Pro-D-Phe-Cys(Acm)-D-Trp-Leu (39). Boc-D-Phe-Cys(Acm)-D-Trp-Leu was coupled with Pro-OMe by the DCCI-HOBt method to give Boc-D-Phe-Cys(Acm)-D-Trp-Leu-Pro-OMe: yield 82%; mp 130-132 °C. Anal. ($C_{43}H_{59}N_7O_9S$) C, H, N, S.

The Boc and the methyl ester groups were cleaved and the free pentapeptide was cyclized by the diphenyl phosphorazidate method to give the cyclic peptide: yield 28%; mp 199-200 °C; MS *m/e* MH^+ 718 ($C_{37}H_{47}N_7O_6S$ requires MH^+ 718).

Cys(Acm)-D-Phe-Cys(Acm)-D-Trp-Leu (40). Boc-Cys(Acm)-D-Phe-Cys(Acm)-D-Trp-Leu-OMe (1.5 g, 1.6 mmol) was converted to Cys(Acm)-D-Phe-Cys(Acm)-D-Trp-Leu and then cyclized by the diphenyl phosphorazidate method. Purification by silica gel column chromatography using 3 and 4% methanol in chloroform as eluants gave the cyclic peptide (160 mg, 13.3%): mp 270-271 °C; $[\alpha]^{25}_D$ -25.07° (*c* 2.05, DMF); AAA Cys 2.1, Leu 1.0, Phe 1.04; MS *m/e* MH^+ 795. Anal. ($C_{38}H_{50}N_8O_7S_2$) C, H, N, S.

Inhibition of Human Renin. Partially purified human renal renin[15] was kindly supplied by Dr. Brenda Leckie (MRC Blood Pressure Unit, Glasgow, U.K.) and was assayed at pH 7.0 in phosphate buffer with human angiotensinogen partially purified from volunteer plasma by ammonium sulfate fractionation and DEAE-Sepharose chromatography.[16] Test compounds were dissolved and diluted as necessary in dimethyl sulfoxide (DMSO). The drug solutions were then diluted in buffer such that the DMSO concentration was 3% v/v. The final incubation mixture (150 μL) contained the following components: 50 mM phosphate buffer, pH 7.0, 3 mM disodium ethylendiaminetetraacetate (EDTA), 3 mM 8-hydroxyquinoline hemisulfate (8-HQ), 0.5 mM phenylmethanesulfonyl fluoride (PMSF), 0.1% w/v sodium azide, 0.1% w/v bovine serum albumin, and 1% v/v DMSO. The substrate concentration used was between 0.3 and 0.5 times the apparent K_m for the reaction and the renin concentration was such that the angiotensin generation rate was 5-10 ng/mL per h. Reactions were allowed to proceed for 120 min at 30 °C and were terminated by cooling on ice. Samples were then assayed for angiotensin I content in the presence of 100 μM pepstatin as described previously with commercially available [^{125}I]angiotensin II (New England Nuclear) and anti-angiotensin I-BSA antibody (Miles Scientific).[17] Under the conditions used, angiotensin-generation rate was linear and at the highest concentrations used the test compounds did not cross-react with the anti-angiotensin antibody. Angiotensin generation rate in the presence of test compound was compared to the control rate in the presence of vehicle. Results were calculated as percentage inhibition of the control reaction rate. IC_{50} values (concentration for 50% inhibition of the renin-catalyzed reaction) were determined from the relationship between percent inhibition and inhibitor concentration. Test concentrations were chosen to bracket the expected IC_{50} and to cover at least 4 orders of magnitude. The presence of 1% DMSO in the incubation mixture had no significant effect on the renin activity.

(15) Brown, J. J.; Davies, D. L.; Lever, A. F.; Robertson, J. I. S.; Tree, M. *Biochem. J.* **1964**, *93*, 594.
(16) Dorer, F. E.; Lentz, K. E.; Kahn, J. R.; Levine, M.; Skeggs, L. *Anal. Biochem.* **1978**, *87*, 11.
(17) Oldham, A. A.; Arnstein, M. J. A.; Major, J. S.; Clough, D. P. *J. Cardiovasc. Pharmacol.* **1984**, *6*, 672.

