Novel Peptidyl Phosphorus Derivatives as Inhibitors of Human Calpain I

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Dipeptidyl phosphorus compounds were synthesized as potential bioisosteric mimics of peptide α-ketoesters and α-ketoacids. α-Ketophosphonate Cbz-Leu-Leu-P(O)$(OCH_3)_2$ (**1b**), containing an α-ketoester bioisostere, inhibits human calpain I with an $IC_{50} = 0.43\ \mu M$. The potency of **1b** compares very favorably with that of α-ketoester Cbz-Leu-Leu-CO_2Et ($IC_{50} = 0.60\ \mu M$). Monomethyl ketophosphonate Cbz-Leu-Leu-P(O)(OH)(OCH_3) (**1a**, $IC_{50} = 5.2\ \mu M$), an α-ketoacid mimic, is less potent. Dibutyl and dibenzyl α-ketophosphonates **1c,e,f** are much less potent calpain inhibitors than dimethyl α-ketophosphonate **1b**. α-Ketophosphinate **1g** ($IC_{50} = 0.37\ \mu M$) and α-ketophosphine oxide **1h** ($IC_{50} = 0.35\ \mu M$) are also potent calpain inhibitors.

Introduction

Calpains, calcium-dependent cysteine proteases,[1] are nonlysosomal protein-degrading enzymes found in most mammalian cells. μ-Calpain, the more calcium-sensitive form, may play critical roles in several pathophysiological processes such as stroke, Alzheimer's disease, amyotrophy, motor neuron damage, and muscular dystrophy.[2]

A number of peptide-based reversible and irreversible calpain inhibitors have been reported including aldehydes, α-ketoacids, α-ketoamides, α-ketoesters, α-diketones, halomethyl ketones, epoxysuccinates, diazomethyl ketones, acyloxymethyl ketones, azapeptide esters, and α-keto heterocycles.[3,4] Reversible, substrate-based calpain inhibitors usually contain an electrophilic carbonyl group in place of the scissile amide moiety of the substrate. For example, α-ketoacids,[3f] α-ketoamides,[3f,g,i] and α-ketoesters[3e] are potent calpain inhibitors. Breuer et al. have shown that the carbonyl groups in nonpeptidic β-amino-α-ketophosphonates are electrophilic.[5] Therefore, in search of novel calpain inhibitors, we introduced α-ketophosphonates, -phosphinates, and -phosphine oxides as bioisosteric replacements of the α-dicarbonyl electrophilic center. We describe here the synthesis and evaluation of α-ketophosphonates, -phosphinates, and -phosphine oxides **1** as calpain inhibitors.

Chemistry

The synthesis of the peptidyl phosphonates, phosphinates, and phosphine oxide is illustrated in Schemes 1 and 2. The reaction of Boc-Leu-H (**2**) with dialkyl phosphites or dialkylphosphine oxide **3** and potassium fluoride in DMF at room temperature afforded diastereomeric mixtures of α-hydroxy phosphonate or phosphine oxide **4** in 75−92% yield.[6] Deprotection of **4** and coupling with Cbz-Leu-OH using BOP or DCC and HOBt activation gave dipeptide α-hydroxy phosphonate and phosphine oxide **6** in 14−71% yield after flash chromatography. Oxidation of **6** with Dess−Martin reagent[7] in methylene chloride gave α-ketophosphonates **1b**,**c** and phosphine oxide **1h**. Compounds **1g**,**i** were prepared by the same procedure as **1c**,**h** but Cbz-Leu-H was utilized as the starting material and deprotection was accomplished by hydrogenolysis over Pd/C. The α-hydroxy dimethyl phosphonate **6b** was monodemethylated with lithium bromide in acetonitrile to monomethyl phosphonate **7**.[5] Dess−Martin oxidation of **7** afforded monomethyl α-ketophosphonate **1a**.

Alternatively, as shown in Scheme 2, the dipeptide aldehyde Cbz-Val-Phe-H was directly converted to peptidyl α-hydroxy phosphonates **6d**−**f** in good yield (62−97%) with dialkyl phosphite and KF. Subsequent oxidation afforded α-ketophosphonates **1d**−**f**. No epimerization in the synthesis of compounds **1a**−**i** was observed by ^{1}H NMR analysis. The diastereomeric purity of **1d** was explicitly confirmed by synthesis of its P_1 D diastereomer. Thus, diastereomerically pure Cbz-Val-D-Phe-H was condensed with dimethyl phosphite. Subsequent Dess−Martin oxidation afforded diastereomerically pure Cbz-Val-D-Phe-P(O)$(OCH_3)_2$, which is spectroscopically distinct from **1d**. ^{1}H NMR spectroscopy disclosed no contamination of **1d** with this P_1 D diastereomer.

