were found to extract quantitatively from plasma with acetonitrile and to inhibit the reconstituted renin assay in a manner similar to the initial primary test system.

Acknowledgment. The assistance of Mary Jo Leveque and Karen Papp with the solubility determinations, Gary Young with the rat experiments, and Carl Nordeen with preparation of **5b** is gratefully acknowledged.

Supplementary Material Available: Tables of fractional coordinates, bond angles, intramolecular distances, and thermal parameters (8 pages). Ordering information is given on any current masthead page.

Inhibition of Aminopeptidases by Peptides Containing Ketomethylene and Hydroxyethylene Amide Bond Replacements

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Inhibitors of aminopeptidase enzymes have been prepared by the synthesis of peptide substrate analogues in which the scissile amide bond has been replaced with the hydrolytically stable ketomethylene ($-COCH_2-$) and hydroxyethylene [$-CH(OH)CH_2-$] functionalities. Two synthetic strategies were used to prepare the inhibitors, and the advantages and disadvantages of each are discussed. The synthesis of peptides that contain the hydroxyethylene isostere was complicated by competing lactone and lactam formation, and attempts to prepare free N-terminal dipeptide hydroxyethylene isostere derivatives were unsuccessful. All ketomethylene isosteres examined were weak inhibitors of both leucine aminopeptidase and aminopeptidase M. However, the ketomethylene inhibitor $Lys^{K}(RS)Phe$ (**58**) (K_i = 4 nM) is a potent inhibitor comparable to the natural product, arphamenine A ($Arg^{K}Phe$; K_i = 2.5 nM). Normal Michaelis–Menten kinetics for inhibition of membrane leucine aminopeptidase are observed in the absence of magnesium ion, but nonlinear kinetics were obtained in the presence of Mg^{2+}.

The aminopeptidases are a diverse group of enzymes that catalyze the hydrolysis of amino-terminal residues from peptide substrates. Since these enzymes appear to be involved in important biological processes, compounds that inhibit the aminopeptidases may have therapeutic applications. Several naturally occurring aminopeptidase inhibitors have been isolated, and their structures are shown in Table I. Bestatin (1), a potent inhibitor of the three enzymes shown in Table I and of other aminopeptidases,[5-9] has been reported to produce important in vitro and in vivo effects. Studies in which bestatin enhances the delayed-type hypersensitivity response in mice to sheep red blood cells[10] suggest that bestatin modulates immune activity. Bestatin also stimulates polysome assembly in T-cell lymphoma (grown in suspension),[11] increases [^{3}H]thymidine incorporation into lymphocytes,[12] and shows promising results in clinical trials with human cancer patients.[13] Other studies have shown that bestatin inhibits metabolism of opioid peptides[14] and potentiates antinociceptive activity.[15] These results suggest that new, potent inhibitors of aminopeptidases may have important medicinal applications.

Our approach to new inhibitors of aminopeptidases was to replace the scissile amide bond in a substrate with a functionality that is hydrolytically stable. Different moieties have been used as isosteric amide bond replacements to provide inhibitors of proteolytic enzymes (Table II).[16-19] Compound **6** is from the work of Szelke and collaborators, who prepared renin inhibitors from substrates by replacing amide bonds with one of four isosteric linkages: the reduced amide bond or aminomethylene group ($-CH_2NH-$); the ketomethylene group ($-COCH_2-$); the hydroxyethylene group [$-CH(OH)CH_2-$]; and the ethylene group ($-CH_2CH_2-$).[17] The hydroxyethylene and ketomethylene compounds **8** and **9** had been prepared as inhibitors of pepsin.[18] When we began work on the preparation of aminopeptidase inhibitors with altered amide bonds, only one example of this approach had been

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(1) Abbreviations: APB, aminopeptidase B or arginyl aminopeptidase; APM, aminopeptidase M or membrane leucine aminopeptidase; Boc, *tert*-butyloxycarbonyl; Cbz, benzyloxycarbonyl; DMAP, (dimethylamino)pyridine; DMF, dimethylformamide; DTT, dithiothreitol; EDC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HMPA, hexamethylphosphoramide; HOBt, 1-hydroxybenzotriazole hydrate; IPA, isopropyl alcohol; LAP, leucine aminopeptidase; MOPS, 4-morpholinepropanesulfonic acid; MPLC, medium-pressure liquid chromatography; NMM, *N*-methylmorpholine; PDC, pyridinium dichromate; RP-HPLC, reversed-phase high-performance liquid chromatography; RT, retention time; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin-layer chromatography; Tris, tris(hydroxymethyl)aminomethane; TsOH, *p*-toluenesulfonic acid; UV, ultraviolet.

(2) Umezawa, H.; Aoyagi, T.; Suda, H.; Hamada, M.; Takeuchi, T. *J. Antiobiot.* **1976**, *29*, 97-99.
(3) Aoyagi, T.; Tobe, H.; Kojima, F.; Hamada, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1978**, *31*, 636-638.
(4) (a) Umezawa, H.; Aoyagi, T.; Ohuchi, S.; Okuyama, A.; Suda, H.; Takita, T.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1983**, *36*, 1572-1575. (b) Umezawa, H.; Nakamura, T.; Fukatsu, S.; Aoyagi, T.; Tatsuta, K. Ibid. **1983**, *36*, 1787-1788.
(5) Hui, K.-S.; Wang, Y.-J.; Lajtha, A. *Biochemistry* **1983**, *22*, 1062-1067.
(6) Röhm, K.-H. *Hoppe-Seyler's Z. Physiol. Chem.* **1982**, *363*, 641-649.
(7) Kawata, S.; Imamura, T.; Ninomiya, K.; Makisumi, S. *J. Biochem.* **1982**, *92*, 1093-1011.
(8) Mantle, D.; Lauffart, B.; McDermott, J. R.; Kidd, A. M.; Pennington, R. J. T. *Eur. J. Biochem.* **1985**, *146*, 307-312.
(9) Morgan, J. G.; Donlon, J. *Eur. J. Biochem.* **1985**, *146*, 429-435.
(10) Umezawa, H.; Ishizuka, M.; Aoyagi, T.; Takeuchi, T. *J. Antibiot.* **1976**, *29*, 857-859.
(11) Mueller, W. E. G.; Zahn, R. K.; Maidof, A.; Umezawa, H. *Cold Spring Harbor Conf. Cell Proliferation* **1979**, *2*, 429-454.
(12) Ishizuka, M.; Masuda, T.; Kanbayashi, N.; Fukasawa, S.; Takeuchi, Y.; Aoyagi, T.; Umezawa, H. *J. Antibiot.* **1980**, *33*, 642-652.

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Table I. Some Naturally Occurring Inhibitors of Aminopeptidases

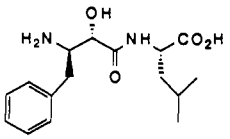
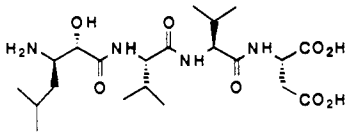
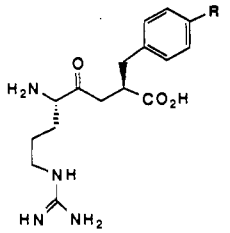
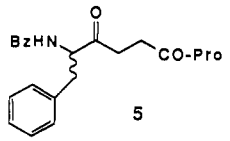
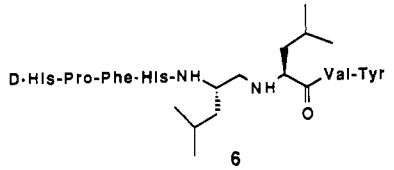
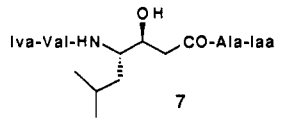
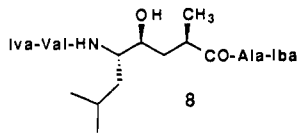
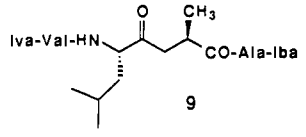
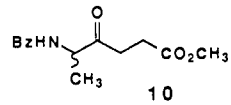
	K_i		
	APM	LAP	APB
 bestatin[2] (1)	4.4 μM	20 nM	14 nM
 amastatin[3] (2)	20 nM	0.22 μM	
 arphamenine A (R = H)[4] (3)		>100 μg/mL	2.5 nM
arphamenine B (R = OH)[4] (4)		>100 μg/mL	0.84 nM

Table II. Examples of Enzyme Inhibitors That Contain Amide Bond Replacements

compd	enzyme	K_i (μM)
 5	ACE[a]	0.1[16]
 6	renin	1.0[17]
 7	pepsin	0.003[18]
 8	pepsin	0.003[18]
 9	pepsin	0.11[18]
 10	PPE[b]	4200[19]

[a] Angiotensin converting enzyme. [b] Porcine pancreatic elastase.

described, that of phenylalanine methyl ketone,[20] which is a poor inhibitor of leucine aminopeptidase (K_i = 2 mM).

(13) Umezawa, H. *Drugs Exp. Clin. Res.* **1984**, *10*, 519–531.

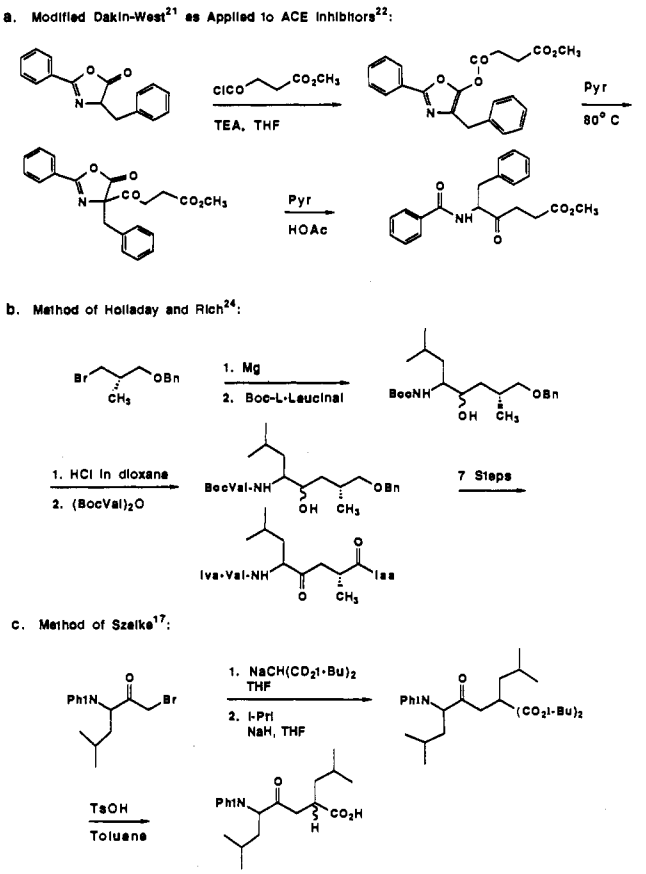


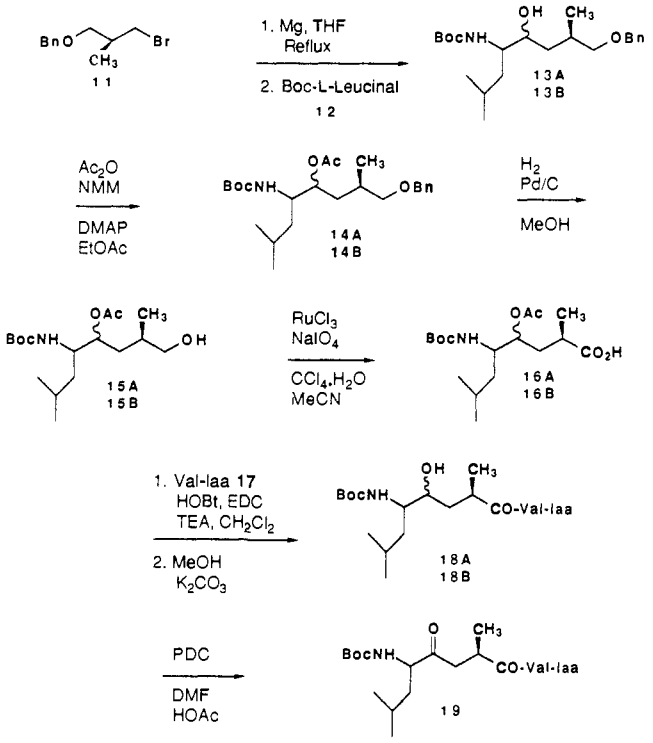
Figure 1. Synthetic routes for the preparation of ketomethylene peptide isosteres.

In the course of our work, however, the arphamenines were shown to be ketomethylene dipeptide isosteres of L-arginyl-L-phenylalanine and L-arginyl-L-tyrosine.[4] Arphamenines **3** and **4** (Table I) are potent inhibitors of arginyl aminopeptidase but do not inhibit leucine aminopeptidase or membrane leucine aminopeptidase.

In this paper we report the synthesis of aminopeptidase inhibitors that were designed by replacing substrate amide bonds with nonhydrolyzable amide bond replacements and the inhibitory potency of these compounds with leucine aminopeptidase, membrane leucine aminopeptidase, and arginyl aminopeptidase.[1] Two different synthetic methodologies were used to prepare these compounds, and the advantages and disadvantages of each method will be discussed. Additional problems with the stability of these

(14) Carenzi, A.; Frigeni, V.; Della-Bella, D. In *Advances in Endogenous and Exogenous Opioids*; Takagi, H., Simon, E. J., Eds.; Elsevier/North-Holland Press: Amsterdam, 1981; p 267 ff.
(15) McKnight, A. T.; Corbett, A. D.; Kosterliz, H. W. *Eur. J. Pharmacol.* **1983**, *86*, 393–402.
(16) Almquist, R. G.; Chao, W.-R.; Ellis, M. E.; Johnson, H. L. *J. Med. Chem.* **1980**, *23*, 1392–1398.
(17) Szelke, M.; Jones, D. M.; Hallett, A. Eur. Pat. Appl. EP 45665, 1982; *Chem. Abstr.* **1982**, *97*, 39405.
(18) Rich, D. H.; Salituro, F. G.; Holladay, M. W.; Schmidt, P. G. In *Conformationally Directed Drug Design*; Vida, J. A., Gordon, M., Eds.; ACS Symposium Series 251; American Chemical Society: Washington, DC, 1984; pp 211–237.
(19) Hori, H.; Yasutake, A.; Minematsu, Y.; Powers, J. C. In *Peptides: Structure and Function. Proceedings of the Ninth American Peptide Symposium* Deber, D. M., Hruby, V. J.; Kopple, K. D., Eds.; Pierce Chemical Co.: Rockford, IL, 1985; pp 812–822.
(20) Fittkau, S. *Wiss. Z.-Martin-Luther-Univ. Halle-Wittenberg, Math.-Naturwiss. Reihe* **1978**, *27*, 81–88.

Scheme I

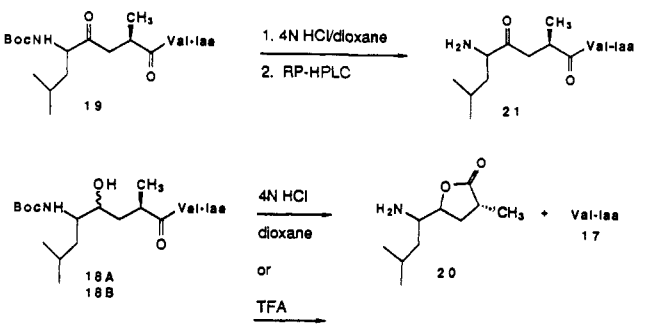


compounds were encountered, and these results will be presented. Although some of the compounds are good inhibitors of leucine aminopeptidase and membrane leucine aminopeptidase, only arginyl aminopeptidase was potently inhibited by peptides incorporating amide bond replacements.

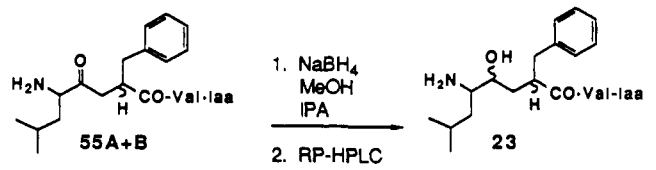
Results

Syntheses. Three procedures have been reported for synthesizing ketomethylene containing peptides (Figure 1). A modified Dakin–West reaction[21] was used by Almquist[22] and Powers[23] to synthesize enzyme inhibitors, but since an achiral intermediate is formed during the reaction, only totally racemic products are obtained via this route (Figure 1a). We have reported a procedure (Figure 1b) that yields products of defined stereochemistry[24] since the asymmetric centers in both the aldehyde and the Grignard reagent are defined. This synthetic strategy was investigated for the preparation of ketomethylene inhibitors of aminopeptidases. The third procedure shown (Figure 1c) was used by Szelke[17] for the synthesis of renin inhibitors, and a modification of this approach was later applied by Umezawa et al. for the synthesis of the arphamenines.[4a] Only the stereochemistry of the bromomethyl ketone is defined; therefore, this procedure gives a product that is composed of two diastereomers. In spite of this disadvantage, we used this methodology to synthesize many of the inhibitors reported herein. The reasons for pursuing this route will be explained.