Inhibitors of Human Renin. Cyclic Peptide Analogues Containing a D-Phe-Lys-D-Trp Sequence

Anand S. Dutta,* James J. Gormley, Peter F. McLachlan, and John S. Major

Chemistry and Bioscience Departments, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire SK10 4TG, U.K.
Received September 26, 1989

Cyclic peptides containing a D-phenylalanine and a D-tryptophan residue have been synthesized and tested as inhibitors of human renin. Most of these are tripeptide derivatives of the type $CO(CH_2)_3CO$-D-Phe-Lys-D-Trp- or $COCH_2NHCH_2CO$-D-Phe-Lys-D-Trp- in which the individual side-chain methylene groups have been replaced with -CHMe-, $-CMe_2-$, -CH(Ph)-, $-CH(CH_2Ph)-$, or $-CH((CH_2)_2CHMe_2)-$ groups. The three amino acid residues and the size of the ring were very important features of these compounds. Reducing the ring size gave much less potent compounds. The most potent analogue of the series, $CO(CH_2)_2CHPhCO$-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$ (**14**, IC_{50} = 26 nM), was obtained by substituting the methylene group nearer to the D-Phe residue by a -CHPh- group. Compound **14** was 15-fold more potent in inhibiting human renin than porcine renin.

The renin-angiotensin system plays an important role in regulating blood pressure. The enzyme renin generates angiotensin I from angiotensinogen. Angiotensin I is then converted by angiotensin converting enzyme to angiotensin II, which is a potent vasoconstrictor and leads to increased blood pressure. Inhibitors of this system have been shown to lower blood pressure in hypertensive animals and patients.[1-3]

A large number of renin inhibitors, based on the renin substrate, have been reported in the literature.[1-3] We have reported novel inhibitors of renin based on a linear tetrapeptide.[4] The structures of two such inhibitors (**1** and **2**) are shown in Table I. Further work on these linear peptides led to potent cyclic peptide inhibitors, e.g. Z-Glu-D-Phe-Lys-D-Trp-Leu-OMe (**3**, IC_{50} = 3.2 μM), of human renin.[5] Structures of these cyclic peptides have been modified further and potent inhibitors of human renin containing a tripeptide unit, X-D-Phe-Lys-D-Trp, are reported here. The main objectives of this work have been (a) to incorporate additional groups which may improve binding to renin and therefore improve potency of these inhibitors, (b) to decrease the molecular weights of the original cyclic penta- and hexapeptide derivatives, and (c) to incorporate changes within the ring structure which may lead to more information on the overall conformation of these peptides. The increased knowledge on the conformation of X-D-Phe-Lys-D-Trp- type of compounds is likely to lead to novel nonpeptide inhibitors of renin.

(1) Kokubu, T.; Hiwada, K. *Drugs Today* **1987**, *23*, 101.
(2) Baldwin, J. J.; Sweet, C. S. *Annu. Rep. Med. Chem.* **1988**, *23*, 59.
(3) Greenlee, W. J. *Pharm. Res.* **1987**, *4*, 364.
(4) Dutta, A. S.; Gormley, J. J.; McLachlan, P. F.; Major, J. S. *J. Chem. Res. (S)* **1990**, 2.
(5) Dutta, A. S.; Gormley, J. J.; McLachlan, P. F.; Major, J. S. *J. Med. Chem.* **1989**, submitted for publication.

0022-2623/90/1833-2560$02.50/0

Table I. Chemical Structures and Renin Inhibitory Activities of the Peptides

no.	compd	inhibn of human renin	
		IC_{50}, μM	% inhibn at 100 μM
1	Boc-D-Phe-Cys(Acm)-D-Trp-Leu-OMe	40	
2	Z-D-Phe-Lys-D-Trp-Leu-OMe	31	
3	Z-Glu-D-Phe-Lys-D-Trp-Leu-OMe	3.2	
4	$CO(CH_2)_2CH(NH\text{-}Z)CO$-D-Phe-Lys-D-Trp-$NHCH_2CH_2CHMe_2$	0.87	
5	$CO(CH_2)_3CO$-D-Phe-Lys-D-Trp-$NHCH_2$-(*S*)-CHMeEt	0.86	
6	$CO(CH_2)_2CO$-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$		53 at 10 μM
7	$COCH_2OCH_2CO$-D-Phe-Lys-D-Trp-$NHCH_2CH_2CHMe_2$	2.3	
8	$COCH_2SCH_2CO$-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$		66, 70
9	$COCMe_2(CH_2)_2CO$-D-Phe-Lys-D-Trp-$NHCH_2$-(*S*)-CHMeEt	1.4	
10	$COCH_2CMe_2CH_2CO$-D-Phe-Lys-D-Trp-$NHCH_2$-(*S*)-CHMeEt		63, 66
11	$CO(CH_2)_2CMe_2CO$-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$	0.88	
12	$COCH_2CHMeCH_2CO$-D-Phe-Lys-D-Trp-$NHCH_2CHMeCH_2Me_2$	0.24	
13	$COCHPh(CH_2)_2CO$-D-Phe-Lys-D-Trp-$NH(CH_2)_2Me_2$	1.5	
14	$CO(CH_2)_2CHPhCO$-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$	0.026	
15	$COCH_2NH$-(*S*)-$CH(CH_2Ph)CO$-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$	0.75	
16	$COCH_2NH$-(*R*)-$CH(CH_2Ph)CO$-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$	2.74	
17	CO-(*S*)-$CH(CH_2Ph)NHCH_2CO$-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$		52
18	CO-(*R*)-$CH(CH_2Ph)NHCH_2CO$-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$		57
19	$COCH_2NH$-(*S*)-$CH(CHMeEt)CO$-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$		84, 86 (45 at 10 μM)
20	CO-(*S*)-$CH(CHMeEt)NHCH_2CO$-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$		58 at 10 μM
21	$COCH_2NH$-(*R*)-$CH(CH_2CHMe_2)CO$-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$		88, 96 (10 at 1 μM)
22	CO-(*R*)-$CH(CH_2CHMe_2)NHCH_2CO$-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$	0.43	

