperature and stirred for 2 h. The reaction was quenched by pouring into H_2O (200 mL). The aqueous layer was extracted with EtOAc (3 x100 mL). The organic layer was dried (Na_2SO_4), and concentrated *in vacuo* to an oil that was chromatographed (SiO_2, 1:1 EtOAc/hexanes) to provide 6.05 g (81%) of 4-(benzyloxy)phosphocinnamic acid diethyl ester as a colorless oil that solidified on standing: mp 43–45 °C; ^1H NMR ($CDCl_3$) δ 7.52–7.32 (m, 8H), 6.97 (d, J = 8.45 Hz, 2H), 6.09 (dd, $J_1 = J_2$ = 17.65 Hz), 5.10 (s, 2H), 4.15 (q, J = 7.35 Hz, 4H), 1.37 (t, J = 7.35 Hz). Anal. ($C_{19}H_{23}O_4P$) C, H.

The above benzyl ether (6.05 g, 17.5 mmol) was dissolved in 50 mL of EtOH. To this solution was added 1.15 g of 5% Pd/C. The mixture was hydrogenated at 40 psi for 3 h. The reaction mixture was then filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to give 4.52 g (99%) of diethyl (4'-hydroxy-2-phenethyl)phosphonate (**2f**) as a colorless oil: ^1H NMR ($CDCl_3$) δ 7.04 (d, J = 8.3 Hz, 2H), 6.80 (d, J = 8.3 Hz, 2H), 4.10 (q, J = 7.1 Hz, 4H), 2.85 (m, 2H), 2.06 (m, 2H), 1.32 (t, J = 7.1 Hz, 6H); FD MS 258. Anal. ($C_{12}H_{19}O_4P$) C, H.

General Procedure for Mitsunobu coupling of 2a–g to (2*S*,4*R*)-*N*-Cbz-4-hydroxyproline. Preparation of (2*S*,4*S*)-*N*-Cbz-4-[4-[(dimethoxyphosphinyl)methyl]phenoxy]proline Methyl ester (3e). To a solution of (2*S*,4*R*)-*N*-Cbz-4-hydroxyproline methyl ester (10.0 g, 35.8 mmol) in 400 mL of anhydrous THF under N_2 at 0 °C were added triphenylphosphine (10.6 g, 39.4 mmol) and dimethyl-(4-hydroxybenzyl)phosphonate (7.9 g, 37.8 mmol). To this mixture was added diethyl azodicarboxylate (6.3 mL, 39.4 mmol) dropwise over a 30 min period. The reaction mixture was then allowed to warm to room temperature and stirred for 18 h. The solvent was then removed *in vacuo*, and the residue was chromatographed (SiO_2, 50–100% EtOAc/hexane) to give 13.3 g (75%) of **3e** as a thick oil: $[\alpha]_D$ −14.2° (c 1.0, MeOH); ^1H NMR ($CDCl_3$) δ (doubling due to amide rotamers) 7.35–7.28 (m, 5H), 7.17 (dd, J = 9.0, 3.0 Hz, 2 H), 6.72 (d, J = 9.0 Hz, 2H), 5.14 (m, 2H), 4.87 (m, 1H), 4.58 and 4.51 (dd, J = 6.0, 2.0 Hz, 1H), 3.81–3.75 (m, 2H), 3.72 and 3.62 (s, 3H), 3.67 (s, 3H), 3.63 (s, 3H), 3.08 (d, J = 21 Hz, 2H), 2.49–2.40 (m, 2H). Anal. ($C_{23}H_{28}NO_8P$) C, H, N.

Data for (2*S*,4*S*)-*N*-Cbz-4-(4-carbethoxyphenoxy)proline methyl ester (3a): isolated in 83% yield by chromatography (SiO_2, 30% EtOAc/hexanes); $[\alpha]_D$ −42.9° (c 1.0, MeOH); ^1H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 7.86 (d, J = 8.5 Hz, 2H), 7.33–7.26 (m, 5H), 6.92 (d, J = 8.5 Hz, 2H), 5.15–4.96 (m, 3H), 4.57–4.48 (m, 1H), 4.23 (q, J = 7.1 Hz, 2H), 3.82 −3.62 (m, 1H), 3.58 and 3.53 (s, 3H), 3.51–3.46 (m, 2H), 2.60 (m, 1H), 2.55 (m, 1H), 1.26 (t, J = 7.1 Hz, 3H). Anal. ($C_{23}H_{25}NO_7$) C, H, N.

Data for (2*S*,4*S*)-*N*-Cbz-4-[4-(2-carbethoxymethyl)phenoxy]proline methyl ester (3b): isolated in 81% yield by chromatography (SiO_2, 30% EtOAc/hexanes); $[\alpha]_D$ −15.8° (c 1.0, MeOH); ^1H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 7.34–7.26 (m, 5H), 7.30 (d, J = 8.4 Hz, 2H), 6.76 (d, J = 8.4 Hz, 2H), 5.12–4.96 (m, 3H), 4.53 and 4.47 (dd, J_1 = 9.0, 1.5 Hz, 1H), 4.02 (q, J = 7.0 Hz, 2H), 3.74 (m, 1H), 3.89 and 3.54 (s, 3H), 3.54 (s, 2H), 3.44–3.30 (m, 1H), 2.52 (m, 1H), 2.23 (m, 1H), 1.14 (t, J = 7.0 Hz, 3H). Anal. ($C_{24}H_{27}NO_7$) C, H, N.

Data for (2*S*,4*S*)-*N*-Cbz-4-[4-(2-carbethoxyethyl)phenoxy]proline methyl ester (3c): isolated in 65% yield by chromatography (SiO_2, 30% EtOAc/hexanes); $[\alpha]_D$ −18.1° (c 1.0, MeOH); ^1H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 7.34–7.26 (m, 5H), 7.09 and 7.08 (d, J = 8.5 Hz, 2H), 6.72 and 6.71 (d, J = 8.5 Hz, 2H), 5.11–4.95 (m, 3H), 4.52 and 4.46 (dd, J = 9.5, 1.6 Hz, 1H), 4.02 (m, 2H), 3.72 (m, 1H), 3.58 and 3.53 (s, 3H), 3.46 (m, 1H), 2.73 (m, 2H), 2.56–2.45 (m, 3H), 2.23 (m, 1H), 1.10 (m, 3H). Anal. ($C_{25}H_{29}NO_7$) C, H, N.

Data for (2*S*,4*S*)-*N*-Cbz-4-[4-(2-carbomethoxyisopropyl)phenoxy]proline methyl ester (3d); isolated as an oil in 58% yield by chromatography (SiO_2, 30% EtOAc/hexanes); $[\alpha]_D$ −15.5° (c 1.0, MeOH); ^1H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 7.30–7.24 (m, 5H), 7.18 and 7.16 (d, J = 8.7 Hz, 2H), 6.76 (d, J = 8.7 Hz, 2H), 5.11–4.97 (m, 3H), 4.53 and 4.44 (dd, J = 8.3, 2.0 Hz, 1H), 3.80–3.61 (m, 1H), 3.59 and 3.54 (s, 3H), 3.53 (s, 3H), 2.55–2.46 (m, 1H), 2.25–2.16 (m, 1H), 1.43 (s, 6H); FD MS 455; high-resolution MS calcd for $C_{25}H_{29}NO_7$ 456.2022, found 456.2013. Anal. ($C_{25}H_{29}NO_7$) H; N; C: calcd, 65.92; found, 64.30; N: calcd, 3.08; found, 3.83.

Data for (2*S*,4*S*)-*N*-Cbz-4-[4-[2-(diethoxyphosphinyl)-ethyl]phenoxy]proline methyl ester (3f): isolated as an oil in 37% yield by chromatography (SiO_2, 75–90% EtOAc/hexanes); $[\alpha]_D$ −11.7° (c 0.9, MeOH); ^1H NMR ($CDCl_3$) δ (doubling due to amide rotamers) 7.38–7.30 (m, 5H), 7.10 (d, J = 8.5 Hz, 2H), 6.72 (d, J = 8.5 Hz, 2H), 5.20–5.11 (m, 2H), 4.90 (m, 1H), 4.61 and 4.58 (dd, J = 6.0, 2.0 Hz, 1H), 4.12 (q, J = 7.0 Hz, 4H), 3.82–3.76 (m, 1H), 3.74 and 3.64 (s, 3H), 2.85 (m, 2H), 2.51–2.45 (m, 2H), 2.04 (m, 2H), 1.32 (t, J = 7.0 Hz, 6H). FD MS 520; high-resolution MS calcd for $C_{26}H_{35}NO_8P$ 520.2100, found 520.2135.. Anal. ($C_{26}H_{34}NO_8P$) H, N; C: calcd, 60.11; found, 59.13.