Scheme II



Scheme III



This first attempt to prepare the desired ketomethylene inhibitors is shown in Scheme I. The chiral bromide **11** was synthesized according to the method of Evans[25] and then was converted to the Grignard reagent and allowed to react with Boc-L-leucinal (**12**).[26] Purification of the product by MPLC separated diastereomers **13A** and **13B**, which had been previously assigned as the 2*R*,4*S*,5*S* and 2*R*,4*R*,5*S* isomers, respectively.[24]

The conversion of **13A** and **13B** to the N-protected carboxylic acids **16A** and **16B** was accomplished in three steps without purification of the intermediate products. The secondary hydroxyl was protected by acetylation and the benzyl ether removed by hydrogenolysis to provide the primary alcohols **15A** and **15B**. These were oxidized to the acids **16A** and **16B** in good yield by using ruthenium chloride.[27] [Attempted oxidation with PDC[28] failed to give any of the desired product, whereas oxidation with potassium permanganate under phase-transfer conditions[29] yielded the desired acids but only in low yields (5%–15%)]. Acids **16A** and **16B** were coupled to L-valine isoamylamide (**17**),[30] and the acetate was removed by methanolysis to provide diastereomers **18A** and **18B**. The protected ketomethylene-containing isostere **19** was obtained by oxidation of alcohol **18A** with PDC.[28]

Deprotection of the protected tetrapeptide hydroxyethylene (**18A** and **18B**) and ketomethylene (**19**) peptides was carried out as shown in Scheme II. The Boc group in **19** was cleaved with 4 N HCl in dioxane,[31] and after purification, the tetrapeptide analogue **21** was obtained in 60% yield as the TFA salt. However, acidolytic cleavage of **18A** and **18B** did not produce the desired product. Instead, treatment with either 4 N HCl in dioxane or TFA afforded two major products, valine isoamylamide **17** (identified by comparison with an authentic sample) and lactone **20**. A hydroxyethylene isostere (**23**) was obtained by first removing the Boc group from the ketomethylene-containing precursor **51** followed by sodium

(21) Steglich, W.; Loefle, G. *Chem. Ber.* **1969**, *102*, 883.
(22) Meyer, R. F.; Nicolaides, E. D.; Tinney, F. J.; Lunney, E. A.; Holmes, A.; Hoefle, M. L.; Smith, R. D.; Essenburg, A. D.; Kaplan, H. R.; Almquist, R. G. *J. Med. Chem.* **1981**, *24*, 964–969.
(23) Hori, H.; Yasutake, A.; Minematsu, Y.; Powers, J. C. In *Peptides: Structure and Function. Proceedings of the Ninth American Peptide Symposium*; Deber, C. M.; Hruby, V. J.; Kopple, K. D., Eds., Pierce Chemical Co.: Rockford, IL, 1985; pp 819–820.
(24) Holladay, M. W.; Rich, D. H. *Tetrahedron Lett.* **1983**, *24*, 4401–4404.
(25) Evans, D. A.; Ennis, M. D.; Mather, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737.
(26) Hamada, Y.; Shioiri, T. *Chem. Pharm. Bull.* **1982**, *30*, 1921.
(27) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936–3938.
(28) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399.
(29) Sims, D. J.; Simmons, H. E. *J. Am. Chem. Soc.* **1972**, *94*, 4024–4025.
(30) Sheehan, J. C.; Cruickshank, P. A.; Boshart, G. L. *J. Org. Chem.* **1961**, *26*, 2525.
(31) Stewart, J. M.; Wooley, D. W. *Nature* **1965**, *206*, 619–620.

Table III. Inhibition Patterns and K_i Values[a] for Synthetic Inhibitors of Aminopeptidases[a]

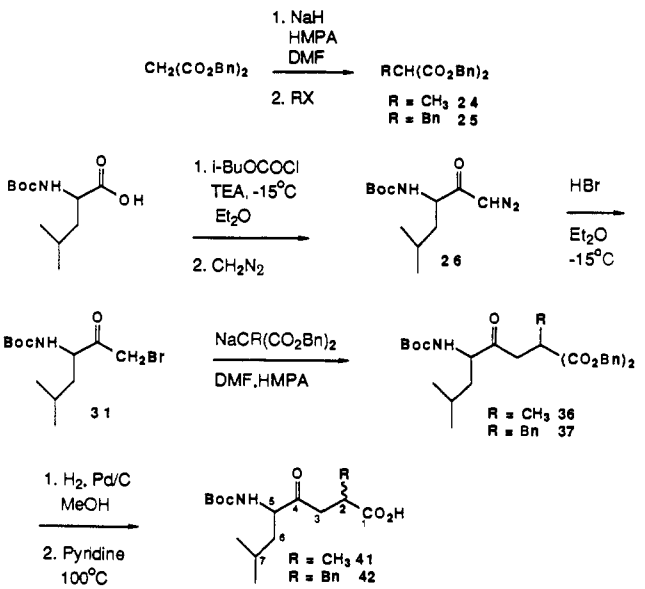
	enzyme[1] compd		
	LAP	APM	APB
ketomethylene isosteres[b]			
Leu^K(*RS*)Ala (**52**)	C K_{is} = 1.1 ± 0.1 mM	NC[c,d] K_{is} = 0.66 ± 0.1 mM K_{ii} = 0.94 ± 0.1 mM	C K_{is} = 0.13 ± 0.02 mM
Leu^K(*RS*)Phe (**53**)	C K_{is} = 57.0 ± 5 μM	NC[e,d] K_{is} = 79.0 ± 12 μM K_{ii} = 0.26 ± 0.04 mM	C K_{is} = 0.17 ± 0.01 μM
D-Leu^K(*RS*)Phe (**54**)	C K_{is} = 0.24 ± 0.02 mM	IC_{50} > 0.8 mM[f]	C K_{is} = 0.77 ± 0.05 μM
Leu^K(*R*)Ala-Val-Iaa (**21**)[g]	C K_{is} = 0.35 ± 0.03 mM	C K_{is} = 0.13 ± 0.01 mM	IC_{50} > 0.4 mM
Leu^K( )Phe-Val-Iaa (**55A**)[h]	C K_{is} = 25.0 ± 0.8 μM	C K_{is} = 8.3 ± 0.4 μM	C[d] K_{is} = 36.0 ± 6 μM
Leu^K( )Phe-Val-Iaa (**55B**)[h]	–	IC_{50} > 0.5 mM[f]	–
Lys^K(*RS*)Ala (**57**)	IC_{50} > 1.0 mM[f]	IC_{50} > 0.2 mM[f]	C K_{is} = 2.0 ± 0.2 μM
Lys^K(*RS*)Ala-Phe (**59**)	IC_{50} > 0.50 mM[f]	C K_{is} = 0.21 ± 0.02 mM	NC(hyper)[d] K_{is} = 8.8 ± 0.7 μM K_{ii} = 2.0 ± 0.6 μM K_{id} = 2.8 ± 1.0 μM
Lys^K(*RS*)Phe (**58**)	IC_{50} > 0.6 mM[f]	IC_{50} > 0.9 mM[f]	C[d] K_{is} = 4.0 ± 0.7 nM
hydroxyethylene isostere[i]			
Leu^{OH}(*RS*)Phe-Val-Iaa (**23**)	IC_{50} > 0.8 mM[f]	C K_{is} = 0.17 ± 0.01 mM	IC_{50} > 0.6 mM[d,f]

[a] Abbreviations: C, competitive; NC, noncompetitive; NC(hyper), noncompetitive, slope linear/intercept hyperbolic. A dash indicates no assays were run. [b] "K" denotes ketomethylene replacement of amide bond. [c] Some negative heterotropic cooperativity in double-reciprocal plot. [d] Denotes slow-binding kinetics. [e] Pronounced negative heterotropic cooperativity when assayed in the presence of 5 mM $MgCl_2$; K_i values determined in the absence of added $MgCl_2$ (cooperativity absent). [f] Less than 50% inhibition observed when [S] ≃ K_m. [g] *R* configuration mimics an L-amino acid residue. [h] Diastereomers were separated; **55A** had significantly greater activity. [i] "OH" denotes hydroxyethylene replacement of amide bond.

borohydride reduction of **55** to the alcohol **23** (Scheme III). This strategy, however, negates any advantage of resolving the hydroxyethylene diastereomers at an early stage of the synthesis and requires an additional resolution step. Although it was not tried, removal of the Boc group by alternative methods[32] might have avoided the acidic conditions that caused the decomposition of **18**. Further work to synthesize diastereomerically pure hydroxyethylene isosteres was discontinued due to the poor inhibitory activity found for the mixture of diastereomers of **23**.

Alkylation of a Bromomethyl Ketone. While this work was in progress, the isolation and synthesis of the arphamenines were reported.[4] These natural ketomethylene analogues were prepared by alkylation of a malonate ester anion with a bromomethyl ketone derived from a suitably protected amino acid by following a synthetic approach first described by Szelke.[17] The synthesis of Boc-L-Leu^K(*RS*)Ala (**41**) and Boc-L-Leu^K(*RS*)Phe (**42**) (Scheme IV) provides an example of this methodology, which was used to prepare all of the inhibitors except for **21**. The mixed anhydride formed from the reaction of Boc-L-leucine with isobutyl chloroformate was reacted with excess diazomethane to give the diazomethyl ketone **26**.[33] This is converted by reaction with HBr in ether to the bromomethyl ketone **31**, which is then reacted with the sodium salt of dibenzyl benzylmalonate (**25**). The substituted malonates **24** and **25** were prepared by alkylation of the anion of dibenzyl malonate with methyl iodide and benzyl bromide (Scheme IV). The dibenzyl ester **37** was purified by MPLC, the esters were cleaved by hydrogenolysis, and the crude diacid was decarboxylated in pyridine at 100 °C. The desired N-protected dipeptide keto-

Scheme IV

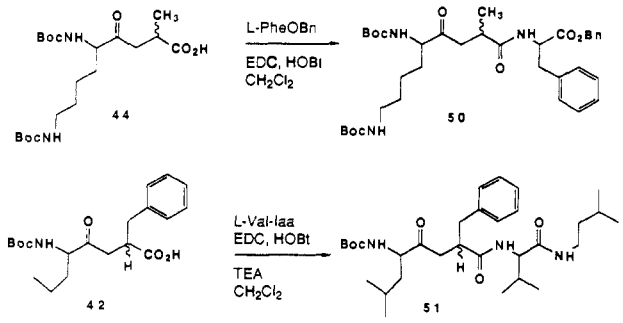


methylene isostere **42** was then purified by MPLC. It should be noted that the product is a mixture of two diastereomers. The configuration at C-5 is determined by the starting amino acid derivative, but the second center (C-2) is racemic.

Two dipeptide isosteres were successfully coupled by using EDC[30] as shown in Scheme V. Compound **44** was coupled to L-phenylalanine benzyl ester to provide the protected tripeptide isostere **50**. Since membrane leucine aminopeptidase is more strongly inhibited by amastatin than bestatin,[34] an analogue of a tetrapeptide inhibitor was prepared in which compound **42** was coupled to L-valine

(32) Scott, R. S.; Chauhan, V. S.; Stammer, C. H. *J. Chem. Soc., Chem. Commun.* **1979**, 495.
(33) Stachowia, K. K.; Khosla, M. C.; Plucinska, K. L.; Khairallah, P. A.; Bumpus, F. M. *J. Med. Chem.* **1979**, *22*, 1128–1130.
(34) Rich, D. H.; Moon, B. J.; Harbeson, S. L. *J. Med. Chem.* **1984**, *27*, 417–422.

Scheme V



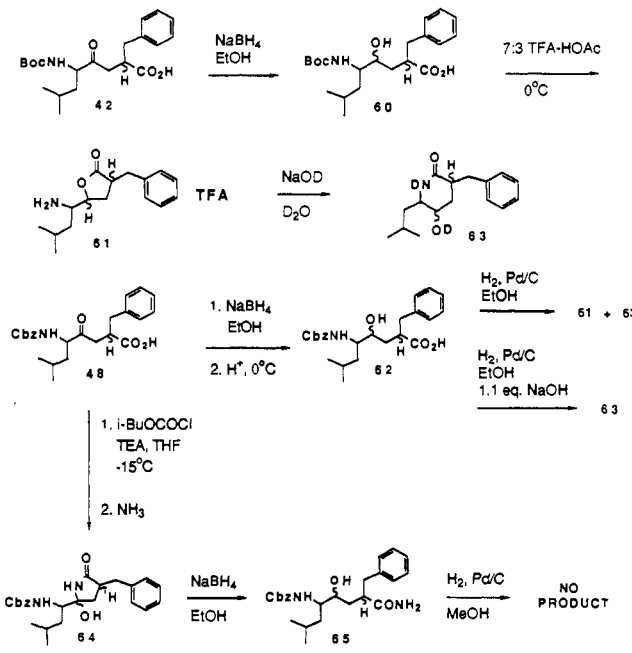
isoamylamide to produce **51**.

Preparation of the Final Products. The final compounds (Table III) were obtained in moderate yields by cleavage of the Boc group with either 4 N HCl in dioxane[31] (compounds **55A**, **55B**, **21**, **52**, **57**, and **58**) or TFA[35] (compounds **53**, **54**, and **59**). In the case of compound **59**, the benzyl ester of **50** was hydrogenolyzed prior to cleavage of the Boc group. Compound **57** was purified by chromatography over LH-20 by eluting with 0.01 N HCl.[4a] No crystalline products were obtained, most likely because most of the products (except **21**) were mixtures of diastereomers. All of the products were very hygroscopic, amorphous solids which were best purified by semipreparative RP-HPLC. The yields of **55A** and **55B** were very low due to the presence of impurities which necessitated discarding fractions on either side of the product peaks and overlapping fractions.

The spectral data for the deprotected isosteres are consistent with the assigned structures. Since the compounds are mixtures of diastereomers (except **21**, **55A**, and **55B**), detailed assignment of the ^{1}H NMR coupling patterns was not attempted, but the integrations are consistent with the structures. The side-chain resonances (i.e., phenyl, isobutyl, and methyl) appear at the expected chemical shifts. The ^{13}C NMR spectra provided more structural information (see Experimental Section). All of the compounds show a ketone resonance at ~205 ppm. The amide and carboxylate resonances are found around 170–179 ppm. The aromatic resonances occur at the expected chemical shifts, and the methine carbons α to an amine and carbonyl are seen at 57–60 ppm. The ^{13}C spectral data for the hydroxyethylene isostere **23** are not reported since this compound is a mixture of four diastereomers and the spectrum is very complex. However, a signal at 60.3 ppm was present, clearly indicating the presence of the secondary alcohol methine; the ketone resonance at 205 ppm was absent.

Dipeptide Hydroxyethylene Isosteres. Attempts to synthesize the free N-terminal dipeptide hydroxyethylene isosteres were unsuccessful (Scheme VI). As expected from the tetrapeptide **18AB** results, the deprotection of hydroxy acid **60** gave the lactone **61**. Keto acid **42** was reduced with $NaBH_4$ to the alcohol **60**, which could be isolated without lactonization by careful acidification at 5 °C. Compound **60** was then treated with 30% HOAc–TFA at 0 °C for 30 min to yield a hygroscopic powder which ^{13}C NMR and TLC indicated to be the lactone **61**. Attempted saponification of lactone **61** (run in deuterated solvents in order to allow direct observation by NMR) yielded a white solid, which was shown by the high-resolution mass spectrum and an absorption at 1680 cm^{-1} in the IR spectrum to be lactam **63**.

Scheme VI



In order to avoid the acid deprotection step, the Cbz-protected ketomethylene dipeptide **48** was first reduced with $NaBH_4$ to provide the crude hydroxy acid **62**, which was then hydrogenated to give a crude product which ^{13}C NMR showed to contain the lactone **61** (C-5 at 78.9 ppm) and a free alcohol (C-5 at 69.6 ppm) (Scheme VI). TLC showed this to be a mixture of the lactone **61** and the lactam **63**. Conversion of the hydroxy acid to the sodium salt prior to hydrogenation gave only the lactam **63**.

In an attempt to prevent the rapid lactonization, the free carboxylic acid **48** was converted to the amide **64** (Scheme VI). It is of interest that no ketone carbonyl for **64** was seen in the ^{13}C NMR but two peaks were found at 89.0 and 88.7 ppm, indicating that the cyclic aminol is formed in chloroform solution. Structure **64** is also supported by the mass spectrum, which shows an ion for $M^+ - 18$ due to the loss of water. The ready formation of structures like **64** could be a problem in other functionalized ketomethylene peptide sequences.