Table II. Inhibition of Human and Porcine Renins by Selected Compounds

	IC_{50}, μM		ratio of porcine/human renin
	porcine renin	human renin	
5	22.0	0.86	25.6
11	12.0	0.88	13.6
14	0.39	0.026	15.0
22	0.75	0.43	1.74

The analogues reported here are listed in Table I. The peptides were tested twice against human renin by using partially purified renal renin and human plasma angiotensinogen[5] at an initial concentration of 100 μM. The percent inhibition figure for the compounds, not fully active at this concentration, is recorded in Table I. Compounds fully active at 100 μM concentration were further tested at lower concentrations and IC_{50} values for such compounds are given in the table. Inhibition of porcine renin by selected compounds (Table II) was tested by the procedure mentioned above except that partially purified porcine renin and porcine angiotensinogen were used.

The inhibition curves for the compounds do not provide any insight to the nature of the inhibitory kinetics. Therefore, the competitive/noncompetitive nature of the inhibitors cannot be predicted on the basis of the present data.

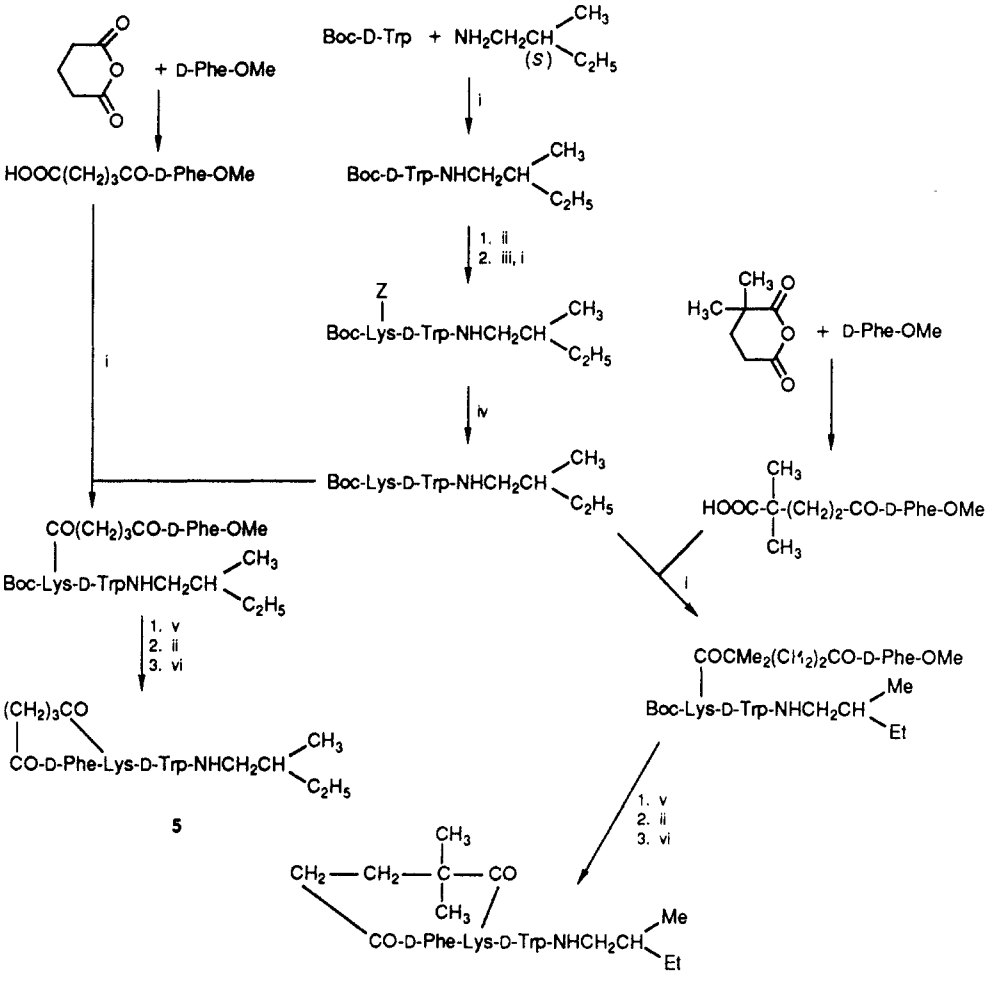
Synthesis

The syntheses of compounds 1–4 have been described in earlier papers.[4,5] Compounds 5 and 6 were prepared by the routes shown in Schemes I and II, respectively. The route shown in Scheme II was also used for compounds 7 and 8. Instead of succinic anhydride used in the synthesis of compound 6, diglycolic anhydride was used for 7 and thiodiglycolic anhydride was used for compound 8.

