(Phosphinyloxy)acyl Amino Acid Inhibitors of Angiotensin Converting Enzyme. 2. Terminal Amino Acid Analogues of (*S*)-1-[6-Amino-2-[[hydroxy(4-phenylbutyl)phosphinyl]oxy]-1-oxohexyl]-L-proline

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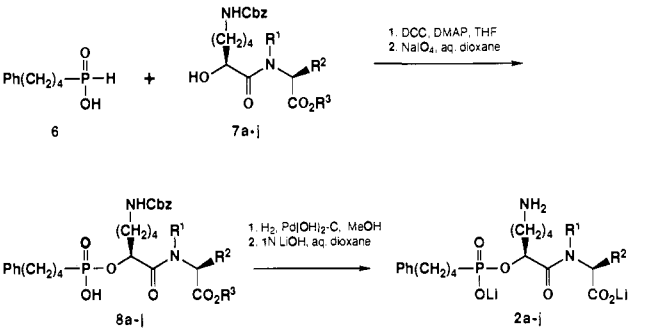
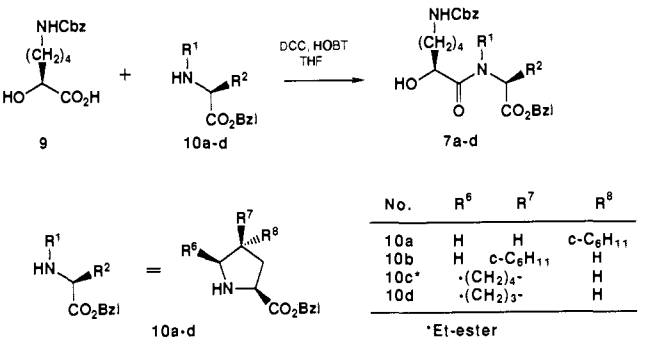
Analogues of (*S*)-1-[6-amino-2-[[hydroxy(4-phenylbutyl)phosphinyl]oxy]-1-oxohexyl]-L-proline (1, SQ 29,852) in which the terminal proline residue has been replaced by a variety of substituted and heteroatom-substituted prolines, *N*-arylglycines, *N*-cycloalkylglycines, and bicyclic amino acids have been synthesized and evaluated as inhibitors of angiotensin converting enzyme in vitro and in vivo. In general, the addition of lipophilic substituents to the 4-position of proline of the parent phosphonate 1 resulted in substantial increases in in vitro activity. The largest improvements were observed in the case of *cis*-benzyl (36-fold) and dithioketal (24-fold) analogues **2r** and **2x**, respectively. These enhancements of in vitro activity were accompanied by modest increases (2–3.5-fold) in in vivo (iv) activity. Among the various terminal amino acid replacements examined in this study, the indoline-based analogue **2i** was by far the most potent compound on iv administration in the normotensive rat.

In part 1 of this series,[1] we reported the discovery and structure–activity relationships of a new class of orally active angiotensin converting enzyme (ACE) inhibitors[2] typified by (*S*)-1-[6-amino-2-[[hydroxy(4-phenylbutyl)-phosphinyl]oxy]-1-oxohexyl]-L-proline (1, SQ 29,852, Chart I). Phosphonate 1 is a potent inhibitor of ACE in vitro and exhibits a remarkably high level of inhibition of the angiotensin I induced pressor response after oral administration to normotensive rats despite the presence of a strongly acidic hydroxyphosphinyl function. This compound differs from our other hydroxyphosphinyl-containing ACE inhibitor fosinopril **3a**[3] in that the parent phosphinic acid **3b** must be administered in the form of a prodrug ester (e.g., **3a**) in order to attain an acceptable level of oral absorption while phosphonate 1 does not. Phosphonate 1 is currently in phase III clinical study. In an effort to prepare more potent analogues of phosphonate 1 and to further extend the structure–activity relationships of this novel class of ACE inhibitors, we have studied the replacement of the terminal proline residue of 1 by various amino acids. The relatively nonspecific binding requirements of the S_2' subsite of ACE have permitted the preparation of many analogues of captopril (**4**)[2,4a,b] and enalapril (**5a**)[2,4b] in which the proline residue has been replaced by a wide variety of N-substituted amino acids. Generally, these substitutions, when compared to the analogous proline-containing inhibitors, increase in vitro potency and sometimes lead to compounds with greater potency or duration of action in vivo. We had previously observed[4b] that substitution of the terminal proline residue of compounds in the phosphinic acid series (e.g., **3b**) resulted in a substantial enhancement of the in vitro and in vivo activities. This marked effect of proline replacement in a moderately active series of proline-based ACE inhibitors has also been reported for (carboxyalkyl)ureas,[5] glutarylprolines,[6] and (trifluoroketoacyl)prolines.[7] In this report we describe the preparation and structure–activity relationships for a series of analogues of 1 in which the proline residue has been replaced by a variety of substituted and heteroatom-substituted prolines, *N*-arylglycines, *N*-cycloalkylglycines, and bicyclic amino acids.

Scheme I



Scheme II



No.	R^6	R^7	R^8
10a	H	H	c-C_6H_{11}
10b	H	c-C_6H_{11}	H
10c*	·$(CH_2)_4$-		H
10d	·$(CH_2)_3$-		H

*Et-ester

Chemistry

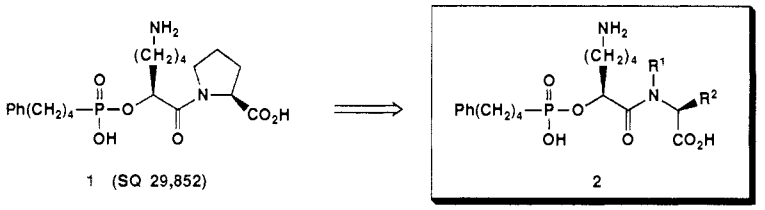
All of the compounds in this study were prepared by one of two general methods. The first (method 1) is a modification of the route used to prepare 1 in which (4-

(1) Karanewsky, D. S.; Badia, M. C.; Cushman, D. W.; DeForrest, J. M.; Dejneka, T.; Loots, M. J.; Perri, M. G.; Petrillo, E. W.; Powell, J. R. *J. Med. Chem.* **1988**, *31*, 204.
(2) (a) Wyvratt, M. J.; Patchett, A. A. *Med. Res. Rev.* **1985**, *5*, 483. (b) Petrillo, E. W.; Ondetti, M. A. *Med. Res. Rev.* **1982**, *2*, 1.
(3) Powell, J. R.; DeForrest, J. M.; Cushman, D. W.; Rubin, B.; Petrillo, E. W. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1984**, *43*, 733.
(4) (a) Smith, E. M.; Swiss, G. F.; Neustadt, B. R.; Gold, E. H.; Sommer, J. A.; Brown, A. D.; Chiu, P. J. S.; Moran, R.; Sybertz, E. J.; Baum, T. *J. Med. Chem.* **1988**, *31*, 875. (b) Krapcho, J.; Turk, C.; Cushman, D. W.; Powell, J. R.; DeForrest, J. M.; Spitzmiller, E. R.; Karanewsky, D. S.; Duggan, M.; Rovnyak, G.; Schwartz, J.; Natarajan, S.; Godfrey, J. D.; Ryono, D. E.; Neubeck, R.; Atwal, K. S.; Petrillo, E. W. *J. Med. Chem.* **1988**, *31*, 1148.
(5) Hayashi, K.; Numami, K.; Sakai, K.; Ozaki, Y.; Kato, J.; Kinashi, K.; Yoneda, N. *Chem. Pharm. Bull.* **1983**, *26*, 3553.
(6) Gruenfield, N.; Stanton, J. L.; Yuan, A. M.; Ebetino, F. H.; Brown, L. J.; Gude, C.; Huebner, C. F. *J. Med. Chem.* **1983**, *26*, 1277.
(7) Gelb, M. H.; Svaren, J. P.; Ables, R. H. *Biochemistry* **1985**, *24*, 1813.

0022-2623/90/1833-1459$02.50/0

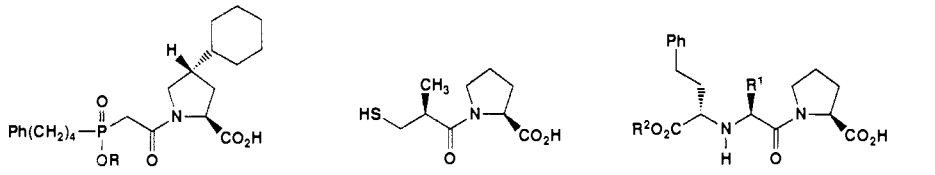
Chart I

Phosphonate ACE Inhibitors



1 (SQ 29,852) 2

Other Clinically Useful ACE Inhibitors

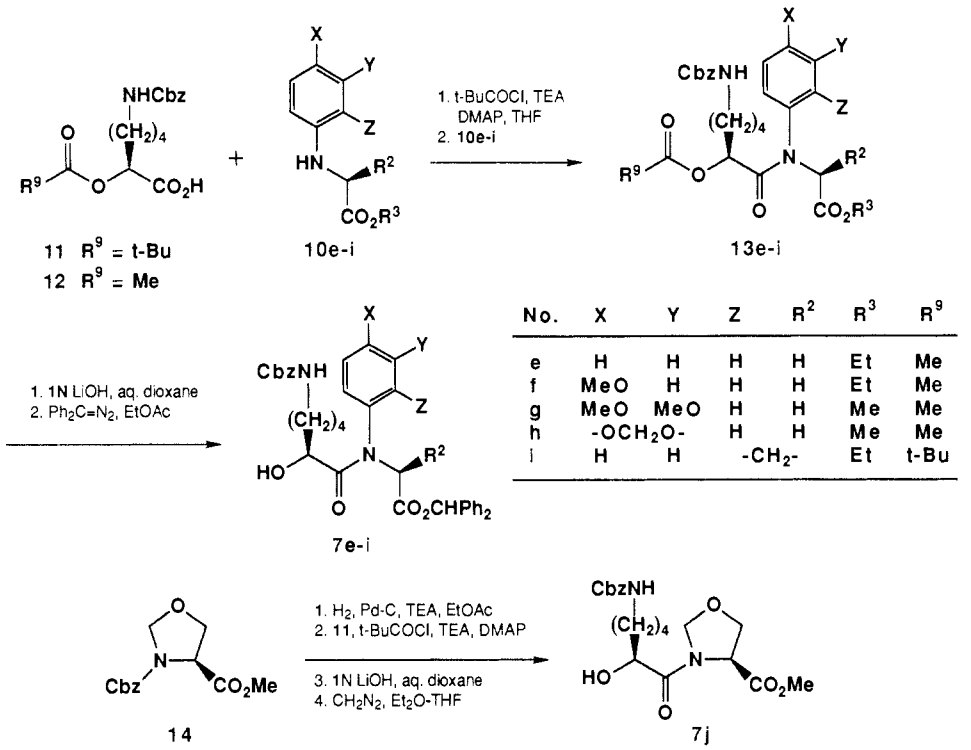


3 a. R = CH(i-Pr)OCOEt (fosinopril)
b. R = H

4 (captopril)

5 a. R^1=Me, R^2=Et (enalapril)
b. R^1=Me, R^2=H (enalaprilat)
c. R^1=$(CH_2)_4NH_2$, R^2=H (lisinopril)

Scheme III



No.	X	Y	Z	R^2	R^3	R^9
e	H	H	H	H	Et	Me
f	MeO	H	H	H	Et	Me
g	MeO	MeO	H	H	Me	Me
h	-OCH_2O-		H	H	Me	Me
i	H	H	-CH_2-		Et	t-Bu