The cyclic lactol **64** was reduced to the protected hydroxyethylene derivative **65** by reaction with $NaBH_4$. This is an extremely slow reduction due to lactol **64**, but apparently in ethanol this cyclic structure is in equilibrium with a small amount of the corresponding ketone needed to afford **65**. Unfortunately, when alcohol **65** was deprotected by hydrogenation, none of the desired product was obtained. At this point, all further attempts to prepare unprotected hydroxyethylene dipeptide isosteres were abandoned.

Inhibition Kinetics. Table III presents the kinetic patterns observed and the K_i values obtained when the compounds were assayed as inhibitors of arginyl aminopeptidase, membrane leucine aminopeptidase, and leucine aminopeptidase. In those cases where less than 50% inhibition was observed when the substrate concentration was approximately at K_m, no K_i values were determined and the IC_{50} value is reported as being greater than the maximum inhibitor concentration tested. The inhibition is reported as either competitive (C) or noncompetitive (NC), and the K_i values are reported for the slope effect (K_{is}) and intercept effect (K_{ii}).[36] The superscripted *d*

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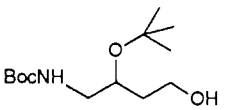
denotes those compounds that demonstrated slow-binding kinetics.[37] A slow-binding inhibitor is defined as one that shows a burst phase when the enzymatic reaction is initiated by addition of enzyme and a lag phase when enzyme and inhibitor are preincubated and the reaction is initiated by addition of substrate.

Discussion

Syntheses of Ketomethylene Isosteres. The oxidation of the primary alcohol of **15** to the carboxylic acid **16** was a serious problem in this synthesis which was not observed in compounds protected at the N-terminus by the phthalyl group or by another amino acid residue. Kaiser and co-workers also encountered this same problem in the attempted oxidation of compound **22**.[38] In their



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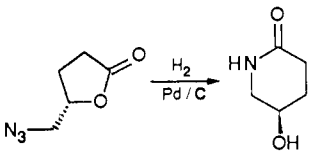
case, neither the use of ruthenium tetroxide nor substitution of the phthalyl for the Boc group improved the yields. The best results were obtained when the oxidation was performed with zinc permanganate in acetone at 0 °C. Due to the successful oxidation with ruthenium tetroxide, the oxidation with zinc permanganate was not attempted in the work presented here.

The method of Holladay and Rich did not work well for synthesizing the ketomethylene isosteres desired for this study. When the Grignard reaction shown in Scheme I was attempted with the aldehyde prepared from N^{α},N^{ϵ}-bis-Boc-lysine, the desired products were not obtained. The anion of the ϵ-BocNH group probably reacts intramolecularly with the aldehyde to form a cyclic aminol which does not undergo the desired addition of the Grignard reagent. This method was found to be incompatible with N^{α}-Cbz derivatives (intramolecular formation of cyclic carbamate) and N^{ϵ}-Boc-lysine (and by inference, the Cbz derivative also) and would also be incompatible with any side chain or protecting group that would be sensitive to a strongly basic, nucleophilic Grignard reagent (i.e., phthalyl, esters, and amides). For these reasons, the α-aminoketomethylene and -hydroxyethylene inhibitors of aminopeptidases were synthesized by another approach.

Alkylation of an α-halomethyl ketone derivative of an amino acid, as suggested by Szelke[17] and applied by Umezawa and co-workers to the synthesis of the arphamenines,[4a] provided a convenient and versatile approach to a variety of ketomethylene isosteres. This work demonstrates that the synthesis of the bromomethyl ketone is compatible with alkyl side chains and either the Boc or Cbz protecting groups on the α-amino group or the ϵ-amino group of lysine. It is also known that a bromomethyl ketone can be prepared from the $(Cbz)_3$ derivative of arginine,[4a] and there is good evidence that the malonate species can be readily altered to fit the synthetic strategy. In this work, both benzyl and ethyl malonate esters were used in the alkylation steps. The cleavage of the esters and decarboxylation proceeded well (the low yield for compound **48** was due to incomplete saponification of **46**). There is also no reason to suspect that a variety of substituents on the malonate ester would not be compatible with this synthetic scheme.

Two disadvantages remain with this synthetic route. The first is that the final products are obtained as a mixture of diastereomers. One optical center (C-5) is determined by the configuration of the starting amino acid and appears to be stable from the observation that the optical rotations of the compounds prepared from D- and L-Boc-Leucine remain equal and opposite. Since the synthetic reactions were performed at different times under varying conditions, a difference in the optical rotations might be expected if C-5 epimerization were occurring. In the original arphamenine synthesis, epimerization was noted only at C-2, not at C-5.[4a] The C-5 center was stable even to the decarboxylation conditions (pyridine, 100 °C). The other problem is the poor yields obtained in the alkylation of the malonate anions with the bromomethyl ketones. The best yields were obtained when the Boc-protected bromomethyl ketone was reacted with a dibenzyl malonyl derivative. The Cbz-protected bromomethyl ketones gave consistently lower yields when reacted with diethyl benzylmalonate although the reaction of Boc-L-leucine bromomethyl ketone **31** with the anion of diethyl benzylmalonate gave only 44% of product (unpublished results), suggesting that the malonate derivative also influences the reaction. Further studies in order to improve the yields of this alkylation reaction are warranted.

Synthesis of Hydroxyethylene Isosteres. The hydroxyethylene-containing peptide **18** was destroyed by lactone formation under the acidic conditions required to cleave the Boc protecting group (Scheme II). Lactone formation also occurs when Boc-Phe^{OH}(*RS*)Phe-Leu benzylamide is treated with HCl.[39] N to O acyl migrations in peptides containing serine and threonine residues are known to be acid catalyzed,[40] and while this reaction is potentially reversible, the acid-catalyzed formation of the five-membered lactones[44] is irreversible due to peptide chain cleavage. The subsequent rearrangement of lactones (e.g., **61**) to lactams (e.g., **63**) under basic conditions has the following precedent:[45]



When Cbz-Leu^{OH}(*RS*)Phe was hydrogenated under neutral conditions, only a mixture of lactone and lactam was obtained. It can be postulated that the zwitterion spontaneously lactonizes and the lactone equilibrates to a mixture of lactone and lactam. The anomalous case arose when the sodium salt of Cbz-Leu^{OH}(*RS*)Phe was hydrogenated and only the lactam was formed. This indicates that either the lactone is not a necessary intermediate in lactam formation or that, under mildly basic conditions, enough of the lactone forms to divert the reaction to the lactam. Finally, there is no explanation for the failure of the hydroxy amide Cbz-Leu^{OH}(*RS*)PheNH_2 (**65**) to yield the

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desired product. Neither the lactone nor lactam was detected in the reaction mixture, but several ninhydrin negative products were formed. Since these could not be the desired product, no further work was carried out to characterize these byproducts. It may become possible to prepare labile, unprotected hydroxyethylene dipeptide analogues if different N- or O-protecting groups[41-43] are used in place of the Boc- or O-acetyl groups.

Enzyme Inhibition. Arginyl Aminopeptidase. The data presented in Table III provide indications of three major structure–activity relationships for inhibition of arginyl aminopeptidase by the ketomethylene dipeptide inhibitors. First, the compounds that contain amino-terminal lysyl (or arginyl) side chains (**57–59** and arphamenine A) are very good inhibitors, which is to be expected since the enzyme specifically removes basic amino acids (arginine and lysine) from the N-terminus of peptide substrates. The positively charged group is a major factor in the binding of the inhibitor to the enzyme since removal of this group from the side chains (as, for example, converting the lysine side chain in **58** to the leucine side chain in **53**) weakens binding to the enzyme by about 50-fold. Nevertheless, Leu^K(*RS*)Phe (**53**) is a surprisingly good inhibitor of arginyl aminopeptidase (K_i = 0.17 μM) and is only approximately 12-fold less active than bestatin (**1**) (K_i = 0.014 μM). Similar results with Leu^K(*RS*)Ala (**52**) indicate that the amino-terminal leucyl side chain is still bound by the enzyme albeit less tightly than the lysyl side chain. Second, the tetrapeptide analogues **21** and **55A** are bound much less tightly than their corresponding dipeptide analogues, **52** and **53**, respectively. This result suggests that the enzyme has a small binding cleft with greater affinity for small peptides. Finally, one of the most interesting results evident in Table III is the obviously increased affinity of arginyl aminopeptidase for compounds bearing an aromatic residue at the P_1' position.[47] Leu^K-(*RS*)Phe (**53**) is bound 760-fold more tightly than Leu^K-(*RS*)Ala (**52**), and Lys^K(*RS*)Phe (**58**) is bound 500-fold more tightly than Lys^K(*RS*)Ala (**57**).

It is also of interest that some compounds demonstrated slow-binding kinetics. Slow-binding behavior does not correlate with inhibitor potency since **58** (K_{is} = 4 mM) and **55A** (K_{is} = 36 μM) are both slow-binding inhibitors despite a difference in affinity of 100-fold. The hydroxyethylene isostere **23** retained slow-binding behavior even though it is a much weaker inhibitor than the analogous ketomethylene inhibitor (**55A**). These transients are probably not due to the slow, enzyme-catalyzed reaction of water or enzyme with the ketone since bestatin (**1**) and **23** demonstrate slow binding and do not contain the ketone functionality. The mechanism leading to the slow-binding phenomena will require further investigation.

Leucine Aminopeptidase. The results obtained with leucine aminopeptidase (Table III) show that peptides incorporating the ketomethylene moiety provide moderately active inhibitors which are several thousand times weaker inhibitors than bestatin (**1**). Previous studies with bestatin and amastatin analogues demonstrated that inhibitor affinity decreased with increasing peptide chain length, but the ketomethylene inhibitors do not show this effect.[34] Compound **21** is two residues longer than the dipeptide isostere **52** but three times more active. If one takes into consideration that **52** is a mixture of two diastereomers, then the two compounds are approximately equipotent. This same pattern holds when the dipeptide

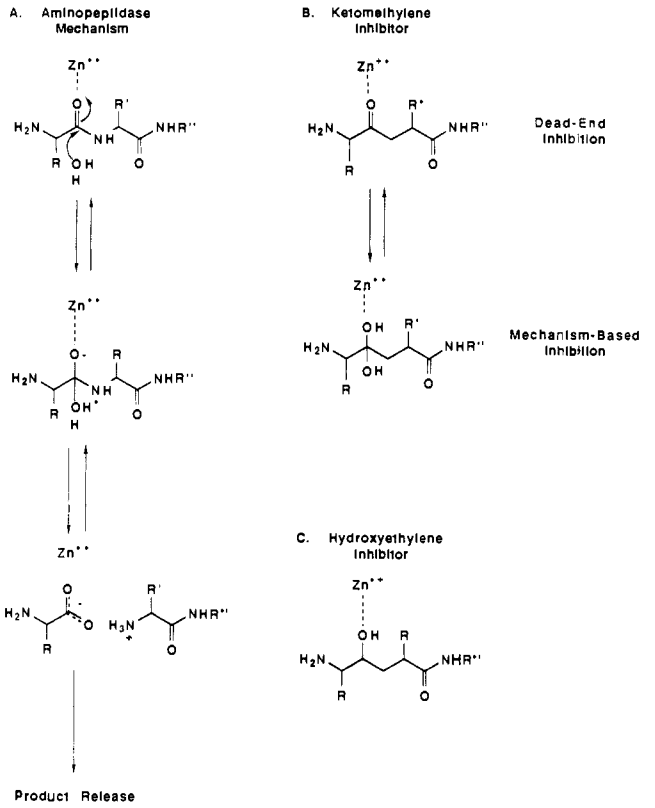

Figure 2. (A) Proposed mechanism for aminopeptidase-catalyzed cleavage of an amide bond. (B) Potential binding modes for ketomethylene inhibitors. (C) Hydroxyethylene inhibitors of aminopeptidases.

isostere **53** is compared with C-terminal-extended analogue **55A**. The enzyme does demonstrate the expected specificity for the amino-terminal residue since lysine side chains are bound very poorly and a D-residue decreases the affinity. Compounds **52** versus **53** and **21** versus **55A** demonstrate that substitution of benzyl for methyl at P_1 results in 14- and 19-fold increases in affinity, respectively. Finally, compound **23** shows that substitution of the hydroxyethylene moiety for the ketomethylene results in a significant loss in inhibitory potency.

Aminopeptidase M. The ketomethylene isosteric amide bond replacement provides modest inhibitors of membrane leucine aminopeptidase that are significantly less active than amastatin (**2**). Leu^{K}Phe-Val-Iaa (**55A**) is the most potent of the inhibitors (K_{is} = 8.3 μM), but this isostere is still 400-fold less active than amastatin. Extending the peptide chain at the C-terminus improves binding to APM, a result that agrees with the structure–activity relationships derived for bestatin and amastatin.[34] Replacement of the ketomethylene moiety by the hydroxyethylene unit appears to decrease inhibitor affinity, but this effect is difficult to quantitate since **23** is a mixture of four diastereomers present in unknown proportions.

Effect of Mg^{2+} on Membrane Leucine Aminopeptidase. The ketomethylene dipeptide isosteres, Leu^K(*RS*)Ala (**52**) and Leu^K(*RS*)Phe (**53**), are slow-binding inhibitors that gave anomalous kinetics when assayed as inhibitors of membrane leucine aminopeptidase. Figure 3 shows the double-reciprocal plot[48] obtained with compound **52**. The mode of inhibition is clearly noncompetitive with linear slope and intercept effects. In addition to the noncompetitive nature of inhibition, there also appears to be a downward curvature in the double-reciprocal

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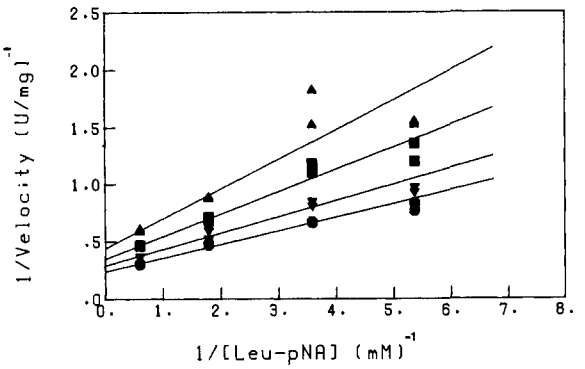

Figure 3. Double-reciprocal plot of the inhibition of aminopeptidase M by Leu^K(*RS*)Ala (**52**). Inhibitor concentrations: 0.0 mM (●); 0.162 mM (▼); 0.406 mM (■); 0.812 mM (▲).

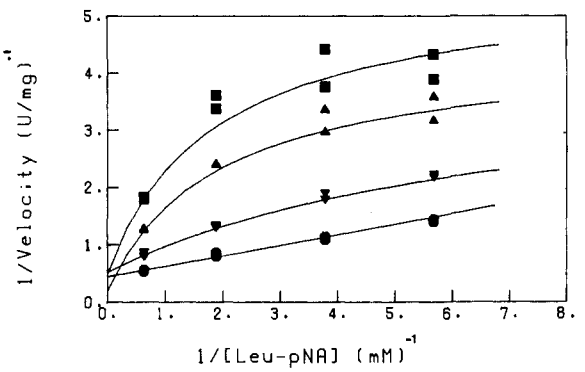

Figure 4. Double-reciprocal plot of the inhibition of aminopeptidase M by Leu^K(*RS*)Phe (**53**). Inhibitor concentrations: 0.0 mM (●); 0.0541 mM (▼); 0.108 mM (■); 0.216 mM (▲).

plot indicating the presence of negative heterotropic cooperativity.[49] This nonlinearity is even more pronounced in the double-reciprocal plot obtained when compound **53** is assayed as an inhibitor of membrane leucine aminopeptidase (Figure 4). As is clearly shown, the curvature in the double-reciprocal plot becomes much more pronounced as the concentration of inhibitor is increased. It should be noted that the inhibitors that had been extended on the C-terminus (**21**, **55A**, and **59**) showed neither nonlinear behavior nor slow-binding kinetics but are competitive inhibitors.

These nonlinear double-reciprocal plots had not been observed in our previous studies with membrane leucine aminopeptidase so that further work to identify the cause was carried out. We found the nonlinearity was caused by Mg^{2+} ions in the assay system. The commercially prepared membrane leucine aminopeptidase is supplied as an ammonium sulfate suspension that contains 10 mM $MgCl_2$, and we routinely assayed the enzyme activity in a 0.1 M MOPS buffer that contained 5 mM $MgCl_2$. When the $MgCl_2$ was removed from the enzyme preparation either by affinity chromatography or by desalting on Sephadex G-25, the non-Michaelis–Menten kinetics were no longer observed with compound **53**. When 5 mM $MgCl_2$ was added back to the magnesium-free membrane leucine aminopeptidase, a slow return of the non-Michaelis–Menten kinetics was observed. The nonlinearity of the double-reciprocal plots was not as pronounced, which allowed the determination of K_i values for **53**. The results in Table IV also show that while $MgCl_2$ has a pronounced effect upon inhibitor binding, it had no effect upon substrate hydrolysis. The observations that Leu^K(*RS*)Phe (**53**) shows linear Michaelin–Menten kinetics in the absence of $MgCl_2$ and that $MgCl_2$ has no effect upon substrate hydrolysis are very good reasons for deleting the magnesium salt from the buffer in order to avoid possible anomalous results when assaying inhibitors with membrane leucine aminopeptidase.