Compounds 9, 10, 12, and 13 were prepared by the route shown in Scheme I. Unlike the reaction of glutaric anhydride with D-Phe-OMe, which can only give one product, the reaction of 2,2-dimethylglutaric anhydride can lead to $HO_2CCMe_2(CH_2)_2CO$-D-Phe-OMe and $HO_2C(CH_2)_2CMe_2CO$-D-Phe-OMe. Under the reaction conditions used, the D-Phe-OMe reacted at the less hindered carbonyl group of 2,2-dimethylglutaric anhydride to give $HO_2CCMe_2(CH_2)_2CO$-D-Phe-OMe in 93% yield. The identity of this product was established by ^{13}C NMR studies. Further reaction of this D-Phe derivative with Boc-Lys-D-Trp-$NHCH_2CHMeEt$ by the DCCI–HOBt method gave Boc-Lys($COCMe_2(CH_2)_2CO$-D-Phe-OMe)-D-Trp-$NHCH_2CHMeEt$, which was then treated first with sodium hydroxide (aqueous) and then with HCl–acetic acid to yield Lys($COCMe_2(CH_2)_2CO$-D-Phe)-D-Trp-$NHCH_2CHMeEt$. Cyclization of this peptide by the diphenyl phosphorazidate method gave compound **9**. For the synthesis of compound **10**, 3,3-dimethylglutaric anhydride was used in place of 2,2-dimethylglutaric anhydride. The $HO_2CCH_2CMe_2CH_2CO$-D-Phe-OMe was obtained in almost quantitative yield (96%). The D-Phe derivative was then converted to compound **10** by the method used for **9**. The synthesis of compound **12** from 3-methylglutaric anhydride, D-phenylalanine, and Boc-Lys-D-Trp-$NHCH_2CHMeEt$ was similar to that of compound **10**. For the synthesis of compound **13**, 2-phenylglutaric anhydride was reacted with D-Phe-OMe. $HO_2CCHPh(CH_2)_2CO$-D-Phe-OMe, also obtained in very high yield (97%), was then reacted with Z-Lys-D-Trp-$NH(CH_2)_2CHMe_2$ to give Z-Lys($COCHPh(CH_2)_2CO$-D-Phe-OMe)-D-Trp-$NH(CH_2)_2CHMe_2$. This peptide was first treated with aqueous sodium hydroxide to cleave the methyl ester group. The benzyloxycarbonyl group was then removed by hydrogenolysis (5% Pd–C) and the resulting product was cyclized by the diphenyl phosphorazidate method to give **13**.

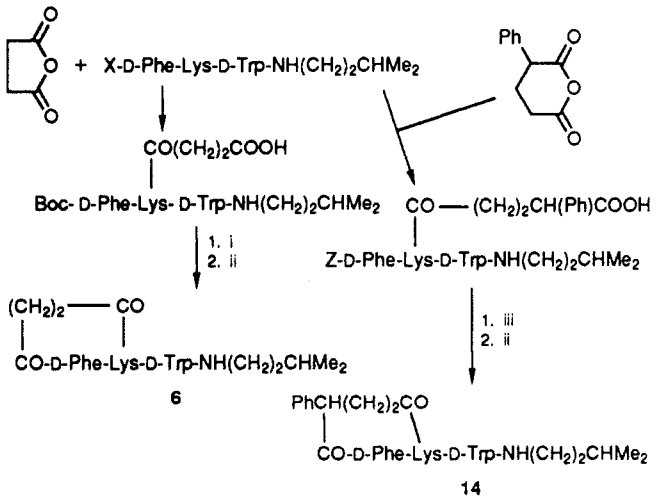
The synthesis of compound **11** is described in Scheme III. The synthesis of this compound was first attempted

Scheme I.[a] Synthesis of Compounds **5**, **9**, **10**, **12**, and **13**



[a] Reagents: (i) DCCI–HOBt; (ii) HCl–acetic acid; (iii) Boc-Lys(Z); (iv) H_2/Pd–C; (v) NaOH; (vi) diphenyl phosphorazidate, $NaHCO_3$.

Scheme II.[a] Synthesis of Compounds **6–8** and **14** (X = Boc or Z)



[a] Reagents: (i) HCl–acetic acid; (ii) diphenyl phosphorazidate, $NaHCO_3$; (iii) Pd–C, ammonium formate.