Data for (2*S*,4*S*)-*N*-Cbz-4-[4-[[2-(triphenylmethyl)-2*H*-tetrazol-5-yl]methyl]phenoxy]proline methyl ester (3g): isolated in 83% yield by chromatography (SiO_2, 5–25% EtOAc/toluene); $[\alpha]_D$ +9.4° (c 1.0, MeOH); ^1H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 7.40–7.26 (m, 20H), 7.09 and 6.97 (d, J = 8.5 Hz, 2H), 6.97 and 6.74 (d, J = 8.5 Hz, 2H), 5.11–4.95 (m, 3H), 4.52 and 4.46 (dd, J = 6.1, 1.5 Hz, 1H), 4.17 (s, 2H), 3.73 (m, 1H), 3.57 and 3.52 (s, 3H), 3.41 (m, 1H), 2.53–2.46 (m, 1H), 2.23 (m, 1H); FD MS 679. Anal. ($C_{41}H_{37}N_5O_5$·0.25EtOAc (from chromatography)) C, H, N.

General Procedure for Deprotection of Proline Esters. (2*S*,4*S*)-4-[4-[(Dimethoxyphosphinyl)methyl]phenoxy]proline Methyl Ester (4e). A solution of **3e** (6.6 g, 13.8 mmol) in 100 mL of 1% concentrated HCl in EtOH was treated with 1.0 g of 10% Pd/C. The mixture was hydrogenated at 40 psi for 2 h and then passed through a pad of Celite to remove the catalyst. The filtrate was concentrated *in vacuo* to an oil and then partitioned between $CHCl_3$ and saturrated $NaHCO_3$ (100 mL each). The layers were separated, and the organic was dried (Na_2SO_4) and concentrated *in vacuo* to give 4.30 g (99%) of the crude deprotected proline ester **4e** as a pale yellow oil. This material was used in subsequent reactions without further purification. $[\alpha]_D$ −6.5° (c 1.0, MeOH); ^1H NMR (DMSO-d_6) δ 7.13 (d, J = 8.5 Hz, 2H), 6.76 (d, J = 8.5 Hz, 2H), 4.80 (m, 1), 3.72 (dd, J = 9.0, 4.3 Hz, 1H), 3.65 (m, 1H), 3.58 (s, 3H), 3.56 (s, 3H), 3.52 (s, 3H), 3.13 (d, J = 21.1 Hz, 1H), 2.36 (m, 1H), 1.97 (m, 1H); FD MS 343.

Data for (2*S*,4*S*)-4-[4-[2-(diethoxyphospinyl)ethyl]phenoxy]proline methyl ester (4f): yield 75%; ^1H NMR ($CDCl_3$) δ 7.09 (d, J = 8.5 Hz, 2H), 6.75 (d, J = 8.5 Hz, 2H), 4.79 (m, 1H), 4.10 (q, J = 6.7 Hz, 4H), 3.82 (dd, J = 9.4, 5.1 Hz, 1H), 3.73 (s, 3H), 3.04 (dd, J = 12.4, 4.2 Hz, 1H), 2.90–2.84 (m,

2H), 2.41 (m, 1H), 2.22 (m, 2H), 2.05–1.96 (m, 2H), 1.32 (t, J = 6.7 Hz, 6H); FD MS 385.

Compounds **4a–d,g** were taken up in anhydrous Et_2O and treated with ethereal HBr until the solution was acidic (Congo red indicator). At this point, the HBr salt precipitated from solution. The solid was collected by filtration and dried *in vacuo*.

Data for (2S,4S)-4-[4-(carbethoxy)phenoxy]proline methyl ester hydrobromide (4a): yield 74%; mp 171–174 °C; $[\alpha]_D$ +15.0° (c 1.0, MeOH); ^{1}H NMR (DMSO-d_6) δ 9.62 (bs, 1H), 7.89 (d, J = 8.7 Hz, 2H), 6.98 (d, J = 8.7 Hz, 2H), 5.26 (m, 1H), 4.69 (dd, J = 9.4, 3.5 Hz, 1H), 4.24 (q, J = 7.0 Hz, 2H), 3.70 (s, 3H), 3.63–3.39 (m, 2H), 2.65–2.35 (m, 2H), 1.26 (t, J = 7.0 Hz, 3H). Anal. ($C_{15}H_{19}NO_5$·1.0HBr) C, H, N.

Data for (2S,4S)-4-[4-(carbethoxymethyl)phenoxy]proline methyl ester hydrobromide (4b): yield 92%; mp 163–165 °C; $[\alpha]_D$ +11.9° (c 1.0, MeOH); ^{1}H NMR (DMSO-d_6) δ 9.58 (bs, 1H), 7.17 (d, J = 8.5 Hz, 2H), 6.81 (d, J = 8.5 Hz, 2H), 5.12 (m, 1H), 4.66 (dd, J = 9.4, 3.5 Hz, 1H), 4.02 (q, J = 7.1 Hz, 2H), 3.71 (s, 3H), 3.56 (s, 2H), 3.51–3.35 (m, 2H), 2.60–2.33 (m, 2H), 1.14 (t, J = 7.1 Hz, 3H). Anal. ($C_{16}H_{21}NO_5$·1.0HBr) C, H, N.

Data for (2S,4S)-4-[4-(2-carbethoxyethyl)phenoxy]proline methyl ester hydrobromide (4c): yield 81%; mp 117–120 °C; $[\alpha]_D$ +10.7° (c 1.0, MeOH); ^{1}H NMR (DMSO-d_6) δ 9.40 (bs, 1H), 7.12 (d, J = 8.5 Hz, 2H), 6.76 (d, J = 8.5 Hz, 2H), 5.10 (m, 1H), 4.66 (dd, J = 9.4, 3.4 Hz, 1H), 3.99 (q, J = 7.1 Hz, 2H), 3.70 (s, 3H), 3.62–3.39 (m, 2H), 2.74 (t, J = 7.4 Hz, 2H), 2.53 (t, J = 7.4 Hz, 2H), 2.50–2.32 (m, 2H), 1.10 (t, J = 7.1 Hz, 3H). Anal. ($C_{17}H_{23}NO_5$·1.0HBr) C, H, N.

Data for (2S,4S)-4-[4-(2-carbomethoxyisopropyl)phenoxy]proline methyl ester hydrobromide (4d): yield 57%; $[\alpha]_D$ +10.5° (c 1.0, MeOH); mp 99–103 °C; ^{1}H NMR (DMSO-d_6) δ 9.40 (bs, 1H), 7.21 (d, J = 8.6 Hz, 2H), 6.82 (d, J = 8.6 Hz, 2H), 5.11 (m, 1H), 4.65 (dd, J = 9.4, 3.4 Hz, 1H), 3.71 (s, 3H), 3.54 (s, 3H), 3.50–3.22 (m, 2H), 2.61–2.32 (m, 2H), 1.44 (s, 6H). Anal. ($C_{17}H_{23}NO_5$·1.0HBr) C, H, N.

Data for (2S,4S)-4-[4-(2H-tetrazol-5-ylmethyl)phenoxy]proline methyl ester hydrobromide (4g): yield 77%; $[\alpha]_D$ +8.6° (c 1.1, MeOH); mp 150–155 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.89 (bs, 1H), 9.23 (bs, 1H), 7.18 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 8.5 Hz, 2H), 5.12 (m, 2H), 4.66 (m, 1H), 4.18 (s, 2H), 3.70 (s, 3H), 3.54–3.39 (m, 2H), 2.59–2.31 (m, 2H); FD MS 304. Anal. ($C_{14}H_{17}N_5O_3$·1.5HBr) C, H, N.

(±)-4-Nitroimidazole-2-octanoic Acid (5). To a suspension of NaH (17.5 g, 0.44 mol, 60% dispersion in mineral oil) in anhydrous DMF (300 mL) under N_2 was added 4-nitroimidazole (49.5 g, 0.44 mol) in small portions such that the internal temperature did not rise above 30 °C. After gas evolution ceased, ethyl 2-bromooctanoate (107 g, 0.426 mol) was introduced dropwise via an addition funnel. After stirring for 2 h at room temperature, the reaction was poured into ice–water (1 L) and extracted with EtOAc (3 × 500 mL). The organic was dried (Na_2SO_4) and concentrated *in vacuo* to an oil. This material was passed through a pad of SiO_2 using 1:1 hexanes/EtOAc as eluant. Concentration provided 125.7 g (100%, contains residual EtOAc) of (±)-ethyl 4-nitroimidazole-2-octanoate as a light yellow liquid. This material was used in the next reaction without further purification.