Table IV. Effect of Magnesium on APM Catalytic Constants

	day 1	day 2
V_{max}, units/mg	4.8 ± 0.1	5.0 ± 0.1
V/K, units/(mg·mM)	13.2 ± 0.7	14.0 ± 0.6
K_m, mM	0.36 ± 0.03	0.35 ± 0.02
I = Leu^K(*RS*)Phe (**53**)		
K_{is}, μM	78.8 ± 12	27.5 ± 7
K_{ii}, μM	260 ± 42	85.9 ± 2

Conclusions. The results presented here have shown that replacing substrate amide bonds with the ketomethylene isostere provides modest inhibitors of leucine aminopeptidase and membrane leucine aminopeptidase but very potent inhibitors of arginyl aminopeptidase. With respect to the possible inhibitory mechanisms, which are shown in Figure 2, these compounds appear to behave as hydrolytically stable substrate analogues that combine in a simple dead-end fashion with leucine aminopeptidase and membrane leucine aminopeptidase. However, because arginyl aminopeptidase is potently inhibited by ketomethylene isosteres, the mechanism of inhibition for arginyl aminopeptidase may be different from that of leucine aminopeptidase and membrane leucine aminopeptidase. The pathway shown in Figure 2, which generates an enzyme-bound tetrahedral species from the ketomethylene isostere, would be a plausible explanation for the potent inhibition observed with the arphamenines (**3** and **4**) and Lys^K(*RS*)Phe (**58**). If the very low K_i values of these compounds are due to the mechanism-based nature of their binding to arginyl aminopeptidase, then a test of this mechanism would be to prepare the ^{13}C inhibitor labeled at the ketone carbonyl and observe the effect of binding to arginyl aminopeptidase upon the ^{13}C chemical shift.[53,54] A large upfield shift from 205 to approximately 90 ppm would provide evidence for the mechanism-based inhibition in Figure 2. Hopefully, these ^{13}C-labeled compounds will be prepared and tested in the future.

Experimental Section

Melting points were determined on a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter (0.999-dm cell). Proton nuclear magnetic resonance spectra in $CDCl_3$ were recorded on a Varian EM-390 (90-MHz) spectrometer whereas spectra run in other solvents and ^{13}C spectra were obtained on a JEOL FX-90Q Fourier transform spectrometer. Chemical shifts were reported as δ units (ppm) relative to TMS as internal standard except for spectra in D_2O, which are reported relative to methanol. Low-resolution mass spectra were obtained on a Finnegan 1015 GC–mass spectrometer interfaced to a Finnegan M6000 data

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system. Reported peaks were greater than 20% of base peak. Microanalyses were performed by Galbraith Laboratories.

TLC was performed on 0.25-mm thickness silica gel plates (Merck, silica gel 60 F-254). TLC solvent systems used were the following: (1) 20% ethyl acetate–Skelly B; (2) 2% acetic acid–chloroform; (3) 85:10:5 chloroform–methanol–acetic acid; (4) 2% methanol–methylene chloride; (5) 33% ethyl acetate–Skelly B; (6) 2% methanol–chloroform; (7) 5% methanol–methylene chloride; (8) 50% ethyl acetate–Skelly B; (9) 5% methanol–methylene chloride (NH_3 vapor); (10) 10% methanol–methylene chloride (NH_3 vapor); (11) 2% ether–Skelly B; (12) 0.1% acetic acid in 4% methanol–methylene chloride; (13) 0.1% acetic acid in 2% methanol–methylene chloride; (14) 25% Skelly B–ethyl acetate; (15) 40% Skelly B–ethyl acetate; (16) 25% ethyl acetate–toluene; (17) 1:1:1:1 butanol–acetic acid–water–ethyl acetate; (18) 5:1:4:3 butanol–acetic acid–water–pyridine; (19) 10% ethyl acetate–Skelly B. The TLC plates were visualized with the following: (1) UV, (2) ninhydrin (0.3 g in 100 mL of 1-propanol and 3 mL of acetic acid, (3) or chlorox–o-tolidine reagent.[50]

High-performance liquid chromatography (HPLC) was performed by use of two Waters Model 510 pumps equipped with extended flow heads, a Waters Model U6K injector, a Waters automatic gradient controller, a Waters Model 441 absorbance detector (214 or 254 nm), and a BBC Servogor 120 recorder. Analytical work was done on a Waters μBondapak C_{18} column (3.9 mm × 30 cm) while semipreparative purifications were performed on a Waters μBondapak C_{18} column (19 mm × 150 mm). Medium-pressure liquid chromatography (MPLC) was done by using an FMI Lab pump and Altex columns (1.5 cm × 100 cm and 2.5 cm × 100 cm) packed with silica gel 60, 230–400 mesh. "Chromatography" refers to gravity flow over silica gel 60, 230–400 mesh.

Solvents and reagents were purified as follows: Skelly B and ethyl acetate were distilled from phosphorous pentoxide, tetrahydrofuran was distilled from sodium–benzophenone, and dioxane was distilled from sodium metal. All other solvents and reagents were either ACS reagent or HPLC grade and were used without further purification. Reagents were obtained from Aldrich, and amino acid derivatives were from BaChem and Sigma.

Enzymatic Assays. Leucine aminopeptidase (EC 3.4.11.1) was obtained from Sigma as a chromatographically purified suspension in 2.9 M ammonium sulfate, 0.1 M Tris, pH 8, and 5 mM magnesium chloride. The enzyme was diluted 10-fold into 0.10 M Tris, pH 8.5, and 5 mM magnesium chloride, and all assays were performed in the same buffer. Aminopeptidase M (EC 3.4.11.2) was also obtained from Sigma as a suspension in 3.5 M ammonium sulfate, pH 7.7, and 10 mM magnesium chloride. The enzyme was diluted 100-fold into 0.10 M MOPS, pH 7.2, and 5 mM magnesium chloride and assayed in the same buffer. Both enzymes were assayed with L-leucine-*p*-nitroanilide hydrochloride as the substrate.

Arginyl aminopeptidase was purified from rat liver by a modification of a literature procedure.[51] Details of the purification are reported in a separate publication.[46] Enzyme activity was monitored spectrophotometrically by using L-arginine *p*-nitroanilide as substrate in 0.10 M PIPES, pH 7.0, containing 0.20 M NaCl and 1 mM DTT.

All initial velocities were measured as the release of *p*-nitroaniline (E = 9620 L/mol·cm)[52] at 405 nm. Assays were performed in semimicro, self-masking cuvettes (1.0-cm path length) at 25 °C. For slow-binding inhibitors, 25 μL of enzyme solution was preincubated with 100 μL of inhibitor solution for 15 min prior to initiation of the enzymatic reaction with 300 μL of substrate solution. Longer preincubation did not alter the results. Inhibitors that did not demonstrate slow-binding kinetics were assayed without preincubation, and hydrolysis was initiated by addition of the enzyme solution.

Kinetic analyses of the data were performed on a Northstar Horizon utilizing RAGASSEK, a regression and graphical analysis package prepared by Dr. Dexter B. Northrop which utilizes a nonlinear regression routine written by Dr. Ronald G. Duggleby.[55] The data were first compiled as initial velocities in units of change in absorbance per minute. These velocities were then converted to units of μmol/(min·mg) (specific activity) and are reported in this form. In all cases, the data were first fit line by line to the hyperbolic form of the Michaelis–Menten equation[56] (eq 1) to generate a double-reciprocal plot. The slope and intercept values were then used in a regression to determine the presence or absence of slope-linear (eq 2), intercept-linear (eq 3), and intercept-hyperbolic effects (eq 7). The data were then refit to the appropriate kinetic pattern: competitive (eq 4); noncompetitive (eq 5); and slope-linear/intercept-hyperbolic noncompetitive (eq 6). These equations show the kinetic values that are reported.

$$v = VS/(K_m + S) \quad (1)$$

$$V/K = V/K_m(1 + I/K_{is}) \quad (2)$$

$$V = V/(1 + I/K_{ii}) \quad (3)$$

$$v = VS/[K_m(1 + I/K_{is}) + S] \quad (4)$$

$$v = VS/[K_m(1 + I/K_{is}) + S(1 + I/K_{ii})] \quad (5)$$

$$v = VS/[K_m(1 + I/K_{is}) + S(1 + I/K_{ii})/(1 + K/K_{id})] \quad (6)$$

$$V = V/(1 + I/K_{ii})/(1 + I/K_{id}) \quad (7)$$

General Procedures. (A) Preparation of N-Protected Amino Diazomethyl Ketones. The N-protected amino acid was dissolved in anhydrous ether (150 mL for 0.04 mol), dried over anhydrous magnesium sulfate, and suction filtered. The filtrate was chilled to −15 °C prior to the addition of TEA (1 equiv) followed by isobutyl chloroformate (1.1 equiv). This mixture was then stirred for 15 min before adding an ethereal, alcohol-free diazomethane solution (approximately 3 g in 200 mL) dropwise with stirring over a 40-min period. The reaction was then stirred 2 h at −15 °C prior to concentration at 30 °C under a nitrogen stream. This residue was then resuspended in ethyl acetate (150 mL) and washed with water followed by saturated $NaHCO_3$. The organic phase was dried over magnesium sulfate, suction filtered, and concentrated in vacuo to a solid, which was then recrystallized from a suitable solvent.

(B) Preparation of N-Protected Amino Bromomethyl Ketones. The diazomethyl ketone was dissolved in anhydrous ether (80 mL) and chilled to 5 °C. A solution of HBr in ether (1.1 equiv) was then added dropwise with stirring over 1 h. The ether solution was then washed with saturated $NaHCO_3$ (1 × 50 mL). The organic phase was dried over magnesium sulfate, suction filtered, and concentrated in vacuo to a solid, which was crystallized from a suitable solvent.

(C) Alkylation of Malonate Anions with Bromomethyl Ketones. A solution of the desired malonate anion was prepared by first rinsing NaH (1.2 equiv 60% oil dispersion) with Skelly B. This slurry was then suspended in DMF (7 mL/10 mmol) and HMPA (1 mL/mmol). A solution of the desired malonate ester (1.2 equiv) in DMF (4 mL) was added (H_2 evolution) and the reaction allowed to stir 40 min at 25 °C under argon. The malonate anion solution was then added dropwise over 30 min with stirring to a solution of the bromomethyl ketone (1 equiv) in DMF (7 mL/8 mmol) and HMPA (1 mL/8 mmol) at 25 °C. After being stirred 2 h, the reaction was poured into water (400 mL) and extracted with ether (3 × 100 mL). The organic phase was washed with water (3 × 100 mL), dried over magnesium sulfate, suction filtered, and concentrated in vacuo to an oil which was purified by MPLC.

(D) Conversion of Diesters to N-Protected Dipeptide Ketomethylene Isosteres. Dibenzyl esters were cleaved by hydrogenolysis of the diester in methanol (1 mL/mmol) on a Parr apparatus at 20 psi H_2 at 25 °C with 10% palladium on carbon (0.1 g/mmol). After 3 h, the reaction was suction filtered through a celite pad (methanol rinse) and the filtrate was concentrated in vacuo to a brittle glass.

Diethyl esters were saponified in ethanol (6 mL) with 1 N NaOH (2.1 equiv). The reaction was stirred until it became a clear solution and, after 15 min more, was diluted into water (150 mL) and washed with ether (1 × 40 mL). The aqueous layer was

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acidified to pH 2 with 1 N HCl and then extracted with ethyl acetate (3 × 50 mL). The organic extract was dried over magnesium sulfate, suction filtered, and concentrated in vacuo to a glassy foam.

In both cases, the crude dicarboxylic acid was decarboxylated without further purification. The crude product was dissolved in pyridine and the resulting solution purged with argon. The solution was heated at 100 °C for the required time, allowed to cool, and then diluted into ethyl acetate and washed with cold 0.5 N HCl. The organic phase was dried over magnesium sulfate, suction filtered, and concentrated in vacuo to give a product which was purified by MPLC.

(E) **Removal of Boc with 4 N HCl in Dioxane.** The Boc-protected compound was dissolved in 4 N HCl in dioxane (10 equiv) and allowed to stir under a dry argon atmosphere at 25 °C for 45 min. The reaction was then concentrated in vacuo and dried under vacuum.

(2*R*,4*RS*,5*S*)-1-(Benzyloxy)-5-[(*tert*-butyloxycarbonyl)-amino]-4-hydroxy-2,7-dimethyloctane (13A and 13B). Magnesium powder (0.90 g, 37 mmol) was placed in a dry 50-mL two-neck round-bottom flask under an argon atmosphere. The magnesium was suspended in dry THF (10 mL), and an iodine crystal was added. Compound **11** (6.0 g, 24.7 mmol) was placed in a 10-mL addition funnel, and 2 drops were added to the magnesium mixture. The reaction was started by gently warming and then maintained at a gentle reflux by dropwise addition of the bromide. The addition was complete in 20 min, and the reaction was stirred 2 h at 25 °C.

The Grignard reagent was chilled to 5 °C before adding a solution of Boc-L-leucinal (**12**) (2.42 g, 11.2 mmol) in THF (10 mL) over a 2-h period. The reaction was stirred 6 h before quenching with aqueous ammonium chloride. The reaction was poured into water (150 mL) and extracted with ether (3 × 40 mL). The organic phase was dried over magnesium sulfate, suction filtered, and concentrated in vacuo to a yellow oil (5.5 g). The product was purified by MPLC (11% ethyl acetate–Skelly B eluent) to provide the separated diastereomers and a mixture of diastereomers. (2*R*,4*S*,5*S*)-**13A** (0.927 g, 2.4 mmol, 22%): R_f(16) 0.44; $[\alpha]_D$ −21.4° (*c* 2.8, chloroform); ^{1}H NMR ($CDCl_3$) δ 0.93 (6 H, m), 1.20–2.27 (18 H, m), 2.97 (1 H, br s), 3.33 (2 H, d, *J* = 6 Hz), 3.40–3.90 (2 H, m), 4.47 (2 H, s), 4.70 (1 H, d, *J* = 9 Hz), 7.30 (5 H, s); mass spectrum, 380 (M^+ + 1), 324, 280, 130, 91. (2*R*,4*R*,5*S*)-**13B** (0.41 g, 1.1 mmol, 9.6%): R_f(16) 0.30; $[\alpha]_D$ −10.6° (C2.89, chloroform); ^{1}H NMR ($CDCl_3$) δ 0.93 (6 H, m), 1.10–1.77 (15 H, m), 1.83–2.30 (1 H, m), 3.20–3.87 (5 H, m), 4.50 (2 H, s), 4.67 (1 H, d, *J* = 9 Hz), 7.30 (5 H, s); mass spectrum, 380 (M^+ + 1), 324, 280, 130, 91. Mixture of **13s**: 0.907 g, 2.4 mmol, 21.3%. Total yield: 2.24 g, 5.9 mmol, 52.5%.

(2*R*,4*S*,5*S*)-4-Acetoxy-5-[(*tert*-butyloxycarbonyl)-amino]-2,7-dimethyloctanoic Acid (16A). Acetylation. 13A (0.927 g, 2.44 mmol) was dissolved in ethyl acetate (3 mL). To this solution were added NMM (0.28 mL, 0.26 g, 0.24 mmol) and DMAP (0.03 g, 0.24 mmol) followed by acetic anhydride (0.70 mL, 0.76 g, 7.4 mmol). The reaction was then stirred 24 h at 25 °C. Saturated $NaHCO_3$ (3 mL) was added and the mixture stirred for 30 min. This was then poured into ethyl acetate (80 mL), and the organic phase was washed with saturated $NaHCO_3$ (3 × 30 mL) and cold 0.5 N HCl (2 × 30 mL), dried over magnesium sulfate, suction filtered, and concentrated in vacuo to give **14A** as an oil (0.984 g, 2.34 mmol, 96%): R_f(16) 0.62; ^{1}H NMR ($CDCl_3$) δ 0.93 (6 H, m), 1.43 (9 H, s), 2.00 (3 H, s), 0.83–2.23 (8 H, m), 3.30 (2 H, d, *J* = 5 Hz), 3.60–4.30 (2 H, m), 4.47 (2 H, s), 4.60 (1 H, d, *J* = 10 Hz), 4.97 (1 H, m), 7.30 (5 H, s); mass spectrum, 422 (M^+ + 1), 366, 336, 322, 306, 262, 230, 186, 130, 91.