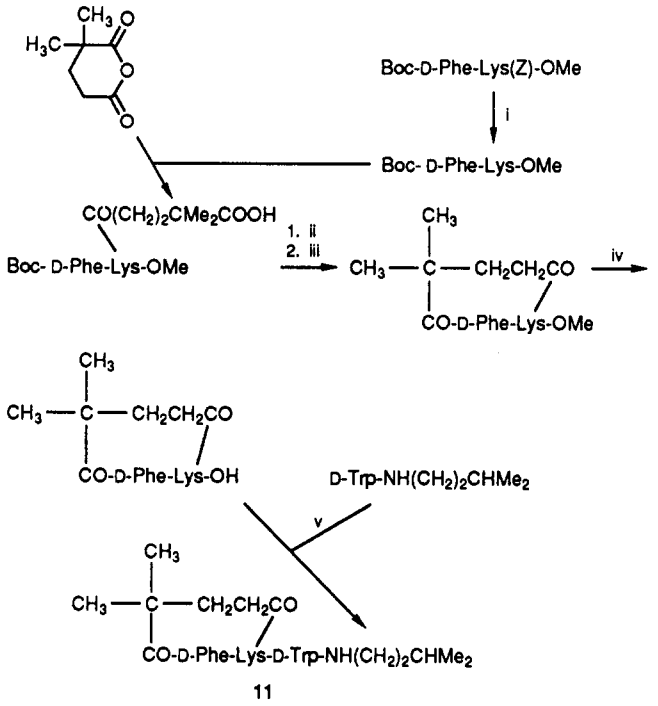
by the reaction of 2,2-dimethylglutaric anhydride with Z-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$. This reaction gave the expected product Z-D-Phe-Lys(CO-$(CH_2)_2CMe_2CO_2H$)-D-Trp-$NHCH_2CH_2CHMe_2$, in high yield. The benzyloxycarbonyl group was then cleaved. The cyclization reaction of D-Phe-Lys(CO-$(CH_2)_2CMe_2CO_2H$)-D-Trp-$NH(CH_2)_2CHMe_2$ did not give the desired cyclic peptide. The only identifiable compound from this reaction mixture was a cyclic urea derivative, $CO(CH_2)_2CMe_2NHCO$-D-Phe-Lys-D-Trp-NH-$(CH_2)_2CHMe_2$, which was characterized by NMR and FAB mass spectrometry. This side reaction was also observed in a number of other cases and made the isolation of the required cyclic amides very difficult. In most cases two purification steps, chromatography on Sephadex LH-20 and silica gel, were required and the final products were obtained in poor yield.

The synthesis of **11** was then started from Boc-D-Phe-Lys-OMe. The reaction of this dipeptide derivative with 2,2-dimethylglutaric anhydride gave Boc-D-Phe-Lys(CO-$(CH_2)_2CMe_2CO_2H$)-OMe, which after deprotection and cyclization gave the desired cyclic peptide CO-$(CH_2)_2CMe_2CO$-D-Phe-Lys-OMe, in 40% yield. No urea derivative was isolated from this reaction (NMR and FAB mass spectrometry data). The cyclic peptide was then extended to compound **11** by the method shown in Scheme III.

Compound **14** was prepared by the route shown in Scheme II.

The syntheses of compounds **15–22** are shown in Scheme IV. The HO_2CCH_2-X-OBzl (X = Phe, D-Phe, Ile, D-Leu) derivatives were first prepared by the reaction of *tert*-butyl bromoacetate and amino acid benzyl esters. The *tert*-butyl ester group was then cleaved by an HCl–acetic acid

Scheme III.[a] Synthesis of Compound 11



[a] Reagents: (i) H_2, Pd–C; (ii) HCl–acetic acid; (iii) diphenyl phosphorazidate, $NaHCO_3$; (iv) NaOH; (v) DCCI–HOBt.

