

# Synthesis of Novel *N*-Phosphonoalkyl Dipeptide Inhibitors of Human Collagenase

John Bird, Rachel C. De Mello, Gregory P. Harper, David J. Hunter, Eric H. Karran, Roger E. Markwell,\*  
Anette J. Miles-Williams, Shahzad S. Rahman, and Robert W. Ward

SmithKline Beecham Pharmaceuticals, Discovery Research, Coldharbour Road, The Pinnacles, Harlow, Essex, CM19 5AD, U.K.

Received August 9, 1993\*

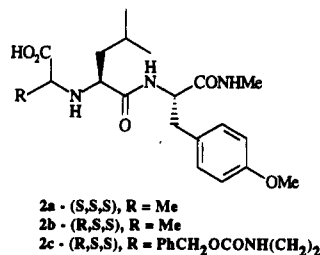
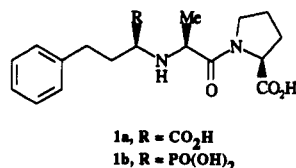
The synthesis of a series of *N*-phosphonoalkyl dipeptides **6** is described. Syntheses were devised that allowed the preparation of single diastereoisomers and the assignment of stereochemistry. The compounds were evaluated *in vitro* for their ability to inhibit the degradation of radiolabeled collagen by purified human lung fibroblast collagenase. Several of the compounds were potent collagenase inhibitors and were at least 10-fold more potent than their corresponding *N*-carboxyalkyl analogues. Activity was lost when the phosphonic acid group  $P(O)(OH)_2$  was replaced by the phosphinic acid groups  $P(O)(H)(OH)$  and  $P(O)(Me)(OH)$ . At the  $P_1$  position, (*R*)- or (*S*)-alkyl groups, especially ethyl and methyl (e.g., **12a,b**, **52a,b**, and **53a,b**), or an (*R*)-phenethyl moiety (**55a**) conferred high potency ( $IC_{50}$  values in the range 0.23–0.47  $\mu M$ ). (*S*)-Stereochemistry was preferred for the  $P_1'$  isobutyl side chain. Structure–activity relationships were also investigated at the  $P_2'$  site, and interestingly, compounds with basic side chains, such as the guanidine **57a**, were equipotent with more lipophilic compounds, such as **52a**. As with other series of collagenase inhibitors, potency was enhanced by introducing bicyclic aromatic  $P_2'$  substituents. The most potent phosphonic acid of the series was the bicyclic aromatic  $P_2'$  tryptophan analogue **59a** ( $IC_{50}$  0.05  $\mu M$ ).

## Introduction

The matrix metalloproteinases (MMPs) are a family of zinc-containing enzymes that are involved in tissue remodeling and are normally produced together with a range of selective, proteinaceous tissue inhibitors of metalloproteinases (TIMPs) to control their proteolytic activities.<sup>1–3</sup> Synthetic inhibitors of matrix metalloproteinases, especially of collagenase (MMP-1)<sup>4</sup> and stromelysin/proteoglycanase (MMP-3),<sup>5</sup> are important targets for drug discovery,<sup>6</sup> since an imbalance between the matrix metalloproteinase synthesis and activation, on the one hand, and the synthesis of the endogenous inhibitors, on the other, is believed to be responsible for the excessive cartilage and bone destruction that occurs in diseases such as rheumatoid arthritis and osteoarthritis. Moreover, low molecular weight (MW) inhibitors are more effective in penetrating cartilage to the sites of connective tissue proteolysis than are high MW inhibitors, such as TIMP, which are excluded because of their size.<sup>7</sup> There are currently no marketed antiarthritic drugs that effectively prevent joint destruction. MMP inhibitors could also be useful in other diseases in which connective tissue degradation is a causative or contributory factor, such as tumor metastasis, corneal ulceration, and periodontal disease.<sup>8</sup>

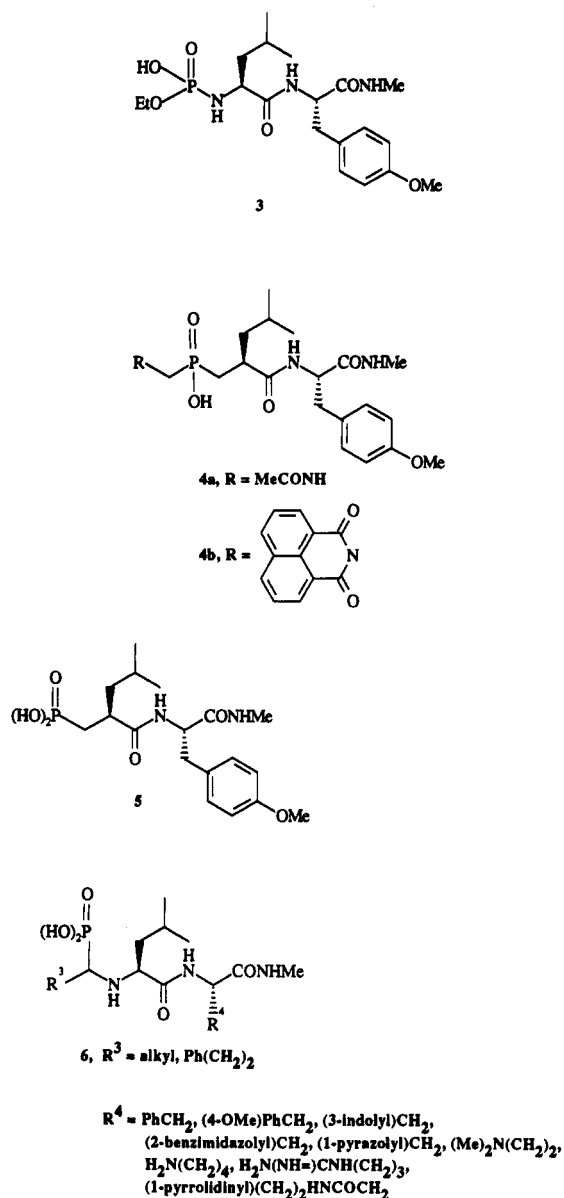
Mammalian collagenase cleaves all three  $\alpha$ -chains of native interstitial collagen at a unique cleavage site (either a Gly-Leu or Gly-Ile bond) to give characteristic one-quarter and three-quarter products.<sup>9</sup> The rational design of low MW collagenase inhibitors, based on the structure of the substrate cleavage site, has recently been reviewed,<sup>8,9</sup> and several potent collagenase inhibitors, with  $IC_{50}$  values in the nanomolar range, have been described. These compounds contain hydroxamic acid<sup>8,10</sup> or  $\beta$ -mercapto-carbonyl<sup>11</sup> ligands that bind, probably in a bidentate manner, to the active site zinc ion in the enzyme. In contrast, substituted *N*-carboxymethyl ligands, which were first used in potent inhibitors of angiotensin-converting

enzyme (ACE)<sup>12,13</sup> such as enalaprilat (**1a**), when used in compounds such as **2** produce less potent collagenase inhibitors ( $IC_{50}$  values in the micromolar range).<sup>8,14,15</sup>



Collagenase inhibitors with phosphorus-containing zinc ligands have also been described. Phosphoramidates such as **3**<sup>16</sup> have  $K_i$  values in the micromolar range against human skin fibroblast collagenase in a synthetic thiopeptolide spectrophotometric assay, but inhibitors of this type are unlikely to be useful drugs because the phosphoramidate moiety has extremely poor *in vivo* stability. Phosphinic acids such as **4a**<sup>17</sup> are relatively weak collagenase inhibitors *in vitro* ( $IC_{50}$  37  $\mu M$ ), but, for reasons that are unclear, potency is enhanced in compounds such as **4b** when phthalimido or naphthalimido groups are incorporated at the  $P_1$  position.<sup>17,18</sup> Positioning a phosphonic acid ligand one methylene spacer unit away from the  $P_1'$  isobutyl side chain leads to inhibitors such as **58a** with micromolar potency. In this paper, we describe the synthesis of a series of *N*-phosphonoalkyl dipeptides **6**, which were evaluated *in vitro* for their ability to inhibit the degradation of radiolabeled collagen by purified human lung fibroblast collagenase.

\* Abstract published in *Advance ACS Abstracts*, December 1, 1993.



## Chemistry

The target molecules in Tables 2 and 3 were prepared according to Schemes 1–6.

The initial synthetic approach to the esters 9a–d (Scheme 1) involved reductive amination of the  $\alpha$ -keto ester 8 with the amino phosphonic esters 7a or 7b<sup>19</sup> and subsequent separation of diastereoisomers to give the single isomers 9a–d. Hydrolysis and coupling with Tyr(Me)-NHMe gave the intermediate phosphonic esters 11a–d, which on treatment with bromotrimethylsilane gave the phosphonic acids 12a–d. Reaction of 7b with the (*R*)-triflate 13 provided an alternative approach to 9b, and subsequently to 10b, and allowed the assignment of the stereochemistry of 9b (*S,S*) and 9d (*S,R*). The stereochemistry of the corresponding enantiomers 9c (*R,R*) and 9a (*R,S*) could then be assigned by NMR analysis. This work allowed the assignment of stereochemistry to the amino phosphonic acids 12a–d.

Scheme 2 shows a different approach: displacement of the triflate group from the dibenzyl phosphonate 15a with *L*-leucine methyl ester (16a) gave a mixture of the esters 17a and 17b in modest yield. Separation of the diastereoisomer 17a and subsequent hydrolysis, coupling with the appropriate P<sub>2</sub>' amino component,<sup>20</sup> and hydrogenol-

ysis gave the phosphonic acids 12a, 26a, and 27a. This preparation of 12a (previously shown to have (*R,S,S*)-stereochemistry) allowed the stereochemistry of 17a and 17b to be assigned as (*R,S*) and (*S,S*), respectively. A more direct approach, involving displacement with the dipeptide 19, gave, after partial separation by chromatography, the dibenzyl ester 20a and the dibenzyl esters 23–25 (inseparable mixtures of two diastereoisomers). Debenzylation provided the amino phosphonic acids 12a and 28–30.

The above routes did not allow the ready variation of P<sub>1</sub> substituents and the preparation of single diastereoisomers. An alternative strategy, involving the thermal addition of a trivalent phosphorus ester to an imine,<sup>21</sup> was therefore investigated. The addition of dimethyl phosphite to the imine prepared from isobutyraldehyde (31) and *L*-leucine methyl ester (16a) (Scheme 3) gave the ester 32, which was elaborated to the amino phosphonic acid 35. This was shown by NMR to be a mixture of four diastereoisomers; presumably, racemization occurs due to the forcing conditions of the phosphite addition reaction. In contrast, the addition of highly reactive dibenzyl trimethylsilyl phosphite,<sup>22</sup> generated *in situ*, to imines at 0 °C (Scheme 4) provided a mild and high-yielding route to single diastereoisomers of the esters 17a–d, 37a,b, 38a,b, and 39a. Subsequent hydrolysis, coupling with the appropriate amino component,<sup>23,24</sup> and hydrogenolysis gave single diastereoisomers of the desired amino phosphonic acids 12a, 52a–d, 53a,b, 54a, 55a,b, 56a,b, and 57a–60a. A comparison of the NMR data for the acids 18a,b of known stereochemistry, and related compounds with different P<sub>1</sub> substituents, allowed the assignment of the stereochemistry and the confirmation of stereochemical purity. The proton  $\alpha$  to the carboxylate group (H<sub>a</sub>) (see Table 1) appears downfield in the NMR spectrum of the (*S,S*)-isomers, relative to the (*R,S*)-isomers. Further confirmation of the stereochemical assignments was provided by an X-ray crystal structure analysis of 50a, which was shown to be the (*R,S,S*)-diastereoisomer.<sup>25</sup>

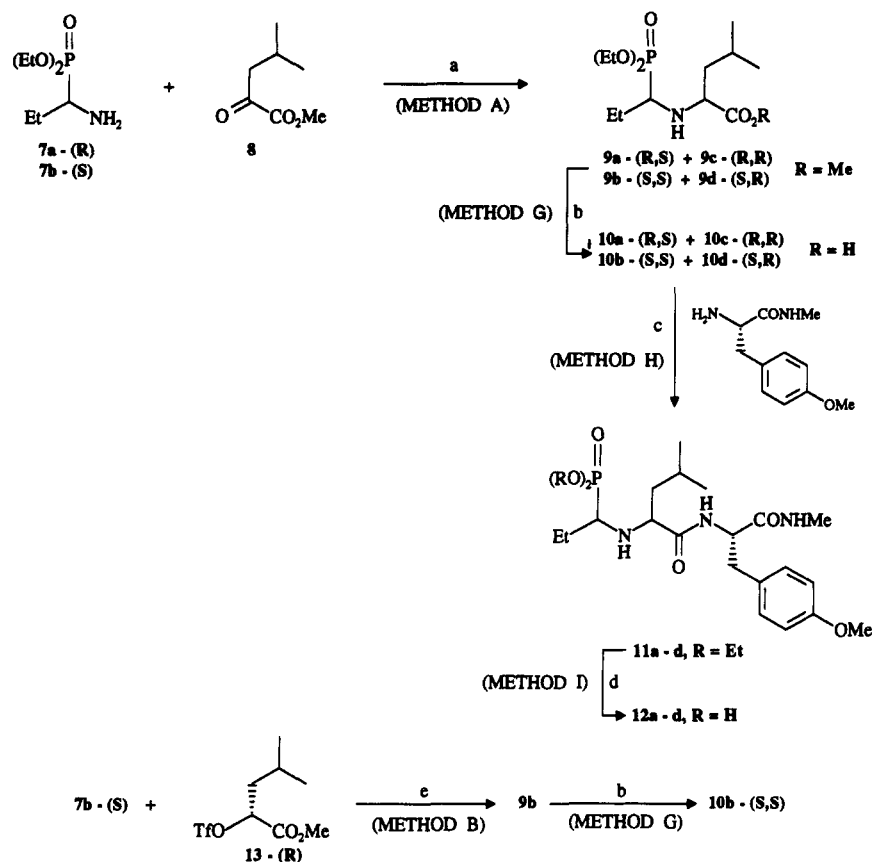
Addition of ethyl (diethoxymethyl)phosphinate (61)<sup>26</sup> to the imine 62 (Scheme 5) gave the ethyl ester 63, which on reductive amination with the  $\alpha$ -keto ester 8 gave the phosphinic ester 66. Similarly, reductive amination of 8 with the phosphinic ester 65, prepared from the acid 64,<sup>27</sup> gave the ester 67. Treatment of 70 with concentrated hydrochloric acid and 71 with bromotrimethylsilane provided the amino phosphonic acids 72 and 73 as mixtures of four diastereoisomers.

The amino carboxylic acids 2a and 2b were prepared, as single diastereoisomers, from the known acids 74a and 74b<sup>14a</sup> (Scheme 6).

## Results and Discussion

**Structure–Activity Relationship (SAR) *in Vitro*.** Compounds were evaluated for their ability to inhibit the degradation of radiolabeled rat skin type I collagen by semipurified human lung fibroblast collagenase. The inhibitory activities (IC<sub>50</sub> values) for the test compounds are shown in Table 3.

**(1) Stereochemical SAR of P<sub>1</sub> and P<sub>1</sub>' Substituents.** (*S*)-Tyr(Me)NHMe and (*S*)-PheNHMe P<sub>2</sub>' residues are known to confer high potency in other series of collagenase inhibitors.<sup>14</sup> Therefore, the initial *N*-phosphonoalkyl dipeptides contained one or the other of these P<sub>2</sub>' moieties

Scheme 1<sup>a</sup>

<sup>a</sup> (a) MeOH, 10% Pd/C, H<sub>2</sub>; (b) NaOH, MeOH, H<sub>2</sub>O; (c) EDC, HOBT, CH<sub>2</sub>Cl<sub>2</sub>; (d) Me<sub>3</sub>SiBr, CH<sub>2</sub>Cl<sub>2</sub>; (e) 1,8-bis(dimethylamino)naphthalene, CH<sub>2</sub>Cl<sub>2</sub>.