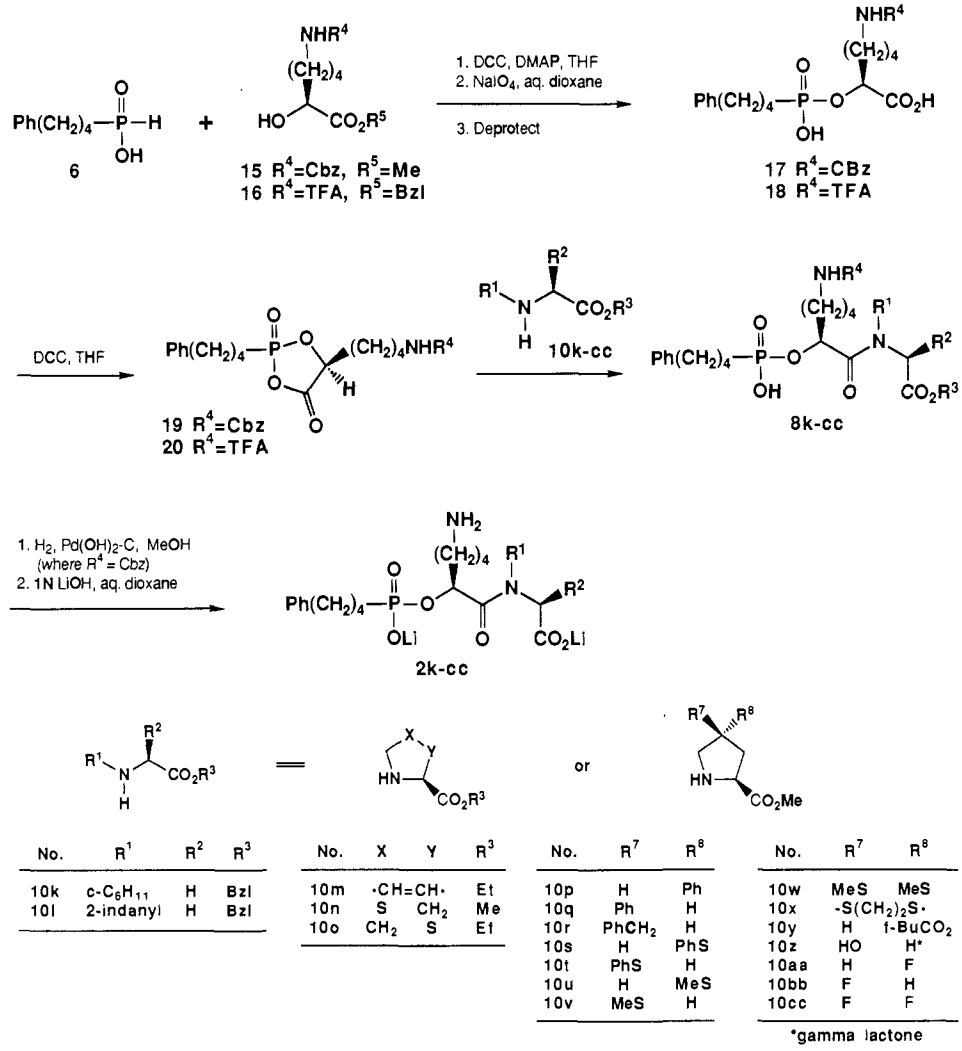
phenylbutyl)phosphonous acid (**6**)[1] was coupled to the hydroxyacyl amino esters **7a–j** to give phosphonous monoesters, which were subsequently oxidized to phosphonic monoesters **8a–j** with sodium metaperiodate in aqueous dioxane.[1,8] Removal of the *N*-Cbz and ester protecting groups gave the desired analogues **2a–j**, isolated as their dilithium salts (Scheme I).

The hydroxyacyl amino esters required for the synthesis of analogues **2a–j** by method 1 were prepared either by a *N,N'*-dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HOBT) mediated coupling of hydroxy acid **9** with amino esters **10a–d** (Scheme II) or coupling of acyloxy acids **11** and **12** to amino esters **10e–i** by using the pivaloyl mixed anhydride method (Scheme III). The latter procedure was required for the coupling of the less nucleophilic *N*-arylglycines **10e–i**. Hydrolysis of the acyloxy and ester protecting groups of **13e–i** followed by reprotection of the carboxylic acid by treatment with diphenyldiazomethane gave the required hydroxyacyl amino acid benzhydryl esters **7e–i**. Protection of the carboxyl group as its sterically hindered benzhydryl ester was necessary to prevent spontaneous lactonization of products **7e–i**. (Hydroxyacyl)oxazolidine **7j** was also prepared by the pivaloyl mixed anhydride method.

A more convergent approach to these compounds (method 2) utilizes the fully elaborated diacid side chains **17** and **18**. These compounds were prepared by a route

(8) Karanewsky, D. S.; Badia, M. C. *Tetrahedron Lett.* **1986**, *27*, 1751.

Scheme IV



No.	R^1	R^2	R^3
10k	c-C_6H_{11}	H	Bzl
10l	2-indanyl	H	Bzl

No.	X	Y	R^3
10m	-CH=CH-		Et
10n	S	CH_2	Me
10o	CH_2	S	Et

No.	R^7	R^8
10p	H	Ph
10q	Ph	H
10r	$PhCH_2$	H
10s	H	PhS
10t	PhS	H
10u	H	MeS
10v	MeS	H

No.	R^7	R^8
10w	MeS	MeS
10x	-S(CH_2)$_2$S-	
10y	H	t-$BuCO_2$
10z	HO	H*
10aa	H	F
10bb	F	H
10cc	F	F

*gamma lactone

analogous to that utilized in method 1 in which (4-phenylbutyl)phosphonous acid (**6**) is coupled to hydroxy esters **15** and **16** and the resulting phosphonous mono esters are oxidized with sodium metaperiodate. Treatment of the resulting diacids **17** and **18** with DCC, 1,1'-carbonyldiimidazole (CDI), or pivaloyl chloride gave cyclic anhydrides **19** and **20**, which reacted regiospecifically with amino esters **10k–cc** to give phosphonic monoesters **8k–cc**. The intermediacy of anhydrides **19** and **20** was demonstrated by ^{13}C NMR.[9] When formed by the CDI method, anhydrides **19** and **20** are apparently in equilibrium with the corresponding carbonylimidazolides.[10] Regardless of the method of formation, cyclic anhydrides **19** and **20** react with amines exclusively at the carbonyl group and with alcohols specifically at phosphorus.[11] The amphoteric reactivity of cyclic phosphonic carboxylic anhydrides **19** and **20** with nitrogen and oxygen nucleophiles has also been observed with cyclic phosphinic carboxylic anhydrides[12] (Scheme IV).

The amino protecting group R^4 in **8** was chosen to be *N*-trifluoroacetyl (TFA) in the case of sulfur-containing amino esters such as **10n–o,s–x** to allow for deprotection of the amino group by nonhydrogenolytic means. Thus, deprotection of phosphonates **8**, where R^4 = TFA, by treatment with 1 N LiOH in dioxane gave the desired dilithium salts **2**.

In the case of *trans*-4-hydroxyproline analogue **2y**, the hydroxy group was protected as the corresponding pivalate, which was removed by base hydrolysis along with the carboxyl and amino protecting groups in the final deprotection step. For the synthesis of the corresponding *cis*-4-hydroxyproline analogue **2z**, the hydroxyl group was internally protected along with the proline carboxyl as the corresponding γ-lactone.[13] Thus, hydrolysis of product **8z** obtained by reaction of anhydride **20** with lactone **10z** gave the desired *cis*-4-hydroxyproline analogue **2z**.

Biological Results

ACE IC_{50}'s were determined against rabbit lung ACE with hippuryhistidylleucine as a substrate.[14] ED_{50}'s were

(9) **17**: ^{13}C NMR (THF-d_8) δ 73.2 ppm (d, J_{P-C} = 7 Hz), methine carbon; **19**: ^{13}C NMR (THF-d_8) δ 78.9 ppm (d, J_{P-C} = 7 Hz) and 80.1 ppm (d, J_{P-C} = 6 Hz), methine carbon (1:1 mixture of diastereomers at phosphorus). For an alternative preparation of **19**, see: Thottathil, J. K.; Wong, M. K. Y. *Tetrahedron Lett.* **1986**, *27*, 5441.

(10) Imidazolide of **17**: ^{13}C NMR (THF-d_8) δ 73.9 ppm (d, J_{P-C} = 7 Hz), methine carbon.

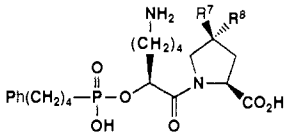
(11) For example, treatment of **19**, prepared by treatment of **17** with *N,N'*-dicyclohexylcarbodiimide at 0 °C in THF, with either EtOH or benzyl alcohol (room temperature, 30 min) gave the corresponding phosphonic diesters in nearly quantitative yields. Karanewsky, D. S.; Badia, M. C., unpublished results.

(12) Crofts, P. C. In *Organic Phosphorus Compounds*; Kosolapoff, G. M., Maier, L.; Eds.; John Wiley & Sons: New York, 1973; Vol. 6, pp 51–52.

(13) Patchett, A. A.; Witkop, B. *J. Am. Chem. Soc.* **1957**, *79*, 185.

(14) Cushman, D. W.; Cheung, H. S. *Biochem. Pharmacol.* **1971**, *20*, 1637.

Table I



no.	R^7	R^8	formula[a]	$[\alpha]_D$, deg (c, MeOH)	ACD I_{50},[a] nM	normotensive rat AI challenge[a] ED_{50}, μmol/kg iv	normotensive rat AI challenge[a] % inhibn at 5.0 μmol/kg po
1	H	H	$C_{21}H_{33}N_2O_6P \cdot H_2O$	−47.5 (1.00)	36	0.063	90
2a	H	c-C_6H_{11}	$C_{27}H_{41}N_2O_6PLi_2 \cdot 2.30H_2O$	−23.4 (0.50)	31	0.110	15
2b	c-C_6H_{11}	H	$C_{27}H_{43}N_2O_6P \cdot 0.75H_2O$	−37.2 (0.50)	11	0.060	33
2p	H	Ph	$C_{27}H_{35}N_2O_6PLi_2 \cdot 1.20H_2O$	−22.0 (0.50)	270	ND[c]	ND
2q	Ph	H	$C_{27}H_{35}N_2O_6PLi_2 \cdot 2.25H_2O$	−14.6 (0.50)	3.5	0.037	58
2r	$PhCH_2$	H	$C_{28}H_{37}N_2O_6PLi_2 \cdot 1.65H_2O$	−38.2 (0.50)	1.0	0.026	14
2s	H	PhS	$C_{27}H_{35}N_2O_6PSLi_2 \cdot 1.30H_2O$	−24.1 (0.58)	2.6	0.066	11
2t	PhS	H	$C_{27}H_{35}N_2O_6PSLi_2 \cdot 1.34H_2O$	−32.3 (0.60)	3.0	0.047	15
2u	H	CH_3S	$C_{22}H_{33}N_2O_6PSLi_2 \cdot 0.80H_2O$	−21.0 (0.50)	9.3	0.031	16
2v	CH_3S	H	$C_{22}H_{33}N_2O_6PSLi_2 \cdot 0.70H_2O$	−19.8 (0.50)	9.7	0.059	8
2w	CH_3S	CH_3S	$C_{23}H_{35}N_2O_6PS_2Li_2 \cdot 1.60H_2O$	−28.8 (0.50)	16	0.140	45
2x	$-S(CH_2)_2S-$		$C_{23}H_{33}N_2O_6PS_2Li_2 \cdot 1.55H_2O$	+15.7 (0.57)	1.5	0.018	51
2y	H	HO	$C_{21}H_{31}N_2O_7PLi_2 \cdot H_2O$	−35.8 (0.50)	140	0.339	ND
2z	HO	H	$C_{21}H_{31}N_2O_7PLi_2 \cdot 2.65H_2O$	−12.4 (0.50)	25	0.033	69
2aa	H	F	$C_{21}H_{32}N_2O_6PF \cdot 0.70H_2O$	−40.8 (0.55)	320	ND	ND
2bb	F	H	$C_{21}H_{32}N_2O_6PF \cdot 0.80H_2O$	−37.3 (0.56)	160	ND	ND
2cc	F	F	$C_{21}H_{29}N_2O_6PF_2Li_2 \cdot 1.20H_2O$	−29.9 (0.64)	170	0.162	49

[a] See the Experimental Section for description of biological assays. [b] All compounds had satisfactory C, H, N, S, and P elemental analysis (±0.4%) and exhibited IR and ^1H and ^{13}C NMR spectra consistent with the structures. [c] ND = not determined.

determined from plots of percent maximal inhibition of the angiotensin I(AI) induced pressor response vs dose after iv administration to the normotensive rat.[15] In general, ED_{50}'s were estimated from plots of at least three doses. Oral activity is reported as percent maximal inhibition of the AI pressor response after a dose of 5.0 μmol/kg po.

Table I shows the biological activity of a number of 4-substituted proline analogues of phenylbutyl phosphonate **1**. Among the various substituents studied, the *cis*-benzyl (**2r**), *cis*-phenyl (**2q**), *cis*- and *trans*-phenylthio (**2t** and **2s**, respectively), and ethylene dithioketal (**2x**) analogues were significantly more potent than the parent unsubstituted analogue **1** with I_{50}'s in the 1–3 nM range. Curiously, *gem*-dimethylthio analogue **2w** was significantly less potent than cyclic dithioketal **2x**. It appears therefore, that the beneficial effect of the ethylene dithioketal is not just an electronic effect and that a cyclic thioketal is essential for full expression of this effect.[16] As was expected on the basis of the in vitro data, the dithioketal (**2x**), *cis*-benzyl (**2r**), and *cis*-phenyl (**2q**) analogues were more potent after intravenous administration in vivo with ED_{50}'s 2–3-fold lower than that of unsubstituted parent compound **1**. Despite enhanced in vitro potency, the *cis*- and *trans*-phenylthio (**2t** and **2s**, respectively) compounds were not significantly more potent than **1** in vivo. On the other hand, *trans*-methylthio analogue **2u** was about 2-fold more active. Unfortunately, all of these 4-substituted proline analogues were less potent than the parent compound **1** after oral administration.