(±)-Ethyl 4-nitroimidazole-2-octanoate (125.7 g crude, 0.425 mol) was dissolved in 120 mL of EtOH. To this solution were added 1.06 L of 2 N NaOH and 100 mL of THF. The resulting mixture was stirred at room temperature for 2 h. The reaction mixture was extracted with Et_2O (2 × 300 mL). The aqueous was then acidified to pH = 3.6 with 5 N HCl. The aqueous layer was extracted with EtOAc (3 x 300 mL). The organic layer was dried (Na_2SO_4) and concentrated *in vacuo* to provide 97.2 g (90%) of **5** as a thick oil that solidified on standing: ^{1}H NMR ($CDCl_3$) δ 8.97 (bs, 1H), 7.91 (s, 1H), 7.72 (s, 1H), 4.80 (dd, J = 9.0, 6.0 Hz, 1H), 2.29 (m, 1H), 2.03 (m, 1H), 1.29–1.19 (m, 8H), 0.85 (t, J = 6.0 Hz, 3 H). Anal. ($C_{11}H_{17}N_3O_4$) C, H, N.

Resolution of 5. A mixture of (±)-**5** (28.75 g, 112 mmol), (−)-cinchonidine (16.5 g, 56 mmol), and triethylamine (5.69 g, 56 mmol) in 330 mL of 1:2 EtOH/H_2O was heated under reflux until a solution was obtained. The solution was allowed to cool and stirred at room temperature for 24 h. The product was collected by filtration, washed with 1:2 EtOH/H_2O (2 × 150 mL), and dried, affording 25.12 g of (*R*)-2-(4-nitro-1*H*-imidazol-1-yl)-octanoic acid-cinchonidine salt as colorless crystals (91.5% e.e.). The product was recrystallized from 330 mL of 1:2 EtOH/H_2O to give 22.3 g (72%, >99% e.e.). A sample of the free acid was generated by partitioning 2.00 g (3.63 mmol) of the salt between 30 mL of EtOAc and 30 mL of 1N HCl. The organic phase was washed with 10 mL of brine, dried ($MgSO_4$), and concentrated *in vacuo* to give 0.93 g (100%) of (*R*)-**5** as an off-white solid.

Data for (*R*)-2-(4-nitro-1*H*-imidazol-1-yl)octanoic acid cinchonidine salt: mp 205 °C dec; $[\alpha]_D$ −111.1° (*c* 1.0, EtOH); ^{1}H NMR ($CDCl_3$) δ 8.81 (d, J = 4.4 Hz, 1H), 8.06 (d, J = 8.1 Hz, 1H), 7.98 (s, 1H), 7.90 (d, J = 8.3 Hz, 1H), 7.67 (m, 2H), 7.53 (s, 1H), 7.43 (m, 1H), 6.40 (bs, 1H), 6.23 (s, 1H), 5.54 (m, 1H), 5.00 (m, 2H), 4.55 (dd, J = 10.0, 5.0 Hz, 1H), 4.28 (m, 1H), 3.34 (m, 2H), 3.18 (m, 1H), 2.97 (m, 1H), 2.63 (m, 1H), 2.22 (m, 1H), 2.00 (m, 5H), 1.76 (m, 1H), 1.24 (m, 9H), 0.83 (t, J = 6.6 Hz, 3H). Anal. ($C_{30}H_{39}N_5O_5$) C, H, N.

Data for (*R*)-5: >99% ee; mp 116–118 °C; $[\alpha]_D$ −32.5° (c 1.0, EtOH); ^{1}H NMR (same as for racemate). Anal. ($C_{11}H_{17}N_3O_4$) C, H, N.

Method for Determination of Enantiomeric Excess (ee). The free acid was esterified with diazomethane in Et_2O and analyzed by chiral HPLC. Analysis conditions: Chiralcel OD column, 85:15 hexane/isopropyl alcohol, flow rate 1 mL/min, λ = 282 nm. t_R: (*S*)-**5**, 5.9 min; (*R*)-**5**, 9.0 min.

General Method for the Synthesis of 6a–e and 7a–e. Preparation of *cis*-4-[4-[(Dimethoxyphosphinyl)methyl]-phenoxy]-(*R*)-1-[1-oxo-2-(4-nitro-1*H*-imidazol-1-yl)octyl]-L-proline Methyl Ester (6e) and *cis*-4-[4-[(Dimethoxyphosphinyl)methyl]phenoxy]-(*S*)-1-[1-oxy-2-(4-nitro-1*H*-imidazol-1-yl)octyl]-L-proline Methyl Ester (7e). (±)-**5** (3.7 g, 14.5 mmol) was dissolved in 25 mL of anhydrous CH_2Cl_2. To this solution was added oxalyl chloride (1.7 mL, 18.9 mmol) followed by 3 drops of DMF. When gas evolution ceased, the solvent was removed *in vacuo* to give the acid chloride as an amber oil that was evaporated from an additional 20 mL of CH_2Cl_2. The acid chloride was used immediately in the next reaction.

To a solution of **4e** in 20 mL of anhydrous CH_2Cl_2 at 10 °C was added *N,N*-diisopropylethylamine (2.7 mL, 15.1 mmol). The acid chloride was then introduced dropwise from an addition funnel as a solution in 10 mL of CH_2Cl_2. The resulting mixture was warmed to room temperature and stirred for 18 h. The reaction mixture was next distributed between EtOAc/H_2O (200 mL ea.). The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 100 mL). The organic layer was combined and washed with brine followed by H_2O. The organic was then dried (Na_2SO_4) and concentrated *in vacuo* to give an oil. The diastereomeric octanoamides were separated by chromatography (SiO_2, 1% MeOH/EtOAc) to give 1.57 g of the (*R,S,S*) isomer **6e** (first isomer to elute) and 1.25 g of the (*S,S,S*) isomer **7e**, along with 1.12 g of a mixed fraction that was rechromatographed to provide an additional 480 mg of **6e** and 565 mg of **7e**. Yield of **6e** is 50%. Yield of **7e** is 44%.

Data for 6e: $[\alpha]_D$ −63.5° (c 1.0, MeOH); R_f 0.27 (95:5, EtOAc/MeOH); ^{1}H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 8.45 and 8.44 (s, 1H), 8.00 and 7.96 (s, 3H), 7.15 and 7.14 (d, J = 8.5 Hz, 2H), 6.77 and 6.75 (d, J = 8.5 Hz, 2H), 5.33 and 5.18 (dd, J_1 = J_2 = 7.5 Hz, 1H), 5.13 and 4.59 (dd, J = 9.3, 1.7 Hz, 1H), 5.11 and 5.00 (m, 1H), 4.01–3.41 (m, 2H), 3.64 and 3.50 (s, 3H), 3.57 (s, 3H), 3.53 (s, 3H), 3.14 (d, J = 21.1 Hz, 2H), 2.53–2.14 (m, 2H), 2.10–1.95 (m, 2H), 1.16–0.80 (m, 11H), 0.19 (m, 3H); FD MS 580. Anal. ($C_{26}H_{37}N_4O_9P$) C, H, N.

Data for 7e: mp 71–75 °C; $[\alpha]_D$ +39.8° (c 1.0, MeOH); R_f 0.20 (95:5, EtOAc/MeOH); ^{1}H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 8.43 and 8.30 (s, 1H), 7.94 and 7.78 (s, 1H), 7.18 and 7.13 (d, J = 8.3 Hz, 2H), 6.82 and 6.71 (d, J = 8.3 Hz, 2H), 5.33 and 5.23 (dd, J_1 = J_2 = 7.4 Hz, 1H), 5.14 and 5.02 (m, 1H), 4.92 and 4.59 (dd, J = 9.1, 2.1 Hz, 1H), 4.20–3.89 (m, 1H), 3.60 and 3.58 (s, 3H), 3.56 and 3.55 (s, 3H), 3.53 and 3.51 (s, 3H), 3.32 (m, 1H), 3.16 and 3.08 (d, J = 21.1 Hz,

2H), 1.21–0.95 (m, 11H), 0.79 (m, 3H); FD MS 580. Anal. ($C_{26}H_{37}N_4O_9P$) C, H, N.