Results and Discussion

Peptidyl phosphorus compounds **1a**−**i** were evaluated as inhibitors of recombinant human calpain I at 20 °C, using Suc-Leu-Tyr-NMA as the substrate, as previously described.[8] The inhibitory activities of these compounds, as well as reference inhibitors Cbz-Leu-Leu-CO_2Et and Cbz-Leu-Leu-COOH, are shown in Table 1. The Cbz-Leu-Leu and Cbz-Val-Phe dipeptide sequences were selected as suitable P_1−P_2 calpain inhibitor addresses based upon the good affinity which these sequences displayed when incorporated into other reversible calpain inhibitors, including aldehydes and α-ketoamides.[3]

Dimethyl α-ketophosphonate **1b**, the bioisosteric replacement of a peptidyl α-ketoester, exhibits potent calpain inhibitory activity ($IC_{50} = 0.43\ \mu M$), comparable with that of the peptide α-ketoester Cbz-Leu-Leu-CO_2Et ($IC_{50} = 0.60\ \mu M$). However, monomethyl α-ketophosphonate **1a** is almost 10 times less potent ($IC_{50} = 5.2\ \mu M$), indicating that the free phosphonic acid functionality is poorly tolerated by the active site of calpain. In contrast, α-ketoacid Cbz-Leu-Leu-COOH is nearly 100 times *more* potent than α-ketoester Cbz-Leu-Leu-COOEt. Since α-ketoacids generally inhibit calpain

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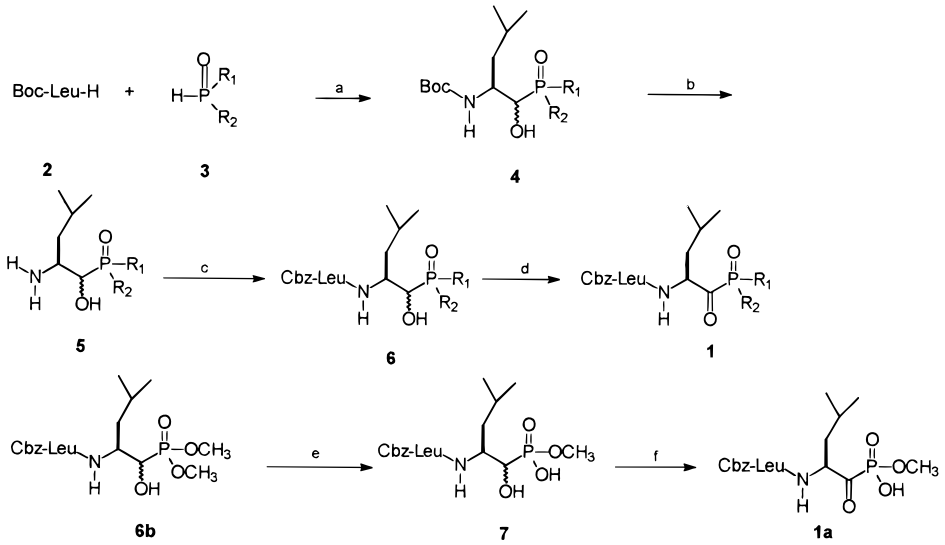
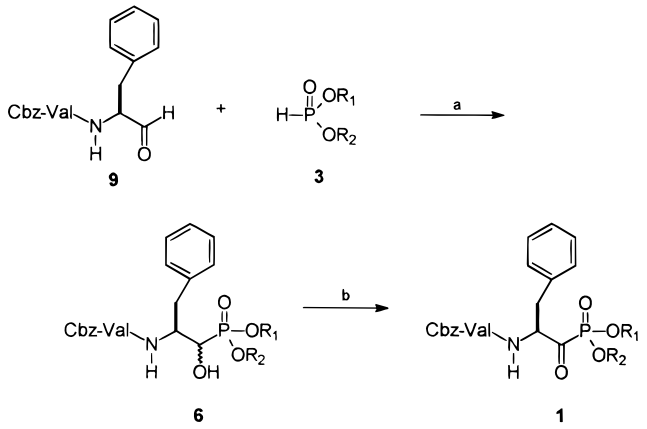
Scheme 1[a]



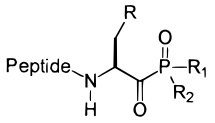
[a] Reagents: (a) KF/DMF; (b) HCl/dioxane; (c) Cbz-Leu-OH, HOBt/BOP/NMM or DCC/HOBt/*i*-Pr_2NEt/DMF; (d) Dess−Martin, CH_2Cl_2; (e) LiBr/CH_3CN; (f) Dess−Martin, CH_2Cl_2.