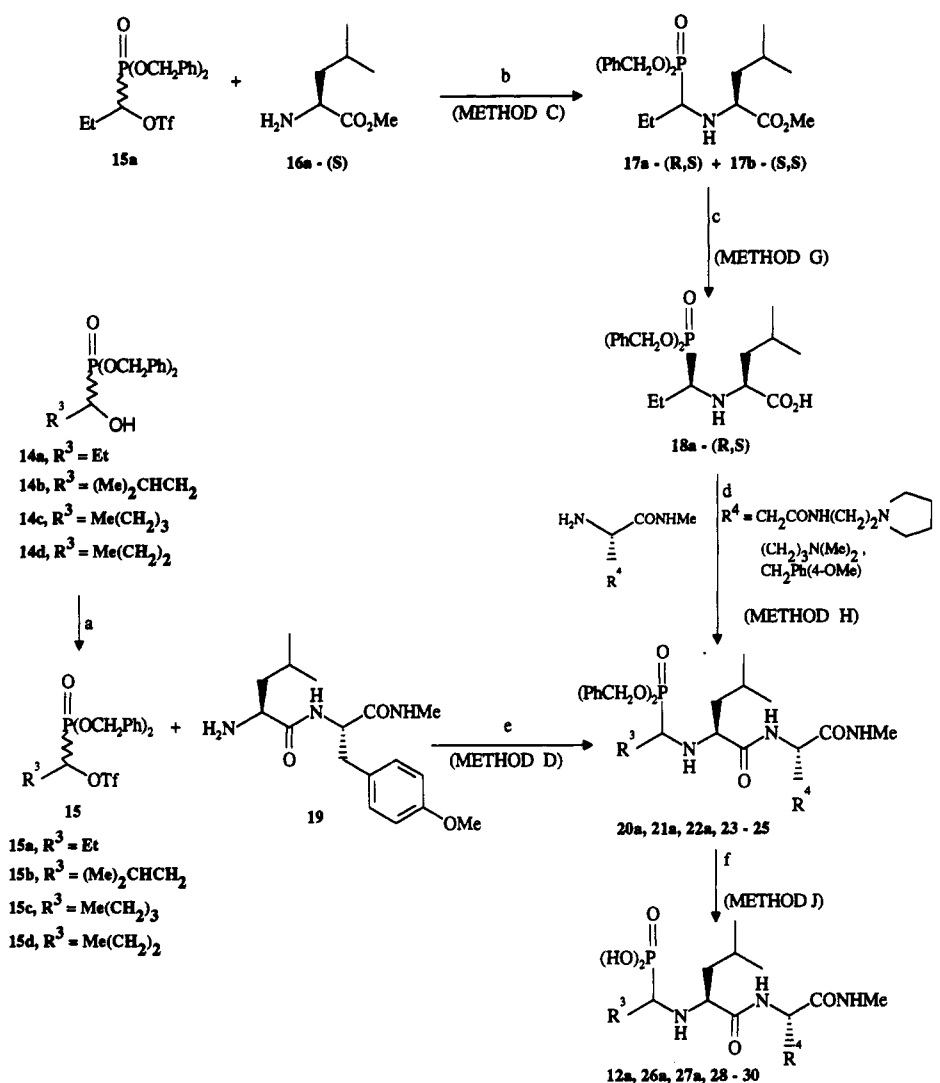
together with an isobutyl substituent at the P<sub>1</sub>' position, and the effect of varying the P<sub>1</sub> substituent was investigated. The preferred stereochemistry at the P<sub>1</sub> and P<sub>1</sub>' centers was unknown, and so, compounds 12a-d, containing an ethyl P<sub>1</sub> moiety and differing in stereochemistry at these centers, were evaluated. Compounds 12a (*R,S,S*), 12b (*S,S,S*) [Tyr(Me)NHMe P<sub>2</sub>' substituent] and 52a (*R,S,S*), 52b (*S,S,S*) [PheNHMe P<sub>2</sub>' substituent] were equipotent, allowing for experimental error (IC<sub>50</sub> values in the range 0.23–0.47 μM); diastereoisomers 12c, 12d, 52c, and 52d (*R*-stereochemistry at the P<sub>1</sub>' position) were greater than 30-fold less potent. Thus, (*S*)-stereochemistry was preferred at the P<sub>1</sub>' center, but the configuration of the P<sub>1</sub> substituent did not markedly influence inhibitory potency.

**(2) SAR of P<sub>1</sub> Substituents.** The effect of varying the size of the P<sub>1</sub> alkyl substituent was also investigated. Compounds 53a and 53b, with methyl P<sub>1</sub> substituents, were equipotent with 12a and 12b (ethyl P<sub>1</sub> substituent), but compounds 28, 29, 30, and 35, with bulkier isobutyl, *n*-butyl, *n*-propyl, and isopropyl substituents, were less potent (IC<sub>50</sub> values 2.9, 41.2, 15.7, and 2.9 μM, respectively). Similarly, compound 54a (isobutyl P<sub>1</sub> substituent) was less potent than its ethyl-substituted analogue 52a. For the alkyl substituents investigated, the inhibitory potency declined in the order: ethyl = methyl > isobutyl = isopropyl > *n*-propyl > *n*-butyl; thus, branched alkyl chains were preferred over their straight-chain congeners, but ethyl or methyl moieties were preferred overall.

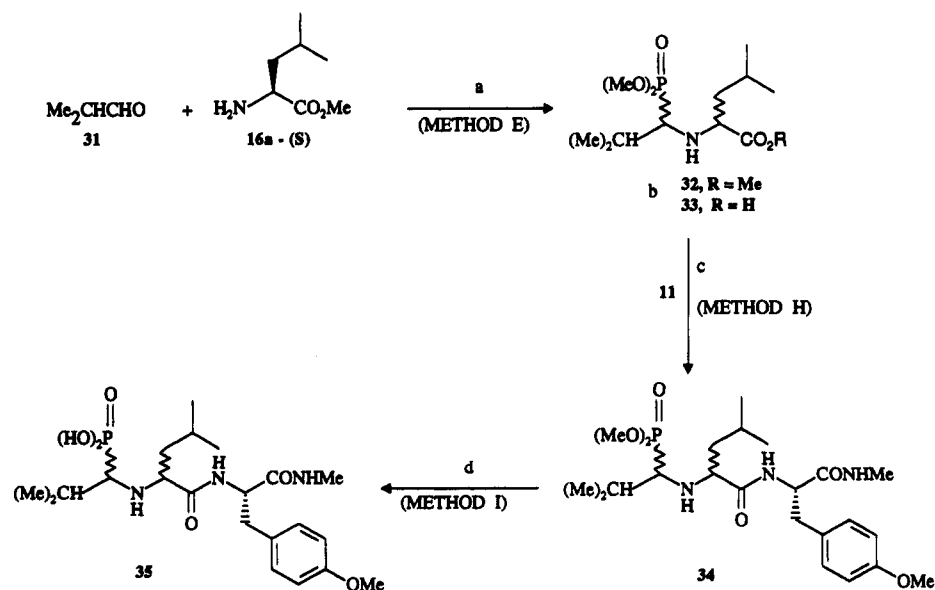
The effect of introducing a phenethyl substituent was also investigated (compounds 55a and 55b), and for the first time, a clear difference in potency between P<sub>1</sub>

diastereoisomers was observed. Compound 55a (*R,S,S*-diastereoisomer; IC<sub>50</sub> 0.4 μM) was approximately equipotent with the original lead compound 52a, but its (*S,S,S*)-diastereoisomer 55b was (4–5)-fold less potent (IC<sub>50</sub> 1.82 μM). It is likely that the relatively poor binding of the (*S,S,S*)-diastereoisomer 55b is a consequence of an unfavorable interaction between the bulky phenethyl side chain and certain residues at the enzyme's active site, and this interaction is absent for the diastereoisomer 55a. This unfavorable interaction is probably not observed with compounds 52b and 53b because of their smaller P<sub>1</sub> substituents. It is unclear why the preferred diastereoisomer 55a (*R,S,S*) has the opposite absolute stereochemistry to the preferred diastereoisomer (*R,S,S*) of the carboxylic acid 2c (CI-1).<sup>14</sup>

**(3) Comparison of Collagenase SAR with ACE SAR.** It was disappointing, but not surprising, that inhibitory potency could not be enhanced by introducing a P<sub>1</sub> phenethyl group (cf. ACE inhibitors<sup>12,13</sup>), and in this respect, the phosphonic acids of the present series mirror the activity profile of the corresponding carboxylic acids 2.<sup>14</sup> One reason for the relatively low potency of compounds 55 is that, unlike ACE, the collagenase enzyme does not appear to possess a S<sub>1</sub> subsite pocket to interact with the P<sub>1</sub> inhibitor side chains; in the natural collagen substrate, the P<sub>1</sub> residue (glycine) lacks an α-side chain. Indeed, it has been shown that a wide variety of substituents is allowed at the P<sub>1</sub> position of collagenase substrates.<sup>9</sup> It is also noteworthy that the preferred stereochemistry (*R*) of the substituted *N*-carboxymethyl ligand in collagenase inhibitors is opposite to that required by ACE.<sup>8,14</sup>

Scheme 2<sup>a</sup>

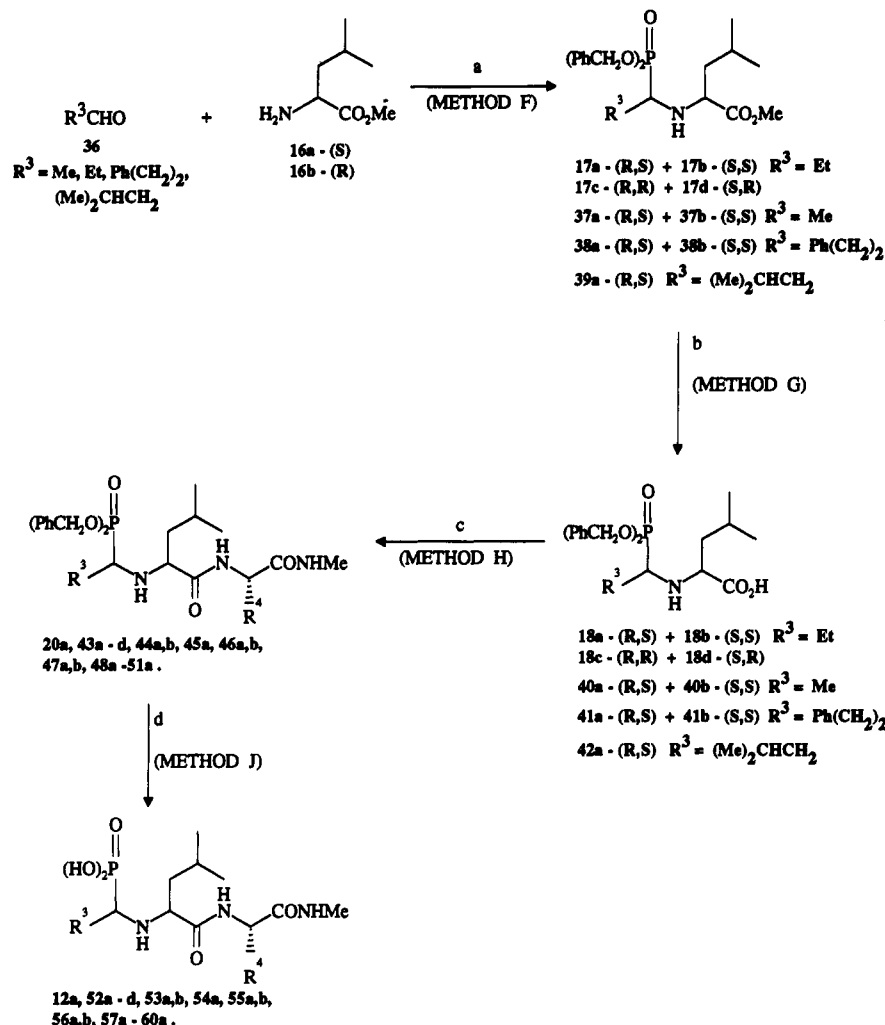
<sup>a</sup> (a) 2,6-Lutidine, (CF<sub>3</sub>SO<sub>2</sub>)<sub>2</sub>O, CH<sub>2</sub>Cl<sub>2</sub>; (b) K<sub>2</sub>CO<sub>3</sub>, MeOH; (c) NaOH, MeOH, H<sub>2</sub>O; (d) EDC, HOBT, CH<sub>2</sub>Cl<sub>2</sub>; (e) 1,8-bis(dimethylamino)naphthalene, CH<sub>2</sub>Cl<sub>2</sub>; (f) EtOH, 10% Pd/C, H<sub>2</sub>.

Scheme 3<sup>a</sup>

<sup>a</sup> (a) Dimethyl phosphite, Δ, toluene; (b) NaOH, MeOH, H<sub>2</sub>O; (c) Tyr(Me)NHMe, EDC, HOBT, CH<sub>2</sub>Cl<sub>2</sub>; (d) Me<sub>3</sub>SiBr, CH<sub>2</sub>Cl<sub>2</sub>.

(4) SAR of P<sub>2</sub>' Substituents. Additional phosphonic acids, 26a, 27a, and 56a-60a, were prepared to investigate

structure-activity relationships at the P<sub>2</sub>' site. It has been reported previously that binding at the P<sub>2</sub>' position involves

Scheme 4<sup>a</sup>

<sup>a</sup> (a) Dibenzyl trimethylsilyl phosphite,  $CH_2Cl_2$ , 0 °C; (b) NaOH, MeOH,  $H_2O$ ; (c)  $H_2NCH(R^4)CONHMe$ , EDC, HOBT,  $CH_2Cl_2$ ; (d) EtOH, 10% Pd/C,  $H_2$ .

Table 1. Proton Chemical Shifts

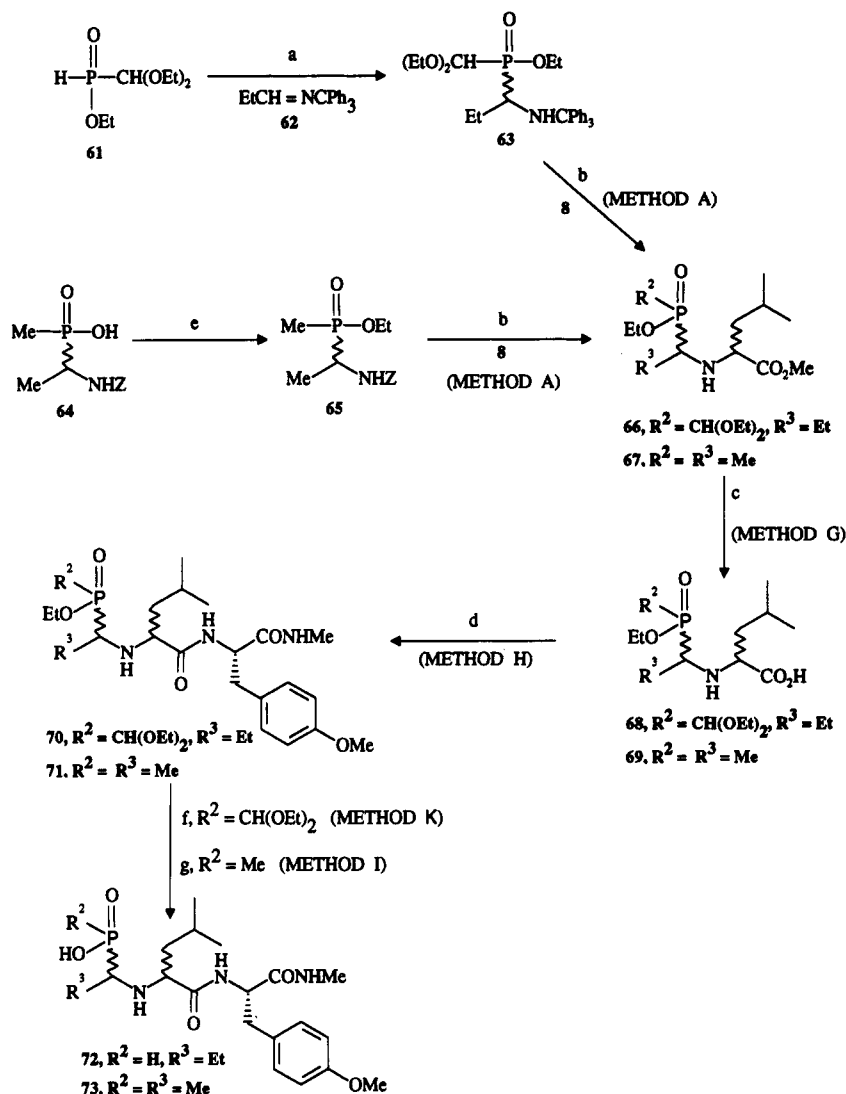
compd	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	chirality	H <sub>4</sub> shift, $\delta$ (CDCl <sub>3</sub> )
10a	EtO	EtO	Et	R,S	3.72
10b	EtO	EtO	Et	S,S	3.42
18a	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Et	R,S	3.75
18b	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Et	S,S	3.40
40a	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Me	R,S	3.65
40b	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Me	S,S	3.40
41a	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Ph(CH <sub>2</sub> ) <sub>2</sub>	R,S	3.75
41b	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Ph(CH <sub>2</sub> ) <sub>2</sub>	S,S	3.37
42a	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	(Me) <sub>2</sub> CHCH <sub>2</sub>	R,S	3.81

an enzyme interaction up to the C $\gamma$  position of the inhibitor side chain and that no upper size limitation exists.<sup>8a</sup> The effect of introducing basic groups at the P<sub>2</sub>' position has not previously been reported in any series of collagenase inhibitors. Compounds 26a, 56a,b, and 57a containing the basic P<sub>2</sub>' side chains Me<sub>2</sub>N(CH<sub>2</sub>)<sub>3</sub>, H<sub>2</sub>N(CH<sub>2</sub>)<sub>4</sub>, and H<sub>2</sub>N(NH=)CNH(CH<sub>2</sub>)<sub>3</sub>, derived from ornithine, lysine, and arginine, respectively, were investigated. Interestingly, the highly basic guanidine 57a ((R,S,S)-stereochemistry) had an IC<sub>50</sub> value of 0.29  $\mu$ M and was equipotent with the corresponding P<sub>2</sub>' phenylalanine analogue 52a.

