Synthesis of α,α-Dimethylaldophosphamide *O*-Methyloxime (11). A solution of hydroperoxide **9** (20 mg, 0.06 mmol) and *O*-methylhydroxylamine hydrochloride (5 mg, 0.06 mmol) in water (1 mL) was stirred overnight at room temperature. The solution was saturated with NaCl and then extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated at reduced pressure. Spectroscopic analysis of the residual material indicated that predominantly (>86%) one diastereomer of **11** (presumably the *E* isomer[38,39]) had been isolated: ^1H NMR (89.55 MHz, $CDCl_3$) δ 7.3 (s, 1 H, N=CH), 4.1-3.2 (m, 10 H, CH_2O and 2 NCH_2CH_2Cl), 3.8 (s, 3 H, OCH_3), 3.1-2.7 (br s, 2 H, NH_2), and 1.13 and 1.11 [2 s, 3 H each, $C(CH_3)_2$].

NMR Measurements in Me_2SO-d_6. A JEOL GX-400 NMR spectrometer was used to record ^1H, ^{13}C, and ^{31}P NMR spectra in Me_2SO-d_6. The instrument was equipped with a variable-temperature unit, which maintained the sample temperature to ±0.1 °C. One-dimensional ^{13}C measurements were made by using bilevel ^1H decoupling (4-W decoupling power during the relaxation delay interval and 9 W during acquisition), and ^{31}P measurements were made with decoupling (9 W) during the acquisition period only so as to suppress possible nuclear Overhauser effects. Measurements for the equilibrium studies were made with a pulse delay of 10 s; in all other cases the pulse delay was 3 s.

^1H-^{31}P selective INEPT data were obtained by using the following pulse sequence: $90°_x(^1H)$-$\Delta_1/2$-$180°_y(^1H)$, $180°_x$-(^{31}P)-$\Delta_1/2$-$90°_{\pm y}(^1H)$, $90°_x(^{31}P)$-$\Delta_2/2$-$180°_x(^1H)$, $180°_x$-(^{31}P)-$\Delta_2/2$-acquire with broad-band decoupling.

Acquisition parameters were as follows: $90°(^1H)$ = 10 ms; $90°(^{31}P)$ = 19 μs; Δ_1 = 20 ms; Δ_2 = 20 ms; pulse delay = 3 s; and total scans = 40.

^{31}P-detected two-dimensional ^1H-^{31}P correlation NMR data were obtained with the pulse sequence: $90°_x(^1H)$-$\Delta_1/2$-$180°_x$-(^{31}P)-$\Delta_1/2$-Δ_2-$90°_x(^1H)$, $90°_x(^{31}P)$-Δ_3-acquire with broad-band decoupling.

Values for Δ_2 and Δ_3 of 10 and 5 ms, respectively, were used for optimum polarization transfer without severe signal loss due to T_2 relaxation. Values for Δ_1 ranged from 0.00 to 6.93 ms in steps of 110 μs for a total of 64 experimental data points in t_1. The block size in t_2 was 1K. The data were collected in ca. 50 min with 16 scans per t_2 slice and a repetition delay of 3 s. The final power spectrum was obtained by using a 3-Hz exponential filter in t_2 and no filtering in t_1.

Acknowledgment. This investigation was supported in part by PHS Grant No. CA21345 [to G.Z. (1-4 years) and S.M.L. (4-6 years)] and CA37323 (to S.M.L.) awarded by the National Cancer Institute, DHHS. We are grateful to Lawrence R. Phillips of the Food and Drug Administration for the electron-impact mass spectrum and we thank Dr. Wojciech J. Stec of the Polish Academy of Sciences for helpful discussions.

(37) A molecular ion was not observed in the EI-MS of a number of C-4 oxidized cyclophosphamide analogues. For example, like **6**, 4-hydroxy-5-methoxycyclophosphamide gave a base peak (*m/z* 239) corresponding to the combined loss of H_2O and $^•CH_2Cl$ from the molecular ion;[19] an analogous species (*m/z* 209) was detected in the EI-MS of 4-hydroperoxycyclophosphamide [Przybylski, M.; Ringsdorf, H.; Lenssen, U.; Peter, G.; Voelcker, G.; Wagner, T.; Hohorst, H. J. *Biomed. Mass Spectrom.* **1977**, *4*, 209]. The presence of ionic species observed at *m/z* 84 and 56 in the EI-MS of **6** was supportive of the expected structural similarities among **6** and other 5,5-dimethyloxazaphosphorines that had been previously investigated [Edmundson, R. S. *Org. Mass Spectrom.* **1982**, *17*, 558].

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Synthetic and Enzyme Inhibition Studies of Pepstatin Analogues Containing Hydroxyethylene and Ketomethylene Dipeptide Isosteres

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Synthetic details for the preparation of a series of hydroxyethylene and ketomethylene dipeptide isosteres with control of stereochemistry at C(2) are described. Incorporation of the isosteres into peptide sequences derived from pepstatin afforded potent inhibitors of the aspartic protease porcine pepsin. When Leu$\overset{OH}{—}$Ala or Leu$\overset{OH}{—}$Phe was substituted for statine ((3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid), inhibitors equipotent to the parent compound were obtained, whereas Leu$\overset{OH}{—}$Gly was a much less effective replacement for statine. A similar trend was evident in the corresponding ketones. The finding that structural features for good substrates do not closely parallel those for good inhibitors is discussed.

The study of natural inhibitors of proteases as a means for understanding enzyme mechanisms and as an aid to the development of potential therapeutic agents has been the subject of recent discussions.[2] Also, particular attention has lately been directed toward isosteric peptide bond replacements in efforts to prepare modified peptides with improved properties as drugs.[3,4] Previous studies from this laboratory[2,5] on the inhibition of the aspartic protease porcine pepsin by analogues of the natural peptide pepstatin have led us to address in some detail the question of how the structure of pepstatin is related to that of a normal substrate for the enzyme.

The pepstatin analogue Iva-Val-Sta-Ala-Iaa (**1**) is a potent (3 nM) inhibitor of pepsin that demonstrates a

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(1) Abbreviations used follow the IUPAC-IUB commission on Biochemical Nomenclature recommendations. Additional abbreviations are as follows: Sta (statine), 3-hydroxy-4-amino-6 methylheptanoic acid; Sto (Statine), 3-oxo-4-amino-6-methylheptanoic acid; Iva, isovaleryl; Iaa, isoamylamide; Iba, isobutylamide; Leu$\overset{OH}{—}$Ala, hydroxyethylene dipeptide isostere; Leu$\overset{K}{—}$Ala, ketomethylene dipeptide isostere; DMF, *N,N*-dimethylformamide; PDC, pyridinium dichromate.

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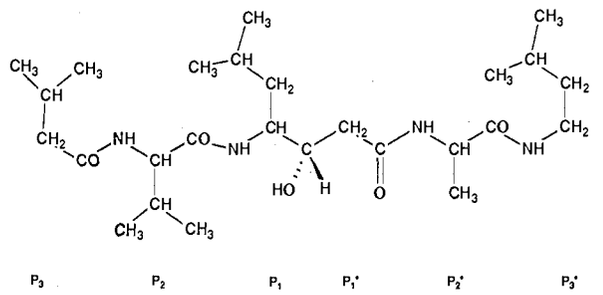
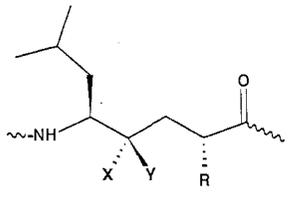
pattern of time-dependent inhibition similar to that of pepstatin[6] and thus has been used by us as a standard upon which to base further studies. At the outset, we did



Iva-Val-Sta-Ala-Iaa, 1

not know whether the statine (Sta) residue of **1** more closely mimicked a single aminoacyl residue or a dipeptidyl residue. Our most recent approach to understanding the role of the statine residue in aspartic protease inhibitors has been to prepare a series of analogues of **1** in which, initially, both -Sta- and -Sta-Ala- were replaced by the hydroxyethylene dipeptide surrogate **2** (R = CH_3).

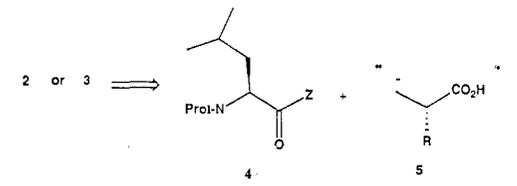
Once it became evident, based on our work[2,5] and that of others,[7-9] that dipeptide surrogate **2** corresponds closely to the Sta residue, and not to Sta-Ala, then further variations in the structure of **2** as replacements for the Sta in **1** were undertaken. Specifically, the side chain R was varied to give the series R = H, R = CH_3, R = CH_2Ph, with the idea of spanning the range between Sta, which has no side chain at this position, and peptide substrates, which optimally contain hydrophobic and preferably aromatic residues at this position.[10] Proceeding one step further, we also have prepared and tested the corresponding ketomethylene analogues of **1**, in which Sta is replaced by **3**. Here we report the results of these studies together



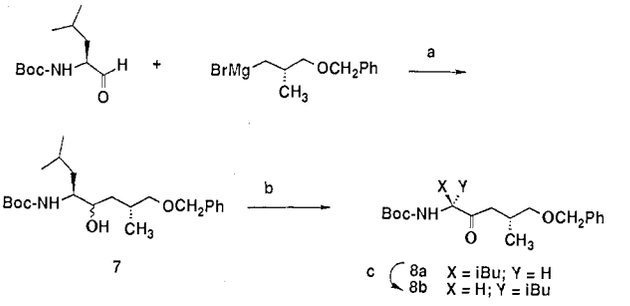
2 X = OH; Y = H
3 X,Y = O

with the details of our synthetic methods. As part of the synthetic studies, some investigation of the chiral stability of model ketomethylene compounds was undertaken, and these results are presented here also.

Scheme I

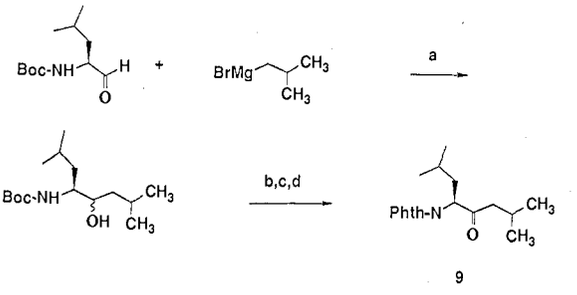


Scheme II[a]



[a] (a) Et_2O, 0 °C. (b) PDC, HOAc. (c) NaOMe, MeOH.

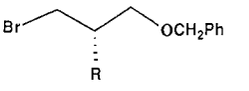
Scheme III[a]



[a] (a) Et_2O, 0 °C. (b) HCl, dioxane. (c) Phthalic anhydide, Δ. (d) PDC, DMF.