Data for *cis*-4-(4-carbethoxyphenoxy)-1-[1-oxo-2(*R*)-(4-nitro-1*H*-imidazol-1-yl)octyl]-L-proline methyl ester (6a): isolated as a solid in 78% yield by chromatography (SiO_2, 30–60% EtOAc/hexanes); $[\alpha]_D$ −81.0° (*c* 1.0, MeOH); R_f 0.30 (75:25, EtOAc/hexanes); ^1H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 8.45 and 8.43 (s, 1H), 7.99 and 7.94 (s, 1H), 7.87 (d, J = 8.6 Hz, 2H), 6.93 (d, J = 8.6 Hz, 2H), 5.32 and 5.22 (dd, $J_1 = J_2$ = 7.3 Hz, 1H), 5.20 and 4.62 (dd, J = 9.0, 1.5 Hz, 2H), 5.18 and 5.15 (m, 1H), 4.24 (q, J = 7.1 Hz, 2H), 4.03 and 3.70 (dd, J = 11.0, 4.0 Hz, 1H), 3.86 and 3.44 (J = 11.0 Hz, 1.5 Hz, 1H), 3.63 and 3.50 (s, 3H), 2.56–2.17 (m, 2H), 2.02–1.95 (m, 2H), 1.24–0.95 (m, 11H), 0.79 (m, 3H). Anal. ($C_{26}H_{34}N_4O_8$) C, H, N.

Data for *cis*-4-(4-carbethoxyphenoxy)-1-[1-oxo-2(*S*)-(4-nitro-1*H*-imidazol-1-yl)octyl]-L-proline methyl ester (7a): isolated as a solid in 81% yield by chromatography (SiO_2, 30–60% EtOAc/hexanes); mp 100–106 °C; $[\alpha]_D$ +47.4° (*c* 1.0, MeOH); R_f 0.14 (75:25, EtOAc/hexanes); ^1H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 8.44 and 8.31 (s, 1H), 7.94 and 7.79 (s, 1H), 7.90 and 7.84 (d, J = 8.5 Hz, 2H), 6.98 and 6.88 (d, J = 8.5 Hz, 2H), 5.27 and 5.24 (dd, $J_1 = J_2$ = 7.8 Hz, 1H), 5.16 and 4.95 (m, 1H), 5.26 and 4.63 (dd, J_1 = 8.4 Hz, J_2 = 1.5 Hz, 1H), 4.28–4.16 (m, 2H), 3.73–3.48 (m, 2H), 3.59 (s, 3H), 2.58–2.20 (m, 2H), 1.95 (m, 2H), 1.29–0.95 (m, 11H), 0.81–0.72 (m, 3H); FD MS 531. Anal. ($C_{26}H_{34}N_4O_8$) C, H, N.

Data for *cis*-4-[4-(carbethoxymethyl)phenoxy]-1-[1-oxo-2(*R*)-(4-nitro-1*H*-imidazol-1-yl)octyl]-L-proline methyl ester (6b): isolated as an oil in 79% yield by chromatography (SiO_2, 30–60% EtOAc/hexanes); $[\alpha]_D$ −67.9° (*c* 1.0, MeOH); R_f 0.49 (75:25 EtOAc/hexanes); ^1H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 8.45 and 8.44 (s, 1H), 7.99 and 7.95 (s, 1H), 7.14 (d, J = 8.4 Hz, 2H), 6.77 and 6.75 (d, J = 8.4 Hz, 2H), 5.33 and 5.22 (dd, $J_1 = J_2$ = 7.4 Hz, 1H), 5.19 and 4.59 (dd, J = 8.5, 1.4 Hz, 1H), 5.14 and 5.01 (m, 1H), 4.06 and 3.59 (m, 1H), 4.03 (q, J = 7.1 Hz, 2H), 3.83 and 3.42 (dd, J = 11.2 Hz, 1.5 Hz, 1H), 3.65 and 3.51 (s, 3H), 3.54 (s, 2H), 2.53–1.96 (m, 4H), 0.80 (m, 3H); FD MS 544. Anal. ($C_{27}H_{36}N_4O_8$) C, H, N.

Data for *cis*-4-[4-(carbethoxymethyl)phenoxy]-1-[1-oxo-2(*S*)-(4-nitro-1*H*-imidazol-1-yl)octyl]-L-proline methyl ester (7b): isolated as a solid in 74% yield by chromatography (SiO_2, 30–60% EtOAc/hexanes); mp 69–73 °C; $[\alpha]_D$ +31.1° (c 1.0, MeOH); R_f 0.24 (75:25 EtOAc/hexanes); ^1H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 8.44 and 8.30 (s, 1H), 7.95 and 7.79 (s, 1H), 7.16 and 7.12 (d, J = 8.4 Hz, 2H), 6.82 and 6.71 (d, J = 8.4 Hz, 2H), 5.32 and 5.23 (dd, $J_1 = J_2$ = 7.5 Hz, 1H), 5.15 and 5.03 (m, 1H), 4.92 and 4.60 (dd, J = 8.9, 2.3 Hz, 1H), 4.20–3.98 (m, 3H), 3.68–3.62 (m, 1H), 3.59 and 3.58 (s, 3H), 3.55 and 3.52 (s, 2H), 2.55–2.15 (m, 2H), 1.97–1.93 (m, 2H), 1.20–0.99 (m, 11H), 0.78 (m, 3H); FD MS 544. Anal. ($C_{27}H_{36}N_4O_8$) C, H, N.

Data for *cis*-4-[4-(2-carbethoxyethyl)phenoxy]-1-[1-oxo-2(*R*)-(4-nitro-1*H*-imidazol-1-yl)octyl]-L-proline methyl ester (6c): isolated as an oil in 74% yield by chromatography (SiO_2, 30–60% EtOAc/hexanes); $[\alpha]_D$ −62.2° (c 1.0, MeOH); R_f 0.41 (75:25, EtOAc/hexanes); ^1H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 8.45 and 8.42 (s, 1H), 7.99 and 7.94 (s, 1H), 7.09 (d, J = 8.2 Hz, 2H), 6.72 and 6.71 (d, J = 8.2 Hz, 2H), 5.33 and 5.17 (dd, $J_1 = J_2$ = 7.5 Hz, 1H), 5.09 and 4.99 (m, 1H), 5.15 and 4.58 (dd, J = 8.7, 1.5 Hz, 1H), 4.00 (q, J = 7.0 Hz, 2H), 3.81–3.40 (m, 2H), 3.65 and 3.50 (s, 3H), 2.74 (t, J = 7.4 Hz, 2H), 2.53 (t, J = 7.4 Hz, 2H), 2.41–2.03 (m, 2H), 1.99–1.94 (m, 2H), 1.27–0.94 (m, 11H), 0.78 (m, 3H). Anal. ($C_{28}H_{38}N_4O_8$) C, H, N.

Data for *cis*-4-[4-(2-carbethoxyethyl)phenoxy]-1-[1-oxo-2(*S*)-(4-nitro-1*H*-imidazol-1-yl)octyl]-L-proline methyl ester (7c): isolated as an oil in 63% yield by chromatography (SiO_2, 30–60% EtOAc/hexanes); $[\alpha]_D$ +32.3° (c 1.0, MeOH); R_f 0.28 (75:25 EtOAc/hexanes); ^1H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 8.43 and 8.30 (s, 1H), 7.94 and 7.78 (s, 1H), 7.12 and 7.07 (d, J = 8.4 Hz, 2H), 6.78 and 6.66 (d, J = 8.4 Hz, 2H), 5.31 and 5.22 (dd, $J_1 = J_2$ = 7.5 Hz, 1H), 5.12 and 5.00 (m, 1H), 4.91 and 4.59 (dd, J = 8.0, 1.5 Hz, 1H), 4.17–3.95 (m, 3H), 3.66–3.43 (m, 1H), 3.59 (s, 3H), 2.77–2.70 (m, 2H), 2.56–2.14 (m, 4H), 2.05–1.95 (m, 2H), 1.17–0.94 (m, 11H), 0.78 (m, 3H). Anal. ($C_{28}H_{38}N_4O_8$) C, H, N.

Data for *cis*-4-[4-(carbomethoxyisopropyl)phenoxy]-1-[1-oxo-2(*R*)-(4-nitro-1*H*-imidazol-1-yl)octyl]-L-proline methyl ester (6d): isolated as an oil in 55% yield by chromatography (SiO_2, 30–60% EtOAc/hexanes); $[\alpha]_D$ −57.2° (*c* 1.0, MeOH); R_f 0.36 (75:25 EtOAc/hexanes); ^1H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 8.45 and 8.43 (s, 1H), 7.99 and 7.95 (s, 1H), 7.18 (d, J = 8.4 Hz, 2H), 6.77 and 6.55 (d, J = 8.4 Hz, 2H), 5.32 and 5.29 (dd, $J_1 = J_2$ = 7.5 Hz, 1H), 5.00 and 4.99 (m, 1H), 5.11 and 4.59 (dd, J = 8.2, 1.5 Hz, 1H), 3.83–3.40 (m, 2H), 3.63 and 3.51 (s, 3H), 3.54 (s, 3H), 2.54 (m, 1H), 2.13–1.98 (m, 3H), 1.44 (s, 6H), 1.18–0.97 (m, 1H), 0.78 (m, 3H); FD MS 588. Anal. ($C_{28}H_{38}N_4O_8$) C, H, N.