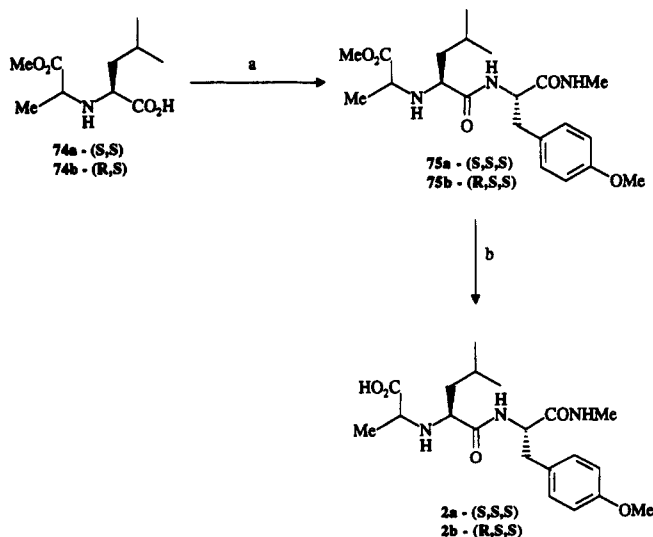
Compounds 26a (R,S,S) and 56b (S,S,S) were only slightly less potent, and 56a (R,S,S) was the least potent with an IC<sub>50</sub> value of 4.3  $\mu$ M. It is not known why diastereoisomers 56a and 56b (lysine P<sub>2</sub>' substituent) showed a 6-fold variation in potency when this was not found for compounds 12a,b and 52a,b with lipophilic P<sub>2</sub>' side chains. Compound 27a, with the bulky extended P<sub>2</sub>' side chain (1-pyrrolidinyl)(CH<sub>2</sub>)<sub>2</sub>NHCOCH<sub>2</sub>, had a still respectable IC<sub>50</sub> value of 1.36  $\mu$ M, confirming the report of no upper size limitation for the P<sub>2</sub>' substituent.<sup>8a</sup>

It is known that aromatic residues, such as tryptophan or (2-naphthyl)alanine, at the P<sub>2</sub>' position increase inhibitory potency.<sup>16,28</sup> In the present series, compounds 58a, 59a, and 60a, with (1-pyrazolyl)CH<sub>2</sub>, (3-indolyl)CH<sub>2</sub>, and (2-benzimidazolyl)CH<sub>2</sub> P<sub>2</sub>' substituents, were compared with the corresponding phenylalanine analogue 52a (all (R,S,S)-stereochemistry). The order of potency was 59a > 60a  $\geq$  52a > 58a; thus, the compound with an indole ring in the P<sub>2</sub>' side chain exhibited increased potency over the phenylalanine analogue 52a, confirming the enhancement of potency with bicyclic aromatic P<sub>2</sub>' substituents reported for other collagenase inhibitor series.<sup>16,28</sup> The P<sub>2</sub>' tryptophan analogue 59a (IC<sub>50</sub> 0.05  $\mu$ M) is the most potent phosphonic acid of the present series.

(5) Comparison of Phosphonic, Phosphinic, and Carboxylic Acid Ligands. It is reported that, for the ACE inhibitor enalaprilat (1a), replacement of the car-

Scheme 5<sup>a</sup>

<sup>a</sup> (a)  $\Delta$ , toluene; (b) MeOH, 10% Pd/C, H<sub>2</sub>; (c) NaOH, MeOH, H<sub>2</sub>O; (d) Tyr(Me)NHMe, EDC, HOBT, CH<sub>2</sub>Cl<sub>2</sub>; (e) EtOH, DCC, DMAP, THF; (f) concentrated HCl,  $\Delta$ ; (g) Me<sub>3</sub>SiBr, CH<sub>2</sub>Cl<sub>2</sub>.

Scheme 6<sup>a</sup>

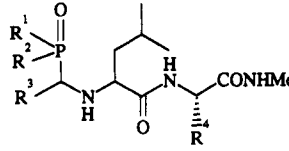
<sup>a</sup> (a) Tyr(Me)NHMe, EDC, HOBT, CH<sub>2</sub>Cl<sub>2</sub>; (b) NaOH, MeOH, H<sub>2</sub>O.

boxylic acid ligand by a phosphonic acid residue (1b) leads to a significant loss of binding affinity.<sup>29</sup> The previously described carboxylic acids 2a (S,S,S) and 2b (R,S,S),<sup>14a</sup>

with methyl P<sub>1</sub> substituents, were also evaluated in our enzyme assay. The diastereoisomer 2b was more potent than 2a (IC<sub>50</sub> values 3.46 and >10  $\mu$ M, respectively), confirming the published patent data.<sup>14a</sup> The corresponding IC<sub>50</sub> values for the analogous phosphonic acids 53a and 54b were 0.52 and 0.26  $\mu$ M, respectively. Thus, in the present inhibitors, a phosphonic acid moiety, as the zinc ligand, generates a more potent collagenase inhibitor than does a carboxylic acid moiety by at least 10-fold. By contrast, compounds 72 and 73, with the phosphonic acid group P(O)(OH)<sub>2</sub> replaced by the phosphinic acid groups P(O)(H)(OH) and P(O)(Me)(OH), were devoid of activity at 100  $\mu$ M. The inactivity of the phosphonic acids 72 and 73 was surprising, as phosphonic acids have been employed previously as zinc ligands in inhibitors of collagenase,<sup>17,18</sup> ACE,<sup>30</sup> and thermolysin.<sup>31</sup> The fact that phosphonic acids are doubly ionized at physiological pH may be an important factor in determining their high potency.<sup>32</sup>

In conclusion, we have described a series of *N*-phosphonoalkyl dipeptide collagenase inhibitors with potent activity *in vitro* against human lung fibroblast collagenase. Several compounds are at least 10-fold more potent than their corresponding *N*-carboxyalkyl analogues. We are presently studying the phosphonic acid collagenase inhibitors *in vivo*, and preliminary studies have indicated

Table 2. Physical Data for Phosphonic Esters



compd	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	chirality	[α] <sub>D</sub> <sup>20</sup> , deg <sup>a</sup>	method <sup>b</sup>	mp, °C	yield, % <sup>c</sup>	formula <sup>d</sup>	FAB-MS, <i>m/e</i> (M + H) <sup>+</sup>
11a	EtO	EtO	Et	(4-MeO)PhCH <sub>2</sub>	<i>R,S,S</i>	-34.8	A,G,H	83-86	81	C <sub>24</sub> H <sub>42</sub> N <sub>3</sub> O <sub>6</sub> P <sup>e</sup>	499/
11b	EtO	EtO	Et	(4-MeO)PhCH <sub>2</sub>	<i>S,S,S</i>	-11.9	A,G,H <sup>f</sup>	oil	89	C <sub>24</sub> H <sub>42</sub> N <sub>3</sub> O <sub>6</sub> P·H <sub>2</sub> O	499/
11c	EtO	EtO	Et	(4-MeO)PhCH <sub>2</sub>	<i>R,R,S</i>	+6.4	A,G,H	oil	77	C <sub>24</sub> H <sub>42</sub> N <sub>3</sub> O <sub>6</sub> P·H <sub>2</sub> O	500
11d	EtO	EtO	Et	(4-MeO)PhCH <sub>2</sub>	<i>S,R,S</i>	+36.4	A,G,H	oil	90	C <sub>24</sub> H <sub>42</sub> N <sub>3</sub> O <sub>6</sub> P·H <sub>2</sub> O	499/
20a	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Et	(4-MeO)PhCH <sub>2</sub>	<i>R,S,S</i>	-35.3	F,G,H <sup>h</sup>	92-93	64	C <sub>34</sub> H <sub>46</sub> N <sub>3</sub> O <sub>6</sub> P	624
21a	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Et	Me <sub>2</sub> N(CH <sub>2</sub> ) <sub>3</sub>	<i>R,S,S</i>		C,G,H	oil	56	C <sub>31</sub> H <sub>46</sub> N <sub>3</sub> O <sub>6</sub> P·0.5H <sub>2</sub> O	599
22a	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Et	(1-pyrrolidinyl)-(CH <sub>2</sub> ) <sub>2</sub> NHCOCH <sub>2</sub>	<i>R,S,S</i>		C,G,H	foam	48	C <sub>34</sub> H <sub>52</sub> N <sub>3</sub> O <sub>6</sub> P·0.5H <sub>2</sub> O	658
23	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	(Me) <sub>2</sub> CHCH <sub>2</sub>	(4-MeO)PhCH <sub>2</sub>	<i>R/S,S,S</i>	mixture	D	oil	25	C <sub>36</sub> H <sub>50</sub> N <sub>3</sub> O <sub>6</sub> P <sup>h</sup>	652
24	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Me(CH <sub>2</sub> ) <sub>3</sub>	(4-MeO)PhCH <sub>2</sub>	<i>R/S,S,S</i>	mixture	D	oil	20	C <sub>36</sub> H <sub>50</sub> N <sub>3</sub> O <sub>6</sub> P <sup>h</sup>	652
25	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Me(CH <sub>2</sub> ) <sub>2</sub>	(4-MeO)PhCH <sub>2</sub>	<i>R/S,S,S</i>	mixture	D	foam	18	C <sub>35</sub> H <sub>48</sub> N <sub>3</sub> O <sub>6</sub> P <sup>h</sup>	637.3293 <sup>i</sup>
34	MeO	MeO	(Me) <sub>2</sub> CH	(4-MeO)PhCH <sub>2</sub>	<i>R/S,R/S,S</i>	mixture	E,G,H	oil	85	C <sub>23</sub> H <sub>40</sub> N <sub>3</sub> O <sub>6</sub> P <sup>h</sup>	486
43a	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Et	PhCH <sub>2</sub>	<i>R,S,S</i>	-40.7	F,G,H	79-81	51	C <sub>33</sub> H <sub>44</sub> N <sub>3</sub> O <sub>6</sub> P	594
43b	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Et	PhCH <sub>2</sub>	<i>S,S,S</i>	-21.8 <sup>m</sup>	F,G,H	oil	56	C <sub>33</sub> H <sub>44</sub> N <sub>3</sub> O <sub>6</sub> P	594
43c	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Et	PhCH <sub>2</sub>	<i>R,R,S</i>	+1.0	F,G,H	113-118	55	C <sub>33</sub> H <sub>44</sub> N <sub>3</sub> O <sub>6</sub> P	594
43d	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Et	PhCH <sub>2</sub>	<i>S,R,S</i>	+38.9	F,G,H	73-76	61	C <sub>33</sub> H <sub>44</sub> N <sub>3</sub> O <sub>6</sub> P	594
44a	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Me	(4-MeO)PhCH <sub>2</sub>	<i>R,S,S</i>	-25.6	F,G,H	123-126	76	C <sub>33</sub> H <sub>44</sub> N <sub>3</sub> O <sub>6</sub> P	610
44b	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Me	(4-MeO)PhCH <sub>2</sub>	<i>S,S,S</i>	-9.4	F,G,H	oil	86	C <sub>33</sub> H <sub>44</sub> N <sub>3</sub> O <sub>6</sub> P <sup>n</sup>	610
45a	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	(Me) <sub>2</sub> CHCH <sub>2</sub>	PhCH <sub>2</sub>	<i>R,S,S</i>	-41.3	F,G,H	oil	86	C <sub>35</sub> H <sub>48</sub> N <sub>3</sub> O <sub>6</sub> P	622
46a	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Ph(CH <sub>2</sub> ) <sub>2</sub>	PhCH <sub>2</sub>	<i>R,S,S</i>	-30.8	F,G,H	foam	66	C <sub>38</sub> H <sub>48</sub> N <sub>3</sub> O <sub>6</sub> P	670
46b	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Ph(CH <sub>2</sub> ) <sub>2</sub>	PhCH <sub>2</sub>	<i>S,S,S</i>		F,G,H	oil	57	C <sub>38</sub> H <sub>48</sub> N <sub>3</sub> O <sub>6</sub> P	670
47a	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Et	PhCH <sub>2</sub> OCONH(CH <sub>2</sub> ) <sub>4</sub>	<i>R,S,S</i>	-34.6 <sup>o</sup>	F,G,H	oil	28	C <sub>38</sub> H <sub>52</sub> N <sub>4</sub> O <sub>7</sub> P·0.25H <sub>2</sub> O	709
47b	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Et	PhCH <sub>2</sub> OCONH(CH <sub>2</sub> ) <sub>4</sub>	<i>S,S,S</i>	-7.3 <sup>p</sup>	F,G,H	oil	59	C <sub>38</sub> H <sub>52</sub> N <sub>4</sub> O <sub>7</sub> P	709
48a	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Et	NO <sub>2</sub> (NH=)CNH(CH <sub>2</sub> ) <sub>3</sub>	<i>R,S,S</i>		F,G,H	oil	51	C <sub>30</sub> H <sub>46</sub> N <sub>7</sub> O <sub>7</sub> P <sup>h</sup>	633
49a	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Et	(1-pyrazolyl)CH <sub>2</sub>	<i>R,S,S</i>		F,G,H	oil	67	C <sub>30</sub> H <sub>46</sub> N <sub>6</sub> O <sub>7</sub> P <sup>h</sup>	584
50a	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Et	(3-indolyl)CH <sub>2</sub>	<i>R,S,S</i> <sup>r</sup>	-47.8	F,G,H	119-121	67	C <sub>35</sub> H <sub>46</sub> N <sub>4</sub> O <sub>6</sub> P	633
51a	PhCH <sub>2</sub> O	PhCH <sub>2</sub> O	Et	(2-benzimidazolyl)CH <sub>2</sub>	<i>R,S,S</i>	-13.8	F,G,H	95-98	65	C <sub>34</sub> H <sub>44</sub> N <sub>6</sub> O <sub>6</sub> P·0.5H <sub>2</sub> O	634
70	EtO	CH(OEt) <sub>2</sub>	Et	(4-MeO)PhCH <sub>2</sub>	<i>R/S,R/S,S</i>	mixture	A,G,H	foam	91	C <sub>27</sub> H <sub>48</sub> N <sub>3</sub> O <sub>6</sub> P·0.25H <sub>2</sub> O	558
71	EtO	Me	Me	(4-MeO)PhCH <sub>2</sub>	<i>R/S,R/S,S</i>	mixture	A,G,H	oil	49	C <sub>22</sub> H <sub>38</sub> N <sub>3</sub> O <sub>6</sub> P <sup>h</sup>	456

<sup>a</sup> (c 1, MeOH) except where indicated. <sup>b</sup> See the Experimental Section for a description of general methods. <sup>c</sup> Percent yield for method D or G. <sup>d</sup> Analyses for C, H, and N within ±0.4% except where characterized by high-resolution mass spectra or as otherwise indicated. <sup>e</sup> N: calcd, 8.41; found, 7.62. <sup>f</sup> EI-MS *m/e* M<sup>+</sup>. <sup>g</sup> Also prepared by methods B, G, and H. <sup>h</sup> Also prepared by methods C, G, and H and method D. <sup>i</sup> Amino component prepared by the procedure described in ref 20. <sup>j</sup> Amino component prepared from BocAspNHMe and 1-(2-aminoethyl)pyrrolidine by EDC coupling. <sup>k</sup> Accurate C, H, and N were not obtained. <sup>l</sup> EI-MS calcd for C<sub>35</sub>H<sub>48</sub>N<sub>3</sub>O<sub>6</sub>P (M<sup>+</sup>) 637.3286. <sup>m</sup> (c 0.2, MeOH). <sup>n</sup> N: calcd, 6.89; found, 7.30. <sup>o</sup> (c 0.4, MeOH). <sup>p</sup> (c 0.5, MeOH). <sup>q</sup> Amino component prepared by the procedure described in ref 23. <sup>r</sup> Structure confirmed by X-ray analysis. <sup>s</sup> Amino component prepared by the procedure described in ref 24.

that compounds from this series are bioavailable in rats following oral administration.<sup>33</sup> Oral absorption of the amino phosphonic acids is likely to be aided by the compounds' good aqueous solubility and relatively low MW.

