of T4 lymphocytes by HIV. Small amounts of HIV are added to cells, and at least two complete cycles of virus reproduction are necessary to obtain the required cell killing. Agents which interact with virions, cells, or virus gene products to interfere with viral activities will protect cells from cytolysis. While every attempt is made to reduce variability, precise values for EC_{50} and IC_{50} are not possible. All tests are compared with a positive (AZT-treated) control done at the same time under identical conditions.

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Phosphoramidate Peptide Inhibitors of Human Skin Fibroblast Collagenase

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An extensive series of *N*-(monoethylphosphoryl)peptides was synthesized and their inhibition of purified human skin fibroblast collagenase examined. At the cleavage site S_1 all reported compounds have the (EtO)(OK)P(O) group and the peptide side chain extended toward the C-terminal end (up to P_5') of the substrate sequence. These phosphoramidates with a tetrahedrally hybridized phosphorus atom are thought to be transition state analogue inhibitors. They exhibited fair inhibitory potency against this vertebrate collagenase having K_i values in the micromolar range. The most potent of these, (EtO)(OK)P(O)-Ile-TrpNHCH$_3$ (**68**), inhibits with a K_i value of 1.5 μM and is nearly 100 times stronger than (EtO)(OK)P(O)-Ile-Ala-GlyOK (**51**) (K_i of 140 μM), which has the sequence matching that of the α_1(I) chain of collagen in P_1', P_2', P_3' after the cleavage site. Several compounds were prepared in an attempt to identify the nature of the S_2', S_3', and S_4' binding sites. Alanine at the P_2' position was replaced by leucine, phenylalanine, tryptophan, or tyrosine derivatives, resulting in K_i values in a significantly lower range, 1.0–40 μM, compared to **51**. No upper size limitation or specificity has been found at this position, yet similar replacements at the P_3' position, which is occupied naturally by a glycine residue, gave weaker inhibitors: (EtO)(OK)P(O)-Ile-Tyr(OBzl)-PheOK (**57**) had a K_i of 120 μM. Hexapeptide derivatives had weaker activities in the 270 μM–2 mM range. All inhibitors were evaluated by using the synthetic thio peptolide spectrophotometric assay.

Design of synthetic collagenase inhibitors is hindered not only because the three-dimensional structure of the enzyme is presently unknown but also because details of the interaction between collagen[1-6] and collagenase[7-13] have not been identified. Most synthetic substrates designed for collagenases have K_m values about 3 orders of magnitude greater than the K_m for collagen.[14-17] This suggests that simple peptides intended as substrates or inhibitors may not have a conformation that is complementary to the enzyme binding site.

A number of inhibitors of mammalian collagenases based on the natural substrate structure have been developed.[18] Peptides containing the thiol,[19] thiol with a methylene spacer,[20,21] *N*-carboxymethyl,[22,23] or hydroxamate[24] ligand group were introduced, giving compounds with activities generally below 10 μM. The best was a hydroxamate analogue of a tripeptide with a K_i of 5 nM[18] for synovial

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Table I. Chemical and Analytical Data for Mono(ethyl esters) of Phosphoramidate Peptides

no.[a]	hydrolysis time,[b] h	column solvent[c]	R_f (solvent system)[d]	mp, °C	% yield	HPLC, min t_R[e]	HPLC, min r_R[f]	^{31}P NMR,[g] ppm	FAB-MS
48	72	18:5:2	0.72 (J)	118–121	63	8.4	10.3	7.12	393 (M + 2K$^+$), 316 (M + 1)
49	72	18:5:2	0.74 (J)	130–132	72	–	10.8	–	329 (M + 2Na$^+$), 284 (M + 1)
50	44	20:3:7	0.68 (J)	166–169	60	9.6	17.1	7.03	521 (M + 2K$^+$), 444 (M + 1), 404 (M – K$^+$)
51	72	20:3:7	0.63 (J)	157–161[h]	47	–	16.8	6.74	444 (M + 1), 365 (M – 2K$^+$)
52	40	20:3:5	0.66 (J)	122–124	39	10.4	–	6.91	536 (M + 2K$^+$), 524 (M + K$^+$), 486 (M + 1)
53	36	20:3:5	0.61 (J)	151–156[h]	74	12.7	–	6.84	533 (M + 2Na$^+$), 488 (M + 1), 464 (M – Na$^+$)
54	44	20:3:4	0.58 (J)	134–137[h]	36	12.1	15.3	7.04	536 (M + 1), 496 (M – K$^+$), 457 (M – 2K$^+$)
55	56	20:6:3	0.12 (G), 0.71 (J)	169–174[h]	59	13.6	11.9	6.91	626 (M + 1), 547 (M – 2K$^+$)
56	56	25:5:4	0.27 (G), 0.83 (J)	187–190	54	20.4	12.4	6.72	709 (M + 2Na$^+$), 664 (M + 1)
57	64	25:4:3	0.17 (G), 0.74 (J)	128–133[h]	43	15.3	–	7.15	716 (M + 1), 637 (M – 2K$^+$)
58	52	20:3:3	0.57 (J)	104–109[h]	39	14.6	17.7	6.84	634 (M + 2K$^+$), 557 (M + 1)
59	48	20:3:5	0.22 (G), 0.60 (J)	123–125	34	11.9	16.1	6.90	636 (M + 2K$^+$), 559 (M + 1), 480 (M – 2K$^+$)
60	34	20:6:3	0.10 (G), 0.54 (J)	131–135[h]	42	15.2	–	7.14	697 (M + 2K$^+$), 620 (M + 1)
61	26	10:4:3.5	0.40 (J)	186–189[h]	46	–	29.3	6.93	632 (M + 1), 592 (M – K$^+$), 553 (M – 2K$^+$)
62	19	20:5:8.5	0.18 (G), 0.76 (J)	172–176	71	17.1	14.8	6.90	651 (M + 1), 604 (M – 2Na$^+$)
63	24	25:5:9	0.26 (G), 0.70 (J)	180–183	37	18.7	–	7.04	811 (M + 1), 733 (M – K$^+$), 694 (M – 2K$^+$)
64	12	10:6:3	0.43 (J)	134–138	21	–	27.2	6.74	760 (M + 1), 720 (M – K$^+$), 681 (M – 2K$^+$)
65	18	16:5:4	0.60 (J)	108–112[h]	40	14.5	15.6	6.80	520 (M + 2Na$^+$), 475 (M + 1)
66	14	20:3:4	0.46 (G), 0.87 (J)	147–152[h]	47	26.4	–	7.33	582 (M + K$^+$), 544 (M + 1)
67	16	20:4:2.5	0.34 (G), 0.80 (J)	162–164	49	27.0	–	7.12	506 (M + K), 468 (M + 1)
68	18	20:3:3	0.50 (G), 0.83 (J)	181–184	39	28.3	12.5	7.13	515 (M + K$^+$), 477 (M + 1)
69[i]	10	20:3:2	0.42 (M), 0.53 (J)	117–121[h]	7	16.7	16.2	6.82	811 (M + 1), 829 (M – K$^+$), 790 (M – 2K$^+$)
70[j]	8	20:2:5	0.60 (M), 0.32 (J)	166–172[h]	12	10.6	31.4	7.03	785 (M + 1), 706 (M – 2K$^+$)
71	8	10:3:2	0.54 (M), 0.42 (J)	114–117	13	13.7	17.8	6.98	785 (M + 1), 706 (M – 2K$^+$)
72	9	10:3:1.6	0.64 (M), 0.27 (J)	142–148[h]	16	–	33.1	7.11	824 (M + 1), 784 (M – K$^+$)

[a] Phosphoramidate peptides were isolated as potassium salts except compounds **49**, **53**, **56**, **62**, and **65**, which are sodium salts. [b] Hydrolysis of parent *N*-(diethylphosphoryl)peptides was done according to general method E described in the Experimental Section. [c] Purification by flash chromatography on silica gel employed 1-propanol/water/concentrated ammonia, v/v. [d] See the Experimental Section for solvent systems. [e] HPLC analyses on a VYDAC (C_{18}) 4.6 mm × 250 mm analytical column. Eluant A, 0.01 N phosphoric acid adjusted to pH 7.0 with triethylamine; eluant B, acetonitrile; initial composition 2% B with a 2% (0–30 min) linear gradient of B; flow rate 1 mL min^{-1}. [f] HPLC analyses on an Aminex AX-10 4.0 mm × 300 mm ion-exchange column. Eluant A, water; eluant B, 0.3 N sodium phosphate buffer at pH 7.0; initial composition 15% B with a 5% (0–30 min) linear gradient of B; flow rate 0.6 mL min^{-1}. [g] Reported ^{31}P NMR spectra are proton decoupled, 85% phosphoric acid was used as an external reference. All compounds had ^{1}H NMR spectra consistent with the structures. [h] Lyophilate (amorphous solid), melting point of this substance is the temperature at which the white solid became a colorless glass. [i] This compound was purified additionally by HPLC (C_{18}): eluant A, water; eluant B, acetonitrile; initial composition 7% B with a 5% min^{-1} (0–45 min) linear gradient of B, flow rate 4 mL min^{-1}. [j] Purification by HPLC (C_{18}): eluant A, water; eluant B, acetonitrile; initial composition 5% B with a 3% min^{-1} (0–30 min) linear gradient of B, flow rate 4 mL min^{-1}.

collagenase. It has been shown that the fibroblast and synovial collagenase are identical.[25] Several cysteine- and penicillamine-containing peptides[26,27] have been described that inhibited human fibroblast collagenase at concentrations between 10 and 100 μM. The ketomethyleneamino peptide Z-Pro-Ala-NHCH$_2$COCH$_2$-Leu-Ala-GlyOEt has a K_i of 60 ± 16 μM[28] against the same enzyme. A series of phosphoramidate and phosphonamidate inhibitors have been synthesized for other bacterial and mammalian zinc metalloproteinases.[29–35] Phosphonamidate analogues of tetrapeptides inhibit human neutrophil collagenase with K_i values of 14–80 μM.[33] The latter approach is based on the assumption that the phosphorus-containing intermediate mimics closely the transition state of the enzyme-catalyzed amide bond hydrolysis.[36,37]

In this paper we describe the synthesis and the inhibitory potency of phosphoramidate peptides for human skin fibroblast collagenase. It was our attention to determine the optimum length of the peptide carrier, to define the specificity of the enzyme binding subsites, and to examine the contribution of different amino acid substituents at the P_1' – P_6' positions.

Chemistry

All of the di-, tri-, and tetrapeptide precursors needed for the target compounds were synthesized by standard peptide-coupling methods (Table I and the Experimental Section). Purified *N*-(diethylphosphoryl)-L-isoleucine (**6**) showed no decomposition over a period of 4 months if stored under an argon atmosphere and was prepared on a large scale, serving as a uniform phosphorylating agent in most of the longer peptide preparations. All reported O-derivatizations of the tyrosine unit in peptides **18–31** were done prior to the final coupling with (EtO)$_2$PO-Ile-OH. The terminal carboxylic methyl esters in all cases were readily removed with 0.25 N KOH, affording free carboxylic acids, which were coupled further (**31**, **44**, and **55**) or purified by recrystallization. In contrast, the phosphoramidate ethyl esters were much more difficult to hydrolyze, requiring at least an equimolar amount of 0.7 N KOH over a period of 12 h to complete the reaction. Fortunately, longer *N*-(diethylphosphoryl)peptides were hydrolyzed somewhat faster than shorter analogues. Other

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reagents used to effect P–OEt bond cleavage under neutral conditions (including trimethylsilyl iodide,[38] chlorotrimethylsilane, and sodium iodide[39] or lithium iodide[40]) were ineffective. The lithium propanethiolate method,[41] although very fast (1–2 h) and effective toward the P–OEt bond, could not be successfully used to the lack of selectivity toward the tyrosine derivatives. For **21**, **24**, **28**, and especially for compounds **31** and **35**, we observed almost the same ratios of cleavage for the P–OEt ester bond and Ph–OR ether bond of tyrosine. Furthermore, all *N*-(diethylphosphoryl)peptides containing tryptophan were subject to a partial oxidation of the indole ring. These highly colored byproducts were very difficult to remove during the ion-exchange chromatography (DEAE-Sephadex A-25, HCO^{3-}, form, and a linear gradient of 0–0.5 M triethylammonium bicarbonate buffer, pH 8.6). For these reasons we have hydrolyzed all *N*-(diethylphosphoryl)-peptides using the aforementioned saponification process. In an effort to isolate the product from the hydrolytic mixture, a simple flash chromatogaphy on silica gel was used with aqueous ammonia present in the solvent system (see the Experimental Section), giving exceptionally good separations within a short period of time. In agreement with other literature reports concerning phosphoramidates,[30,42,43] the deprotected phosphoramidates **48–73** were somewhat labile in our hands. The hydrolytic sensitivity of the phosphoramidate bond towards acids is well known;[43,44] therefore care was taken to avoid exposing the deprotected phosphoramidates to acidic conditions. However, even with these precautions some decomposition occurred (3–7% based on ^1H NMR and HPLC) during the hydrogenation and lyophilization procedures affording deprotected phosphoramidates contaminated with several byproducts including $EtOPO_2H$.