Scheme 2[a]



[a] Reagents: (a) KF/DMF; (b) Dess−Martin, CH_2Cl_2.

Table 1. Inhibition of Calpain I by Peptidyl Phosphonates, Phosphinates, and Phosphine Oxides[a]



1

compd	peptidyl phosphorus compound	IC_{50} (μM)
1a	Cbz-Leu-Leu-P(O)(OH)OCH_3	5.2
1b	Cbz-Leu-Leu-P(O)$(OCH_3)_2$	0.43
1c	Cbz-Leu-Leu-P(O)$(OC_4H_9)_2$	>10
1d	Cbz-Val-Phe-P(O)$(OCH_3)_2$	7.4
1e	Cbz-Val-Phe-P(O)$(OC_4H_9)_2$	>10
1f	Cbz-Val-Phe-P(O)$(OCH_2C_6H_5)_2$	6.0
1g	Cbz-Leu-Leu-P(O)(Ph)OEt	0.37
1h	Cbz-Leu-Leu-P(O)$(C_6H_4$-*p*-Cl$)_2$	0.35
1i	Cbz-Leu-Leu-P(O)$(CH_3)_2$	>10
	Cbz-Leu-Leu-CO_2Et	0.60
	Cbz-Leu-Leu-COOH	0.007

[a] $n \geq 3$ in all cases. Replicate determinations of IC_{50} agree within 25%.

more potently than their esters,[3f] the diminished affinity of monomethyl α-ketophosphonate **1a** compared with dimethyl α-ketophosphonate **1b** was unanticipated. The greater potency of α-ketoacids compared to their esters might be ascribed to Coulombic attraction with the histidine residue of the catalytic triad. Perhaps the tetrahedral geometry of phosphorus in α-ketophosphonate **1a**, in contrast to the planar-trigonal geometry of the carboxylate of an α-ketoacid, confines the anionic oxygen of the phosphonic acid to a position in which favorable interaction with the active site histidine is impossible. Variation of the peptide address had a dramatic effect on the inhibitory potencies of α-ketophosphonates **1b**,**d** toward calpain. Cbz-Val-Phe-P(O)$(OCH_3)_2$ (**1d**) is ∼17 times less potent than Cbz-Leu-Leu-P(O)$(OCH_3)_2$ (**1b**).

Dibutyl α-ketophosphonates **1c**,**e** are essentially inactive, and dibenzyl α-ketophosphonate **1f** is a weak inhibitor, indicating unfavorable P′ interactions. In contrast, butyl and benzyl α-ketoamides and α-ketoesters, as well as analogues with more sterically demanding P′ substituents, retain potency as calpain inhibitors.[3f,g,i]

The α-ketophosphine oxide functionality of **1h** was envisioned as a bioisostere of the α,β-diketone group, known to be equipotent with α-ketoesters.[3e] α-Ketophosphinate **1g** (IC_{50} = 0.37 μM) and α-ketophosphine oxide **1h** (IC_{50} = 0.35 μM) are potent calpain inhibitors. Despite their aromatic substituents, inhibitors **1g**,**h** evidently do not produce unfavorable steric interactions with calpain. In contrast, dimethyl α-ketophosphine oxide **1i** (IC_{50} > 10 μM) is essentially inactive.

In summary, α-ketophosphonate **1b**, α-ketophosphinate **1g**, and α-ketophosphine oxide **1h**, α-ketoester bioisosteres, inhibit calpain with somewhat greater potency than the corresponding α-ketoester Cbz-Leu-Leu-CO_2Et. These compounds constitute a novel and interesting class of α-dicarbonyl mimic inhibitors of calpain.

Experimental Section

General Aspects. NMR spectra were recorded in $CDCl_3$ solvent at 300 MHz using a General Electric QE Plus-300 instrument. Electrospray mass spectra were recorded on a VG platform II instrument (Fisons Instruments). Elemental analyses were performed by Quantitative Technologies Inc. of

Whitehouse, NJ. High-resolution mass spectroscopy was performed by M-Scan Inc. of West Chester, PA.

