Stabilized Analogs of Thymopentin. 2. 1,2- and 3,4-Ketomethylene Pseudopeptides

Joseph I. DeGraw,*[,†] Ronald G. Almquist,[†] Charles K. Hiebert,[†] Amrit K. Judd,[†] Linda Dousman,[†] R. Lane Smith,[‡] William R. Waud,[§] and Itsuo Uchida[||]

Bio-Organic Chemistry Laboratory, SRI International, Menlo Park, California 94025, Orthopedic Research Laboratory, Stanford University Medical School, Palo Alto, California 94305, Southern Research Institute, Birmingham, Alabama 35205, and Pharmaceutical Research Laboratory, Japan Tobacco Inc., Tokyo 227, Japan

Received November 1, 1995[⊗]

In this second paper in a series of three studies of stable analogs of thymopentin (Arg[1]-Lys[2]-Asp[3]-Val[4]-Tyr[5]), the synthesis of analogs stablized at peptide bonds 1,2 and 3,4 via insertion of ketomethylene units is described. A tris(carbobenzyloxy)arginyl(k)norleucine pseudopeptide was synthesized and coupled to Asp-Val-Phe-resin units followed by HF cleavage to prepare Arg(k)Nle-Asp-Val-Phe analogs. Preparation of *N*-BOC Asp(k)Val and *N*-BOC Asp(k)Ala units followed by coupling to Phe- or Tyr-resin units provided resin-bound pseudotripeptide substrates for attachment of various arginyl dipeptides. Cleavage from the resin afforded 3,4-ketomethylene-stabilized pseudopeptide analogs of thymopentin. The Arg-Lys-Asp(k)Val-Phe and Arg-Lys-Asp(k)Val-Tyr analogs were more strongly bound to CEM cells than thymopentin itself. There was significant enhancement of stability in serum for the analogs, especially those containing Arg(k)Nle or Arg-NMeLys moieties at the 1,2-peptide bond.

In the accompanying first paper of this series,[1] we presented the background for the pentapeptide, thymopentin (Arg[1]-Lys[2]-Asp[3]-Val[4]-Tyr[5]), with resepect to scientific observations and clinical developments. While the peptide has clear potential for treatment of inflammatory and infectious diseases its stability in plasma presents a major limitation. Our approach to increase the half-life of thymopentin has been to insert hydrolytically stable ketomethylene bonds at key points in the peptide chain. In the first paper we reported our structure−activity investigation following substitution at the peptide bond linking amino acids 4 and 5 as in formula **1**. In this second paper we report the results of our related investigation of ketomethylene substitution at the 1,2- (formula **2**) and 3,4-peptide bonds (formula **3**).

Chemistry

Previous investigations had indicated that the Arg−Lys bond was the most sensitive to cleavage and that stabilization at this juncture would greatly enhance the half-life of thymopentin in serum.[1−3] However, arginyl ketomethylene dipeptides present considerable synthetic difficulties in view of the polyfunctional nature of this amino acid. We initially elected to investigate the preparation of arginyl ketomethylene norleucine subunits and their pseudopentapeptides. Ultimately we confined our study to this substitution pattern because of synthetic difficulty encountered in synthesis of Arg-(k)Lys moieties.

Arg-X-Asp-Y(k)Z

1 X = Lys, Nle, Pro, Ala, Leu, D-Lys, *N*-MeNle
Y = Val, Ala
Z = Phe, Val

Arg(k)Nle-Asp-X-Phe

2 X = Val, D-Val

Arg-X-Asp(k)Y-Z

3 X = Nle, Lys, Pro, *N*-MeLys
Y - Val, Ala
Z = Phe, Tyr

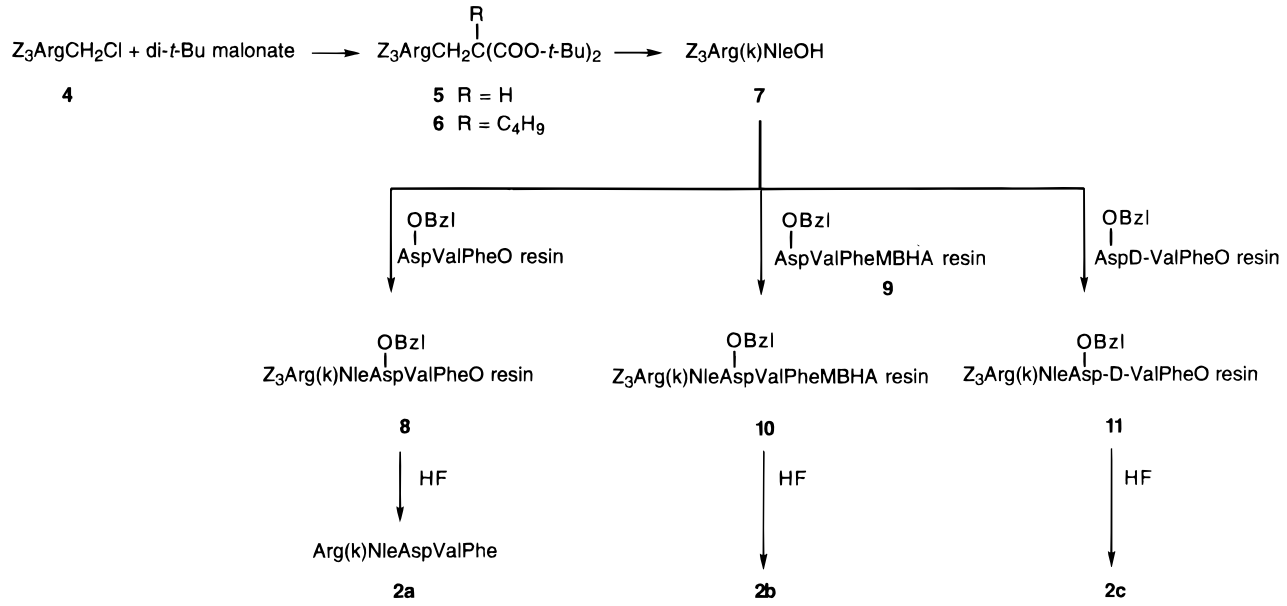
The synthetic route for Arg(k)Nle analogs **2a**−**c** is outlined in Scheme 1 and is based upon general procedures previously reported.[4−6] N^{α},N^{δ},N^{ω}-Tris(carbobenzyloxy)-L-lysine was converted to the chloromethyl ketone **4** in 35% overall yield by treatment with isobutyl chloroformate and diazomethane in a modified version of the Arndt−Eistert reaction. The intermediate diazo ketone in this process was transformed to the chloromethyl ketone via reaction with LiCl in 80% HOAc, a method developed[7] for acid-sensitive halomethyl ketones. The chloromethyl ketone **4** was further activated by conversion to the iodo ketone and reacted *in situ* with sodio di-*tert*-butyl malonate in dimethoxyethane to afford the arginyl ketomethylene malonate **5**. The solution containing **5** was treated with an equivalent of NaH and the subsequently formed sodio salt was further alkylated by *n*-butyl iodide to ultimately give the α-butyl ketomethylene malonate **6** in 68% yield. The di-*tert*-butyl ester was cleaved to the diacid by exposure to trifluoroacetic acid (TFA) at room temperature. The diacid was subsequently decarboxylated by heating at 100 °C in pyridine to afford the tris(carbobenzyloxy)-arginylnorleucine ketomethylene dipeptide **7** as a mixture of diastereomers. Chromatographic separation yielded pure isomers A (24%) and B (27%).

Compound **7**, isomer A, was then coupled with aspartyl(β-benzyl ester)-valyl-phenylalanine bound to Merrifield resin via ester formation at the Phe carboxyl

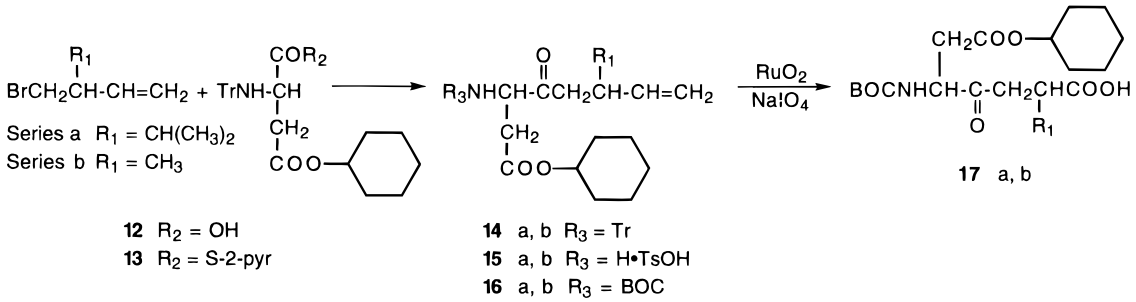
† SRI International.
‡ Stanford University Medical School.
§ Southern Research Institute
|| Japan Tobacco Inc.
⊗ Abstract published in *Advance ACS Abstracts,* June 15, 1997.