Data for *cis*-4-[4-(carbomethoxyisopropyl)phenoxy]-1-[1-oxo-2(*S*)-(4-nitro-1*H*-imidazol-1-yl)octyl]-L-proline methyl ester (7d): isolated as an oil in 53% yield by chromatography (SiO_2, 30–60% EtOAc/hexanes); $[\alpha]_D$ +31.1° (*c* 1.0, MeOH); R_f 0.26 (75:25, EtOAc/hexanes); ^1H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 8.43 and 8.30 (s, 1H), 7.94 and 7.78 (s, 1H), 7.20 and 7.15 (d, J = 8.7 Hz, 2H), 6.83 and 6.71 (d, J = 8.7 Hz, 2H), 5.32 and 5.29 (dd, $J_1 = J_2$ = 7.6 Hz, 1H), 5.14 and 5.02 (m, 1H), 4.93 and 4.60 (dd, J = 9.5, 3.0 Hz, 1H), 4.19 and 3.64 (m, 1H), 3.48–3.45 (m, 1H), 3.60 and 3.59 (s, 3H), 3.54 and 3.52 (s, 3H), 2.55 (m, 1H), 2.19–1.92 (m, 3H), 1.44 and 1.42 (s, 6H), 1.28–1.07 (m, 11H), 0.78 (m, 3H); FD MS 588. Anal. ($C_{28}H_{38}N_4O_8 \cdot 0.40CH_2Cl_2$ (transfer solvent)) C, H, N.

***cis*-4-[4-(2*H*-Tetrazol-5-ylmethyl)phenoxy]-1-[1-oxo-2(*R*)-(4-nitro-1*H*-imidazol-1-yl)octyl]-L-proline Methyl Ester (6g).** To a solution of **4g** (0.58 g, 1.38 mmol) in 13.0 mL of DMF at room temperature was added *N,N*-diisopropylethylamine (0.72 mL, 4.14 mmol). After stirring for 30 min, (R)-**5** (0.39 g, 1.52 mmol) was added along with hydroxybenzotriazole (HOBT) (0.20 g, 1.52 mmol). After stirring an additional 10 min, dicyclohexylcarbodiimide (DCC) (0.37 g, 1.79 mmol) was added in small portions. The resulting mixture was stirred for 16 h after which time the reaction mixture was diluted with EtOAc (75 mL) and the precipitated dicyclohexylurea (DCU) was removed by filtration. The filtrate was washed with H_2O (4 × 50 mL). The organic was dried (Na_2SO_4) and concentrated *in vacuo* to an oil that was chromatographed (SiO_2, 98:2 $CHCl_3$/MeOH) to provide 570 mg (76%) of **6g** as a colorless oil: $[\alpha]_D$ −58.9° (c 1.0, MeOH); ^1H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 8.45 and 8.42 (s, 1H), 7.99 and 7.94 (s, 1H), 7.15 (d, J = 8.4 Hz, 2H), 6.78 and 6.77 (d, J = 8.4 Hz, 2H), 5.32 and 5.18 (dd, $J_1 = J_2$ = 7.3 Hz, 1H), 5.17 and 4.58 (dd, J = 8.3, 1.5 Hz, 1H), 5.12 and 5.01 (m, 1H), 4.17 and 3.64 (s, 3H), 4.00 and 3.68 (dd, J = 11.2, 4.5 Hz, 1H), 3.81 and 3.43 (11.2, 1.5 Hz, 1H), 2.85 and 2.69 (s, 2H), 2.52–2.16 (m, 3H), 2.07–1.87 (m, 2H), 1.77–0.99 (m, 8H), 0.79 (m, 3H); FD MS 540. Anal. ($C_{25}H_{32}N_8O_6 \cdot 0.30CHCl_3$ (from chromatography)) C, H, N.

Data for *cis*-4-Phenoxy-1-[1-oxo-2(*R*)-(4-nitro-1*H*-imidazol-1-yl)octyl]-L-proline Methyl Ester (6h) and *cis*-4-Phenoxy-1-[1-oxo-2(*S*)-(4-nitro-1*H*-imidazol-1-yl)octyl]-L-proline Methyl Ester (7h). Compounds **6h** and **7h** were prepared exactly as described for **6a–d,e** and **7a–d,e**. **Data for 6h:** isolated by chromatography (SiO_2, hexanes/EtOAc) as a semisolid in 78% yield; $[\alpha]_D$ −64.1° (*c* 1.0, MeOH); ^1H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 8.45 and 8.43 (s, 1H), 7.99 and 7.95 (s, 1H), 7.25 and 6.92 (m, 3H), 6.81–6.79 (m, 2H), 5.32 and 5.19 (dd, $J_1 = J_2$ = 7.2 Hz, 1H), 5.18 and 4.59 (dd, J = 11.3, 1.7 Hz, 1H), 5.14 and 5.03 (m, 1H), 4.02 and 3.63 (dd, J = 11.3, 3.5 Hz, 1H), 3.82 and 3.42 (dd, J = 11.3, 1.0 Hz, 1H), 3.65 and 3.51 (s, 3H), 2.46–1.95 (m, 4H), 1.18–0.92 (m, 8H), 0.79 (m, 3H). Anal. ($C_{23}H_{30}N_4O_6$) C, H, N.

Data for 7h: isolated by chromatography (SiO_2, hexanes/EtOAc) as a white solid in 64% yield; recrystallized from hexanes/EtOAc; mp 98–100 °C; $[\alpha]_D$ +34.4° (c 1.0, MeOH); ^1H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 8.44 and 8.30 (s, 1H), 7.94 and 7.79 (s, 1H), 7.21 and 6.95 (m, 3H), 6.88 and 6.76 (d, J = 8.1 Hz, 2H), 5.32 and 5.20 (dd, $J_1 = J_2$ = 7.2 Hz, 1H), 5.19 and 5.05 (m, 1H), 4.93 and 4.60 (dd, J = 9.1, 1.2 Hz, 1H), 4.17 and 3.48 (dd, J = 11.3, 4.6 Hz, 1H), 3.65 and

3.48 (dd, J = 11.3, 4.6 Hz, 1H), 3.59 (s, 3H), 2.56–1.95 (m, 4H), 1.19–0.90 (m, 8H), 0.78 (m, 3H). Anal. ($C_{23}H_{30}N_4O_6$) C, H, N.

General Method for the Preparation of 8a–e,g. Preparation of *cis*-4-[4-[(Dimethoxyphosphinyl)methyl]phenoxy]-1-[1-oxo-2(*R*)-[4-[(2-sulfobenzoyl)amino]-1*H*-imidazol-1-yl]octyl]-L-proline Methyl Ester (8e). To a solution of **6e** (4.00 g, 6.90 mmol) in 50 mL of absolute EtOH was added 1.0 g of 5% Pd/C. The mixture was hydrogenated at 40 psi for 30 min. The catalyst was then removed by passing the mixture through a pad of Celite. The filtrate was concentrated to an amber oil that was evaporated twice from anhydrous THF.

In a separate flask, sulfobenzoic anhydride (1.40 g, 7.60 mmol) was dissolved in 5 mL of anhydrous THF under N_2. To this solution was added the above aminoimidazole as a solution in 5 mL of anhydrous THF. After stirring for 30 min, the solution was triturated with Et_2O/hexanes to yield 4.70 g (93%) of the sulfonic acid **8e** as a light yellow solid that was collected by filtration. This product was used in the next reaction without further purification or characterization: mp 110 °C dec; FAB MS 735.2.

Preparation of (2(*R*)-[4-[(2-Sulfobenzoyl)amino]-1*H*-imidazol-1-yl]octanoic Acid (9). (*R*)-**5** (16.0 g, 63.0 mmol) was dissolved in 1.0 L of anhydrous MeOH along with 300 mg of pTsOH. The reaction was heated to reflux for 16 h. Upon cooling, the solvent was removed *in vacuo* to give an oil that was partitioned between EtOAc/saturated $NaHCO_3$ solution (300 mL each). The layers were separated, and the organic layer was dried (Na_2SO_4) and concentrated *in vacuo* to provide 13.2 g (78%) of (*R*)-methyl [4-[(2-sulfobenzoyl)-amino]-1*H*-imidazol-1-yl]octanoate as an amber oil: $[\alpha]_D$ −16.8° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.91 (s, 1H), 7.53 (s, 1H), 4.74 (dd, J = 7.1, 4.5 Hz, 1H), 3.81 (s, 3H), 2.22 (m, 1H), 2.03 (m, 1H), 1.20–1.18 (m, 8H), 0.84 (t, J = 7.1 Hz, 3H); FD MS 269. Anal. ($C_{12}H_{19}N_3O_4$) C, H, N.