***N*-(*tert*-Butoxycarbonyl)-L-leucinal (2, Boc-Leu-H)** was prepared from (*tert*-butoxycarbonyl)-L-leucine *N*-methyl-*O*-methylcarboxamide with lithium aluminum hydride in ether according to Goel's procedure.[9a] The ^{1}H NMR spectrum agrees with the literature spectrum.[9a] The compound was stored under N_2 in a freezer prior to use.

Ethyl *N*-(benzyloxycarbonyl)-L-leucyl-L-leucinecarboxylate (Cbz-Leu-Leu-CO_2Et), prepared by the lithiated ethyl vinyl ether procedure,[3e] was hydrolyzed to *N*-(benzyloxycarbonyl)-L-leucyl-L-leucinecarboxylic acid (Cbz-Leu-Leu-CO_2H) with NaOH in methanol.

***N*-(Benzyloxycarbonyl)-L-valyl-L-phenylalaninal (9, Cbz-Val-Phe-H)**[3e,n] was prepared by coupling *N*-(benzyloxycarbonyl)-L-valine and L-phenylalanine *N*-methyl-*O*-methylamide HCl salt with isobutyl chloroformate and *N*-methylmorpholine in DMF as previously described.[9b] The resulting *N*-(benzyloxycarbonyl)-L-valyl-L-phenylalanine *N*-methyl-*O*-methylamide was reduced with lithium aluminum hydride in ether according to Goel's procedure.[9a] Crystallization from EtOAc/hexane gave one pure isomer, *N*-(benzyloxycarbonyl)-L-valyl-L-phenylalaninal (**9**): ^{1}H NMR δ 0.87 (d, J = 6 Hz, 3H), 0.94 (d, J = 6 Hz, 2H), 2.11 (septet, J = 6 Hz, 1H), 3.11 (d, 2H), 4.01 (t, 2H), 4.74 (q, 1H), 5.08 (s, 2H), 5.22 (d, 1H), 6.34 (d, 1H), 7.1−7.4 (m, 5H), 9.60 (s, 1H).

^{1}H NMR spectroscopy revealed that this sample was diastereomerically pure by comparison with ***N*-(benzyloxycarbonyl)-L-valyl-D-phenylalaninal (Cbz-Val-D-Phe-H):** ^{1}H NMR δ 0.83 (d, J = 6 Hz, 3H), 0.88 (d, J = 6 Hz, 2H), 2.09 (septet, J = 6 Hz, 1H), 3.11 (d, 2H), 4.02 (t, 2H), 4.75 (q, 1H), 5.08 (s, 2H), 5.20 (d, 1H), 6.35 (d, 1H), 7.1−7.4 (m, 5H), 9.60 (s, 1H). In pH 7.0 phosphate buffer (50 mM), Cbz-Val-Phe-H epimerizes to Cbz-Val-D-Phe-H with a half-life of 4 days at 32 °C.

Representative Procedures for the Preparation of Compounds 4 and 6d−f. Method A: Dimethyl (*2S*)-2-[(*tert*-Butoxycarbonyl)amino]-1-hydroxy-4-methylpentylphosphonate (4b). 4b was prepared from *N*-(*tert*-butoxycarbonyl)-L-leucinal (Boc-L-Leu-H) (2.15 g, 10 mmol) and dimethyl phosphite (1.10 g, 10 mmol) with KF (2.91 g, 50 mmol) in DMF at room temperature for 24 h according to the procedure described by Texier-Boullet et al.[6] Purification by flash chromatography (10% MeOH in CH_2Cl_2) afforded 3.0 g (92%) of **4b**: ^{1}H NMR δ 0.94 (d, 6H), 1.42 (s, 9H), 1.68 (m, 1H), 3.82 (m, 7H); MS m/z 348 (M + Na)$^+$.

Dibutyl (*2S*)-2-[(*tert*-butoxycarbonyl)amino]-1-hydroxy-4-methylpentylphosphonate (4c): *N*-(*tert*-butoxycarbonyl)-L-leucinal (Boc-Leu-H), dibutyl phosphite, 24 h; yield, 42%; ^{1}H NMR δ 0.93 (m, 12H), 1.30−1.77 (m, 11H), 1.42 (s, 9H), 3.95 (m, 1H), 4.15(m, 4H), 4.42 (m, 1H); MS m/z 410 (M + H)$^+$.

Ethyl (2*S*)-[2-[(benzyloxycarbonyl)amino]-1-hydroxy-4-methylpentyl](phenyl)phosphinate (4g): *N*-(benzyloxycarbonyl)-L-leucinal (Cbz-Leu-H), ethyl phenylphosphinate,[10] 23 h; yield, 75%; MS 420 m/z (M + H)$^+$, 442 (M + Na)$^+$.