## Experimental Section

Melting points were determined on a Buchi 510 machine and are uncorrected. Proton magnetic resonance (<sup>1</sup>H NMR) spectra were recorded on a Bruker AM400, a Bruker AC250, a JEOL GX270, or a Varian EM360A spectrometer using Me<sub>4</sub>Si as an internal standard. Mass spectra (MS) data were obtained on a JEOL DX303 spectrometer using electrical or chemical (NH<sub>3</sub>) ionization procedures or fast atom bombardment (FAB) using glycerol as the matrix. Elemental analyses were within 0.4% of the theoretical values unless otherwise stated. All evaporations of solvents were carried out under reduced pressure. Unless otherwise stated, organic solutions were dried over Na<sub>2</sub>SO<sub>4</sub> and for column chromatography, the silica gel used was Merck Kieselgel 60. Brine refers to saturated sodium chloride solution.

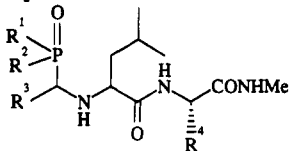
**Collagenase Inhibitor Assay.** The test is essentially as described by Cawston and Barrett.<sup>34</sup> The test compounds were dissolved in MeOH by sonication and then were serially diluted as necessary. Trypsin-activated, semipurified human collagenase was obtained from culture supernatants of the WI-38 human lung fibroblast cell line and was added together with diluent/buffers. Assay tubes were cooled to 4 °C, and either <sup>3</sup>H- or <sup>14</sup>C-acetylated rat skin type I collagen (100 μg/tube) was added. The choice of radiolabel did not alter the ability of collagenase to degrade the collagen substrate. Following incubation of the assay

tubes at 37 °C for 1000 min, the tubes were centrifuged at 12 000 rpm for 15 min at 4 °C. Undigested radiolabeled collagen was pelleted, while digested radiolabeled collagen fibrils were found as soluble peptides in the supernatant. Aliquots of the supernatant were taken for liquid scintillation counting. A collagenase standard curve demonstrated a linear relationship between enzyme concentration and collagen degradation up to 70% of the total collagen degraded. For inhibitor assays, an amount of enzyme was added such that 70% of the total collagen would be degraded during the course of the assay. Different preparations of collagenase and collagen were tested to ensure assay comparability. The activity of the test compounds (IC<sub>50</sub>) is expressed as that concentration of compound that inhibited a known concentration of enzyme by 50%.

**Method A. *N*-[(*R*)-1-(Diethoxyphosphinyl)propyl]-(*S*)-leucine, Methyl Ester (9a).** A solution of 7a<sup>19</sup> (5.63 g, 29 mmol) and methyl 4-methyl-2-oxopentanoate (8) (12.47 g, 87 mmol) in EtOH (200 mL) was hydrogenated at room temperature and atmospheric pressure over 10% Pd/C. After 72 h, the catalyst was filtered off and the solvent evaporated. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub>, washed successively with saturated NaHCO<sub>3</sub>, 5% citric acid, and brine, and then dried, and the solvent was evaporated. The residue was purified by column chromatography on silica gel, eluting with ether to give 9a (1.72 g, 18%) as a colorless oil: [α]<sub>D</sub><sup>20</sup> -32.4° (c 0.2, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.94 (6 H, t, *J* = 6 Hz), 1.06 (3 H, t, *J* = 5 Hz), 1.32 (3 H, t, *J* = 5 Hz), 1.34 (3 H, t, *J* = 5 Hz), 1.40-2.00 (6 H, m), 2.68 (1 H, m), 3.70 (3 H, s), 3.78 (1 H, t, *J* = 5 Hz), 4.13 (4 H, m); MS *m/e* 323 (M)<sup>+</sup>.

Further elution gave 9c (1.26 g, 13%) as a colorless oil: [α]<sub>D</sub><sup>20</sup> +6.4° (c 0.4, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.90 (6 H, dd, *J* = 3,

Table 3. Physical Data and Inhibitory Potency of Phosphonic Acids and Related Compounds



compd	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	chirality	[α] <sub>D</sub> <sup>20</sup> , deg <sup>a</sup>	method <sup>b</sup>	mp, °C	yield, %	formula <sup>c</sup>	FAB-MS, <i>m/e</i> (M + H) <sup>+</sup>	IC <sub>50</sub> , μM (n) <sup>d</sup>
12a	HO	HO	Et	(4-MeO)PhCH <sub>2</sub>	<i>R,S,S</i>	-12.7	I	162–165	40	C <sub>20</sub> H <sub>34</sub> N <sub>3</sub> O <sub>6</sub> P · H <sub>2</sub> O	444	0.47 ± 0.26 (7)
12b	HO	HO	Et	(4-MeO)PhCH <sub>2</sub>	<i>S,S,S</i>	+4.1	I	154–157	52	C <sub>20</sub> H <sub>34</sub> N <sub>3</sub> O <sub>6</sub> P	444.2270 <sup>e</sup>	0.30 ± 0.05 (6)
12c	HO	HO	Et	(4-MeO)PhCH <sub>2</sub>	<i>R,R,S</i>	-8.4	I	141–145	56	C <sub>20</sub> H <sub>34</sub> N <sub>3</sub> O <sub>6</sub> P	444.2219 <sup>e</sup>	15.6 ± 12.7 (3)
12d	HO	HO	Et	(4-MeO)PhCH <sub>2</sub>	<i>S,R,S</i>	+4.3	I	219–223	70	C <sub>20</sub> H <sub>34</sub> N <sub>3</sub> O <sub>6</sub> P	444.2267 <sup>e</sup>	39.3 ± 34.4 (2)
26a	HO	HO	Et	Me <sub>2</sub> N(CH <sub>2</sub> ) <sub>3</sub>	<i>R,S,S</i>	-25.8 <sup>f</sup>	J	88–90	74	C <sub>17</sub> H <sub>37</sub> N <sub>4</sub> O <sub>6</sub> P	409.2605 <sup>g</sup>	0.49
27a	HO	HO	Et	(1-pyrrolidinyl)-(CH <sub>2</sub> ) <sub>2</sub> NHCOCH <sub>2</sub>	<i>R,S,S</i>	-19.2	J	foam	95	C <sub>20</sub> H <sub>34</sub> N <sub>3</sub> O <sub>6</sub> P · 1.5H <sub>2</sub> O <sup>h</sup>	478.2722 <sup>i</sup>	1.36
28	HO	HO	(Me) <sub>2</sub> CHCH <sub>2</sub>	(4-MeO)PhCH <sub>2</sub>	<i>R/S,S,S</i>	mixture	J	foam	91	C <sub>22</sub> H <sub>38</sub> N <sub>3</sub> O <sub>6</sub> P	472.2561 <sup>j</sup>	2.90 ± 1.42 (3)
29	HO	HO	Me(CH <sub>2</sub> ) <sub>3</sub>	(4-MeO)PhCH <sub>2</sub>	<i>R/S,S,S</i>	mixture	J	foam	95	C <sub>22</sub> H <sub>38</sub> N <sub>3</sub> O <sub>6</sub> P	472.2527 <sup>j</sup>	41.2
30	HO	HO	Me(CH <sub>2</sub> ) <sub>2</sub>	(4-MeO)PhCH <sub>2</sub>	<i>R/S,S,S</i>	mixture	J	foam	88	C <sub>21</sub> H <sub>36</sub> N <sub>3</sub> O <sub>6</sub> P	480.2260 <sup>k</sup>	15.7
35	HO	HO	(Me) <sub>2</sub> CH	(4-MeO)PhCH <sub>2</sub>	<i>R/S,R/S,S</i>	mixture	I	foam	76	C <sub>21</sub> H <sub>36</sub> N <sub>3</sub> O <sub>6</sub> P	376.2603 <sup>l</sup>	2.90 ± 0.41 (2)
52a	HO	HO	Et	PhCH <sub>2</sub>	<i>R,S,S</i>	-21.8	J	172–174	83	C <sub>19</sub> H <sub>32</sub> N <sub>3</sub> O <sub>6</sub> P · H <sub>2</sub> O <sup>h</sup>	414.2169 <sup>m</sup>	0.23 ± 0.05 (12)
52b	HO	HO	Et	PhCH <sub>2</sub>	<i>S,S,S</i>	-3.1	J	143–145	88	C <sub>19</sub> H <sub>32</sub> N <sub>3</sub> O <sub>6</sub> P · 2H <sub>2</sub> O <sup>h</sup>	414.2175 <sup>m</sup>	0.24 ± 0.08 (3)
52c	HO	HO	Et	PhCH <sub>2</sub>	<i>R,R,S</i>	-26.8	J	151–154	97	C <sub>19</sub> H <sub>32</sub> N <sub>3</sub> O <sub>6</sub> P · H <sub>2</sub> O <sup>h</sup>	414.2167 <sup>m</sup>	>10
52d	HO	HO	Et	PhCH <sub>2</sub>	<i>S,R,S</i>	-1.1	J	152–156	89	C <sub>19</sub> H <sub>32</sub> N <sub>3</sub> O <sub>6</sub> P · 0.5H <sub>2</sub> O <sup>h</sup>	414.2169 <sup>m</sup>	>10
53a	HO	HO	Me	(4-MeO)PhCH <sub>2</sub>	<i>R,S,S</i>	-9.0	J	166–168	96	C <sub>19</sub> H <sub>32</sub> N <sub>3</sub> O <sub>6</sub> P · H <sub>2</sub> O <sup>h</sup>	430.2111 <sup>n</sup>	0.52 ± 0.25 (2)
53b	HO	HO	Me	(4-MeO)PhCH <sub>2</sub>	<i>S,S,S</i>	+1.6	J	162–164	91	C <sub>19</sub> H <sub>32</sub> N <sub>3</sub> O <sub>6</sub> P · H <sub>2</sub> O <sup>h</sup>	430.2086 <sup>n</sup>	0.26
54a	HO	HO	(Me) <sub>2</sub> CHCH <sub>2</sub>	PhCH <sub>2</sub>	<i>R,S,S</i>	-29.8	J	149–152	73	C <sub>21</sub> H <sub>38</sub> N <sub>3</sub> O <sub>6</sub> P · 0.25H <sub>2</sub> O <sup>h</sup>	442	1.56
55a	HO	HO	Ph(CH <sub>2</sub> ) <sub>2</sub>	PhCH <sub>2</sub>	<i>R,S,S</i>	-26.2	J	147–150	95	C <sub>26</sub> H <sub>38</sub> N <sub>3</sub> O <sub>6</sub> P · 0.5H <sub>2</sub> O <sup>h</sup>	490	0.40 ± 0.14 (5)
55b	HO	HO	Ph(CH <sub>2</sub> ) <sub>2</sub>	PhCH <sub>2</sub>	<i>S,S,S</i>	-13.7	J	141–144	97	C <sub>26</sub> H <sub>38</sub> N <sub>3</sub> O <sub>6</sub> P · 2H <sub>2</sub> O <sup>h</sup>	512.2366 <sup>o</sup>	1.82 ± 0.42 (2)
56a	HO	HO	Et	H <sub>2</sub> N(CH <sub>2</sub> ) <sub>4</sub>	<i>R,S,S</i>	-37.9	J	219–222	97	C <sub>16</sub> H <sub>32</sub> N <sub>4</sub> O <sub>6</sub> P · 0.5H <sub>2</sub> O <sup>h</sup>	395.2429 <sup>p</sup>	4.32 ± 2.51 (4)
56b	HO	HO	Et	H <sub>2</sub> N(CH <sub>2</sub> ) <sub>4</sub>	<i>S,S,S</i>	-46.9	J	161–165	95	C <sub>16</sub> H <sub>32</sub> N <sub>4</sub> O <sub>6</sub> P · 2H <sub>2</sub> O <sup>h</sup>	395.2433 <sup>p</sup>	0.74 ± 0.20 (4)
57a	HO	HO	Et	H <sub>2</sub> N(NH=)CNH(CH <sub>2</sub> ) <sub>3</sub>	<i>R,S,S</i>	-3.9	J	foam	74	C <sub>16</sub> H <sub>32</sub> N <sub>6</sub> O <sub>6</sub> P · CH <sub>3</sub> CO <sub>2</sub> H	423.2466 <sup>q</sup>	0.29 ± 0.06 (6)
58a	HO	HO	Et	(1-pyrazolyl)CH <sub>2</sub>	<i>R,S,S</i>	-60.1 <sup>r</sup>	J	156–159	83	C <sub>16</sub> H <sub>30</sub> N <sub>6</sub> O <sub>6</sub> P	404.2074 <sup>s</sup>	0.76
59a	HO	HO	Et	(3-indolyl)CH <sub>2</sub>	<i>R,S,S</i>	-45.0	J	foam	62	C <sub>21</sub> H <sub>33</sub> N <sub>4</sub> O <sub>6</sub> P · 1.5H <sub>2</sub> O <sup>h</sup>	453.2296 <sup>t</sup>	0.05 ± 0.03 (2)
60a	HO	HO	Et	(2-benzimidazolyl)CH <sub>2</sub>	<i>R,S,S</i>	+28.4	J	foam	91	C <sub>20</sub> H <sub>32</sub> N <sub>5</sub> O <sub>6</sub> P	454.2227 <sup>u</sup>	0.15
72	HO	H	Et	(4-MeO)PhCH <sub>2</sub>	<i>R/S,R/S,S</i>	mixture	K	foam	95	C <sub>20</sub> H <sub>34</sub> N <sub>3</sub> O <sub>6</sub> P · HCl <sup>h</sup>	428.2316 <sup>v</sup>	>100
73	HO	Me	Me	(4-MeO)PhCH <sub>2</sub>	<i>R/S,R/S,S</i>	mixture	I	foam	58	C <sub>20</sub> H <sub>34</sub> N <sub>3</sub> O <sub>6</sub> P	428.2310 <sup>w</sup>	>100
2a	x	x	x	x	<i>S,S,S</i>	+7.1	w	195–197	61	C <sub>20</sub> H <sub>31</sub> N <sub>3</sub> O <sub>5</sub> · 0.2H <sub>2</sub> O	394	>10
2b	x	x	x	x	<i>R,S,S</i>	+6.6	w	199–201	71	C <sub>20</sub> H <sub>31</sub> N <sub>3</sub> O <sub>5</sub> · 0.2H <sub>2</sub> O	394	3.46 ± 1.71 (4)
2c	x	x	x	x	<i>R,S,S</i>							0.35

<sup>a</sup> (c 1, MeOH) except where indicated. <sup>b</sup> See the Experimental Section for a description of general methods. <sup>c</sup> Analyses for C, H, and N within ±0.4% except where characterized by high-resolution FAB-MS. <sup>d</sup> Values are mean ± SEM of the number of experiments indicated in parentheses. <sup>e</sup> Calcd for C<sub>20</sub>H<sub>35</sub>N<sub>3</sub>O<sub>6</sub>P (M + H)<sup>+</sup> 444.2263. <sup>f</sup> (c 0.4, MeOH). <sup>g</sup> Calcd for C<sub>17</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub>P (M + H)<sup>+</sup> 409.2580. <sup>h</sup> Characterized by elemental analysis as well as by high-resolution FAB-MS. <sup>i</sup> Calcd for C<sub>20</sub>H<sub>41</sub>N<sub>3</sub>O<sub>6</sub>P (M + H)<sup>+</sup> 478.2794. <sup>j</sup> Calcd for C<sub>22</sub>H<sub>39</sub>N<sub>3</sub>O<sub>6</sub>P (M + H)<sup>+</sup> 472.2576. <sup>k</sup> Calcd for C<sub>21</sub>H<sub>36</sub>N<sub>3</sub>O<sub>6</sub>PNa (M + Na)<sup>+</sup> 480.2239. <sup>l</sup> Calcd for C<sub>21</sub>H<sub>37</sub>N<sub>3</sub>O<sub>6</sub>P (M + H - H<sub>2</sub>PO<sub>3</sub>)<sup>+</sup> 376.2600. <sup>m</sup> Calcd for C<sub>19</sub>H<sub>33</sub>N<sub>3</sub>O<sub>6</sub>P (M + H)<sup>+</sup> 414.2157. <sup>n</sup> Calcd for C<sub>19</sub>H<sub>33</sub>N<sub>3</sub>O<sub>6</sub>P (M + H)<sup>+</sup> 430.2107. <sup>o</sup> Calcd for C<sub>25</sub>H<sub>36</sub>N<sub>3</sub>O<sub>6</sub>PNa (M + Na)<sup>+</sup> 512.2290. <sup>p</sup> Calcd for C<sub>16</sub>H<sub>36</sub>N<sub>4</sub>O<sub>6</sub>P (M + H)<sup>+</sup> 395.2423. <sup>q</sup> Calcd for C<sub>16</sub>H<sub>36</sub>N<sub>6</sub>O<sub>6</sub>P (M + H)<sup>+</sup> 423.2485. <sup>r</sup> (c 0.3, MeOH). <sup>s</sup> Calcd for C<sub>16</sub>H<sub>31</sub>N<sub>5</sub>O<sub>6</sub>P (M + H)<sup>+</sup> 404.2063. <sup>t</sup> Calcd for C<sub>21</sub>H<sub>34</sub>N<sub>4</sub>O<sub>6</sub>P (M + H)<sup>+</sup> 453.2267. <sup>u</sup> Calcd for C<sub>20</sub>H<sub>33</sub>N<sub>5</sub>O<sub>6</sub>P (M + H)<sup>+</sup> 454.2220. <sup>v</sup> Calcd for C<sub>20</sub>H<sub>35</sub>N<sub>3</sub>O<sub>6</sub>P (M + H)<sup>+</sup> 428.2314. <sup>w</sup> See the Experimental Section. <sup>x</sup> For the carboxylic acids, see text and Scheme 4 for structures.