The hydroxylated and fluorinated proline analogues **2y–cc** were prepared in an attempt to enhance the inhibitory potency of **1** by the addition of a relatively small substituents without greatly increasing the overall lipophilicity of the compound. With the exception of *cis*-hydroxy analogue **2z**, hydroxylation or fluorination of the 4-position of proline had a marked detrimental effect on the inhibitory potency of these compounds. *cis*-Hydroxy analogue **2z** showed a 2-fold increase in iv potency while most of the oral activity was retained.

Replacement of the terminal proline residue by an *N*-alkyl- or *N*-arylglycine in several cases resulted in analogues with in vitro and in vivo (iv) potencies comparable to or exceeding those of the parent proline compound (Table II). The most potent of these analogues were 4-methoxy- and 3,4-dimethoxyphenyl compounds **2f** and **2g**. Both of the compounds were less active than phosphonate **1** when administered orally.

Table III shows a series of proline analogues in which one of the carbon atoms has been replaced by a heteroatom or where a fused carbocyclic ring has been introduced. 3,4-Dehydroproline analogue **2m** retains most of the biological activity of parent phosphonate **1** while oxazolidine **2j** is significantly less active. Thiazolidines **2n** and **2o** were slightly less active than parent proline derivative **1**. 5,5-Bicyclic analogue **2d** appears to be more potent on iv administration than either 5,6-bicyclic analogue **2c** or parent proline compound **1**. The most potent analogue in this series of compounds was indoline derivative **2i**. Compound **2i** is at least 5-fold more potent than parent phosphonate **1** on iv administration yet was considerably less active when administered orally.

Discussion

Studies on the hydrolysis of synthetic substrates by ACE[17] and inhibition of ACE by dipeptides[18] and hippuryl di- and tripeptides[19] have demonstrated that the enzyme

(15) Rubin, B.; Laffin, R. J.; Kotter, D. G.; O'Keefe, E. H.; DeMaio, D. A.; Goldberg, M. E. *J. Pharmacol. Exp. Ther.* **1978**, *204*, 271.

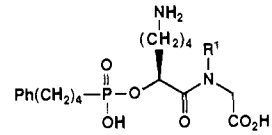
(16) This observation is consistent with results obtained by workers at Schering in a series of 4-substituted proline analogues of captopril (see ref 4a).

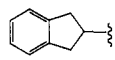
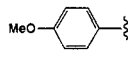
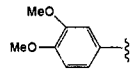
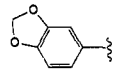
(17) Bunning, P.; Holmquist, B.; Riordan, J. G. *Biochemistry* **1983**, *22*, 103.

(18) Cheung, H. S.; Wang, F. L.; Ondetti, M. A.; Sabo, E. F.; Cushman, D. W. *J. Biol. Chem.* **1980**, *255*, 401.

(19) Rohrbach, M. S.; Williams, E. B.; Rolstad, R. A. *J. Biol. Chem.* **1981**, *256*, 225.

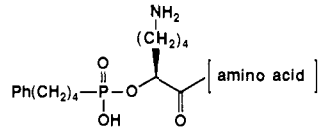
Table II

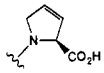
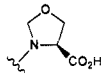
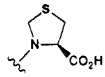
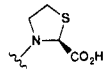
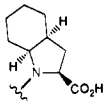
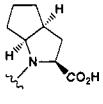
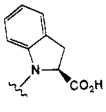


no.	R^1	formula[b]	$[\alpha]_D$, deg (c, MeOH)	ACE I_{50},[a] nM	normotensive rat AI challenge[a] ED_{50}, μmol/kg iv	normotensive rat AI challenge[a] % inhibn at 5.0 μmol/kg po
2k		$C_{24}H_{37}N_2O_6PLi_2 \cdot 2.40H_2O$	−16.2 (0.50)	22	0.106	31
2l		$C_{27}H_{35}N_2O_6PLi_2 \cdot 2.40H_2O$	−19.6 (0.50)	27	0.196	ND[c]
2e		$C_{24}H_{31}N_2O_6PLi_2 \cdot 0.70H_2O$	+47.2 (0.46)	29	0.136	53
2f		$C_{25}H_{33}N_2O_7PLi_2 \cdot 1.20H_2O$	+63.6 (0.50)	39	0.043	40
2g		$C_{26}H_{35}N_2O_8PLi_2 \cdot 1.35H_2O$	+50.8 (0.50)	35	0.040	62
2h		$C_{25}H_{31}N_2O_8PLi_2 \cdot 1.60H_2O$	+37.3 (0.48)	24	0.100	69

[a] See the Experimental Section for description of biological assays. [b] All compounds had satisfactory C, H, N, S, and P elemental analysis (±0.4%) and exhibited IR and ^1H and ^{13}C NMR spectra consistent with the structures. [c] ND = not determined.

Table III



no.	amino acid	formula[b]	$[\alpha]_D$, deg (c, MeOH)	ACE I_{50},[a] (nM)	normotensive rat AI challenge[a] ED_{50}, μmol/kg iv	normotensive rat AI challenge[a] % inhibn at 5.0 μmol/kg po
2m		$C_{21}H_{29}N_2O_6PLi_2 \cdot 1.80H_2O$	−119.8 (0.50)	43	0.105	73
2j		$C_{20}H_{31}N_2O_7P \cdot 0.75H_2O$	−53.9 (0.56)	145	0.677	ND[d]
2n		$C_{20}H_{29}N_2O_6PSLi_2 \cdot 1.43H_2O$	−79.2 (0.50)	91	0.124	46
2o		$C_{20}H_{29}N_2O_6PSLi_2 \cdot 1.33H_2O$	−42.0 (0.66)	81	0.163	50
2c		$C_{25}H_{37}N_2O_6PLi_2 \cdot 1.50H_2O$	−29.8 (0.50)	28	0.100	14
2d		$C_{24}H_{35}N_2O_6PLi_2 \cdot H_2O$	−1.4 (0.50)	26	0.035	41
2i		$C_{25}H_{31}N_2O_6PLi_2 \cdot 1.25H_2O$	−88.4 (0.54)[c]	4.4	<0.014 (74%)	13

[a] See the Experimental Section for description of biological assays. [b] All compounds have satisfactory C, H, N, S, and P elemental analysis (±0.4%) and exhibited IR and ^1H and ^{13}C NMR spectra consistent with the structures. [c] Determined in H_2O. [d] ND = not determined.

will tolerate considerable variation at the P_2' position of the substrate, with aromatic amino acids being especially preferred. The naturally occurring snake venom peptide ACE inhibitors provided the first indication that proline was especially well-tolerated in the P_2' position of inhibitors.[20] The relatively nonspecific binding requirements

of the S_2' subsite of ACE have permitted the preparation of many analogues of captopril and enalapril in which the proline residue has been replaced by thiazolidinecarboxylic acids,[21a,b] *N*-alkyl- and *N*-arylglycines,[22a,b] indoline carboxylic acids,[22b] and isoquinoline carboxylic acids.[23] Many of these compounds have shown enhanced in vitro and greater potency and duration of action in vivo. With moderately active proline-based inhibitors, such as [(hydroxyalkylphosphinyl)acetyl]-[4b] and glutarylprolines,[6] replacement of the proline residue with more lipophilic imino acids resulted in a marked enhancement of in vitro and in vivo activities. With the exception of the [(hydroxyalkylphosphinyl)acetyl]prolines, the effect of proline replacement has only been studied with inhibitors based on the alanyl-proline terminal dipeptide sequence. The present work extends the observed activity-enhancing effect of proline replacement to a series of phosphonate ACE inhibitors based on the lysyl-proline terminal dipeptide sequence. The SAR observed for this series of inhibitors, in most cases, parallels that reported for the alanyl-proline-based inhibitors, suggesting that there is no overlap of the P_2' residue of the inhibitors with the S_1' subsite of the enzyme which, in the case of the lysyl-proline based inhibitors, must be occupied by the aminobutyl side chain.

Consistent with what we had observed in the mercaptan, carboxyalkyl dipeptide, and phosphinic acid inhibitor series,[4b] the addition of lipophilic substituents to the 4-position of proline of the parent phosphonate **1** resulted in substantial increases in in vitro activity. The largest improvements were observed in the case of *cis*-benzyl (36-fold) and dithioketal (24-fold) analogues **2r** and **2x**, respectively. These enhancements of in vitro activity were accompanied by modest increases (2–3.5-fold) in in vivo (iv) activity. In some cases, increases in inhibitory potency were not accompanied by an increase in in vivo potency (e.g., **2s** and **2t**). With the exception of *trans*-phenyl compound **2p**, the stereochemistry of the proline substituent had little effect on inhibitory potency. The reason for the anomalous behavior of **2p** is unclear, but the results are consistent with what we had observed in the phosphinic acid inhibitor series.[4b]

The replacement of proline by various *N*-cycloalkyl- and *N*-arylglycines has been extensively studied in the mercaptan series by several research groups.[22a,b] The particular N-substituted glycines chosen for the present study were based on the SAR developed in these studies. As expected on the basis of the mercaptan series, all of the N-substituted glycine analogues of phosphonate **1** described here had in vitro potency comparable to that of parent proline derivative. Methoxy-substituted phenylglycines **2f** and **2g** were the most potent analogues on the basis of their iv in vivo activity with ED_{50}'s approximately 1.5-fold lower than that of proline analogue **1**.

In the present study, indoline-based inhibitor **2i** was by far the most potent compound on iv administration in the normotensive rat. This proline replacement has also proven to be particularly effective in the mercaptan,[22b] carboxyalkyl dipeptide,[24] and trifluoromethyl ketone[7] inhibitor series. The relatively poor oral activity displayed by this and several of the other analogues which show enhanced intrinsic activity compared to that of parent proline derivative **1** is surprising. However, this phenomenon is consistent with the rather stringent structural requirements for maximum oral activity we had observed in our initial investigation in the phosphonate inhibitor series.[1] This further supports the hypothesis that a carrier-mediated transport mechanism is responsible for the excellent oral activity displayed by phosphonate **1**. Recently, Friedman and Amidon[25] reported the results of a study of the intestinal absorption of several ACE inhibitors, including **1**, in fasted rats using the single-pass perfusion method. The study indicated that the absorption of **1** is concentration dependent and inhibited by cephradine. These results are consistent with the absorption of **1** being mediated by the peptide transport mechanism. The present study suggests that an unsubstituted terminal proline residue is essential for optimal recognition by this transport system.

Experimental Section

Proton NMR spectra were determined at 270 MHz on a JEOL FX-270 spectrometer. ^{13}C NMR spectra were determined at 15 MHz on a JEOL FX-60Q spectrometer. Chemical shifts (ppm) are reported relative to internal tetramethylsilane (^1H, 0.00 ppm), $CDCl_3$ (^{13}C, 77.0 ppm), CD_3OD (^{13}C, 49.0 ppm), CD_3CN (^{13}C, 1.30 ppm), or dioxane (^1H, 3.53 ppm; ^{13}C, 66.5 ppm) in the case of spectra run in D_2O. P–C coupling constants (Hz) are shown in parentheses. Infrared spectra were recorded on a Perkin-Elmer Model 137 spectrophotometer. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Tetrahydrofuran (THF) and diethyl ether were distilled from potassium benzophenone. Dichloromethane was distilled from P_2O_5. Flash chromatography was performed on Whatman LPS-1 silica gel (13–24 μm). MCI Gel CHP-20P is a highly porous polystyrene–divinylbenzene copolymer resin (75–150 μm) supplied by Mitsubishi Chemical Industries Ltd. The preparation of amino esters **10a–x** and **10z–bb** has been described elsewhere.[26] All other compounds were prepared by methods identical with those described below.