(2*S*)-[2-[(*tert*-Butoxycarbonyl)amino]-1-hydroxy-4-methylpentyl]bis(4-chlorophenyl)phosphine oxide (4h): *N*-(*tert*-butoxycarbonyl)-L-leucinal (Boc-Leu-H), bis(4-chlorophenyl)phosphine oxide (Organometallics, Inc.), 2.5 h; yield, 83%; MS m/z 486 (M + H)$^+$.

(2*S*)-[2-[(*tert*-Butoxycarbonyl)amino]-1-hydroxy-4-methylpentyl](dimethyl)phosphine oxide (4i): *N*-(*tert*-Butoxycarbonyl-L-leucinal (Boc-Leu-H), dimethylphosphine oxide,[11] 2.5 h; yield, 75%; MS m/z 316 (M + Na)$^+$.

Representative Procedures for the Preparation of Compounds 5 and 6b,c,g−i. Method B: Dimethyl *N*-(Benzyloxycarbonyl)-L-leucyl-(*2S*)-2-amino-1-hydroxy-4-methylpentylphosphonate (6b). Dimethyl (*2S*)-2-[(*tert*-butoxycarbonyl)amino]-1-hydroxy-4-methylpentylphosphonate (**4b**) was treated with 4 N HCl in dioxane for 2 h. The solvent was evaporated in vacuo, and the residue was triturated with diethyl ether. The crude white solid, dimethyl (*2S*)-2-amino-1-hydroxy-4-methylpentylphosphonate HCl salt (**5b**), was used directly for the next step.

To a solution of *N*-(benzyloxycarbonyl)-L-leucine (Cbz-Leu-OH) (2.0 mmol) in DMF (4 mL) was added dimethyl 2-amino-1-hydroxy-4-methylpentylphosphonate HCl salt (2.0 mmol) (**5b**), *i*-Pr_2NEt or NMM (4.0 mmol), HOBt (2.0 mmol), and DCC or BOP (2.0 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 5 min and for 2−24 h at room temperature. Dicyclohexylurea was filtered off (when DCC was used), and the solvent was evaporated. The residue was dissolved in ethyl acetate (20 mL) and washed with 3% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$. Purification by flash chromatography (5% MeOH in CH_2Cl_2) gave 0.65 g (69%) of **6b**: ^{1}H NMR δ 0.90 (t, 12H), 1.41−1.80 (m, 6H), 3.80 (d, 6H), 4.02 (m, 2H), 4.22 (m, 1H), 4.95 (m, 1H), 5.10 (s, 2H), 5.39 (d, 1H), 6.92 (d, 1H), 7.39 (s, 5H); MS m/z 473 (M + H)$^+$.

The following compounds were prepared according to the representative procedures above. The starting materials, reaction times, purification methods, and yields are given in abbreviated format.

Dibutyl *N*-(benzyloxycarbonyl)-L-leucyl-(2S)-2-amino-1-hydroxy-4-methylpentylphosphonate (6c): method B, *N*-(benzyloxycarbonyl)-L-leucine (Cbz-Leu-OH), dibutyl (*2S*)-2-amino-1-hydroxy-4-methylpentylphosphonate HCl salt (**5c**), 36 h, flash chromatograghy (40% hexane in EtOAc); yield, 71%; MS m/z 557 (M + H)$^+$, 579 (M + Na)$^+$.

Dimethyl *N*-(benzyloxycarbonyl)-L-valyl-(*2S*)-2-amino-1-hydroxy-3-phenylpropylphosphonate (6d): method A, *N*-(benzyloxycarbonyl)-L-valyl-L-phenylalaninal (Cbz-Val-Phe-H), dimethyl phosphite, 24 h; yield, 97%; ^{1}H NMR δ 0.70−0.94 (m, 6H), 2.08 (m, 1H), 3.04 (m, 2H), 3.63−4.21 (m, 9H), 5.12 (s, 2H), 5.38 (m, 1H), 5.43 (d, 1H), 7.02 (d, 1H), 7.16−7.35 (m, 10H); MS m/z 515 (M + Na)$^+$.