5 Hz), 1.05 (3 H, t, *J* = 5 Hz), 1.32 (3 H, t, *J* = 5 Hz), 1.34 (3 H, t, *J* = 5 Hz), 1.40–1.95 (6 H, m), 2.76 (1 H, m), 3.48 (1 H, t, *J* = 5 Hz), 3.72 (3 H, s), 4.12 (4 H, m); MS *m/e* 323 (M)<sup>+</sup>.

Compounds 9b and 9d were prepared from 7b<sup>19</sup> by the same procedure. Column chromatography, as above, gave 9d: [α]<sub>D</sub><sup>22</sup> +27.3° (c 1, MeOH); <sup>1</sup>H NMR and MS data were identical to 9a. Further elution gave 9b: [α]<sub>D</sub><sup>22</sup> +1.0° (c 1, MeOH); <sup>1</sup>H NMR and MS data were identical to 9c.

Compound 66 was obtained by method A from 63<sup>26</sup> as a mixture of four stereoisomers after column chromatography with EtOAc as eluent: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.92 (12 H, m), 1.03 (6 H, m), 1.20–1.37 (18 H, m), 1.40–2.00 (12 H, m), 2.85 (2 H, m), 3.50–3.97 (10 H, m), 3.78 (6 H, s), 4.21 (4 H, m), 4.96 (2 H, dd, *J* = 7, 15 Hz); MS *m/e* 382 (M + H)<sup>+</sup>.

Similarly, 67 was prepared from 65 as a mixture of four stereoisomers after column chromatography with EtOAc/MeOH (20:1) as eluent: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.91 (12 H, m), 1.17–1.40 (22 H, m), 1.75 (4 H, m), 2.93 (2 H, m), 3.46 (2 H, m), 3.72 (6 H, m), 4.10 (4 H, m); FAB-MS *m/e* 280 (M + H)<sup>+</sup>.

**Method B. *N*-[(*S*)-1-(Diethoxyphosphinyl)propyl]-(*S*)-leucine, Methyl Ester (9b).** The triflate 13 (1.33 g, 4.77 mmol) was prepared by the addition of trifluoromethanesulfonic anhydride (0.92 mL, 5.47 mmol) to methyl L-2-hydroxy-4-methylpentanoate (0.69 g, 4.77 mmol) and 2,6-lutidine (0.68 mL) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) at -60 °C. After the temperature had risen to 0 °C, a solution of 7b<sup>19</sup> (0.93 g, 4.77 mmol) in CHCl<sub>3</sub> (10 mL) was added followed by 1,8-bis(dimethylamino)naphthalene (1.02 g, 4.77 mmol). The mixture was stirred at room temperature for

4 days, filtered, washed with 10% citric acid and water, and then dried with  $\text{MgSO}_4$ . The solvent was evaporated and the residue purified by column chromatography, eluting with a gradient of 0–2%  $\text{MeOH}/\text{EtOAc}$ , to give **9b** (0.69 g, 47%) as a pale yellow oil:  $[\alpha]_D^{25} +0.3^\circ$  (c 1.1,  $\text{MeOH}$ );  $^1\text{H}$  NMR and MS data were identical to **9b** prepared by method A.

**1-[Bis(benzyloxy)phosphinyl]propanol (14a).** Dibenzyl phosphite (36.9 g, 141 mmol) and propionaldehyde (8.2 g, 141 mmol) were stirred together at room temperature, and alumina (70 g) was added in one portion, following a literature procedure.<sup>35</sup> After 24 h,  $\text{CHCl}_3$  was added, the mixture was filtered, and the solvent was evaporated. The residue was purified by column chromatography, eluting with a gradient of 0–5%  $\text{MeOH}/\text{Et}_2\text{O}$ , to give **14a** (27.8 g, 64%) as white needles: mp 81–82 °C ( $\text{Et}_2\text{O}/\text{pentane}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.04 (3 H, t,  $J = 7$  Hz), 1.60–1.95 (2 H, m), 3.81 (1 H, m), 5.07 (4 H, m), 7.35 (10 H, s). Anal. ( $\text{C}_{17}\text{H}_{21}\text{O}_4\text{P}$ ) C, H.

Compounds **14b–d** were prepared by the same procedure from dibenzyl phosphite and the appropriate aldehyde.

**Compound 14b:** mp 71–73 °C ( $\text{Et}_2\text{O}/\text{pentane}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.85 (3 H, d,  $J = 6$  Hz), 0.92 (3 H, d,  $J = 6$  Hz), 1.47 (1 H, m), 1.60–1.78 (2 H, m), 1.90 (1 H, br s), 3.97 (1 H, m), 5.08 (4 H, m), 7.35 (10 H, s). Anal. ( $\text{C}_{15}\text{H}_{24}\text{O}_4\text{P}$ ) C, H.

**Compound 14c:** colorless oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.86 (3 H, t,  $J = 6$  Hz), 1.20–1.80 (6 H, m), 2.60 (1 H, br s), 3.87 (2 H, m), 5.06 (4 H, m), 7.35 (10 H, s).

**Compound 14d:** mp 61–65 °C ( $\text{Et}_2\text{O}/\text{pentane}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.90 (3 H, d,  $J = 7$  Hz), 1.25–1.80 (4 H, m), 2.40 (1 H, br s), 3.90 (1 H, m), 5.08 (4 H, m), 7.35 (10 H, s). Anal. ( $\text{C}_{18}\text{H}_{23}\text{O}_4\text{P}$ ) C, H.

**Method C. *N*-[(*R*)-1-(Bis(benzyloxy)phosphinyl)propyl]-(*S*)-leucine, Methyl Ester (17a).** The triflate **15a** (1.35 g, 3 mmol), prepared by a literature procedure<sup>36</sup> from the alcohol **14a**, in  $\text{MeOH}$  (2 mL) was added to **16a** (0.43 g, 3 mmol) and  $\text{K}_2\text{CO}_3$  (1 g) in  $\text{MeOH}$  (3 mL), and the reaction mixture was heated at 50 °C for 4 h. After the mixture was stirred at room temperature for 18 h, the solvent was evaporated,  $\text{CHCl}_3$  was added, and the mixture was filtered. The solvent was evaporated and the residue purified by column chromatography, eluting with  $\text{Et}_2\text{O}/\text{pentane}$  (1:1), to give **17a** (0.15 g, 11%) as a colorless oil:  $[\alpha]_D^{25} -32.1^\circ$  (c 0.9,  $\text{MeOH}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.86 (3 H, d,  $J = 6$  Hz), 0.91 (3 H, d,  $J = 6$  Hz), 1.02 (3 H, t,  $J = 7$  Hz), 1.42 (2 H, m), 1.50–1.95 (4 H, m), 2.74 (1 H, m), 3.70 (3 H, s), 3.78 (1 H, t,  $J = 7$  Hz), 5.03 (4 H, m), 7.37 (10 H, s); MS  $m/e$  448 ( $\text{M} + \text{H}$ )<sup>+</sup>. Anal. ( $\text{C}_{24}\text{H}_{34}\text{NO}_5\text{P}$ ) C, H, N.

Further elution gave **17b** (0.14 g, 10%) as a colorless oil:  $[\alpha]_D^{25} -1.1^\circ$  (c 1,  $\text{MeOH}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.88 (3 H, d,  $J = 3$  Hz), 0.90 (3 H, d,  $J = 3$  Hz), 1.00 (3 H, t,  $J = 7$  Hz), 1.44 (2 H, t,  $J = 6$  Hz), 1.50–1.95 (4 H, m), 2.80 (1 H, m), 3.49 (1 H, t,  $J = 7$  Hz), 3.66 (3 H, s), 5.03 (4 H, m), 7.35 (10 H, s); MS  $m/e$  448 ( $\text{M} + \text{H}$ )<sup>+</sup>. Anal. ( $\text{C}_{24}\text{H}_{34}\text{NO}_5\text{P}$ ) C, H, N.

**Method D. *N*-[*N*-(*R*)-1-(Bis(benzyloxy)phosphinyl)propyl]-(*S*)-leucyl]-*O*-methyl-(*S*)-tyrosine *N*-Methyl Amide (20a).** The triflate **15a**<sup>36</sup> (0.42 g, 0.93 mmol), 1,8-bis(dimethylamino)naphthalene (0.20 g, 0.93 mmol), and **19** (0.3 g, 0.93 mmol) in  $\text{MeOH}$  (1 mL) were stirred at room temperature for 10 days, with the exclusion of light. The solvent was evaporated, and the residue was dissolved in  $\text{CHCl}_3$ , washed with 10% citric acid and water, and then dried. The solvent was evaporated and the residue partially purified by column chromatography, eluting with a gradient of 0–5%  $\text{MeOH}/\text{CHCl}_3$ . Further chromatography, eluting with a gradient of 0–5%  $\text{MeOH}/\text{EtOAc}$ , gave a mixture of **20a** and the corresponding (*S,S,S*)-diastereoisomer (0.23 g, 41%).

Further elution gave pure **20a** (0.02 g, 4%) as a white crystalline solid: mp 92–93 °C ( $\text{EtOAc}$ );  $[\alpha]_D^{25} -35.3^\circ$  (c 1,  $\text{MeOH}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.80 (3 H, d,  $J = 3$  Hz), 0.83 (3 H, d,  $J = 3$  Hz), 0.96 (3 H, t,  $J = 6$  Hz), 1.13–1.32 (2 H, m), 1.40–1.60 (2 H, m), 1.60–1.86 (2 H, m), 2.58 (1 H, m), 2.72 (3 H, d,  $J = 5$  Hz), 2.99 (2 H, m), 3.60 (1 H, m), 3.74 (3 H, s), 4.50 (1 H, m), 4.97 (4 H, m), 6.08 (1 H, br s), 6.79 (2 H, d,  $J = 7$  Hz), 7.10 (2 H, d,  $J = 7$  Hz), 7.33 (11 H, s); MS  $m/e$  624 ( $\text{M} + \text{H}$ )<sup>+</sup>. Anal. ( $\text{C}_{34}\text{H}_{46}\text{N}_3\text{O}_6\text{P}$ ) C, H, N.

Compounds **23–25** were prepared, as mixtures of two diastereoisomers, from **19** and the corresponding alcohol by the same procedure.

**Ethyl [1-((Benzyloxycarbonyl)amino)ethyl]-methylphosphinate (65).** *N,N*-Dicyclohexylcarbodiimide (1.08 g, 5.2 mmol) was added to a stirred solution of the phosphinic acid **64**<sup>27</sup> (1.06 g, 4.1 mmol) in dry THF (25 mL). 4-(Dimethylamino)pyridine (0.06 g, 0.5 mmol) and ethanol (0.26 g, 5.7 mmol) were added, and the mixture was stirred for 18 h. The filtered solution was washed with saturated  $\text{NaHCO}_3$  and dried and the solvent evaporated to give a colorless oil which was purified by column chromatography, eluting with  $\text{EtOAc}$ , to give **65** (0.8 g, 68%);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.25–1.50 (9 H, m), 4.05 (3 H, m), 5.12 (2 H, s), 5.35 (1 H, br d), 7.33 (5 H, s); MS  $m/e$  285 ( $\text{M}$ )<sup>+</sup>.

**Method E. *N*-[(*R,S*)-1-(Dimethoxyphosphinyl)-2-methylpropyl]-(*R,S*)-leucine, Methyl Ester (32).** A solution of **31** (1.08 g, 15 mmol) and **16a** (2.17 g, 15 mmol) in toluene (100 mL) was heated at reflux for 2 h with azeotropic removal of water. Dimethyl phosphite (1.65 g, 15 mmol) was added and the solution refluxed for a further 18 h. The solution was allowed to cool to room temperature, the solvent was evaporated, and the residue was purified by column chromatography, eluting with  $\text{EtOAc}$ , to give **32** (2.9 g, 63%) as a yellow oil (mixture of four stereoisomers):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.92 (12 H, m), 1.06 (12 H, m), 1.60 (4 H, m), 1.76 (4 H, m), 2.10 (2 H, t), 2.77 (2 H, m), 3.37 (1 H, t,  $J = 7$  Hz), 3.75 (19 H, m); MS  $m/e$  309 ( $\text{M}$ )<sup>+</sup>.

**Method F. *N*-[(*R*)-1-(Bis(benzyloxy)phosphinyl)propyl]-(*S*)-leucine, Methyl Ester (17a).** Dibenzyl trimethylsilyl phosphite (7.25 g, 22 mmol) was prepared from dibenzyl phosphite (5.75 g, 22 mmol), triethylamine (4.7 g, 22 mmol), and chlorotrimethylsilane (3.6 g, 22 mmol) by a literature procedure.<sup>22</sup>

Propionaldehyde (1.2 g, 20 mmol) was added to **16a** (2.9 g, 20 mmol) at 0 °C. Magnesium sulfate (10 g) was added and the mixture stirred at room temperature for 1 h. After addition of  $\text{CH}_2\text{Cl}_2$  (20 mL), the solution was filtered and added to dibenzyl trimethylsilyl phosphite (7.35 g, 22 mmol) under  $\text{N}_2$  at 0 °C. The solution was allowed to warm to room temperature and stirred for 18 h, washed with water, 10% citric acid, and saturated  $\text{NaHCO}_3$ , and then dried. The solvent was evaporated to give a yellow oil which was purified by column chromatography, eluting with  $\text{Et}_2\text{O}/\text{pentane}$  (1:1), to give **17a** (2.7 g, 30%) as a colorless oil:  $[\alpha]_D^{25} -32.1^\circ$  (c 0.9,  $\text{MeOH}$ );  $^1\text{H}$  NMR and MS data were identical to **17a** prepared by method C. Anal. ( $\text{C}_{24}\text{H}_{34}\text{NO}_5\text{P}$ ) C, H, N.

Further elution gave **17b** (2.2 g, 25%) as a colorless oil:  $[\alpha]_D^{25} -1.1^\circ$  (c 1,  $\text{MeOH}$ );  $^1\text{H}$  NMR and MS data were identical to **17b** prepared by method C. Anal. ( $\text{C}_{24}\text{H}_{34}\text{NO}_5\text{P}$ ) C, H, N.

Compounds **17c** and **17d** were prepared from propionaldehyde and **16b** by the same procedure.

**Compound 17c:**  $^1\text{H}$  NMR and MS data were identical to **17b**.

**Compound 17d:**  $^1\text{H}$  NMR and MS data were identical to **17a**.

The following compounds were prepared from **16a** and the appropriate aldehyde by the same procedure.

**Compound 37a:**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.85 (3 H, d,  $J = 6$  Hz), 0.90 (3 H, d,  $J = 6$  Hz), 1.16–1.46 (5 H, m), 1.53–1.80 (2 H, m), 2.90 (1 H, m), 3.65 (1 H, t,  $J = 6$  Hz), 3.70 (3 H, s), 5.03 (4 H, m), 7.31 (10 H, s); MS  $m/e$  434 ( $\text{M} + \text{H}$ )<sup>+</sup>.

**Compound 37b:**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.90 (6 H, t,  $J = 6$  Hz), 1.19–1.48 (5 H, m), 1.73 (1 H, m), 1.93 (1 H, m), 3.09 (1 H, m), 3.48 (1 H, t,  $J = 6$  Hz), 3.69 (3 H, s), 5.03 (4 H, m), 7.31 (10 H, s); MS  $m/e$  434 ( $\text{M} + \text{H}$ )<sup>+</sup>.