We had difficulties separating analytically pure samples of mono(ethyl esters) of phosphoramidate peptides as well as their sodium or potassium salts (**48–73**). These compounds had to be protected from any significant decomposition immediately after isolation by the addition of a small excess of NaOH or KOH (this excess, however, interfered with the elemental analyses). Therefore, inhibitors were tested with the small degree of the inorganic salt contamination and their homogeneity was determined by TLC and HPLC analysis, followed by ^1H NMR, ^{31}P NMR, and FAB/MS spectra without microanalysis.

The synthetic substrate of vertebrate collagenase **5b**[17] was obtained by esterification of *N*-acetyl-L-prolyl-L-leucylglycine with (*S*)-*N*-[(2-mercaptoisopentyl)-carbonyl]-L-leucylglycine ethyl ester (**4b**) in the presence of 4-(dimethylamino)pyridine. Thiol **4b**, with the same optical geometry as L-leucine, was prepared by the thioacetylation of (*R*)-2-bromo-4-methylpentanoic acid (**1a**) followed by the coupling with L-leucylglycine ethyl ester. To further confirm the optical geometry of **4b**, the corresponding *R* isomer **4a** of this thiol was synthesized, as well as (*R,S*)-, (*R*)-, and (*S*)-*N*-[(2-thioacetylisopentyl)-carbonyl]-L-leucylglycine ethyl esters **3**, **3a**, and **3b**, which were prepared independently. The HPLC analyses and CD spectra proved the optical purity (97%) of compound **4b**.

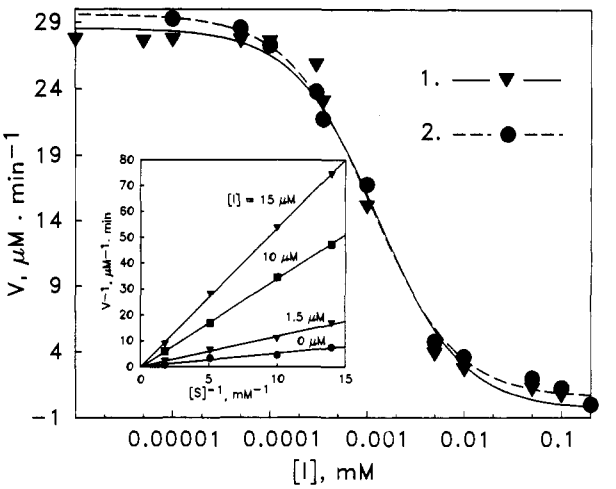

Figure 1. Inhibition of human skin fibroblast collagenase by (EtO)(OK)P(O)-Ile-Trp-$NHCH_3$ (**68**) using the thiol ester substrate, (▼) at pH 6.5 and the amide substrate assay (●) at pH 7.5. Inset: a Lineweaver–Burk plot of the same inhibitor **68** using the thiol ester substrate.

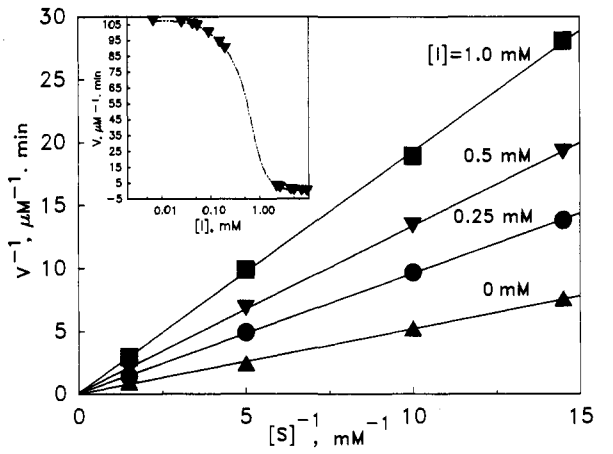

Figure 2. A Lineweaver–Burk plot of inhibition of human skin fibroblast collagenase by (EtO)(OK)P(O)-Ile-Ala-Gly-Gln-Arg-GlyOK (**71**) using the thiol ester substrate at pH 6.5. Inset: the IC_{50} determination for this inhibitor from thiol ester substrate method.

Results and Discussion

The IC_{50} and K_i determination for all inhibitors were performed at pH 6.5 using the Weingarten thiol ester substrate[17] with velocities of the enzyme-catalyzed reaction corrected for the spontaneous hydrolysis. The sensitivity of this fast spectrophotometric assay is higher by over 200-fold when compared to some collagen-based assays.[45-47] In addition compounds **50**, **56**, and **68** (Table II) were assayed with a second synthetic substrate[18] (hexapeptide) at pH 7.5 which did not suffer from the spontaneous hydrolysis. Figure 1 shows that IC_{50} curves obtained from both assays for the inhibitor (EtO)(OK)P(O)-Ile-Trp-$NHCH_3$ (**68**) were in good agreement. The pH dependence of K_i is not known for inhibitors for this vertebrate col-

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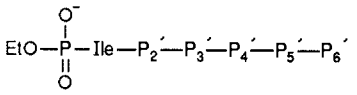
Table II. Inhibitory Potency of Phosphoramidate Peptide Analogues



no.[a]	P_2'	P_3'	P_4'	P_5'	P_6'	K_i,[b] μM
48[a]						990
49[a,c]						950
50	Ala	Gly				130 (147)[d]
51[a]	Ala	Gly				140
52	Leu	Gly				49
53	Phe	Gly				29
54	Tyr	Gly				22
55	Tyr(OBzl)	Gly				6.7 (0.2)[e]
56	Tyr($OBzlCl_2$)	Gly				5.7 (13)[d]
57	Tyr(OBzl)	Phe				120
58	Ala	Gly	Leu			240
59	Trp	Gly				13
60	Tyr(OTHP)	Gly				25
61	Tyr(OCH_2COOH)	Gly				32
62	Tyr($OCH_2CONHCH_2Ph$)	Gly				16
63	Tyr[$OCH_2CON(CH_2Ph)_2$]	Gly				27
64	TyrOCH_2CO-Lys	Gly				50
65	*p*-NH_2-Phe	Gly				19
66	Tyr(OBzl)	$NHCH_3$				18
67	Tyr(OMe)	$NHCH_3$				31
68[f]	Trp	$NHCH_3$				1.5 (0.9)[d] (700)[e]
69	Ala	Gly	Gln	$Arg(NO_2)$		1900
70	Ala	Gly	Glu	$Arg(NO_2)$	Gly	270
71	Ala	Gly	Gln	Arg	Gly	460
72	Ala	Gly	Glu	Arg	Gly	403

[a] Reported inhibitors have the isoleucyl substituent at the P_1' position except compounds **49**, **51**, and **70**, which are leucyl analogues. All inhibitors have an unprotected carboxy terminal group except compounds **66–68**, which are *N*-methylamides. [b] Inhibitors were assayed by using the spectrophotometric assay with the thiol ester substrate[17,45] at pH 6.5 and K_i's were determined from Lineweaver–Burk and Dixon plots from two independent experiments, and agreed within ±50%. [c] Compounds **48** and **49** are *N*-(monoethylphosphoryl)-L-isoleucine and -L-leucine, respectively. [d] The K_i determined from the amide substrate assay at pH 7.5 using fluorescamine detection. [e] Inhibition of angiotensin converting enzyme; see the Experimental Section for the assay procedure. [f] Compound **68** inhibits bacterial collagenase from *Clostridium histolyticum* with $IC_{50} > 5$ mM.

lagenase. Phosphorus-containing inhibitors of the zinc protease carboxypeptidase A, however, are 2–3-fold weaker at pH 7.5 compared to pH 6.5.[48] Phosphoramidate peptide inhibitors were stable at the thiol ester assay conditions (50 mM Hepes buffer, pH 6.5). ^{31}P NMR spectra of selected inhibitors (hexapeptide analogue (EtO)(OK)P(O)-Ile-Ala-Gly-Gln-Arg-GlyOK (**71**), dipeptide (EtO)(OK)P(O)-Ile-Trp$NHCH_3$ (**68**), and tripeptide (EtO)(OK)P(O)-Ile-Tyr(OTHP)-GlyOK (**60**)) taken in the assay buffer at 25 °C showed no signs of decomposition even over a 5-day period. Only the original resonances were observed. Acidification of the solution containing inhibitor **60** to the pH 2 caused rapid hydrolysis. Representative examples of Lineweaver–Burk and Dixon plots for this series of inhibitors are shown in Figures 1–3 (compounds **68** and **71**).

Inhibitors (EtO)(OK)P(O)-Leu-Ala-GlyOK (**51**) and (EtO)(OK)P(O)-Ile-Ala-GlyOK (**50**) have a similar K_i value around 140 μM. No apparent preference of the enzyme for leucine or isoleucine at the P_1' position was observed. Based primarily on the conclusions from the reported series of closely related phosphonamidate inhibitors,[33] it was assumed that lengthening of the peptide chain, which has the same amino acid sequence as that existing in the natural substrate, should enhance the inhibition. This was not confirmed in our series of longer phosphoramidates. The K_i (240 μM) of the tetrapeptide analogue **58**, which contains the leucine unit at the P_4' position, and the unexpectedly high K_i values (0.27–0.50 mM) for all three phosphoramidates **70–72** containing an amino acid sequence up to the P_6' position are nearly 5 times higher than for the tripeptide inhibitor **50** ($K_i \sim 140$ μM). The pentapeptide inhibitor *N*-(ethylphosphoryl)-L-isoleucyl-L-alanylglycyl-L-glutaminyl-N^G-nitro-L-arginine (**69**) inhibits the enzyme with a K_i of 1.9 mM. Such weak inhibition is unexpected considering the increased number of subsites that could be occupied on the enzyme. The thus far reported[49] small peptide inhibitors, 2-mercapto-4-methylpentanoyl tetrapeptides (containing the Ala-Gly-Gln-D-Arg-NH_2 sequence), for tadpole back spin collagenase in-

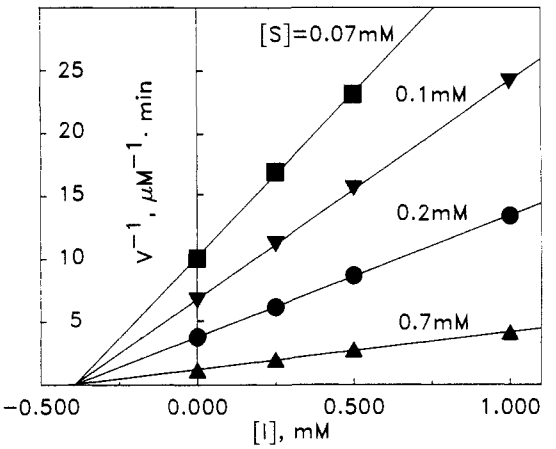


Figure 3. A Dixon plot of inhibition of human skin fibroblast collagenase by (EtO)(OK)P(O)-Ile-Ala-Gly-Gln-Arg-GlyOK (**71**) using the thiol ester substrate at pH 6.5.

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hibit this enzyme with an IC_{50} of ~10 μM. However, shorter mercapto peptide derivatives have not been tested and it is unknown whether the tetrapeptide sequence has the optimum length or whether shorter inhibitors would be as potent. In the similar series of 2-mercapto-4-methylpentanoyl peptides,[19] the increase of the inhibitor length also did not significantly increase the potency.