Dibutyl *N*-(benzyloxycarbonyl)-L-valyl-(*2S*)-2-amino-1-hydroxy-3-phenylpropylphosphonate (6e): method A, *N*-(benzyloxycarbonyl)-L-valyl-L-phenylalaninal (Cbz-Val-Phe-H), dibutyl phosphite, 24 h; yield, 84%; ^{1}H NMR δ 0.90 (m, 16H), 1.36 (m, 3H), 1.63 (m, 3H), 2.12 (m, 1H), 3.04 (m, 1H), 3.90−4.34 (m, 5H), 5.10 (m, 3H), 5.34 (d, 1H), 6.76 (d, 1H), 7.27−7.36 (m, 10H); MS m/z 577 (M + H)$^+$.

Dibenzyl *N*-(benzyloxycarbonyl)-L-valyl-(*2S*)-2-amino-1-hydroxy-3-phenylpropylphosphonate (6f): method A, *N*-(benzyloxycarbonyl)-L-valyl-L-phenylalaninal (Cbz-Val-Phe-H), dibenzyl phosphite, 24 h; yield, 62%; ^{1}H NMR δ 0.82 (d, 3H), 0.84 (d, 3H), 2.03 (m, 1H), 2.98 (m, 2H), 3.97 (m, 2H), 4.22 (m, 1H), 4.67 (m, 1H), 4.92−5.20 (m, 6H), 5.25 (d, 1H), 6.75 (d, 1H), 7.05−7.34 (m, 20H); MS m/z 645 (M + H)$^+$. Anal. ($C_{36}H_{41}N_2O_7P$) C, H, N.

Ethyl [*N*-(benzyloxycarbonyl)-L-leucyl-(2*S*)-2-amino-1-hydroxy-4-methylpentyl](phenyl)phosphinate (6g): hydrogenation over 10% Pd/C in ethanol; yield, 81%; product, **5g**; MS m/z 286 (M + H)$^+$. Method B: *N*-(benzyloxycarbonyl)-L-leucine (Cbz-Leu-OH), ethyl (2*S*)-(2-amino-1-hydroxy-4-methylpentyl)(phenyl)phosphonate (**5g**), 4 h, flash chromatography (ethyl acetate); yield, 67%; product, **6g**; MS m/z 533 (M + H)$^+$, 555 (M + Na)$^+$.

[(Benzyloxycarbonyl)-L-leucyl-(2*S*)-2-amino-1-hydroxy-4-methylpentyl]bis(4-chlorophenyl)phosphine oxide (6h): method B, (2*S*)-[2-[(*tert*-butoxycarbonyl)amino]-1-hydroxy-4-methylpentyl]bis(4-chlorophenyl)phosphine oxide (**4h**) was treated with 25% trifluoroacetic acid in dichloromethane for 1 h; yield, 97%; product, **5h**; MS m/z 386 (M+H)$^+$. *N*-(Benzyloxycarbonyl)-L-leucine (Cbz-Leu-OH), [(2*S*)-2-amino-1-hydroxy-4-methylpentyl]bis(4-chlorophenyl)phosphine oxide (**5h**), 14 h, flash chromatography (50% ethyl acetate/hexane); yield, 48%; product, **6h**; MS m/z 633 (M + H)$^+$, 655 (M + Na)$^+$.

[*N*-(Benzyloxycarbonyl)-L-leucyl-(2*S*)-2-amino-1-hydroxy-4-methylpentyl](dimethyl)phosphine oxide (6i): hydrogenation over 10% Pd/C in ethanol; product, **5i**; MS m/z 194 (M + H)$^+$. *N*-(Benzyloxycarbonyl)-L-leucine (Cbz-Leu-OH), [(2*S*)-2-amino-1-hydroxy-4-methylpentyl](dimethyl)phosphine oxide (**5i**), 14 h, flash chromatography (50% ethyl acetate/hexane); yield, 41%; product, **6i**; MS m/z 463 (M + Na)$^+$.

Methyl Hydrogen *N*-(Benzyloxycarbonyl)-L-leucyl-

(2*S*)-2-amino-1-hydroxy-4-methylpentylphosphonate (7). A solution of dimethyl *N*-(benzyloxycarbonyl)-L-leucyl-(2*S*)-2-amino-1-hydroxy-4-methylpentylphosphonate (**6b**) (0.3 mmol, 142 mg) in CH_3CN (1.2 mL) was stirred at room temperature under Ar as lithium bromide (0.95 mmol, 25 mg) was added. The reaction mixture was stirred overnight. The solvent was removed, and the crude product **7** was used directly for the next step.