S0022-2623(95)00804-1 CCC: $14.00

Scheme 1



Scheme 2

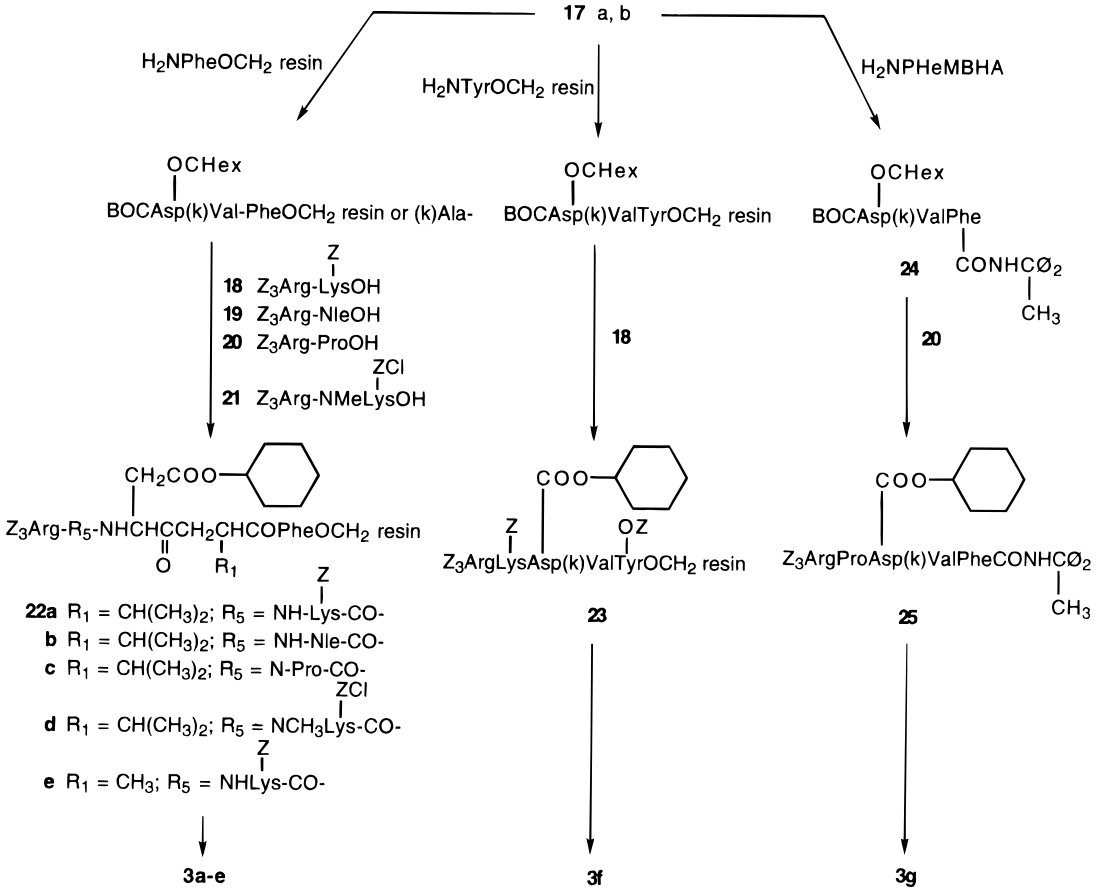


group. The blocked pentapeptide **8** so obtained was then deblocked and cleaved from the resin carrier by treatment with HF/anisole to yield the Arg(k)Nle-Asp-Val-Phe compound (**2a**) as a mixture of two isomers designated **2a-A** and **2a-B**. Similar coupling of **7**, isomer B, afforded **2a-C** and **2a-D**. These additional isomeric pairs were apparently generated by racemization about the α-carbon of the arginyl ketone moiety. Similar coupling of compound **7** (isomer A) with Asp(*β*-*O*-benzyl)-Val-Phe-methybenzhydrylamine resin (**9**) gave the Arg(k)Nle-Asp-Val-Phe amide (**2b**) as a mixture of isomers (A and B) following cleavage/deblocking of **10** with HF. Compound **7** (isomer A) was also coupled as above with Asp(*β*-*O*-benzyl)-D-Val-Phe-resin to afford **2c** (isomers A and B), following HF treatment of the intermediate blocked pentapeptide (**11**).

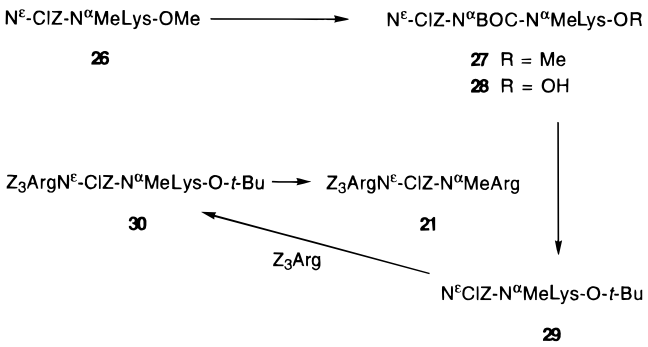
For synthesis of analogs in series **3** it was necessary to first prepare suitable blocked aspartyl ketomethylene valyl and alanyl subunits as shown in Scheme 2, formula **17a,b**. *N*-BOC-aspartic acid *β*-cyclohexyl ester was treated with TFA in $MeCl_2$ to remove the BOC group followed by tritylation to give *N*-tritylaspartic acid *β*-cyclohexyl ester (**12**). Reaction with 2-pyridinethiol with carboxyl activation by dicyclohexylcarbodiimide (DCC) afforded the blocked α-2-thiopyridine ester (**13**) in 83% yield. Subsequent reaction of **13** with the Grignard reagent prepared from 4-methyl-3-(bromomethyl)-1-pentene gave the blocked aspartyl keto olefin (**14a**) in 89% yield following chromatography on silica gel. The ketone carbonyl group was confirmed by a signal at 209.40 ppm in the ^{13}C-NMR spectrum. The trityl group was readily cleaved by exposure of **14a** to an equivalent of *p*-toluenesulfonic acid in acetonitrile, and the amine salt **15a** was treated with $(BOC)_2O-Et_3N$ to produce the *N*-BOC keto olefin (**16a**) in 79% yield as an isomeric mixture. Oxidation of the olefin with $RuO_2 \cdot xH_2O/NaIO_4$ gave the key *N*-BOC-*β*-*O*-cyclohexyl-Asp(k)Val dipeptide (**17a**) in 69% yield as a diastereomeric mixture showing ketone carbonyl signals at 206.90 and 207.23 ppm in the ^{13}C-NMR. Similarly compound **13** was treated with the Grignard reagent from 1-bromo-2-methyl-3-butene to afford the *N*-trityl olefinic ketone (**14b**). Further processing in a manner similar to that described for **17a** yielded the *N*-BOC-*β*-*O*-cyclohexyl-Asp(k)Ala dipeptide (**17b**).

Coupling of **17a** (Scheme 3) with phenylalanine (bound to resin via carboxylic ester linkage), removal of the *N*-BOC group from the aspartyl moiety by TFA, and subsequent coupling of the resin-bound Asp(k)Val-Phe tripeptide unit with tris(carbobenzyloxy)arginyl-N^{ϵ}-(chlorobenzyloxycarbonyl)lysine (**18**) gave the blocked ketomethylene resin-bound pentapeptide (**22a**). The coupling of protected dipeptides to the ketomethylene-containing pseudotripeptide was necessitated by reasons discussed in the previous paper of this series.[1] The use of HOBt/DCC or HOSu/DCC activation strategies were also employed to minimize racemizations as discussed in paper 1. After cleavage/deblocking with HF/anisole the target analog peptide **3a** was obtained as a mixture of isomers (A, B) separated via preparative HPLC. Analogs **3b**−**e** were similarly obtained as TFA salts by coupling of appropriate blocked arginyl dipeptide units

Scheme 3



Scheme 4



(**18**−**21**) with Asp(k)Val-Phe-resin or Asp(k)Ala-Phe-resin units as generated from **17a** or **17b**, respectively, followed by cleavage with HF/anisole. Coupling of **17a** with *p*-(carbobenzyloxy)tyrosine bound to resin, removal of *N*-BOC with TFA, and coupling with **18** gave the Arg-Lys-Asp(k)Val-Tyr analog (**3f**) after cleavage of the resin-bound pentapeptide (**23**) with HF. Coupling of **17a** with phenylalanine bound to methylbenzhydrylamine resin (**24**) and subsequent coupling with **20** yielded the resin-bound blocked pentapeptide (**25**). Treatment with HF caused cleavage from the resin, removal of blocking groups, and cleavage of the methylbenzhydryl moiety to afford the Arg-Lys-Asp(k)Val-Phe amide (**3g**).

Arginyl dipeptide substrates **18**−**20** were prepared by conventional methods; however, the arginyl *N*-methyllysine dipeptide (**21**) was synthesized via the method in Scheme 4. N^{ϵ}-(2-Chlorocarbobenzoxy)-*N*-methyllysine methyl ester (**26**) was prepared by the Diels−Alder/Et_3SiH process described by Grieco and Bahsas[8] for the corresponding N^{ϵ}-(carbobenzyloxy)-*N*-methyllysine ester. The α-NH was blocked with BOC, and the methyl ester (**27**) was saponified. Treatment of the acid (**28**) with isobutylene−H_2SO_4 in dioxane effected cleavage of the *N*-BOC group and concommitant formation of the *tert*-butyl ester to give **29**. Coupling with tricarbobenzyloxy arginine and removal of the *t*-Bu ester in **30** gave the desired blocked dipeptide substrate (**21**).

Biological Evaluation

Receptor Binding. Compounds in series **2** and **3** were assayed for their ability to bind to CEM cell receptors as described for series **1** analogs in the preceeding paper.[1] The binding assay was conducted as a competition for displacement of radiolabeled thymopentin from the receptor. Each analog was analyzed in terms of the mean level of radioactivity remaining bound to CEM cells in the presence of increasing concentration of the test compound. Binding efficacy of the analog represents a comparison with the nature of authentic thymopentin; counts remaining after competition were subtracted from total binding of thymopentin and divided by the counts reduced by authentic thymopentin (Table 1).

The results shown in Table 1 indicate that in series **2** analogs the insertion of a ketomethylene group at the 1,2-bond was not beneficial in terms of receptor binding. The examples **2a**−**c** feature Arg(k)Nle rather than Arg(k)Lys substitutions, thus obviating a direct comparison with thymopentin at this locus. However, substitution of norleucine for lysine did not seriously depress binding in comparable examples in series **1** as seen in the accompanying paper.[1] The presence of an amide at the

Table 1. Thymopentin Analog Binding to CEM Cells[a]

compd tested	no. of trials	binding competition mean counts ± SEM	efficacy (%)
none	47	3078 ± 49	
2a-A, Arg(k)Nle-Asp-Val-Phe	12	2222 ± 172	44
2a B, Arg(k)Nle-Asp-Val-Phe	12	2176 ± 248	47
2a-C, Arg(k)Nle-Asp-Val-Phe	18	2687 ± 122	20
2a-D, Arg(k)Nle-Asp-Val-Phe	18	2333 ± 137	39
2b-A, Arg(k)Nle-Asp-Val-PheNH$_2$	6	3627 ± 324	0
2b-B, Arg(k)Nle-Asp-Val-PheNH$_2$	6	3648 ± 338	0
2c-B, Arg(k)Nle-Asp-D-Val-Phe	8	1713 ± 207	71
3a-A, Arg-Lys-Asp(k)Val-Phe	7	844 ± 86	115
3a-B, Arg-Lys-Asp(k)Val-Phe	8	1091 ± 93	103
3b-A, Arg-Nle-Asp(k)Val-Phe	6	1232 ± 100	96
3b-B, Arg-Nle-Asp(k)Val-Phe	6	1447 ± 110	85
3c-B, Arg-Pro-Asp(k)Val-Phe	6	1686 ± 196	72
3d-A, Arg-NMeLys-Asp(k)Val-Phe	6	2820 ± 121	13
3e-A, Arg-Lys-Asp(k)Ala-Phe	6	2172 ± 111	47
3e-B, Arg-Lys-Asp(k)Ala-Phe	6	2606 ± 129	24
3f-A, Arg-Lys-Asp(k)Val-Tyr	6	653 ± 57	126
3f-B, Arg-Lys-Asp(k)Val-Tyr	9	1781 ± 179	67
3g-A, Arg-Pro-Asp(k)Val-PheNH$_2$	6	2692 ± 223	20
thymopentin	75	1150 ± 60	100

[a] CEM cells were resuspended at a final concentration of 5×10^6 cells/0.1 mL of binding buffer. For competition assays, dilutions of TP-5 or TP-5 analogs were added to the cells over a concentration range from 10^{-6} to 10^{-3} M. Radiolabeled TP-5 (548 000 cpm) was added, and the cells were incubated for 30 min at 25 °C. At the end of incubation, the cells were diluted with 1 mL of ice-cold binding buffer and washed two times by centrifugation. Radioactivity was determined by scintillation counting.

carboxy terminus (**2b**) resulted in loss of binding, but surprisingly the insertion of D-valine at position 4 (**2c-B**) was well tolerated.