***trans*-4-(2,2-Dimethyl-1-oxopropoxy)-1-[(phenylmethoxy)carbonyl]-L-proline, Methyl Ester.** To a solution of *trans*-4-hydroxy-1-[(phenylmethoxy)carbonyl]-L-proline, methyl ester (5.127 g, 18.4 mmol), in dry THF (25 mL) at room temperature under argon were added trimethylacetyl chloride (2.5 mL, 20.2 mmol), triethylamine (2.8 mL, 20.2 mmol), and 4-(dimethylamino)pyridine (0.298 g, 1.8 mmol). After stirring at room temperature for 3.0 h, the mixture was partitioned between 1.0 N HCl and EtOAc. The organic phase was washed with saturated $NaHCO_3$ and saturated NaCl solutions, dried over anhydrous Na_2SO_4, and evaporated to a pale yellow oil. The crude product

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(21) (a) Oya, M.; Baba, T.; Kato, E.; Kawashima, Y.; Watanabe, T. *Chem. Pharm. Bull.* **1982**, *30*, 440. (b) Oya, M.; Kato, E.; Iwao, J.; Yatsuoka, N. *Chem. Pharm. Bull.* **1982**, *30*, 484.
(22) (a) Suh, J. T.; Skiles, J. W.; Williams, B. E.; Youssefyeh, R. D.; Jones, H.; Loev, B.; Neiss, E. S.; Schwab, A.; Mann, W. S.; Khandwala, A.; Wolf, P. S.; Weinryb, I. *J. Med. Chem.* **1985**, *28*, 57. (b) Stanton, J. L.; Guenfeld, N.; Babiarz, J. E.; Ackerman, M. H.; Friedmann, R. C.; Yuan, A. M.; Macchia, W. *J. Med. Chem.* **1983**, *26*, 1267.
(23) Kim, D. H.; Guinosso, C. J.; Buzby, G. C.; Herbst, D. R.; McCaully, R. J.; Wicks, T. C.; Wendt, R. L. *J. Med. Chem.* **1983**, *26*, 394.
(24) Ksander, G. M. Abstracts, 19th National Medicinal Chemistry Symposium, Tucson, AZ, June 17–21, 1984, p 20.
(25) Friedman, D. I.; Amidon, G. L. *Pharm. Res.* **1988**, 5 (supplement), 104S.
(26) The amino esters were prepared as described in the following references: **10a,b,p–v,x** see ref 4b. **10c**, see: Vincent, M.; Remond, G.; Portevin, B.; Serkiz, B.; Laubie, M. *Tetrahedron Lett.* **1982**, *23*, 1677. **10d**, see: Teetz, V.; Geiger, R.; Gaul, H. *Tetrahedron Lett.* **1984**, *25*, 4479. Urbach, H.; Henning, R. *Tetrahedron Lett.* **1985**, *26*, 1839. **10e–i,k,l**, see ref 22b. **10m**, see: Dormoy, J.-R. *Synthesis* **1982**, 753. **10n**, see: Ratner, S.; Clarke, H. T. *J. Am. Chem. Soc.* **1937**, *59*, 200. **10o**, see: Johnson, R. L.; Smissman, E. E. *J. Med. Chem.* **1978**, *21*, 165. **10w,aa,bb**, see ref 4a. **10z**, see ref 13.

was purified by flash chromatography on silica gel (200 g) eluting with hexane/EtOAc (2:1) to give the title compound (6.298 g, 94%) as a clear, colorless oil.

***trans*-4-(2,2-Dimethyl-1-oxopropoxy)-L-proline, Methyl Ester, 4-Methylbenzenesulfonate (1:1) Salt (10y).** A solution of *trans*-4-(2,2-dimethyl-1-oxopropoxy)-1-[(phenylmethoxy)carbonyl]-L-proline, methyl ester (6.298 g, 17.3 mmol), and *p*-toluenesulfonic acid monohydrate (3.39 g, 17.8 mmol) in EtOAc (75 mL) was degassed with argon, treated with 10% Pd/C (0.630 g), and hydrogenated in a Parr apparatus at a pressure of 40 psi for 2.5 h. Catalyst was removed by filtration through Celite and the filtrate was evaporated to a white solid. Trituration with Et_2O and drying in vacuo gave pure **10y** (5.304 g, 76%) as a white granular solid: mp 109–112 °C; TLC (CH_2Cl_2/CH_3OH, 9:1) R_f 0.66; ^{13}C NMR ($CDCl_3$) δ 21.3 (CH_3), 26.8 (3 × CH_3), 34.5, 38.5 (C), 51.3, 52.8 (CH_3), 58.7 (CH), 71.8 (CH), 125.9 (CH), 128.8 (CH), 140.4 (C), 141.4 (C), 167.9 (C), 177.5 (C); $[\alpha]_{365}$ +2.4° (c 0.50, MeOH).

4,4-Difluoro-1-[(phenylmethoxy)carbonyl]-L-proline, Methyl Ester. A solution of 4-oxo-1-[(phenylmethoxy)carbonyl]-L-proline[4b] (6.57 g, 25 mmol) in Et_2O (50 mL) at 0 °C was treated with ethereal diazomethane in portions until TLC indicated consumption of the starting acid. The solution was evaporated to dryness to give the crude methyl ester as a colorless oil: TLC (EtOAc/hexane; 1:1) R_f 0.40.

The crude methyl ester was taken up in methylene chloride (100 mL), treated with (diethylamido)sulfur trifluoride (DAST, 6.6 mL, 54 mmol) and stirred at room temperature for 16 h. The mixture was poured into an ice/water mixture and stirred vigorously for 30 min. The organic phase was separated, washed with water, and evaporated to dryness. The crude product was purified by flash chromatography on silica gel (100 g) eluting with EtOAc/hexane (1:9) to give the title compound as a colorless oil: TLC (EtOAc/hexane; 1:1) R_f 0.53; ^{13}C NMR ($CDCl_3$) δ 38.0 (b t, $J_{C-F} \cong 26$ Hz), 52.4 (CH_3), 53.2 (t, J_{C-F} = 33 Hz), 56.9 (CH), 67.5, 127.9 (CH), 128.1 (CH), 128.4 (CH), 135.9 (C), 153.9 (C), 170.8 (C).

4,4-Difluoro-L-proline, Methyl Ester, 4-Methylbenzenesulfonate (1:1) Salt (10cc). A solution of 4,4-difluoro-1-[(phenylmethoxy)carbonyl]-L-proline, methyl ester (6.635 g, 22.2 mmol) and *p*-toluenesulfonic acid monohydrate (4.13 g, 22.4 mmol) in methanol (100 mL) was treated with 10% Pd/C (0.60 g) and hydrogenated in a Parr apparatus at a pressure of 30 psi for 1.5 h. Catalyst was removed by filtration through Celite and the filtrate was evaporated to a white solid. Trituration with Et_2O and drying in vacuo gave crude **10cc** (7.38 g, 99%) as a white solid. Recrystallization from acetonitrile/Et_2O gave pure **10cc** (6.995 g, 94%) as white needles: mp 99–101 °C; TLC (CH_2Cl_2/CH_3OH, 9:1) R_f 0.57; ^{13}C NMR (CD_3OD) δ 21.3 (CH_3), 37.4 (t, J_{C-F} = 26 Hz), 52.2 (t, J_{C-F} = 32 Hz), 54.3 (CH_3), 58.9 (CH), 126.8 (CH), 129.9 (CH), 141.8 (C); $[\alpha]_D$ −7.8° (c 0.50, MeOH).

[1(*R),2α,4α]-4-Cyclohexyl-1-[2-hydroxy-1-oxo-6-[[(phenylmethoxy)carbonyl]amino]hexyl]-L-proline, Phenylmethyl Ester (7b).** To a solution of *cis*-4-cyclohexyl-L-proline, phenylmethyl ester, monohydrochloride (**10b**, 1.562 g, 4.83 mmol), (*S*)-2-hydroxy-6-[[(phenylmethoxy)carbonyl]amino]hexanoic acid (**9**,[1] 1.36 g, 4.83 mmol), and triethylamine (0.71 mL, 5.07 mmol) in dry THF (15 mL) at room temperature under argon were added hydroxybenzotriazole (0.718 g, 5.31 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (1.10 g, 5.31 mmol). After stirring at room temperature for 2 h, the mixture was diluted with EtOAc and filtered. The filtrate was washed successively with 5% $KHSO_4$, saturated $NaHCO_3$, and saturated NaCl solutions, dried over anhydrous Na_2SO_4, and evaporated to an oil. The crude product was purified by flash chromatography on silica gel eluting with hexane/EtOAc (7:3) to give pure **7b** (2.315 g, 87%) as a colorless, viscous oil: TLC (CH_2Cl_2/MeOH, 9:1) R_f 0.38; ^{13}C NMR (CD_3CN) δ 22.4, 26.8, 27.1, 30.4, 32.0, 32.6, 34.2, 41.5, 41.7 (CH), 46.0 (CH), 52.0, 60.7 (CH), 66.7, 67.2, 69.8 (CH), 157.4 (C), 172.7 (C), 174.0 (C).

The following compounds were also prepared by this method: **7a** (100%), **17c** (78%), and **7d** (75%).

[*S*-(*R,*R**)]-1-[2-(2,2-Dimethyl-1-oxopropoxy)-1-oxo-6-[[(phenylmethoxy)carbonyl]amino]hexyl]-2,3-dihydro-1*H*-indole-2-carboxylic Acid, Ethyl Ester (13i).** To a solution of hydroxy acid **9** (1.40 g, 4.98 mmol) and triethylamine (1.52 mL, 11.0 mmol) in dry THF (30 mL) at 0 °C (ice bath) under argon was added trimethylacetyl chloride (1.36 mL, 11.0 mmol) followed by 4-(dimethylamino)pyridine (0.10 g, 0.82 mmol). After stirring at 0 °C for 1 h, (*S*)-2,3-dihydro-1*H*-indole-2-carboxylic acid, ethyl ester monohydrochloride (**10i**, 1.48 g, 6.47 mmol), and triethylamine (0.90 mL, 6.5 mmol) were added; the mixture was allowed to warm to room temperature and stirred for 16 h. The mixture was partitioned between EtOAc and 1 N HCl, the organic phase washed successively with 1 N HCl, saturated $NaHCO_3$, and saturated NaCl solutions, dried over Na_2SO_4, and evaporated to dryness. The crude product was purified by flash chromatography on silica gel (100 g) eluting with acetone/hexanes (15:85) to give pure **13i** (1.715 g, 64%) as a colorless glass: TLC (acetone/hexanes, 3:7) R_f 0.33; ^1H NMR ($CDCl_3$) δ 1.25 (12 H, m), 1.45–1.68 (4 H, m), 2.04 (1 H, b s), 3.05–3.33 (3 H, m), 3.45–3.72 (1 H, m), 4.05–4.28 (2 H, m), 5.06 (2 H, s), 5.24 (1 H, b d), 5.57 (1 H, b m), 7.00–7.40 (9 H, m).

[*S*-(*R,*R**)]-2,3-Dihydro-1-[2-hydroxy-1-oxo-6-[[(phenylmethoxy)carbonyl]amino]hexyl]-1*H*-indole-2-carboxylic Acid, Diphenylmethyl Ester (7i).** A solution of diester **13i** (1.698 g, 3.15 mmol) in dioxane (15 mL) was treated with 1.0 N LiOH solution (8.0 mL, 8.0 mmol) and stirred at room temperature under argon for 16 h. The mixture was partitioned between EtOAc and 5% $KHSO_4$; the organic phase was washed with saturated NaCl solution, dried over anhydrous Na_2SO_4 and concentrated to a volume of ca. 30 mL. The solution was immediately treated with diphenyldiazomethane (1.90 g, 9.78 mmol) and stirred at room temperature for 1.5 h. The mixture was evaporated to dryness and the purple residue was purified by flash chromatography on silica gel (100 g) eluting EtOAc/hexanes (30:70) to give pure **7i** (1.401 g, 75%) as a white foam: TLC (EtOAc/hexanes, 35:65, two developments) R_f 0.34; ^{13}C NMR ($CDCl_3$) δ 21.7, 29.3, 33.3, 40.4, 60.2 (CH), 66.3, 69.7 (CH), 78.3 (CH), 111.5 (C), 124.3 (CH), 126.5 (CH), 126.9 (CH), 127.9 (CH), 128.3 (CH), 136.6 (C), 139.1 (C), 156.4 (C), 169.8 (C), 173.3 (C).

(*S*)-2-(Acetyloxy)-6-[[(phenylmethoxy)carbonyl]amino]-hexanoic Acid (12). To a solution of hydroxy acid **9** (5.62 g, 20.0 mmol) in dry THF (40 mL) at 0 °C (ice bath) under argon was added triethylamine (5.6 mL, 40.0 mmol) and acetyl chloride (2.84 mL, 40.0 mmol) and the resulting mixture was stirred at 0 °C for 2 h. The suspension was filtered, cooled to 0 °C, treated with half-saturated $NaHCO_3$ (40 mL), and stirred at 0 °C for 1 h. The mixture was partitioned between EtOAc and 5% $KHSO_4$, the organic phase washed with saturated NaCl, dried over anhydrous Na_2SO_4 and concentrated in vacuo to give 8.20 g of crude **12** as a yellow oil.

The crude acid was purified by conversion to its 1-adamantanamine salt. Thus, crude acid **12** was taken up in Et_2O (25 mL) and treated with a solution of 1-adamantanamine (3.00 g, 19.8 mmol) in Et_2O (20 mL). The resulting white precipitate was collected, washed with Et_2O, and dried in vacuo to give **12**·adamantanamine salt (8.53 g, 90% overall from **9**) as a white solid.