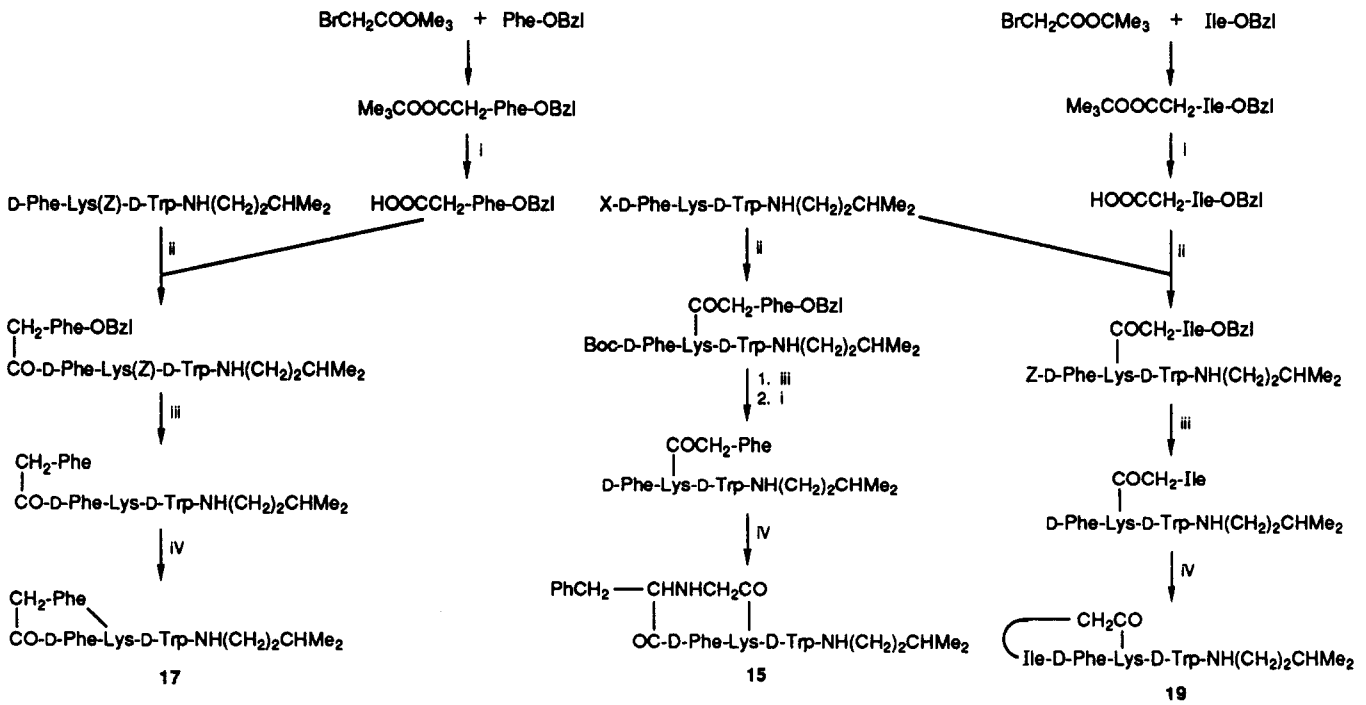
treatment to give the *N*-carboxymethyl amino acid benzyl esters. The reaction of *N*-(carboxymethyl)phenylalanine benzyl ester with Boc-D-Phe-Lys-D-Trp-$NHCH_2CH_2CHMe_2$ by the DCCI–HOBt method, followed by deprotection and cyclization, gave compound **15**. The above sequence of reactions using *N*-(carboxymethyl)-D-phenylalanine benzyl ester and Boc-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$ gave compound **16**. For the syntheses of compounds **17**, **18**, **20**, and **22**, the required *N*-carboxymethyl derivatives of Phe, D-Phe, Ile, and D-Leu benzyl esters were reacted with D-Phe-Lys(Z)-D-Trp-$NH(CH_2)_2CHMe_2$. The benzyl and benzyloxycarbonyl protecting groups were cleaved in one step and the linear peptides were cyclized by the diphenyl phosphorazidate method. Compound **19** was prepared from HO_2CCH_2-Ile-OBzl and Z-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$ by the steps shown in the scheme. The preparation of compound **21** was similar except that HO_2CCH_2-D-Leu-OBzl was used in place of the Ile derivative used for **19**.

Results and Discussion

The work reported earlier on linear tetrapeptides, e.g. Boc-D-Phe-Cys(Acm)-D-Trp-Leu-OMe (**1**), and cyclic pentapeptides, e.g. Z-Glu-D-Phe-Lys-D-Trp-Leu-OMe (**3**), indicated that (a) the D-phenylalanine and the D-tryptophan residues were very important for the renin inhibitory activity, (b) the Leu-OMe residue could be replaced by various other amino acid and non amino acid residues, (c) the N-terminal benzyloxycarbonyl or *tert*-butoxycarbonyl groups could be replaced by a large number of groups, and (d) in the case of cyclic peptides, a 15-membered ring structure was necessary for potent renin inhibitory activity.[4,5] On the basis of this information, the cyclic peptides reported here contain a D-Phe-Lys-D-Trp-NH-$(CH_2)_2CHMe_2$ or a D-Phe-Lys-D-Trp-$NHCH_2CHMeEt$ residue. The linking group between the α-amino group of the D-Phe and the N^{ϵ}-amino group of the Lys residue has been varied, but the overall size of the ring has been kept to 15 atoms.