In series **3** the presence of the ketomethylene bond at the 3,4-position was either well tolerated (**3b-A**, **3b-B**, **3c-B**) or was responsible for increased binding (**3a-A**, **3a-B**, **3f-A**). It is interesting that both diastereomers of **3a** were more tightly bound than thymopentin, but in the case of **3f** the B isomer binding decreased considerably compared to the very potent A isomer. It is also noteworthy that the presence of lysine or norleucine was comparable for binding purposes. Even the structurally dissimilar proline at this position was reasonably tolerated. *N*-Me-lysine at position 2 was clearly unfavorable to binding as was the presence of the carboxy terminal amide in **3g**. It was surprising that replacement of the valine at position 4 with alanine was not well tolerated. This is in contrast to the experience in series **1**.[1]

Stability Studies. A few of the analogs in series **2** and **3** were examined for their stability in human and/or mouse plasma in comparison to thymopentin itself. The half-lives of the compounds were determined following incubation in plasma for variable times, using quantitation by HPLC as mentioned in paper 1.[1] Mouse plasma was prepared by EDTA or heparin treatment of serum. Human plasma was prepared by heparin treatment. The results for selected compounds are shown in Table 2.

As previously seen[1] for compounds in series **1** the analogs showed a significant improvement in their half-lives when compared with thymopentin under the same experimental conditions. Compounds **3a-A** and **3f-A** which were particularly impressive in the binding assay above had virtually identical half-lives in the heparinized mouse plasma, some 3-fold longer than thymopentin itself. This result suggests that the Val−Phe and Val−Tyr bonds are about equally susceptible to degradation. Unfortunately **3f-A** was not measured in human plasma, but **3a-A** was nearly twice as long lived as thymopentin in this medium. As expected the susceptible 1,2-peptide bond was protected by the insertion of the ketomethylene group in **2a** with a profound increase in half-life (12.5 min) being observed for **2a-A**. As previously observed in series **1**, *N*-methylation of the amino acid at position 2 (compound **3d-A**) completely protected the pentapeptide from degradation in plasma. This is a curious result and suggests that the *N*-methyl moiety has an effect on the entire molecule since the C-terminal 4,5-peptide bond should have been susceptible to degradation as in **2a-A**. An alternate explanation is that the Asp(k)Val moiety exerts an extended effect at the 4,5-bond once the particularly vulnerable 1,2-bond is protected by the *N*-Me-lysine.

Table 2. Stability of Thymopentin and Analogs in Mouse and Human Plasma

compd tested	half-life[a] (min)		
	mouse		human
	EDTA-treated	heparinized	heparinized
thypentin	3.1	0.8	1.5
2a-A			12.5
3a-A	5.8	2.4	2.7
3f-A		2.4	
3d-A		not degraded	not degraded

[a] Half-life of TP-5 and TP-5 analogs were determined by HPLC methods that permitted quantitative resolution of the decrease of TP-5 and the appearance of degradation products by absorbance. EDTA-treated and heparinized serum was collected to prevent removal of clotting components.

Synthetic difficulties prevented us from conducting an extensive exploration of series **2** analogs. However, the results of our limited investigation suggest that binding with T-cell-related receptors is unacceptably compromised by insertion of ketomethylene at the 1,2-peptide bond even though stability in plasma is considerably enhanced. The structural variation introduced in series **3** with ketomethylene at the 3,4-bond was well tolerated, if not more beneficial, in terms of receptor binding. Plasma half-life was significantly, if not overwhelmingly, improved, and compounds such as **3a-A,B**, **3f-A**, and **3b-A,B** would seem to have potential for improved results *in vivo* as compared with thymopentin. The evaluation of compounds of series **1**−**3** *in vivo* is presented in paper 3 of this series.

Experimental Section

Elemental analyses were obtained from Galbraith Laboratories, Knoxville, TN. Values were within 0.4% except as noted in parentheses following the element. ^{1}H- and ^{13}C-NMR spectra were determined with a Varian 400A or a JEOL FX 90Q spectrometer. Mass spectra were obtained with a Ribermag R10-10C MS or a ZAB-2EQ VG reverse geometry high-resolution instrument in the case of final target peptides. Reverse phase HPLC analyses were conducted on a Waters Associates Novapak C18 column. The solvent systems were 10−40% CH_3CN/0.1% TFA.

Designation of isomers were made arbitrarily on the basis of chromatographic behavior at the first point of separation whether intermediate or final product. Isomer A was assigned to the faster moving component and B to the slower for thin layer or HPLC chromatograms.

Less common abbreviations used in the experimental description or test are as follows: ClZ (*p*-chlorobenzyloxycarbonyl), cHex (cyclohexyl), $(BOC)_2O$ (di-*tert*-butyl dicarbonate),

DIEA (diisopropylethylamine), MBHA (*p*-methylbenzhydrylamine resin), BOP [(benzotriazol-1-yloxy)tris(dimethylamino)-phosphonium hexafluorophosphate].

1-Chloro-3-amino-6-guanidino-2-hexanone Tris(carbobenzyloxy)amide (4). To a solution of Z_3-L-Arg (10.0 g, 17.4 mmol) in THF (50 mL) cooled with an ice bath was added Et_3N (1.94 g, 19.0 mmol) followed by isobutyl chloroformate (2.59 g, 19.0 mmol). In 20 min the Et_3N·HCl was removed by filtration and washed with THF (15 mL). Ethereal CH_2N_2 (~25 mmol in 40 mL of Et_2O) was added at 0−5 °C, and the solution was kept at ambient temperature for 63 h. Within the first 1.5 h, a copious precipitate formed which redissolved at room temperature. The reaction mixture was evaporated *in vacuo* to a white solid, which was partitioned between $CHCl_3$ and H_2O. The organic layer was washed with saturated $NaHCO_3$ and evaporated, and the residue was recrystallized twice from EtOAc−petroleum ether to obtain the diazo ketone intermediate (5.93 g, 57%): mp 123−124 °C; $[\alpha]_D$ −15.2 (*c* = 1.0, DMF); IR (KBr) 2105 cm^{-1} (C*H*N_2).

Following the literature method of Aplin et al.[7] for acid sensitive halo ketones, the diazo ketone (9.85 g, 16.4 mmol) was dissolved in 80% aqueous HOAc (250 mL) containing anhydrous LiCl (35 g, 0.83 mol) at 0 °C. The mixture was allowed to warm to ambient temperature overnight. Addition of 100 mL of H_2O and 75 mL of EtOAc with vigorous stirring caused precipitation of a white solid which was collected by filtration and dessicator-dried, overnight. Recrystallization from THF−Et_2O−petroleum gave the product **4** (6.18 g, 62%): mp 132−134 °C; $[\alpha]_D$ −17.2° (*c* = 1.0, DMF). Anal. Calcd for $C_{31}H_{33}ClN_4O_7$: C, H, N.

***tert*-Butyl 2-(*tert*-Butyloxycarbonyl)-2-butyl-4-oxo-5(*S*)-[(benzyloxycarbonyl)amino]-8-bis(benzyloxycarbonyl)-guanidinooctanoate (6).** To a suspension of NaH, 50% in mineral oil (135 mg, 2.8 mmol) in dimethoxyethane (DME) (25 mL), was added di-*tert*-butyl malonate (603 mg, 2.8 mmol), and the resulting solution was stirred under argon until it clarified. Z_3-Arg-CH_2Cl (**4**) (1.53 g, 2.5 mmol) and NaI (380 mg, 2.54 mmol) were suspended in DME, and the mixture was stirred for 15−20 min. During this time most of the material dissolved and NaCl precipitated. The malonate solution was then transferred to the resulting iodomethyl ketone solution via a cannula. TLC indicated that the reaction was complete after 1 h to afford the monoalkylated malonate **5**. The reaction mixture was transferred via cannula to a flask containing NaH, 50% in mineral oil (150 mg, 3.13 mmol). When the transfer was complete, 1-iodobutane (3.45 g, 18.8 mmol) was added to the mixture. After 3 h, TLC indicated that the reaction was complete. The reaction was quenched with a few drops of AcOH, and the solvent was removed *in vacuo*. The crude material was dissolved in $MeCl_2$ (100 mL), washed with 0.1 N HCl (2 × 50 mL) and saturated NaCl (250 mL), dried over $MgSO_4$, and evaporated. The oily residue was flash chromatographed, eluting with hexane, EtOAc−hexane (1:9), and EtOAc−hexane (1:3). Interesting fractions were pooled and concentrated to give 1.442 g (68%) of product **6**: TLC R_f 0.58 (EtOAc−hexane, 35:65); ^{1}H-NMR ($CDCl_3$) δ 0.85 (t, 3H, CH_3), 1.15 (m, 4H), 1.43 (s, 18H, CO_2t-*Bu*), 1.45−1.98 (m, 6H), 3.05 (s, 2H, COCH_2), 3.91 (m, 2H, Arg δ CH_2), 4.26 (m, 1H, Arg α C*H*), 5.02 (s, 2H, ArCH_2), 5.11 (s, 2H, ArCH_2), 5.20 (s, 2H, ArCH_2), 5.74 (d, 1H, N*H*), 7.30 (s, 5H, Ar*H*), 7.36 (s, 10H, Ar*H*), 9.31 (bd, 2H, N*H*).

2-Butyl-4-oxo-5(*S*)-[(benzyloxycarbonyl)amino]-8-bis-(benzyloxycarbonyl)guanidinooctanoate (7). Compound **6** (1.44 g, 1.7 mmol) was treated with TFA (20 mL) for 45 min and then evaporated to a thick gum. The residual material was evaporated twice from heptane. Pyridine (20 mL) was added to the diacid, the reaction flask was immersed in an oil bath preequilibrated at 100 °C, and the reaction mixture was heated for 30 min. The pyridine was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (100 mL), washed with 1 N HCl (1 × 50 mL) and saturated NaCl (2 × 50 mL), dried over $MgSO_4$, and evaporated. The material was purified, and the diastereomers were separated by flash chromatography eluting with $CHCl_3$, $CHCl_3$−MeOH (98:2), $CHCl_3$−MeOH (96:4), and $CHCl_3$−MeOH (94:6). Fractions for the respective isomers were pooled and evaporated, giving 286 mg of isomer A of **7** and 314 mg of isomer B.

7, isomer A: TLC R_f 0.62 (MeOH−$CHCl_3$, 1:9); ^{1}H-NMR ($CDCl_3$) δ 0.89 (t, 3H, CH_3), 1.12−1.98 (m, 10H), 2.32 (m, 1H, CHCO_2H), 2.92 (m, 2H, COCH_2CH), 3.5−4.15 (m, 2H, Arg δ CH_2), 4.43 (m, 1H, Arg α C*H*), 5.09 (s, 2H, ArCH_2), 5.13 (d, 2H, ArCH2), 5.25 (s, 2H, ArCH_2), 5.74 (d, 1H, N*H*), 7.36 (m, 15H, Ar*H*), 9.31 (bs, 2H, N*H*).

7, isomer B: TLC, R_f 0.57 (MeOH−$CHCl_3$, 1:9); ^{1}H-NMR ($CDCl_3$) δ 0.89 (t, 3H, CH_3), 1.05−1.90 (m, 10H), 2.46 (m, 1H, CHCO_2H), 2.90 (m, 2H, COCH_2CH), 3.90 (m, 2H, Arg δ CH_2), 4.24 (m, 1H, Arg α C*H*), 5.08 (s, 2H, ArCH_2), 5.13 (s, 2H, ArCH_2), 5.23 (s, 2H ArCH_2), 5.89 (d, 1H, N*H*), 7.35 (m, 15H, Ar*H*), 9.32 (bs, 2H, N*H*). ^{13}C-NMR shows weak absorbances at 208 ppm for the ketone carbons of the mixture of the two isomers.