To regenerate the free acid, the salt (8.53 g) was partitioned between EtOAc and 1 N HCl, and the organic phase was washed with 1 N HCl (2 × 30 mL) and saturated NaCl, dried over Na_2SO_4, and evaporated to give pure **12** (5.80 g, 91%) as a colorless, viscous oil: TLC (CH_2Cl_2/MeOH/AcOH, 20:1:1) R_f 0.82; ^{13}C NMR (CD_3OD) δ 22.6, 24.3, 27.9, 33.5, 36.6, 40.2, 56.0 (CH_3), 71.2 (CH), 115.8 (C), 126.6 (CH), 129.3 (CH), 130.5 (CH), 136.4 (CH), 143.6 (C), 160.8 (C), 174.5 (C).

(*S*)-[[2-(Acetyloxy)-1-oxo-6-[[(phenylmethoxy)-carbonyl]amino]hexyl](3,4-dimethoxyphenyl)amino]acetic Acid, Methyl Ester (13g). To a solution of (*S*)-2-(acetyloxy)-6-[[(phenylmethoxy)carbonyl]amino]hexanoic acid (**12**, 1.29 g, 4.0 mmol) in dry THF (10 mL) at 0 °C (ice bath) under argon was added triethylamine (0.61 mL, 4.40 mmol), trimethylacetyl chloride (0.54 mL, 4.40 mmol), and 4-(dimethylamino)pyridine (0.20 g, 1.6 mmol) and the mixture was stirred at 0 °C for 2 h. [(3,4-Dimethoxyphenyl)amino]acetic acid, methyl ester (**10g**, 0.98 g, 4.40 mmol), was added and the mixture was allowed to warm to room temperature. After stirring at room temperature for 16 h, pyridine (4 mL) was added and the resulting solution was stirred for an additional 24 h. The mixture was evaporated and the residue partitioned between EtOAc and 1 N HCl. The organic phase was washed with saturated NaCl, dried over anhydrous

$MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel (150 g) eluting with acetone/hexane (3:7) to give pure **13g** (0.67 g, 33%) as a colorless glass: TLC (EtOAc) R_f 0.70; ^{13}C NMR (CD_3CN) δ 20.9 (CH_3), 22.9, 30.0, 31.4, 41.4, 52.4, 52.7 (CH_3), 56.7 (2 × CH_3), 66.8, 71.8 (CH), 112.9 (C), 113.2 (C), 121.5 (C), 128.8 (CH), 129.5 (CH), 135.5 (CH), 138.6 (C), 150.7 (C), 150.4 (C), 157.4 (C), 170.4 (C), 171.4 (C).

The following compounds were also prepared by this method: **13e** (29%), **13f** (43%), and **13h** (28%).

(*S*)-[[2-Hydroxy-1-oxo-6-[[(phenylmethoxy)carbonyl]amino]hexyl](3,4-dimethoxyphenyl)amino]acetic Acid, Diphenylmethyl Ester (7g). To a solution of **13g** (0.67 g, 1.3 mmol) in dioxane (7 mL) at room temperature under argon was added 1 N LiOH (7.0 mL, 7.0 mmol) and the resulting mixture was stirred for 1 h. The reaction mixture was partitioned between EtOAc (200 mL) and 10% $KHSO_4$, the organic phase washed with water and saturated NaCl and then dried over anhydrous $MgSO_4$ and concentrated to a volume of ca. 40 mL: TLC (CH_2Cl_2/CH_3OH/HOAc, 20:1:1) R_f 0.29. Diphenyldiazomethane (0.50 g, 2.6 mmol) was added and the mixture was stirred at room temperature under argon for 48 h. The mixture was concentrated to a volume of ca. 10 mL and flash chromatographed on silica gel (200 g) eluting with EtOAc/hexanes (7:3) to give pure **7g** (0.61 g, 74%) as a colorless glass: TLC (EtOAc) R_f 0.78; ^{13}C NMR (CD_3CN) δ 22.8, 30.1, 35.1, 41.4, 53.3, 56.6 (2 × CH_3), 66.8, 68.9 (CH), 79.0 (CH), 112.8 (2 × CH_3), 121.4 (CH), 127.8 (CH), 128.8 (CH), 129.0 (CH), 129.6 (CH), 135.3 (C), 138.5 (C), 141.2 (C), 150.3 (C), 150.8 (C), 157.4 (C), 169.4 (C), 176.9 (C).

The following compounds were also prepared by this method: 7e (83%), **17f** (74%), and **7h** (86%).

(*S*)-3-[(Phenylmethoxy)carbonyl]-4-oxazolidinecarboxylic Acid, Methyl Ester (14). A solution of L-serine (2.10 g, 20.0 mmol) in a mixture of 2 N NaOH solution (10.0 mL, 20.0 mmol) and 37% formaldehyde (1.70 mL, 22.7 mmol) was stirred at 5 °C for 18 h. The mixture was cooled in an ice bath, the pH was adjusted to 9.5 with concentrated HCl and then treated with benzyl chloroformate (3.0 mL, 14.7 mmol) in 0.5-mL portions at 15-min intervals. Throughout the reaction, the pH was maintained between 8.5 and 9.5 by the addition of 2 N NaOH solution. After the pH had stabilized, the reaction mixture was diluted with water and washed with two portions of Et_2O. The aqueous phase was acidified with concentrated HCl and extracted with EtOAc. The organic phase was washed with saturated NaCl, dried over anhydrous Na_2SO_4, and evaporated to give a colorless oil: TLC (*i*-PrOH/NH_4OH/H_2O, 7:2:1) R_f 0.88.

The crude acid was taken up in Et_2O (30 mL), cooled to 0 °C (ice bath), and treated with an ethereal solution of diazomethane in portions until TLC indicated the consumption of the starting acid. Excess diazomethane was discharged with a few drops of AcOH and the mixture was evaporated to dryness. The crude product was purified by flash chromatography on silica gel (100 g) eluting with Et_2O/hexanes (4:6) to give pure 14 (4.502 g, 85%) as a colorless oil: TLC (EtOAc/hexanes, 1:1) R_f 0.59; ^{13}C NMR ($CDCl_3$) δ 52.1 (CH_3), 56.9 (CH), 67.1, 70.3, 79.1, 127.7 (CH), 127.9 (CH), 128.2 (CH), 129.1 (C).

[*S*-(*R,*R**)]-3-[2-(2,2-Dimethyl-1-oxopropoxy)-1-oxo-6-[[(phenylmethoxy)carbonyl]amino]hexyl]-4-oxazolidinecarboxylic Acid, Methyl Ester.** A solution of (*S*)-3-[(phenylmethoxy)carbonyl]-4-oxazolidinecarboxylic acid, methyl ester (14, 1.32 g, 4.98 mmol), in a mixture of triethylamine (1.82 mL, 13.0 mmol) and EtOAc (20 mL) was treated with 20% $Pd(OH)_2/C$ (0.34 g) and stirred under a hydrogen atmosphere for 1.5 h. Concurrently, a solution of (*S*)-2-hydroxy-6-[[(phenylmethoxy)carbonyl]amino]hexanoic acid (**9**, 1.40 g, 4.98 mmol) and triethylamine (1.52 mL, 10.9 mmol) in dry THF (30 mL) at 0 °C under argon was treated with trimethylacetyl chloride (1.36 mL, 11.0 mmol) followed by 4-(dimethylamino)pyridine (0.10 g, 0.82 mmol). After stirring at 0 °C for 1.5 h, the hydrogenolysis reaction mixture was filtered into the solution of the mixed anhydride with 10 mL of THF to rinse the catalyst, and the resulting mixture was allowed to warm to room temperature. After stirring at room temperature for 16 h, the mixture was partitioned between EtOAc and 5% $KHSO_4$. The organic phase was washed with saturated $NaHCO_3$ and saturated NaCl solutions, dried over anhydrous Na_2SO_4, and evaporated to an oil. The crude product was purified by flash chromatography on silica gel (100 g) eluting with EtOAc/hexanes (4:6) to give 1.051 g of impure product as a colorless, viscous oil. TLC (acetone/hexanes, 3:7) shows two spots: R_f 0.21 (desired) and 0.14. The mixture was further purified by flash chromatography on silica gel eluting with acetone/hexanes (3:7) to give the title compound (0.483 g, 20%) as a clear, colorless oil: TLC (acetone/hexane, 3:7) R_f 0.21; ^{13}C NMR ($CDCl_3$) δ 21.5, 26.9 (3 × CH_3), 29.4, 30.2, 38.4 (C), 40.4, 52.5 (CH_3), 56.5, 66.4, 69.4, 71.8 (CH), 78.7, 127.9 (CH), 128.3 (CH), 136.6 (C), 156.4 (C), 166.9 (C), 169.9 (C), 177.0 (C).

[*S*-(*R,*R**)]-3-[2-Hydroxy-1-oxo-6-[[(phenylmethoxy)carbonyl]amino]hexyl]-4-oxazolidinecarboxylic Acid, Methyl Ester (7j).** To a solution of [*S*-(*R**,*R**)]-3-[2-(2,2-dimethyl-1-oxopropoxyl)-1-oxo-6-[[phenylmethoxy)carbonyl]amino]hexyl]-4-oxazolidinecarboxylic acid, methyl ester (0.483 g, 1.01 mmol) in dioxane (5.0 mL) at room temperature under argon was added a 1.0 N LiOH solution (2.5 mL, 2.5 mmol). After stirring at room temperature for 16 h, the mixture was partitioned between EtOAc and 5% $KHSO_4$. The organic phase was washed with saturated NaCl solution, dried over anhydrous Na_2SO_4, and evaporated in vacuo to give the crude acid as a colorless oil.

The crude acid was dissolved in dry THF (15 mL), cooled to 0 °C (ice bath), and treated with an ethereal solution of diazomethane in portions until TLC indicated consumption of the starting acid. Evaporation of solvents and purification of the residue by flash chromatography on silica gel (60 g) eluting with acetone/hexane (1:1) gave pure **7j** (0.318 g, 80%) as a colorless oil: TLC (acetone/hexane, 1:1) R_f 0.14; ^{13}C NMR (CD_3CN) δ 22.5, 30.3, 34.6, 41.4, 53.1 (CH_3), 57.8 (CH), 66.7, 70.0, 71.1 (CH), 79.6, 128.7 (CH), 129.4 (CH), 139.0 (C), 157.4 (C), 171.3 (C), 172.3 (C).

[*S*-(*R,*R**)]-2,3-Dihydro-1-[2-[[hydroxy(4-phenylbutyl)-phosphinyl]oxy]-1-oxo-6-[[(phenylmethoxy)carbonyl]amino]hexyl]-1*H*-indole-2-carboxylic Acid, Diphenylmethyl Ester (8i).** To a solution of benzhydryl ester **7i** (0.688 g, 1.16 mmol) and (4-phenylbutyl)phosphonous acid (**6**, 0.350 g, 1.77 mmol) in dry THF (5.0 mL) at room temperature under argon was added *N*,*N*′-dicyclohexylcarbodiimide (0.350 g, 1.70 mmol) and 4-(dimethylamino)pyridine (0.040 g, 0.33 mmol). After stirring at room temperature for 2 h, the mixture was filtered and diluted with EtOAc. The solution was washed with 5% $KHSO_4$, saturated $NaHCO_3$, and saturated NaCl solutions, dried over anhydrous Na_2SO_4, and evaporated to dryness: TLC (acetone/hexane, 1:1) R_f 0.29 (R_f of **7i** 0.44).

The crude phosphonous ester was taken up in dioxane (8.0 mL) and treated with a solution of $NaIO_4$ (0.275 g, 1.29 mmol) in water (3.0 mL) and the mixture was stirred at room temperature for 16 h. The mixture was partitioned between EtOAc and 5% $KHSO_4$, the organic phase was washed with dilute $NaHSO_3$ and saturated NaCl solutions, dried over anhydrous Na_2SO_4, and evaporated to dryness. The crude product was purified by filtration through a short pad of silica gel (25 g) eluting with AcOH/MeOH/CH_2Cl_2 (1:1:40) to give pure phosphonic monoester **8i** (0.736 g, 80%) as a white foam: TLC (AcOH/MeOH/CH_2Cl_2, 1:1:20) R_f 0.33; ^{13}C NMR ($CDCl_3$) δ 20.8 (5 Hz), 21.5, 26.0 (133 Hz), 32.0 (18 Hz), 34.0, 35.2, 40.4, 60.5 (CH), 66.3, 74.9 (CH), 78.1 (CH), 113.5 (C), 117.5 (C), 124.3 (CH), 125.6 (CH), 126.7 (CH), 127.9 (CH), 128.2 (CH), 136.6 (C), 139.3 (C), 141.9 (C), 156.4 (C), 168.3 (C), 170.0 (C).