Benzyl Ether Cleavage. The benzyl ether was then removed by catalytic-transfer hydrogenation. **14A** (0.984 g, 2.34 mmol) was dissolved in 1:1 IPA–acetic acid (30 mL), and this solution was flushed with argon before adding ammonium formate (1.5 g, 23.8 mmol) and 10% palladium on carbon (0.60 g). This mixture was stirred 6 h at 25 °C and then filtered through a Celite pad (ethyl acetate wash). The filtrate was diluted with ethyl acetate (200 mL) and washed with water (3 × 100 mL) and saturated $NaHCO_3$ (3 × 100 mL). The organic phase was then dried over magnesium sulfate, suction filtered, and concentrated in vacuo to give **15A** as an oil, which was used without further purification (0.712 g, 2.15 mmol, 92%): R_f(8) 0.52; ^{1}H NMR ($CDCl_3$) δ 0.93 (6 H, m), 1.43 (9 H, s), 2.06 (3 H, s), 1.07–1.97 (6 H, m), 2.80 (1 H, br s), 3.40 (2 H, m), 3.80 (1 H, m), 4.67 (1 H, d, *J* = 10 Hz), 4.93 (1 H, m); mass spectrum, 332 (M^+ + 1), 286, 232, 216, 186, 157, 130, 91.

Oxidation. The primary alcohol **15A** (0.38 g, 1.15 mmol) was oxidized to the carboxylic acid by dissolving in 1:1 CCl_4–MeCN (4.5 mL) and then adding water (3.4 mL), sodium periodate (0.724 g, 3.4 mmol), and ruthenium chloride (4.5 mg, 0.02 mmol). The reaction mixture was stirred vigorously for 2.5 h and then diluted into cold 0.5 N HCl (100 mL). The aqueous phase was extracted with ether (3 × 40 mL), and the extract was dried over magnesium sulfate, suction filtered, and concentrated in vacuo to an oil which was purified by chromatography (20 g, 2% methanol–0.5% acetic acid–methylene chloride eluent) to provide **16A** as a glassy foam (0.264 g, 0.76 mmol, 66%): R_f(7) 0.34; ^{1}H NMR ($CDCl_3$) δ 0.93 (6 H, d, *J* = 6 Hz), 1.43 (9 H, s), 2.07 (3 H, s), 1.03–2.13 (8 H, m), 2.53 (1 H, m), 3.77 (1 H, m), 4.57 (1 H, d, *J* = 11 Hz), 5.0 (1 H, m), 10.5 (1 H, br s); mass spectrum, 346 (M^+ + 1), 246, 230, 212, 186, 142, 130, 86.

(2*R*,4*R*,5*S*)-4-Acetoxy-5-[(*tert*-butyloxycarbonyl)-amino]-2,7-dimethyloctanoic Acid (16B). Acetylation. 13B (0.41 g, 1.08 mmol) was treated as described for **13A** starting with ethyl acetate (1.5 mL), NMM (0.13 mL, 0.12 g, 0.11 mmol), DMAP (13 mg, 0.10 mmol), and acetic anhydride (0.31 mL, 0.34 g, 3.3 mmol) to yield **14B** as an oil (0.421 g, 1.0 mmol, 92%): R_f(16) 0.55; ^{1}H NMR ($CDCl_3$) δ 0.93 (6 H, m), 1.43 (9 H, s), 2.00 (3 H, s), 1.03–2.27 (8 H, m), 3.30 (2 H, t, *J* = 7 Hz), 3.73–4.27 (2 H, m), 4.47 (3 H, br s), 4.97 (1 H, m), 7.27 (5 H, s); mass spectrum, 422 (M^+ + 1), 366, 336, 322, 306, 262, 230, 186, 130, 91.

Benzyl Ether Cleavage. The benzyl ether was also removed by catalytic-transfer hydrogenation as described for **15A**, starting with **14B** (0.421 g, 1.0 mmol) in 1:1 IPA–acetic acid (15 mL) and reacting it with ammonium formate (0.65 g, 10.3 mmol) and 10% palladium on carbon (0.5 g). **15B** was obtained as an oil, which was oxidized to the carboxylic acid without further purification (0.254 g, 0.766 mmol, 77%): R_f(8) 0.45; ^{1}H NMR ($CDCl_3$) δ 0.97 (6 H, m), 1.43 (9 H, s), 2.03 (3 H, s), 1.03–2.00 (9 H, m), 2.33 (1 H, br s), 3.47 (2 H, m), 3.93 (1 H, m), 4.57 (1 H, d, *J* = 9 Hz), 4.93 (1 H, m); mass spectrum, 332 (M^+ + 1), 286, 232, 216, 186, 157, 130, 91.

Oxidation. The primary alcohol **15B** (0.221 g, 0.67 mmol) was oxidized in a mixture of CCl_4 (1.3 mL), MeCN (1.3 mL), and water (2 mL) with sodium periodate (0.421 g, 2.0 mmol) and ruthenium chloride (2.6 mg, 0.01 mmol). The crude product was purified by chromatography (11 g, 2% methanol–0.5% acetic acid–methylene chloride eluent) to yield **16B** as a solid (0.112 g, 0.32 mmol, 49%): R_f(7) 0.41; mp 125–128 °C; $[\alpha]_D$ +25.4° (*c* 0.49, chloroform); ^{1}H NMR ($CDCl_3$) δ 0.93 (6 H, dd, *J* = 3, 6.4 Hz), 1.20 (3 H, d, *J* = 6.8 Hz), 1.44 (9 H, s), 2.05 (3 H, s), 1.15–2.17 (5 H, m), 2.40 (1 H, m), 3.99 (1 H, m), 4.79 (2 H, m), 10.5 (1 H, br s); mass spectrum, 346 (M^+ + 1), 246, 230, 212, 186, 142, 130, 86.

[(2*R*,4*S*,5*S*)-5-[(*tert*-Butyloxycarbonyl)amino]-4-hydroxy-2,7-dimethyloctanoyl]-L-valine Isoamylamide (18A). Compound **66** (0.213 g, 0.744 mmol) was deprotected according to General Procedure E. The solvent was removed in vacuo to give a brittle foam (assume a quantitative yield).

The carboxylic acid **16A** (0.264 g, 0.764 mmol) was dissolved in methylene chloride (4 mL) and added to the above amine hydrochloride. This mixture was chilled to 5 °C before adding HOBt (0.13 g, 0.85 mmol), TEA (0.11 mL, 0.08 g, 0.79 mmol), and EDC (0.163 g, 0.85 mmol). After stirring 12 h at 5 °C, the reaction was diluted into ethyl acetate (80 mL) and washed with cold 0.5 N HCl (3 × 30 mL), saturated $NaHCO_3$ (3 × 30 mL), and saturated NaCl (1 × 30 mL). The organic phase was dried over magnesium sulfate, suction filtered, and concentrated in vacuo to a glassy solid (0.360 g, 0.701 mmol, 94%). This material was deacetylated without further purification.

The acetate (0.36 g, 0.701 mmol) was dissolved in MeOH (7 mL), anhydrous K_2CO_3 (0.68 g, 4.9 mmol) was added, and the mixture was stirred for 12 h at 25 °C under argon. The reaction mixture was then diluted into water (80 mL) and extracted with ethyl acetate (3 × 30 mL). The organic phase was dried over magnesium sulfate, suction filtered, and concentrated in vacuo to give a crude product (0.28 g), which was purified by trituration with cold ethyl acetate to provide **18A** as a white solid (0.21 g,

0.44 mmol, 64%): R_f(14) 0.53; mp 140–143 °C; $[\alpha]_D$ –61.1° (*c* 0.842, methanol); ^{1}H NMR ($CDCl_3$) δ 0.67–2.90 (40 H, m), 3.07–3.80 (4 H, m), 4.03–4.43 (2 H, m), 4.83 (1 H, m), 6.83 (1 H, m), 7.17 (1 H, d, *J* = 9 Hz); mass spectrum, 472 (M^+ + 1), 416, 372, 329, 283, 267, 230, 186, 168, 130, 112, 99, 88, 72.

Anal. ($C_{25}H_{49}N_3O_5$) C, H, N.

[(2*R*,4*R*,5*S*)-5-[(*tert*-Butyloxycarbonyl)amino]-4-hydroxy-2,7-dimethyloctanoyl]-L-valine Isoamylamide (18B). This isomer was prepared in a manner similar to the procedure for **18A**, starting with the deprotection of **66** (0.068 g, 0.238 mmol).

The amine hydrochloride was then coupled to **16B** (0.075 g, 0.217 mmol) in methylene chloride (1 mL) with HOBt (0.037 g, 0.24 mmol), TEA (0.031 mL, 0.022 g, 0.22 mmol), and EDC (0.046 g, 0.24 mmol) as previously described. The crude product (0.11 g, 0.214 mmol) was then deacetylated without further purification in MeOH (2 mL) with anhydrous K_2CO_3 (0.208 g, 1.5 mmol). This product was also purified by trituration with ethyl acetate to yield **18B** as a white solid (0.068 g, 0.14 mmol, 67%): R_f(14) 0.45; mp 138–140 °C; $[\alpha]_D$ –47.0° (*c* 0.908, methanol); ^{1}H NMR ($CDCl_3$) δ 0.77–2.80 (40 H, m), 3.23 (2 H, q, *J* = 7 Hz), 3.50–3.93 (3 H, m), 4.13 (1 H, m), 4.73 (1 H, d, *J* = 9 Hz), 6.37 (1 H, m), 6.60 (1 H, d, *J* = 9 Hz); mass spectrum, 472 (M^+ + 1), 416, 372, 329, 283, 267, 230, 186, 168, 130, 112, 99, 88, 72.

Anal. ($C_{25}H_{49}N_3O_5$) C, H, N.

[(2*R*,5*S*)-5-[(*tert*-Butyloxycarbonyl)amino]-2,7-dimethyl-4-oxooctanoyl]-L-valine Isoamylamide (19). Compound **18A** (0.10 g, 0.212 mmol) was dissolved in 1:1 DMF–acetic acid (2 mL). To this solution was added PDC (0.641 g, 1.70 mmol), and the mixture was stirred 12 h at 25 °C. The reaction was then diluted into water (100 mL) and extracted with methylene chloride (3 × 50 mL). The organic phase was washed with cold 0.5 N HCl (3 × 50 mL), saturated $NaHCO_3$ (3 × 50 mL), and saturated NaCl (1 × 50 mL), dried over magnesium sulfate, suction filtered, and concentrated in vacuo to a white solid (0.094 g). This material was purified by trituration with 33% ethyl acetate–Skelly B to give **19** as a white solid (0.074 g, 0.16 mmol, 74%): R_f(15) 0.56; mp 152–154 °C; $[\alpha]_D$ –68.0° (*c* 0.44, methanol); ^{1}H NMR ($CDCl_3$) δ 0.61–1.96 (37 H, m), 2.08–3.01 (3 H, m), 3.24 (2 H, q, *J* = 7 Hz), 4.17 (2 H, m), 4.97 (1 H, m), 5.93–6.60 (2 H, m); mass spectrum, 470 (M^+ + 1), 414, 355, 299, 256, 228, 169, 130, 114, 98, 86, 72.

Anal. ($C_{25}H_{47}N_3O_5$) C, H, N.

5(*S*)-[1(*S*)-Amino-3-methylbutyl]-3(*R*)-methyldihydrofuran-2(3*H*)-one Trifluoroacetate (20). Compound **18A** (97 mg, 0.21 mmol) was dissolved in 3 mL of TFA and stirred for 30 min at 25 °C under argon. The excess reagent was removed in vacuo to give an oil which was dissolved in 10% acetonitrile–water and lyophilized. An oil was obtained (133 mg) which was purified by semipreparative RP-HPLC [gradient elution: solvent A (0.1% aqueous TFA) and solvent B (0.1% TFA in 1:1 acetonitrile–water); 70% A to 10% A, 45 min, 4 mL/min] to yield two products after lyophilization. L-valine isoamylamide trifluoroacetate (**17**) (48.7 mg, 0.16 mmol, 77%): R_f(10) 0.63; ^{1}H NMR (CD_3OD) δ 0.80–1.13 (12 H, m), 1.10–1.78 (3 H, m), 2.17 (1 H, m), 3.25 (2 H, m), 3.57 (1 H, d, *J* = 6 Hz); ^{13}C NMR (CD_3OD) δ 18.3, 18.9, 22.7, 26.8, 31.4, 38.8, 39.2, 59.7, 168.3.

Lactone **20** (20.1 mg, 0.07 mmol, 33%): R_f(10) 0.52; ^{13}C NMR (CD_3OD) δ 16.2, 21.9, 23.6, 33.3, 35.1, 39.3, 54.4, 78.9, 180.6; ^{1}H NMR (CD_3OD) δ 1.01 (6 H, m), 1.29 (3 H, d, *J* = 7.3 Hz), 1.37–1.87 (3 H, m), 2.04 (2 H, m), 2.24 (1 H, m), 2.81 (1 H, m), 3.40 (1 H, m), 4.57 (1 H, m); mass spectrum, unstable to conditions.

Anal. ($C_{12}H_{20}F_3NO_4 \cdot H_2O$) C, H, N.

[(2*R*,5*S*)-5-Amino-2,7-dimethyl-4-oxooctanoyl]-L-valine Isoamylamide Trifluoroacetate (21). Compound **19** (0.057 g, 0.12 mmol) was deprotected according to General Procedure E. The solvent was removed in vacuo to give a crude product (0.055 g). This material was purified by semipreparative RP-HPLC [gradient elution: solvent A (0.1% TFA) and solvent B (0.1% TFA in 1:1 water–acetonitrile); 70% A to 10% A, 50 min, 3 mL/min] to provide **21** after lyophilization as a white powder (0.035 g, 0.072 mmol, 60%): R_f(9) 0.36; $[\alpha]_D$ –9.04° (*c* 0.432, methanol); ^{13}C NMR (CD_3OD) δ 18.2, 18.8, 19.5, 21.3, 22.6, 23.1, 25.5, 26.6, 31.7, 36.3, 38.4, 39.0, 39.7, 43.2, 57.9, 60.3, 173.1, 177.3, 205.5; ^{1}H NMR (CD_3OD) δ 0.80–2.24 (28 H, m), 2.37–3.36 (5 H, m), 4.07 (2 H m); mass spectrum, 370 (M^+ + 1), 283, 247, 220, 176, 122, 98, 86, 72.

Anal. ($C_{22}H_{40}F_3N_3O_5$) C, H, N.

(3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-diazo-5-methyl-2-oxohexane (26). Boc-L-leucine hydrate (10.2 g, 40.9 mmol) was reacted according to General Procedure A. The product was recrystallized from Skelly B to provide **26** as a pale yellow solid (7.1 g, 27.8 mmol, 68%): R_f(1) 0.25; mp 85–87 °C; $[\alpha]_D$ –79.7° (*c* 1.12, methanol); ^{1}H NMR ($CDCl_3$) δ 0.93 (6 H, d, *J* = 6 Hz), 1.43 (9 H, s), 1.40–1.80 (3 H, br m), 4.20 (1 H, br q, *J* = 7 Hz), 5.10 (1 H, br d, *J* = 7 Hz), 5.48 (1 H, s); mass spectrum, 256 (M^+ + 1), 200, 157, 130, 114, 86.

Anal. ($C_{12}H_{21}N_3O_3$) C, H, N.

(3*R*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-diazo-5-methyl-2-oxohexane (27). General Procedure A was followed, starting with Boc-D-leucine hydrate (9.1 g, 36.5 mmol). Recrystallization from Skelly B provided a light yellow solid (7.56 g, 29.6 mmol, 81%): R_f(1) 0.25; mp 87–89 °C; $[\alpha]_D$ +79.0° (*c* 1.12, methanol).

Anal. ($C_{12}H_{21}N_3O_3$) C, H, N.

(3*S*)-1-Bromo-3-[(*tert*-butyloxycarbonyl)amino]-5-methyl-2-oxohexane (31). Compound **26** (7.0 g, 27.4 mmol) was reacted according to General Procedure B, and the product was recrystallized from Skelly B to provide **31** as a white, crystalline solid (6.92 g, 22.5 mmol, 82%): R_f(1) 0.48; mp 76–78 °C; $[\alpha]_D$ –45.7° (*c* 0.975, methanol); ^{1}H NMR ($CDCl_3$) δ 0.97 (6 H, d, *J* = 6 Hz), 1.42 (9 H, s), 1.40–1.80 (3 H, m), 4.07 (1 H, s), 4.55 (1 H, m), 4.97 (1 H, br d, *J* = 7 Hz); mass spectrum, 310 (M^+ + 2), 308 (M^+), 254, 252, 236, 234, 210, 208, 130, 86.

Anal. ($C_{12}H_{22}BrNO_3$) C, H, N.