A sample of the ester was hydrolyzed to the acid with 2 equiv of NaOH in MeOH/H_2O. This material was determined to be 98% ee using the analytical method described earlier.

To a solution of the ester (13.0 g, 45.7 mmol) in EtOH (150 mL) was added 10% Pd/C (2.0 g). The mixture was hydrogenated at 40 psi for 2 h. The catalyst was removed by passing the mixture through a pad of Celite. The filtrate was concentrated to a yellow oil and then dissolved in anhydrous THF (100 mL). To this solution was added KOAc (4.44 g, 45 mmol), K_2CO_3 (3.12 g, 22.5 mmol) followed by sulfobenzoic anhydride (8.83 g, 47.7 mmol). After stirring for 4 h, a white precipitate formed. The mixture was diluted with THF (100 mL) and the solid collected by filtration to provide 22.5 g of crude 2(*R*)-[4-[(2-sulfobenzoyl)amino]-1*H*-imidazol-1-yl]octanoate potassium salt. This material was dissolved in a mixture of H_2O (200 mL) and EtOH (100 mL). To this solution was added 1 N NaOH (53 mL). After stirring at room temperature for 3 h, the solution was concentrated *in vacuo* to remove the EtOH. The aqueous solution was then acidified to pH = 1.5 with 5 N HCl. This solution was then extracted with 9:1 EtOAc/EtOH (3 × 200 mL). The organic was dried (Na_2SO_4) and concentrated *in vacuo* to give 8.65 g (46% for two steps) of **9** as a white solid: mp 250–260 °C dec; $[\alpha]_D$ −8.3° (c 1.0, MeOH); ^{1}H NMR (DMSO-d_6) δ 12.07 (s, 1H), 8.63 (s, 1H), 7.86 (d, J = 6.4 Hz, 1H), 7.67 (m, 2H), 7.54–7.49 (m, 2H), 5.16 (dd, $J_1 = J_2$ = 7.4 Hz, 1H), 2.10 (m, 2H), 1.20–1.09 (m, 8H), 0.81 (m, 3H). Anal. ($C_{18}H_{23}N_3O_6S$) C, H, N.

Preparation of *cis*-4-[4-[2-(Diethoxyphosphinyl)ethyl]-phenoxy]-1-[1-oxo-2(*R*)-[4-[(2-sulfobenzoyl)amino]-1*H*-imidazol-1-yl]octyl]-L-proline Methyl Ester (8f). To a solution of **4f** (1.88 g, 4.90 mmol) and **9** (2.00 g, 4.90 mmol) in 15.0 mL of dry DMF under N_2 was added HOBT (0.73 g, 5.4 mmol). After the mixture was stirred for 10 min, DCC (1.13 g, 5.4 mmol) was added in small portions. The resulting mixture was stirred at room temperature for 18 h. The reaction mixture was then diluted with 50 mL of EtOAc, and the DCU precipitate was filtered off. The filtrate was washed with H_2O (4 × 50 mL). The organic was dried (Na_2SO_4) and concentrated *in vacuo* to a thick oil. Chromatography (SiO_2, 5% MeOH/$CHCl_3$) provided 1.33 g (30%) of **8f** as an amber solid. mp 150–155 °C; ^{1}H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 11.81 and 11.68 (s, 1H), 7.99 (bs, 1H), 7.88 (m, 1H), 7.68–7.40 (m, 1H), 7.61 and 7.57 (s, 1H), 7.46 (m, 2H), 7.14 (d, J = 8.6 Hz, 2H), 6.76 and 6.72 (d, J = 8.6 Hz, 2H), 5.21–4.98 (m, 2H), 5.03 and 4.59 (dd, J = 9.4, 1.9 Hz, 1H), 4.05–3.49 (m, 2H), 3.92 (q, J = 7.0 Hz, 4H), 3.94 and 3.67 (s, 3H), 2.67 (m, 2H), 2.51–2.10 (m, 4H), 2.04–1.88 (m, 2H), 1.20–1.01 (m, 14H), 0.82 (m, 3H). Anal. ($C_{36}H_{49}N_4O_{11}$-PS·0.8MeOH) C, H, N.

General Method for the Preparation of 1a–d,g. Preparation of *cis*-4-[4-(Carboxymethyl)phenoxy]-1-[1-oxo-2(*R*)-[4-[(2-sulfobenzoyl)amino]-1*H*-imidazol-1-yl]octyl]-L-proline (1b). To a solution of **8b** (1.10 g, 1.60 mmol) in 100 mL of THF at room temperature was added 1 N NaOH (5.0 mL). The reaction mixture was stirred for 3 h, after which time the THF was removed *in vacuo*. The aqueous layer was diluted with H_2O (10 mL) and acidified to pH = 1.5 using 5 N HCl. The aqueous was extracted extracted with 90:10 EtOAc/EtOH (3 × 15 mL). The organic was dried (Na_2SO_4) and concentrated *in vacuo* to a solid that was triturated from CH_3-CN/Et_2O. Vacuum filtration provided 610 mg (58%) of **1b** as a white solid: mp 160–175 °C; $[\alpha]_D$ −14.1° (*c* 1.0, MeOH); ^{1}H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 12.00 and 11.90 (s, 1H), 8.50 (bs, 1H), 7.87–7.41 (m, 5H), 7.14 and 7.13 (d, J = 8.2 Hz, 2H), 6.81 and 6.76 (d, J = 8.2 Hz, 2H), 5.35 and 5.13 (dd, $J_1 = J_2$ = 7.2 Hz, 1H), 5.11 and 4.96 (m, 1H), 5.02 and 4.45 (d, J = 9.4, 2.0 Hz, 1H), 3.45 (s, 2H), 4.02 and 3.73 (dd, J = 11.4, 3.5 Hz, 1H), 3.89 and 3.38 (dd, J = 11.4, 1.0 Hz, 1H), 2.55–1.89 (m, 4H), 1.18–0.96 (m, 8H), 0.81 (m, 3H). Anal. ($C_{31}H_{36}N_4O_{10}S$) C, H, N.

Data for *cis*-4-(4-carboxyphenoxy)-1-[1-oxo-2(*R*)-[4-[(2-sulfobenzoyl)amino]-1*H*-imidazol-1-yl]octyl]-L-proline (1a): isolated in 61% yield; mp 185–195 °C; $[\alpha]_D$ −6.2° (c 1.0, MeOH); ^{1}H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 12.05 and 11.93 (s, 1H), 8.59 (bs, 1H), 7.87–7.46 (m, 7H), 6.95 and 6.88 (d, J = 8.6 Hz, 2H), 5.41 and 5.16 (dd, $J_1 = J_2$ = 7.2 Hz, 1H), 5.24 and 5.09 (m, 1H), 5.10 and 4.47 (dd, J = 9.4 Hz, 1.0 Hz, 1H), 4.05 and 3.74 (dd, J = 13.2, 4.6 Hz, 1H), 3.97 and 3.50 (dd, J = 13.2, 1.0 Hz, 1H), 2.58–1.93 (m, 4H), 1.31–0.97 (m, 8H), 0.80 (m, 3H). Anal. ($C_{30}H_{34}N_4O_{10}S$) C, H, N.

Data for *cis*-4-[4-(2-carboxyethyl)phenoxy]-1-[1-oxo-2(*R*)-[4-[(2-sulfobenzoyl)amino]-1*H*-imidazol-1-yl]octyl]-L-proline (1c): isolated in 48% yield; mp 140–148 °C; $[\alpha]_D$ −14.4° (c 1.0, MeOH); ^{1}H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 12.04 and 11.92 (s, 1H), 8.57 (bs, 1H), 7.86–7.52 (m, 3H), 7.50 (m, 2H), 7.16 (d, J = 8.5 Hz, 2H), 6.78 and 6.73 (d, J = 8.5 Hz, 2H), 5.38 and 5.16 (dd, $J_1 = J_2$ = 7.4 Hz, 1H), 5.06 and 5.03 (m, 1H), 5.02 and 4.46 (d, J = 11.1, 1.5 Hz, 1H), 4.04 and 3.65 (m, 1H), 3.99 and 3.46 (dd, J = 11.1 Hz, 1.5 Hz, 1H), 2.70 (m, 2H), 2.46–1.87 (m, 6H), 1.35–0.91 (m, 8H), 0.81 (m, 3H). Anal. ($C_{32}H_{38}N_4O_{10}S$) C, H, N.