The following compounds were also prepared by this method: **8b** (85%), **8c** (55%), **8d** (90%), **8e** (74%), **8f** (90%), **8g** (75%), **8h** (53%), and **8j** (92%).

[*S*-(*R,*R**)]-2,3-Dihydro-1-[6-amino-2-[[hydroxy(4-phenylbutyl)phosphinyl]oxy]-1-oxohexyl]-1*H*-indole-2-carboxylic Acid, Dilithium Salt (2i).** A solution of phosphonic monoester **8i** (0.721 g, 0.91 mmol) in methanol (20 mL) was treated with 20% $Pd(OH)_2/C$ (0.170 g) and stirred under a hydrogen atmosphere (balloon) for 2 h. The mixture was filtered through Celite, diluted with water (40 mL), and washed with Et_2O. The aqueous phase was evaporated to dryness to give the crude amino diacid (0.410 g) as a colorless glass. The crude product was taken up in 1.0 N LiOH solution (2.2 mL) and purified on CHP-20 (50-mL bed volume, 1 in. diameter column) eluting first with water (400 mL) and then with 20% acetonitrile/water (250 mL). The product-containing fractions were combined and evaporated to dryness. The residue was taken up in water, filtered, and lyophilized to give dilithium salt **2i** (0.416 g, 76%) as a fluffy white solid: TLC (*i*-PrOH/NH_4OH/H_2O, 7:2:1) R_f 0.27; $[\alpha]_D$ −88.4° (c

0.54, H_2O); ^{13}C NMR (CD_3OD) δ 23.0, 24.1 (4 Hz), 28.0, 28.8 (140 Hz), 35.6 (18 Hz), 35.8, 36.6, 40.5, 61.7 (CH), 74.5 (CH), 115.5 (C), 118.5 (C), 125.7 (CH), 126.6 (CH), 129.3 (CH), 131.0 (CH), 143.7 (C), 144.1 (C), 172.3 (C), 174.8 (C). Anal. ($C_{25}H_{31}N_2O_6PLi_2 \cdot 1.25H_2O$) C, H, N, P.

The following compounds were also prepared by this method: **2b** (80%, isolated as the amino diacid), **2c** (81%), **2d** (32%), **2e** (81%), **2f** (49%), **2g** (73%), **2h** (35%), and **2j** (82%, isolated as the amino diacid).

(*S*)-2-Hydroxy-6-[[(phenylmethoxy)carbonyl]amino]-hexanoic Acid, Methyl Ester (15). To a solution of hydroxy acid **9** (4.0 g, 14.2 mmol) and methyl iodide (0.97 mL, 15.6 mmol) in dry DMF (15 mL) at room temperature under argon was added powdered potassium carbonate (2.55 g, 18.5 mmol) and the resulting mixture was stirred for 2 h. The mixture was partitioned between 5% $KHSO_4$ and EtOAc, the organic phase was washed with water, saturated $NaHCO_3$, and saturated NaCl solutions, dried over anhydrous Na_2SO_4, and evaporated to give methyl ester **15** (3.703 g, 88%) as a viscous, pale yellow oil: TLC (EtOAc/hexane, 9:1) R_f 0.69; ^{13}C NMR ($CDCl_3$) δ 21.7, 29.1, 33.4, 40.5, 51.9 (CH_3), 66.1, 70.0 (CH), 127.6 (CH), 128.1 (CH), 136.4 (C), 156.3 (C), 175.1 (C).

(*S*)-2-[[Hydroxy(4-phenylbutyl)phosphinyl]oxy]-6-[[(phenylmethoxy)carbonyl]amino]hexanoic Acid (17). To a solution of hydroxy ester **15** (3.323 g, 11.3 mmol) and (4-phenylbutyl)phosphonous acid (**6**, 3.37 g, 17 mmol) in dry THF (30 mL) at room temperature under argon was added *N,N'*-dicyclohexylcarbodiimide (3.51 g, 17 mmol) and 4-(dimethylamino)pyridine (0.208 g, 1.7 mmol). After stirring at room temperature for 40 min, the mixture was diluted with EtOAc and filtered. The filtrate was washed with 5% $KHSO_4$, saturated $NaHCO_3$, and saturated NaCl solutions, dried over anhydrous Na_2SO_4, and evaporated to dryness. The crude product was pufified by chromatography on a pad of SilicAR CC-7 silica gel eluting with EtOAc/hexane (9:1) to give the crude phosphonous ester (5.77 g) as a viscous oil: TLC (EtOAc/hexane, 9:1) R_f 0.23.

To a solution of the crude phosphonous ester (5.77 g) in dioxane (60 mL) at room temperature under argon was added a solution of $NaIO_4$ (2.78 g, 13.0 mmol) in water (15 mL) and the resulting mixture was stirred for 16 h. The mixture was filtered and partitioned between 1% $KHSO_4$ and EtOAc, and the organic phase was washed with water, dilute $NaHSO_3$, and saturated NaCl solutions, dried over Na_2SO_4, and evaporated to dryness.

The crude product was purified by way of its 1-adamantanamine salt. Thus, to a solution of the crude product in Et_2O (20 mL)/EtOAc (5 mL) was added a solution of 1-adamantanamine (1.71 g, 11.3 mmol) in Et_2O (5.0 mL). The solution was diluted with hexane and placed in an ice bath. The solvent was decanted from the precipitated 1-adamantanamine salt which was immediately partitioned between 1.0 N HCl and EtOAc. The organic phase was washed with saturated NaCl solution, dried over anhydrous Na_2SO_4, and evaporated to give 17-monomethyl ester (5.043 g, 85%) as a viscous, pale yellow oil: TLC (*i*-PrOH/NH_4OH/H_2O, 20:1:1) R_f 0.37; ^{13}C NMR ($CDCl_3$) δ 19.7, 21.4, 28.5 (92 Hz), 28.7, 31.5 (13 Hz), 32.1, 34.9, 40.3, 52.0 (CH_3), 66.0, 72.3 (C, 7 Hz), 125.4 (CH), 127.5 (CH), 127.9 (CH), 136.4 (C), 141.2 (C), 156.1 (C), 170.2 (C).

To a solution of the methyl ester (5.00 g, 10.2 mmol) in dioxane (15 mL) at room temperature under argon was added 2.0 N NaOH solution (11.2 mL, 22.4 mmol) and the resulting mixture was stirred for 2 h. The mixture was acidified with concentrated HCl (2 mL) and extracted with EtOAc. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, and evaporated to give **17** (4.80 g, 99% from ester) as a clear, colorless, viscous oil: TLC (CH_2Cl_2/HOAc/MeOH, 20:1:1) R_f 0.10; ^{13}C NMR ($CDCl_3$) δ 21.5, 25.8 (143 Hz), 28.9, 31.3 (16 Hz), 32.5, 35.1, 40.6, 52.0 (CH_3), 66.3, 72.7 (C, 7 Hz), 125.5 (CH), 127.7 (CH), 128.1 (CH), 136.4 (C), 141.7 (C), 156.4 (C), 170.5 (C, 3 Hz).

(*S*)-2-Hydroxy-6-[(trifluoroacetyl)amino]hexanoic Acid, Phenylmethyl Ester (16). To a suspension of (*S*)-6-amino-2-hydroxyhexanoic acid[1] (4.50 g, 30.6 mmol) in dry acetonitrile (60 mL) at room temperature under argon was added bis(trimethylsilyl)trifluoroacetamide (24.0 mL, 96.2 mmol). After stirring at room temperature for 2 h, the resulting clear solution was cooled to 0 °C (ice bath), treated with trifluoroacetic anhydride (5.30 mL, 37.2 mmol), and allowed to warm to room temperature. The mixture was evaporated to dryness; the residue was taken up in acetonitrile (50 mL), treated with water (10 mL), and stirred at room temperature for 30 min. The mixture was evaporated to dryness; the residue was taken up in half-saturated $NaHCO_3$ and washed with Et_2O. The aqueous phase was acidified with concentrated HCl, saturated with solid NaCl, and extracted with EtOAc. The organic phase was washed with saturated NaCl solution, dried over Na_2SO_4, and evaporated. The residue was triturated with hexane to give crude (*S*)-2-hydroxy-6-[(trifluoroacetyl)amino]hexanoic acid (7.414 g, 100%) as a white solid: TLC (AcOH/MeOH/CH_2Cl_2, 1:1:20) R_f 0.28.

The crude acid (7.414 g) was dissolved in dry DMF (30 mL), treated with benzyl bromide (3.95 mL, 33.2 mmol) and powdered potassium carbonate (3.35 g, 33.5 mmol), and stirred at room temperature under argon for 5 h. The mixture was partitioned between EtOAc and water, and the organic phase was washed with water (2 × 50 mL) and saturated NaCl solution, dried over anhydrous Na_2SO_4, and evaporated to dryness. Trituration of the crude product with hexane gave benzyl ester **16** (9.44 g, 93% overall) as a white, crystalline solid: mp 60–61 °C; TLC (acetone/CH_2Cl_2, 15:85) R_f 0.50; $[\alpha]_D$ −12.4° (*c* 1.25, MeOH); ^{13}C NMR ($CDCl_3$) δ 21.9, 28.2, 33.4, 39.6, 67.3, 70.2 (CH), 128.3 (CH), 128.6 (CH), 157.0 (C, J_{C-F} = 37 Hz).

(*S*)-2-[[Hydroxy(4-phenylbutyl)phosphinyl]oxy]-6-[(trifluoroacetyl)amino]hexanoic Acid (18). To a solution of benzyl ester **16** (9.24 g, 27.7 mmol) and (4-phenylbutyl)phosphonous acid (**6**, 8.30 g, 41.9 mmol) in dry THF (60 mL) at 0 °C (ice bath) under argon was added *N,N'*-dicyclohexylcarbodiimide (8.57 g, 41.6 mmol) and 4-(dimethylamino)pyridine (1.0 g, 8.2 mmol). The reaction mixture was allowed to warm to room temperature, stirred for 2 h, and filtered. The filtrate was diluted with EtOAc (150 mL) and washed successively with 5% $KHSO_4$, saturated $NaHCO_3$, and saturated NaCl solutions, dried over anhydrous Na_2SO_4, and evaporated to dryness: TLC (acetone/Et_2O, 2:8) R_f 0.60.

The crude phosphonous monoester was taken up in dioxane (80 mL), treated with a solution of $NaIO_4$ (6.89 g, 32.2 mmol) in water (40 mL), and stirred at room temperature under argon for 18 h. The mixture was partitioned between 1% $KHSO_4$ and EtOAc (150 mL each), the organic phase was washed with water, dilute $NaHSO_3$, and saturated NaCl solutions, dried over Na_2SO_4, and evaporated to give the crude phosphonic monoester (16.04 g, theoretical yield 14.68 g) as a pale yellow viscous oil suitable for use in the next step: TLC (AcOH/MeOH/CH_2Cl_2, 1:1:20) R_f 0.22.

The crude phosphonic acid (16.04 g) was taken up in methanol (75 mL) and water (7.5 mL), treated with 10% Pd/C (1.0 g), and hydrogenated on a Parr apparatus at a pressure of 30 psi for 2 h. The catalyst was removed by filtration through Celite, and the filtrate was evaporated to dryness. The residue was taken up in half-saturated $NaHCO_3$ (75 mL) and washed with EtOAc. The aqueous phase was acidified with concentrated HCl and extracted with EtOAc. The EtOAc extract was washed with saturated NaCl, dried over anhydrous Na_2SO_4, and evaporated to give slightly impure diacid: TLC (AcOH/MeOH/CH_2Cl_2, 1:1:8) R_f 0.32, R_f of impurity = 0.59.

The crude diacid was taken up in Et_2O (100 mL), treated with a solution of 1-adamantanamine (8.45 g, 56 mmol) in Et_2O (40 mL), and evaporated to dryness. The residue was triturated with hexane to give crude diadamantanamine salt (21.71 g) as a white, crystalline solid; mp 65–90 °C. The crude salt was triturated with water (200 mL), collected, and air-dried. Trituration of the salt with two 100-mL portions of Et_2O gave purified diadamantanamine salt (17.51 g, 85% overall) as a white, crystalline solid; mp 103–106 °C.

The purified diadamantanamine salt (1.255 g, 1.69 mmol) was partitioned between EtOAc and 1.0 N HCl (50 mL each); the organic phase was washed with 1.0 N HCl and saturated NaCl solutions, dried over anhydrous Na_2SO_4, and evaporated to give pure diacid **18** (0.722 g, 97%) as a colorless, viscous oil: TLC (AcOH/MeOH/CH_2Cl_2, 1:1:8) R_f 0.32; ^{13}C NMR ($CDCl_3$) δ 21.6, 21.7 (4 Hz), 25.7 (143 Hz), 27.8, 31.8, 32.0 (16 Hz), 35.2, 39.6, 73.2 (CH), 125.8 (CH), 128.3 (CH), 141.8 (C), 157.6 (C, J_{C-F} = 37 Hz), 171.9 (C), 174.3 (C).