Arg(k)Nle-Asp-Val-Phe (2a). Compound **7** (isomer A, 286 mg, 0.41 mmol) in $MeCl_2$ (5 mL) and HOBt (69 mg, 0.45 mmol) in DMF (2 mL) were combined and stirred at 0 °C. DCC (104 mg, 0.5 mmol) was added dropwise over a 5 min period. The solution was stirred at 0 °C for 10 min and then at room temperature for 15 min. Meanwhile, the BOC group was removed from BOC-L-Asp(OBzl)-L-Val-L-Phe resin (0.80 g of 0.52 mequiv/g [theoretical], 0.42 mmol), prepared by standard solid-phase peptide synthesis. The reaction was stirred in $MeCl_2$ for 15 min followed by treatment with 40% TFA/10% anisole/50% CH_2Cl_2 for 30 min to remove the BOC group. The resin was washed numerous times with $MeCl_2$ and 2-P_2OH, neutralized with 10% disopropylethylamine, and washed with $MeCl_2$. The activated ester of isomer A of **7** was filtered into the resin reaction vessel, and the mixture was shaken overnight. The resin-bound peptide (**8**) was cleaved from the resin by stirring with 10% anisole in anhydrous HF at 0−5 °C for 90 min. After evaporation of HF, the resin was washed with anhydrous Et_2O (250 mL) and the peptide was extracted with 20% CH_3CN containing 0.5% TFA (8 × 25 mL). The extracts containing peptide were combined and lyophilized to give 286 mg of crude material. The crude material was purified using a 90-min linear preparative gradient of 10−40% acetonitrile in water with 0.1% TFA. Fractions were pooled and lyophilized to give 37.6 mg (98% pure) of isomer A of **2a** and 31.6 mg (99% pure) of isomer B.

2a, isomer A: FABS-MS *m*/*e* 648 (M + H^+); ^{1}H-NMR (CD_3-OD) δ 0.99 (m, 9H), 1.15−1.80 (m, 9H), 1.85−2.25 (m, 3H), 2.50−3.30 (m, 8H), 4.28 (m, 2H), 4.50 (m, 1H), 4.81 (m, 1H), 7.22 (m, 5H). Anal. Calcd for $C_{31}H_{49}N_7O_8$·1.5CF_3COOH·$^3/_4H_2O$: C, H (0.5), N, F.

2a, isomer B: FABS-MS *m*/*e* 648 (M + H^+); ^{1}H-NMR (CD_3-OD) δ 0.92 (m, 9H), 1.20−1.85 (m, 9H), 1.85−2.23 (m, 3H), 2.55−3.30 (m, 8H), 7.22 (m, 5H). Anal. Calcd for $C_{31}H_{49}O_8N_7$·2.1CF_3COOH: C, H, N, F (0.5).

Compound **7** (Isomer B, 314 mg, 0.46 mmol) was similarly coupled with Asp (OBzl)-Val-Phe-resin to afford **2a** isomers C (49 mg) and D (51 mg) following chromatography.

2a, isomer C: FABS-MS *m*/*e* 648 (M + H^+), 455 (M − 193 + H^+), 356 (M − 292 + H^+). Anal. Calcd for $C_{31}H_{49}N_7O_8$·2CF_3-CO_2H·H_2O: C, H, N, F (0.5).

2a, isomer D: FABS-MS *m*/*e* 648 (M + H^+). Anal. Calcd for $C_{31}H_{49}N_7O_8$·1.9 CF_3COOH·H_2O: C (0.6), H, N, F.

***N*$^\alpha$-(2-Butyl-4-oxo-5(*S*)-amino-8-guanidinooctanoyl)-L-aspartyl-L-valyl-L-phenylalanine Amide (Arg(k)Nle-Asp-Val-PheNH_2) (2b).** Following the procedure described previously compound **7** (isomer A) (365.5 mg, 0.53 mmol), HOBt (82.0 mg, 0.54 mmol), BOP (236.5 mg, 0.54 mmol), and *N*-methylmorpholine (87.5 μL, 0.80 mmol) were mixed in DMF (10 mL) at room temperature for 30 min. TFA·Asp(OBZl)-L-Val-L-Phe-MBHA resin (**9**) (1.30 g, 0.56 mmol at 0.43 mequiv/g), prepared by standard solid-phase peptide synthesis, was neutralized with 10% diisopropylethylamine (2×), the resin washed with $MeCl_2$ (3×), and the activated ketomethylene dipeptide above was added. After the reaction vessel was shaken overnight, a Kaiser test indicated that coupling was not complete. The reaction was allowed to proceed 7 days, and the resin was washed and dried, yielding 1.58 g of resin-bound blocked peptide **10**. HF removal of peptide from resin yielded 393 mg of crude peptide **2b**. The product was partially purified

by preparative HPLC using a 10−30% gradient of acetonitrile in water containing 0.1% TFA, which gave 55 mg (98% pure) of **2b**, isomer A. A second preparative HPLC purification for isomer B using a 17−30% gradient of acetonitrile gave 70 mg of 100% pure isomer B.

2b, isomer A: ^{1}H-NMR (D_2O) δ 0.83 (d, 3H), 0.86 (t, 3H), 0.96 (d, 3H), 1.24 (m, 4H), 1.48 (m, 3H), 1.67 (m, 1H), 1.93 (m, 1H), 2.06 (m, 2H), 2.73 (dd, 1H), 2.80 (m, 2H), 2.90 (dd, 1H), 2.99 (dd, 1H), 3.05 (dd, 1H), 3.13 (dd, 1H), 3.20 (t, 2H), 4.03 (d, 1H), 4.31 (dd, 1H), 4.55 (dd, 1H), 4.74 (dd, 1H), 7.21−7.37 (m, 5H); ^{13}C-NMR (D_2O) δ 207.57 (ketone).

2b, isomer B: FABS-MS *m/e* 647 (M + H$^+$); ^{1}H-NMR (D_2O) δ 0.76 (d, 3H), 0.79 (d, 3H), 0.82 (t, 3H), 1.23 (m, 4H), 1.49 (m, 3H), 1.66 (m, 1H), 1.95 (dd, 1H), 2.04 (m, 1H), 2.68−2.86 (m, 4H), 2.96 (dd, 1H), 3.03 (dd, 1H), 3.17 (dd, 1H), 3.23 (t, 2H), 4.03 (d, 1H), 4.29 (dd, 1H), 4.59 (d, 1H), 4.65 (t, 1H), 7.24−7.36 (m, 5H); ^{13}C-NMR (D_2O) δ 206.58 (ketone). Anal. Calcd for $C_{28}H_{43}N_7O_8 \cdot 1.65CF_3COOH \cdot 2.5H_2O$: C, H (0.6), N, F.

***N*$^\alpha$-(2-Butyl-4-oxo-5(*S*)-amino-8-guanidinooctanoyl)-L-aspartyl-D-valyl-L-phenylalanine (Arg(k)Nle-Asp-D-Val-Phe) (2c).** Following the procedure described previously compound **7** (isomers A and B) (731 mg, 1.06 mmol), HOBt (164.0 mg, 1.07 mmol), BOP (473 mg, 1.07 mmol), and *N*-methylmorpholine (175 μL, 1.59 mmol) were mixed in DMF (15 mL) at room temperature for 30 min. TFA·ASP(OBzl)-D-Val-L-Phe-*O*-resin (**11**) (1.10 g, 0.61 mmol 0.55 mequiv/g), prepared by standard solid-phase synthesis using Merrifield Phe-resin, was neutralized with 10% diisopropylethylamine (2×), the resin was washed with $MeCl_2$ (3×), and the activated ketomethlylene dipeptide was added. The reaction was allowed to proceed 7 days, and the resin was washed and dried, yielding 1.31 g. HF removal of peptide from resin yielded 387 mg of crude peptide. The product was partially purified by preparative HPLC using a 10−30% gradient of acetonitrile in water containing 0.1% TFA. Isomer A coeluted with Asp-Val-Phe byproduct. A second preparative HPLC purification for isomer B using a 17−30% gradient of acetonitrile gave 37 mg (97% pure) of **2c** isomer B.

2c, isomer B: FABS-MS *m/e* 648 (M + H$^+$); ^{1}H NMR (D_2O) δ 0.61 (d, 3H), 0.69 (d, 3H), 0.80 (t, 3H), 1.21 (m, 4H), 1.49 (m, 3H), 1.65 (m, 1H), 1.82−1.95 (m, 2H), 2.05 (m, 1H), 2.71 (dd, 1H), 2.77 (dd, 1H), 2.82 (m, 1H), 2.86 (dd, 1H), 2.93 (dd, 1H), 3.04 (dd, 1H), 3.21 (t, 2H), 3.25 (dd, 1H), 4.15 (m, 1H), 4.28 (dd, 1H), 4.62−4.69 (m, 2H), 7.22−7.35 (m, 5H); ^{13}C-NMR (D_2O) δ 206.82 (ketone). Anal. Calcd for $C_{31}H_{49}N_7O_8 \cdot 1.5CF_3COOH \cdot 2.5H_2O$: C, H (0.5), N, F.

***N*$^\alpha$-Trityl-L-aspartic Acid β-Cyclohexyl Ester (12).** *N*$^\alpha$-BOC-L-aspartic acid β-cyclohexyl ester (23.7 g, 75.1 mmol) was dissolved in $MeCl_2$TFA (1:1, 200 mL), and the resulting solution was stirred for 45 min at room temperature. The solvent was removed under reduced pressure, and the resulting oil was diluted with Et_2O (100 mL) and precipitated with heptane (100 mL). The supernatant was decanted, and the residual solvent was removed *in vacuo* to give a white solid. The material was suspended in $MeCl_2$ (300 mL), Et_3N (10.5 mL, 75 mmol) and trimethylsilyl chloride (33.4 mL, 262 mmol) were added, and the resulting mixture was heated at reflux for 30 min. The reaction mixture was cooled to room temperature, additional Et_3N (36.8 mL, 262 mmol) was added, and the reaction was refluxed for 45 min. The reaction vessel was then cooled to 0 °C, and MeOH (4.5 mL, 0.11 mol) in $MeCl_2$ (80 mL) was added dropwise over a 10 min period. The reaction mixture was stirred for an additional 10 min and then warmed to room temperature. Trityl chloride (21.0 g, 75 mmol) in $MeCl_2$ (50 mL) was added, and the reaction mixture was stirred for 3 h. MeOH (50 mL) was added to the reaction, and the material was stirred at room temperature for 30 min. The reaction mixture was concentrated under vacuum to a viscous yellow oil, which was dissolved in Et_2O (500 mL) and extracted with 1 N NaOH (4 × 250 mL). The combined aqueous extracts were cooled to 0 °C and neutralized with solid citric acid. The product was extracted from the aqueous layer with Et_2O (5 × 200 mL); the extracts were washed with saturated NaCl (2 × 200 mL), dried over $MgSO_4$, and evaporated to a light yellow foam to give 28.8 g (84%) of **12**: MS *m/e* 413 (M − CO_2); ^{1}H-NMR ($CDCl_3$) δ 1.15−1.90 (m, 11H, cHex and Asp β C*H*), 2.56 (dd, 1H, Asp β C*H*), 3.58 (m, 1H, Asp α C*H*), 4.70 (m, 1H, OC*H*), 7.15−7.57 (m, 15H, Ar*H*).