(3*R*)-1-Bromo-3-[(*tert*-butyloxycarbonyl)amino]-5-methyl-2-oxohexane (32). Compound **27** (7.5 g, 29.4 mmol) was reacted according to General Procedure B, and the product was recrystallized from Skelly B to provide a white solid (7.55 g, 24.5 mmol, 83%): R_f(1) 0.48; mp 76–78 °C; $[\alpha]_D$ +45.9° (*c* 0.996, methanol); ^{1}H NMR ($CDCl_3$) δ 0.97 (6 H, d, *J* = 6 Hz), 1.42 (9 H, s), 1.40–1.80 (3 H, m), 4.07 (1 H, s), 4.55 (1 H, m), 4.97 (1 H, br d, *J* = 7 Hz); mass spectrum, 310 (M^+ + 2), 308 (M^+), 254, 252, 236, 234, 210, 208, 130, 86.

Anal. ($C_{12}H_{22}BrNO_3$) C, H, N.

Dibenzyl Benzylmalonate (25). NaH (0.80 g, 60% oil dispersion, 20 mmol) was placed in a dry 50-mL flask and rinsed with Skelly B (3 mL) under an argon atmosphere. The solvent was decanted before adding DMF (20 mL) and HMPA (2 mL). Dibenzyl malonate (5.0 mL, 5.7 g, 20 mmol) was then slowly added with stirring. When the addition was complete (approximately 10 min), the reaction was stirred 30 min at 25 °C before adding benzyl bromide (2.6 mL, 3.7 g, 22 mmol) at once. The reaction was stirred 3 h at 25 °C under argon and then diluted into ethyl acetate (300 mL). The organic phase was washed with water (4 × 100 mL), dried over magnesium sulfate, suction filtered, and concentrated in vacuo to a yellow oil (9.3 g). The crude product was purified by MPLC (both columns in series, 6% ethyl acetate–Skelly B eluent) to yield **25** as a clear, colorless oil (3.97 g, 10.6 mmol, 53%): R_f(19) 0.58; ^{1}H NMR ($CDCl_3$) δ 3.20 (2 H, d, *J* = 8 Hz), 3.73 (1 H, t, *J* = 8 Hz), 5.07 (4 H, s), 6.97–7.37 (15 H, m); mass spectrum, 374 (M^+), 339, 247, 239, 221, 193, 180, 107, 91.

Anal. ($C_{24}H_{22}O_4$) C, H.

Dibenzyl Methylmalonate (24). The procedure for preparation of **25** was repeated but with methyl iodide (1.4 mL, 3.2 g, 22 mmol). The crude product was purified by MPLC (both columns in series, 10% ethyl acetate–Skelly B eluent) to provide **24** as a clear, colorless oil (4.06 g, 13.6 mmol, 68%): R_f(19) 0.46; ^{1}H NMR ($CDCl_3$) δ 1.43 (3 H, d, *J* = 7.5 Hz), 3.50 (1 H, q, *J* = 7.5 Hz), 5.10 (4 H, s), 7.27 (10 H, m); mass spectrum, 299 (M^+ + 1), 207, 181, 107, 91.

Anal. ($C_{18}H_{18}O_4$) C, H.

Benzyl (5*S*)-2-(Benzyloxycarbonyl)-5-[(*tert*-butyloxycarbonyl)amino]-2,7-dimethyl-4-oxooctanoate (36). General Procedure C was followed by reacting NaH (0.404 g, 10.0 mmol) with **24** (3.0 g, 10.0 mmol). The anion was then alkylated with **31** (2.58 g, 8.4 mmol). Purification by chromatography (100 g, 10:1 Skelly B–ethyl acetate eluent) provided **36** as a viscous, clear, colorless oil (2.2 g, 4.2 mmol, 50%): R_f(1) 0.45; $[\alpha]_D$ –31.2° (*c* 1.51, methanol); ^{1}H NMR ($CDCl_3$) δ 0.90 (6 H, m), 1.40 (9 H, s), 1.57 (3 H, s), 1.20–1.90 (3 H, m), 3.20 (2 H, s), 4.20 (1 H, m), 4.83 (1 H, m), 5.10 (4 H, s), 7.27 (10 H, br s); mass spectrum, 526 (M^+ + 1), 470, 426, 186, 181, 142, 130, 91.

Anal. ($C_{30}H_{39}NO_7$) C, H, N.

(2*RS*,5*S*)-5-[(*tert*-Butyloxycarbonyl)amino]-2,7-dimethyl-4-oxooctanoic Acid (41). Compound **36** (2.1 g, 4.0 mmol) was hydrogenated according to General Procedure D to provide the diacid (1.42 g), which was then decarboxylated in pyridine (15 mL) for 1.5 h. Purification (40 g, 2% methanol–methylene chloride eluent) provided **41** as a glass (0.97 g, 3.2 mmol, 80%): R_f(13) 0.39; ^{1}H NMR ($CDCl_3$) δ 0.95 (6 H, m), 1.23 (3 H, d, J = 7 Hz), 1.43 (9 H, s), 1.30–1.83 (3 H, m), 2.37–3.17 (3 H, m), 4.27 (1 H, m), 5.07 (1 H, m), 10.5 (1 H, br s); mass spectrum, 302 (M^+ + 1), 246, 228, 202, 130, 86.

Anal. ($C_{15}H_{27}NO_5$) C, H, N.

(2*RS*,5*S*)-5-Amino-2,7-dimethyl-4-oxooctanoic Acid Hydrochloride Salt (52). Compound **41** (0.215 g, 0.71 mmol) was dissolved in 4 N HCl in dioxane (2 mL) and allowed to stir 30 min at 25 °C. The solvent was removed in vacuo, and the residue was then triturated with ether. The crude product was then purified by semipreparative RP-HPLC [gradient elution: solvent A (0.1% THF) and solvent B (0.1% TFA in 1:1 acetonitrile–water); 90% A to 10% A, 60 min, 6 mL/min] and then lyophilized from dilute aqueous HCl to provide a hygroscopic glass (0.088 g, 0.37 mmol, 52%): R_f(18) 0.77; ^{1}H NMR (CD_3OD) δ 1.03 (6 H, m), 1.25 (3 H, d, J = 7 Hz), 1.42–2.07 (3 H, m), 2.47–3.13 (3 H, m), 4.20 (1 H, m); ^{13}C NMR (CD_3OD) δ 17.3, 21.7, 23.2, 25.9, 35.8, 36.5, (39.7, 40.0), (43.2, 43.6), (58.7, 59.0), 178.6, 205.2; mass spectrum, 218 (M^+ + 1), 144, 130, 86.

Anal. ($C_{10}H_{20}ClNO_3 \cdot 0.5H_2O$) C: calcd, 48.68; found, 49.11.

Benzyl (5*S*)-2-Benzyl-2-(benzyloxycarbonyl)-5-[(*tert*-butyloxycarbonyl)amino]-7-methyl-4-oxooctanoate (37). General Procedure C was followed by reacting NaH (0.29 g, 7.2 mmol) with **25** (2.69 g, 7.2 mmol). The anion was then alkylated with **31** (1.85 g, 6.0 mmol). Purification as described for compound **36** provided **37** as a colorless oil (1.97 g, 3.3 mmol, 54%): R_f(1) 0.46; $[\alpha]_D$ −26.8° (c 0.98, methanol); ^{1}H NMR ($CDCl_3$) δ 0.83 (6 H, d, J = 6 Hz), 1.43 (9 H, s), 1.40–1.80 (3 H, m), 3.07 (2 H, s), 4.17 (1 H, m), 4.80 (1 H, d, J = 7 Hz), 5.10 (4 H, s), 6.80–7.23 (5 H, m), 7.30 (10 H, s); mass spectrum, 602 (M^+ + 1), 546, 502, 186, 180, 130, 91, 86.

Anal. ($C_{36}H_{43}NO_7$) C, H, N.

Benzyl (5*R*)-2-Benzyl-2-(benzyloxycarbonyl)-5-[(*tert*-butyloxycarbonyl)amino]-7-methyl-4-oxooctanoate (38). General Procedure C was followed by reacting NaH (0.32 g, 8.0 mmol) with **25** (3.0 g, 8.0 mmol). The anion was then alkylated with **32** (2.07 g, 6.7 mmol). Purification as described for **36** provided **38** as a colorless oil (2.42 g, 4.0 mmol, 60%): R_f(1) 0.46; $[\alpha]_D$ +26.2° (c 1.15, methanol); ^{1}H NMR ($CDCl_3$) δ 0.83 (6 H, d, J = 6 Hz), 1.43 (9 H, s) 1.40–1.80 (3 H, m), 3.07 (2 H, s), 4.17 (1 H, m), 4.80 (1 H, d, J = 7 Hz), 5.10 (4 H, s), 6.80–7.23 (5 H, m), 7.30 (10 H, s); mass spectrum, 602 (M^+ + 1), 546, 502, 186, 180, 130, 91, 86.

Anal. ($C_{36}H_{43}NO_7$) C, H, N.

(2*RS*,5*S*)-2-Benzyl-5-[(*tert*-butyloxycarbonyl)amino]-7-methyl-4-oxooctanoic Acid (42). Compound **37** (2.0 g, 3.3 mmol) was hydrogenated according to General Procedure D to provide the diacid (1.37 g), which was then decarboxylated in pyridine (14 mL) for 30 min. Purification as for compound **41** provided a brittle glass (0.90 g, 2.4 mmol, 72%): R_f(2) 0.36; ^{1}H NMR ($CDCl_3$) δ 0.90 (6 H, m), 1.40 (9 H, s), 1.20–1.90 (3 H, m), 2.30–3.37 (5 H, m), 4.20 (1 H, m), 5.03 (1 H, br d, J = 7 Hz), 7.23 (5 H, m); mass spectrum, 378 (M^+ + 1), 304, 278, 260, 186, 174, 130, 91, 86.

Anal. ($C_{21}H_{31}NO_5$) C, H, N.

(2*RS*,5*R*)-2-Benzyl-5-[(*tert*-butyloxycarbonyl)amino]-7-methyl-4-oxooctanoic Acid (43). Compound **38** (2.3 g, 3.8 mmol) was hydrogenated according to General Procedure D to provide the diacid (1.58 g), which was then decarboxylated in pyridine (14 mL) for 30 min. Purification as for compound **41** provided a brittle glass (1.14 g, 3.0 mmol, 79%): R_f(2) 0.36; ^{1}H NMR ($CDCl_3$) δ 0.90 (6 H, m), 1.40 (9 H, s), 1.20–1.90 (3 H, m), 2.30–3.37 (5 H, m), 4.20 (1 H, m), 5.03 (1 H, br d, J = 7 Hz), 7.23 (5 H, m); mass spectrum, 378 (M^+ + 1), 304, 278, 260, 186, 174, 130, 91, 86.

Anal. ($C_{21}H_{31}NO_5$) C, H, N.

(2*RS*,5*S*)-5-Amino-2-benzyl-7-methyl-4-oxooctanoic Acid Trifluoroacetate Salt (53). Compound **42** (0.151 g, 0.40 mmol) was dissolved in TFA (2 mL) and allowed to stir 45 min at 25 °C. TFA was removed in vacuo to provide a crude product (200 mg), which was purified by semipreparative RP-HPLC [gradient elution: solvent A (0.1% aqueous TFA) and solvent B (0.1% TFA in 1:1 water–acetonitrile); 75% A to 10% A, 45 min, 4.5 mL/min] to provide after lyophilization a white, very hygroscopic powder (0.113 g, 0.29 mmol, 72%): R_f(3) 0.23, 0.31; ^{13}C NMR (CD_3OD) δ 21.7, 23.1, 25.8, 38.4, (39.7, 39.9), (40.6, 41.0), (43.0, 43.5), 58.6, 59.0, 127.8, 129.6, 130.1, 139.8, 176.8, 205.3; ^{1}H NMR (CD_3OD) δ 1.0 (6 H, m), 1.37–1.83 (3 H, m), 2.47–3.30 (6 H, m), 4.1 (1 H, m), 7.27 (5 H, m); mass spectrum, 278 (M^+ + 1), 260, 216, 174, 140, 117, 91, 86.

Anal. ($C_{16}H_{23}NO_3 \cdot H_2O \cdot CF_3CO_2H$) C, H, N.

(2*RS*,5*R*)-5-Amino-2-benzyl-7-methyl-4-oxooctanoic Acid Trifluoroacetate Salt (54). Compound **43** (0.227 g, 0.60 mmol) was deprotected and then purified as described for the 5*S* isomer **42** to provide **54** as a pale yellow solid (0.16 g, 0.41 mmol, 68%).

Anal. ($C_{16}H_{23}NO_3 \cdot CF_3CO_2H$) C, H, N.

[(2*RS*,5*S*)-2-Benzyl-5-[(*tert*-butyloxycarbonyl)amino]-7-methyl-4-oxooctanoyl]-L-valine Isoamylamide (51). Compound **66** (82 mg, 0.28 mmol) was deprotected according to General Procedure E. The reaction was concentrated to a glassy foam in vacuo. To this were added methylene chloride (2 mL), HOBt (45 mg, 0.29 mmol), TEA (38 μL, 28 mg, 0.28 mmol), and compound **42** (100 mg, 26.5 mmol). The mixture was cooled to 5 °C, and EDC (56 mg, 0.29 mmol) was added and the reaction allowed to stir 24 h. The mixture was diluted into ethyl acetate (70 mL) and washed with cold 0.5 N HCl (2 × 40 mL), saturated $NaHCO_3$ (2 × 40 mL) and finally with saturated NaCl (1 × 40 mL). The organic phase was dried over magnesium sulfate, suction filtered, and concentrated in vacuo to an oil (137 mg), which was purified by chromatography (6 g, 2% methanol–methylene chloride eluent) to provide **51** as a glassy foam (102 mg, 0.187 mmol, 70%): R_f(4) 0.22, 0.28; ^{1}H NMR ($CDCl_3$) δ 0.50–1.07 (18 H, m), 1.17–1.73 (15 H, m), 1.97–2.33 (1 H, br m), 2.43–3.37 (7 H, m), 4.10 (2 H, m), 5.0 (1 H, m), 5.73 (1 H, m), 6.67 (1 H, m), 7.20 (5 H, m); mass spectrum, 546 (M^+ + 1), 490, 459, 359, 304, 241, 154, 130, 86, 72.

Anal. ($C_{31}H_{51}N_3O_5$) C, H, N.

[(2*RS*,5*S*)-5-Amino-2-benzyl-7-methyl-4-oxooctanoyl]-L-valine Isoamylamide Trifluoroacetate Salt (55A and 55B). Compound **51** (100 mg, 0.18 mmol) was deprotected by treatment with 4 N HCl in dioxane (3 mL) at 25 °C for 40 min. Solvent was removed in vacuo to provide the crude product (91 mg), which was purified by semipreparative RP-HPLC [gradient elution: solvent A (0.1% aqueous TFA) and solvent B (0.1% TFA in 1:1 water–acetonitrile); 75% A to 10% A, 60 min, 3.6 mL/min] to provide two diastereomers. **55A** (15.1 mg, 0.027 mmol, 15%): RP-HPLC (isocratic elution, 0.1% TFA in 35% acetonitrile–water, 1.6 mL/min) RT = 9.1 min; R_f(18) 0.74; $[\alpha]_D$ +15.1° (c 0.225, methanol); ^{13}C NMR nCD_3OD) δ 18.96, 19.77, 21.56, 22.86, 23.40, 25.90, 26.98, 32.18, 38.73, 39.38, 39.60, 39.98, 41.71, 43.99, 58.23, 60.51, 127.80, 129.64, 130.24, 139.82, 173.14, 176.18, 206.08; ^{1}H NMR (CD_3OD) δ 0.96 (18 H, m), 1.12–2.10 (7 H, m), 2.26–3.25 (7 H, m), 3.92–4.21 (2 H, m), 7.24 (5 H, s); mass spectrum, 446 (M^+ + 1), 359, 260, 241, 154, 126, 72.

Anal. ($C_{26}H_{43}N_3O_3 \cdot CF_3CO_2H \cdot H_2O$) C, H, N.

55B (15.5 mg, 0.0277 mmol, 15.4%): RP-HPLC (isocratic elution, 0.1% TFA in 35% acetonitrile–water, 1.6 mL/min) RT = 12.6 min; R_f(18) 0.73; $[\alpha]_D$ −20.5° (c 0.205, methanol); ^{13}C NMR (CD_3OD) δ 18.47, 19.66, 21.62, 22.81, 23.30, 25.84, 27.03, 31.58, 38.84, 39.38, 39.71, 42.09, 44.80, 58.83, 60.57, 127.85, 129.69, 130.24, 140.87, 174.01, 177.20, 207.08; ^{1}H NMR (CD_3OD) δ 0.70 (6 H, d, J = 5.4 Hz), 0.96 (12 H, m), 1.15–2.05 (7 H, m), 2.82 (3 H, m), 3.23 (4 H, m), 4.03 (2 H, m), 7.26 (5 H, s); mass spectrum, 446 (M^+ + 1), 359, 260, 241, 154, 126, 72.