Changes made at the P_2' position naturally occupied by the alanine residue enhanced the inhibitory potency. This observation was first made by Delaisse et al.[50] with a L-leucyl-*O*-methyltyrosyl methylamide derivative of a carboxyalkyl ligand moiety (K_i = 0.3 μM against bone tissue collagenase). This carboxyalkyl compound also showed the high activity achievable with only a dipeptide sequence. We found K_i's of 49 μM for *N*-(ethylphosphoryl)-L-isoleucyl-L-leucylglycine (**52**) and 29, 22, and 13 μM for *N*-(ethylphosphoryl)-L-isoleucyl-L-phenylglycine (**53**), *N*-(ethylphosphoryl)-L-isoleucyl-L-tyrosylglycine (**54**), and *N*-(ethylphosphoryl)-L-isoleucyl-L-tryptophylglycine (**59**), respectively. The best inhibitor of the series, *N*-(ethylphosphoryl)-L-isoleucyl-L-tryptophan methylamide (**68**), has a K_i value of 1.5 μM and binds as tightly to the collagenase as the natural substrate does (K_m = 1.5 μM[16]). The structural requirements of the P_2' subsite were investigated further with a series of 14 tripeptide analogues **52–65**. The replacements were selected on the basis of their size and different chemical properties in an attempt to define the S_2' binding site. In the series of following substitutions, Leu, Phe, Tyr, *p*-NH_2-Phe, Tyr(OTHP), Trp, Tyr(OBzl), and Tyr($OBzlCl_2$), the K_i values were decreased by factors of 3 (Leu), 7 (*p*-NH_2-Phe), 10 (Trp), and 23 (Tyr($OBzlCl_2$)). Substituents with different hydrophobicity and hydrogen-bonding capabilities such as Tyr(OCH_2COOH), Tyr($OCH_2CONHCH_2Ph$), Tyr(OCH_2CO-Lys) were still accommodated and corresponding phosphoramidates inhibited the enzyme more strongly than (EtO)(OK)P(O)-Ile-Ala-GlyOK (**50**). No upper limitation of the substituent size has been found for the S_2' subsite. It is conceivable that the side chain at this position can point toward the solvent rather than the enzyme. There is also evidence for the hydrophobic character of the S_2' binding site, since compounds containing more hydrophobic amino acid residues at the P_2' position are better inhibitors. The enzyme site S_3' appears to incorporate only small side-chain substituents. When the glycine residue at the P_3' position of *N*-(ethylphosphoryl)-L-isoleucyl-*O*-benzyl-L-tyrosylglycine (**55**) was replaced by phenylalanine to give *N*-(ethylphosphoryl)-L-isoleucyl-*O*-benzyl-L-tyrosyl-L-phenylalanine (**57**), the K_i value increased by more than 20-fold. All three analogues of the dipeptide methylamides **66**, **67**, and **68** are more potent inhibitors than their corresponding phosphorylated tripeptides **54**, **55**, and **59**. This further suggests that the S_3' binding subsite of the enzyme might be a small pocket in nature.

The increase of the inhibitory power of phosphoramidates **53–56** and **65–68**, in the comparison with the potency of compounds **50** and **51**, appears to be primarily a consequence of favorable interaction in ancillary binding sites of the enzyme. Thus the tetrahedral phosphoramidate mono(ethyl ester) moiety may not coordinate optimally to the active site of the enzyme. A phosphoramidate, having slightly longer bonds[51–53] and greater flexibility than a carboxamide because of the lack of the double-bond character in the P–N linkage,[53] may permit the peptide analogue to adopt conformations somewhat different from those found in the natural substrate. Additional conformational changes may occur in phosphoramidates compared to carboxamides due to the different hydrogen-bonding capabilities of the former.[54]

Inhibitors **55** and **68**, with K_i's of 7 and 1.5 μM, respectively, for human skin fibroblast collagenase, were also evaluated against angiotensin converting enzyme. *N*-(Monoethylphosphoryl)-L-isoleucyl-*O*-benzyl-L-tyrosylglycine (**55**) inhibits converting enzyme better (K_i = 0.2 μM) than collagenase (K_i = 6.7 μM). *N*-(Monoethylphosphoryl)-L-isoleucyl-L-tryptophan methylamide (**68**) is selective for human skin fibroblast collagenase compared to converting enzyme (IC_{50} = 700 μM) by 400-fold. These results confirm the requirement for an unprotected carboxy terminal group in inhibitors of converting enzyme.[55] The best yet reported[34] phosphonamidate tripeptide inhibitor (K_i = 20 μM) of bacterial collagenase from *Clostridium histolyticum*,[56] *N*-(isoamylphosphonyl)glycyl-L-prolyl-L-alanine, inhibits human skin fibroblast collagenase with a K_i greater than 5 mM. Conversely, *N*-(monoethylphosphoryl)-L-isoleucyl-*O*-benzyl-L-tyrosylglycine (**55**) is a very weak inhibitor for this bacterial collagenase (IC_{50} > 5 mM) but inhibits human skin fibroblast collagenase with a K_i of about 7 μM. These selective inhibition potencies point out the different requirements at the bond cleavage site (P_1–P_1') for bacterial and vertebrate collagenase.

Our experiments demonstrate that human fibroblast collagenase is inhibited by *N*-(monoethylphosphoryl)-peptides of a carboxy terminal sequence related to the type I collagen substrate. The enzyme prefers a hydrophobic group at the S_2' subsite and changes at this position maximize the potency. These findings are similar and consistent with the inhibition data reported earlier for human synovial collagenase.[18] All 14 substituents selected for the P_2' position gave more active inhibitors than those with the natural residue, alanine. The nonspecific nature of this subsite might indicate that P_2' substituents can extend into the solvent in the enzyme–inhibitor complex. The subsite S_3' appears to be a small binding area and larger substituents at this position are detrimental for the inhibitory potency. Extension of the peptide chain beyond the P_3' position gave weakly active compounds.

Experimental Section

Materials and Methods. Melting points were determined on a hot stage and are uncorrected. ^1H NMR spectra were recorded on a Varian EM-390 and XL-200 or XL-300 spectrometer. Chemical shifts are reported in ppm downfield from internal TMS or DSS standards for ^1H NMR spectra and in ppm downfield from an external 85% phosphoric acid reference for ^{31}P NMR spectra. All ^{31}P NMR proton decoupled spectra were acquired at the Magnetic Resonance Imaging and Spectroscopy Center at the University of Kentucky. TLC was performed on 0.25-mm-thickness silica gel plates (Merck, silica gel 60, F-254). Compounds were visualized by the following methods: ninhydrin (0.5 g in 100 mL of methanol) for deprotected peptides; exposure to hydrochloric acid fumes followed by ninhydrin for phosphorylated amines and N-protected peptides;[57] ultraviolet light; iodine vapor. For purification of protected intermediates, gravity or flash column

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chromatography (performed according to the method of Still et al.[58]) was carried out on Merck silica gel 60, 70–230 mesh, in ethyl acetate/*n*-hexane or ethyl acetate/methanol. The purification of labile deprotected phosphoramidate peptides was accomplished by flash chromatography on silica gel employing an 1-propanol/water/ammonia system as an eluant in various proportions. An excess of 1-propanol and ammonia in collected fractions was easily evaporated at room temperature under vacuum. During the entire process of evaporation, 0.5 N KOH was being added to maintain pH 8–10. The aqueous residue was then lyophilized. High-performance liquid chromatography (HPLC) employing various gradients and isocratic solvent systems (from 5% to 85%, v/v, of acetonitrile and water or 0.01 N phosphoric acid adjusted to pH 7.0 with triethylamine) was done on a Varian 5000 liquid chromatograph system, using Waters μ-Bondapak or VYDAC (C_{18}) 4.6 mm × 250 mm analytical columns. The HPLC ion-exchange chromatography was performed with an Aminex AX-10 (4.0 mm × 300 mm) column (Bio-Rad Laboratories). For preparative HPLC, the reversed-phase VYDAC C_{18} (50 mm × 300 mm) column and a gradient of water/acetonitrile as an eluant was used. Absorbance was monitored at 220 nm. Mass spectral analyses using fast atom bombardment (FAB-MS) were performed by the Midwest Center for Mass Spectrometry, University of Nebraska–Lincoln, Department of Chemistry, Lincoln, NE. The molecular weight of protected intermediates was determined from mass spectra taken on a Finnigan 3300 GC-MS spectrometer at 150 eV, methane chemical ionization (CI-MS). Commercially available chemicals and solvents were reagent grade and were purchased from Aldrich Chemical Co., Milwaukee, WI, or Sigma Chemical Co., St. Louis, MO, unless stated otherwise. THF was freshly distilled over $LiAlH_4$ prior to use. All amino acids were the L enantiomers unless otherwise specified.

Solvent Systems: A, $CHCl_3$; B, THF/$CHCl_3$; C, EtOAc/*n*-hexane (3:1); D, EtOAc; E, C_6H_6; F, EtOAc/MeOH (9:1); G, EtOAc/MeOH/NH_4OH (90:10:5); H, MeOH; I, EtOAc/EtOH (3:1); J, 1-propanol/H_2O/NH_4OH (20:5:2); K, $CHCl_3$/MeOH/AcOH (85:10:5); L, $CHCl_3$/acetone/AcOH (20:6:7); M, 1-butanol/pyridine/AcOH (10:2:5).

General Synthetic Methods. Method A. Synthesis of the Short Peptide Fragments. The preparation of the majority of simple intermediate peptides described in this paper was accomplished by using well-established, sequential methods; dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HOBt),[59] mixed anhydride,[60] or *N,N'*-carbonyldiimidazole (CDI)[61,62] couplings. All fully protected peptides used in further synthetic steps appeared as a single spot on TLC, were at least 92% pure by HPLC analysis, and had consistent MS and ^1H NMR spectra. Synthesis of all modified peptides and peptolides that required individualized methodology are described later in this section.

Method B. Removal of the *tert*-Butyloxycarbonyl Protecting Groups with Gaseous or Methanolic HCl. The solution of a *N*-*tert*-butyloxycarbonyl-peptide benzyl ester (15 mmol) in dry CH_2Cl_2 was saturated with gaseous HCl at 0 °C for 90 min. When the reaction was completed (as monitored by TLC), the excess of HCl was removed by passing a stream of nitrogen through the mixxture. The solvent was evaporated under vacuum, leaving a residue of the crude product which was recrystallized from MeOH/ether or EtOAc/ether, giving the product as a solid. When the solubility of the protected peptide in CH_2Cl_2 was limited (tri- or tetrapeptide methyl esters), to the solution of a starting peptide (15 mmol) in MeOH (10 mmol) were added anisole (18 mmol) and 2.2 N methanolic HCl (25 mL). After the mixture was stirred at room temperature for 3 h, the solvent was removed in vacuo to afford the crude product, which was recrystallized from MeOH/ether.

Method C. Removal of the *tert*-Butyloxycarbonyl Protecting Groups with Trifluoroacetic Acid. The solution of a *N*-(*tert*-butyloxycarbonyl)-peptide benzyl ester (12 mmol) in trifluoroacetic acid (15 mmol) was stirred at room temperature for 90 min. The excess acid was evaporated under vacuum and the residue treated with anhydrous ethyl ether, causing solidification of the product, which was washed several times with ethyl ether. Dried TFA salts were used in the next step without further purification or were treated as follows: a crude product was dissolved in EtOAc (150 mL) and the solution adjusted to pH 8 with saturated $NaHCO_3$ (100 mL). The organic layer was washed with 50% aqueous NaCl (100 mL) and H_2O (100 mL), dried over $MgSO_4$, and evaporated to dryness, giving the free amine as an oil. This was dissolved in ethyl ether (100 mL) and treated with saturated ethereal HCl (100 mL) to yield the hydrochloride salt as a solid.

Method D. Saponification of Carboxy Terminal Methyl Esters. To the solution of tetra-, tri-, or dipeptide methyl ester (10 mmol) in a THF/H_2O mixture (50 mL) was added 0.25 N KOH (12 mmol). After stirring at room temperature for 90 min, the reaction mixture was diluted with H_2O (20 mL) and THF was evaporated at room temperature under vacuum. The aqueous solution was washed with EtOAc (2 × 30 mL), the pH was then adjusted to 2 with 10% citric acid, and the water phase extracted again with EtOAc (4 × 30 mL). These extracts were dried ($MgSO_4$) and after removal of the solvent afforded the product as a solid.

Method E. Hydrolysis of *N*-(Diethylphosphoryl)peptides. Diethyl phosphoramidate peptide (10 mmol) was suspended in 2 mL of water and ethanol was added dropwise until complete dissolution. An equimolar amount of 1.0 N KOH was added in three portions over a period of 12 h. The progress of the hydrolysis was monitored by TLC (suitable systems: G, J, K). Upon completion of the reaction (12–72 h) the mixture was lyophilized and the residue was purified by flash chromatography on a silica gel column with 1-propanol/water/ammonia system as an eluant in various proportions (typically 20:5:2). The excess of 1-propanol and ammonia was evaporated from collected fractions under vacuum at room temperature. During the entire process of evaporation, 0.2 N KOH was added to maintain pH 8–10. The solid potassium salt of the mono(ethyl ester) phosphoramide peptide could then be purified further by preparative HPLC or by repeated precipitation from absolute EtOH.