Methyl hydrogen *N*-(benzyloxycarbonyl)-L-leucyl-L-leucylphosphonate (1a): method C, methyl hydrogen *N*-(benzyloxycarbonyl)-L-leucyl-(2*S*)-2-amino-1-hydroxy-4-methylpentylphosphonate (**7**), Dess−Martin periodinane, 16 h; yield, 65%; mp 57 °C dec; ^{1}H NMR δ 0.92 (m, 6H), 1.40−1.78 (m, 6H), 3.81 (m, 6H), 4.02 (m, 1H), 4.22 (m, 1H), 5.12 (s, 2H), 5.40 (m, 1H), 7.38 (s, 5H); MS *m*/*z* 455 $(M - H)^-$. Anal. ($C_{21}H_{33}N_2O_7P$) C, H, N.

Dimethyl *N*-(Benzyloxycarbonyl)-L-leucyl-L-leucylphosphonate (1b). Method C: A solution of *N*-(benzyloxycarbonyl)-L-leucyl-(2*S*)-2-amino-1-hydroxy-4-methylpentylphosphonate (**6b**) (94.4 mg, 0.2 mmol) and *t*-BuOH (34.5 mg, 0.41 mmol) in CH_2Cl_2 (5 mL) was stirred as Dess−Martin periodinane (169.6 mg, 0.4 mmol) was added. The reaction was stirred at room temperature for 2−3 h. The reaction progress was followed by TLC. The reaction mixture was diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed with 10% $Na_2S_2O_3$ and dried over $MgSO_4$ to afford 80.0 mg (85%) of **1b**: ^{1}H NMR δ 0.90 (t, 12H), 1.41−1.80 (m, 6H), 3.80 (d, 6H), 4.22 (m, 1H), 4.86 (m, 1H), 5.10 (m, 3H), 6.51 (d, 1H), 7.34 (s, 5H); MS *m*/*z* 471 $(M + H)^+$; FAB *m*/*z* $(M + H)^+$ calcd for ($C_{22}H_{36}N_2O_7P$) 471.2260, observed *m*/*z* 471.2274.

Dibutyl *N*-(benzyloxycarbonyl)-L-leucyl-L-leucylphosphonate (1c): method C, dibutyl *N*-(benzyloxycarbonyl)-L-leucyl-(2*S*)-2-amino-1-hydroxy-4-methylpentylphosphonate (**6c**), Dess−Martin periodinane; 5 h, yield, 13% (after preparative HPLC); ^{1}H NMR δ 0.90 (m, 22H), 1.42 (m, 5H), 1.70 (m, 5H), 4.20 (m, 5H), 4.98 (m, 1H), 5.18 (s, 2H), 5.20 (d, 1H), 6.61 (d, 1H), 7.40 (s, 5H); MS *m*/*z* 555 $(M + H)^+$, 577 $(M + Na)^+$. Anal. ($C_{28}H_{47}N_2O_7P \cdot 0.6H_2O$) C, H, N.

Dimethyl *N*-(benzyloxycarbonyl)-L-valyl-L-phenylalanylphosphonate (1d): method C, dimethyl *N*-(benzyloxycarbonyl)-L-valyl-(2*S*)-2-amino-1-hydroxy-3-phenylpropylphosphonate (**6d**), Dess−Martin periodinane, 2 h; yield, 88%; ^{1}H NMR δ 0.79 (d, J = 6.5 Hz, 6H), 0.87 (d, J = 6.5 Hz, 6H), 2.07 (m, 1H), 3.01 (dd, 1H), 3.30 (dd, 1H), 3.82 (d, J = 4.5 Hz, 3H), 3.85 (d, J = 4.5 Hz, 3H), 4.05 (m, 1H), 5.06 (d, 2H), 5.12 (m, 1H), 5.41 (d, NH), 6.88 (d, 1H), 7.11−7.40 (m, 10H); MS *m*/*z* 513 $(M + Na)^+$. Anal. ($C_{24}H_{31}N_2O_7P$) C, H, N.

Dimethyl *N*-(benzyloxycarbonyl)-L-valyl-D-phenylalanylphosphonate was prepared by the same procedure from *N*-(benzyloxycarbonyl)-L-valyl-D-phenylalaninal (Cbz-Val-D-Phe-H): ^{1}H NMR δ 0.74 (d, J = 6.8 Hz, 6H), 0.82 (d, 6.8 Hz, 6H), 2.03 (m, 1H), 3.05 (dd, 1H), 3.31 (dd, 1H), 3.82 (d, J = 6.0 Hz, 3H), 3.86 (d, J = 6.0 Hz, 3H) 4.02 (m, 1H), 5.09 (s, 2H), 5.16 (m, 1H), 6.41 (d, 1H), 7.15−7.38 (m, 11H).