As a first step to simplify the structures of the cyclic tetrapeptide derivatives, the Z-Glu residue in compound **4** was first replaced by a glutaric residue. This change does not effect the ring size but reduces the molecular weight very significantly. The glutaryl analogue **5** was equipotent to the Z-Glu analogue **4**, indicating that the benzyloxycarbonyl group and the amino group of the Glu residue were not important for the biological activity. Reducing the size of the ring, i.e. by replacing the glutaric acid residue by a succinic acid residue, led to compound **6**, which was at least 10-fold less potent than **5**. This loss in potency (10-fold) was much less than that seen in the

Scheme IV.[a] Syntheses of Compounds 15–22 (X = Boc or Z)



[a] Reagents: (i) HCl–acetic acid; (ii) DCCI–HOBt; (iii) H_2/Pd–C; (iv) diphenyl phosphorazidate, $NaHCO_3$.

Z-Glu-D-Phe-Lys-D-Trp-Leu-OMe (3) series of analogues. In this series, reducing the ring size by one methylene group gave Z-Asp-D-Phe-Lys-D-Trp-Leu-OMe and Z-Glu-D-Phe-Orn-D-Trp-Leu-OMe analogues, which were at least 100-fold less potent than 3.

Introduction of a heteroatom in the glutaryl part of the cyclic structure did not lead to an improvement in potency. Compound 7, in which the $-COCH_2CH_2CH_2CO-$ residue of 5 was replaced by a $-COCH_2OCH_2CO-$ group, was about 3-fold less potent than 5. The corresponding analogue containing a $-COCH_2SCH_2CO-$ group in the side chain (8) was about 50–100-fold less potent than 5.

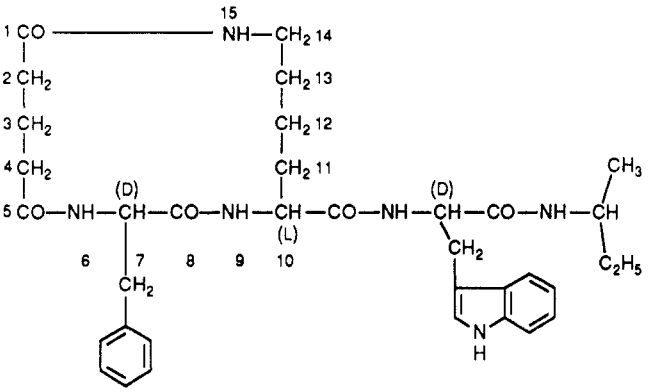
Conformational restraints were then introduced into the glutaryl part of the ring structure. The three methylene groups were substituted by CHMe, CMe_2, or CHPh groups (9–14). Compounds 9 and 11 containing a CMe_2 group in place of the methylene group nearer to either D-Phe or Lys were equipotent to 5, but compound 10, in which the middle $-CH_2-$ group has been replaced by a CMe_2 group, was about 100-fold less potent. Introduction of a single methyl group in this position gave a very potent analogue. Compound 12 was about 4-fold more potent than 5. Replacement of the $-CH_2-$ group nearer to the Lys residue by a –CHPh– group led to a marginal reduction in potency. Compound 13 was about $^1/_2$ as potent as 5. Compound 14 containing a –CHPh– group in place of the $-CH_2-$ group nearer to the D-Phe residue gave rise to the most potent analogue reported in this paper. This analogue (14) was about 30-fold more potent than 13 and about 60-fold more potent than 5.

The overall conformation of the ring structure was further explored by incorporating amino acid residues in the linking group. In order to keep the size of the linking groups similar, *N*-carboxymethyl amino acid derivatives were substituted for the $-CO(CH_2)_3CO-$ group. A comparison of compounds 15 and 17 shows that when a $-CH(CH_2Ph)-$ (from L-Phe) group was substituted in place of a methylene group nearer to the D-Phe or the Lys residue, the two analogues were very different in their biological activity. Compound 15, with a $-CH(CH_2Ph)-$ group nearer to the D-Phe residue, was about 100-fold more potent than 17. Similar conclusions can be drawn from comparisons of compounds 16 and 18. Here again the analogue with a $-CH(CH_2Ph)-$ group (from a D-Phe residue) nearer to the Lys residue (18) was about 50-fold less potent than 16. The results were somewhat different when bigger aliphatic groups were substituted in place of the side-chain methylene groups. Analogues 19 and 20 were nearly equipotent, but compound 22 was at least 10-fold more potent than 21. This result was the opposite of the results for compounds 9, 11, and 13-18. In all these compounds the analogues with a $-CMe_2-$, –CHPh–, or a $-CH(CH_2Ph)-$ group in place of the methylene group nearer to the D-Phe amino group were more potent (11 and 14–16) than the analogues in which the methylene group nearer to the side chain amino group of the Lys residue was substituted (compounds 9, 13, 17, and 18).