Synthesis[11]

The construction of the carbon skeleton in **2** and **3** is logically achieved by a condensation of an appropriate derivative (**4**) of L-leucine and a donor synthon (**5**) of a 2-substituted propionic acid (Scheme I). A number of variations in the details of Scheme I can be envisioned.[12] We have developed the use of chiral synthons of **5**, in the form of Grignard reagents from the bromo ethers **6**, which



6a R = CH_3
6b R = CH_2Ph

(5) Rich, D. N.; Salituro, F. G.; Holladay, M. W.; Schmidt, P. G. In *Conformationally Directed Drug Design*; Vida, J. A., Gordon, M., Eds.; American Chemical Society: Washington, DC, 1984; pp 211–237.
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(11) A preliminary account of the synthetic work has been communicated: Holladay, M. W.; Rich, D. H. *Tetrahedron Lett.* **1983**, *24*, 4401–4404.
(12) Szelke et al. have developed useful syntheses of the required carbon skeleton via α-halo or α-hydroxy ketones derived from chiral amino acids: (a) Reference 13. (b) Hudson, D.; Sharpe, R.; Szelke, M. U.S. Patent 4 198 398, 1980. (c) Sharpe, R.; Szelke, M. British Patent 1 587 809, 1981. (d) Szelke, M.; Jones, D. M.; Hallett, A. U.S. Patent 4 424 207, 1984.
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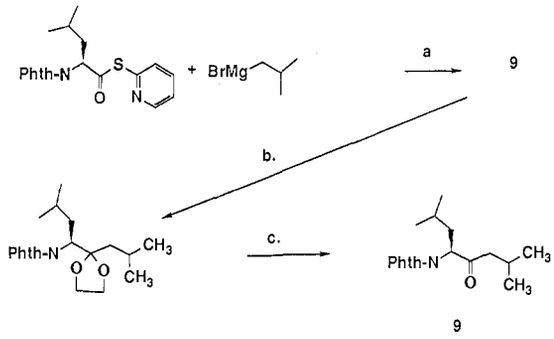
Scheme IV[a]



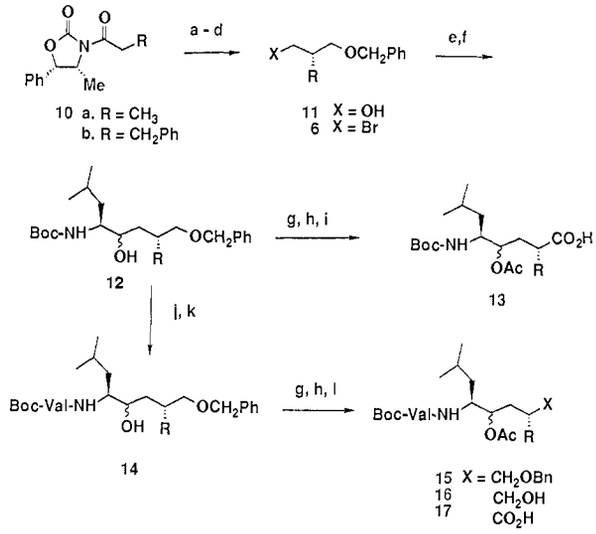
[a] (a) THF. (b) $MeOCH(OCH_2)_2$, TsOH, Δ. (c) 90% aqueous CF_3CO_2H.

are obtainable via the elegant chemistry of Evans et al.[14] For Leu-Gly isosteres (**2** and **3**, R = H) we used the Grignard reagent from 4-bromo-1-butene for the synthon **5** (R = H). Jennings-White and Almquist have developed chemistry using 2-alkyl-4-bromo-1-butenes as readily available achiral precursors to **5** (R = alkyl) synthons and the ethylene ketal of 3-bromopropanol as a **5** (R = H) synthon.[16,17]

For the L-leucine derivative **4**, we chose Boc-L-leucinal in analogy to the methodology developed earlier in this laboratory for the synthesis of statine.[18] We used the two-step conversion of Boc-Leu-OMe to the aldehyde as reported by Hamada and Shioiri[19] in preference to the one-step Dibal-H method,[18] which is less clean. We found that Boc-L-leucinal prepared by the former method retained its chemical and chiral integrity even after storage at 0 °C for several months. Almquist et al.[7] have introduced the use of 2-pyridyl thio esters of *N*-phthaloyl amino acids to afford, after treatment with Grignard reagents, protected precursors to ketomethylene isosteres. Johnson and Miller have modified this approach by carrying out the reaction on the 2-pyridyl thio esters of *N*-trityl amino acids.[20] In the course of our synthetic studies, we wished to assess the chiral integrity of the ketomethylene C(5) chiral center obtained either by the Grignard reaction on Boc-leucinal followed by oxidation to ketone or by Grignard addition to phthaloyl-L-leucine-2-pyridyl thio ester, with subsequent ketone protection and deprotection steps. As described below, we can report that all of these steps proceed essentially racemization-free; the stability of the chiral α-center to prolonged acid conditions at elevated temperature is particularly noteworthy.

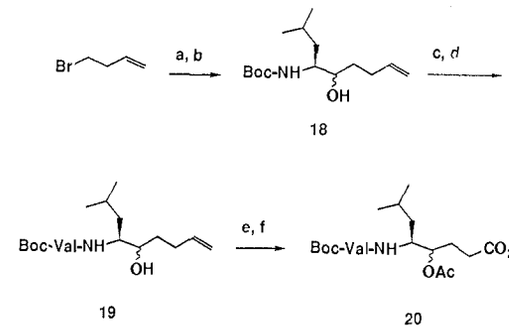
For this study, the model reactions shown in Scheme II–IV were carried out. A priori one might consider that racemization could occur at any of the following steps: (a) reaction of a Grignard reagent with a chiral Boc-amino aldehyde (Schemes II and III) or with a chiral pyridyl thio ester (Scheme IV), with the realization that in the latter case, unlike the former, enolization is possible in the product as well as the starting material; (b) during oxidation of a hydroxyethylene derivative to the ketomethylene analogue (Schemes II and III); (c) during the relatively strong acid conditions used for protection and deprotection of the ketone as a ketal (Scheme IV). In the preparation of **8a** (Scheme II), care was taken to retain potential minor isomeric components during purifications after steps a and b; even so, isomer **8b** could not be detected by HPLC, TLC (1:1 Et_2O–hexane), or 90-MHz NMR. But after treatment with NaOMe in MeOH (room temperature, overnight), the product (**8a** + **8b**) showed a clear doubling of the benzylic protons by 90-MHz NMR ($\Delta\delta$ = 2.5 Hz) and two spots (**8a**, R_f 0.56; **8b**, R_f 0.62) by TLC (1:1 Et_2O–hexane). Thus, given the limits of detectability by TLC and NMR, <5% racemization could have occurred during the Grignard reaction and oxidation steps. Scheme III shows the preparation of a phthaloyl-protected ketomethylene derivative **9** by steps that now may be considered essentially free of racemization. When **9** is prepared from the pyridyl thio ester (Scheme IV), a comparison of specific rotation indicates a minimum of racemization (<10%). Ketalization under prolonged acidic conditions followed by hydrolysis back to **9** (Scheme IV) under the conditions described by Almquist et al.[16] led to no loss of optical activity in recovered **9**.

Scheme V[a]



[a] (a) $LiN(Me_3Si)_2$. (b) $BrCH_2OCH_2Ph$. (c) $Ca(BH_4)_2$. (d) PPh_3, NBS. (e) Mg, Et_2O. (f) Boc-L-leucinal. (g) Ac_2O, DMAP. (h) H_2, Pd–C. (i) $KMnO_4$, C_6H_6, H_2O, *n*-Bu_4NBr. (j) HCl, dioxane. (k) $(Boc\text{-}Val)_2O$. (l) PDC, DMF.

Scheme VI[a]



[a] (a) Mg, Et_2O. (b) Boc-L-leucinal. (c) HCl, dioxane. (d) Boc-Val-*S*-2-pyridyl. (e) Ac_2O, DMAP. (f) $KMnO_4$, C_6H_6, H_2O, *n*-Bu_4NBr.

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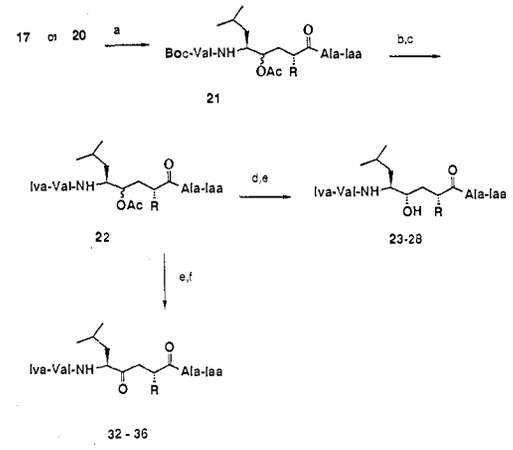
Scheme VII[a]



[a] (a) H-Ala-Iaa, DCC, HOBt. (b) HCl, dioxane. (c) $(Iva)_2O$. (d) Silica gel chromatography. (e) MeOH, K_2CO_3. (f) PDC, HOAc.

Our synthetic routes to hydroxyethylene and ketomethylene peptide analogues are shown in Scheme V–VII. A number of aspects deserve some comment. For the alkylation of the acyloxazolidinone **10a** (Scheme V, steps a and b), we obtained essentially the same results with distilled commercially available benzyl chloromethyl ether as the alkylating agent as with the bromomethyl ether that was recommended.[14] In the case of R = CH_2Ph, the alkylation was more sluggish, so the bromomethyl ether was the preferred reagent; we found it convenient to convert chloromethyl ether to bromomethyl ether by treatment of the former with 1 equiv of LiBr in anhydrous THF (followed by evaporation, dissolution in Et_2O, and filtration to remove LiCl) prior to addition to the enolate.