Synthesis of the Thio Peptolide, Synthetic Substrate of Collagenase. (*R*,*S*)-2-Bromo-4-methylpentanoic Acid (1). To a solution of sodium (16.7 g, 0.725 g-atom) in 400 mL of absolute ethanol stirred under nitrogen was added diethyl malonate (11.6 g, 0.725 mol). The mixture was allowed to react for 15 min, and 100 g of isobutyl bromide was added dropwise. After 8 h of reflux, NaBr was filtered off, ethanol evaporated, and 400 mL of ethyl ether added to the residue. The solution was washed with 3% $NaHCO_3$ and water. The organic phase, after drying over $MgSO_4$ and evaporation of the solvent, was distilled under reduced pressure, giving 62 g (71% yield) of pure diethyl isobutylmalonate: bp 119–120 °C (16 torr); ^1H NMR ($CDCl_3$) δ 0.86 (d/d, 6 H, 2 CH_3), 1.64 (d/t, 6 H, 2 CH_3), 1.94 (m, 3 H, $CHCH_2$), 3.05 (t, 1 H, CH), 4.11 (d/q, 4 H, 2 OCH_2). The hydrolysis of the diethyl ester was affected in a mixture of 2 N aqueous NaOH/EtOH (1:1), giving the corresponding diacid: ^1H NMR (DMSO-d_6) δ 1.74 (m, 6 H, 2 CH_3), 2.50 (m, 3 H, $CHCH_2$), 3.90 (t, 1 H, CH), 12.4 (s, 2 H, COOH). Decarboxylation at 100 °C (neat) gave 34 g (67% yield) of 4-methylpentanoic acid: ^1H NMR ($CDCl_3$) δ 0.87 (d, 6 H, 2 CH_3), 1.54 (m, 3 H, $CHCH_2$), 2.30 (t, 2 H, CH_2), 11.30 (s, 1 H, COOH). 4-Methylpentanoic acid, dried under high vacuum, was treatd with 19.5 mL (32 g, 0.35 mol) of bromine and 1.2 mL of PCl_3 was cautiously added. The mixture was heated to 80–85 °C until the bromine decolorized (about 3 h). The temperature was then increased to 100 °C and kept at this range for 1 h. After cooling, the mixture was distilled, and five fractions were collected. The second fraction (24.3 g, bp 76–79 °C (0.2 torr)) contained pure product: ^1H NMR ($CDCl_3$) δ 0.98 (d/d, 6 H, 2 CH_3), 1.72–2.10 (m, 3 H, $CHCH_2$), 4.26 (t, 1 H, CHBr), 10.80 (s, 1 H, COOH).

(*R*)- and (*S*)-2-Bromo-4-methylpentanoic Acid (1a and 1b). Freshly powdered $NaNO_2$ (8.5 g, 0.60 mol) was added over 2.5 h to a rapidly stirred solution of optically pure D- or L-leucine (10 g, 0.072 mol) dissolved in 6 N HBr (100 mL) of 0–5 °C. Stirring was continued for 14 h at 4 °C. The mixture was extracted with

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ethyl ether, dried over $CaCl_2$, and fractionally distilled. The product (57% yield), (*R*)- or (*S*)-2-bromo-4-methylpentanoic acid, was collected as an oil: bp 67–70 °C (1.5 torr); ^1H NMR ($CDCl_3$), δ 0.97 (d/d, 6 H, 2 CH_3), 1.72–2.10 (m, 3 H, $CHCH_2$), 4.26 (t, 1 H, CHBr), 10.80 (s, 1 H, COOH).

(*S*,*R*)-, (*R*)-, or (*S*)-2-Thioacetyl-4-methylpentanoic Acid (2, 2a, 2b). (*R*,*S*)-, (*S*)-, or (*R*)-2-Bromo-4-methylpentanoic acid (5 g, 25.6 mmol) was dissolved in 5 mL of ethanol and was carefully neutralized with 1.44 g (26.0 mmol) of concentrated KOH (1.44 g of KOH was dissolved in 0.25 mL of H_2O and 1.5 mL of EtOH). Thiolacetic acid (1.85 mL, 0.026 mol) was neutralized in the same manner (1.45 g of KOH/0.25 mL of H_2O/1.5 mL of EtOH). The neutralized acid was added dropwise to the solution of 1, **1b**, or **1a** and the resulting mixture was gently refluxed for 20 min and then stirred at room temperature for an additional 3 h. Precipitated KBr was filtered off, ethanol was partially evaporated, and 20 mL of water was added. The resulting solution was acidified to pH 2 with 6 N HCl and the oily residue extracted with ethyl ether (2 × 50 mL). Combined ethereal extracts were washed with water and dried over $MgSO_4$. After evaporation of the solvent, the crude product was fractionally distilled, giving 5.2 g (64% yield) of the pure thioacetyl acid **2**, **2a**, or **2b**: bp 91–94 °C (0.4 torr); ^1H NMR ($CDCl_3$) δ 0.90 (d/d, 6 H, 2 CH_3), 1.32–2.00 (m, 3 H, $CHCH_2$), 2.31 (s, 3 H, $COCH_3$), 4.17 (t, 1 H, COCH), 11.10 (s, 1 H, SCOOH).

(*R*,*S*)-, (*R*)-, or (*S*)-*N*-[(2-Thioacetylisopentyl)carbonyl]-L-leucylglycine Ethyl Ester (3, 3a, or 3b). In 30 mL of anhydrous DMF were dissolved L-leucylglycine ethyl ester trifluoroacetate salt (2.53 g, 0.01 mol; prepared according to the general methods A, B, C, and D) and *N*-ethylmorpholine (1.3 mL), followed by the acid **2**, **2a**, or **2b** (1.9 g, 0.01 mol), respectively, at 0 °C. DCC dissolved in 5 mL of DMF was added after 10 min and the reaction mixture stirred at 4 °C for 20 h. The precipitated DCU was filtered off, the solvent removed under reduced pressure, andthe residue dissolved with stirring in 80 mL of EtOAc/*n*-hexane mixture. The next portion of DCU was filtered off and the clear filtrate washed with water, 5% $NaHCO_3$, and 3% citric acid, dried over $MgSO_4$, and evaporated to dryness. The solid residue was recrystallized from an ethyl ether/*n*-hexane mixture, giving 2.10 g (averaged yield from three preparations of **3** was 54%) of the corresponding peptolides **3**, **3a**, or **3b**: ^1H NMR (DMSO-d_6) δ 0.85–0.93 (m, 12 H, 4 CH_3), 1.02 (s, 3 H, CH_3), 1.1–1.7 (m, 6 H, 2 $CHCH_2$), 2.12 (s, 3 H, $COCH_3$), 3.67 (d, 2 H, CH_2, from glycine), 3.90 (q, 2 H, OCH_2), 4.0–4.3 (m, 2 H, 2 CH), 8.0 (t, 1 H, NH, from glycine), 8.3 (d, 1 H, NH, from leucine).

(*R*)- and (*S*)-*N*-[(2-Mercaptoisopentyl)carbonyl]-L-leucylglycine Ethyl Ester (4a and 4b). To a solution of 0.53 g (1.36 mol) of **3a** or **3b** in 20 mL of anhydrous EtOH was added 2.71 mL of 0.5 N NaOEt under nitrogen. After 20 min of stirring at room temperature the solution was neutralized with 6 N HCl. Ethanol was evaporated and the oily residue dissolved in EtOAc (50 mL), washed with saturated NaCl solution, and dried over $MgSO_4$. Evaporation of the solvent gave a waxy solid, which was recrystallized from AcOEt/*n*-hexane, giving 0.32 g (58%) of **4a**, mp 74–76 °C. The deprotection of **3b** was accomplished with 59% yield. The attempted recrystallization failed and the oily residue was purified on a silica gel column (EtOAc/*n*-hexane, 20:0.5), giving the product **4b** as a foam: ^1H NMR (DMSO-d_6) δ 0.9 (m, 12 H, 4 CH_3), 1.0 (t, 3 H, CH_3), 1.05–1.30 (m, 6 H, 2 $CHCH_2$), 3.66 (d, 2 H, CH_2, from glycine), 3.7 (q, 2 H, OCH_2), 3.95–4.10 (m, 3 H, 2 CH, SH), 7.7 (t, 1 H, NH from glycine), 7.99 (d, 1 H, NH from leucine). The purity of both isomers was checked by HPLC (C_{18}): t_R 16.7 min for **4a** and t_R 15.9 min for **4b**; eluant A, water; eluant B, CH_3CN; flow rate 2 mL/min; initial composition 11% B with a 2% min^{-1} linear gradient of B.

(*R*)- or (*S*)-*N*-[2-[(*N*-Acetyl-L-prolyl-L-leucylglycyl)-thio]-1-oxoisopentyl]-L-leucylglycine Ethyl Ester (5a and 5b). To the stirred solution of *N*-acetyl-L-prolyl-L-leucylglycine (prepared by methods A, B, C, and D; 3.27 g, 10 mmol) and **4a** or **4b** (3.50 g, 10 mmol) in 15 mL of DMF at 0 °C were added DCC (2.10 g, 11 mmol) and 4-(dimethylamino)pyridine (50 mg). The resulting mixture was stirred at 4 °C for 12 h. Solvent was evaporated under high vacuum at room temperature and the residue redissolved in 60 mL of an EtOAc/*n*-hexane mixture. The organic layer was washed with 1.5% $NaHCO_3$ in saline, dilute hydrochloric acid in saline, 10% $CuSO_4$ in saline, and finally a saturated solution of NaCl. The ethyl acetate layer was dried over $MgSO_4$ and the solvent evaporated to give 1.8 g (77%) of a glassy residue of crude **5a** or **5b**. The HPLC analysis of this material showed about 82% of the desired product. The attempts to recrystallize these products led to the partial decomposition with the main byproduct being the starting mercaptans **4a** or **4b**. Similar decomposition was observed during the attempted flash chromatography on a preequilibrated silica gel column. Satisfactory purification was achieved on a reversed-phase preparative column. HPLC (C_{18}): t_R of **5a** 21.6 min, t_R of **5b** 23.0 min; eluant A, H_2O; eluant B, CH_3CN; flow rate 5 mL/min; starting composition 20% B with a 1.2% min^{-1} linear gradient of B). Purified products showed no decomposition if stored under a nitrogen atmosphere (the purity was checked by HPLC over a period of 3 weeks). The purified isomeric thio peptolides had correct amino acid analyses and consistent mass spectral data: FAB–MS m/z 656 (M + 1), 253 (100).

The hexapeptide substrate Ac-Pro-Leu-Gly-Leu-Leu-Gly-OEt used in the amide substrate assay was prepared by the sequential method, as described in the general methods A–D. The crude peptide was purified to apparent homogeneity by (C_{18}) reversed-phase HPLC: t_R 19.1 min; eluant A, water; eluant B, methanol; initial composition 50% B with a 10% linear gradient of B; flow rate 7 mL min^{-1}.

***N*-(Diethylphosphoryl)-L-isoleucine (6).** To a solution of L-isoleucine benzyl ester hydrochloride (7.21 g, 27 mmol) and 2,6-lutidine (6.6 mL, 56 mmol) in anhydrous CH_2Cl_2 (40 mL) was added dropwise diethyl chlorophosphate (4.1 mL, 27 mmol) in the same solvent (10 mL) at −15 °C. The mixture was kept under nitrogen and allowed to reach room temperature within a 2-h period and then was diluted with CH_2Cl_2 (50 mL) and washed with 25-mL portions of 3% citric acid, 10% $NaHCO_3$, and water. The organic layer was dried over $MgSO_4$ and concentrated under vacuum, and the oily residue was purifed on a silica gel column (EtOAc/*n*-hexane, 4:1), giving the benzyl ester of **6** (81% yield) as a colorless oil: R_f 0.54 (A); ^1H NMR ($CDCl_3$) δ 0.86 (m, 6 H), 1.21 (d/t, 6 H, 2 CH_3), 1.76 (7, 1 H), 3.21 (t, 1 H), 3.60–4.02 (m, 5 H), 5.10 (s, 2 H, $PhCH_2$), 7.34 (m, 5 H, Ph). Hydrogenation of the benzyl ester (5.8 g, 22 mmol) was accomplished in methanol (50 mL) at atmospheric pressure with 10% Pd/C, giving **6** (4.9 g, 96%) as a crystalline solid: mp 74–76 °C; ^1H NMR ($CDCl_3$) δ 0.96 (m, 6 H), 1.35 (d/t, 6 H), 1.84 (m, 1 H), 3.72 (d/d, 2 H), 3.96–4.32 (m, 5 H), 4.70 (d, 1 H, NH), 11.6 (s, 1 H, COOH). In a similar reaction, *N*-(diethylphosphoryl)-L-leucine (**7**) was obtained (79% overall yield) as a crystalline solid: mp 79 °C dec; R_f 0.40 (K); CI-MS (CH_4) m/z 268 (M + 1), 266 (M − 1), 247 (M − 18); HPLC (C_{18}) t_R 12.6 min for **6** and t_R 11.9 min for **7**, eluant A water, eluant B CH_3CN, initial composition 10% B with a gradient of 10–60% B (25 min), flow rate 1 mL min^{-1}.