***N*-Tritylaspartic Acid α-(2-Mercaptopyridyl)-β-cyclohexyl Diester (13).** Compound **12** (27.5 g, 60.0 mmol) and 2-mercaptopyridine (6.90 g, 62.0 mmol) were dissolved in EtOAc (100 mL). Argon was bubbled through the solution to reduce oxidation of the thiol. The mixture was cooled to 0 °C, and DCC (13.7 g, 66 mmol) in EtOAc (50 mL) was added dropwise over a 10 min period. The reaction was kept at 0 °C for 2 h and then allowed to stir at room temperature for 2 days. The reaction mixture was cooled and then filtered to remove the urea byproduct. The solvent was removed under reduced pressure to give a thick yellow oil. The product was purified using flash chromatography eluting with EtOAc−hexanes (1.5:8.5). The fractions of interest were pooled and evaporated to give an off-white solid: yield 27.54 g (83%); TLC (silica gel) R_f 0.47 (EtOAc−hexane, 1:4); ^{1}H NMR ($CDCl_3$) δ 1.13 (dd, 1H, Asp β C*H*), 1.15−1.85 (m, 10H, cHex), 2.45 (dd, 1H, Asp β C*H*), 3.42 (d, 1H, N*H*), 3.78 (m, 1H, Asp α C*H*), 4.61 (m, 1H, OC*H*), 7.10−7.83 (m, 18H, Ar*H*), 8.63 (m, 1H, Ar*H*).

Cyclohexyl 3(*S*)-3-(Tritylamino)-4-oxo-6-isopropyloct-7-enoate (14a). A suspension of Mg turnings (4.83 g, 0.2 mol) in Et_2O (30 mL) was treated dropwise with 1,2-dibromoethane (11.8 g, 63 mmol) in Et_2O (30 mL) at a rate to maintain a steady reflux. The reaction was kept at reflux while a solution of 4-methyl-3-(bromomethyl)-1-pentene (11.2 g, 63 mmol) in Et_2O (30 mL) was added dropwise over 3 h. The reaction was refluxed an additional hour and then cooled to room temperature. The resulting Grignard solution was added in portions via cannula to a solution of **13** (11.06 g, 20 mmol) in THF (50 mL) at −10 °C. The reaction, followed by TLC, was judged to be complete before all of the Grignard reagent had been added. The reaction mixture was poured into a mixture of saturated NH_4Cl (200 mL) and Et_2O (200 mL). The organic layer was washed with saturated $NaHCO_3$ (3 × 200 mL) and saturated NaCl (2 × 200 mL), dried over $MgSO_4$, and evaporated to a yellow oil. The material was purified on a flash column, eluting with EtOAc−hexane (1:9) to give 9.62 g (89%) of **14a** as an oil: TLC R_f 0.78 (EtOAc−hexane, 1:4); ^{1}H-NMR ($CDCl_3$) δ 0.77 (m, 6H, CH_3), 1.1−1.9 (m, 12H, cHex and C*H*C*H*), 1.95−2.62 (m, 4H, Asp β CH_2 and COCH_2), 3.54 (m, 2H, Asp α C*H* and N*H*), 4.72 (m, 1H, OC*H*), 4.75−5.02 (m, 2H, C=CH_2), 5.22−5.67 (m, 1H, C*H*=C), 7.02−7.48 (m, 15H, Ar*H*); ^{13}C-NMR ($CDCl_3$) δ 209.40 (ketone).

Cyclohexyl 3-(Tritylamino)-4-oxo-6-methyl-7-octenoate (14b). To magnesium turnings (1.30 g, 54.0 mmol) in Et_2O (20 mL) was added 1,2-dibromoethane (3.4 g, 18.0 mmol) at a rate that maintained reflux. The resulting mixture was refluxed for an additional hour, followed by the dropwise addition of 1-bromo-2-methyl-3-butene (2.70 g, 18.0 mmol) in Et_2O (10 mL) over a 30-min period. Reflux was continued for 1 h; then the mixture was cooled to room temperature. The Grignard reagent was transferred via cannula to a cooled (0 °C) solution of **13** (3.30 g, 6.0 mmol) in THF (30 mL). After being stirred at 0 °C for 2 h, the reaction mixture was poured into a biphasic mixture of saturated NH_4Cl and Et_2O (400 mL each). The organic layer was washed with saturated NaCl (2 × 200 mL), dried over $MgSO_4$, filtered, and evaporated to give crude product. The material was purified on flash chromatography using 15% EtOAc in hexane as the eluant to give 2.68 g (88%) of **14b** as a light yellow syrup: DCI-MS *m/e* 510 (M + H$^+$); ^{1}H-NMR ($CDCl_3$) δ 0.92 (m, 3H), 1.20−2.10 (m, 11H), 2.15−2.72 (m, 4H), 3.60 (d, 1H), 3.65 (m, 1H), 5.70−6.05 (m, 3H), 4.63 (m, 1H), 7.18−7.65 (m, 15H); ^{13}C-NMR ($CDCl_3$) δ 209.46 (ketone).

Cyclohexyl 3(*S*)-[(*tert*-Butyloxycarbonyl)amino]-4-oxo-6-isopropyloct-7-enoate (16a). A solution of *p*-TsOH·H_2O (4.38 g, 23 mmol) in CH_3CN (50 mL) was added to a solution of the trityl olefin (**14a**) (11.8 g, 21.9 mmol) in CH_3CN (100 mL). After stirring 30 min, the tosylate salt (**15a**) was collected, suspended in $MeCl_2$ (100 mL), treated with Et_3N (4.56 g, 44.5 mmol) and $(BOC)_2O$ (9.72 g, 44.5 mmol), and then stirred at room temperature for 3 h. The reaction mixture was diluted with additional $MeCl_2$ (100 mL), washed with ice-cold 0.1 N HCl (2 × 100 mL), ice-cold saturated $NaHCO_3$ (2 × 100 mL), and saturated NaCl (2 × 100 mL), dried over $MgSO_4$,

and evaporated. The crude material was flash chromatographed, eluting with EtOAc−hexane (15:85). Pooling product fractions gave 6.80 g (79%) of **16a** as a gum: TLC R_f 0.38 (EtOAc−hexane, 15:85); ^{1}H-NMR ($CDCl_3$) δ 0.86 (2d, 6H, CH_3), 1.1−1.9 (m, 12H, cHex and C*H*C*H*), 1.2−1.9 (m and s at 1.46, 21H, BOC, cHex and C*H*C*H*), 2.60 (m, 2H, COCH_2), 2.76 (m, 2H, Asp β CH_2), 4.36 (m, 1H, Asp α C*H*), 4.73 (m, 1H, OC*H*), 4.8−5.2 (m, 2H, C=CH_2), 5.4−5.85 (m, 2H, C*H*=C and N*H*); ^{13}C-NMR ($CDCl_3$) δ 207.45 and 207.56 (ketone).

Cyclohexyl 3-[(*tert*-Butyloxycarbonyl)amino]-4-oxo-6-methyl-7-octenoate (16b). Compound **14b** (2.24 g, 4.4 mmol) was dissolved in Et_2O (30 mL) and treated with *p*-TsOH·H_2O (860 mg, 4.5 mmol); the resulting solution was stirred for 3 h. The precipitated salt (**15b**) was filtered, and the white solid was suspended in $MeCl_2$ (40 mL). Di-*tert*-butyl dicarbonate (1.95 g, 8.8 mmol) was added to the reaction followed by Et_3N (1.22 mL, 8.8 mmol). The reaction mixture was stirred for 4 h and then evaporated to a yellow oil. Filter-pad chromatography using a step gradient of CCl_4 and $MeCl_2$ (0%, 25%, 50%, 75%, 100% $MeCl_2$) was used to obtain 1.36 g (84%) of **16b** as a clear syrup; DCI-MS *m*/*e* 368 (M + H$^+$); ^{1}H-NMR ($CDCl_3$) δ 0.91 (d, 3H), 1.02−1.83 (m, 11H), 1.34 (s, 9H), 2.51 (m, 2H), 2.71 (m, 2H), 4.30 (m, 1H), 4.63 (m, 1H), 4.70−4.98 (m, 2H), 5.48−5.84 (m, 2H); ^{13}C-NMR ($CDCl_3$) δ 207.07 (ketone). Anal. Calcd for $C_{20}H_{33}NO_5$: C, H, N.

Cyclohexyl 3(*S*)-[(*tert*-Butyloxycarbonyl)amino]-4-oxo-6-carboxy-7-methyloctanoate (17a). A solution of olefin **16a** (1.15 g, 2.9 mmol) in acetone (40 mL) was cooled to 0 °C with stirring while a solution of $NaIO_4$ (3.6 g, 16.8 mmol) and RuO_2·*x*H_2O (59.3% Ru; 18 mg) in H_2O (10 mL) was added dropwise. Once addition was complete, the reaction was allowed to warm to room temperature and stir for 2 h. The mixture was filtered through Celite, and the pad was washed with acetone. The combined filtrates were saturated with NaCl and then extracted with EtOAc (2 × 100 mL). The organic extracts were combined, washed with 10% $NaHSO_3$ (2 × 50 mL) and H_2O (1 × 50 mL), dried over Na_2SO_4, and evaporated to a foam to yield 830 mg (69%) of **17a**: ^{1}H-NMR ($CDCl_3$) δ 0.96 (m, 6H, CH_3), 1.12−2.23 (m, 20H, cHex, BOC and C*H*(CH_3)$_2$), 2.35−3.15 (m, 5H, Asp β CH_2, COCH_2C*H*CO_2), 4.45 (m, 1H, Asp α C*H*), 4.74 (m, 1H, OC*H*), 5.70 (bd, 1H, N*H*); ^{13}C-NMR ($CDCl_3$) δ 206.90, 207.23 (ketone).

Cyclohexyl 3-[(*tert*-Butyloxycarbonyl)amino]-4-oxo-6-carboxyheptanoate (17b). Compound **16b** (1.24 g, 3.4 mmol) was similarly oxidized with $NaIO_4$ (2.9 g, 13.6 mmol) and RuO_2·*x*H_2O (6 mg) in H_2O (10 mL)−acetone (40 mL) as described for **17a** to give 1.30 g (100%) of **17b** as a solid: DCI-MS *m*/*e* 386 (M + H$^+$); ^{1}H NMR ($CDCl_3$) δ 1.18 (d, 3H), 1.20−1.93 (m, 10H), 1.40 (s, 9H), 2.53−3.18 (m, 5H), 4.42 (m, 1H), 4.70 (m, 1H), 5.68 (d, 1H), 10.28 (bs, 1H); ^{13}C-NMR ($CDCl_3$) δ 206.21, 206.48 (ketone). Anal. Calcd for $C_{19}H_{31}NO_7$: C, H, N.