[1(*R),2α,4α]-4-Fluoro-1-[2-[[hydroxy(4-phenylbutyl)-phosphinyl]oxy]-1-oxo-6-[[(phenylmethoxy)carbonyl]-amino]hexyl]-L-proline, Methyl Ester (8bb).** A solution of

diacid 17 (0.720 g, 1.51 mmol) in dry THF (6.0 mL) at 0 °C (ice bath) under argon was treated with 1,1'-carbonyldiimidazole (0.290 g, 1.79 mmol) and stirred at 0 °C for 1 h. To the resulting solution were added *cis*-4-fluoro-L-proline, methyl ester 4-methylbenzenesulfonate (1:1) salt (**10bb**, 0.625 g, 1.96 mmol), and triethylamine (0.55 mL, 3.95 mmol), and the mixture was allowed to warm to room temperature. After stirring at room temperature for 18 h, the mixture was partitioned between EtOAc and 1.0 N HCl. The organic phase was washed succesively with 1.0 N HCl, half-saturated $NaHCO_3$ (3 × 30 mL), 5% $KHSO_4$, and saturated NaCl solutions, dried over anhydrous Na_2SO_4, and evaporated to give pure **8bb** (0.724 g, 79%) as a white foam: TLC (*i*-PrOH/NH_4OH/H_2O, 7:2:1) R_f 0.65; ^{13}C NMR (CD_3CN) δ 22.0, 22.7, 22.9 (6 Hz), 26.8 (140 Hz), 30.2, 32.9 (16 Hz), 33.2 (7 Hz), 35.9, 36.0 (J_{C-F} = 20 Hz), 41.4, 52.9 (CH_3), 54.2 (J_{C-F} = 23 Hz), 58.5 (CH), 66.8, 73.5 (CH, 7 Hz), 94.0 (CH, J_{C-F} = 176 Hz), 126.8 (CH), 128.1 (CH), 128.7 (CH), 129.4 (CH), 138.5 (C), 143.3 (C), 157.5 (C), 170.1 (C), 172.0 (C).

The following compounds were also prepared by this method: **8aa** (80%) and **8cc** (66%).

[1(*R),2α,4α]-1-[6-Amino-2-[[hydroxy(4-phenylbutyl)phosphinyl]oxy]-1-oxohexyl]-4-fluoro-L-proline (2bb).** To a solution of methyl ester **8bb** (0.724 g, 1.19 mmol) in dioxane (5.0 mL) at room temperature under argon was added 1.0 N LiOH solution (3.0 mL, 3.0 mmol). After stirring at room temperature for 1 h, the mixture was partitioned between EtOAc and 5% $KHSO_4$; the organic phase was washed with saturated NaCl, dried over anhydrous Na_2SO_4, and evaporated to give the crude Cbz diacid (0.694 g) as a colorless glass: TLC (*i*-PrOH/NH_4OH/H_2O, 7:2:1) R_f 0.52.

The Cbz diacid (0.694 g) was taken up in methanol (15 mL), treated with 20% Pd/C (0.15 g), and stirred under a hydrogen atmosphere (balloon) for 2 h. The mixture was filtered through Celite and evaporated to give 0.522 g of crude product as a colorless glass. The product was taken up in water, filtered, and evaporated. The residue was triturated first with CH_3CN and then with Et_2O to give pure **2bb** (0.489 g, 89%) as a white, crystalline solid: mp 145–160 °C softens, 206 °C dec; TLC (*i*-PrOH/NH_4OH/H_2O, 7:2:1) R_f 0.20; $[\alpha]_D$ −37.3° (c 0.56, MeOH); ^{13}C NMR (CD_3OD) δ 22.6, 24.4 (6 Hz), 28.0, 29.0 (137 Hz), 33.5, 34.1 (17 Hz), 36.1 (J_{C-F} = 15 Hz), 36.6, 40.4, 54.3 (J_{C-F} = 30 Hz), 59.1 (CH), 72.1 (CH), 94.1 (J_{C-F} = 176 Hz), 126.6 (CH), 128.3 (CH), 130.4 (CH), 143.6 (C), 172.9 (C), 174.3 (C); ^1H NMR (CD_3OD) δ 1.45–1.90 (12 H, m), 2.37 (1 H, b m), 2.47 (1 H, b m), 2.63 (2 H, b t), 2.95 (2 H, b t), 3.85 (1 H, dd, J = 12 Hz, J_{H-F} = 24 Hz), 4.12 (1 H, ddd, J = 5, 12 Hz; J_{H-F} = 32 Hz), 4.75 (1 H, m), 4.87 (1 H, m), 5.33 (1 H, b d, $J_{H-F} \cong$ 50 Hz), 7.05–7.30 (5 H, m). Anal. ($C_{21}H_{32}N_2O_6PF \cdot 0.80H_2O$) C, H, N, P, F.

The following compounds were also prepared by this method: **2aa** (85%) and **2cc** (82%, isolated as the corresponding dilithium salt after treatment with 1 N LiOH followed by CHP-20 chromatography).

(*S*)-[Cyclohexyl[2-[[hydroxy(4-phenylbutyl)phosphinyl]oxy]-1-oxo-6-[[(phenylmethoxy)carbonyl]amino]hexyl]amino]acetic Acid, Phenylmethyl Ester (8k). To a mixture of diacid 17 (1.040 g, 2.18 mmol), (cyclohexylamino)acetic acid, phenylmethyl ester monohydrochloride (**10k**, 0.926 g, 3.27 mmol), and triethylamine (0.46 mL, 3.30 mmol) in dry THF (20 mL) at room temperature under argon was added *N,N'*-dicyclohexylcarbodiimide (1.555 g, 7.54 mmol). After stirring at room temperature for 6 h, the mixture was diluted with EtOAc and filtered. The filtrate was washed with 5% $KHSO_4$, saturated $NaHCO_3$, and saturated NaCl solutions, dried over anhydrous Na_2SO_4, and evaporated to an oil. The crude product was purified by filtration through silica gel eluting with CH_2Cl_2, then CH_2Cl_2/acetone (8:2), then CH_2Cl_2/CH_3OH (8:2) to give **8k** (0.800 g, 52%) as a pale yellow oil: TLC (*i*-PrOH/NH_4OH/H_2O, 7:2:1) R_f 0.72; ^{13}C NMR ($CDCl_3$) δ 21.6, 22.6, 25.4 (3 × CH_3), 26.3 (143 Hz), 28.9, 32.5 (17 Hz), 32.8, 35.4, 40.3, 43.5, 56.0 (CH), 66.2, 66.6, 72.4 (CH), 125.4 (CH), 127.7 (CH), 128.0 (CH), 135.3 (C), 136.6 (C), 142.1 (C), 156.4 (C), 169.2 (C), 171.3 (C).

Compound **8l** (69%) was also prepared by this method.

(*S*)-[[6-Amino-2-[[hydroxy(4-phenylbutyl)phosphinyl]oxy]-1-oxohexyl]cyclohexylamino]acetic Acid, Dilithium Salt (2k). A solution of diester **8k** (0.800 g, 1.13 mmol) in methanol (20 mL) was treated with 20% Pd/C (0.120 g) and stirred under a hydrogen atmosphere (balloon) for 3 h. The catalyst was removed by filtration through Celite, and the filtrate was evaporated. The residue was taken up in 1.0 N LiOH (5 mL, 5.0 mmol), filtered, concentrated to a small volume, and chromatographed on a CHP-20P column (200-mL bed volume, 25 mm diameter column) eluting with a linear gradient of H_2O (100%) → CH_3CN (100%). The product-containing fractions were pooled and evaporated to dryness. The residue was taken up in water (50 mL), filtered, and lyophilized to give dilithium salt **2k** (0.421 g, 69%) as a white solid: TLC (*i*-PrOH/NH_4OH/H_2O, 7:2:1) R_f 0.29; $[\alpha]_D$ −16.2° (*c* 0.50, MeOH); ^{13}C NMR (D_2O/dioxane ref) δ 21.4, 22.7, 23.0, 25.6 (3 Hz), 26.5 (143 Hz), 29.5, 32.5 (13 Hz), 34.9, 39.2, 46.0, 46.9, 54.6 (CH), 56.7 (CH), 70.9 (CH), 71.7 (CH), 125.5 (CH), 128.3 (CH), 142.5 (C), 142.7 (C), 172.2 (C), 173.0 (C), 173.2 (C), 175.9 (C). Anal. ($C_{24}H_{37}N_2O_6PLi_2 \cdot 2.4OH_2O$) C, H, N, P.

Compound **2l** (69%) was also prepared by this method.

[1(*R),2α,4β]-1-[2-[[Hydroxy(4-phenylbutyl)phosphinyl]oxy]-1-oxo-6-[(trifluoroacetyl)amino]hexyl]-4-(phenylthio)-L-proline, Methyl Ester (8s).** To a solution of diacid 18 (0.661 g, 1.51 mmol) in dry THF (8.0 mL) at 0 °C (ice bath) under argon was added 1,1'-carbonyldiimidazole (0.270 g, 1.67 mmol). After stirring at 0 °C for 1 h, *trans*-4-(phenylthio)-L-proline, methyl ester monohydrochloride (**10s**, 0.490 g, 1.81 mmol), and triethylamine (0.46 mL, 3.32 mmol) were added, and the mixture was allowed to warm to room temperature. After stirring at room temperature for 4.5 h, the mixture was partitioned between EtOAc and 1.0 N HCl; the organic phase was washed with 1.0 N HCl and saturated NaCl solutions, dried over anhydrous Na_2SO_4, and evaporated to give **8s** (0.874 g, 89%) as a white foam: TLC (AcOH/MeOH/CH_2Cl_2, 1:1:8) R_f 0.59; ^{13}C NMR ($CDCl_3$) δ 21.0, 21.6 (5 Hz), 26.0 (137 Hz), 27.6, 31.3, 31.9 (17 Hz), 34.4 (CH), 35.0, 39.1, 44.9, 52.1, 58.4 (CH), 72.6 (CH, 7 Hz), 125.5 (CH), 127.8 (CH), 128.0 (CH), 129.1 (CH), 132.1 (CH), 132.9 (C), 141.6 (C), 157.1 (C, 37 Hz), 168.6 (C, 3 Hz), 171.6 (C).

The following compounds were also prepared by this method: **8m** (92%), **8n** (81%), **8o** (40%), **8p** (83%), **8q** (79%), **8r** (87%), **8t** (41%), **8u** (84%), **8v** (82%), and **8w** (66%).

[1(*R),2α,4β]-1-[6-Amino-2-[[hydroxy(4-phenylbutyl)phosphinyl]oxy]-1-oxohexyl]-4-(phenylthio)-L-proline, Dilithium Salt (2s).** A solution of **8s** (0.874 g, 1.33 mmol) in dioxane (6.0 mL) was treated with a 1.0 N LiOH solution (4.7 mL, 4.7 mmol) and the mixture was stirred under argon at room temperature for 2 h. The mixture was filtered and evaporated to dryness. The residue was taken up in water (5.0 mL) and chromatographed on a CHP-20P column (200-mL bed volume, 25 mm diameter column) eluting with a linear gradient of water (100%) → CH_3CN (100%). The product-containing fractions were pooled and evaporated to dryness. The residue was taken up in water (50 mL), filtered, and lyophilized to give dilithium salt **2s** (0.677 g, 87%) as a white solid: TLC (*i*-PrOH/NH_4OH/H_2O, 7:2:1) R_f 0.22; $[\alpha]_D$ −24.1° (c 0.58, MeOH); ^{13}C NMR (D_2O/dioxane ref) δ 20.9, 22.7 (6 Hz), 26.4, 27.3 (137 Hz), 31.3, 31.9 (17 Hz), 34.4, 35.0, 39.2, 44.6 (CH), 52.5, 61.4 (CH), 71.8, 125.6 (CH), 127.2 (CH), 128.3 (CH), 129.2 (CH), 131.0 (CH), 133.7 (C), 142.6 (C), 171.5 (C), 171.7 (C), 178.0 (C); ^1H NMR (CD_3OD) δ 1.45–1.90 (12 H, m), 2.17 (1 H, m), 2.26 (1 H, m), 2.60 (2 H, b t), 2.92 (2 H, m), 3.80 (1 H, dd, J = 6, 9 Hz), 3.90–4.10 (2 H, m), 4.53 (1 H, dd, J = 6, 9 Hz), 4.90 (1 H, m), 7.05–7.50 (10 H, m). Anal. ($C_{27}H_{35}N_2O_6SPLi_2 \cdot 1.3H_2O$) C, H, N, S, P.

The following compounds were also prepared by this method: **2m** (74%), **2n** (57%), **2o** (78%), **2p** (83%), **2q** (88%), **2r** (77%), **2t** (80%), **2u** (90%), **2v** (78%), and **2w** (44%).