For the preparation of **11** by reductive removal of the chiral oxazolidinone auxiliary, we found $Ca(BH_4)_2$ (readily formed in situ from $NaBH_4$ and $CaCl_2$)[22-25] in a EtOH–THF mixture to give in some cases slightly superior yields and cleaner reaction than $LiAlH_4$ in ether or $LiBH_4$ in THF.[14] The ratio of EtOH to THF (ca. 3:2)[19] apparently is important, since the presence of THF suppresses protonolysis of the reducing agent by EtOH, whereas the presence of EtOH serves to accelerate the reaction to a convenient rate; in fact, we have observed that when such reactions are proceeding slowly or not at all, the addition of more EtOH is sufficient to induce a convenient rate of reaction. The optically active compounds **11a** and **6a** had been prepared previously[21] from β-hydroxyisobutyric acid, which was obtained by bacterial oxidation of isobutyric acid. Although the rotation of **11a** prepared by us via the chiral oxazolidinone was somewhat lower than the literature value,[21] no enantiomer was detected in chiral shift NMR experiments under conditions that were found to resolve the racemic mixture ($Eu(Fod)_3$, CCl_4). The bromide **6a** from **11a**, on the other hand, had a rotation $[[\alpha]^{24}_D$ +13.0° (*c* 1.0, EtOH)] that compared quite favorably with the literature value $[[\alpha]^{24}_D$ +12.48° (*c* 0.89, EtOH)].[21]

In the reaction of chiral Grignard reagent with Boc-L-leucinal to form **12**, the yield apparently is influenced by the size of R, since consistently lower yields (ca. 30%) were obtained with R = CH_2Ph compared to 50–65% yields when R = CH_3. In subsequent steps, the most convenient route to the target compounds required extension of the N-terminus by one amino acid residue (**12** → **14**) prior to elaboration of the C-terminus. The direct conversion of **12** to **13** results in a less straightforward protection scheme, since the acetyl group must be removed before extension of the N-terminus is attempted, otherwise rapid intramolecular O → N transacetylation occurs. In addition, the final oxidation to carboxylic acid from primary alcohol was cleaner for the preparation of **15** (step 1) than for **13** (step i); indeed, we were unable to apply the conditions that were successful for the oxidation of **16** to **17** (PDC in DMF) to the preparation of **13**. However, phase-transfer permanganate oxidation[26] did provide a useful means to prepare **13**. We speculate that the additional Val residue may inhibit interactions between the C-terminal carbon and the nitrogen six atoms away as the reaction progresses through the aldehyde oxidation state.

The only remaining synthetic step that deserves some comment is the final oxidation of hydroxyethylene compounds to the corresponding ketomethylene derivatives (Scheme VII, step f). Although PDC in DMF was satisfactory, albeit slow, for some peptides, the reaction failed to go to completion in some cases, particularly for longer peptides, even with large excesses of PDC. Replacement of DMF with HOAc, however, led to a marked acceleration of the rate of reaction, such that even the larger hydroxyethylene peptides were readily oxidized when this modification was used.

Finally, it should be mentioned that with at least one ketomethylene compound (specifically the model analogue (Iva-Leu$\overset{K}{—}$Ala-Iaa), some instability (conversion to new spots on TLC) was noted under basic (10% NEt_3 in

Table I. Inhibition of Porcine Pepsin by Pepstatin Analogues

no.	compound	K_i, nM	slow binding
1	Iva-Val-Sta-Ala-Iaa	3[a]	+
23	Iva-Val-Leu$\overset{OH}{—}$Ala-Iaa	27[b]	–
24	Iva-Val-Leu$\overset{OH}{—}$Gly-Ala-Iaa	50[c]	–
25	Iva-Val-Leu $\overset{OH}{—}$Ala-Ala-Iaa	3	+
26	Iva-Val-Leu$\overset{OH}{—}$Phe-Ala-Iaa	2.5[c]	+
27	Iva-Val-Leu$\overset{OH}{—}$Ala-Ala-Iba	3[c,d]	+
28	Iva-Val-Leu$\overset{OH}{—}$D-Phe-Ala-Iaa	140	–
29	Iva-Val-Leu$\overset{OH}{—}$Ala-Gly-Iba	53[d]	–
30	Iva-Val-Sta-Gly-Iaa	56[d]	–
31	Iva-Val-Sto-Ala-Iaa	56[e]	–
32	Iva-Val-Leu$\overset{K}{—}$Gly-Ala-Iaa	2140	–
33	Iva-Val-Leu$\overset{K}{—}$Ala-Ala-Iaa	27	–
34	Iva-Val-Leu$\overset{K}{—}$Phe-Ala-Iaa	72[c,d]	–
35	Iva-Val-Leu$\overset{K}{—}$Ala-Ala-Iba	110[c,d]	–
36	Iva-Val-Leu$\overset{K}{—}$D-Phe-Ala-Iaa	850	–

[a] Previously reported in ref 6. [b] Previously reported in ref 27. [c] Previously reported in ref 2. [d] Previously reported in ref 5. [e] Previously reported in ref 28.

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Table II. Kinetic Constants for Selected Substrates of Porcine Pepsin[a]

Z-His-Phe-X-OMe, X	$k_{cat.}/K_M$, ms^{-1} mM^{-1}	K_m, mM	$k_{cat.}$, ms^{-1}
Gly	1.3	1.6	2.1
Ala	2.1	1.8	3.7
Leu	10	0.5	5.2
Phe	520	0.33	170
D-Phe (inhibitor)		K_I = 0.28	

[a] Data from ref 10.

$CHCl_3$) and acidic (90% CF_3CO_2H) conditions, or even on prolonged standing in MeOH. Although the new products were not characterized, preliminary evidence suggests that the C-terminal amide nitrogen is involved, since similar compounds lacking this nitrogen were stable under the same conditions.

Enzyme-Inhibition Results

The K_i values for inhibition of porcine pepsin by the pepstatin-based hydroxyethylene and ketomethylene analogues are collected in Table I. Also indicated in Table I are those inhibitors that exhibited slow binding inhibition characteristic of the pepstatin–pepsin interaction.

In the series of inhibitors containing Leu$\overset{OH}{—}$X [X = Gly (**24**), Ala (**25**), Phe (**26**)], it is evident that the lack of a side chain at the presumed P_1' site as in **24** leads to a 15- to 20-fold loss of potency and loss of slow binding inhibitory properties. On the other hand, the increasing size, hydrophobicity, and aromatic character of the side chain on replacing CH_3 (**25**) with CH_2Ph (**26**) appear to have little effect on the inhibitory potency. In the corresponding ketomethylene series (**32** vs. **33** vs. **34**), the lack of side chain in the Leu$\overset{K}{—}$Gly analogue **32** results in a considerably more pronounced loss of potency (ca. 80-fold) compared to the hydroxyethylene analogues. Interestingly, inhibitor **34**, which bears a CH_2Ph substituent at the presumed P_1' site, is slightly weaker (ca. 3-fold) than the CH_3-bearing analogue **33**, despite the known preference of pepsin for hydrophobic, aromatic side chains near the cleavage sites of substrates.[10]

Compound **27** represents the deletion of one carbon from the C-terminus as compensation for adding the additional carbon in the isostere portion; thus **1** has one less carbon in the backbone than does **25**, whereas **1** and **27** have the same number of atoms in the backbone. For the hydroxyethylene analogues, the extra carbon has no apparent effect (**25** vs. **27**), but in the ketomethylene analogues, the extra carbon in **33** provides a slightly more potent inhibitor (ca. 4-fold) than the Iba compound **35**.

To probe the effect of stereochemistry at the isostere C-2 side chain, the inhibitors containing Leu$\overset{OH}{—}$D-Phe (**28**) and Leu$\overset{K}{—}$D-Phe (**36**) were prepared. In both cases, weaker inhibitors were obtained, although the loss was more pronounced in the hydroxyethylene analogues (ca. 60-fold) than in the ketomethylene series (ca. 12-fold).

Discussion

Two main aspects of these results merit further discussion. First is the low correlation between the binding of the isostere-containing inhibitors to pepsin compared to the apparent binding of peptide substrates to the enzyme. Table II shows kinetic constants for a series of pepsin substrates Z-His-Phe-X-OMe, where X = Gly, Ala, Leu, Phe.[10] If k_{cat}/K_m is taken as a measure of the efficiency of the enzyme action on a particular substrate, then it is evident that the substrate with X = Phe is cleaved ca. 250 times more efficiently than the substrates with X = Gly or X = Ala. It also is apparent that most of this difference derives from differences in $k_{cat.}$ and not in K_m.

Neither $k_{cat.}/K_m$ nor K_m as a function of side chain for substrates shows a close correlation to K_i with respect to side chain for the inhibitors in Table I. Thus, the K_m is essentially the same for substrates with X = Gly vs. X = Ala, but the additional methyl group has a profound effect on K_i for the Leu$\overset{K}{—}$Gly (**32**) vs. Leu$\overset{K}{—}$Ala (**33**) inhibitors. Similarly, for the hydroxyethylene isosteres, the methyl group (**24** vs. **25**) causes 10-fold decrease in K_i binding and the onset of the slow-binding property. In the substrates, a change from CH_3 to CH_2Ph in the P_1' position gives a ca. 5-fold decrease in K_m and a 45-fold increase in $k_{cat.}$, but the parallel changes in the hydroxyethylene (**25** vs. **26**) and ketomethylene (**33** vs. **34**) inhibitors cause little or no change in inhibitory potency. The change in the substrate P_1' position from Phe to D-Phe causes little change in K_m, whereas the hydroxyethylene (**26** vs. **28**) and ketomethylene (**34** vs. **36**) inhibitors, the change in stereochemistry at the analogous position causes considerable loss of binding potency.

Thompson[29] and Bartlett and Marlowe[30] have proposed equations to relate K_i to either K_m or $k_{cat.}/K_m$ as diagnostics for multisubstrate vs. transition-state analogue inhibitors of elastase and thermolysin. Excellent correlations of inhibitor K_i with substrate $k_{cat.}/k_m$ were found as predicted for transition-state analogue inhibitors. An analysis of the presently reported data for pepsin with regard to the results of Thompson and Barlett already has been presented.[2] Briefly, good correlations in the elastase and thermolysin examples may indicate that a single step dominates the catalytic transformations, whereas in the case of pepsin, stopped-flow methods have shown that $k_{cat.}$ includes at least two distinct steps, one step incorporating amide bond cleavage and one step incorporating release of product.[31] When $k_{cat.}$ is determined by steady-state methods, it is not possible to distinguish the individual steps. Thus, attempts to associate an inhibitor structure with a single stage along the catalytic pathway by correlation of the K_i with kinetic parameters that are dependent on $k_{cat.}$, and determined under steady-state conditions, are likely to fail when more than one step significantly influences $k_{cat.}$.

A minimal kinetic mechanism for proteolytic amide bond hydrolysis (eq 1) contains several steps, each with a corresponding transition-state and low-energy complex, and it can be supposed that the pepstatin analogue–enzyme complexes could mimic any of these. It also is possible that small changes in inhibitor structure could affect which intermediate or transition state might be represented by a particular enzyme–inhibitor complex.

$$E + A + B \rightleftharpoons EAB \rightleftharpoons EX \rightleftharpoons EPQ \rightleftharpoons EQ + P \rightarrow E + Q \quad (1)$$

Since the assumption that $K_m \cong K_s$ apparently is valid for pepsin-catalyzed hydrolysis of the substrates in Table II,[10] then a good correlation of inhibitor K_i with substrate K_m as a function of P_1' side chain would provide support for a multisubstrate mode of inhibition.[30] The poor correlation that is observed then suggests, at least, that these inhibitors do not act uniformly or exclusively as multisubstrate inhibitors. In any event, it is evident that at-

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tempts to design optimal enzyme inhibitors based solely on a knowledge of structures for the best substrates should proceed with caution.