Tris(carbobenzyloxy)-L-arginyl-*N*$^\epsilon$-(carbobenzyloxy)-L-lysine (18). *N*-(Carbobenzyloxy)-L-lysine (5.60 g, 20 mmol) was suspended in a mixture of dioxane (25 mL) and isobutylene (50 mL). Concentrated H_2SO_4 (2 mL) was added carefully, and the reaction allowed to proceed at room temperature while employing a dry ice condenser. After 4 h, the condenser was removed and the excess isobutylene allowed to evaporate. The reaction was poured into ice-cold 1 N NaOH (200 mL) and extracted with Et_2O (3 × 100 mL). The combined organic extracts were washed with saturated $NaHCO_3$ (2 × 100 mL) and saturated brine (2 × 100 mL), dried ($MgSO_4$), and evaporated to leave 3.72 g (55%) of *N*$^\epsilon$-Cbz-lysine *tert*-butyl ester as an oil, which was used directly: ^{1}NMR σ 1.46 (m, 13H), 3.19 (m, 3H), 4.85 (bs, 1H), 5.09 (s, 2H), 7.43 (s, 5H).

Tris(carbobenzyloxy)-L-arginine (5.76 g, 10 mmol) was dissolved in THF (40 mL) and cooled to −10 °C with an ice−methanol bath. *N*-Methylmorpholine (1.05 mL, 11 mmol) and isobutyl chloroformate (1.55 mL, 12 mmol) were added successively. After the mixture was strred for 15 min at −10 °C, a solution of the *tert*-butyl ester above (3.72 g, 11 mmol) in THF (10 mL) was added. The reaction mixture was stirred an additional 2 h, and then it was poured into a 50% saturated brine solution (200 mL) to precipitate a gum. The aqueous material was decanted, and the gum was dissolved in $MeCl_2$ (200 mL), washed with saturated $NaHCO_3$ (100 mL) and saturated brine (2 × 100 mL), dried, and evaporated to give a white solid. The material was crystallized from THF−Et_2O to give the Z_3-Arg-*N*$^\epsilon$-Z-Lys-*t*-Bu ester intermediate, yield 7.67 g (85.7%).

Tris(benzyloxycarbonyl)-L-arginyl-L-norleucine (19). Tris(carbobenzyloxy)arginine (2.88 g, 5.0 mmol) was dissolved in THF (40 mL) and cooled to −10° (ice−MeOH). To the amino acid solution was added *N*-methylmorpholine (0.53 mL, 5.5 mmol) and isobutyl chloroformate (0.78 mL, 6.0 mmol) in succession. After 15 min of stirring at −10 °C, a solution of norleucine *tert*-butyl ester (1.43 g, 7.6 mmol) in THF (10 mL) was added followed by *N*-methylmorpholine (0.53 mL, 5.5 mmol). The reaction mixture was stirred at room temperature for 2 h and then the product precipitated with Et_2O (200 mL). The crude product was collected by filtration, dissolved in $MeCl_2$ (100 mL), washed with 5% citric acid (2 × 50 mL) and saturated brine (2 × 100 mL), dried over $MgSO_4$, and evaporated to a solid. Recrystallization from EtOAc−Et_2O gave 3.10 g (83.3%) of Z_3-Arg-Nle *tert*-butyl ester: FABS-MS *m*/*e* 690 (M + H$^+$). The ester (920 mg/1.23 mmol) was dissolved in TFA−$MeCl_2$ (1:1) (50 mL), and the reaction mixture was stirred for 30 min at room temperature. The material was evaporated to an oil. The residue was dissolved in $MeCl_2$ (100 mL) and washed with water (50 mL) and saturated brine (2 × 50 mL), dried over $MgSO_4$, and evaporated to give 793 mg (93.4%) of **19**; FABS-MS *m*/*e* 746 (M + H$^+$); ^{1}H-NMR ($CDCl_3$) δ 0.83 (m, 3H), 1.12−1.38 (m, 4H), 1.5−1.83 (m, 6H), 3.75−4.05 (m, 2H), 4.38 (m, 2H), 5.08 (s, 2H), 5.10 (m, 2H), 5.20 (s, 2H), 6.08 (d, 1H), 6.50 (d, 1H), 7.23−7.45 (m, 15H), 8.8−9.7 (bs, 2H). Anal. Calcd for $C_{36}H_{43}N_5O_9$: C, H, N.

Tris(benzyloxycarbonyl)-L-arginyl-L-proline (20). Tribenzyloxycarbonyl arginine (2.88 g. 5.0 mmol) in THF (40 mL) was cooled to −10 °C (ice−MeOH) and *N*-methylmorpholine (0.53 mL, 5.5 mmol) and isobutyl chloroformate (0.78 mL, 6.0 mmol) were added successively. After the mixture was stirred at -10 °C for 20 min, a solution of proline *tert*-butyl ester (2.31 g) in THF (10 mL) was added followed by *N*-methylmorpholine (0.53 mL, 5.5 mmol). After being stirred at room temperature for 2 h, the reaction mixture was evaporated to an oil. The crude material was dissolved in $MeCl_2$ (100 mL), washed with 5% citric acid (2 × 100 mL) and saturated brine (2 × 100 mL), dried over $MgSO_4$, and evaporated to a foam. Purification by flash chromatography using a step gradient of 20%, 30%, and 40% EtOAc in hexane gave 3.20 g (87.7%) of Z_3-Arg-Pro-*t*-Bu ester: FABS-MS *m*/*e* 730 (M + H$^+$).

The *tert*-butyl ester (1.06 g, 1.45 mmol) was dissolved in TFA−$MeCl_2$ (50 mL, 1:1), and the reaction mixture was stirred for 30 min at room temperature. The material was evaporated to an oil. The residue was dissolved in $MeCl_2$ (100 mL), washed with water (50 mL) and saturated brine (2 × 50 mL), dried over $MgSO_4$, and evaporated to give 869 mg (92.6%) of **20**: FAB-MS *m*/*e* 674 (M + H$^+$); NMR ($CDCl_3$) δ 1.50−2.05 (m, 8H), 3.52 (m, 2H), 3.87 (m, 2H), 4.50 (m, 2H), 5.04 (s, 2H), 5.13 (s, 2H), 5.16 (s, 2H), 5.90 (d, 1H), 7.20−7.45 (m, 15H), 9.20 (bs, 2H). Anal. Calcd for $C_{35}H_{39}N_5O_9$: C, H, N.

***N*$^\alpha$-(*tert*-Butyloxycarbonyl)-*N*$^\alpha$-methyl-*N*$^\epsilon$-(2-chlorobenzyloxycarbonyl)-L-lysine Methyl Ester (27).** *N*$^\epsilon$-(2-Chlorobenzloxycarbonyl)-*N*$^\alpha$-methyllysine methyl ester (**26**) was prepared in 50% yield from *N*$^\epsilon$-(2-chlorobenzyloxycarbonyl)-lysine methyl ester by the method of Grieco and Bahsas[8] as described for the corresponding *N*$^\epsilon$-carbobenzoxy derivative. The material was obtained as a clear syrup. Compound **26** (4.3 g, 10 mmol) was dissolved in $MeCl_2$ (50 mL) and treated with di-*tert*-butyl dicarbonate (4.80 g, 22.0 mmol). After being stirred for 4 h, the mixture was evaporated to an oil and subjected to filter-pad chromatography using a step gradient with CCl_4 and $MeCl_2$ as the eluants. Product fractions were combined and evaporated to give 4.69 g (54%) of **27** as a clear syrup: DCI-MS *m*/*e* 443 (M + H$^+$); ^{1}H-NMR ($CDCl_3$) δ 1.10−2.15 (m, 6H, CH_2), 1.40 (s, 9H, *t*-Bu), 2.79 (s, 3H, NCH_3), 3.22 (m, 2H, CH_2N), 3.68 (s, 3H, OCH_3), 4.54 (m, 1H, α-CH), 5.00 (m, 1H, NH), 5.20 (s, 2H, CH_2O), 7.15−7.45 (m, 4H, Ar). Anal. Calcd for $C_{21}H_{31}N_2O_6Cl$: C, H, N.

***N*$^\alpha$-(*tert*-Butyloxycarbonyl)-*N*$^\alpha$-methyl-*N*$^\epsilon$-(2-chlorobenzyloxycarbonyl)-L-lysine (28).** Compound **27** (4.42 g, 10.0

mmol) was dissolved in MeOH (100 mL) and cooled to 0 °C (ice bath). To this mixture was added a solution of 1 N NaOH (15 mL, 15 mmol). The reaction mixture was stirred at 0 °C for 2 h and then overnight at room temperature. The solution was concentrated to an oil and treated with the mixture of ice-cold 0.1 N HCl (100 mL) and $MeCl_2$ (100 mL). The aqueous layer was extracted with additional $MeCl_2$ (2 × 100 mL), and the combined organic extracts were washed with saturated NaCl (100 mL), dried over $MgSO_4$, and evaporated to give 4.26 g (92%) as a clear oil: trimethylsilylated DCI-MS *m/e* 451 (M + H^+); ^{1}H-NMR ($CDCl_3$) δ 1.10−1.95 (m, 6H, CH_2), 1.40 (s, 9H, *t*-Bu), 2.79 (s, 3H, NCH_3), 3.24 (m, 2H, CH_2N), 4.57 (m, 1H, α-CH), 5.05 (m, 1H, NH), 5.22 (s, 2H, CH_2O), 7.15−7.45 (m, 4H, Ar).

Tris(benzyloxycarbonyl)-L-arginyl-N^α-methyl-N^ϵ-(2-chlorobenzyloxycarbonyl)-L-lysine *tert*-Butyl Ester (30). Compound **28** (4.00 g, 8.0 mmol) was added to a mixture of concentrated H_2SO_4 (1.5 mL) in dioxane (35 mL) and stirred for 10 min under argon. Isobutylene (30 mL) was condensed into the reaction mixture using a Dewar reflux condenser. The mixture was stirred for 6 h and then poured into 1 N NaOH (250 mL). The aqueous mixture was extracted with Et_2O (3 × 100 mL). The combined Et_2O extracts were washed with saturated $NaHCO_3$ (100 mL) and saturated NaCl (2 × 100 mL), dried over $MgSO_4$, and evaporated to give 2.05 g of crude *tert*-butyl ester **29**. Tris(benzyloxycarbonyl)-L-arginine (2.90 g, 5.0 mmol) and isobutyl chloroformate (0.78 mL, 6.0 mmol) were added successively. After 20 min of stirring, a solution of **29** in $MeCl_2$ (25 mL) was added to the reaction, followed by *N*-methylmorpholine (0.53 mL, 5.5 mmol). The reaction mixture was allowed to come to room temperature and stirred for 5 h. The mixture was evaporated to an oil and subjected to flash chromatography using a step gradient of acetone in hexane yielding 2.89 g (30%) of **30** as a syrup: ^{1}H-NMR ($CDCl_3$) δ 1.10−2.15 (m, 10H), 1.38 (s, 9H), 2.87 (s, 3H), 3.19 (m, 2H), 3.71 (m, 1H), 3.94 (m, 1H), 4.35 (m, 0.5H), 4.72 (m, 1.5H), 4.95−5.40 (m, 7H), 5.84 (m, 1H), 7.15−7.49 (m, 19H), 9.38 (bs, 2H). Anal. Calcd for $C_{49}H_{51}N_6O_{11}Cl$: C, H, N.