Data for *cis*-4-[4-(carbomethoxyisopropyl)phenoxy]-1-[1-oxo-2(*R*)-[4-[(2-sulfobenzoyl)amino]-1*H*-imidazol-1-yl]octyl]-L-proline (1d): isolated by filtration of aqueous solution in 88% yield; mp 182–187 °C; $[\alpha]_D$ −29.4 ° (c 0.5, MeOH); ^{1}H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 12.10 and 11.98 (s, 1H), 8.70 (bs, 1H), 7.86–7.48 (m, 5H), 7.24 and 7.21 (d, J = 8.5 Hz, 2H), 6.83 and 6.78 (d, J = 8.5 Hz, 2H), 5.40 and 5.18 (dd, $J_1 = J_2$ = 7.4 Hz, 1H), 5.10 and 4.96 (m, 1H), 5.08 and 4.46 (dd, J = 9.0, 1.2 Hz, 1H), 4.01 and 3.69 (dd, J = 13.0 Hz, 3.5 Hz, 1H), 3.93 and 3.46 (d, J = 13.0 Hz, 1.2 Hz, 1H), 2.54–1.96 (m, 4H), 1.41 (s, 6H), 1.36–1.08 (m, 8H), 0.81 (m, 3H). Anal. ($C_{33}H_{40}N_4O_{10}S$·0.6HCl) C, H, N.

Data for *cis*-4-[4-(2*H*-tetrazol-5-ylmethyl)phenoxy]-1-[1-oxo-2(*R*)-[4-[(2-sulfobenzoyl)amino]-1*H*-imidazol-1-yl]-octyl]-L-proline (1g): isolated by filtration of aqueous solution in 52% yield; a sample suitable for characterization was purified by reverse-phase (C_{18}) HPLC (1% HOAc/30% CH_3CN/60% H_2O); mp 165–172 °C; $[\alpha]_D$ −19.6° (c 1.0, MeOH); ^{1}H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 11.51 and 11.32 (s, 1H), 8.86 and 8.68 (s, 1H), 7.89–7.47 (m, 5H), 7.18 and 7.14 (d, J = 8.2 Hz, 2H), 6.84 and 6.79 (d, J = 8.2 Hz, 2H), 5.16–4.41 (m, 3H), 4.18 (s, 2H), 4.05 and 3.79 (d, J = 11.7, 4.1 Hz,

1H), 3.81 and 3.45 (d, J = 11.7 Hz, 1.0 Hz, 1H), 2.58–1.87 (m, 4H), 1.24–1.03 (m, 8H), 0.82 (m, 3H). Anal. ($C_{31}H_{36}N_8O_8S$) C, H, N.

General Method for the Preparation of *cis*-4-[4-(Phosphonomethyl)phenoxy]-1-[1-oxo-2(*R*)-[4-[(2-sulfobenzoyl)amino]-1*H*-imidazol-1-yl]octyl]-L-proline (1e) and *cis*-4-[4-(2-Phosphonoethyl)phenoxy]-1-[1-oxo-2(*R*)-[4-[(2-sulfobenzoyl)amino]-1*H*-imidazol-1-yl]octyl]-L-proline (1f). To a solution of **8e** (4.70 g, 6.5 mmol) in 25 mL of anhydrous CH_2Cl_2 at 0 °C was added trimethylsilyl bromide (5.0 g, 32.4 mmol) dropwise over a 15 min period. The resulting mixture was warmed to room temperature and stirred for 1 h. The solvent was then removed *in vacuo*, and the residue was dissolved in 16 mL of 2 N NaOH. After stirring for 1 h, the reaction mixture was acidified to pH = 1.0 with 5 N HCl. The aqueous was extracted with 10% EtOH/EtOAc (3 × 50 mL). The organic was dried (Na_2SO_4) and concentrated to give a solid residue that was dissolved in minimal absolute EtOH and triturated with Et_2O/hexanes. Isolation by filtration provided 2.79 g (61%) of the phosphonic acid **1e** as a pale yellow solid: mp 190°C decs $[\alpha]_D$ −28.5° (c 1.0, MeOH); ^{1}H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 11.87 and 11.72 (s, 1H), 8.08 (s, 1H), 7.84–7.44 (m, 5H), 7.11 (d, J = 8.2 Hz, 2H), 6.76 and 6.72 (d, J = 8.2 Hz, 2H), 5.42 and 5.26 −4.41 (m, 3H), 4.03 and 3.58 (d, J = 11.5, 2.1 Hz, 1H), 3.75 and 3.45 (d, J = 11.5, 1.0 Hz, 1H), 2.83 (d, J = 21.1 Hz, 2H), 2.45–1.91 (m, 4H), 1.19–0.96 (m, 8H), 0.80 (m, 3H); FAB MS 693.5. Anal. ($C_{30}H_{37}N_4O_{11}PS$) C, H, N.

Data for 1f: isolated 26% yield by filtration of the acidified reaction mixture; mp 230–234 °C dec; $[\alpha]_D$ −28.4° (c 1.1, MeOH); ^{1}H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 12.13 and 12.01 (s, 1H), 8.74 (bs, 1H), 7.86–7.46 (m, 5H), 7.15 and 7.05 (d, J = 8.4 Hz, 2H), 6.78 and 6.74 (d, J = 8.4 Hz, 2H), 5.41 and 5.22 (dd, $J_1 = J_2$ = 7.0 Hz, 1H), 5.09 and 4.95 (m, 1H), 5.07 and 4.46 (dd, J = 9.4, 1.0 Hz, 1H), 4.01 and 3.67 (dd, J = 13.0, 4.5 Hz, 1H), 3.94 and 3.46 (dd, J = 13.0, 1.0 Hz, 1H), 2.68 (m, 2H), 2.55–1.96 (m, 4H), 1.71 (m, 2H), 1.31–0.98 (m, 8H), 0.81 (m, 3H); FD MS 707. Anal. ($C_{31}H_{39}N_4O_{11}PS$·1.5HCl) C, H, N.

***cis*-4-[4-(Carboxymethyl)phenoxy]-1-[1-oxo-2(*R*)-[4-[(2-sulfobenzoyl)amino]-1*H*-imidazol-1-yl]octyl]-L-proline (41) and *cis*-4-[3-(2-Carboxyethyl)phenoxy]-1-[1-oxo-2(*R*)-[4-[(2-sulfobenzoyl)amino]-1*H*-imidazol-1-yl]octyl]-L-proline (42).** Compounds **41** and **42** were prepared exactly as described for **1a–d.**

Data for 41: isolated in 58% yield; mp 166–171 °C; $[\alpha]_D$ −15.9° (c 1.0, MeOH); ^{1}H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 12.14 and 12.07 (s, 1H), 8.86 and 8.84 (s, 1H), 7.86–7.46 (m, 5H), 7.24–7.12 (m, 2H), 6.97–6.84 (m, 2H), 5.43 and 5.23 (dd, $J_1 = J_2$ = 7.0 Hz, 1H), 5.14 and 4.97 (m, 1H), 5.11 and 4.45 (dd, J = 9.7, 2.6 Hz, 1H), 4.08 and 3.76 (dd, J = 13.0, 3.9 Hz, 1H), 3.85 and 3.52 (dd, J = 13.0, 1.3 Hz, 1H), 3.43 and 3.40 (s, 2H), 2.59–2.0 (m, 4H), 1.31–0.96 (m, 8H), 0.81 (m, 3H). Anal. ($C_{31}H_{36}N_4O_{10}S$) C, H, N.

Data for 42: isolated in 48% yield; mp 153–167 °C; $[\alpha]_D$ −27.9° (c 1.1, MeOH); ^{1}H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 12.04 and 11.92 (s, 1H), 8.56 (bs, 1H), 7.86–7.15 (m, 5H), 6.87–6.70 (m, 4H), 5.37–4.45 (m, 3H), 4.09–3.60 (m, 2H), 3.49 (s, 2H), 2.44–1.98 (m, 4H), 1.39–0.97 (m, 8H), 0.81 (m, 3H). Anal. ($C_{31}H_{36}N_4O_{10}S$) C, H, N.

X-ray Crystallographic Analysis of 7h. X-ray crystallographic analysis of **7h** was carried out by the X-ray Crystallography Department at Lilly Research Laboratories using a Siemens R3m/V automated four-circle diffractometer. The structure was solved by direct methods using the Siemens SHELXTL PLUS (VMS) system (Sheldrick, G. M. Shelxtl, Rev 4, Instrument Corporation, 1983). A summary of crystal parameters data collection and refinement is provided in Table 3.