Anal. ($C_{26}H_{43}N_3O_3 \cdot CF_3CO_2H \cdot H_2O$) C, H, N.

[(2*RS*,4*RS*,5*S*)-Amino-2-benzyl-4-hydroxy-7-methyloctanoyl]-L-valine Isoamylamide Trifluoroacetate Salt (23). Compound **51** (0.130 g, 0.24 mmol) was deprotected according to General Procedure E. The solvent was removed in vacuo, and the residue was dissolved in 33% methanol–IPA. $NaBH_4$ (30 mg, 0.70 mmol) was added, and the reaction was stirred 20 min at 25 °C. The excess $NaBH_4$ was quenched by addition of 10% aqueous acetic acid, and the solvent was removed in vacuo to yield the crude product (0.14 g). The desired product was isolated by semipreparative RP-HPLC [gradient elution: solvent A (0.1% aqueous TFA) and solvent B (0.1% TFA in 1:1 water–acetonitrile); 75% to 5% A, 60 min, 3.6 mL/min]. Compound **23** was isolated as a white solid following lyophilization (79 mg, 0.141 mmol, 59%):

R_f(10) 0.35, 0.38; ^{1}H NMR (CD_3OD) δ 0.51–1.09 (18 H, m), 1.18–2.02 (10 H, m), 2.73–3.27 (4 H, m), 3.56–4.17 (2 H, m), 7.21 (5 H, br s); mass spectrum, unstable to conditions.

Anal. ($C_{26}H_{45}N_3O_3 \cdot CF_3CO_2H \cdot H_2O$) C, H, N.

(3*S*)-3,7-Bis[(*tert*-butyloxycarbonyl)amino]-1-diazo-2-oxoheptane (28). Bis-Boc-L-Lysine (16.7 g, 48.2 mmol) was reacted according to General Procedure A. A yellow oil was obtained, which was purified by MPLC (2.5 × 100 cm column, 20% ethyl acetate–Skelly B eluent) to provide a pale yellow crystalline solid (11.0 g, 29.7 mmol, 62%): R_f(5) 0.24; mp 81–84 °C; $[\alpha]_D$ −20.0° (*c* 0.98, methanol); ^{1}H NMR ($CDCl_3$) δ 1.43 (18 H, s), 1.23–1.97 (6 H, m), 3.10 (2 H, q, J = 6 Hz), 4.17 (1 H, m), 4.70 (1 H, br t, J = 6 Hz), 5.33 (1 H, d, J = 9 Hz), 5.53 (1 H, s); mass spectrum, unstable to conditions.

Anal. ($C_{17}H_{30}N_4O_5$) C, H, N.

(3*S*)-1-Bromo-3,7-bis[(*tert*-butyloxycarbonyl)amino]-2-oxoheptane (33). Compound **28** (11.0 g, 29.7 mmol) was dissolved in a mixture of ether (70 mL) and dioxane 20 mL) and reacted according to General Procedure B. The product was recrystallized from ether–Skelly B to provide a white, crystalline solid (11.1 g, 26.2 mmol, 88%): R_f(5) 0.42; mp 102–104 °C; $[\alpha]_D$ +1.5° (*c* 0.98, chloroform); ^{1}H NMR ($CDCl_3$) δ 1.43 (18 H, s), 1.33–1.73 (6 H, m), 3.10 (2 H, br q, J = 6 Hz), 4.07 (2 H, s), 4.53 (1 H, m), 5.25 (1 H, br d, J = 9 Hz); mass spectrum, 424 (M^+ + 2), 422 (M^+), 369, 367, 325, 323, 313, 311, 269, 267, 169, 145, 128, 84.

Anal. ($C_{17}H_{31}BrN_2O_5$) C, H, N, Br.

Benzyl (5*S*)-2-(Benzyloxycarbonyl)-5,9-bis[(*tert*-butyloxycarbonyl)amino]-2-methyl-4-oxononanoate (39). Compound w33 (1.56 g, 3.7 mmol) was reacted with **24** (1.29 g, 4.3 mmol) according to General Procedure C. Purification by MPLC (1.5 × 100 cm column, 17% ethyl acetate–Skelly B eluent) provided a viscous oil (1.06 g, 1.7 mmol, 45%): R_f(5) 0.41; $[\alpha]_D$ −19.5° (*c* 0.845, methanol); ^{1}H NMR ($CDCl_3$) δ 1.42 (18 H, s), 1.57 (3 H, s), 1.20–1.83 (6 H, m), 3.05 (2 H, q, J = 6 Hz), 3.17 (2 H, s), 4.20 (1 H, m), 4.60 (1 H, m), 5.10 (5 H, br s), 7.30 (10 H, s); mass spectrum, unstable to conditions.

Anal. ($C_{35}H_{48}N_2O_9$) C: calcd, 65.61; found, 65.19.

Benzyl (5*S*)-2-Benzyl-2-(benzyloxycarbonyl)-5,9-bis[(*tert*-butyloxycarbonyl)amino]-4-oxononanoate (40). Compound **33** (1.16 g, 2.7 mmol) was reacted with **25** (1.59 g, 4.2 mmol) according to General Procedure C. The product was purified as with compound **39** to provide a colorless syrup (1.07 g, 1.5 mmol, 53%): R_f(5) 0.57; $[\alpha]_D$ +4.7° (*c* 0.148, chloroform); ^{1}H NMR ($CDCl_3$) δ 1.43 (18 H, s), 1.20–1.80 (6 H, m), 3.03 (4 H, m), 3.43 (2 H, s), 4.13 (1 H, m), 4.53 (1 H, m), 5.07 (1 H, m), 5.13 (4 H, s), 6.77–7.23 (5 H, m), 7.30 (10 H, s); mass spectrum, unstable to conditions.

Anal. ($C_{41}H_{52}N_2O_9$) C, H, N.

(2*RS*,5*S*)-5,9-Bis[(*tert*-butyloxycarbonyl)amino]-2-methyl-4-oxononanoic Acid (44). Compound **39** (1.0 g, 1.6 mmol) was hydrogenated according to General Procedure D to provide the diacid (0.65 g), which was then decarboxylated in pyridine (6 mL) for 1.5 h. Purification by chromatography (20 g, 2% methanol–methylene chloride eluent) provided a glassy foam (0.423 g, 1.02 mmol, 64%): R_f(6) 0.20, 0.27; ^{1}H NMR ($CDCl_3$) δ 1.23 (3 H, d, J = 7 Hz), 1.40 (18 H, s), 1.30–1.97 (6 H, m), 2.37–3.27 (5 H, m), 4.27 (1 H, m), 4.80 (1 H, m), 5.37 (1 H, m), 7.93 (1 H, br s); mass spectrum, 417 (M^+ + 1), 361, 261, 128, 84.

Anal. ($C_{20}H_{36}N_2O_7$) C, H, N.

(2*RS*,5*S*)-2-Benzyl-5,9-bis[(*tert*-butyloxycarbonyl)amino]-4-oxononanoic Acid (45). Compound **40** (1.0 g, 1.3 mmol) was hydrogenated according to General Procedure D to provide the diacid (0.73 g), which was then decarboxylated in pyridine (6 mL) for 40 min. Purification by chromatography (40 g, 3% methanol–methylene chloride eluent) provided a glassy foam (0.559 g, 1.13 mmol, 87%): R_f(7) 0.59; ^{1}H NMR ($CDCl_3$) δ 1.17–1.90 (24 H, m), 2.33–3.37 (7 H, m), 4.17 (1 H, m), 4.77 (1 H, m), 5.27 (1 H, m), 7.20 (5 H, m), 8.50–9.17 (1 H, br); mass spectrum, 493 (M^+ + 1), 437, 356, 319, 300, 209, 184, 165, 128, 91, 84.

Anal. ($C_{26}H_{40}N_2O_7$) C, H, N.

[(2*RS*,5*S*)-5,9-Bis[(*tert*-butyloxycarbonyl)amino]-2-methyl-4-oxononanoyl]-L-phenylalanine Benzyl Ester (50). The free base of L-phenylalanine benzyl ester (65 mg, 0.25 mmol) was formed from the TsOH salt by treatment with saturated $NaHCO_3$ followed by extraction with ethyl acetate. The amine component was dissolved in a mixture of methylene chloride (2 mL) and DMF (0.5 mL), and to this was added **44** (100 mg, 0.24 mmol) and HOBt (40 mg, 0.26 mmol). The solution was chilled to 0 °C, and EDC (52 mg, 0.27 mmol) was added. The reaction was stirred 24 h in an ice bath, diluted into ethyl acetate (70 mL), and washed with cold 0.5 N HCl (2 × 40 mL), saturated $NaHCO_3$ (2 × 40 mL), and saturated NaCl (1 × 40 mL). The organic phase was dried over magnesium sulfate, suction filtered, and concentrated in vacuo to a crude product (146 mg), which was purified by chromatography (7 g, 2% methanol–methylene chloride eluent) to provide a colorless glass (133 mg, 0.20 mmol, 85%): R_f(4) 0.28; ^{1}H NMR ($CDCl_3$) δ 1.10 (3 H, m), 1.40 (18 H, s), 1.23–1.63 (6 H, m), 2.07–3.20 (7 H, m), 4.20 (1 H, m), 4.40–5.30 (5 H, m), 6.17 (1 H, m), 6.93–7.50 (10 H, m); mass spectrum, 654 (M^+ + 1), 554, 498, 435, 372, 352, 262, 145, 128, 91, 84.

Anal. ($C_{36}H_{51}N_3O_8$) C, H, N.

(2*RS*,5*S*)-5,9-Diamino-2-methyl-4-oxononanoic Acid Dihydrochloride Salt (57). Compound **44** (0.10 g, 0.24 mmol) was dissolved in 4 N HCl in dioxane (1 mL) and allowed to stir 30 min at 25 °C under argon. The solvent was removed in vacuo, and the residue was dissolved in 0.01 N HCl and chromatographed on LH-20 (40 mL, 25–100-μm beads) eluting with the same solvent. The fractions containing the product were pooled and lyophilized to yield a hygroscopic glass (44.4 mg, 0.14 mmol, 60%): R_f(17) 0.40; ^{13}C NMR (CD_3OD) δ (17.44, 17.61), (22.64, 23.02), 28.12, (30.07, 30.23), (35.59, 36.30), 40.36, (43.18, 43.61), (59.65, 60.08), 179.0, 205.5; ^{1}H NMR (CD_3OD) δ 1.07–2.30 (9 H, M), 2.46–3.25 (5 H, M), 4.23 (1 H, M); mass spectrum, 217 (M^+ + 1), 200, 180, 152, 112, 84.

Anal. ($C_{10}H_{20}N_2O_3 \cdot 2HCl \cdot H_2O$) H: Calcd, 9.12; found, 8.70.

(2*RS*,5*S*)-2-Benzyl-5,9-diamino-4-oxononanoic Acid Bis(trifluoroacetate) Salt (58). Compound **45** (80 mg, 0.16 mmol) was deprotected in 4 N HCl in dioxane (2 mL) for 40 min at 25 °C under argon. The solvent was removed in vacuo, and the residue was purified by semipreparative RP-HPLC [gradient elution: solvent A (0.1% aqueous TFA) and solvent B (0.1% TFA in 1:1 water–acetonitrile); 85% A to 50% A, 60 min, 4.5 mL/min] to yield an extremely hygroscopic, light yellow solid (59 mg, 0.11 mmol, 71%): R_f(17) 0.57; ^{13}C NMR (CD_3OD) δ (22.6, 23.0), 28.1, (30.0, 30.2), 38.4, 40.2, (40.5, 41.1), (42.8, 43.5), (59.5, 60.1), 127.7, 129.6, 130.2, 139.8, 177.4, 205.5; ^{1}H NMR (CD_3OD) δ 1.07–2.30 (9 H, m), 2.46–3.25 (5 H, m), 4.23 (1 H, m); mass spectrum, unstable to conditions.

Anal. ($C_{16}H_{24}N_2O_3 \cdot 2CF_3CO_2H \cdot 1.5H_2O$) C, H, N.

[(2*RS*,5*S*)-5,9-Diamino-2-methyl-4-oxononanoyl]-L-phenylalanine Bis(trifluoroacetate) Salt (59). Compound **50** (0.13 g, 0.20 mmol) was dissolved in MeOH (10 mL), and the solution was purged with argon; 10% palladium on carbon (30 mg) was added, and the mixture was hydrogenated for 12 h at 25 °C on a Parr hydrogenation apparatus (25 psi H_2). The reaction mixture was purged with argon and suction filtered through a Celite pad (MeOH rinse). The filtrate was concentrated in vacuo to give a glass.

This product was deprotected in TFA (2 mL) for 40 min at 25 °C. The reaction was concentrated in vacuo to a hygroscopic glass, which was purified by semipreparative RP-HPLC [gradient elution: solvent A (0.1% aqueous TFA) and solvent B (0.1% TFA in 1:1 water–acetonitrile] to yield a hygroscopic white powder (69 mg, 0.11 mmol), 56%): R_f(12) 0.45; ^{13}C NMR (CD_3OD) δ (18.5, 18.8), (22.2, 23.2), (28.0, 28.3), (30.1, 30.2), (36.3, 37.6), (38.3, 38.6), (40.1, 40.8), (43.4, 43.6), 55.4, (59.4, 60.4), 127.7, 129.5, 130.4, 138.5, 174.8, 177.6, 205.3; ^{1}H NMR (CD_3OD) δ 0.96 (1 H, m), 1.18 (2 H, d J = 7 Hz), 1.08–2.17 (6 H, m), 2.48–3.22 (7 H, m), 4.17 (1 H, br t, J = 4 Hz), 4.49 (1 H, br t, J = 7 Hz), 7.27 (5 H, m).

Anal. ($C_{19}H_{29}N_3O_4 \cdot 2CF_3CO_2H \cdot 1.5H_2O$) C, H, N.

(3*S*)-3-[(Benzyloxycarbonyl)amino]-1-diazo-5-methyl-2-oxohexane (29). Cbz-L-Leucine (10.0 g, 37.7 mmol) was reacted according to General Procedure A. The product was recrystallized from ethyl acetate–Skelly B to give a light yellow solid (7.37 g, 25.5 mmol, 68%): R_f(5) 0.48; mp 63–65 °C; $[\alpha]_D$ −57.5° (*c* 0.905, methanol); ^{1}H NMR ($CDCl_3$) δ 0.93 (6 H, d, J = 6 Hz), 1.37–1.93 (3 H, m), 4.27 (1 H, m), 5.07 (2 H, s), 5.23 (1 H, m), 5.37 (1 H, s), 7.33 (5 H, s); mass spectrum, 290 (M^+ + 1), 262, 220, 176, 91.

Anal. ($C_{15}H_{19}N_3O_3$) C, H, N.

(3*S*)-3-[(Benzyloxycarbonyl)amino]-1-bromo-5-methyl-2-oxohexane (34). Compound **29** (5.03 g, 17.4 mmol) was reacted

according to General Procedure B to provide a clear, colorless oil, which required no further purification (5.7 g, 16.7 mmol, 96%): R_f(5) 0.64; $[\alpha]_D$ −32.4° (*c* 0.855, methanol); ^{1}H NMR ($CDCl_3$) δ 0.93 (6 H, m), 1.37–1.90 (3 H, m), 4.03 (2 H, s), 4.60 (1 H, m), 5.10 (2 H, s), 5.27 (1 H, d, *J* = 7.5 Hz), 7.33 (5 H, s); mass spectrum, 342 (M^+), 298, 234, 176, 91.

Anal. ($C_{15}H_{20}BrNO_3$) C, H, Br, N.

Ethyl (5*S*)-2-Benzyl-5-[(benzyloxycarbonyl)amino]-2-(ethoxycarbonyl)-7-methyl-4-oxooctanoate (46). General Procedure C was followed by reacting the bromomethyl ketone **34** (3.0 g, 8.8 mmol) with diethyl benzyl malonate (2.46 mL, 2.62 g, 10.5 mmol). The product was purified by chromatography (130 g, 9% ethyl acetate–Skelly B eluent) to provide **46** as a colorless oil (1.67 g, 3.3 mmol, 37%): R_f(1) 0.30; $[\alpha]_D$ −16.4° (*c* 1.12, methanol); ^{1}H NMR ($CDCl_3$) δ 0.90 (6 H, d, *J* = 6 Hz), 1.23 (6 H, t, *J* = 7.5 Hz), 1.10–1.93 (3 H, m), 3.03 (2 H, s), 3.40 (2 H, s), 4.20 (4 H, q, *J* = 7.5 Hz), 4.27 (1 H, m), 5.10 (2 H, s), 6.93–7.33 (5 H, m), 7.37 (5 H, s); mass spectrum, 512 (M^+ + 1), 468, 422, 360, 245, 176, 145, 91.