The other point that should be emphasized concerns the relationship of statine to hydroxyethylene isosteres as components of aspartic protease inhibitors. Table I shows the comparison between the replacement of the Sta-Ala portion of **1** with either Leu$\overset{OH}{—}$Ala (**23**) or Leu$\overset{OH}{—}$Ala-Ala (**25**). Since **25** is equipotent with **1** and also exhibits slow binding, whereas **23** is almost 10-fold less potent and does not exhibit slow binding, it is likely that Leu$\overset{OH}{—}$Ala and Sta are serving similar roles. This conclusion is reinforced by the finding that inhibitors containing Sta-Gly (**30**) and Leu$\overset{OH}{—}$Ala-Gly (**29**) display almost identical enzyme inhibitory properties; that is, the effect of removing the methyl group of the C-terminal Ala residues of both **1** and **25** is the same for both inhibitors. While these results point strongly to the role of statine as spanning the distance of a dipeptidyl segment of an intermediate in the pathway for enzyme-catalyzed substrate hydrolysis, results from the Merck[9] and Szelke[13] groups allow, in addition, comparisons between substrates and statine-containing inhibitors. Both groups have prepared inhibitors in which statine replaced either only the P_1 residue or both the P_1 and P_1' residues in sequences related to the renin substrate. A somewhat more potent inhibitor was obtained in the Szelke series (but not in the Merck series) when Sta was incorporated as a replacement of only the P_1 residue relative to replacement of both P_1 and P_1'. Thus, it may be asserted[13] that statine can, in some cases, effectively serve as a replacement for a single amino acid residue. However, it is still logical to suppose also in this example that statine would span the distance of a dipeptide-derived segment and that additional residues on the C-terminal end of the inhibitor promote enhanced binding by virtue of favorable interactions with other sites on the enzyme. Returning to the comparison of statine with hydroxyethylene isosteres, it is interesting to note that whereas Leu$\overset{OH}{—}$Ala effectively replaces Sta in pepsin inhibitors, Leu$\overset{OH}{—}$Gly (cf. **24**) is not nearly as effective, since **24** is 15- to 20-fold less potent than **1** or **25** and does not exhibit slow binding. It is likewise surprising that addition of substituents (e.g., benzyl) to the C(2) position of statine in renin inhibitors does not significantly increase inhibitory potency,[32] although there presumably is a binding site for the side chain of the P_1' residue of renin substrate that would accommodate such a substituent. Thus it is remarkable that unsubstituted statine, which lacks both a backbone atom and a side chain relative to either a dipeptide or a corresponding hydroxyethylene isostere (e.g., **25** or **26**), apparently contains all of the elements necessary for highly potent binding to the enzyme when contained within a minimal substrate sequence.

From a medicinal chemistry viewpoint, statine thus appears as a readily synthesized entity that, under the appropriate circumstances, can mimic the structural features of a dipeptidyl unit as effectively as a synthetically more demanding isosteric replacement. In view of the current attention to modifying peptides for the purpose of exploiting their potential as therapeutic agents, it is interesting to speculate whether the appropriate β-hydroxy or β-keto-γ-amino acids might effectively replace certain dipeptide units in other biologically active peptides, e.g., peptide hormones or neurotransmitters.

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Experimental Section

Melting points were determined with a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer Model 241 automatic polarimeter (0.999-dm cell). Proton nuclear magnetic resonance spectra were recorded on a Varian EM-390 (90 MHz) or a Bruker WH-270 spectrometer (270 MHz). Chemical shifts were reported as δ units (ppm) relative to tetramethylsilane as internal standard. TLC was performed on 0.25-mm-thickness silica gel plates (Merck, silica gel 60 F-254).

Standard workup from an organic solution refers to successive washes with approximately equal volumes of (once each) saturated aqueous $KHSO_4$, H_2O, saturated aqueous $NaHCO_3$, H_2O, and brine, drying of the resultant organic solution ($MgSO_4$ unless otherwise specified), then filtration, evaporation under reduced pressure, and drying under high vacuum. Column chromatography was carried out with either Merck silica gel 60 (70–230 mesh, gravity) or Merck Kieselgel 60 (230–400 mesh, flash).

Enzyme-inhibition studies were carried out on porcine pepsin (Sigma) as described previously.[6]

(***R***)-**3-(Benzyloxy)-2-methylpropanol (11a).** Dried, crushed $CaCl_2$ (3.74 g, 34 mmol) was stirred under N_2 with 50 mL of absolute EtOH until mostly dissolved (ca. 0.5 h), and then the mixture was cooled in an ice bath and 2.6 g (68 mmol) of $NaBH_4$ was added. Then (4*S*,5*S*)-3-[3-(benzyloxy)-2-methylpropionyl]-4-methyl-5-phenyl-1-oxazolidinone (**10a**)[14] (12.0 g, 34 mmol) in 30 mL of dry THF was added dropwise with stirring over ca. 20 min. Stirring was continued at 0 °C until the reaction was complete by TLC (generally 2–4 h; if reaction is sluggish, the rate is increased by addition of more ethanol). The mixture was diluted with EtOAc (ca. 100 mL) and H_2O, and then 20% HOAc was carefully added to destroy excess active hydride. After addition of 2 N HCl to pH ~2, the layers were separated, and the aqueous phase was extracted with 2 × 100 mL of EtOAc. The combined organic phases were washed with saturated $NaHCO_3$ (1×) and brine (1×), dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was treated with 50 mL of 7:1 hexane/Et_2O and filtered to remove most of the byproduct oxazolidinone. After evaporation of the filtrate the product was Kugelrohr distilled [bp 99–105 °C (0.5 mm)]: yield 70–85%; NMR (CCl_4) δ 0.90 (d, J = 8 Hz, 3 H), 2.1 (m, 1 H), 2.5 (br s, 1 H), 3.3–3.7 (m, 4 H), 4.4 (s, 2 H), 7.2 (s, 5 H); $[\alpha]^{23}_D$ −3.5° (*c* 1, EtOH) [lit.[21] $[\alpha]_D$ −4° (*c* 1.1, EtOH)].

(***S***)-**3-(Benzyloxy)-1-bromo-2-methylpropane (6a).** The literature procedure[21] was modified slightly. To a mechanically stirred solution of **11a** (8.4 g, 46 mmol) and triphenylphosphine (12.2 g, 46 mmol) in 80 mL of CH_2Cl_2, cooled in an ice/H_2O bath, was added over 0.5 h, in portions, 8.3 g (46 mmol) of solid *N*-bromosuccinimide (recrystallized from HOAc) such that the internal temperature remained between 10 and 18 °C. After addition was complete, the mixture was stirred at room temperature for 12 h. TLC (Et_2O) showed a trace of starting material, so the mixture was cooled and an additional 1.2 g of PPh_3 and 0.83 g of NBS were added. After being stirred for 3 h at room temperature, the mixture was poured onto ice, and then the aqueous layer was neutralized with $NaHCO_3$. The layers were separated, the aqueous phase was extracted with an equal volume of CH_2Cl_2, and then the combined organic fractions were concentrated. Hexane (80 mL) and H_2O (80 mL) were added to the residue, and the mixture was stirred until triphenylphosphine oxide formed a solid, which could be filtered. After suction filtration, the hexane layer was subjected to standard workup. The resultant residue was chromatographed on 200 g of silica gel, eluting with 9:1 → 1:1 hexane–$CHCl_3$ (product elution at ca. 800–2000 mL of eluate) to provide 9.2 g of colorless liquid, which was Kugelrohr distilled [110–120 °C (1.1 mm)] to give 8.84 g (78%) of pure product: $[\alpha]^{24}_D$ +13.0° (*c* 1, EtOH) [lit.[21] $[\alpha]^{24}_D$ +12.48 (*c* 0.89, EtOH)]; NMR ($CDCl_3$) δ 1.0 (d, J = 6.5 Hz, 3 H), 2.15 (m, 1 H), 3.4 (d, J = 6 Hz, 2 H), 3.5 (d, J = 5 Hz, 2 H), 4.5 (s, 2 H), 7.35 (s, 5 H).

***N*-(*tert*-Butyloxycarbonyl)-(3*R*,4*RS*,5*S*)-5-amino-1-(benzyloxy)-2,7-dimethyloctan-4-ol (12a).** Into a dry, three-neck, 200-mL, round-bottom flask equipped with a Teflon-coated

stirring magnet and fitted with a thermometer, a serum cap, and a condenser bearing a gas inlet were introduced under a stream of dry N_2 890 mg (36.6 mmol) of Mg powder (Alfa) and a few small crystals of I_2. The flask was warmed briefly with a heat gun until I_2 vapors were visible and then allowed to cool to ambient temperature. Then 10 mL of dry THF was added via syringe followed by a few drops (50–100 μL) of **6a**. The mixture was warmed (heat gun) without stirring until the reaction began (disappearance of yellow color), and the remainder of the bromo ether (total 5.9 g, 24.2 mmol) in ca. 10 mL of dry THF was added dropwise with stirring such that the temperature remained between 35 and 40 °C. After addition was complete (ca. 1–1.5 h), the mixture was stirred under N_2 for 12 h, and then a THF solution of 2.05 g (9.5 mmol) of Boc-L-leucinal[19] was added dropwise over 2 h with good stirring. After being stirred for an additional 3.5 h at room temperature, the mixture was poured into saturated aqueous NH_4Cl. Ethyl acetate was added and the mixture was acidified (cooling and stirring) with 2 N HCl. After standard workup from EtOAc, the residue was chromatographed on 320 g of silica gel, eluting with 9:1 PhMe–EtOAc. The desired compound was eluted starting at ca. 1950 mL of eluate to afford 1.4 g (39%) of the higher R_f (2*R*,4*S*,5*S*) isomer, 0.57 g (16%) of a mixture of isomers, and 0.38 g (10%) of the lower R_f (2*R*,4*R*,5*S*) isomer.

2*R*,4*S*,5*S* isomer: syrup; $[\alpha]^{24}_D$ −22° (*c* 2.8, $CHCl_3$); TLC (4:1 PhMe–EtOAc) R_f 0.31; NMR ($CDCl_3$) δ 0.95 (m, 9 H), 1.2–1.75 (m, 14 H) [includes δ 1.45 (s, 9 H)], 2.05 (m, 1 H), 2.9 (br s, 1 H), 3.35 (m, 2 H), 3.55 (m, 1 H), 3.7 (m, 1 H), 4.5 (s, 2 H), 4.65 (br d, 1 H, *J* = 10 Hz), 7.3 (m, 5 H). Anal. ($C_{22}H_{37}NO_4$) C, H, N.