Table 3. Single-Crystal X-ray Crystallographic Analysis of **7h**

empirical formula	$C_{23}H_{30}N_4O_6$
formula weight	458.5
color; habit	CLEAR/TABULAR
crystal system	orthorhombic
space group	$P2_12_12_1$
unit cell dimensions	a = 9.0980(10) Å
	b = 10.694(2) Å
	c = 25.388(6) Å
volume	2470.0(9) Å^3
Z	4
density (calcd)	1.233 mg/m^3
absorption coefficient	0.709 mm^{-1}
F(000)	976
system used	Siemens SHELXTL PLUS (VMS)
solution	direct methods
refinement method	full-matrix least-squares
temperature	22 °C
radiation	Cu Kα (λ = 1.541 78 Å)
2θ range	0.0–116.0°
reflections collected	1968
independent reflections	1943 (R_{int} = 0.00%)
observed reflections	1338 ($R > 4.0\sigma(F)$)
final R indicies (obs. data)	R = 7.27%, R_w = 8.29%

Pharmacological Methods. *In Vitro* Antagonism of Ang II (Rabbit Aorta). New Zealand white rabbits (Hazelton, 2–3 kg) were sacrificed by cervical dislocation, and thoracic aortae were removed and cleaned of excess fat and connective tissue. Rings of tissue (3 mm wide) were mounted in 10 mL tissue baths between L-shaped stainless steel hooks. The lower hook was attached to a stationary rod and the upper hook to a force displacement transducer. The bath chambers were maintained at 37 °C, aerated with 95% O_2/5% CO_2, and contained physiological solution of the following composition (mM): NaCl, 117; glucose, 5.6; NaH_2PO_4, 1.0; $MgSO_4$, 0.7; KCl, 5.2; $CaCl_2$, 1.8; $NaHCO_3$, 26; and phentolamine hydrochloride, 0.003. Rings were equilibrated for 1 h with 2 g of tension. During the equilibration period, the tissues were washed by overflow every 15 min. Rings were then exposed to 10 nM Ang II and were allowed to contract until a steady state was reached. This challenge was repeated an hour later. Tissues were then washed every 15 min for 1 h. Cumulative concentration–response curves to Ang II (0.1 nM to 10 μM) were then obtained. At the conclusion of the concentration–response curve, tissues were washed every 2 min until baseline tension was reached and then every 15 min for 30 min. Ang II antagonists were dissolved in DMSO, added in a 10 μL volume, and allowed to incubate for 30 min before repeating the concentration response curve to Ang II. Contractions to Ang II were expressed as a percent of the maximum contraction obtained in the control curve (the first Ang II concentration–response curve). EC_{50}s (concentration that contracted the tissue to half the control maximum) for each were calculated using a four-parameter logistics model (NLIN, SAS Institute, Cary, NC).

Computation and Analysis of K_B. According to Waud, a reasonable function to model an empirical dose-response curve is the three-parameter logistic:

$$\text{response} = \text{max}/[1 + (\text{ED}_{50}(1/a))^s] \quad (1)$$

where max = the maximum possible response, a = the agonist concentration, and s = steepness of the sigmoidal curve.[28] If a second dose response curve is generated after adding a competitive antagonist, then Waud (ref 30, eq 14) indicates the following equation relates equally effective agonist concentrations:

$$1/a = (1/A)(1 + (B/K_B)) \quad (2)$$

where B = antagonist concentration, K_B = dissociation constant of the antagonist, and A = agonist concentration equally effective in the presence of antagonist. Equation 2 may be substituted into eq 1 giving the following dose response equation in the presence of a competitive antagonist:

$$\text{response} = \text{max}/[1 + (\text{ED}_{50}(1/A)(1 + (B/K_b))^s] \quad (3)$$

If a second dose response curve is generated after adding a noncompetitive antagonist, Kenakin suggests the following modification to eq 2:

$$1/a = [(1/A)(1 + (B/K_B)) + \text{int}] \quad (4)$$

where int = intercept term for the linear equation.[31] Equation 4 may be substituted into eq 1, giving the following dose response equation in the presence of a noncompetitive antagonist:

$$\text{response} = \text{max}/[1 + (\text{ED}_{50}((1/A)(1 + (B/K_B)) + \text{int}))^s] \quad (5)$$

For competitive antagonists, eqs 1 and 3 were simultaneously fit to pairs of dose response curves without and with antagonist, respectively. For noncompetitive antagonists, eqs 1 and 5 were fit simultaneously. The curve fitting and estimation of K_B were done by the nonlinear least squares methodology available in the software package JMP.[32] The estimated K_B values (after logarithmic transformation) were compared among compounds using analysis of variance with the Tukey-Kramer method for all pairwise comparisons.

***In Vivo* Antagonism of Ang II (Pithed Rats).** Male Sprague–Dawley rats (Harlan Industries, Indianapolis, IN), 240–280 g, were anesthetized with isoflurane. The trachea was intubated with PE240 tubing and the rats pithed by insertion of a steel rod (1.5 mm diameter) down the spinal canal. Rats were immediately respired at a rate of 80 cycles/min at a volume of 0.6 mL/100 g of body weight. The right carotid artery and right jugular vein were cannulated for blood pressure monitoring and injection of drugs. Animals were allowed to equilibrate 15 min before a noncumulative iv dose response curve to Ang II (10 ng/kg to 10 μg/kg) was obtained. For oral studies, rats were dosed by gavage 4 h prior to pithing. The *in vivo* K_B for each compound was calculated using either competitive or noncompetitive kinetic modeling as described above.

Conscious SHR Monitored by Telemetry. Spontaneously hypertensive male rats [Tac:N(SHR)fBR] were obtained at 12–20 weeks of age from Taconic Farms (Germantown, NY) and housed under a 12 h diurnal cycle. Approximately 1 week after arrival, rats were anesthetized with im ketamine (60 mg/kg) followed in 5 min with ip pentobarbital (21 mg/kg). Under sterile conditions, the abdomen was shaved and prepped with 2-propanol, and a 4.5 cm abdominal incision was made beginning just caudal to the level of kidneys. The abdominal aorta was isolated and gently cleaned of connective tissue with a sterile cotton swab. A small spatula was used to raise a portion of the aorta away from the vena cava in an area just rostal to the iliac bifurcation. Two bulldog clamps isolated a portion of the aorta between the bifurcation and the left renal artery. The aorta was punctured near the iliac bifurcation clamp using a 21 G needle (bent at a 45° angle and with the bevel down). A fluid-filled catheter (0.7 mm OD, 8 cm in length) attached to a hermetically sealed sensor and radio transmitter (model TA11PA-C40, Data Sciences, St. Paul, MN) was inserted up to the rostal clamp using the bent needle as a guide. The needle was removed and the area dried with a cotton swab, and one drop of tissue adhesive was applied at the entry point while the clamps are removed. The entry point was then further sealed using tissue adhesive and a cellulose fiber patch (Vetbond, 3M Co.). The body of the transmitter was then sutured to the muscles of the inner abdominal wall using nonabsorbable 4-0 silk. The muscle layers were then approximated with interrupted knots using sterile 3-0 silk, and the final incision closed with sterile metal wound clips. All animals were administered 10 000 units of penicillin im (Ambi-Pen, Butler, Columbus, OH) and housed individually with food and water ad lib. All animals were allowed to recover for at least one week before study.

Food access was restricted for the next 12 h while the animal's blood pressure and heart rate were monitored by telemetry. At the time of study, rats were 13–50 weeks of age and weighed from 300 to 450 g. In some experiments, animals were briefly sedated with isoflurane (Aerrane, Anaquest, Madison, WI) and administered about 0.3 mL of Lasix (furosemide, 10 mg/kg sc, Sigma, St. Louis, MO) in a vehicle consisting of 10% DMSO and PEG 200. Access to drinking water was restricted from the time of Lasix administration (about 5:00 P. M.) until the conclusion of the study. Twenty four hours after Lasix dosing, the rats were again briefly sedated with isoflurane and administered test compounds or vehicle by oral gavage (0.1 mL of 0.1 N NaOH per 30 mg of compound then diluted with distilled water so as to administer a volume of approximately 2 mL). Pressure signals were acquired for 30 s every 10 min using a Data Sciences-provided software package (Dataquest IV, version 2.0). The digitized values were stored and manipulated using a Compac Deskpro 486/33M computer and were corrected for ambient pressures. Six readings over a 1 h period were averaged for analysis. Mean pressures for the 2 h period before dosing were taken as baseline value. Statistical analysis was performed using JMP software (SAS Institute, Cary, NC). A comparison of hourly postdrug mean pressure to baseline values within groups was performed using a paired sample Student's *t* test. ANOVA was used for statistical comparisons among groups using the Tukey–Kramer HSD test for multiple comparisons. All pairwise comparisons were made at each time interval up to 12 h.

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Supplementary Material Available: Atomic coordinates, bond lengths, bond angles, anisotropic displacement coefficients, and hydrogen coordinates for **7h** (5 pages). Ordering information is given on any current masthead page.

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