[*S*-(*R,*R**)]-7-[2-[[Hydroxy(4-phenylbutyl)phosphinyl]oxy]-1-oxo-6-[(trifluoroacetyl)amino]hexyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic Acid, Methyl Ester (8x).** To a solution of diacid **18** (0.660 g, 1.50 mmol) and triethylamine (0.46 mL, 3.32 mmol) in dry THF (8.0 mL) at 0 °C (ice bath) under argon was added trimethylacetyl chloride (0.20 mL, 1.62 mmol). After stirring at 0 °C for 30 min, 1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, methyl ester monohydrochloride (**10x**, 0.460 g, 1.80 mmol), and triethylamine (0.25 mL, 1.8 mmol) were added. After stirring at 0 °C for 30 min and at room temperature for 2 h, the mixture was partitioned between EtOAc and 1.0 N HCl. The organic phase was washed successively with 1.0 N HCl, saturated $NaHCO_3$, 1.0 N HCl, and saturated NaCl solutions,

dried over anhydrous Na_2SO_4, and evaporated to dryness. The crude product was purified by flash chromatography on silica gel (10 g) eluting with acetone/CH_2Cl_2 (5:95), then MeOH/CH_2Cl_2 (2:98), and finally with AcOH/MeOH/CH_2Cl_2 (1:1:20) to give purified **8x** (0.561 g, 58%) as a white foam: TLC (AcOH/MeOH/CH_2Cl_2, 1:1:8) R_f 0.69; ^{13}C NMR ($CDCl_3$) δ 21.0, 21.7 (4 Hz), 26.0 (135 Hz), 27.6, 31.7, 32.0 (17 Hz), 35.1, 39.3 (2 × CH_2), 39.9, 44.1, 52.3 (CH_3), 59.3, 61.2, 66.9 (C), 72.8 (CH, 6 Hz), 116.0 (CF_3, J_{C-F} = 291 Hz), 125.6 (CH), 128.1 (CH), 141.7 (C), 157.2 (C, J_{C-F} = 36 Hz), 168.4 (C, 3 Hz), 170.9 (C).

[*S*-(*R,*R**)]-7-[6-Amino-2-[[hydroxy(4-phenylbutyl)-phosphinyl]oxy]-1-oxohexyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic Acid, Dilithium Salt (2x).** To a solution of **8x** (0.561 g, 0.876 mmol) in dioxane (4.0 mL) at room temperature under argon was added a 1.0 N LiOH solution (3.1 mL, 3.1 mmol). After stirring at room temperature for 2.5 h, the mixture was diluted with water (50 mL) and extracted with EtOAc (30 mL). The aqueous phase was filtered and evaporated to dryness. The residue was taken up in water (5.0 mL) and chromatographed on a CHP-20P column (200-mL bed volume, 25 mm diameter column) eluting with a linear gradient of H_2O (100%) → CH_3CN/H_2O (90:10). The product-containing fractions were pooled and evaporated to dryness. The residue was taken up in water (50 mL), filtered, and lyophilized to give dilithium salt **2x** (0.401 g, 80%) as a white solid: TLC (*i*-PrOH/NH_4OH/H_2O, 7:2:1) R_f 0.29; $[\alpha]_D$ +15.7° (c 0.57, MeOH); ^{13}C NMR (D_2O, dioxane ref) δ 20.8, 22.7 (4 Hz), 26.4, 27.0, 32.3 (17 Hz), 32.6, 38.9, 39.3 (2 × CH_2), 43.8, 61.5, 62.4 (CH), 66.9 (C), 71.7 (CH, 5 Hz), 125.9 (CH), 128.6 (CH), 143.0 (C), 171.2 (C), 177.3 (C); ^1H NMR (CD_3OD) δ 1.45–1.90 (12 H, m), 2.40 (1 H, dd, J = 9, 12 Hz), 2.60 (2 H, b t), 2.70 (1 H, dd, J = 8, 12 Hz), 2.95 (2 H, b t), 3.37 (4 H, s), 3.91 (1 H, d, J = 12 Hz), 4.28 (1 H, d, J = 12 Hz), 4.41 (1 H, dd, 1 H, J = 7, 9 Hz), 4.92 (1 H, m), 7.06–7.25 (5 H, m). Anal. ($C_{23}H_{33}N_2O_6S_2PLi_2$·1.55 H_2O) C, H, N, P, S.

[1(*R),2α,4α]-1-[6-Amino-2-[[hydroxy(4-phenylbutyl)-phosphinyl]oxy]-1-oxohexyl]-4-hydroxy-L-proline, Dilithium Salt (2z).** To a solution of diacid **18** (1.41 g, 3.21 mmol) in dry THF (10 mL) at 0 °C (ice bath) under argon was added 1,1′-carbonyldiimidazole (0.624 g, 3.85 mmol). After stirring at 0 °C for 1 h, (1*S*,4*S*)-2-oxa-5-azabicyclo[2.2.1]heptane-3-one, monohydrobromide salt (**10z**, 0.747 g, 3.85 mmol), and triethylamine (0.54 mL, 3.85 mmol) were added, and the mixture was allowed to warm to room temperature. After stirring at room temperature for 2 h, the mixture was partitioned between EtOAc and 1.0 N HCl. The organic phase washed successively with saturated $NaHCO_3$, 1.0 N HCl, and saturated NaCl solutions, dried over anhydrous Na_2SO_4, and evaporated to give the crude product (1.257 g, 73%) as a colorless glass: TLC (CH_2Cl_2/MeOH/HOAc, 8:1:1) R_f 0.31; ^{13}C NMR ($CDCl_3$) δ 21.3 (2 × CH_2), 25.8 (138 Hz), 27.5, 31.8 (17 Hz), 32.3, 34.9 (2 × CH_2), 39.0, 50.1, 57.6 (CH), 74.8 (CH), 77.7 (CH), 125.6 (CH), 128.0 (CH), 141.4 (C), 157.1 (C, J_{C-F} = 36 Hz), 168.1 (C), 170.5 (C).

To a solution of the above crude product (1.257 g, ca. 2.35 mmol) in dioxane (15 mL) at room temperature under argon was added 1.0 N LiOH solution (8.2 mL, 8.2 mmol). After stirring at room temperature for 1.5 h, the mixture was diluted with water and washed with EtOAc. The aqueous phase was filtered and evaporated to dryness. The residue was dissolved in water (5.0 mL) and chromatographed on a CHP-20P column eluting with a linear gradient of H_2O (100%) → CH_3CN (100%). The product-containing fractions were pooled and evaporated to dryness. The residue was taken up in water (50 mL), filtered, and lyophilized to give dilithium salt **2z** (0.807 g, 66%) as a white solid: TLC (*i*-PrOH/NH_4OH/H_2O, 7:2:1) R_f 0.21; $[\alpha]_D$ −12.4° (c 0.50, MeOH); ^{13}C NMR (D_2O/dioxane ref) δ 22.0, 23.6, 27.6, 28.2 (137 Hz), 32.7, 33.3 (18 Hz), 35.9, 37.4, 40.3, 55.5, 61.9 (CH), 71.0 (CH), 72.1 (CH), 126.8 (CH), 129.5 (CH), 143.8 (C), 172.9 (C), 180.0 (C); ^1H NMR (CD_3OD) δ 1.45–1.90 (12 H, m), 1.95 (1 H, dt, J = 2, 12 Hz), 2.33 (1 H, m), 2.61 (2 H, b t), 2.93 (2 H, b t), 3.61 (1 H, dd, J = 2, 12 Hz), 3.98 (1 H, dd, J = 5, 12 Hz), 4.26–4.40 (2 H, m), 4.92 (1 H, m), 7.05–7.30 (5 H, m). Anal. ($C_{21}H_{31}N_2O_7PLi_2$·2.65H_2O) C, H, N, P.

[1(*R),2α,4β]-1-[6-Amino-2-[[hydroxy(4-phenylbutyl)-phosphinyl]oxy]-1-oxohexyl]-4-hydroxy-L-proline, Dilithium Salt (2y).** To a solution of diacid **18** (1.355 g, 3.09 mmol) in dry THF (15 mL) at 0 °C (ice bath) under argon was added 1,1′-carbonyldiimidazole (0.602 g, 3.71 mmol). After stirring at 0 °C for 1 h, amino ester·TsOH **10y** (1.49 g, 3.71 mmol) and triethylamine (1.50 mL, 10.8 mmol) were added and the mixture was allowed to warm to room temperature. After stirring at room temperature for 4 h, the mixture was partitioned between EtOAc and 1.0 N HCl. The organic phase was washed successively with 1.0 N HCl, saturated $NaHCO_3$, and saturated NaCl solutions, dried over anhydrous Na_2SO_4, and evaporated to give the crude product (1.211 g, 61%) as a colorless glass: TLC (CH_2Cl_2/MeOH/HOAc, 8:1:1) R_f 0.52; ^{13}C NMR ($CDCl_3$) δ 20.9, 21.4, 26.4 (3 × CH_3), 27.4, 31.6, 32.1, 33.7, 34.8, 38.2 (C), 38.9, 51.9, 57.8 (CH), 72.3 (2 × CH), 125.3 (CH), 127.9 (CH), 141.4 (C), 156.9 (C, J_{C-F} = 37 Hz), 168.6 (C, 2 Hz), 171.2 (C), 177.3 (C).

To a solution of the above crude product (1.211 g, ca. 1.86 mmol) in dioxane (15 mL) at room temperature under argon was added 1.0 N LiOH solution (9.3 mL, 9.3 mmol). After stirring at room temperature for 16 h, the mixture was diluted with water and washed with EtOAc. The aqueous phase was evaporated to dryness. The residue was taken up in water (5.0 mL) and chromatographed on a CHP-20P column eluting with a linear gradient of H_2O (100%) → CH_3CN (100%). The product-containing fractions were pooled and evaporated to dryness. The residue was taken up in water (50 mL), filtered, and lyophilized to give dilithium salt **2y** (0.709 g, 78%) as a white solid: TLC *i*-PrOH/NH_4OH/H_2O, 7:2:1) R_f 0.21; $[\alpha]_D$ −35.8° (*c* 0.50, MeOH); ^{13}C NMR (D_2O, dioxane ref) δ 22.0, 23.6, 27.5, 28.2 (137 Hz), 33.3 (17 Hz), 33.8, 35.8, 38.1, 40.3, 56.0, 61.9 (CH), 71.0 (CH), 72.4 (CH), 126.8 (CH), 129.6 (CH), 143.9 (C), 172.8 (C), 179.8 (C); ^1H NMR (CD_3OD) δ 1.45–1.90 (12 H, m), 2.01 (1 H, m), 2.23 (1 H, m), 2.61 (2 H, b t), 2.92 (2 H, m), 3.72 (1 H, dd, J = 4, 11 Hz), 3.90 (1 H, d, J = 11 Hz), 4.44 (1 H, b m), 4.48 (1 H, t, J = 8 Hz), 4.92 (1 H, m), 7.05–7.25 (5 H, m). Anal. ($C_{21}H_{31}N_2O_7PLi_2$·H_2O) C, H, N, P.

In Vitro Inhibition of Angiotensin Converting Enzyme from Rabbit Lung. The conditions for the assay of inhibition of ACE are those we reported previously[14] in which hippuric acid liberated from the synthetic substrate hippurylhistidylleucine by rabbit lung ACE is quantitated by a spectrophotometric method (see ref 14 for details).

Angiotensin Converting Enzyme Inhibitor Screen in Vivo. Male Sprague–Dawley rats (225–275 g) were equipped with indwelling abdominal aorta and vena caval catheters by using a modification of the method of Weeks and Jones.[27] The animals were allowed to recover for at least 2 weeks before experimentation, during which they were housed individually and maintained on rat chow and tap water ad libitum. On the day of experimentation, aortic blood pressures were monitored directly by pressure transducers and recorded on a Beckman dynograph. The venous catheter was used for drug injections. During all experiments the rats were conscious and unrestrained in their cages. Pressor responses were obtained for angiotensin I (310 ng/kg iv) and angiotensin II (100 ng/kg iv) before administration of the compounds. For intravenous testing, compounds were administered in 0.1 mL of water or 5% $NaHCO_3$ and angiotensin I and II pressor responses were evaluated for up to 70 min. For oral testing, compounds were administered in 0.1 mL of water, 5% $NaHCO_3$, or 1% agar suspension and angiotensin I and II pressor responses were evaluated for up to 280 min. Maximum percent inhibition was determined as the mean of the responses for four animals per dose. The dose required to produce 50% inhibition of the response was estimated by interpolation of a plot of maximum inhibition versus dose.

(27) Weeks, J. R.; Jones, J. A. *Proc. Soc. Exp. Biol. Med.* **1960**, *104*, 646.