2*R*,4*R*,5*S* isomer: waxy solid, mp 77–81 °C; $[\alpha]^{24}_D$ −26° (*c* 2.8, $CHCl_3$); TLC (4:1 (PhMe–EtOAc) R_f 0.25; NMR ($CDCl_3$) δ 0.95 (m, 9 H), 1.1–1.5 (m, 13 H) (includes δ 1.45 (s, 9 H), 1.6 (m, 1 H), 2.0 (m, 1 H), 3.3 (dd, 1 H, *J* = 8 Hz, 18 Hz), 3.4 (dd, *J* = 6 Hz, 8 Hz), 3.5–3.75 (cmplx, 2 H), 4.5 (s, 2 H), 4.65 (br d, 1 H, *J* = 8 Hz), 7.3 (m, 5 H). Anal. ($C_{22}H_{37}NO_4$) C, H, N.

Determination of C(4) Stereochemistry for Isomers of 12a. Each C(4) epimer of **12a** was converted to the corresponding oxazolidinone as follows: A mixture of 48 mg (0.13 mmol) of the Boc-amino hydroxy ether and 17 mg (0.15 mmol) of KO-*t*-Bu in 1.5 mL of dry THF was heated at reflux for 0.75 h. The cooled product (one spot by TLC) was then analyzed by ^1H NMR ($CDCl_3$).

Oxazolidinone from major (higher R_f) starting material: δ 0.95 (m, 9 H), 1.1–2.3 (cmplx, 6 H), 3.4 (cmplx, 3 H), 4.2 (m, 1 H), 4.5 (s, 2 H), 6.6 (br s, 1 H), 7.3 (s, 5 H). Irradiation at δ 1.65 caused the absorption at 4.2 ppm to become a doublet, *J* = 6.3 Hz.

Oxazolidinone from minor (lower R_f) starting material: δ 0.95 (m, 9 H), 1.1–2.3 (cmplx, 6 H), 3.4 (d, *J* = 6 Hz, 2 H), 3.75 (m, 1 H), 4.5 (s, 2 H), 4.7 (m, 1 H), 6.3 (br s, 1 H), 7.3 (s, 5 H). Irradiation at δ 1.9 caused the absorption at 4.7 ppm to become a doublet, *J* = 9 Hz.

According to Futagawa et al.[33] cis coupling constants for a series of oxazolidinones were in the range 9–10, whereas trans values were in the range 4.5–6. This is consistent with the major product (higher R_f) having trans stereochemistry (4*S*,5*S*) and minor product (lower R_f) being cis (4*R*,5*S*).

***N*-[(*tert*-Butyloxycarbonyl)-L-valyl]-(2*S*,4*RS*,5*S*)-5-amino-1-(benzyloxy)-2,7-dimethyloctan-4-ol (14a).** Boc was removed from 1.34 g (3.5 mmol) of a ca. 4:1 4*S*–4*R* mixture of **12a** by treatment with 15 mL of 4 N HCl–dioxane for 0.5 h. After evaporation of volatile components under reduced pressure, Et_2O was added and evaporated three times, and then the residue was kept under high vacuum over KOH overnight. The hydrochloride was dissolved in 10 mL of CH_2Cl_2, cooled to 0 °C, and neutralized with 0.5 mL (363 mg, 3.6 mmol) of NEt_3. To the stirred solution was added $(Boc\text{-}Val)_2O$ (prepared from Boc-Val-OH (2.28 g, 10.5 mmol) and DCC (1.08 g, 5.25 mmol)[32] in ca. 10 mL of DMF. The mixture was stirred at 0 °C for 6 h during which time an additional 0.6 mL of NEt_3 was added to keep the solution slightly basic and then treated with 5 mL of 2 M NaOH and concentrated to remove CH_2Cl_2. Then EtOH (ca. 20 mL) was added to give a homogeneous mixture, which was stirred for 1 h, concentrated in vacuo, diluted with EtOAc, and subjected to standard workup. The residue was chromatographed on 100 g of silica gel, eluting with 3:1 hexane–EtOAc (350 mL) and then 2:1 Skelly B–EtOAc (product elution began at ca. 400 mL of eluate) to afford 1.48 g (89%) of product as a colorless syrup. The diastereomers were observed to be separable by TLC with 1:1 EtOAc–hexane. **14a**: NMR ($CDCl_3$) δ 0.95 (m, 15 H), 1.2–2.3 (cmplx, 16 H) [includes δ 1.45 (s, 9 H)], 3.4 (m, 2 H), 3.6–4.2 (cmplx, 4 H), 4.6 (s, 2 H), 5.05 (br d, 1 H), 6.1 (br d, 1 H), 7.4 (s, 5 H). Anal. ($C_{27}H_{46}N_2O_5$) C, H, N.

Isolation 4*S* isomer: 270-MHz NMR δ 0.95 (m, 15 H), 1.3–1.7 (m, 14 H) [includes δ 1.45 (s, 9 H)], 2.0 (m, 1 H), 2.1 (m, 1 H), 3.35 (m, 2 H), 3.75 (br d, *J* = 8 Hz, 1 H), 3.85 (dd, *J* = 8.4 Hz, 8 Hz, 1 H), 4.5 (s, 2 H), 5.05 (br d, 1 H, *J* = 8 Hz), 6.2 (d, 1 H, *J* = 10 Hz), 7.3 (m, 5 H).

***N*-[(*tert*-Butyloxycarbonyl)-L-valyl]-(2*R*,4*RS*,5*S*)-4-acetoxy-5-amino-1-(benzyloxy)-2,7-dimethyloctane (15a).** To a solution of **14a** (1.48 g, 3.09 mmol) in 25 mL of EtOAc were added 583 μL (0.63 g, 6.18 mmol) of Ac_2O, 862 μL (0.62 g, 6.18 mmol) of NEt_3, and 0.04 g (0.3 mmol) of 4-(dimethylamino)-pyridine. After the mixture was stirred for 5 h at room temperature, 20 mL of saturated aqueous $NaHCO_3$ was added, the mixture was stirred for 1 h, and then the layers were separated. The EtOAc layer was subjected to standard workup to afford 1.58 g (98%) of yellowish syrup, which was used without further purification (one spot TLC). **15a**: TLC (2:1 hexane–EtOAc) R_f 0.42. In 3:2 Et_2O–hexane, the diastereomers were separated: 2*R*,4*S*,5*S*, R_f 0.44; 2*R*,4*R*,5*S*, R_f 0.40; NMR ($CDCl_3$) δ 0.90 (cmplx, 15 H), 1.1–2.3 (cmplx, 19 H) [includes δ 1.4 (s, 9 H), 2.0 (s, 4*R* OAc), and 2.05 (s, 4*S* OAc)], 3.3 (d, *J* = 6 Hz, 2 H), 3.8 (m, 1 H, 4.2 (78 1 H), 5.0 (cmplx, 2 H), 4.5 (s, slightly split, 2 H), 5.8 (m, 1 H), 7.35 (s, 5 H). Anal. ($C_{29}H_{48}N_2O_6$) C, H, N.

***N*-[(*tert*-Butyloxycarbonyl)-L-valyl]-(2*R*,4*RS*,5*S*)-5-amino-2,7-dimethyloctan-1-ol (16a).** Powdered ammonium formate (1.51 g, 24 mmol) and 10% Pd–C (Aldrich, 600 mg) were added to a solution of **15a** in 40 mL of 1:1 *i*-PrOH–HOAc under N_2. The mixture was magnetically stirred until TLC (3:2 Et_2O–hexane) indicated complete reaction. The mixture was filtered through Celite while being rinsed with EtOAc. An approximately equal volume of H_2O was added, the layers were separated, and the aqueous phase was extracted with an additional ca. 100 mL of EtOAc. The combined EtOAc layers were subjected to standard workup to afford 1.04 g (100%) of an amorphous colorless solid, which was carried to the next step without further treatment. **16a**: TLC (3:2 Et_2O–hexane) R_f 0.12 (4*S* isomer), 0.10 (4*R* isomer); NMR ($CDCl_3$) δ 0.95 (cmplx, 15 H), 1.05–2.3 (cmplx, 20 H) [includes δ 1.35 (s, 9 H), and δ 2.05 (s with shoulder, 3 H)], 3.4 (m, 2 H), 3.8 (m, 1 H), 4.25 (m, 1 H), 5.0 (cmplx, 3 H, 6.0 (m, 1 H). Anal. ($C_{22}H_{42}N_2O_6 \cdot H_2O$) C, H, N.

***N*-[(*tert*-Butyloxycarbonyl)-L-valyl]-(3*R*,4*RS*,5*S*)-4-acetoxy-5-amino-2,7-dimethyloctanoic Acid (17a).** To **11a** (1.0 g, 2.3 mmol) in 15 mL of dry DMF was added pyridinium dichromate (6.1 g, 16.2 mmol) (prepared according to Corey and Schmidt, *Tetrahedron. Lett.* **1979**, 399), and the mixture was stirred under N_2 for 35 h. The mixture was diluted with 100 mL of H_2O followed by 100 mL of EtOAc, and while being cooled and stirred, the mixture was acidified (pH 1–2) with solid $KHSO_4$ added in portions. The layers were separated, and the aqueous phase was extracted with 2 × 75 mL of EtOAc. The combined EtOAc extracts were washed with 3 × 100 mL of 1 M $KHSO_4$ and 2 × 100 mL of H_2O to give a nearly colorless organic phase, which was concentrated to a small volume. The concentrate was diluted with Et_2O and extracted with 6 × 30 mL of 2.5% aqueous $NaHCO_3$. The combined aqueous layers were back-extracted with 20 mL of Et_2O and then, with cooling and stirring, acidified (pH ~2) with solid $KHSO_4$. The layers were separated, and the aqueous phase was extracted with 2 × 100 mL of EtOAc. The combined EtOAc layers were washed once each with water and brine, dried ($MgSO_4$), filtered, and evaporated to 850 mg (83%) of a solid foam: TLC (50:25:1 hexane–EtOAc–HOAc) R_f 0.18; mp 68–70 °C; NMR ($CDCl_3$) δ 0.95 (cmplx, 12 H), 1.1–2.6 (cmplx, 18 H) [includes δ 1.2 (d, *J* = 6 Hz, 3 H, C(2)-CH_3), 1.4 (s, 9 H), 2.0 (s, 4*R* OAc), 2.1 (s, 4*S* OAc), 2.5 (cmplx, 1 H)], 3.9 (m, 1 H, C(5)–H), 4.2 (m, 1 H, Val α-H), 5.0 (cmplx, 2 H, Boc NH, C(4)-H), 6.2 (m, 1 H). Anal. ($C_{22}H_{40}N_2O_7$), C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-(2*R*,4*RS*,5*S*)-4-acetoxy-5-amino-2,7-dimethyloctanoic Acid (13a).** To **12a** (507 mg, 1.33

(33) Futagawa, S.; Inui, T.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 3308–3310.

mmol) in ca. 10 mL of dry EtOAc were added Ac_2O (252 μL, 273 mg, 2.66 mmol), NEt_3 (372 μL, 270 mg, 2.66 mmol), and 4-(dimethylamino)pyridine (16 mg, 0.13 mmol). The solution was stirred for 2 h at room temperature, then 5 mL of saturated aqueous $NaHCO_3$ was added, and the mixture was stirred for 1.5 h. The layers were separated, and the organic phase was subjected to the standard workup from EtOAc to yield 551 mg (98%) of desired product as a pale yellow syrup, which was used without further purification: TLC (2:1 hexane–EtOAc) starting material R_f 0.44, product R_f 0.56. Diastereomers were separable in 1:1 Et_2O–hexane (R_f 0.49, 0.53): NMR ($CDCl_3$) δ 0.95 (cmplx, 9 H), 1.05–2.2 (cmplx, 18 H) [includes δ 1.4 (s, 9 H) and 2.0 (s, 3 H)], 3.3 (d, J = 6 Hz, 2 H), 3.8 (m, 1 H), 4.5 (m, 1 H and s, 2 H), 5.0 (m, 1 H), 7.3 (s, 5 H).