Preparation of *N*-(Diethylphosphoryl)peptides. Tri- and Tetrapeptide Diethylphosphoramidates 8–17. To the solution of *N*-(diethylphosphoryl)-L-isoleucine (1.34 g, 5 mmol) and 1-hydroxybenzotriazole (0.59 g, 5 mmol) in anhydrous DMF (4 mL) was added DCC (1.1 g, 5.3 mmol) in the same solvent (1 mL) at 0 °C. The hydrochloride salt of the corresponding di- or tripeptide benzyl or methyl ester (5 mmol) dissolved in dry DMF (2 mL) was neutralized with *N*-ethylmorpholine (5 mmol) at 0 °C. Both mixtures were combined, and stirring was continued at 4 °C for 10 h. The solvent was removed under vacuum, and the residue diluted with $CHCl_3$ (70 mL) and washed with 25-mL portions of 3% citric acid, 10% $NaHCO_3$, and water. The organic layer then dried was over $MgSO_4$ and concentrated to give the crude product. Chromatographic purification on a silica gel column afforded the product as a solid, which was either recrystallized or purified further by HPLC. By this method a series of 10 compounds of the type $(EtO)_2P(O)$Ile-R was prepared, where R = Ala-Gly-OBzl **8** and **9** (compound **9** was the *N*-(diethylphosphoryl)-L-leucyl derivative), Leu-Gly-OBzl **10**, Phe-Gly-OBzl **11**, Tyr-Gly-OBzl **12**, Tyr(OBzl)-Gly-OMe **13**, Tyr(OBzlCl_2)-GlyOMe **14**, Tyr(OBzl)-Phe-OMe **15**, Trp-Gly-OBzl **16**, Ala-Gly-Leu-OBzl **17**.

***N*-(Diethylphosphoryl)-L-isoleucyl-*O*-(tetrahydro-2-pyranyl)-L-tyrosylglycine Ethyl Ester (18).** A solution containing *N*-CBZ-L-tyrosylglycine ethyl ester, prepared by the DCC/HOBT method (3.0 g, 75 mmol), in dry CH_2Cl_2 (80 mL) and 3,4-dihydro-2*H*-pyran (5 mL) with dried pyridinium *p*-toluenesulfonate[63] (1.7 g, 20 mmol) was stirred at 35 °C under

a nitrogen atmosphere for 72 h to complete the etherification. The mixture was diluted with $CHCl_3$ (50 mL), and the resulting solution was washed with saturated aqueous $NaHCO_3$ and dried over $MgSO_4$. The solvent was evaporated and the product purified by column chromatography on silica gel (EtOAc/*n*-hexane, 20:3) to give *N*-CBZ-*O*-(tetrahydro-2-pyranyl)-L-tyrosylglycine ethyl ester (**19**) (3.5 g, 85%) as colorless needles: mp 59–61 °C; R_f 0.64 (C); ^1H NMR ($CDCl_3$) δ 1.14 (t, 3 H), 1.35–2.10 (m, 6 H), 2.91 (m, 2 H), 3.52 (t, 1 H), 3.80 (d, 2 H), 4.05 (q, 2 H), 4.43 (d/d, 1 H), 4.89 (s, 2 H), 5.21 (t, 1 H, NH), 5.72 (d, 1 H, NH), 6.62 (d, 2 H), 6.89 (d, 2 H), 7.20 (m, 5 H, Ph). The benzyl carbamate group of this derivative (3.1 g, 64 mmol) was cleaved by hydrogenolysis (10% Pd/C, 50 mg) at atmospheric pressure (180 min) in MeOH (60 mL), giving the corresponding free amine **20** (2.1 g, 93%) as a hygroscopic oil: R_f 0.32 (F), R_f 0.57 (K); ^1H NMR (DMSO-d_6) 1.12 (t, 3 H), 1.40–2.10 (7, 6 H), 2.87 (7, 2 H), 3.54 (t, 1 H), 3.94 (d, 2 H), 4.12 (q, 2 H), 5.14 (m, 2 H, NH_2), 6.87 (d, 2 H), 7.10 (d, 2 H), 8.15 (d, 1 H, NH). Amine **20** was dried under high vacuum and immediately added to the previously prepared mixture of **6** (1.59 g, 6.0 mmol) and *N*,*N*′-carbonyldiimidazole (0.97 g, 6.0 mmol) in anhydrous DMF (20 mL) at −10 °C. The reaction mixture was allowed to warm up to room temperature and stirred for 3 h. The solvent was evaporated and the residue was purified on a silica gel column (EtOAc/MeOH, 20:0.5), affording the product **18** (2.9 g, 80%) as a white solid: mp 77–80 °C dec; R_f 0.70 (F), R_f 0.44 (D); ^1H NMR (DMSO-d_6) δ 0.89 (m, 6 H), 1.40 (t/t, 6 H), 1.60 (t, 3 H), 1.72–2.30 (m, 6 H; overlapped with m, 1 H), 2.90 (m, 2 H), 3.61 (t, 1 H), 3.97 (d, 2 H), 4.17–4.60 (q, 2 H; overlapped with m, 4 H), 5.05–5.30 (m, 6 H), 5.81 (d, 1 H, NH), 6.86 (d, 2 H), 7.08 (d, 2 H), 8.10 (m, 1 H, NH), 8.74 (m, 1 H, NH); FAB-MS *m*/*z* 600 (M + 1), 598 (M − 1), 307 (100); HPLC (C_{18}) t_R 18.6 min, eluant A water, eluant B CH_3CN, initial composition 15% B with a 2% min^{-1} linear gradient of B, flow rate 2 mL min^{-1}.

***N*-(Diethylphosphoryl)-L-isoleucyl-*O*-[(benzyloxycarbonyl)methyl]-L-tyrosylglycine Methyl Ester (21).** To a solution of *N*-(*tert*-butyloxycarbonyl)-L-tyrosylglycine methyl ester (4.6 g, 13 mmol, prepared by general methods A, B, and C) in anhydrous acetone (distilled over P_2O_5, 70 mL) was added anhydrous, freshly powdered Na_2CO_3 (0.16 g, 15 mmol) followed by dropwise addition of benzyl 2-bromoacetate (3.5 g, 15 mmol). Rigorously dry reaction conditions were maintained, and the mixture was refluxed for 8 h with stirring. Then the solvent was evaporated under vacuum and the residue treated with a CH_2Cl_2/*n*-hexane mixture (100 mL). The suspension was filtered and the solid residue was extracted with CH_2Cl_2 (2 × 25 mL). Combined extracts and the filtrate were washed with 10% $NaHCO_3$ and water, dried over $MgSO_4$, and evaporated. The crude product, slightly contaminated with the starting dipeptide, was purified on a silica gel column (EtOAc/*n*-hexane, 20:1.5) to give *N*-(*tert*-butyloxycarbonyl)-*O*-[(benzyloxycarbonyl)-methyl]-L-tyrosylglycine methyl ester (**22**) (5.8 g, 92%) as a white solid: mp 91–93 °C; R_f 0.24 (E), R_f 0.72 (F); ^1H NMR (DMSO-d_6) δ 1.26 (s, 9 H), 1.74 (m, 1 H), 2.94–3.15 (m, 3 H, CH_2 and NH), 3.43 (s, 3 H), 3.72 (d, 2 H), 4.61 (s, 2 H), 4.94 (s, 2 H), 6.84 (d, 2 H), 7.12 (d, 2 H), 7.20 (m, 5 H), 8.12 (d, 1 H, NH). Removal of the *tert*-butyloxycarbonyl group was done according to the general method B, to afford the corresponding hydrochloride salt **23** (4.7 g, 87%) as a white solid: mp 112–115 °C; R_f 0.36 (F), R_f 0.70 (K). Hydrochloride **23** (1.0 g, 2.29 mmol) was dissolved in DMF (10 mL) and neutralized with *N*-ethylmorpholine (0.3 mL), and *N*-(diethylphosphoryl)-L-isoleucine (**6**) (0.61 g, 2.3 mmol) and DCC (0.5 g, 2.3 mmol) were added at 0 °C. The coupling was done as described in general method A, and a crude product was purified on a silica gel column (EtOAc/MeOH, 20:0.7), giving **21** as a white solid: mp 84–85 °C; R_f 0.18 (B), R_f 0.59 (F); ^1H NMR ($CDCl_3$) δ 0.63–1.00 (m, 6 H), 1.17 (t, 1 H), 1.72 (m, 1 H), 2.82–3.16 (m, 3 H), 3.40–4.22 (m, 9 H, overlapped with t, 3 H), 4.51 (s, 2 H), 4.79 (d/d, 1 H), 5.18 (s, 2 H), 6.72 (d, 2 H), 7.12 (d, 2 H), 7.20 (m, 5 H), 7.56–7.64 (m, 2 H, 2 NH); FAB-MS *m*/*z* 650 (M + 1), 648 (M − 1), 314 (100); HPLC (C_{18}) t_R 17.3 min, eluant A water, eluant B CH_3CN, initial composition 15% B with a 2% min^{-1} linear gradient of B, flow rate 2 mL min^{-1}.

***N*-(Diethylphosphoryl)-L-isoleucyl-*O*-[[(*N*-benzylamino)carbonyl]methyl]-L-tyrosylglycine Methyl Ester (24).** Hydrogenolysis (10% Pd/C, 20 mg) of **22** (3.1 g, 6.2 mmol) in methanol (60 mL) gave *N*-[*tert*-butyloxycarbonyl)-*O*-(carboxymethyl)-L-tyrosylglycine methyl ester (**25**) as a white solid: mp 84–87 °C; R_f 0.43 (K); ^1H NMR ($CDCl_3$) δ 1.42 (s, 9 H), 2.87–3.15 (m, 2 H), 3.58 (s, 3 H), 3.92 (d, 2 H), 4.62 (d/d, 1 H), 4.74 (s, 2 H), 5.51 (m, 1 H, NH), 8.24 (d, 1 H, NH), 10.34 (s, 1 H, COOH). A solution of **25** (2.5 g, 6.1 mmol) in anhydrous CH_2Cl_2 (20 mL) was treated with *N*,*N*′-carbonyldiimidazole (1.0 g, 6.1 mmol) at −15 °C, and after 2 h, freshly distilled benzylamine (0.7 mL, 6.5 mmol) was added. After 3 h of stirring at 0 °C, the solvent was evaporated and the residue purified on a silica gel column (EtOAc/MeOH, 20:3), giving *N*-(*tert*-butyloxycarbonyl)-*O*-[[(*N*-benzylamino)carbonyl]methyl]-L-tyrosylglycine methyl ester (**26**) (2.44 g, 80%) as a colorless foam: R_f 0.57 (I), R_f 0.74 (J); ^1H NMR ($CDCl_3$) 1.28 (s, 9 H), 3.58 (s, 3 H), 3.84 (d, 2 H), 4.21–4.56 (m, 5 H), 5.17 (d, 1 H, NH), 6.60 (d, 2 H), 6.89–7.16 (m, 2 H, 2 NH), 7.26 (m, 5 H). Cleavage of the Boc group of **26** was done according to the general method B, furnishing the corresponding amine hydrochloride **27** (2.10 g, 98%) as a hygroscopic solid: mp 139–143 °C dec; R_f 0.52 (K); ^1H NMR (DMSO-d_6) δ 2.85–3.05 (m, 2 H), 3.46 (s, 3 H), 3.64 (d/d, 2 H), 3.90–4.08 (m, 2 H), 4.31 (d, 2 H), 4.46 (d, 2 H), 5.54 (m, 1 H, CH), 6.76 (d, 2 H), 7.04 (d, 2 H), 7.15 (m, 5 H), 8.32 (m, 3 H, NH_2HCl), 8.64 (t, 1 H, NH), 9.17 (t, 1 H, NH). A solution of **27** (1.75 g, 4 mmol) in DMF (8 mL) was treated with *N*-ethylmorpholine (0.51 mL, 6 mmol) at 0 °C, DCC (0.85 g, 6 mmol) was added followed by **6** (1.1 g, 6 mmol), and stirring was continued overnight. The solvent was evaporated and an oily residue redissolved in EtOAc (70 mL), filtered, and evaporated again. A crude product was purified on a silica gel column (EtOAc/MeOH, 20:0.7) to give **24** (1.82 g, 70%) as a white solid: mp 97–100 °C; R_f 0.53 (F), R_f 0.62 (G), R_f 0.94 (K); ^1H NMR ($CDCl_3$) 0.86 (m, 6 H), 1.18 (t, 6 H), 1.91–2.12 (m, 2 H), 2.97–3.18 (m, 2 H), 3.37–3.64 (m, 4 H), 3.72 (s, 3 H), 3.78–4.20 (m, 5 H), 4.37–4.52 (m, 4 H), 4.74 (d/d, 1 H), 6.79 (d, 2 H), 7.05 (d, 2 H), 7.12 (m, 5 H), 5.05–7.31 (m, 2 H, 2 NH); FAB-MS *m*/*z* 671 (M + Na^+), 649 (M + 1), 312 (100); HPLC (C_{18}) t_R 20.7 min, eluant A water, eluant B CH_3CN, starting composition 15% B with a 2% min^{-1} linear gradient of B, flow rate 2 mL min^{-1}.