Anal. ($C_{29}H_{37}NO_7$) C, H, N.

(2*RS*,5*S*)-2-Benzyl-5-[(benzyloxycarbonyl)amino]-7-methyl-4-oxooctanoic Acid (48). Diethyl ester **46** (1.4 g, 2.7 mmol) was saponified according to General Procedure D to provide the diacid (1.1 g), which was then decarboxylated in pyridine (12 mL) for 30 min. Purification (35 g, 2% methanol–methylene chloride eluent) provided an oil (0.37 g, 0.90 mmol, 33%): R_f(12) 0.50; ^{1}H NMR ($CDCl_3$) δ 0.88 (6 H, m), 1.18–1.75 (3 H, m), 2.45 (1 H, m), 2.72 (2 H, m), 3.12 (2 H, m), 3.98–4.40 (1 H, m), 5.02 (2 H, s), 5.18 (1 H, m), 7.00–7.38 (10 H, m); mass spectrum, 412 (M^+ + 1), 394, 350, 304, 260, 176, 91.

Anal. ($C_{24}H_{49}NO_5$) C, H, N.

(3*S*)-3,7-Bis[(benzyloxycarbonyl)amino]-1-diazo-2-oxoheptane (30). Bis-Cbz-L-lysine (10.0 g, 24.1 mmol) in dry THF (90 mL) was reacted according to General Procedure A. An oily product was obtained. This material (1 g) was purified by chromatography (20 g, 20% ethyl acetate–Skelly B eluent) to provide a sample for characterization; the remaining material was used without further purification: R_f(8) 0.43; $[\alpha]_D$ −27.4° (*c* 1.06, methanol); ^{1}H NMR ($CDCl_3$) δ 1.20–1.90 (6 H, m), 3.16 (2 H, q, *J* = 6 Hz), 4.16 (1 H, m), 5.07 (5 H, m), 5.38 (1 H, s), 5.77 (1 H, d, *J* = 7.5 Hz), 7.33 (10 H, s); mass spectrum, unstable to conditions.

Anal. ($C_{23}H_{26}N_4O_5$) C, H, N.

(3*S*)-3,7-Bis[(benzyloxycarbonyl)amino]-1-bromo-2-oxoheptane (35). Compound **30** (10.6 g, 24.2 mmol) was dissolved in 1:1 THF–ether (80 mL) and reacted according to General Procedure B. The product was recrystallized from ethyl acetate–Skelly B to provide a white solid (8.66 g, 17.6 mmol, 73%): R_f(8) 0.58; mp 89–90 °C; $[\alpha]_D$ −16.3° (*c* 1.02, methanol); ^{1}H NMR ($CDCl_3$) δ 1.13–1.97 (6 H, m), 3.13 (2 H, q, *J* = 6 Hz), 4.03 (2 H, s), 4.57 (1 H, m), 4.90 (1 H, m), 5.07 (4 H, m), 5.63 (1 H, m), 7.36 (10 H, s); mass spectrum, 491 (M^+ + 1), 447, 367, 218, 181, 174, 160, 107, 91.

Anal. ($C_{23}H_{27}BrN_2O_5$) Br: calcd, 16.26; found, 15.60.

Ethyl (5*S*)-2-Benzyl-5,9-bis[(benzyloxycarbonyl)amino]-2-(ethoxycarbonyl)-4-oxononanoate (47). General Procedure C was followed by reacting bromomethyl ketone **35** (5.0 g, 10.2 mmol) with diethyl benzylmalonate (3.6 mL, 3.8 g, 15.3 mmol). Purification by chromatography (230 g, 25% ethyl acetate–Skelly B eluent) provided a clear, colorless oil (2.02 g, 3.1 mmol, 30%): R_f(8) 0.58; $[\alpha]_D$ −8.97° (*c* 1.06, methanol); ^{1}H NMR ($CDCl_3$) δ 1.22 (6 H, t, *J* = 8 Hz), 1.07–2.03 (6 H, m), 3.00 (2 H, m), 3.13 (2 H, q, *J* = 6 Hz), 3.40 (2 H, s), 4.20 (4 H, q, *J* = 8 Hz), 4.10–4.50 (1 H, m), 4.87 (1 H, m), 5.07 (4 H, m), 5.43 (1 H, d, *J* = 7 Hz), 6.90–7.37 (5 H, m), 7.33 (10 H, s); mass spectrum, 661 (M^+ + 1), 617, 599, 553, 509, 463, 245, 218, 174, 145, 91.

Anal. ($C_{37}H_{44}N_2O_9$) C, H, N.

(2*RS*,5*S*)-Benzyl-5,9-bis[(benzyloxycarbonyl)amino]-4-oxononanoic Acid (49). Diethyl ester **47** (2.0 g, 3.0 mmol) was saponified according to General Procedure D to provide the diacid (1.68 g), which was then decarboxylated in pyridine (16 mL) for 30 min. Purification by chromatography (30 g, 0.1% acetic acid in 2% methanol–methylene chloride eluent) provided a clear, colorless glass (1.40 g, 2.5 mmol, 82%): R_f(12) 0.56, 0.62; ^{1}H NMR ($CDCl_3$) δ 1.00–1.83 (6 H, m), 2.17–3.37 (7 H, m), 4.23 (1 H, br m), 5.00 (5 H, br s), 5.73 (1 H, m), 7.00–7.50 (15 H, br s); mass spectrum, unstable to conditions.

Anal. ($C_{32}H_{36}N_2O_7$) C, H, N.

5(*RS*)-[1(*S*)-Amino-3-methylbutyl]-3(*RS*)-benzyldihydrofuran-2(3*H*)-one Trifluoroacetate Salt (61). Keto acid **42** (128 mg, 0.34 mmol) was dissolved in ethanol (2 mL) and reacted with $NaBH_4$ (20 mg, 0.54 mmol). TLC (0.1% HOAc in 2% MeOH–CH_2Cl_2) showed rapid reduction of the ketone to the alcohol **60**, which could be isolated without lactonization by careful acidification at 5 °C. Compound **60** was then treated with 30% HOAc–TFA (1 mL) at 0 °C for 30 min. The reaction was then diluted into cold water (30 mL) and lyophilized to a hygroscopic powder which ^{13}C NMR and TLC indicated to be the lactone **61**. This material was then purified by RP-HPLC [gradient elution: solvent A (0.1% aqueous TFA) and solvent B (0.1% TFA in 50% water–acetonitrile); 65% A to 10% A, 6 mL/min, 40 min] to yield a hygroscopic white solid (95 mg, 0.25 mmol, 74%): R_f(18) 0.80; ^{13}C NMR (D_2O) δ 20.8, 22.4, 22.7, 23.9, 27.2, 27.9, 29.6, 30.8, 34.6, 35.2, 35.6, 36.1, 36.3, 37.1, 37.5, 41.5, 41.7, 41.9, 51.1, 51.6, 53.4, 53.7, 78.7, 79.1, 79.4, 127.5, 129.3, 129.5, 138.1, 138.3, 180.9, 181.7; ^{1}H NMR (D_3OD) δ 1.00 (6 H, m), 1.44–1.96 (3 H, m), 2.05–3.08 (5 H, m), 3.66 (1 H, m), 4.62 (1 H, m), 7.27 (5 H, m); mass spectrum, 261 (M^+), 208, 175, 145, 91.

Anal. ($C_{16}H_{23}NO_2 \cdot CF_3CO_2H \cdot 0.75H_2O$) C, H, N.

3(*RS*)-Benzyl-5(*RS*)-hydroxy-6(*S*)-isopropyl-2-piperidone (63). Lactone **61** (63 mg, 0.17 mmol) was dissolved in D_2O (1 mL) to which was added a 40% solution of NaOD in D_2O (0.17 g, 0.12 mL, 1.7 mmol). A white solid formed immediately. A portion of this mixture (0.7 mL) was removed and neutralized to pH 7.2 with 0.2 N HCl at 0 °C. The mixture was lyophilized to a white solid, which was purified by semipreparative RP-HPLC (10% acetonitrile–water to 60% acetonitrile–water, 40 min, 4.5 mL/min) to provide a white solid (13 mg, 0.05 mmol, 48%); in absorption at 1680 cm^{-1} in the IR spectrum confirmed the lactam structure **63**: R_f(18) 0.77; ^{13}C NMR (D_3COD) δ 22.3, 23.4, 25.0, 25.4, 34.4, 36.8, 35.6, 37.3, 38.2, 40.2, 41.6, 54.5, 55.8, 58.1, 69.9, 70.9, 127.3, 127.6, 129.5, 130.3, 141.6; ^{1}H NMR (CD_3OD) δ 0.89 (6 H, m), 1.12–1.91 (5 H, m), 2.30–3.25 (4 H, m), 3.73 (1 H, m), 7.20 (5 H, m); mass spectrum, 261 (M^+), 218, 204, 175, 170, 131, 91, 86; exact mass calcd for $C_{16}H_{23}NO_2$, *m/e* 261.1729, obsd 261.1727.

5(*RS*)-[1(*S*)-[(Benzyloxycarbonyl)amino]-3-methylbutyl]-5(*RS*)-hydroxy-3(*RS*)-benzyl-2-pyrrolidone (64). Compound **48** (0.89 g, 2.2 mmol) was reacted with isobutyl chloroformate (0.29 g, 0.28 mL, 2.2 mmol) and TEA (0.22 g, 0.3 mL, 2.2 mmol) in dry THF (5 mL) at −15 °C for 15 min. Anhydrous ammonia was then bubbled through the reaction mixture for 10 min. After standing at room temperature for 1 h, the reaction was diluted into water (100 mL) and extracted with ethyl acetate to provide the crude product **64** (0.72 g). A portion of this material was purified for characterization: R_f(7) 0.35; ^{13}C NMR ($CDCl_3$) δ 179.7, 179.4, 157.2, 157.0, 139.0, 138.8, 136.3, 129.0, 128.8, 128.5, 128.1, 128.0, 126.4, 89.0, 88.7, 67.1, 57.0, 55.7, 42.1, 41.5, 38.5, 36.3, 36.0, 24.9, 24.5, 23.7, 21.3; mass spectrum, 392 (M^+ − 18), 301, 284, 257, 220, 176, 91.

Anal. ($C_{24}H_{30}N_2O_4$) C, H, N.

2(*RS*)-Benzyl-5(*S*)-[(benzyloxycarbonyl)amino]-4-(*RS*)-hydroxy-7-methyloctanamide (65). The remaining crude product **64** (0.61 g) was reduced with $NaBH_4$ (114 mg, 3.1 mmol) in ethanol (4 mL) for 4 h before TLC (4% methanol–methylene chloride) showed the reaction to be complete. The reaction was quenched with acetic acid and the product isolated by extractive workup. Purification by chromatography (25 g, 4% methanol–methylene chloride eluent) provided **65** as a white solid (0.35 g, 0.84 mmol, 45%): R_f(7) 0.27, 0.31; mp 146–150 °C; ^{13}C NMR (DMSO-d_6) δ 176.6, 156.0, 140.3, 137.3, 128.8, 128.2, 127.8, 127.5, 127.4, 125.6, 70.8, 65.0, 53.8, 42.8, 36.2, 24.2, 23.7, 21.3; ^{1}H NMR (DMSO-d_6) δ 0.83 (6 H, m), 1.05–1.75 (5 H, br m), 2.68 (3 H, br s) 3.38 (3 H, m), 4.60 (1 H, m), 5.03 (3 H, br s), 6.65 (1 H, m), 6.83–7.82 (10 H, m); mass spectrum, 413 (M^+ + 1), 395, 304, 260, 192, 176, 91.

Anal. ($C_{24}H_{32}N_2O_4$) C: Calcd, 69.88; found, 69.46.

***N*-(*tert*-Butyloxycarbonyl)-L-valine Isoamylamide (66).** Boc-L-valine (2.17 g, 10.0 mmol), HOBt (1.68 g, 11 mmol), and isoamylamine (1.68 mL, 0.87 g, 10.0 mmol) were dissolved in methylene chloride (20 mL) and chilled to 0 °C. EDC (2.11 g, 11 mmol) was added, and the reaction was allowed to stir 24 h.

The reaction mixture was diluted into methylene chloride (200 mL) and washed with cold 0.5 N HCl (3 × 100 mL), saturated $NaHCO_3$ (2 × 100 mL), and saturated NaCl (1 × 100 mL). The organic phase was dried over magnesium sulfate, suction filtered, and concentrated in vacuo to obtain a white solid (2.54 g, 8.9 mmol, 89%): R_f(4) 0.42; mp 127-129 °C; $[\alpha]_D$ -23.5° (c 1.01, MeOH); 1H NMR ($CDCl_3$) δ 0.93 (12 H, m), 1.57 (9 H, s), 1.23-1.77 (3 H, m), 2.10 (1 H, m), 3.23 (2 H, br q, J = 9 Hz), 3.83 (1 H, dd, J = 9, 6 Hz), 5.17 (1 H, br d, J = 9 Hz), 6.23 (1 H, br m); mass spectrum, 286 (M^+), 213, 172, 116, 72.

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Probes for Narcotic Receptor Mediated Phenomena. 15.[1] (3*S*,4*S*)-(+)-*trans*-3-Methylfentanyl Isothiocyanate, a Potent Site-Directed Acylating Agent for the δ Opioid Receptors in Vitro

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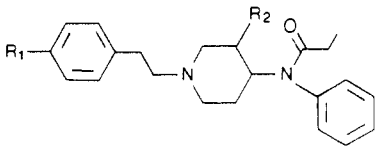
Recently we reported the synthesis of the first enantiomeric pair of irreversible opioid ligands [(3*S*,4*R*)-(-)- and (3*R*,4*S*)-(+)-*cis*-4, SUPERFIT] and specific interaction of the latter with the δ receptor. Here we report another enantiomeric pair of irreversible opioid ligands, (+)-*trans*- and (-)-*trans*-3-methylfentanyl isothiocyanates [(3*S*,4*S*)-(+)-*trans*- and (3*R*,4*R*)-(-)-*trans*-4]. A single-crystal X-ray analysis of the 2,4,6-trinitrobenzenesulfonic acid salt of (+)-*trans*-3-methyl-*N*-phenyl-4-piperidinamine [(+)-*trans*-8] revealed it (and, therefore, 4) to have the trans configuration and the absolute configuration of (+)-*trans*-8 to be 3*S*,4*S*. The (+)-trans enantiomer of 4 was shown to be highly potent and about 10-fold more selective as an acylating agent than (-)-*trans*-4 for the higher affinity [^{3}H]DADL (δ) binding site in rat brain membranes. In that assay, (+)-*trans*-4 and (+)-*cis*-4 were essentially equipotent as affinity ligands, and the levo enantiomers were considerably less potent. (+)-*trans*-4 was, thus, a potent, subtype-selective acylating agent for the δ opioid receptor in vitro. With membranes from NG108-15 neuroblastoma × glioma hybrid cells, containing only δ receptors, (+)-*cis*-4 was found to be a little more potent than (+)-*trans*-4. Similarly, (+)-*cis*-4 is the most effective inhibitor of adenylate cyclase in these membranes, (+)-*trans*-4 has weak activity, and the levo enantiomers are inactive. Only (+)-*cis*-4 was found to have antinociceptive activity in vivo.

Since the discovery of saturable, high-affinity, stereospecific receptors for opioid drugs and their antagonists in the mammalian central nervous system[2] and the identification of endogenous peptide ligands[3] for these receptors, much effort has been devoted toward elucidating their structure and function. These efforts have firmly established[4] the existence of distinct μ, δ, and κ subtypes and validated the hypothesis of heterogeneity of opioid receptors advanced more than two decades ago.[5] Additionally, on the basis of a variety of in vitro and in vivo evidence, an "opioid receptor complex" consisting of distinct yet interacting μ and δ binding sites has been postulated.[6-8]

A number of groups have synthesized irreversible ligands as tools for the study of opiod receptor subtypes.[9-13] Some of these ligands were used for the purification to homogeneity of μ[14] and δ[15] receptor subtypes. Other irreversible ligands have facilitated the study of the mechanisms of tolerance and dependence,[16] interactions among opioid receptor subtypes,[17] and examination of the hypothesis of multiple μ receptors,[18] as well as developing conditions for the autoradiographic visualization and mapping of the anatomical distributions of opioid receptor subtypes.[19-21]

As part of our ongoing opioid receptor studies, we described recently the μ-selective acylating agent BIT based on etonitazene and the δ-specific ligand FIT (2), a derivative of the potent opioid agonist fentanyl (1).[22,23] We also



1: $R_1 = R_2 = H$
2: R_1 = NCS, $R_2 = H$
3: $R_1 = H$, $R_2 = CH_3$
4: R_1 = NCS, $R_2 = CH_3$

reported the synthesis, absolute configuration, and bio-

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