To a solution of the residue from the previous reaction (510 mg, 1.21 mmol) in 35 mL of *i*-PrOH, flushed with N_2, was added 250 mg of 10% Pd–C (Aldrich). The mixture was shaken for 46 h in a Parr apparatus under 40 psi of H_2, at which time TLC (1:1 Et_2O–hexane; product R_f 0.16, 0.13) showed essentially complete consumption of starting material. Minor impurities (R_f 0.18 and 0.19) also were visible. The solution was filtered through Celite, rinsing with EtOAc, then solvents were evaporated, and the residue was dried under high vacuum to 369 mg of oily residue. Flash chromatography of 361 mg of the crude product on 75 g of silica gel, eluting with 150 mL of 1:1 EtOAc–hexane, followed by 3:2 EtOAc–hexane afforded 305 mg (78%) of the desired product (mixture of diastereomers) [NMR ($CDCl_3$) δ 0.95 (cmplx, 9 H), 1.05–2.0 (cmplx, 16 H), [includes δ 1.4 (s, 9 H)], 2.0 (s, 3 H), 3.5 (m, 2 H), 3.85 (br m, 1 H), 4.3 (br m, 1 H), 4.9 (m, 1 H)] and 28 mg (7%) of secondary O → primary O transacetylation product [NMR ($CDCl_3$) δ 0.95 (cmplx, 9 H), 1.05–2.3 (cmplx, 16 H) [includes δ 1.4 (s, 9 H) and 2.05 (s, 3 H)], 3.65 (cmplx, 2 H), 4.0 (m, 2 H), 4.6 (m, 1 H)].

To a solution of the above primary alcohol (138 mg, 0.42 mmol) in ca. 0.5 mL of C_6H_6 were added 2.5 mL of H_2O, 332 mg (2.1 mmol) of $KMnO_4$, and 33 mg (0.1 mmol) of *n*-Bu_4NBr. After the mixture was stirred at room temperature for 5 h, 0.5 mL of HOAc was added and stirring was continued for an additional 3 h. The mixture was cooled, and saturated aqueous $NaHSO_3$ was added to destroy excess $KMnO_4$. The organic phase was diluted with EtOAc, H_2O was added, and the mixture was acidified while being cooled and stirred with solid $KHSO_4$. The layers were separated, and the aqueous phase was extracted with 2 × 35 mL of EtOAc. The combined organic layers were concentrated to small volume and extracted with 5 × 20 mL of 2.5% aqueous $NaHCO_3$. The combined aqueous extracts were back-extracted with 10 mL of Et_2O, then an approximately equal volume of EtOAc was added, and the mixture was acidified while being cooled and stirred with solid $KHSO_4$ to pH 1–2. The EtOAc layer was separated, the aqueous phase was extracted with 2 × 25 mL of EtOAc, and the combined EtOAc layers were washed with H_2O and then brine, dried ($MgSO_4$), filtered, and evaporated to 121 mg of oily residue. Flash chromatography on 8 g of silica gel, eluting with 3:1:0.04 hexane–EtOAc–HOAc, afforded 100.5 mg (70%) of the desired acid as a colorless syrup: TLC (2:1:0.04 hexane–EtOAc–HOAc) R_f 0.28; NMR ($CDCl_3$) δ 0.95 (d, J = 6 Hz, 6 H), 1.1–2.2 (cmplx, 18 H) [includes δ 1.2 (d, J = 7 Hz), 1.45 (s, 9 H), 2.1 (s, 3 H)], 2.5 (m, 1 H), 3.5 (m, ca. 0.4 H), 3.85 (m, ca. 0.6 H), 4.55 (br d, J = 11 Hz, ca. 0.4 H), 5.0 (m, ca. 0.6 H), 5.6 (m, ca. 0.4 H), 10.8 (br s, 1 H). Anal. ($C_{17}H_{31}NO_6$), C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-(5*RS*,6*S*)-6-amino-8-methyl-1-nonen-5-ol (18).** A dry three-neck, round-bottom flask fitted with a reflux condenser and nitrogen inlet was charged with Mg powder (1.28 g, 52.6 mmol) and a crystal of I_2, warmed (heat gun) to volatilize the I_2, and then allowed to cool. Then 10 L of dry THF was added followed by introduction of ca. 0.5 mL of neat 4-bromo-1-butene. The reaction was initiated by brief warming, and then the remainder of a total of 4.7 g (35 mmol) of the bromide in 3 mL of THF was added dropwise over 0.5 h as the mixture was maintained at reflux. After the addition was complete, the mixture was heated at reflux for an additional 15 min and then allowed to cool. To the stirred Grignard reagent was added dropwise over 2 h a solution of Boc-L-leucinal in 10 mL of dry THF, and then the mixture was stirred an additional 4.5 h at room temperature (21–22 °C). The mixture was poured onto ice, 150 mL of EtOAc was added, and then with cooling and stirring, 2 H HCl was added to pH 1–2. The separated aqueous phase was extracted with 150 mL of EtOAc, and then the combined organic phases were subjected to standard workup.

The crude product was chromatographed on 250 g of silica gel, eluting with 6:1 PhMe–Et_2O (product elution at ca. 1100 mL), to afford 2.3 g (62%) of the product as a colorless semisolid: TLC (5% MeOH–$CHCl_3$) R_f 0.68; NMR ($CDCl_3$) δ 0.95 (d, J = 6 Hz, 6 Hz), 1.1–1.9 (cmplx, 13 H), [includes δ 1.45 (s, 9 H)], 1.9–2.6 (cmplx, 4 H), 3.5 (cmplx, 2 H), 4.8 (br d, J = 9 Hz, 1 H), 4.95 (m, 2 H), 5.75 (m, 1 H). Anal. ($C_{15}H_{29}NO_3$) C, H, N.

***N*-[(*tert*-Butyloxylcarbonyl)-L-valyl]-(5*RS*,6*S*)-6-amino-8-methyl-1-nonen-5-ol (19).** Compound **18** (291 mg, 1.07 mmol) was treated with 4 N HCl/dioxane (3 mL) for 30 min, and then volatile compounds were evaporated. To the hydrochloride suspended in ca. 2 mL of dioxane were added 150 μL (111 mg, 1.1 mmol) of NEt_3 and 341 mg (1.1 mmol) of Boc-valyl-2-pyridyl thio ester. The mixture was stirred under N_2 for 2 h, then an additional 152 μL of NEt_3 was added, and stirring was continued for 21 h. Excess thio ester was destroyed by addition of 1 mL of 2 N NaOH and stirring for 1.5 h. The mixture was diluted with EtOAc and subjected to standard workup to afford 334 mg (84%) of a white solid, essentially pure by TLC and ^1H NMR and used without further purification: TLC (5% MeOH–$CHCl_3$) R_f 0.6; NMR ($CDCl_3$) δ 0.93 (m, 9 H), 1.1–1.8 (cmplx, 13 H), [includes δ 1.45 (s, 9 H)], 1.9–2.4 (cmplx, 5 H), 2.4–4.3 (cmplx, 3 H), 5.05 (m, 2 H), 5.5 (br d, J = 7.5 Hz, 1 H), 5.8 (m, 1 H), 7.9 (br d, J = 9 Hz, 1 H).

An analytical sample was obtained by recrystallization from EtOAc–Skelly B: mp 143–146 °C. Anal. ($C_{20}H_{38}N_2O_4$) C, H, N.

***N*-[(*tert*-Butyloxycarbonyl)-L-valyl]-(4*RS*,5*S*)-3-acetoxy-4-amino-7-methyloctanoic Acid (20).** Compound **19** (325 mg, 0.88 mmol) was acetylated by treating a stirred EtOAc solution (ca. 3 mL) with Ac_2O (167 μL, 180 mg, 1.76 mmol), NEt_3 (245 μL, 178 mg, 1.76 mmol), and 4-(dimethylamino)pyridine (10 mg, 0.08 mmol). After 2 h at room temperature, saturated aqueous $NaHCO_3$ was added, the mixture was stirred for 0.5 h, the layers were separated, and then the organic fraction was subjected to standard workup from EtOAc. The crude product, essentially pure by ^1H NMR and TLC, was obtained in quantitative yield and carried to the next step without further purification: TLC (2:1 hexane–EtOAc) R_f 0.36; NMR ($CDCl_3$) δ 0.95 (m, 12 H), 1.15–1.85 (cmplx, 13 H) [includes δ 1.45 (s, 9 H)], 1.85–2.4 (cmplx, 7 H) [includes δ 2.05 (s, 3 H)], 2.8 (m, 1 H), 4.25 (m, 1 H), 4.85–5.3 (cmplx, 4 H), 5.7 (m, 1 H, 6.05 (br d, J = 9 Hz, 1 H).

To 1.6 g (3.9 mmol) of acetoxyalkene in 20 mL of benzene were added 4 mL of HOAc, 15 mg (0.04 mmol) of tetrabutylammonium bromide, and 20 mL of H_2O. The mixture was cooled in an ice bath, then 2.1 g (13.4 mmol) of $KMnO_4$ was added, the ice bath was removed, and the mixture was vigorously stirred (magnetic) for 2 h. Solid $NaHSO_3$ was added in portions until a colorless mixture was obtained, and then saturated aqueous $KHSO_4$ was added with cooling and stirring to pH 1–2. The separated aqueous phase was extracted twice with EtOAc, and then the combined organic solutions were washed once with H_2O and then evaporated. The residue in a small volume of ether was extracted three times with equal volumes of 5% $NaHCO_3$, and the aqueous extracts were back-washed once with a small volume of EtOAc and then covered with a layer of fresh EtOAc, cooled, and stirred as solid $KHSO_4$ was added to pH 1–2. The layers were separated, the aqueous phase was extracted twice more with EtOAc, and then the combined organic phases were washed with H_2O and brine, dried ($MgSO_4$), filtered, and evaporated. The foam obtained under high vacuum collapsed in air to a clear, colorless analytically pure glass: 1.3 g (78%); TLC (50:25:1 hexane–EtOAc–HOAc) R_f 0.12; NMR ($CDCl_3$) δ 0.95 (m, 12 H), 1.1–1.6 (cmplx, 13 H) [includes δ 1.45 (s, 9 H)], 1.7–2.2 (m, 5 H) [includes δ 2.0 (s, minor OAc) and 2.1 (s, major OAc)], 2.35 (m, 2 H), 3.85 (m, 1 H), 4.3 (m, 1 H), 5.0 (m, 1 H), 5,3 (major) and 5.6 (minor) (2 d, J = 9 Hz, 1 H), 6.35 (d, J = 10 Hz, 1 H). Anal. ($C_{21}H_{38}N_2O_7$) C, H, N.