***N*-(Diethylphosphoryl)-L-isoleucyl-*O*-[[(*N*,*N*-dibenzylamino)carbonyl]methyl]-L-tyrosylglycine Methyl Ester (28).** To a solution of **25** (1.15 g, 2.8 mmol) in anhydrous CH_2Cl_2 (30 mL) was added *N*,*N*′-carbonyldiimidazole (0.45 g, 2.8 mmol) at −15 °C and the mixture was stirred at this temperature for 2 h. Then freshly distilled dibenzylamine (0.6 mL, 3 mmol) was added and the stirring was continued for an additional 3 h. The mixture was diluted with $CHCl_3$ (50 mL) and washed with 3% citric acid, 10% $NaHCO_3$, and water. The solvent was evaporated and the crude product was purified on a silica gel column (EtOAc/MeOH, 20:0.6) to give *N*-(*tert*-butyloxycarbonyl)-*O*-[[(*N*,*N*-dibenzylamino)carbonyl]methyl]-L-tyrosylglycine methyl ester (**29**) (1.3 g, 81%) as a colorless foam: R_f 0.31 (I), R_f 0.62 (J); ^1H NMR ($CDCl_3$) δ 1.37 (s, 9 H), 3.02 (d, 2 H), 3.68 (s, 3 H), 3.94 (d, 2 H), 4.24–4.66 (m, 5 H), 4.83 (s, 2 H), 5.14 (d, 1 H, NH), 6.58 (t, 1 H, NH), 6.78 (d, 2 H), 7.05–7.43 (m, 12 H). Removal of the Boc protecting group was done as described in general method B, giving the amine hydrochloride **30** (87%) as a white solid: mp 127–129 °C dec; R_f 0.44 (K). This salt (0.81 g, 1.5 mmol) was coupled with **6** (0.41 g, 1.5 mmol) in a manner described for **24** and the crude product was purified on a silica gel column (EtOAc/MeOH, 20:0.3) to give **28** (0.96 g, 86%) as a colorless solid: mp 107–111 °C dec; R_f 0.67 (F), R_f 0.74 (G); ^1H NMR ($CDCl_3$) δ 0.86 (m, 6 H), 1.21 (t, 6 H), 1.93 (m, 1 H), 3.18 (m, 4 H), 3.51–3.64 (m, 3 H), 3.61 (s, 3 H), 3.74–4.15 (m, 6 H), 4.42–4.71 (m, 3 H), 4.70 (s, 6 H), 6.65 (d, 2 H), 7.02–7.41 (m, 13 H); FAB-MS *m*/*z* 755 (M + 1), 674, 312 (100); HPLC (C-18) t_R 26.7 min, eluant A water, eluant B CH_3CN, starting composition 15% B with a 2% min^{-1} linear gradient of B and flow rate 2 mL min^{-1}.

***N*-(Diethylphosphoryl)-L-isoleucyl-*O*-[[[[1-(benzyloxycarbonyl)-5-(carbobenzoxyamino)pentyl]amino]carbonyl]methyl]-L-tyrosylglycine Methyl Ester (31).** A solution of **25** (3.0 g, 7.3 mmol) in anhydrous CH_2Cl_2 (50 mL) was treated with *N*,*N*′-carbonyldiimidazole (1.18 g, 7.3 mmol) at −15 °C and kept at this temperature for 3 h. Then N^{ϵ}-carbobenzoxy-L-lysine benzyl ester hydrochloride (3.0 g, 7.3 mmol), prepared according to the

(63) Miyashita, M.; Yoshikashi, A.; Grieco, P. *J. Org. Chem.* **1977**, *42*, 3722–3744.

procedure of Bergmann et al.,[64] was added and the mixture stirred at 4 °C for the next 20 h. After dilution with $CHCl_3$ (50 mL), the mixture was washed with 3% citric acid, 10% $NaHCO_3$, and water and dried over $MgSO_4$ and the solvent was evaporated. A crude product was purified on a silica gel column (EtOAc/$CHCl_3$/MeOH, 10:10:0.3), giving *N*-(*tert*-butyloxycarbonyl)-*O*-[[[[1-(benzyloxycarbonyl)-5-(carbobenzoxyamino)pentyl]-amino]carbonyl]methyl]-L-tyrosylglycine methyl ester (**32**) as a colorless foam: R_f 0.43 (I), R_f 0.70 (G). This was deprotected according to the general method B, giving corresponding amine hydrochloride (4.6 g, 90%) as a waxy hygroscopic solid. A crude salt was dissolved in anhydrous MeOH (40 mL) and combined with a solution of sodium (0.15 g, 6.6 × 10^{-3} g-atom) in MeOH (10 mL). Ether was added and the precipitated NaCl filtered off. The filtrate was concentrated under vacuum, dissolved in $CHCl_3$ (50 mL), and concentrated again to remove methanol. The free amine, after drying under high vacuum for 2 h, was dissolved in anhydrous CH_2Cl_2 (10 mL). *N*-(Diethylphosphoryl)-L-isoleucine (**6**) (0.48 g, 1.8 mmol; dissolved in anhydrous CH_2Cl_2 (20 mL) was treated with *N,N'*-carbonyldiimidazole (0.29 g, 1.8 mmol) at −15 °C for 2 h. Both solutions were combined, and after a few hours at −15 °C, the reaction mixture was permited to warm up to room temperature and then to stand overnight. The solvent was evaporated and the oily residue purified on a silica gel column (EtOAc/MeOH, 4:1), giving **31** (0.84 g, 51%) as a white solid: mp 112–113 °C; R_f 0.41 (I), R_f 0.59 (K); ^{1}H NMR (DMSO-d_6) δ 0.74 (m, 6 H), 1.21 (t, 6 H), 1.26–1.94 (m, 10 H), 2.05 (s, 3 H), 2.64–3.80 (m, 1 H), 3.96–4.24 (m, 12 H), 4.96 (s, 2 H), 5.12 (s, 2 H), 5.59 (s, 2 H), 5.43 (d, 2 H, NH), 6.72 (d, 2 H), 7.09 (d, 2 H), 7.14 (m, 5 H), 7.20 (m, 5 H), 7.74 (d, 1 H, NH), 7.93 (m, 1 H, NH), 8.28 (m, 1 H, NH); FAB-MS *m/z* 912 (M + 1), 876, 217 (100); HPLC (C_{18}) t_R 29.1 min, eluent A water, eluant B CH_3CN, initial composition 15% B with a 2% min^{-1} linear gradient of B, flow rate 2 mL min^{-1}.

***N*-(Diethylphosphoryl)-L-isoleucyl-4-amino-L-phenylalanlylglycine Methyl Ester (33).** A solution of **6** (2.16 g, 8 mmol) in anhydrous DMF (10 mL) was treated with *N,N'*-carbonyldiimidazole (1.3 g, 8 mmol) at −20 °C for 6 h. Meanwhile, 4-nitro-L-phenylalanylglycine methyl ester hydrochloride (2.56 g, 8 mmol) was dissolved in MeOH (40 mL) and combined with a solution of sodium (0.184 g, 8 × 10^{-3} g-atom) in MeOH (15 mL). Ether was added and the precipitated NaCl filtered off. The filtrate was concentrated under vacuum, and the residue was dissolved in $CHCl_3$ (50 mL) and, after filtration, concentrated again. The dried residue was dissolved in DMF (5 mL) and added to the reaction mixture from above. After 1 h at −20 °C, the reaction mixture was permitted to warm to room temperature slowly and then to stand overnight. The solvent was evaporated under reduced pressure and the residual oil heated with EtOAc. The solid obtained upon cooling was recrystallized from an EtOAc/*n*-hexane mixture to give *N*-(diethylphosphoryl)-L-isoleucyl-4-nitro-L-phenylalanylglycine methyl ester (**34**) (3.72 g, 87%) was a white solid: mp 128–129 °C dec; R_f 0.37 (F), R_f 0.74 (K); ^{1}H NMR (DMSO-d_6) δ 0.84 (m, 6 H), 1.43 (t, 6 H), 1.43–2.00 (m, 5 H), 2.94–3.62 (m, 3 H), 3.71 (s, 3 H), 3.81–4.17 (m, 5 H), 4.32 (d/d, 1 H), 4.1 (m, 1 H), 5.31 (d/d, 1 H, NH), 7.39 (d, 2 H), 7.81 (d/d, 1 H, NH), 8.15 (d, 2 H), 8.37 (t, 1 H, NH). A solution of **34** (1.4 g, 2.7 mmol) in MeOH (20 mL) was hydrogenated (40 mg of 10% Pd/C) at atmospheric pressure for 90 min. The solvent was evaporated and the crude product applied to a (C_{18}) reversed-phase column and eluted with 30% aqueous CH_3CN, to afford **33** (470 mg): mp 144–146 °C; ^{1}H NMR (DMSO-d_6) δ 0.58 (m, 6 H), 1.16 (m, 6 H), 3.62–4.08 (m, 5 H), 4.50–4.93 (m, 2 H), 6.54 (d, 2 H), 7.02 (d, 2 H), 7.62 (m, 2 H, NH_2), 7.73 (d/d, 1 H, NH), 8.40 (t, 1 H, NH); FAB-MS *m/z* 501 (M + 1), 306 (100); HPLC (C_{18}) t_R of **33** 17.3 min, t_R of **34** 26.8 min, eluant A water, eluant B CH_3CN, initial composition 17% B with a 2% min^{-1} linear gradient of B and flow rate 2 mL min^{-1}.

***N*-(Diethylphosphoryl)-L-isoleucyl-*O*-benzyl-L-tyrosine Methylamide (35).** A solution of *N*-(*tert*-butyloxycarbonyl)-*O*-benzyl-L-tyrosine (3.71 g, 10 mmol) in anhydrous CH_2Cl_2 (50 mL) was treated with *N,N'*-carbonyldiimidazole (1.62 g, 10 mmol) at −10 °C for 2 h. Then a stream of gaseous methylamine was passed through the mixture for 5 min and the reaction flask was kept tightly covered at 0 °C. An excess of the amine was removed with a stream of nitrogen, the solution was washed with 3% citric acid, 10% $NaHCO_3$, and water and dried over $MgSO_4$, and the solvent was evaporated. The crude product was recrystallized from an EtOAc/*n*-hexane mixture, giving *N*-(*tert*-butyloxycarbonyl)-*O*-benzyl-L-tyrosine methylamide (**36**) (3.8 g, 96%) as a white solid: mp 94–95 °C; ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 9 H), 2.63 (d, 3 H), 2.93 (d, 2 H), 4.23 (d/d, 1 H), 4.96 (s, 2 H), 5.17 (d, 1 H, NH), 5.87–6.23 (m, 1 H, NH), 6.84 (d, 2 H), 7.03 (d, 2 H), 7.34 (m, 5 H). This was deprotected according to method B, giving *O*-benzyl-L-tyrosine methylamide hydrochloride (**37**) (3.10 g, 97%) as a white solid: mp 117–119 °C; R_f 0.51 (G), R_f 0.69 (K). This amide (1.6 g, 5 mmol) was coupled with **6** (1.38 g, 5 mmol) via the DCC/HOBt coupling method as described in an illustrative procedure in ref 57, to give after purification on a silica gel column (EtOAc/MeOH, 20:0.2) the amide **35** (1.08 g, 71%) as a white solid: mp 69–71 °C dec; R_f 0.42 (D), R_f 0.68 (F); ^{1}H NMR (DMSO-d_6) δ 0.79 (m, 6 H), 1.16 (t, 6 H), 1.86–2.04 (m, 2 H), 2.46 (d, 3 H), 2.94 (d, 2 H), 3.76–3.95 (m, 5 H), 4.12–4.23 (m, 3 H), 4.37 (d/d, 1 H), 5.14 (s, 2 H), 6.82 (d, 2 H), 7.03 (d, 2 H), 7.29 (m, 5 H), 8.09 (m, 1 H, NH); CI-MS (CH_4) *m/z* 534 (M + 1), 532 (M − 1), 527, 515; HPLC (C_{18}) t_R 19.4 min, eluant A water, eluant B CH_3CN, initial co mposition 20% B with a 2% min^{-1} linear gradient of B, flow rate 2 mL min^{-1}.