Dibutyl *N*-(benzyloxycarbonyl)-L-valyl-L-phenylalanylphosphonate (1e): method C, dibutyl *N*-(benzyloxycarbonyl)-L-valyl-(2*S*)-2-amino-1-hydroxy-3-phenylpropylphosphonate (**6e**), Dess−Martin periodinane, 14 h; yield, 84%; ^{1}H NMR δ 0.88 (m, 12H), 1.40 (m, 4H), 1.62 (m, 4H), 2.03 (m, 1H), 3.20 (ABq, 2H), 4.12 (m, 5H), 4.98 (m, 1H), 5.22 (m, 2H), 5.55 (d, 1H), 6.80 (d, 1H), 7.11−7.40 (m, 10H); MS *m*/*z* 577 $(M + H)^+$, 599 $(M + Na)^+$. Anal. ($C_{30}H_{439}N_2O_7P \cdot 0.4CH_2Cl_2$) C, H, N.

Dibenzyl *N*-(benzyloxycarbonyl)-L-valyl-L-phenylalanylphosphonate (1f): method C, dibenzyl *N*-(benzyloxycarbonyl)-L-valyl-(2*S*)-2-amino-1-hydroxy-3-phenylpropylphosphonate (**6f**), Dess−Martin periodinane, 1 h; yield, 62%; ^{1}H NMR δ 0.76 (d, 2H), 0.855 (d, 2H), 2.04 (m, 1H), 3.11 (ABq, 2H), 3.95 (m, 1H), 5.16 (m, 5H), 6.34 (d, 1H), 7.03 (d, 1H), 7.20−7.33 (m, 10H); MS *m*/*z* 643 $(M + H)^+$, 665 $(M + Na)^+$. Anal. ($C_{36}H_{39}N_2O_7P \cdot 1.5H_2O$) C, H, N.

Ethyl [*N*-(benzyloxycarbonyl)-L-leucyl-L-leucyl](phenyl)phosphinate (1g): method C, ethyl [(benzyloxycarbonyl)-L-leucyl-(2*S*)-2-amino-1-hydroxy-4-methylpentyl](phenyl)phosphinate (**6g**), Dess−Martin periodinane, 4 h; yield, 92%; ^{1}H NMR δ 0.92 (m, 12H), 1.40 (t, 3H), 1.40−1.80(m, 6H), 4.20 (m, 3H), 5.21 (m, 3H), 6.58 (d, 1H), 6.82 (d, 1H), 7.18−7.88 (m, 10H); MS *m*/*z* 531 $(M + H)^+$, 553 $(M + Na)^+$. Anal. ($C_{28}H_{39}Cl_2N_2O_6P \cdot 0.3\ H_2O$) C, H, N.

[(Benzyloxycarbonyl)-L-leucyl-L-leucyl]bis(4-chlorophenyl)phosphine oxide (1h): method C, [*N*-(benzyloxycarbonyl)-L-leucyl-(2*S*)-2-amino-1-hydroxy-4-methylpentyl]bis(4-chlorophenyl)phosphine oxide (**6h**), Dess−Martin periodinane, 1 h; yield, 30%; ^{1}H NMR δ 0.90 (dd, 6H), 1.21−1.75 (m, 6H), 4.10 (m, 1H), 5.02 (m, 3H), 7.20−7.65 (m, 13H); MS *m*/*z* 631 $(M + H)^+$, 652, $(M + Na)^+$. Anal. ($C_{32}H_{37}Cl_2N_2O_5P \cdot 1.5H_2O$) C, H, N.

***[N*-(Benzyloxycarbonyl)-L-leucyl-L-leucyl](dimethyl)phosphine oxide (1i):** method C, [*N*-(benzyloxycarbonyl)-L-leucyl-(2*S*)-2-amino-1-hydroxy-4-methylpentyl](dimethyl)phosphine oxide (**6i**), Dess−Martin periodinane, 3 h; yield, 87%; ^{1}H NMR δ 0.91 (bs, 12H), 1.41−1.75 (m, 12H), 4.12 (m, 1H), 4.57 (m, 1H), 5.15 (s, 2H), 5.32 (d, 1H), 6.42 9d, 1H), 7.33 (s, 5H). MS *m*/*z* 461 $(M + Na)^+$. Anal. ($C_{22}H_{35}Cl_2N_2O_5P \cdot 0.5H_2O \cdot 0.2CH_2CH_2$) C, H, N.

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