(4*S*,5*S*)-3-[(2*S*)-2-Benzyl-3-(benzyloxy)propionyl]-4-methyl-5-phenyloxazolidinone. To a solution of (4*R*,5*S*)-3-(3-phenylpropionyl)-4-methyl-5-phenyl-2-oxazolidinone (**10b**)[34] (25 g, 81 mmol) in dry THF (30 mL) at −78 °C under N_2 was added

(34) Evans, D. A.; Mathre, D. J. *J. Org. Chem.* **1985**, *50*, 1830–1835.

a 1 M solution of lithium bis(trimethylsilyl)amide in THF (84 mmol) dropwise via syringe over a period of 0.5 h. The mixture was stirred for an additional 0.5 h before benzyl bromomethyl ether (240 mmol) in THF (20 ml) was added over a period of 0.5 h via a pressure-equalized addition funnel. The mixture was stirred at −78 °C for 0.5 h, warmed to −45 °C (acetonitrile–dry ice) for 4 h, and then warmed to 0 °C for 1 h. To the stirred mixture were added dry pyridine (18 mL, 228 mmol) and acetic anhydride (14.4 mL, 152 mmol). The mixture was stirred at room temperature for 3 h and then diluted with 190 mL of 2 M aqueous $KHCO_3$. The mixture was stirred until gas evolution ceased and then concentrated in vacuo to about 200 mL. The residue was extracted with methylene chloride (2 × 150), and then the organic phase was washed with 1 N HCl, saturated $NaHCO_3$, and saturated brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was recrystallized from hexane to yield pure product in 65% yield (2 crops): mp 85–86 °C; TLC (4:1 CH_2Cl_2–hexane) R_f 0.20; $[\alpha]^{24}_D$ −23.0° (*c*, 1.02, CH_2Cl_2); NMR ($CDCl_3$) δ 0.81 (d, *J* = 7 Hz, 3 H), 2.93 (d, *J* = 7.5 Hz, 2 H), 3.55–3.97 (m, 2 H), 4.38–4.85 (m, 4 H, includes singlet δ 4.52), 5.27 (d, *J* = 7.5 Hz, 1 H), 7.10–7.50 (m, 15 H). Anal. ($C_{27}H_{27}N_1O_4$) C, H, N.

(2*R*)-2-Benzyl-3-(benzyloxy)propanol (11b) was prepared from **10b** (7 g, 17.3 mmol) in a manner analogous for preparation of **11a** from **10a**. The crude product was treated with Et_2O to precipitate recyclable oxazolidinone. The filtrate was purified by MPLC (400 g of SiO_2, 40% Et_2O–hexane, flow rate ca. 6 mL/min) to afford the product as a clear oil in 90% yield: TLC (1:1 EtOAc–hexane) R_f 0.40; $[\alpha]^{24}_D$ +23.6° (*c* 0.72, EtOH); NMR ($CDCl_3$) δ 2.12 (m, 1 H), 2.40 (m, 1 H), 3.54 (d, *J* = 7.5 Hz, 2 H), 3.40–3.75 (m, 4 H), 4.48 (s, 2 H), 7.05–7.44 (m, 10 H). Anal. ($C_{17}H_{20}O_2$) C, H.

(2*S*)-2-Benzyl-3-(benzyloxy)-1-bromopropane (6b) was prepared from **11b** (3.7 g, 14.4 mmol) analogously to the preparation of **6a**. The crude product was flash chromatographed on 200 g of silica gel, eluting with 5% Et_2O–hexane, to afford product as a clear liquid in 80% yield: TLC (1:1 EtOAc–hexane) R_f 0.57; $[\alpha]^{24}_D$ −2.75° (*c* 0.8, EtOH); NMR ($CDCl_3$) δ 2.24 (m, 1 H), 3.68 (d, *J* = 7.5 Hz, 2 H), 3.30–3.55 (m, 4 H), 4.42 (s, 2 H), 7.05–7.40 (m, 10 H). Anal. ($C_{17}H_{19}BrO$) C, H.

(2*R*,4*RS*,5*S*)-2-Benzyl-1-(benzyloxy)-5-[(*tert*-butyloxycarbonyl)amino]-7-methyloctan-4-ol (12b). Into a dry, two-neck, 25-mL, round-bottom flask under a stream of dry nitrogen were added magnesium powder (170 mg, 7.1 mmol) and a few crystals of iodine. The flask was heated with a heat gun until I_2 vapors were visible and then allowed to cool to room temperature. Dry THF (2 mL) was added via syringe followed by 1 drop of (2*S*)-2-benzyl-3-(benzyloxy)-1-bromopropane (**6b**). The mixture was warmed until reaction began, and then the remainder of the bromo ether (total 1.75 g, 5.48 mmol) in THF (1 mL) was added over a period of 1.5 h. The mixture was then stirred for 4 h before Boc-leucinal (591 mg, 2.75 mmol) in THF was added over a period of 2 h. After being stirred for an additional 4 h at room temperature, the mixture was poured into saturated aqueous NH_4Cl. Ethyl acetate was added and the mixture was acidified with 2 N HCl. The organic layer was then washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and removed in vacuo. The residue was chromatographed over 50 g of silica gel, eluting with 15% EtOAc–hexane under positive pressure (~5 mL/min). Product was obtained as a mixture of C(4) epimers (4*S*-4*R* = 3:2) in about 30% yield as an oil: TLC (1:4 EtOAc–hexane) R_f 0.15; NMR ($CDCl_3$) δ 0.84 (d, *J* = 6 Hz, 6 H), 1.15–1.68 (m, 14 H), 2.38 (m, 1 H), 2.70 (dd, *J* = 7 Hz, *J* = 3 Hz, 2 H), 3.04 (m, 1 H), 3.37–3.88 (m, 4 H), 4.51 (s, 2 H), 4.71 (m, 1 H), 7.10–7.40 (m, 5 H), 7.42 (s, 5 H). Anal. ($C_{28}H_{41}NO_4$), C, H, N.

***N*-[(*tert*-Butyloxycarbonyl)-L-valyl]-(2*R*,4*RS*,5*S*)-5-amino-2-benzyl-1-(benzyloxy)-7-methyloctan-4-ol (14b).** Compound **12b** (110 mg, 0.24 mmol) was deprotected according to the procedure for the preparation of **14a**. The resulting hydrochloride was coupled with Boc-valine anhydride (0.48 mmol) with methylene chloride as solvent. The crude compound was chromatographed on 10 g of silica gel (MPLC grade) and eluted with 1% MeOH/$CHCl_3$ under positive pressure (~5 mL/min). Pure compound was obtained as an oil in 75% yield: TLC (5% MeOH–$CHCl_3$) R_f 0.66; NMR ($CDCl_3$) δ 0.85–1.0 (m, 12 H), 1.15–1.75 (m, 14 H), 1.95–2.33 (m, 2 H), 2.61 (m, 2 H), 3.22–3.91 (m, 6 H), 4.46 (s, 2 H), 5.00 (m, 1 H), 5.97 (m, 1 H), 6.90–7.39 (m, 10 H). Anal. ($C_{33}H_{50}N_2O_5$) C, H, N.

***N*-[(*tert*-Butyloxycarbonyl)-L-valyl]-(2*R*,4*RS*,5*S*)-4-acetoxy-5-amino-2-benzyl-1-(benzyloxy)-7-methyloctane (15b)** was prepared from **14b** (70 mg, 0.125 mmol) analogously to the preparation of **15a** from **14a**. The crude product was chromatographed over 10 g of silica gel, eluting with 1:1 Et_2O–hexane. Pure product was obtained as an oil in quantitative yield: TLC (1:1 Et_2O–hexane) R_f 0.28 (4*S* isomer), 0.22 (4*R* isomer); NMR ($CDCl_3$) δ 0.75–1.10 (m, 12 H), 1.12–1.75 (m, 14 H), 1.90–2.18 (m, 5 H includes singlet δ 2.08), 2.40–3.0 (m, 2 H), 3.37 (d, *J* = 5 Hz, 2 H), 3.70–3.95 (m, 1 H), 4.19 (m, 1 H), 4.48 (s, 2 H), 4.92–5.21 (m, 2 H), 5.90 (d, *J* = 10 Hz, 1 H), 7.05–7.30 (m, 5 H), 7.32 (s, 5 H). Anal. ($C_{35}H_{52}N_2O_6$) C, H, N.

***N*-[(*tert*-Butyloxycarbonyl)-L-valyl]-(2*S*,4*RS*,5*S*)-4-acetoxy-5-amino-2-benzyl-7-methyloctan-1-ol (16b)** was prepared from **15b** (76 mg, 0.13 mmol) analogously to the preparation of **16a** and **15a**. The crude product was flash chromatographed on 10 g of silica gel, eluting with 3:2 EtOAc–hexane. Pure product was obtained as an oil in 95% yield: TLC (3:2 Et_2O–hexane) R_f 0.1; NMR ($CDCl_3$) δ 0.75–1.10 (m, 12 H), 1.20–1.75 (m, 14 H), 1.90–2.25 (m, 5 H includes singlet & 2.04), 2.51–2.79 (m, 3 H), 3.54 (m, 1 H), 3.82 (m, 1 H), 4.20 (m, 1 H), 5.03 (m, 1 H), 5.20–5.65 (m, 2 H), 6.16 (d, *J* = 10 Hz, 1 H), 7.24 (s, 5 H). Anal. ($C_{28}H_{46}N_2O_6 \cdot 0.5H_2O$) C, H, N.

***N*-[(*tert*-Butyloxycarbonyl)-L-valyl]-(2*R*,4*RS*,5*S*)-4-acetoxy-5-amino-2-benzyl-7-methyloctanoic acid (17b)** was prepared from **16b** (61 mg, 0.12 mmol) analogously to the preparation of **17a** from **16a**. The crude product, obtained in 95% yield, was used without further purification: NMR ($CDCl_3$) δ 0.75–1.10 (m, 12 H), 1.21–1.62 (m, 13 H), 1.80–2.25 (m, 5 H), 2.55–3.14 (m, 3 H), 3.72 (m, 1 H), 4.10 (m, 1 H), 5.00 (m, 1 H), 5.29 (m, 1 H), 7.20 (s, 5 H), 9.00 (m, 1 H).