***N*-(Diethylphosphoryl)-L-isoleucyl-*O*-methyl-L-tyrosine Methylamide (38).** To a solution of *N*-(*tert*-butyloxycarbonyl)-L-tyrosine (6.25 g, 23 mmol) in anhydrous DMF (20 mL) was added freshly powdered K_2CO_3 (7.8 g, 58 mmol), followed by MeI (10 g). The mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, the residue was treated with $CHCl_3$ (100 mL), and the resulting suspension was filtered and the solid residue extraced with $CHCl_3$ (2 × 20 mL). Combined extracts and the filtrate were washed with water, dried over $MgSO_4$, and evaporated. A crude product was purified on a silica gel column (EtOAc/MeOH, 20:0.7), giving *N*-(*tert*-butyloxycarbonyl)-*O*-(methyl)-L-tyrosine methylamide (**39**) (5.47 g, 68%) as a white solid: mp 76–77 °C; R_f 0.33 (D), R_f 0.72 (F); ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9 H), 2.64 (d, 3 H), 2.87 (d, 2 H), 3.66 (s, 3 H), 4.26 (d/d, 1 H), 5.37 (d, 1 H, NH), 6.41 (m, 1 H, NH), 6.75 (d, 2 H), 7.06 (d, 2 H). The Boc group of **39** was cleaved as described in general method B, giving L-*O*-methyltyrosine methylamide hydrochloride (5.16 g, 96%), which was suspended in CH_2Cl_2/Et_2O (80 mL, 2:1), and a stream of ammonia was passed through the mixture at 0 °C. This was filtered and the solvent evaporated at 20 °C, leaving the oily residue of *O*-(methyl)-L-tyrosine methylamide (**40**) (4.86 g, 97%), which solidified under high vacuum: mp 66–69 °C; R_f 0.38 (G); ^{1}H NMR ($CDCl_3$) δ 1.54 (m, 2 H, NH_2), 2.84 (d, 3 H), 3.05–3.17 (m, 2 H), 3.62 (s, 3 H), 4.13 (d/d, 1 H), 5.70 (d, 1 H, NH), 6.70 (d, 2 H), 7.10 (d, 2 H). Free amine **40** (2.08 g, 10 mmol) was coupled with **6** (2.67 g, 10 mmol) via the DCI coupling method according to general method A, and the crude product was purified on a silica gel column (EtOAc/MeOH, 20:0.7), giving diethyl phosphoramidate **38** (1.83 g, 69%) as a white solid: mp 79–83 °C; R_f 0.46 (D), R_f 0.71 (F); ^{1}H NMR ($CDCl_3$) δ 0.74 (m, 6 H), 1.15 (t, 6 H), 1.24–1.60 (m, 2 H), 2.67 (d, 3 H), 3.00 (d, 2 H), 3.07–3.31 (m, 2 H), 3.38–3.62 (m, 3 H), 3.59 (s, 3 H), 3.77–4.12 (m, 4 H), 4.66 (d/d, 1 H), 6.74 (d, 2 H), 7.13 (d, 2 H); FAB-MS *m/z* 458 (M + 1), 306 (100); HPLC (C-18) t_R 22.7 min, eluant A water, eluant B CH_3CN, initial composition 20% B with a 2% min^{-1} linear gradient of B, flow rate 2 mL min^{-1}.

***N*-(Diethylphosphoryl)-L-isoleucyl-L-tryptophan Methylamide (41).** With *N*-(*tert*-butyloxycarbonyl)-L-tryptophan as starting material, methylamide **42** was prepared in the same way as described above for **36**, and after purification on a silica gel column (EtOAc/MeOH, 20:0.3), amide **42** (92%) was obtained as a white solid: mp 77–79 °C; R_f 0.54 (D), R_f 0.81 (F); ^{1}H NMR (DMSO-d_6) δ 1.15 (s, 9 H), 2.64 (d, 3 H), 3.08 (m, 2 H), 4.02–4.35 (m, 1 H), 6.67–7.52 (m, 6 H, aromatics and NH), 10.84 (s, 1 H, NH). Amide **42** was deprotected as described for **39**, giving the oil L-tryptophan methylamide **43**: R_f 0.44 (G); ^{1}H NMR ($CDCl_3$) δ 1.52 (m, 2 H, NH_2), 2.64 (d, 3 H), 2.86–3.38 (m, 2 H), 3.72 (d/d, 1 H), 6.84–7.21 (m, 5 H), 7.54 (m, 1 H, NH), 8.35 (m, 1 H, NH). This was coupled with **6**, giving crude **41** as an oil. This material was purified by HPLC, on a reversed-phase (C_{18}) preparative column, giving 380 mg of **41** as a white solid: mp 107–108 °C dec;

(64) Bergmann, M.; Zervas, L. *J. Biol. Chem.* **1935**, *21*, 245–253.

R_f 0.55 (G), R_f 0.84 (K); ^{1}H NMR ($CDCl_3$/DMSO-d_6, 1:1) δ 0.82 (m, 6 H), 1.18 (t, 6 H), 1.32–1.74 (m, 2 H), 2.64 (d, 3 H), 3.00–3.65 (m, 4 H), 3.71–4.02 (m, 5 H), 4.26–4.70 (m, 2 H), 6.73–7.12 (m, 3 H), 7.14–7.51 (m, 2 H), 7.97 (d, 1 H, NH), 10.72 (s, 1 H, NH); FAB-MS m/z 467 (M + 1), 301 (100); HPLC (C_{18}) t_R 21.4 min, eluant A water, eluant B CH_3CN with initial composition 20% B and a linear gradient of 2% min^{-1}, flow rate 2.5 mL min^{-1}.

N-(Diethylphosphoryl)-L-isoleucyl-L-alanylglycyl-L-glutaminyl-N^G-nitro-L-arginylglycine Benzyl Ester (44). The title compound was prepared sequentially by two carbodiimide couplings followed by the active ester method: *N*-(*tert*-butyloxycarbonyl)-L-glutamine (5.66 g, 23 mmol) was coupled to the trifluoroacetate salt of N^G-nitro-L-arginylglycine benzyl ester[65] (10.7 g, 23 mmol), neutralized earlier with *N*-ethylmorpholine (2.93 mL), by treatment with DCC (4.95 g, 24 mmol) in DMF (30 mL) at 0 °C. This gave a crude tripeptide in 20% yield (87% pure by HPLC) after recrystallization from MeOH/H_2O mixture. Deprotection of this material (2.49 g, 4.18 mmol) as above was done accordingly to general method C, giving the trifluoroacetate salt, which was used in the active ester coupling without further purification. *N*-(Diethylphosphoryl)-L-isoleucyl-L-alanylglycine (obtained after hydrolysis of **8** by use of method D) and *N*-hydroxysuccinimide were coupled according to method A to give the corresponding succinimido ester (69%) after recrystallization from CH_2Cl_2 (mp 141–144 °C). This material was used directly in the following coupling.

To a stirred solution of the above trifluoroacetate salt (2.33 g, 3.93 mmol) in DMF (5 mL) containing triethylamine (40 mg, 3.95 mmol) was added the succinimido ester (1.93 g, 3.93 mmol). After 18 h, the mixture was diluted with EtOAc/*n*-hexane (90 mL, 1:1), causing precipitation of a crude product as a white gel. This was separated by centrifugation and chromatographed on a silica gel column (EtOAc/MeOH, 5:3). Combination of selected fractions provided 1.16 g (34%) of desired product **44**: mp 107–109 °C dec; R_f 0.14 (F), R_f 0.41 (G), R_f 0.64 (K); ^{31}P NMR (CD_3OD) 23.7°; FAB-MS m/z 857 (M + 1), 765, 312 (100); HPLC (C_{18}) t_R 37.4 min, eluant A 100 mM phosphoric acid adjusted to pH 7 with triethylamine, eluant B CH_3CN; initial composition 20% B with a 3% min^{-1} (0–30 min) and 1% min^{-1} (30–60 min) linear gradient of B, flow rate 2 mL min^{-1}.

N-(Diethylphosphoryl)-L-isoleucyl-L-alanylglycyl-5-benzyl-L-glutamyl-N^G-nitro-L-arginylglycine Benzyl Ester (45). This preparation was done as described for **44** except that the DCC/HOBt method was used in the final coupling: *N*-(*tert*-butyloxycarbonyl)-L-glutamic acid 5-(benzyl ester) (8.5 g, 25 mmol) was coupled to the trifluoroacetate salt of N^G-nitro-L-arginylglycine benzyl ester (11.63 g, 25 mmol) as described above, giving a crude tripeptide (8.7 g, 37%), which was deprotected without purification. This gave the trifluoroacetate salt (4.82 g, 76%), used in the following coupling.

To a stirred solution of *N*-(diethylphosphoryl)-L-isoleucyl-L-alanylglycine (2.77 g, 7 mmol) in DMF (5 mL) and HOBt (0.95 g, 7 mmol) was added DCC (1.5 g, 7.3 mmol) at 0 °C in two portions. This mixture was combined with a solution of the above trifluoroacetate salt in DMF (5 mL) containing triethylamine (0.1 mL, 7.4 mmol), and the reaction mixture was stirred overnight. After filtration, the solvent was removed under vacuum and the solid residue was chromatographed on a silica gel column (EtOAc/MeOH, 5:3) to give the product **45** (1.94 g, 29%) as a white foam: R_f 0.27 (F), R_f 0.63 (G), R_f 0.82 (K); ^{31}P NMR (CD_3OD) δ 21.8; FAB-MS m/z 947 (M + 1), 209 (100); HPLC (C^{18}) t_R 40.5 min, elution conditions as described for **44**.

N-(Diethylphosphoryl)-L-isoleucyl-L-alanylglycyl-L-glutaminyl-L-arginylglycine (46). A solution of **44** (0.94 g, 1.1 mmol) in MeOH (20 mL) was hydrogenated (200 mg, 10% Pd/C) at atmospheric pressure and the progress of the reaction was monitored by TLC. Starting compound (R_f 0.64 K)) was not detectable after 1 h. Two components: R_f 0.31 (K) (UV positive) and R_f 0.12 (K) (major spot, UV negative) were found after 9 h of hydrogenation. When the faster band R_f 0.31 (K) was no longer detected (14 h), the mixture was diluted with MeOH (40 mL), the catalyst was filtered off, and the solvent was evaporated. The crude product was then purified on a short (150 mm) silica gel column (*n*-propanol/water/ammonia, 50:2:6), giving the desired product **46** (0.7 g, 74%) as a white foam: R_f 0.40 (L), R_f 0.55 (M); ^{31}P NMR (D_2O/NaOD) δ 19.7; FAB-MS m/z 722 (M + 1), 263 (100); HPLC (C_{18}) t_R 29.0 min, elution conditions as described for **44**.

N-(Diethylphosphoryl)-L-isoleucyl-L-alanylglycyl-L-glutamyl-L-arginylglycine (47). Hydrogenation of **45** (1.7 g, 1.8 mmol) was done similarly to the procedure above in MeOH (40 mL) and was conducted for 11 h. The solid residue after evaporation of the solvent contained two components: R_f 0.36 (L), with absorption at ϵ_{268} 8760 characteristic for peptides containing the NO_2-N^G-Arg unit, and R_f 0.19 (L), with no absorbance at 268 nm. The faster spot corresponded to *N*-(diethylphosphoryl)-L-isoleucyl-L-alanylglycyl-L-glutamyl-N^G-nitro-L-arginylglycine (**47a**). This was purified by reversed-phase HPLC (C_{18}) at 7 mL/min using CH_3CN/water mobile phases. Two major peaks were collected and fractions lyophilized to give: 340 mg of **47a** (24%) and 563 mg of **47** (43%), respectively; FAB-MS m/z of **47a** 767 (M + 1), 219 (100); for **47** 722 (M + 1), 187 (100); ^{31}P NMR (D_2O/NaOD) δ 22.6 **47a** and 21.3 for **47**; HPLC (C_{18}) t_R of **47a** 22.4 min; t_R of **47** 179.1 min, elution conditions as described for **44**.

Preparation of Mono(ethyl esters) of Phosphoramidate Peptides (48–72). N-(Ethylphosphoryl)-L-isoleucyl-L-alanylglycine Dipotassium Salt (50). A solution of **8** (1 g, 2.06 mmol) in MeOH (20 mL) was hydrogenated (30 mg, 10% Pd/C) at atmospheric pressure for 1 h. When the catalyst was filtered off and the solvent evaporated, the carboxylic acid obtained (mp 212–215 °C) was held under high vacuum for 5 h. Further hydrolysis was done according to general method E. Following purification on a silica gel column (1-propanol/water/ammonia, 20:3:7) gave **50** (0.63 g, 69%) as a white amorphous solid (lyophilate), mp 163–169 °C. This was treated with anhydrous EtOH (15 mL) and water was added until dissolution (0.12 mL). This solvent was partially (10 mL) evaporated under vacuum at room temperature, causing crystallization of the product (0.54 g, 60%): mp 166–169 °C; FAB-MS m/z 444 (M + 1), 404 (M − K^+); ^{31}P NMR (D_2O) δ 7.04; ^{1}H NMR (D_2O) δ 0.84 (m, 6 H), 1.14 (t, 3 H), 1.32 (d, 3 H), 1.89 (m, 1 H), 3.56–3.74 (m, 5 H), 3.91 (q, 2 H), 4.17 (m, 1 H).

The following mono(ethyl esters) of phosphoramidate peptides were also prepared by this method of hydrolysis: **48** (63%), **49** (72%), **51** (47%), **52** (39%), **53** (74%), **54** (36%), **55** (59%), **56** (54%), **57** (43%), **58** (39%), and **59** (34%). Yields quoted are of the pure dipotassium or disodium salts after purification by chromatography and/or recrystallization of lyophilates.