Tris(benzyloxycarbonyl)-L-arginyl-N^α-methyl-N^ϵ-(2-chlorobenzyloxycarbonyl)-L-lysine (21). Compound **30** (1.42 g, 1.50 mmol) was dissolved in a mixture of TFA (10 mL) and $MeCl_2$ (10 mL) and stirred for 40 min. The material was concentrated to an oil, diluted in $MeCl_2$ (50 mL), washed with H_2O (4 × 50 mL) and saturated NaCl (50 mL), dried over $MgSO_4$, and evaporated to give 1.16 g (87%) of a white foam: ^{1}H-NMR ($CDCl_3$) δ 1.10−2.15 (m, 10H), 2.87 (s, 3H), 3.09 (m, 2H), 3.71 (m, 1H), 3.94 (m, 1H), 4.37 (m, 0.5H), 4.78 (m, 1.5H), 4.95−5.40 (m, 7H), 5.84 (m, 1H), 7.15−7.49 (m, 19H), 9.45 (bs, 3H). Anal. Calcd for $C_{45}H_{43}N_6O_{11}Cl$: C, H, N.

Arg-Lys-Asp(k)Val-Phe (3a). *N*-BOC-L-phenylalanine resin (373 mg of 0.67 mequiv/g resin, 0.25 mmol) was twice treated with 40% TFA/10% anisole in $MeCl_2$ for 5 min and then 30 min to remove the N-terminal BOC group. During removal of the BOC from the resin, ketomethylene dipeptide **17a** (208 mg, 0.50 mmol), BOP (222 mg, 0.50 mmol), and HOBt (76.9 mg, 0.50 mmol) were dissolved in DMF (2 mL). The mixture was treated with *N*-methylmorpholine (0.9 mL, 0.82 mmol), and the resulting solution was allowed to stir for 30 min. The Phe-resin was washed numerous times with $MeCl_2$ and 2-PrOH, neutralized with 5% diisopropylethylamine in $MeCl_2$, and washed again with $MeCl_2$. The activated ester solution of **17a** from above was added to the resin and the reaction allowed to proceed overnight. A Kaiser test suggested that the coupling was incomplete. A second coupling was performed using more ketomethylene subunit **17a** (312 mg, 0.75 mmol) with BOP (333 mg, 0.75 mmol), HOBt (115 mg, 0.75 mmol), and *N*-methylmorpholine (1.40 mL, 1.30 mmol). After the activated ketomethylene depeptide was allowed to react with the resin overnight, a Kaiser test suggested that coupling was still incomplete. Unreacted resin was therefore "capped" by treatment with Ac_2O (1 mL) and pyridine (0.1 mL) in $MeCl_2$ for 30 min. Next, the BOC group of the Asp unit was removed with 40% TFA/10% anisole in $MeCl_2$. Tris(carbobenzyloxy)-L-arginyl-N^ϵ-(carbobenzyloxy)-L-lysine **18** (840 mg, 1.0 mmol) and HOBt (153.3 mg, 1.0 mmol) were dissolved in DMF (2 mL) in a separate flask and cooled to 0 °C. Dicyclohexylcarbodiimide (228.2 mg, 1.1 mmol) in $MeCl_2$ (5 mL) was added, and the reaction mixture was stirred at 0 °C for 15 min and then at room temperature for 30 min. The peptide-resin was washed alternately with $MeCl_2$ and 2-PrOH to remove residual TFA. The activated dipeptide prepared in the separate flask was added to the peptide-resin, followed by addition of diisopropylethylamine (175 μL, 1.0 mmol). The reaction vessel was then shaken overnight. The coupling was checked for completion (negative Kaiser test) and the peptide-resin washed with $MeCl_2$. The blocked pseudopentapeptide (**22a**) so obtained was cleaved from the resin by stirring with 10% anisole in anhydrous HF at 0−5 °C for 1 h. After evaporation of the HF, the resin was washed with Et_2O and $CHCl_3$, and then the peptide was eluted with 20% acetonitrile in water with 0.5% TFA present. The extract was frozen and lyophilized to give 128 mg of crude **3a**. The isomers were initially separated by HPLC using a preparative gradient of 10−30% acetonitrile in water with 0.1% TFA. The separated isomers were further purified. Isomer A was chromatographed under isocratic conditions using 18% acetonitrile in water with 0.1% TFA and isomer B using 22% acetonitrile in water with 0.1% TFA. This resulted in a recovery of 25.4 mg (>99% pure) of isomer A of **3a** and 20.5 mg (>90% pure) of isomer B of **3a**.

Isomer A: FAB-MS *m/e* 663; ^{1}H-NMR (D_2O) δ 0.80 (d, 6H), 1.41 (m, 3H), 1.50−1.70 (m, 4H), 1.74 (m, 2H), 1.87 (m, 2H), 2.48 (m, 1H), 2.59 (dd, 1H), 2.66 (dd, 1H), 2.81 (m, 2H), 2.93 (m, 2H), 2.98 (dd, 1H), 3.14 (dd, 1H), 3.15 (m, 2H), 4.00 (t, 1H), 4.32 (dd, 1H), 4.44 (dd, 1H), 4.59 (dd, 1H), 7.22−7.35 (m, 5H). ^{13}C-NMR (D_2O) δ 207.60 (ketone). Anal. Calcd for $C_{31}H_{50}N_8O_8 \cdot 2.5CF_3COOH \cdot 2H_2O$: C, H, N (0.7), F (0.5).

Isomer B: ^{1}H-NMR (D_2O) δ 0.55 (d, 3H), 0.61 (d, 3H), 0.8 (m, 1H), 1.39 (m, 2H), 1.57 (m, 2H), 1.61 (m, 2H), 1.87 (m, 2H), 2.48 (m, 1H), 2.59 (dd, 1H), 2.77 (m, 1H), 2.80 (m, 1H), 2.86 (m, 1H), 2.91 (m, 3H), 3.14 (t, 2H), 3.20 (dd, 1H), 3.99 (t, 1H), 4.31 (dd, 1H), 4.58 (dd, 1H), 4.63 (dd, 1H), 7.20−7.33 (m, 5H); ^{13}C-NMR (D_2O) δ 207.64 (ketone).

Arg-Nle-Asp(k)Val-Phe (3b). Pseudopeptide **3b** was similarly prepared from **17a** by coupling of the resin-bound Asp(k)Val-Phe unit with the activated ester of **19** to afford **22b**. Starting from 793 mg of **19** the weight of crude **3b** as an isomeric mixture was 694 mg following HF cleavage of **22b**. The mixture was purified by HPLC using a 10−30% acetonitrile−H_2O−0.1% TFA gradient to afford 145 mg of isomer A and 144 mg of isomer B.

Isomer A: FABS-MS *m/e* 648 (M + H^+), 357 (M − 291 + H^+); ^{1}H-NMR (D_2O) δ 0.83 (d, 6H), 0.87 (m, 3H), 1.32 (m, 4H), 1.63 (m, 2H), 1.76 (m, 3H), 1.90 (m, 2H), 2.51 (m, 1H), 2.63 (m, 1H), 2.66 (dd, 1H), 2.83 (dd, 1H), 2.86 (dd, 1H), 3.02 (dd, 1H), 3.17 (m, 1H), 3.19 (m, 2H), 4.03 (t, 1H), 4.32 (t, 1H), 4.48 (dd, 1H), 4.65 (dd, 1H), 7.26−7.39 (m, 5H); ^{13}C-NMR (D_2O) δ 207.60 (ketone). Anal. Calcd $C_{31}H_{49}N_7O_8 \cdot 1.8CF_3COOH \cdot 0.5H_2O$: C, H, N, F.

Isomer B: FABS-MS *m/e* 648 (M + H^+), 375 (M − 291 + H^+); ^{1}H-NMR (D_2O) δ 0.70 (d, 3H), 0.75 (d, 3H), 0.95 (m, 3H), 1.39 (m, 4H), 1.69 (m, 3H), 1.81 (m, 2H), 1.96 (m, 2H), 2.55 (m, 1H), 2.76 (dd, 1H), 2.78−3.40 (m, 4H), 3.20 (t, 2H), 3.26 (dd, 1H), 4.01 (t, 1H), 4.30 (dd, 1H), 4.61 (m, 1H), 7.12−7.23 (m, 5H); ^{13}C-NMR (D_2O) δ 207.79 (ketone). Anal. Calcd $C_{31}H_{49}N_7O_8 \cdot 1.6CF_3COOH \cdot 0.5H_2O$: C, H, N, F.

Arg-Pro-Asp(k)Val-Phe (3c). Peptide **3c** was also prepared from **17a** and the blocked dipeptide **20** proceeding through the resin-bound-blocked pentapeptide **22c**. From 798 mg of **20** was obtained 694 mg of crude **3c** following HF cleavage of **22c**. Purification by HPLC (as for **3b** above) yielded 170 mg of **3c** isomer B. A fraction containing isomer A (90% pure) could not be adequately purified for analytical purposes, but was identified by mass spectrometry.

Isomer A: FABS-MS *m/e* 632 (M + H^+), 341 (M − 291 + H^+).

Isomer B: FABS-MS *m/e* 632 (M + H^+), 341 (M − 291 + H^+). Anal. Calcd for $C_{30}H_{45}N_7O_8 \cdot 2CF_3CO_2H$: C, H, N, F.

Arg-NMeLys-Asp(k)Val-Phe (3d). Peptide **3d** was likewise prepared from **17a** and the tris(carbobenzyloxyarginyl)-*N*-Me-lysyl dipeptide unit (**21**) via the resin-bound blocked pentapeptide intermediate **22d**. From 1.06 g of **21** there was obtained 329 mg of crude **3d**, whose HPLC analysis indicated

a mixture of four compounds. Purification was achieved via preparative HPLC with a gradient of 10−30% acetonitrile−H_2O−0.1% TFA, followed by rechromatography with an isocratic elution by 17% acetonitrile−0.1% TFA and finally a gradient elution with 12−25% acetonitrile−0.1% TFA. Evaporation and lyophilization gave 23 mg of **3d**, isomer A: FAB-MS *m/e* 677 ($M + H^+$); ^{1}H-NMR (D_2O) δ 0.86 (d, 6H), 1.37 (m, 2H), 1.62−2.00 (m, 9H), 2.55 (m, 1H), 2.70 (m, 3H), 2.83 (m, 2H), 3.04 (m, 5H), 3.16 (m, 1H), 3.21 (m, 2H), 4.56 (m, 3H), 5.03 (m, 1H), 7.26−7.42 (m, 5H).

Arg-Lys-Asp(k)-Ala-Phe (3e). Peptide **3e** was similarly prepared from the aspartyl ketomethylene alanine dipeptide unit (**17b**) and the blocked Arg−Lys dipeptide (**18**) proceeding through the resin-bound pentapeptide (**22e**). From 1.13 g of **18** there was obtained 396 mg of crude **3e**. Preparative HPLC using a 0−20% acetonitrile−H_2O−0.1% TFA gradient afforded 64 mg of **3e** (isomer A) and 79 mg of **3e** (isomer B).