**Compound 38a:**  $[\alpha]_D^{25} -13.7^\circ$  (c 1,  $\text{MeOH}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.89 (3 H, d,  $J = 6$  Hz), 0.90 (3 H, d,  $J = 6$  Hz), 1.40 (2 H, m), 1.61–1.90 (3 H, m), 2.10 (1 H, m), 2.65 (1 H, m), 2.83 (2 H, m), 3.63 (3 H, s), 3.75 (1 H, t,  $J = 7$  Hz), 4.97 (4 H, m), 7.25 (15 H, m); MS  $m/e$  ( $\text{M} + \text{H}$ )<sup>+</sup>. Anal. ( $\text{C}_{24}\text{H}_{34}\text{NO}_5\text{P}$ ) H, N. C: calcd, 68.82; found, 69.72.

**Compound 38b:**  $[\alpha]_D^{25} -5.3^\circ$  (c 1,  $\text{MeOH}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.87 (3 H, d,  $J = 3$  Hz), 0.90 (3 H, d,  $J = 3$  Hz), 1.40 (2 H, m), 1.75 (2 H, m), 2.05 (1 H, m), 2.65 (1 H, m), 2.83 (3 H, br m), 3.42 (1 H, t,  $J = 7$  Hz), 3.59 (3 H, s), 4.94 (4 H, m), 7.25 (15 H, m); MS  $m/e$  524 ( $\text{M} + \text{H}$ )<sup>+</sup>. Anal. ( $\text{C}_{24}\text{H}_{34}\text{NO}_5\text{P}$ ) C, H, N.

**Compound 39a:**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.79 (3 H, d,  $J = 6$  Hz), 0.83 (3 H, d,  $J = 6$  Hz), 0.88 (3 H, d,  $J = 6$  Hz), 1.32–1.74 (5 H, m), 1.84 (1 H, br s), 2.10 (1 H, m), 2.87 (1 H, m), 3.63 (3 H, s), 3.80 (1 H, t,  $J = 7$  Hz), 4.99 (4 H, m), 7.31 (10 H, m); MS  $m/e$  476 ( $\text{M} + \text{H}$ )<sup>+</sup>.

**Method G. *N*-[(*S*)-1-(Diethoxyphosphinyl)propyl]-(*S*)-leucine (10b).** A solution of **9b** (1.08 g, 5.2 mmol) and sodium

hydroxide (0.17 g, 4.2 mmol) in EtOH (30 mL) and water (20 mL) was stirred for 24 h at room temperature. The EtOH was evaporated, and the aqueous solution was acidified with 5 N HCl, extracted with CH<sub>2</sub>Cl<sub>2</sub>, dried, and evaporated to give 10b (1.05 g, 89%) as a white crystalline solid: mp 59–60 °C (pentane); [ $\alpha$ ]<sub>D</sub><sup>25</sup> +4.1° (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.95 (6 H, d, *J* = 6 Hz), 1.05 (3 H, t, *J* = 6 Hz), 1.35 (6 H, t, *J* = 6 Hz), 1.43–1.98 (5 H, m), 2.78 (1 H, m), 3.42 (1 H, dd, *J* = 3, 5 Hz), 4.16 (4 H, m), 5.70 (1 H, br s); MS *m/e* 309 (M)<sup>+</sup>. Anal. (C<sub>13</sub>H<sub>22</sub>NO<sub>5</sub>P) C, H, N.

The same procedure was used to prepare the following compounds.

**Compound 10a:** mp 90–92 °C (CHCl<sub>3</sub>); [ $\alpha$ ]<sub>D</sub><sup>25</sup> –26.5° (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.94 (3 H, d, *J* = 3 Hz), 0.98 (3 H, d, *J* = 3 Hz), 1.08 (3 H, t, *J* = 6 Hz), 1.35 (6 H, t, *J* = 6 Hz), 1.40–1.96 (5 H, m), 2.73 (1 H, m), 3.72 (1 H, dd, *J* = 7, 10 Hz), 4.16 (4 H, m), 5.40 (1 H, br s); FAB-MS *m/e* 310 (M + H)<sup>+</sup>.

**Compound 10c:** [ $\alpha$ ]<sub>D</sub><sup>25</sup> –4.3° (c 1, MeOH); <sup>1</sup>H NMR and MS data were identical to 10b.

**Compound 10d:** [ $\alpha$ ]<sub>D</sub><sup>25</sup> +23.7° (c 1, MeOH); <sup>1</sup>H NMR and MS data were identical to 10a.

**Compound 18a:** mp 112–114 °C (Et<sub>2</sub>O); [ $\alpha$ ]<sub>D</sub><sup>25</sup> –12.6° (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.88 (6 H, t, *J* = 5 Hz), 1.03 (3 H, t, *J* = 6 Hz), 1.35–1.97 (6 H, m), 2.72 (1 H, m), 3.75 (1 H, t, *J* = 7 Hz), 5.02 (4 H, m), 7.31 (10 H, s); MS *m/e* 434 (M + H)<sup>+</sup>. Anal. (C<sub>23</sub>H<sub>32</sub>NO<sub>5</sub>P·1.5H<sub>2</sub>O) C, H, N.

**Compound 18b:** mp 71–73 °C (Et<sub>2</sub>O); [ $\alpha$ ]<sub>D</sub><sup>25</sup> +0.5° (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.90 (3 H, t, *J* = 6 Hz), 0.92 (3 H, d, *J* = 6 Hz), 1.33–1.90 (6 H, m), 2.75 (1 H, m), 3.40 (1 H, dd, *J* = 5, 7 Hz), 5.01 (4 H, m), 7.33 (10 H, s); MS *m/e* 434 (M + H)<sup>+</sup>. Anal. (C<sub>23</sub>H<sub>32</sub>NO<sub>5</sub>P) C, H, N.

**Compound 18c:** [ $\alpha$ ]<sub>D</sub><sup>25</sup> +0.2° (c 0.9, MeOH); <sup>1</sup>H NMR and MS data were identical to 18b. Anal. (C<sub>23</sub>H<sub>32</sub>NO<sub>5</sub>P) C, H, N.

**Compound 18d:** [ $\alpha$ ]<sub>D</sub><sup>25</sup> +12.4° (c 0.9, MeOH); <sup>1</sup>H NMR and MS data were identical to 18a. Anal. (C<sub>23</sub>H<sub>32</sub>NO<sub>5</sub>P) C, H, N.

**Compound 33:** mixture of four stereoisomers; <sup>1</sup>H NMR  $\delta$  0.90 (12 H, m), 1.03 (12 H, m), 1.59 (4 H, m), 1.76 (4 H, m), 2.10 (2 H, m), 2.75 (2 H, m), 3.40 (1 H, t, *J* = 7 Hz), 3.73 (13 H, m); MS *m/e* 296 (M + H)<sup>+</sup>.

**Compound 40a:** [ $\alpha$ ]<sub>D</sub><sup>25</sup> –13.6° (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.96 (3 H, d, *J* = 3 Hz), 0.99 (3 H, d, *J* = 3 Hz), 1.35 (3 H, dd, *J* = 6, 16 Hz), 1.40–1.60 (2 H, m), 1.75 (1 H, m), 3.08 (1 H, m), 3.65 (1 H, dd, *J* = 5, 7 Hz), 5.04 (4 H, m), 7.34 (10 H, s); MS *m/e* 420 (M + H)<sup>+</sup>. Anal. (C<sub>22</sub>H<sub>30</sub>NO<sub>5</sub>P·0.5H<sub>2</sub>O) C, H, N.

**Compound 40b:** [ $\alpha$ ]<sub>D</sub><sup>25</sup> –7.0° (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.90 (6 H, t, *J* = 5 Hz), 1.31 (3 H, dd, *J* = 6, 16 Hz), 1.43 (2 H, m), 1.62 (2 H, m), 2.97 (1 H, m), 3.40 (1 H, dd, *J* = 5, 7 Hz), 5.04 (4 H, m), 7.33 (10 H, s); MS *m/e* 420 (M + H)<sup>+</sup>. Anal. (C<sub>22</sub>H<sub>30</sub>NO<sub>5</sub>P·0.5H<sub>2</sub>O) C, H, N.

**Compound 41a:** [ $\alpha$ ]<sub>D</sub><sup>25</sup> –12.1° (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.87 (3 H, d, *J* = 3 Hz), 0.90 (3 H, d, *J* = 3 Hz), 1.47 (2 H, m), 1.76 (2 H, m), 2.12 (1 H, m), 2.63 (1 H, m), 2.87 (2 H, m), 3.75 (1 H, t, *J* = 7 Hz), 5.02 (4 H, m), 7.08 (2 H, d, *J* = 7 Hz), 7.20 (3 H, m), 7.33 (10 H, m); MS *m/e* 510 (M + H)<sup>+</sup>. Anal. (C<sub>29</sub>H<sub>36</sub>NO<sub>5</sub>P·0.5H<sub>2</sub>O) C, H, N.

**Compound 41b:** <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.88 (6 H, t, *J* = 5 Hz), 1.39 (1 H, m), 1.62 (2 H, m), 1.85 (1 H, m), 2.10 (1 H, m), 2.70 (1 H, m), 2.82 (2 H, m), 3.37 (1 H, dd, *J* = 5, 7 Hz), 5.03 (4 H, m), 7.06 (2 H, d, *J* = 7 Hz), 7.22 (3 H, m), 7.33 (10 H, m); MS *m/e* 510 (M + H)<sup>+</sup>.

**Compound 42a:** [ $\alpha$ ]<sub>D</sub><sup>25</sup> –28.3° (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.79 (3 H, d, *J* = 6 Hz), 0.89 (6 H, t, *J* = 7 Hz), 0.90 (3 H, d, *J* = 6 Hz), 1.32–1.60 (4 H, m), 1.73 (1 H, m), 1.83 (1 H, m), 2.92 (1 H, m), 3.81 (1 H, t, *J* = 7 Hz), 5.03 (4 H, m), 7.34 (10 H, d), 7.20 (3 H, m); MS *m/e* 461 (M)<sup>+</sup>. Anal. (C<sub>25</sub>H<sub>36</sub>NO<sub>5</sub>P·0.25H<sub>2</sub>O) C, H, N.

**Compound 68:** mixture of four stereoisomers; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.96 (12 H, m), 1.08 (6 H, t, *J* = 6 Hz), 1.22–1.40 (18 H, m), 1.40–2.00 (12 H, m), 2.85–3.04 (2 H, m), 3.43 (1 H, m), 3.58 (1 H, t, *J* = 6 Hz), 3.70 (5 H, m), 3.88 (4 H, m), 4.24 (4 H, m), 4.87 (2 H, dd, *J* = 7, 15 Hz), 5.70 (2 H, br s); MS *m/e* 368 (M + H)<sup>+</sup>.

**Compound 69:** mixture of four stereoisomers; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.89 (12 H, m), 1.23 (12 H, m), 1.41 (12 H, m), 1.75 (2 H, m), 2.94 (2 H, m), 3.40 (4 H, br s), 3.99 (4 H, m); FAB-MS *m/e* 266 (M + H)<sup>+</sup>.

**Method H.** *N*-[*N*-((*S*)-1-(Diethoxyphosphinyl)propyl)-(*S*)-leucyl]-*O*-methyl-(*S*)-tyrosine *N*-Methyl Amide (11b). A solution of the diethyl ester 10b (0.25 g, 0.81 mmol) and 1-hydroxybenzotriazole monohydrate (0.12 g, 0.9 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (25 mL) at 0 °C, under N<sub>2</sub>, was treated with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (0.17 g, 0.9 mmol). After 1 h at 0 °C, a solution of *O*-methyl-L-tyrosine *N*-methyl amide (0.2 g, 0.9 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added. The reaction was stirred at 0 °C for 1 h and then at room temperature for 18 h. The solution was washed with water, saturated NaHCO<sub>3</sub>, 5% citric acid, and brine and then dried. The solvent was evaporated and the residue purified by column chromatography, eluting with Et<sub>2</sub>O/MeOH (20:1), to give the single diastereoisomer 11b (0.36 g, 89%) as a colorless oil: [ $\alpha$ ]<sub>D</sub><sup>25</sup> –11.9° (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.84 (3 H, d, *J* = 5 Hz), 0.88 (3 H, d, *J* = 5 Hz), 1.01 (3 H, t, *J* = 7 Hz), 1.16 (1 H, m), 1.34 (3 H, t, *J* = 6 Hz), 1.35 (3 H, t, *J* = 6 Hz), 1.40–1.90 (5 H, m), 2.65 (1 H, m), 2.77 (3 H, d, *J* = 5 Hz), 2.91 (1 H, dd, *J* = 10, 14 Hz), 3.23 (2 H, m), 3.77 (3 H, s), 4.10 (4 H, m), 4.77 (1 H, m), 6.80 (2 H, d, *J* = 8 Hz), 7.11 (2 H, d, *J* = 8 Hz), 7.68 (1 H, br s), 7.72 (1 H, br s); MS *m/e* 499 (M)<sup>+</sup>. Anal. (C<sub>24</sub>H<sub>42</sub>N<sub>3</sub>O<sub>6</sub>P·H<sub>2</sub>O) C, H, N.

Compounds 11a,c,d were prepared from the corresponding diethyl esters 10a,c,d by the same procedure.

**Compound 11a:** mp 83–86 °C (EtOAc); [ $\alpha$ ]<sub>D</sub><sup>25</sup> –34.8° (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.89 (6 H, d, *J* = 6 Hz), 1.00 (3 H, t, *J* = 7 Hz), 1.25 (1 H, m), 1.33 (6 H, t, *J* = 6 Hz), 1.46–1.90 (5 H, m), 2.57 (1 H, m), 2.73 (3 H, d, *J* = 5 Hz), 3.02 (2 H, m), 3.60 (1 H, br s), 3.77 (3 H, s), 4.12 (4 H, m), 4.52 (1 H, m), 6.12 (1 H, br s), 6.82 (2 H, d, *J* = 8 Hz), 7.13 (2 H, d, *J* = 8 Hz), 7.45 (1 H, d, *J* = 8 Hz); MS *m/e* 499 (M)<sup>+</sup>. Anal. (C<sub>24</sub>H<sub>42</sub>N<sub>3</sub>O<sub>6</sub>P) C, H, N: calcd, 8.41; found, 7.62.

**Compound 11c:** [ $\alpha$ ]<sub>D</sub><sup>25</sup> +6.4° (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.87 (3 H, d, *J* = 2 Hz), 0.89 (3 H, d, *J* = 2 Hz), 1.00 (3 H, t, *J* = 7 Hz), 1.25–1.85 (6 H, m), 1.34 (3 H, t, *J* = 6 Hz), 1.36 (3 H, t, *J* = 6 Hz), 2.53 (1 H, m), 2.73 (3 H, d, *J* = 5 Hz), 3.00 (1 H, dd, *J* = 8, 14 Hz), 3.15 (1 H, m), 3.22 (1 H, dd, *J* = 8, 14 Hz), 3.78 (3 H, s), 4.13 (4 H, m), 4.62 (1 H, m), 6.80 (2 H, d, *J* = 8 Hz), 6.88 (1 H, br s), 7.07 (1 H, d, *J* = 8 Hz), 7.13 (2 H, d, *J* = 8 Hz); FAB-MS *m/e* 500 (M + H)<sup>+</sup>. Anal. (C<sub>24</sub>H<sub>42</sub>N<sub>3</sub>O<sub>6</sub>P·H<sub>2</sub>O) C, H, N.

**Compound 11d:** [ $\alpha$ ]<sub>D</sub><sup>25</sup> +36.4° (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.87 (3 H, d, *J* = 3 Hz), 0.90 (3 H, d, *J* = 3 Hz), 1.02 (3 H, t, *J* = 6 Hz), 1.25–1.80 (6 H, m), 1.34 (3 H, t, *J* = 7 Hz), 1.36 (3 H, t, *J* = 7 Hz), 2.60 (1 H, m), 2.71 (3 H, d, *J* = 5 Hz), 2.97 (1 H, dd, *J* = 6, 14 Hz), 3.10 (1 H, dd, *J* = 6, 14 Hz), 3.58 (1 H, t, *J* = 6 Hz), 3.79 (3 H, s), 4.12 (4 H, m), 4.60 (1 H, m), 6.24 (1 H, br s), 6.83 (2 H, d, *J* = 8 Hz), 7.13 (3 H, d, *J* = 8 Hz); MS *m/e* 499 (M)<sup>+</sup>. Anal. (C<sub>24</sub>H<sub>42</sub>N<sub>3</sub>O<sub>6</sub>P·H<sub>2</sub>O) C, H, N.

The same procedure was also used to prepare compounds 34, 70, and 71 from the esters 33, 68, and 69, respectively.