N-(Ethylphosphoryl)-L-isoleucyl-O-[[(N-benzylamino)carbonyl]methyl]-L-tyrosylglycine Disodium Salt (62). This compound was prepared by hydrolysis of **24** (1.14 g, 1.76 mmol) according to general method E, using 2 molar equiv of 0.8 N NaOH (4.4 mL), and the progress of the hydrolysis has been monitored by TLC (systems G and J). Saponification of the carboxy-terminal methyl ester was completed after 1 h, while cleavage of the P–OEt ester required an additional 18 h at room temperature. After lyophilization of the crude reaction mixture and purification on a column (1-propanol/water/ammonia, 20:5:8.5), lyophilized product (0.83 g, 73%) was recrystallized from EtOH as described above, giving 0.71 g of **62** as a crystalline salt: mp 172–176 °C; FAB-MS m/z 651 (M + 1), 604 (M − 2Na^+), 183 (100); ^{31}P NMR (D_2O) δ 6.90; ^{1}H NMR (D_2O) δ 0.77 (m, 6 H), 1.15 (t, 3 H), 1.59 (m, 1 H), 2.93 (m, 2 H), 4.43 (s, 2 H), 6.69 (d, 2 H), 7.03 (d, 2 H), 7.19 (m, 4 H).

The following compounds were prepared analogously: **60** (42%), **61** (46%), **63** (37%), **64** (21%), **65** (40%), **66** (47%), **67** (49%), **68** (39%), **69** (7%), **70** (12%), **71** (13%), and **72** (16%). Yields quoted are of the compounds purified by flash chromatography and/or recrystallization of the lyophilates, except **68** and **69**, which were purified by HPLC.

Enzyme Preparation and Biochemical Assays. Purified human skin fibroblast procollagenase was a gift from Dr. John Jeffrey, Division of Dermatology, Washington University School of Medicine, St. Louis, MO. Procollagenase (1.2 μg) was activated to collagenase by incubating for 20 min at room temperature with trypsin (1.0 μg) in 20 μL of tris(hydroxymethyl)aminomethane hydrochloride (50 mM, pH 7.5), 10 mM in $CaCl_2$. The activation

(65) Hofmann, K.; Peckham, W.; Rheiner, A. *J. Am. Chem. Soc.* **1961**, *33*, 728–733.

was terminated by the addition of 4 μL of soybean trypsin inhibitor diluted to 2.4 μg/μL in the same buffer. Electrophoresis of our activated human skin fibroblast collagenase on a polyacrylamide gel containing gelatin[46] showed a major band (collagenase) digesting the gelatin substrate at a molecular weight estimated to be 50 kD. A faint band near the marker bovine serum albumin (68 kD) also digested the gelatin substrate and could be gelatinase. (Note that molecular weights estimated with this gelatin-containing gel are not necessarily accurate[46]). Gelatinase cleaves the thiol ester substrate at least as well as collagenase.[45] However, we estimate the amount of gelatinase in our collagenase to be negligible. The faint band observed on the gelatin polyacrylamide gel that may be gelatinase appeared to be about 1% of the intensity of the collagenase band. Human skin fibroblast gelatinase cleaves gelatin more than 30 times better than human skin fibroblast collagenase (Collier et al.[66]). Therefore, our collagenase is contaminated by at most 0.03% gelatinase, an insignificant amount.

Spectroscopic Assay. Collagenase inhibitors were assayed against human skin fibroblast collagenase using the spectrophotometric assay of Weingarten.[17] In this method cleavage of the thiol ester substrate is instantaneously and continuously detected by reaction with an excess of 4,4'-dithiodipyridine to yield 4-thiopyridone, ϵ_{324} 19 800. The assay was performed in a 0.5 cm path length quartz cuvette at 25 °C in 1.0 mL of 50 mM Hepes buffer adjusted to pH 6.5 with hydrochloric acid and 10 mM in calcium chloride, with a Cary 2200 spectrophotometer. 4,4'-Dithiopyridine was made up to 100 mM in ethanol. Inhibitors were dissolved in the buffer at a concentration at least 100 times their K_i, solubility permitting. Final concentrations of the substrate in the cuvette ranged from 70 μM to 700 μM for determinations of K_i and were either 100 μM or 200 μM for determinations of IC_{50}. Final 4,4'-Dithiodipyridine concentration was 1 mM and final enzyme concentration was 1-2 nM. K_m was found to be 3.4 ± 0.4 mM and k_{cat} 110 ± 20 s^{-1} (K_m 3.9 mM and k_{cat} 103 s^{-1} [17,45]). The substrate, an inhibitor, and the buffer were incubated in the cuvette in the spectrophotometer for several minutes to ensure temperature equilibrium before the reaction was initiated by the addition of 20 μL or less of activated enzyme. The velocity of the enzyme-catalyzed reaction was always corrected for the spontaneous hydrolysis of the thiol ester substrate, which was never more that 10% of the uninhibited enzyme catalyzed velocity at the low enzyme concentrations employed here. At high degrees of inhibition the spontaneous hydrolysis velocity became a significant proportion of the observed velocity. At the low enzyme concentrations employed, initial velocities were linear. All velocities were measured in duplicate and averaged. IC_{50}'s were determined one or more times for each inhibitor, as were K_i's. Each K_i determination involved averaging the K_i's calculated from a Lineweaver–Burk and Dixon plot. At the low substrate concentration the mode of inhibition (competitive or not) could not be determined.[67]

Amide Substrate Assay. Compounds **50**, **57**, and **68** were also assayed with the substrate Ac-Pro-Leu-Gly-Leu-Leu-Gly-OEt as used by Johnson et al.[18] to assay inhibitors against human synovial collagenase, detecting the amino group of the product, Leu-Leu-Gly-OEt, with fluorescamine. IC_{50}'s and K_i's were determined at 0.5 mM substrate in up to 1 mL of 10 mM HEPES buffer at pH 7.5 containing 10 mM calcium chloride. Enzyme concentration was 1–2 nM. After 1–3 h of reaction, 100–500-μL aliquots were diluted into 1.4 to 1.0 mL of 0.2 M boric acid adjusted to pH 8.0 with sodium hydroxide, 0.5 mL of fluorescamine in acetone (0.5 mg/mL) was added with vortexing, and the fluorescent product was measured in an Aminco filter fluorimeter with excitation at 390 nm and emission at 475–490 nm. The tripeptide Leu-Leu-Gly-OEt was used to generate a standard curve of fluorescence units per nanomole of product. K_m was 0.66 mM (lit. 1.2 mM,[45] 0.57 mM[18]), k_{cat} was 580 min^{-1} (lit.[45] 350 min^{-1}).

ACE Assay. Angiotensin converting enzyme was purified from rabbit lungs and assayed with the substrate furylacryloyl-Phe-Gly-Gly with a Cary 2200 spectrophotometer at a wavelength of 328 nm as previously described.[68,69]

Collagenase Assay. *Clostridium histolyticum* collagenase (Sigma Chemical Co. C-0773) was assayed by the spectrophotometric method of Van Wart and Steinbrink[70] using 2-furylacryloyl-L-leucylglycyl-L-prolyl-L-alanine.

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Registry No. **1**, 42990-24-9; **1a**, 42990-28-3; **1b**, 28659-87-2; **2**, 122899-89-2; **2a**, 122999-38-6; **2b**, 122999-39-7; **3a**, 122900-71-4; **3b**, 122900-72-5; **4a**, 122900-73-6; **4b**, 98992-75-7; **5a**, 122999-40-0; **5b**, 99828-55-4; **6**, 122899-90-5; **6** (benzyl ester), 122900-74-7; **7**, 122899-91-6; **8**, 122899-92-7; **9**, 122899-93-8; **10**, 122899-94-9; **11**, 122924-11-2; **12**, 122899-95-0; **13**, 122899-96-1; **14**, 122899-97-2; **15**, 122899-98-3; **16**, 122899-99-4; **17**, 122900-00-9; **18**, 122900-01-0; **19**, 122900-02-1; **20**, 122900-03-2; **21**, 122900-04-3; **22**, 122900-05-4; **23**, 122900-06-5; **24**, 122900-07-6; **25**, 122900-08-7; **26**, 122900-09-8; **27**, 122900-10-1; **28**, 122900-11-2; **29**, 122900-12-3; **30**, 122900-13-4; **31**, 122924-12-3; **32**, 122900-14-5; BOC-deblocked **32**·HCl, 122900-78-1; **33**, 122900-15-6; **34**, 122900-16-7; **35**, 122900-17-8; **36**, 96136-08-2; **37**, 101363-62-6; **38**, 122900-18-9; **39**, 122900-19-0; **40**, 96136-31-1; **40**·HCl, 105027-32-5; **41**, 122900-20-3; **42**, 122900-21-4; **43**, 53708-63-7; **44**, 122900-22-5; **45**, 122900-23-6; **46**, 122900-24-7; **47**, 122924-13-4; **47a**, 122900-88-3; **48**, 122924-14-5; **48** (free acid), 122900-46-3; **49**, 122900-25-8; **49** (free acid), 122900-47-4; **50**, 122924-15-6; **50** (free acid), 122900-48-5; **51**, 122900-26-9; **51** (free acid), 122900-49-6; **52**, 122900-27-0; **52** (free acid), 122900-50-9; **53**, 122900-28-1; **53** (free acid), 122900-51-0; **54**, 122900-29-2; **54** (free acid), 122900-52-1; **55**, 122900-30-5; **55** (free acid), 122900-53-2; **56**, 122900-31-6; **56** (free acid), 122900-54-3; **57**, 122900-32-7; **57** (free acid), 122900-55-4; **58**, 122900-33-8; **58** (free acid), 122900-56-5; **59**, 122900-34-9; **59** (free acid), 122900-57-6; **60**, 122900-35-0; **60** (free acid), 122900-58-7; **61**, 122900-36-1; **61** (free acid), 122900-59-8; **62**, 122923-55-1; **62** (free acid), 122900-60-1; **63**, 122900-37-2; **63** (free acid), 122900-61-2; **64**, 122900-38-3; **64** (free acid), 122900-62-3; **65**, 122900-39-4; **65** (free acid), 122900-63-4; **66**, 122900-40-7; **66** (free acid), 122900-64-5; **67**, 122900-41-8; **67** (free acid), 122900-65-6; **68**, 122900-42-9; **68** (free acid), 122900-66-7; **69**, 122900-43-0; **69** (free acid), 122900-67-8; **70**, 122924-16-7; **70** (free acid), 122900-68-9; **71**, 122900-44-1; **71** (free acid), 122900-69-0; **72**, 122900-45-2; **72** (free acid), 122900-70-3; $CH_2(COOEt)_2$, 105-53-3; *i*-BuBr, 78-77-3; $Me_2CHCH_2CH(COOEt)_2$, 10203-58-4; $Me_2CHCH_2CH(COOH)_2$, 4361-06-2; $Me_2CHCH_2CH_2COOH$, 646-07-1; H-D-Leu-OH, 328-38-1; H-Leu-OH, 61-90-5; AcSH, 507-09-5; H-Leu-Gly-OEt·TFA, 87215-58-5; Ac-Pro-Leu-Gly-OH, 89626-38-0; $ClP(O)(OEt)_2$, 814-49-3; H-Ile-OBzl·HCl, 103310-88-9; H-Ala-Gly-OBzl·HCl, 63649-10-5; H-Leu-Gly-OBzl·HCl, 60079-62-1; H-Phe-Gly-OBzl·HCl, 49759-88-8; H-Tyr-Gly-OBzl·HCl, 122900-75-8; H-Tyr(Bzl)-Gly-OMe·HCl, 55033-35-7; H-Tyr(BzlCl$_2$)-Gly-OMe·HCl, 122900-76-9; H-Tyr(Bzl)-Phe-OMe·HCl, 67470-40-0; H-Trp-Gly-OBzl·HCl, 112689-73-3; H-Ala-Gly-Leu-OBzl·HCl, 122900-77-0; Cbz-Tyr-Gly-OEt, 41888-91-9; BOC-Tyr-Gly-OMe, 7733-20-2; $BrCH_2COOBzl$, 5437-45-6; $BzlNH_2$, 100-46-9; $(Bzl)_2NH$, 103-49-1; H-Lys(Cbz)-OBzl, 24458-14-8; H-Phe(4-NO_2)-Gly-OMe·HCl, 122900-79-2; BOC-Tyr(Bzl)-OH, 2130-96-3; BOC-Tyr-OH, 3978-80-1; BOC-Gln-OH, 13726-85-7; H-Arg(NO_2)-Gly-OBzl·TFA, 110523-21-2; BOC-Gln-Arg(NO_2)-Gly-OBzl, 122900-80-5; H-Gln-Arg(NO_2)-Gly-OBzl·TFA, 122900-82-7; $(EtO)_2P(O)$-Ile-Ala-Gly-OH, 122900-83-8; $(EtO)_2P(O)$-Ile-Ala-Gly-ONSu, 122900-84-9; BOC-Glu(OBzl)-OH, 13574-13-5; BOC-Glu(OBzl)-Arg(NO_2)-Gly-OBzl, 122900-85-0; H-Glu(OBzl)-Arg(NO_2)-Gly-OBzl·TFA, 122900-87-2; 3,4-dihydro-2*H*-pyran, 110-87-2; collagenase, 9001-12-1.

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