Isomer A: FABS-MS *m/e* 635 ($M + H^+$); ^{1}H-NMR (D_2O) δ 1.06 (d, 3H), 1.44 (m, 2H), 1.58−1.76 (m, 4H), 1.82 (m, 2H), 1.93 (m, 2H), 2.59 (dd, 1H), 2.70 (dd, 1H), 2.76 (dd, 1H), 2.81 (m, 2H), 2.91 (dd, 1H), 2.99 (t, 2H), 3.04 (dd, 1H), 3.21 (t, 2H), 3.23 (dd, 1H), 4.07 (t, 1H), 4.37 (t, 1H), 4.54 (dd, 1H), 4.64 (dd, 1H), 7.28−7.42 (m, 5H); ^{13}C-NMR (D_2O) δ 208.85 (ketone). Anal. Calcd for $C_{29}H_{46}N_8O_8 \cdot 3CF_3COOH \cdot 0.5H_2O$: C, H, N, F.

Isomer B: FABS-MS *m/e* 635 ($M + H^+$); ^{1}H-NMR (D_2O) δ 0.90 (d, 3H), 1.43 (m, 2H), 1.63 (m, 2H), 1.69 (m, 2H), 1.79 (m, 2H), 1.92 (m, 2H), 2.61 (dd, 1H), 2.73−2.87 (m, 3H), 2.91−3.20 (m, 4H), 3.20 (t, 2H), 3.25 (dd, 1H), 4.05 (t, 1H), 4.36 (t, 1H), 4.64 (m, 2H), 7.25−7.40 (m, 5H); ^{13}C-NMR (D_2O) δ 208.95 (ketone).

Arg-Lys-Asp(k)Val-Tyr (3f). Compound **17a** (566 mg, 1.38 mmol), HOBt (217 mg, 1.41 mmol), BOP (617 mg, 1.40 mmol), and *N*-methylmorpholine (467 μL, 4.27 mmol) were combined in $MeCl_2$ (10 mL) and stirred at room temperature for 30 min. Meanwhile, the BOC group was removed from BOC-L-Tyr-(OCBZ)-*O*-resin (2.59 g, 1.50 mmol at 0.58 mequiv/g) with 40% TFA/10% anisole in the usual manner, and the resin was washed with $MeCl_2$ (3×), neutralized with 10% diisopropylethylamine in $MeCl_2$ (2×), and washed with $MeCl_2$ (3×). The activated ketomethylene was added to the resin and shaken for 7 days. The untreated amines were capped using acetic anhydride (1.0 mL) and pyridine (0.1 mL) in $MeCl_2$ (9 mL). The BOC group was removed from the Asp portion as usual, leaving the resin-bound material as the TFA salt. Meanwhile, Z_3-L-Arg-N^{ϵ}-Z-L-Lys (**18**, 1.26 g, 1.50 mmol) and HOBt (230 mg, 1.50 mmol) were dissolved in $MeCl_2$ and cooled to 0 °C, after which DCC (340 mg, 1.65 mmol) was added. The resin was neutralized (2×) with 5% diisopropylethylamine and washed with $MeCl_2$ (3×) just before the activated dipeptide was added. The vessel was then shaken overnight. The reaction was determined to be complete (negative Kaiser test), and the resin was washed and dried to give 3.43 g of peptide-resin. HF removal of peptide from resin yielded 588 mg of crude peptide. The products were separated and partially purified by preparative HPLC using a 0−17% gradient of acetonitrile in water containing 0.1% TFA. Isomer A was further purified by preparative HPLC using a 3−12% gradient of acetonitrile in water containing 0.1% TFA. Fractions were pooled, evaporated of acetonitrile, frozen, and lyophilized; yield, 92 mg (99% pure) of **3f** (isomer A). Isomer B was further purified by preparative HPLC using a 7−17% gradient of acetonitrile in water containing 0.1% TFA to yield 31 mg (98% pure) of **3f** (isomer B).

Isomer A: FABS-MS *m/e* 679 ($M + H^+$); ^{1}H-NMR (D_2O) δ 0.81 (d, 3H), 0.82 (d, 3H), 1.42 (m, 2H), 1.52−1.82 (m, 7H), 1.89 (q, 2H), 2.49 (m, 1H), 2.60 (dd, 1H), 2.67 (dd, 1H), 2.78−2.97 (m, 5H), 3.07 (dd, 1H), 3.17 (m, 2H), 4.03 (t, 1H), 4.34 (dd, 1H), 4.47 (dd, 1H), 4.54 (dd, 1H), 6.80 (m, 2H), 7.12 (m, 2H); ^{13}C-NMR (D_2O) 209.38 (ketone). Anal. Calcd for $C_{31}H_{50}N_8O_9 \cdot 2.6CF_3COOH \cdot 3H_2O$: C, H, N, F.

Isomer B: FABS-MS *m/e* 679 ($M + H^+$); ^{1}H-NMR (D_2O) δ 0.56 (d, 3H), 0.64 (d, 3H), 0.79−0.92 (m, 1H), 1.41 (m, 2H), 1.53−1.71 (m, 5H), 1.78 (m, 2H), 1.90 (q, 2H), 2.52 (m, 1H), 2.62 (dd, 1H), 2.79 (dd, 1H), 2.81−2.87 (m, 2H), 2.89 (dd, 1H), 2.96 (t, 2H), 3.18 (m, 3H), 4.02 (t, 1H), 4.34 (t, 1H), 4.57 (dd, 1H), 4.66 (dd, 1H), 6.80 (m, 2H), 7.14 (m, 2H); ^{13}C-NMR (D_2O) δ 212.11 (ketone). Anal. Calcd for $C_{31}H_{50}N_8O_9 \cdot 2.4CF_3COOH \cdot 3H_2O$: C, H (0.6), N, F.

Arg-Pro-Asp(k)Val-PheNH$_2$ (3g). Compound **17a** (668 mg, 1.62 mmol) and *N*-hydroxysuccinimide (260 mg, 1.70 mmol) were dissolved in $MeCl_2$ and cooled to 0 °C (ice bath). DCC (351 mg, 1.70 mmol) was added, and the reaction was stirred at 0 °C for 2 h and then refrigerated overnight. The precipitated DCU was removed and the filtrate evaporated to give the succinimide ester. The BOC group was removed from BOC-Phe-methylbenzhydrylamine resin (2.5 g, 1.3 mmol at 0.5 mequiv/g) using the standard protocol, and the resin was neutralized and washed. The succinimide ester was dissolved in $MeCl_2$ (10 mL) and added to the resin along with a catalytic amount of HOBt (5 mg). The reaction was allowed to proceed 7 days with periodic monitoring using the Kaiser test. The resin was then capped using acetic anhydride (1 mL) and pyridine (0.2 mL) in $MeCl_2$ (10 mL). The BOC was removed as usual, leaving the resin as the TFA salt. Meanwhile, Z_3-L-Arg-L-Pro (**20**, 1.05 g, 1.56 mmol) and HOBt (250 mg, 1.64 mmol) were dissolved in $MeCl_2$ and cooled to 0 °C, after which DCC (350 mg, 1.70 mmol) was added. The resin was neutralized (2×) with 5% diisopropylethylamine and washed with $MeCl_2$ (3×) just before the activated dipeptide was added. The vessel was then shaken overnight. The reaction was determined to be complete (negative Kaiser test), and the resin was washed and dried to give 2.81 g. HF removal of peptide from resin yielded 352 mg of crude peptide. The product was partially purified by preparative HPLC using a 10−25% gradient of acetonitrile in water containing 0.1% TFA. Further preparative HPLC purification was performed in an isocratic system using 22% acetonitrile in water containing 0.1% TFA. Fractions containing isomer A were pooled, evaporated of acetonitrile, frozen, and lyophilized, yield 42 mg (98% pure) of **3g** (isomer A). Isomer B appeared unstable, giving rise to multiple peaks in the analytical HPLC.

Isomer A: FAB-MS *m/e* 631 ($M + H^+$); ^{1}H-NMR (D_2O) δ 0.43 (d, 3H), 0.58 (d, 3H), 1.45 (m, 1H), 1.68 (m, 2H), 1.91 (m, 3H), 2.00 (m, 2H), 2.33 (m, 2H), 2.72 (m, 1H), 2.79−2.91 (m, 4H), 3.19 (t, 2H), 3.00 (dd, 1H), 3.57 (m, 1H), 3.72 (m, 1H), 4.36 (t, 3H), 4.48 (dd, 1H), 4.59 (dd, 1H), 4.63 (t, 1H), 7.22−7.34 (m, 5H); ^{13}C-NMR (D_2O) δ 210.94 (ketone). Anal. Calcd for $C_{30}H_{45}N_8O_7 \cdot 1.8CF_3COOH \cdot 2.5H_2O$: C, H, N, F.

Acknowledgment. This work was sponsored by Japan Tobacco Co., Ltd. We thank Dr. D. Thomas for mass spectrometric analyses and Mr. G. Detre for certain NMR analyses.

References

(1) DeGraw, J. I.; Almquist, R. G.; Hiebert, C. K.; Colwell, W. T.; Crase, J.; Hayano, T.; Judd, A. K.; Dousman, L.; Smith, R. L.; Waud, W. R.; Uchida, I. Stabilized analogs of thymopentin. 1. 4,5-Ketomethylene pseudopeptides. *J. Med. Chem.* **1997**, *40*, 2386−2397.

(2) Tishio, T. P.; Patrick, J. E.; Weintraub, H. S.; Chasin, M.; Goldstein, G. Short *in vitro* half-life of thymopentin pentapeptide in human plasma. *Int. J. Pept. Protein Res.* **1983**, *14*, 479−484.

(3) Sisto, A.; Mariotti, S.; Groggia, A.; Marcozzi, G.; Villa, L.; Neucioni, L.; Silvestri, S.; Verdini, A.; Pessi, A. Biologically active retro-inverso analogs of thypentin. *Pept.: Struct., Biol. Proc. Am. Pept. Symp., 11th* **1989**, 772−773.

(4) Garcia-Lopez, M. T.; Gonzalez-Muniz, R.; Harto, J. R. Synthesis of ketomethylene dipeptides containing basic amino-acid analogs at C-terminus. *Tetrahedron* **1988**, *44*, 5131−5138.

(5) Garcia-Lopez, M. T.; Gonzalez-Muniz, R.; Harto, J. R. A simple and versatile route to ketomethylene dipeptide analogs. *Tetrahedron Lett.* **1988**, *29*, 1577−1580.

(6) Umezawa, H.; Nakamura, T.; Fukatsu, S.; Aoyagi, T.; Tatsuta, K. Synthesis of arphamenine-A and epi-arphamenine-A. *J. Antibiot.* **1983**, *36*, 1787−1788.

(7) Aplin, R. T.; Christiansen, J.; Young, G. T. Amino-acids and peptides. 48. Synthesis of bradykinyl-chloromethane. *Int. J. Pept. Protein Res.* **1983**, *21*, 555−561.

(8) Grieco, P. A.; Bahsas, A. Immonium ion based synthetic methodology. A novel method for N-methylation of dipeptides and amino acid derivatives via retro aza Diels-Alder reactions. *J. Org. Chem.* **1987**, *52*, 5465−5749.

JM9508043