*N*-[*N*-((*R*)-1-(Bis(benzyloxy)phosphinyl)propyl)-(*S*)-leucyl]-(*S*)-phenylalanine *N*-Methyl Amide (43a). The dibenzyl ester 18a (2.0 g, 4.61 mmol) and L-phenylalanine *N*-methyl amide (0.92 g, 5.16 mmol) were coupled by the procedure described above, to give, after column chromatography with EtOAc as eluent, 43a (2.6 g, 95%) as a single diastereoisomer: mp 79–81 °C (EtOAc); [ $\alpha$ ]<sub>D</sub><sup>25</sup> –40.7° (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.80 (3 H, d, *J* = 3 Hz), 0.82 (3 H, d, *J* = 3 Hz), 0.95 (3 H, t, *J* = 7 Hz), 1.20 (2 H, m), 1.51 (2 H, m), 1.80 (2 H, m), 2.56 (1 H, m), 2.71 (3 H, d, *J* = 5 Hz), 2.97 (1 H, dd, *J* = 6, 14 Hz), 3.12 (1 H, dd, *J* = 6, 14 Hz), 3.61 (1 H, m), 4.60 (1 H, m), 4.97 (4 H, m), 6.15 (1 H, br s), 7.18 (5 H, m), 7.34 (11 H, s); FAB-MS *m/e* 594 (M + H)<sup>+</sup>. Anal. (C<sub>33</sub>H<sub>44</sub>N<sub>3</sub>O<sub>6</sub>P) C, H, N.

Compounds 43b–d were prepared from the corresponding dibenzyl esters 18b–d and L-phenylalanine *N*-methyl amide by the same procedure.

**Compound 43b:** [ $\alpha$ ]<sub>D</sub><sup>25</sup> –21.8° (c 0.2, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.79 (6 H, d, *J* = 5 Hz), 0.94 (3 H, t, *J* = 7 Hz), 1.02–1.87 (6 H, m), 2.61 (1 H, m), 2.75 (3 H, d, *J* = 5 Hz), 2.90 (1 H, dd, *J* = 6, 14 Hz), 3.15 (1 H, m), 3.34 (1 H, dd, *J* = 6, 14 Hz), 4.82 (1 H, m), 5.00 (4 H, m), 7.20 (5 H, m), 7.33 (10 H, s), 7.77 (1 H, d, *J* = 7 Hz), 7.82 (1 H, br s); FAB-MS *m/e* 594 (M + H)<sup>+</sup>. Anal. (C<sub>33</sub>H<sub>44</sub>N<sub>3</sub>O<sub>6</sub>P) C, H, N.

**Compound 43c:** mp 113–118 °C (EtOAc); [ $\alpha$ ]<sub>D</sub><sup>25</sup> +1.0° (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.83 (3 H, d, *J* = 3 Hz), 0.86 (3 H,

d,  $J = 3$  Hz), 0.94 (3 H, t,  $J = 6$  Hz), 1.22 (2 H, t,  $J = 6$  Hz), 1.37 (1 H, br s), 1.50 (2 H, m), 1.65–1.85 (2 H, m), 2.52 (1 H, m), 2.73 (3 H, d,  $J = 5$  Hz), 2.99 (1 H, dd,  $J = 8, 14$  Hz), 3.04 (1 H, t,  $J = 7$  Hz), 3.27 (1 H, dd,  $J = 6, 14$  Hz), 4.65 (1 H, m), 4.88 (1 H, dd,  $J = 6, 13$  Hz), 5.00 (3 H, m), 6.90 (1 H, br s), 7.10 (1 H, d,  $J = 8$  Hz), 7.19 (5 H, m), 7.32 (5 H, s), 7.34 (5 H, s); FAB-MS  $m/e$  594 (M + H)<sup>+</sup>. Anal. (C<sub>33</sub>H<sub>44</sub>N<sub>3</sub>O<sub>5</sub>P) C, H, N.

**Compound 43d:** mp 73–76 °C (EtOAc);  $[\alpha]_D^{25} +38.9^\circ$  (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.80 (6 H, d,  $J = 3$  Hz), 0.97 (3 H, t,  $J = 6$  Hz), 1.27 (2 H, m), 1.40–1.80 (4 H, m), 2.61 (1 H, m), 2.70 (3 H, d,  $J = 5$  Hz), 2.99 (1 H, dd,  $J = 6, 14$  Hz), 3.15 (1 H, dd,  $J = 6, 14$  Hz), 3.15 (1 H, dd,  $J = 6, 14$  Hz), 3.15 (1 H, dd,  $J = 6, 14$  Hz), 3.55 (1 H, t,  $J = 7$  Hz), 4.64 (1 H, m), 4.97 (4 H, m), 6.29 (1 H, br s), 7.09 (1 H, d,  $J = 8$  Hz), 7.20 (5 H, m), 7.34 (10 H, s); FAB-MS  $m/e$  594 (M + H)<sup>+</sup>. Anal. (C<sub>33</sub>H<sub>44</sub>N<sub>3</sub>O<sub>5</sub>P) C, H, N.

Compounds 20a (also prepared by method D), 21a, 22a, 23–25, 44a, b, 45a, 46a, b, 47a, b, and 48a–51a were prepared by the same procedure from the corresponding dibenzyl ester and the appropriate amino component.

**Method I. N-[N-((S)-1-Phosphonopropyl)-(S)-leucyl]-O-methyl-(S)-tyrosine N-Methyl Amide (12b).** Bromotrimethylsilane (1.32 g, 8.6 mmol) was added to a solution of 11b (0.54 g, 1.08 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL). After the mixture was stirred at room temperature for 24 h, the solvent was evaporated and the residue was dissolved in MeOH (100 mL) and stirred for 1 h at room temperature. The solvent was evaporated, and the residue was purified by column chromatography on reverse-phase silica, eluting with a gradient of 5–30% MeOH in water, to give 12b (0.25 g, 52%) as a single diastereoisomer: mp 154–157 °C (MeOH/Et<sub>2</sub>O);  $[\alpha]_D^{25} +4.1^\circ$  (c 1, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  0.78 (3 H, d,  $J = 6$  Hz), 0.82 (3 H, d,  $J = 6$  Hz), 0.90 (3 H, t,  $J = 7$  Hz), 1.32 (2 H, m), 1.50 (2 H, m), 1.72 (1 H, m), 2.53 (1 H, m), 2.60 (3 H, s), 2.77 (1 H, dd,  $J = 10, 14$  Hz), 3.00 (1 H, dd,  $J = 6, 14$  Hz), 3.61 (1 H, m), 3.65 (3 H, s), 4.49 (1 H, m), 6.73 (2 H, d,  $J = 8$  Hz), 7.08 (2 H, d,  $J = 8$  Hz); HR FAB-MS calcd for C<sub>20</sub>H<sub>35</sub>N<sub>3</sub>O<sub>6</sub>P (M + H)<sup>+</sup> 444.2263, found 444.2270.

Compounds 12a, c, d were prepared from the corresponding diethyl esters 11a, c, d by the same procedure.

**Compound 12a:** mp 162–165 °C (MeOH/Et<sub>2</sub>O);  $[\alpha]_D^{25} -12.7^\circ$  (c 1, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  1.02 (9 H, m), 1.50–2.00 (5 H, m), 2.48 (1 H, m), 2.80 (3 H, s), 2.96 (1 H, dd,  $J = 10, 14$  Hz), 3.15 (1 H, dd,  $J = 6, 14$  Hz), 3.87 (3 H, s), 4.15 (1 H, t,  $J = 7$  Hz), 4.66 (1 H, m), 6.96 (2 H, d,  $J = 8$  Hz), 7.29 (2 H, d,  $J = 8$  Hz); FAB-MS  $m/e$  444 (M + H)<sup>+</sup>. Anal. (C<sub>20</sub>H<sub>34</sub>N<sub>3</sub>O<sub>6</sub>P·H<sub>2</sub>O) C, H, N.

**Compound 12c:** mp 141–145 °C (MeOH/Et<sub>2</sub>O);  $[\alpha]_D^{25} -8.4^\circ$  (c 1, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  0.65 (3 H, d,  $J = 6$  Hz), 0.69 (3 H, d,  $J = 6$  Hz), 0.89 (1 H, m), 0.99 (3 H, t,  $J = 7$  Hz), 1.15–1.90 (4 H, m), 2.65 (3 H, s), 2.74 (2 H, m), 3.07 (1 H, dd,  $J = 5, 14$  Hz), 3.66 (3 H, s), 3.85 (1 H, t,  $J = 7$  Hz), 4.52 (1 H, m), 6.75 (2 H, d,  $J = 8$  Hz), 7.03 (2 H, d,  $J = 8$  Hz); HR FAB-MS calcd for C<sub>20</sub>H<sub>35</sub>N<sub>3</sub>O<sub>6</sub>P (M + H)<sup>+</sup> 444.2263, found 444.2219.

**Compound 12d:** mp 219–223 °C (MeOH/Et<sub>2</sub>O);  $[\alpha]_D^{25} +4.3^\circ$  (c 1, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  0.72 (3 H, d,  $J = 6$  Hz), 0.80 (3 H, d,  $J = 6$  Hz), 1.12 (3 H, t,  $J = 7$  Hz), 1.20–2.10 (5 H, m), 2.70 (1 H, m), 2.75 (3 H, s), 2.90 (1 H, m), 3.27 (1 H, dd,  $J = 5, 14$  Hz), 3.77 (3 H, s), 4.31 (1 H, m), 4.60 (1 H, m), 6.86 (2 H, d,  $J = 8$  Hz), 7.17 (2 H, d,  $J = 8$  Hz); HR FAB-MS calcd for C<sub>20</sub>H<sub>35</sub>N<sub>3</sub>O<sub>6</sub>P (M + H)<sup>+</sup> 444.2263, found 444.2267.

Compounds 35 and 73 were prepared from 34 and 71, respectively, by the above procedure.

**Method J. N-[N-((R)-1-Phosphonopropyl)-(S)-leucyl]-O-methyl-(S)-phenylalanine N-Methyl Amide (52a).** A solution of the dibenzyl ester 43a (2.8 g, 4.7 mmol) in EtOH (100 mL) was hydrogenated at room temperature and atmospheric pressure over 10% Pd/C. After 8 h, the catalyst was filtered off and the solvent evaporated to give 52a (1.6 g, 83%) as a single diastereoisomer: mp 172–174 °C (MeOH/Et<sub>2</sub>O);  $[\alpha]_D^{25} -21.8^\circ$  (c 1, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  0.88 (3 H, t,  $J = 6$  Hz), 0.95 (3 H, d,  $J = 5$  Hz), 1.00 (3 H, d,  $J = 5$  Hz), 1.68 (4 H, m), 1.84 (1 H, m), 2.40 (1 H, m), 2.70 (3 H, s), 2.95 (1 H, dd,  $J = 8, 14$  Hz), 3.10 (1 H, dd,  $J = 5, 14$  Hz), 4.44 (1 H, t,  $J = 6$  Hz), 4.67 (1 H, m), 7.31 (5 H, s); HR FAB-MS calcd for C<sub>19</sub>H<sub>32</sub>N<sub>3</sub>O<sub>5</sub>P (M + H)<sup>+</sup> 414.2157, found 414.2169. Anal. (C<sub>19</sub>H<sub>32</sub>N<sub>3</sub>O<sub>5</sub>P·H<sub>2</sub>O) C, H, N.

Compounds 52b–d were prepared from the corresponding dibenzyl esters 43b–d by the same procedure.

**Compound 52b:** mp 143–145 °C (MeOH/Et<sub>2</sub>O);  $[\alpha]_D^{25} -3.1^\circ$  (c 1, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  0.91 (3 H, t,  $J = 5$  Hz), 1.00 (6 H, m), 1.50–1.90 (5 H, m), 2.69 (3 H, s), 2.80 (1 H, m), 3.00 (1 H, dd,  $J = 8, 14$  Hz), 3.12 (1 H, dd,  $J = 6, 14$  Hz), 4.04 (1 H, m), 4.70 (1 H, m), 7.30 (5 H, s); HR FAB-MS calcd for C<sub>19</sub>H<sub>32</sub>N<sub>3</sub>O<sub>5</sub>P (M + H)<sup>+</sup> 414.2157, found 414.2175. Anal. (C<sub>19</sub>H<sub>32</sub>N<sub>3</sub>O<sub>5</sub>P·2H<sub>2</sub>O) C, H, N: calcd, 8.07; found, 6.95.

**Compound 52c:** mp 151–154 °C (MeOH/Et<sub>2</sub>O);  $[\alpha]_D^{25} -26.8^\circ$  (c 1, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  0.60 (3 H, d,  $J = 5$  Hz), 0.64 (3 H, d,  $J = 5$  Hz), 0.78 (1 H, m), 1.02 (3 H, t,  $J = 6$  Hz), 1.38 (2 H, t,  $J = 6$  Hz), 1.60–1.90 (2 H, m), 2.67 (3 H, s), 2.76 (1 H, dd,  $J = 10, 14$  Hz), 2.88 (1 H, m), 3.16 (1 H, dd,  $J = 5, 14$  Hz), 4.00 (1 H, t,  $J = 6$  Hz), 4.60 (1 H, m), 7.20 (5 H, s); HR FAB-MS calcd for C<sub>19</sub>H<sub>32</sub>N<sub>3</sub>O<sub>5</sub>P (M + H)<sup>+</sup> 414.2157, found 414.2167. Anal. (C<sub>19</sub>H<sub>32</sub>N<sub>3</sub>O<sub>5</sub>P·H<sub>2</sub>O) C, H, N.

**Compound 52d:** mp 152–156 °C (MeOH/Et<sub>2</sub>O);  $[\alpha]_D^{25} -1.1^\circ$  (c 1, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  0.70 (3 H, d,  $J = 5$  Hz), 0.80 (3 H, d,  $J = 5$  Hz), 0.94 (1 H, m), 1.10 (3 H, t,  $J = 6$  Hz), 1.45 (2 H, t,  $J = 6$  Hz), 1.73 (1 H, m), 2.01 (1 H, m), 2.77 (3 H, s), 2.86 (2 H, m), 3.35 (1 H, dd,  $J = 5, 14$  Hz), 4.34 (1 H, t,  $J = 6$  Hz), 4.65 (1 H, m), 7.30 (5 H, s); HR FAB-MS calcd for C<sub>19</sub>H<sub>32</sub>N<sub>3</sub>O<sub>5</sub>P (M + H)<sup>+</sup> 414.2157, found 414.2169. Anal. (C<sub>19</sub>H<sub>32</sub>N<sub>3</sub>O<sub>5</sub>P·0.5H<sub>2</sub>O) C, H, N.

The same procedure was used to prepare compounds 12a (also prepared by method I from 11a), 26a, 27a, 28–30, 53a, b, 54a, 55a, b, 56a, b, and 57a–60a from the corresponding dibenzyl esters.

**Method K. N-[N-((R,S)-1-Phosphinopropyl)-(R,S)-leucyl]-O-methyl-(S)-tyrosine N-Methyl Amide (73).** A solution of 70 (0.4 g, 0.72 mmol) in 36% HCl (10 mL) was refluxed under N<sub>2</sub> for 4 h. The solution was allowed to cool and evaporated to give 73 (0.29 g, 95%), after recrystallization from MeOH/Et<sub>2</sub>O, as the hydrochloride salt (mixture of four diastereoisomers): <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  0.88 (12 H, m), 1.09 (24 H, m), 1.15–1.30 (12 H, m), 1.47–2.20 (12 H), 2.65 (12 H, br s), 2.80–3.05 (8 H, m), 3.38 (4 H, m), 3.85 (12 H, br s), 4.05 (2 H, m), 4.25–4.50 (2 H, m), 4.77–4.98 (4 H, m), 6.97 (8 H, m), 7.28 (8 H, m); HR FAB-MS calcd for C<sub>20</sub>H<sub>35</sub>N<sub>3</sub>O<sub>5</sub>P (M + H)<sup>+</sup> 428.2314, found 428.2316.