Coupling at C-Terminus with DCC–HOBt. In a typical procedure, a mixture of Boc-Val-Leu-$\overset{OAc}{\text{—}}$-Ala-OH (**17a**) (0.1 mmol), HCl·Ala-Iaa (1.1 equiv), NEt_3 (1.1 equiv), and HOBt·H_2O (1.1 equiv) in ca. 1 mL of CH_2Cl_2 was cooled in an ice bath and then treated with DCC (1.1 equiv). The mixture was stirred at 0 °C for 2–4 h and then at room temperature overnight. The reaction mixture was checked periodically for appropriate pH, and additional NEt_3 was added if necessary to maintain slightly basic conditions. After completion of the reaction, the mixture was diluted with EtOAc, filtered, and then subjected to standard workup. The crude product was taken up in a small volume of EtOAc and filtered to remove DCU. The resulting crude product usually contained traces of DCU but otherwise appeared quite clean by TLC (5% MeOH–$CHCl_3$) or NMR. It was thus most convenient to use this material in the next step without further treatment.

For analytical purposes, Boc-Val-Leu-$\overset{(RS)OAc}{\text{—}}$-Ala-Ala-Iaa (**21a**) was purified by flash chromatography (10 g of SiO_2, 2% MeOH–$CHCl_3$): TLC (5% MeOH–$CHCl_3$) R_f 0.49 (4*S*), 0.43 (4*R*); ^1H NMR (90 MHz, $CDCl_3$) δ 0.75–2.55 (cmplx, 45 H) [includes δ 1.45 (s), 2.0 (s, minor OAc), 2.05 (s, major)], 3.2 (q, *J* = 7 Hz, 2 H), 3.8 (m, 1 H), 4.1 (m, 1 H), 4.45 (m, 1 H), 4.85 (m, 1 H, isostere C(4)-H), 5.15 and 5.35 (2 d, *J* = 8 Hz, 1 H), 6.2 (d, *J* = 9 Hz, 1 H), 6.7 (t, *J* = 5 Hz, 1 H), 6.9 (d, *J* = 7 Hz, 1 H). Anal. ($C_{30}H_{56}N_4O_7$) C, H, N.

Extension of N-Terminus by Deprotection and Symmetrical Anhydride Couplings. This sequence was carried out according to standard procedures (Boc deprotection with 4 N HCl–dioxane followed by treatment with a symmetrical anhydride in DMF with NEt_3 or *N*-methylmorpholine as base).[35]

Separation of Diastereomers by Flash Chromatography. In a typical procedure, crude Iva-Val-Leu-$\overset{R,S\text{-}OAc}{\text{—}}$-Ala-Ala-Iaa (**22a**) obtained from 0.11 mmol of **20a** was dissolved in a minimum of $CHCl_3$ and applied to a column (8 × 24 mm) of silica gel (230–400 mesh) packed in $CHCl_3$. The product was then eluted with 2% EtOH–$CHCl_3$, appearing at between ca. 100 and 300 mL of elute. In this manner, 33 mg of pure 4*S* isomer, 8 mg of mixed isomers, and 10 mg of pure 4*R* isomer were obtained (82% from **20a**): TLC

(35) Rich, D. H.; Bernatowicz, M. S. *J. Med. Chem.* **1982**, *25*, 791–795.

Table III. Physical Data for Synthetic Inhibitors

compd	yield,[a] %	mp,[b] °C	R_f[c]	$[\alpha]^{24}_D$,[d] deg	microanal.
23	67	238–239 (MeOH–Et_2O)	0.62	–51 (0.1, MeOH)	C, H, N
24	81	212–213 (MeOH–Et_2O)	0.43	–71 (0.1, MeOH)	C, H, N
25	54	279–280 (HOAc–Et_2O)	0.58	–95 (0.07, MeOH)	C, H, N
26	87	>250	0.21	–33 (0.21, MeOH)	C, H, N[e]
27	52	284 (HOAc–MeOH–Et_2O)	0.55	–36 (0.06, MeOH)	C, H, N
28	95	220–223	0.50	–64 (0.24, MeOH)	C, H, N
29	55	234–234.5 (MeOH–Et_2O)	0.49	–93 (0.07, MeOH)	C, H, N
32	70	227–228 (MeOH–Et_2O)	0.38	–93 (0.76, MeOH)	C, H, N
33	40	229–230 (MeOH–$CHCl_3$–hexane)	0.71	–80 (0.1, MeOH)	C, H, N[f]
34	83	238–239 (MeOH–Et_2O)	0.63	–21.5 (0.2, MeOH)	C, H, N[g]
35	51	252–244 (MeOH–$CHCl_3$–hexane)	0.62	–81 (0.08, MeOH)	C, H, N
36	81	222–223 (hexane)	0.57	–83 (0.54, MeOH)	C, H, N

[a] From acetoxy precursor **22**. [b] (Recrystallization solvent). [c] In 10% MeOH–$CHCl_3$. [d] (Concentration, solvent). [e] Calcd for $C_{34}H_{58}N_4O_5 \cdot H_2O$. [f] Calcd for $C_{28}H_{52}N_4O_5 \cdot 0.5MeOH$. [g] Calcd for $C_{34}H_{56}N_4O_5 \cdot 1.5MeOH$.

(5% MeOH–$CHCl_3$) R_f 0.33 (4*S*), 0.30 (4*R*). 270-MHz NMR of 4*S* isomer ($CDCl_3$): δ 0.6–1.7 (cmplx, 30 H) [includes δ 1.10 (d, J = 5.7 Hz, isostere C(2)-CH_3), 1.35 (d, J = 6 Hz, Ala-CH_3)], 1.85 (m, 2 H), 2.0–2.13 (cmplx, 7 H) [includes δ 2.10 (s, OAc)], 2.35 (m, 2 H), 3.25 (m, 2 H, Iaa α-CH_2), 4.2 (2 m, 2 H, Val-Hα and isostere C(5)-H), 4.4 (m, 1 H, Ala-H_2), 4.9 (m, 1 H, isostere C(4)-H), 5.95 (d, J = 8.5 Hz, 1 H), 6.17 (d, J = 10 Hz, 1 H), 6.3 (t, J = 6 Hz, Iaa-NH), 6.6 (d, J = 8.5 Hz, 1 H). Anal. ($C_{30}H_{56}N_4O_6$) C, H, N. NMR of 4*R* isomer ($CDCl_3$): δ 2.05 (s, OAc).

Preparation of Hydroxyethylene Peptides. An ca. 0.1 M solution of acetoxy peptide in MeOH was vigorously stirred with 10 molar equiv of anhydrous K_2CO_3 until the starting material was consumed (TLC, 5% MeOH–$CHCl_3$). Ordinarily 15–20 h of reaction time was allowed. Then 5–10 volumes of H_2O was added, the mixture was chilled, and the precipitated peptide was collected by filtration and washed with H_2O. Yields for crude product were usually >90%. Recrystallization from an appropriate solvent system (see Table III) afforded product used for characterization and for enzyme-inhibition studies. Physical data are collected in Table III. 360-MHz NMR (MeOH-d_4) of Iva-Val-Leu$\underline{^{OH}}$Ala-Ala-Iaa (**25**): δ 0.8–1.0 (cmplx, 24 H), 1.1 (d, J = 6.6 Hz, 3 H, isostere ((2)-CH_3), 1.3 (d, J = 6.6 Hz, 3 H, Ala-CH_3), 1.31–1.72 (cmplx, 7 H), 2.02 (m, 1 H, Val-β), 2.1 (m, 2 H, Iva-CH_2), 2.61 (m, 1 H, isostere C(2)-H), 3.18 (m, 2 H, Iaa α-CH_2), 3.68 (m, 1 H, isostere C(4)-H), 3.88 (m, 1 H, isostere C(5)-H), 4.1 (d, J = 7.8 Hz, 1 H, Val-Hα), 4.25 (m, 1 H, Ala-Hα).

Oxidation of the Hydroxyethylene Moiety to the Ketomethylene Moiety. Ordinarily, crude products from deacetylation of (*R*,*S*)-OAc precursors were used as the starting materials. To an ca. 0.02–0.04 M solution of starting hydroxyethylene peptide in glacial HOAc was added pyridinium dichromate (3 mmol/mol of peptide). The mixture was stirred until starting material was consumed (typically 5–25 h) and then diluted with equal volumes of $CHCl_3$ and H_2O. The layers were separated, and then the organic phase was subjected to standard workup. The product was purified by flash chromatography (ca. 200:1 w/w SiO_2–peptide), eluting with 1% MeOH–$CHCl_3$, followed by recrystallization from an appropriate solvent system. Physical data are collected in Tables III and IV. 360-MHz NMR (MeOH-d_4) for Iva-Val-Leu$\underline{^{K}}$Ala-Ala-Iaa (**33**): δ 0.8–1.0 (cmplx, 24 H), 1.1 (d, J = 6.6 Hz, 3 H, isostere C(2)-CH_3), 1.3 (d, J = 6.6 Hz, 3 H, Ala-CH_3), 1.32–1.78 (cmplx, 7 H), 2.02 (m, 1 H, Val-β), 2.1 (m, 2 H, Iva-CH_2), 2.57 (m, 1 H, isostere C(2)-H), 2.85 (m, 2 H, isostere C(3)-H), 3.18 (m, 2 H, Iaa α-CH_2), 4.2 (cmplx, 2 H, Ala-Hα and Val-Hα), 4.4 (m, 1 H, isostere C(5)-H).

Acknowledgment. Financial support from the National Institutes of Health (AM 20100) and Merck Sharp and Dohme is is gratefully acknowledged. We thank Professor David Evans for communicating detailed procedures for the synthesis of **11** and Cyndy Davis and Joan Doerrer for their assistance in preparing the manuscript.

Structure–Activity Studies of 5-[[4-(4,5-Dihydro-2-oxazolyl)phenoxy]alkyl]-3-methylisoxazoles: Inhibitors of Picornavirus Uncoating

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A series of substituted phenyl analogues of 5-[[4-(4,5-dihydro-2-oxazolyl)phenoxy]alkyl]-3-methylisoxazoles has been synthesized and evaluated in vitro against several human rhinovirus (HRV) serotypes. Substituents in the 2-position greatly enhanced activity when compared to the unsubstituted compound. Many of these compounds exhibited mean MICs ($\overline{MIC}$) against five serotypes as low as 0.40 μM. The mean MIC correlated well (r = 0.83) with the MIC_{80} (the concentration that inhibited 80% of the serotypes tested). A quantitative structure–activity relationship study indicated a strong dependency of $\overline{MIC}$ on lipophilicity (log P) in combination with inductive effects (σ_m) and bulk factors (MW).

Compound **1** is a broad-spectrum antipicornavirus agent[1] that inhibits replication of 36 out of 45 rhinovirus serotypes at levels ranging from 0.3 to 3.0 μM and is also effective against several enteroviruses.[2,3] When administered orally,[4] **1** reduces mortality in mice infected intracerebrally

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0022-2623/87/1830-0383$01.50/0