**N-[N-((S)-1-(Methoxycarbonyl)ethyl)-(S)-leucyl]-O-methyl-(S)-tyrosine N-Methyl Amide (75a).** 75a was prepared from the acid 74a<sup>14a</sup> (4.14 g, 19 mmol) and O-methyl-L-tyrosine N-methyl amide (4.22 g, 19 mmol) by the coupling procedure described in method H. Column chromatography, eluting with EtOAc/pentane (1:1), gave 75a (2.4 g, 31%) as a white crystalline solid: mp 108–109 °C (EtOAc/Et<sub>2</sub>O);  $[\alpha]_D^{25} -40.4^\circ$  (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.90 (6 H, t,  $J = 6$  Hz), 1.27 (3 H, d,  $J = 6$  Hz), 1.33 (1 H, m), 1.67 (1 H, m), 1.82 (2 H, br s), 2.75 (3 H, d,  $J = 5$  Hz), 3.00 (3 H, m), 3.13 (1 H, q,  $J = 6$  Hz), 3.71 (3 H, s), 3.79 (3 H, s), 4.50 (1 H, q,  $J = 6$  Hz), 5.93 (1 H, br s), 6.82 (2 H, d,  $J = 8$  Hz), 7.12 (2 H, d,  $J = 8$  Hz), 7.72 (1 H, d,  $J = 8$  Hz); FAB-MS  $m/e$  408 (M + H)<sup>+</sup>. Anal. (C<sub>21</sub>H<sub>33</sub>N<sub>3</sub>O<sub>6</sub>) C, H, N.

**N-[N-((R)-1-(Methoxycarbonyl)ethyl)-(S)-leucyl]-O-methyl-(S)-tyrosine N-Methyl Amide (75b).** 75b was prepared from the acid 74b<sup>14a</sup> in the same way as described for 75a. Column chromatography, eluting with EtOAc/pentane (1:1), gave 75b (2.2 g, 28%): mp 98–100 °C (EtOAc/Et<sub>2</sub>O);  $[\alpha]_D^{25} -9.3^\circ$  (c 1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.88 (3 H, d,  $J = 2$  Hz), 0.90 (3 H, d,  $J = 2$  Hz), 1.10 (1 H, m), 1.31 (3 H, d,  $J = 6$  Hz), 1.35–1.62 (3 H, m), 1.76 (1 H, br s), 2.77 (3 H, d,  $J = 5$  Hz), 3.01 (2 H, m), 3.18 (1 H, dd,  $J = 6, 14$  Hz), 3.38 (1 H, q,  $J = 6$  Hz), 3.70 (3 H, s), 3.79 (3 H, s), 4.63 (1 H, m), 6.75 (1 H, br s), 6.81 (2 H, d,  $J = 8$  Hz), 7.11 (2 H, d,  $J = 8$  Hz), 7.71 (1 H, d,  $J = 8$  Hz); FAB-MS  $m/e$  408 (M + H)<sup>+</sup>. Anal. (C<sub>21</sub>H<sub>33</sub>N<sub>3</sub>O<sub>6</sub>) C, H, N.

**N-[N-((S)-1-Carboxyethyl)-(S)-leucyl]-O-methyl-(S)-tyrosine N-Methyl Amide (2a).** A solution of 75a (0.51 g, 1.25 mmol) and KOH (0.21 g, 3.75 mmol) in MeOH (3 mL) and water (1.5 mL) was stirred at room temperature. After 3 h, excess acetic acid was added and the solution evaporated. The residue was purified by column chromatography on reverse-phase silica, eluting with a gradient of 0–10% MeOH/H<sub>2</sub>O, to give 2a (0.3 g, 61%): mp 195–197 °C (H<sub>2</sub>O);  $[\alpha]_D^{25} +7.1^\circ$  (c 0.9, MeOH); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  0.79 (3 H, d,  $J = 5$  Hz), 0.83 (3 H, d,  $J = 5$  Hz), 1.11 (3 H, d,  $J = 6$  Hz), 1.18 (2 H, m), 1.58 (1 H, m), 2.61 (3 H, d,  $J = 4$  Hz), 2.74 (1 H, dd,  $J = 6, 10$  Hz), 2.91 (2 H, m), 2.98 (1 H, t,  $J = 5$  Hz), 3.71 (3 H, s), 4.47 (1 H, m), 6.80 (2 H, d,  $J = 6$  Hz), 7.07 (2 H, d,  $J = 6$  Hz), 7.88 (1 H, d,  $J = 6$  Hz), 7.98

(1 H, d,  $J = 3$  Hz); FAB-MS  $m/e$  394 ( $M + H$ )<sup>+</sup>. Anal. ( $C_{20}H_{31}N_3O_5 \cdot 0.2H_2O$ ) C, H, N.

**N-[N-((R)-1-Carboxyethyl)-(S)-leucyl]-O-methyl-(S)-tyrosine N-Methyl Amide (2b).** 2b was prepared from 75b (0.51 g, 1.25 mmol) by the method described for 2a. The residue was recrystallized from MeOH/H<sub>2</sub>O to give 2b (0.35 g, 71%): mp 199–201 °C (H<sub>2</sub>O);  $[\alpha]_D^{25} + 6.6^\circ$  (c 1, MeOH); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  0.78 (3 H, d,  $J = 5$  Hz), 0.83 (3 H, d,  $J = 5$  Hz), 1.08 (3 H, d,  $J = 5$  Hz), 1.20 (2 H, t,  $J = 5$  Hz), 1.55 (1 H, m), 2.58 (3 H, d,  $J = 3$  Hz), 2.69 (1 H, dd,  $J = 8, 11$  Hz), 2.76 (1 H, m), 2.90 (1 H, dd,  $J = 4, 10$  Hz), 3.14 (1 H, t,  $J = 5$  Hz), 3.70 (3 H, s), 4.46 (1 H, m), 6.79 (2 H, d,  $J = 6$  Hz), 7.11 (2 H, d,  $J = 6$  Hz), 7.88 (1 H, d,  $J = 4$  Hz), 8.20 (1 H, d,  $J = 6$  Hz); FAB-MS  $m/e$  394 ( $M + H$ )<sup>+</sup>. Anal. ( $C_{20}H_{31}N_3O_5 \cdot 0.2H_2O$ ) C, H, N.

**Acknowledgment.** We gratefully acknowledge Lisa Brown and Guinevere Hodges for their skillful technical assistance and the members of the Department of Analytical Sciences for spectroscopic determinations and analytical data.

## References

- Docherty, A. J. P.; Murphy, G. The Tissue Metalloproteinase Family and the Inhibitor TIMP: a Study using cDNAs and Recombinant Proteins. *Ann. Rheum. Dis.* 1990, 49, 469–479.
- Cawston, T. E.; Murphy, G.; Mercer, E.; Galloway, W. A.; Hazelman, B. L.; Reynolds, J. J. The Interaction of Purified Rabbit Bone Collagenase with Purified Rabbit Bone Metalloproteinase Inhibitor. *Biochem. J.* 1983, 211, 313–318.
- Stetler-Stephenson, W. G.; Kruttsch, H. C.; Liotta, L. A. Tissue Inhibitor of Metalloproteinase (TIMP-2) A New Member of the Metalloproteinase Inhibitor Family. *J. Biol. Chem.* 1989, 264, 17374–17378.
- Stricklin, G. P.; Bauer, E. A.; Jeffrey, J. J.; Eisen, A. Z. Human Skin Collagenase: Isolation of Precursor and Active Forms from Both Fibroblast and Organ Cultures. *Biochemistry* 1977, 16, 1607–1615.
- Okada, Y.; Nagase, H.; Harris, E. D. A Metalloproteinase from Human Rheumatoid Synovial Fibroblasts that Digests Connective Tissue Matrix Components. *J. Biol. Chem.* 1986, 261, 14245–14255.
- Henderson, B.; Docherty, A. J. P.; Beeley, N. R. A. Design of Inhibitors of Articular Cartilage Destruction. *Drugs of the Future* 1990, 15, 495–508.
- (a) Maroudas, A. Distribution and Diffusion of Solutes in Articular Cartilage. *Biophys. J.* 1970, 10, 365–379. (b) Andrews, H. J.; Plumpton, T. A.; Harper, G. P.; Cawston, T. E. A Synthetic Peptide Metalloproteinase Inhibitor, but not TIMP, Prevents the Breakdown of Proteoglycan Within Articular Cartilage *in vitro*. *Agents Actions* 1992, 37, 147–154.
- (a) Johnson, W. H.; Roberts, N. A.; Borkakoti, N. Collagenase Inhibitors: Their Design and Potential Therapeutic Use. *J. Enzyme Inhib.* 1987, 2, 1–22. (b) Schwartz, M. A.; Van Wart, H. E. Synthetic Inhibitors of Bacterial and Mammalian Interstitial Collagenases. *Prog. Med. Chem.* 1992, 29, 271–334. (c) Birkedal-Hansen, H.; Moore, W. G. I.; Boddon, M. K.; Windsor, L. J.; Birkedal-Hansen, B.; DeCarlo, A.; Engler, J. A. Matrix Metalloproteinases: A Review. *Crit. Rev. Oral Biol. Med.* 1993, 4, 197–250.
- Netzel-Arnett, S.; Fields, G.; Birkedal-Hansen, H.; Van Wart, H. E. Sequence Specificities of Human Fibroblast and Neutrophil Collagenases. *J. Biol. Chem.* 1990, 265, 6747–6755.
- (a) Galloway, W. A.; Crimmin, M. J.; DiMartino, M. J. Synthetic Inhibitors of Collagenase: Design of Potent Inhibitors with Anti-Arthritic Properties. *J. Cell. Biochem., Suppl. 15 (Part E)* 1991, 180. (b) Davidson, A. H.; Dickens, J. P.; Crimmin, M. J. Hydroxamic Acid based Collagenase Inhibitors. Patent Application WO 9005-716-A, 1990. (c) Campion, C.; Davidson, A. H.; Dickens, J. P.; Crimmin, M. J. Hydroxamic Acid Based Collagenase Inhibitors. Patent Application WO 9005-719-A, 1990.
- Besant, B.; Bird, J.; Gaster, L. M.; Harper, G. P.; Hughes, I.; Karan, E. H.; Markwell, R. E.; Miles-Williams, A. J.; Smith, S. A. Synthesis of Novel Dipeptide Inhibitors of Human Collagenase:  $\beta$ -Mercapto Carboxylic Acid Derivatives. *J. Med. Chem.* 1993, 36, 4030–4039.
- Wyvrat, M. J.; Patchett, A. A. Recent Developments in the Design of Angiotensin-Converting Enzyme Inhibitors. *Med. Res. Rev.* 1985, 5, 483–531.
- Patchett, A. A.; Harris, E.; Tristram, E. W.; Wyvrat, M. J.; Wu, M. T.; Taub, D.; Peterson, E. R.; Ikeler, T. J.; tenBroeke, J.; Payne, L. G.; Ondeyka, D. L.; Thorsett, E. D.; Greenlee, W. J.; Lohr, N. S.; Hoffsommer, R. D.; Joshua, H.; Ruyle, W. V.; Rothrock, J. W.; Aster, S. D.; Maycock, A. L.; Robinson, F. M.; Hirschmann, R.; Sweet, C. S.; Ulm, E. H.; Gross, D. M.; Vassil, T. C.; Stone, C. A. A New Class of Angiotensin Converting Enzyme Inhibitors. *Nature* 1980, 288, 280–283.
- (a) McCullagh, K.; Wadsworth, H.; Hann, M. Carboxyalkyl Peptide Derivatives. European Patent Application 126,974, 1984. (b) Brannstrom, M.; Woessner, F. J., Jr.; Koos, R. D.; Sear, C. H. J.; LeMaire, W. J. Inhibitors of Mammalian Tissue Collagenase and Metalloproteinases Suppress Ovulation in the Perfused Rat Ovary. *Endocrinology* 1988, 122, 1715–1721. (c) Delaisse, J. M.; Eeckhout, Y.; Sear, C.; Galloway, K. M.; Vaes, G. A New Synthetic Inhibitor of Mammalian Tissue Collagenase Inhibits Bone Resorption in Culture. *Biochem. Biophys. Res. Commun.* 1985, 133, 483–490.
- Wadsworth, H. J. Unpublished data.
- Kortylewicz, Z. P.; Galarzy, R. E. Phosphoramidate Peptide Inhibitors of Human Skin Fibroblast Collagenase. *J. Med. Chem.* 1990, 33, 263–273.
- Broadhurst, M. J.; Handa, B. K.; Johnson, W. H.; Lawton, G.; Machin, P. J. Phosphinic Acid Derivatives. European Patent Application 276,436, 1987.
- Galarzy, R. E.; Grobelny, D.; Kortylewicz, Z. P.; Poncz, L. Inhibition of Human Skin Fibroblast Collagenase by Phosphorus-Containing Peptides. *Metalloproteinases Inhib., Proc. Matrix Metalloproteinase Conf.*, 1989, *Matrix Suppl. n1* 1992, 259–262.
- Jacquier, R.; Ouazzani, F.; Roumestant, M.-L.; Viallefont, P. Synthesis of Enantiomerically Pure Aminophosphonic Acids. *Phosphorus Sulfur* 1988, 36, 73–77.
- Benoit, L. Amino Acids and Peptides. II. Synthesis of  $\epsilon$ -N-Methyl-L-Lysine and Related Compounds. *Can. J. Chem.* 1964, 42, 2043–2047.
- (a) Fields, E. K. The Synthesis of Esters of Substituted Amino Phosphonic Acids. *J. Am. Chem. Soc.* 1952, 74, 1528–1531. (b) Tyka, R. Novel Synthesis of  $\alpha$ -Aminophosphonic Acids. *Tetrahedron Lett.* 1970, 9, 677–680.
- Afarinkia, K.; Rees, C. W.; Cadogan, J. I. G. Synthesis of Organophosphorus Compounds via Silyl Esters of Phosphorus Acids. *Tetrahedron* 1990, 46, 7175–7196.
- Arnold, L. D.; Kalantar, T. H.; Vederas, J. C. Conversion of Serine to Stereochemically Pure  $\beta$ -Substituted  $\alpha$ -Amino Acids via  $\beta$ -Lactones. *J. Am. Chem. Soc.* 1985, 107, 7105–7109.
- Nestor, J. J., Jr.; Horner, B. L.; Ho, T. L.; Jones, G. H.; McRae, G. I.; Vickery, B. H. Synthesis of a Novel Class of Heteroaromatic Amino Acids and Their Use in the Preparation of Analogues of Leutinizing Hormone-Releasing Hormone. *J. Med. Chem.* 1984, 27, 320–325.
- Eggleston, D. S. Unpublished data.
- (a) McCleery, P. P.; Tuck, B. Synthesis of 1-Aminoalkylphosphonic Acids. Part 2. An Alkylation Approach. *J. Chem. Soc., Perkin Trans. I* 1989, 1319–1329. (b) Gallagher, M. J.; Honegger, H. Dialkoxymethylation of Phosphorus with Trialkyl Orthoformates: Reaction of Phosphonic and Phosphinic Acids via Their Trivalent Tautomers. *Tetrahedron Lett.* 1977, 34, 2987–2990. (c) Gallagher, M. J.; Honegger, H. Organophosphorus Intermediates. VI. The Acid Catalyzed Reaction of Trialkyl Orthoformate with Phosphinic Acid. *Aust. J. Chem.* 1980, 33, 287–294.
- Petrillo, E. W., Jr. Amino and Substituted Aminophosphinylalkanoyle Compositions. European Patent Application 63,896, 1982.
- Darlak, K.; Miller, R. B.; Stack, M. S.; Spatola, A. F.; Gray, R. D. Thiol-based Inhibitors of Mammalian Collagenase. *J. Biol. Chem.* 1990, 265, 5199–5205.
- Flynn, G. A.; Giroux, E. L. The Synthesis of an Aminophosphonic Acid Converting Enzyme Inhibitor. *Tetrahedron Lett.* 1986, 27, 1757–1758.
- 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, W. P., Jr. Angiotensin-Converting Enzyme Inhibitors. Mercaptan, Carboxyalkyl Dipeptides, and Phosphinic Acid Inhibitors Incorporating 4-Substituted Prolines. *J. Med. Chem.* 1988, 31, 1148–1160.
- Marlowe, C. K.; Bartlett, P. A. Phosphoramidates as Transition-State Analog Inhibitors of Thermolysin. *Biochemistry* 1983, 22, 4618–4624.
- Baylis, E. K.; Campbell, C. D.; Dingwall, J. G. 1-Aminoalkylphosphonous Acids. Part 1. Isosteres of the Protein Amino Acids. *J. Chem. Soc., Perkin Trans. I* 1984, 2845–2853.
- Murphy, J. A.; Muxlow, N.; Rice, A. Unpublished data.
- Cawston, T. E.; Barrett, A. J. A Rapid and Reproducible Assay for Collagenase Using [<sup>14</sup>C]Acetylated Collagen. *Anal. Biochem.* 1979, 99, 340–345.
- Texier-Boulet, F.; Foucaud, A. Synthesis of 1-Hydroxyalkane-phosphonic Esters on Alumina. *Synthesis* 1982, 916.
- Geary, X.; Underiner, T. L. Stabilization Demands of Diethyl Phosphonate Substituted Carbocations as Revealed by Substituent Effects. *J. Org. Chem.* 1985, 20, 2165–2170.