All the analogues containing a nitrogen atom in the linking group (15–22) were less potent than the substituted glutaryl analogue (14). This may be due to the presence of an –NH– in compounds 15–22 in place of a methylene in compound 14. The lower potencies of these compounds may also be due to side-chain differences.

Four of the analogues reported here were also tested as inhibitors of porcine renin (Table II). All the four analogues (5, 11, 14, and 22) did inhibit porcine renin, but the IC_{50} values against porcine renin were 2–25-fold higher than the IC_{50} values against human renin. Compound 14 ($CO(CH_2)_2CHPhCO$-D-Phe-Lys-D-Trp-$NHCH_2CH_2CHMe_2$), which was the most potent analogue of this series against human renin (IC_{50} = 26 nM), was about 15-fold less potent against porcine renin. In comparison to Me_3CCH_2-Glu-D-Phe-Lys-D-Trp-NH-$(CH_2)_2CHMe_2$ (reported in an earlier paper), compound 14 was much less selective. Both these analogues are potent inhibitors of human renin (IC_{50} = 26 and 63 nM, respectively).

Chart I. Chemical Structure of Compound 5



The structure–activity data reported here and in earlier papers[4,5] on the linear and cyclic peptide inhibitors of renin leads to the following conclusions (the numbering system shown in Chart I for compound 5 has been used for this part of the discussion).

i. The minimum structure required for potent renin inhibitory activity in this class of peptides appears to be a tripeptide (D-Phe-Lys-D-Trp) sequence. Since in an earlier series of compounds a tripeptide derivative, Z-Glu-D-Phe-Lys-$NH(CH_2)_2$-indol-3-yl (IC_{50} = 100 μM), was shown to have some renin inhibitory activity, it is likely that a dipeptide derivative of the type X-D-Phe-Lys-NH-$(CH_2)_2$-indol-3-yl could be developed, with suitable modifications in positions 1–5 and 10–15 (Chart I), which may also be a potent inhibitor of renin.

ii. The three methylene groups (positions 2–4) do not seem to be essential for biological activity. These can be substituted with –CHMe–, $-CMe_2-$, –CHPh–, $-CH(CH_2Ph)-$, $-CH((CH_2)_2CHMe_2)-$, and $-CH(CH_2CHMeEt)-$ groups. The methylene group in position 3 could also be replaced by an O, N, or S atom. When a –CHPh– or $-CH(CH_2Ph)-$ group was substituted in place of the methylene groups in positions 2 or 4, the position 4 modified analogues were more potent than the position 2 modified analogues. The methylene groups in positions 11–14 have not yet been modified.

iii. Only one of the amide bonds (between positions 4 and 7) has been replaced by a $-CH_2NH-$ group. The resulting compound was a very poor inhibitor of renin (unpublished results). The two other amide bonds within the ring structure (between positions 2 and 14 and 7 and 10) have not yet been modified.

Conclusions

The results reported here indicate that potent inhibitors of human renin can be obtained from peptides unrelated to the angiotensinogen sequence. The binding mode of this novel series of inhibitors, based on Boc-D-Phe-Cys-(Acm)-D-Trp-Leu-OMe (1), to renin is not known at this moment. Efforts have been concentrated to synthesize analogues which may provide information about the overall

conformation of the more potent members of this series. The linear peptides (1 and 2) are likely to be extremely flexible, but in cyclic peptides (3–5) the conformational freedom is somewhat restricted. This may be one of the reasons for the improved potency of these analogues. These cyclic peptides have a 15-membered ring with seven methylene groups and three amide bonds within the ring structure. Three of the methylene groups have been substituted with various aliphatic and aromatic groups to obtain further conformationally restrained analogues. The information obtained from these analogues is being used presently in molecular modeling and energy-minimization studies. Chemical modifications of the four lysine side chain methylene groups are also being investigated. The results obtained from all these studies may lead to better understanding of the overall conformation of this series of peptides and may also shed some light on the binding interactions with renin. Hopefully, this may also suggest some modified peptide or nonpeptide structures which may lead to useful drugs for the treatment of hypertension.

Experimental Section

Details of the various coupling and deblocking procedures have only been given in a few cases. In all the other examples the methods have been mentioned by name. The details of TLC systems, spray reagents, amino acid analysis, and other general considerations have been described earlier.[6,7] The syntheses of some linear peptide intermediates and compounds 1–4 (Table I) can be found in earlier papers on renin inhibitors.[4,5] An experimental procedure for the renin inhibitory test has been reported earlier.[4]

$HO_2C(CH_2)_3CO$-D-Phe-OMe. Glutaric anhydride (1.71 g, 15 mmol) in DMF (5 mL) was cooled (0 °C) and added to a solution of D-Phe-OMe·HCl (2.15 g, 10 mmol) and triethylamine (2.1 mL, 1.5 mmol) in DMF (30 mL) also at 0 °C. The reaction was allowed to reach room temperature and stirring was continued for 16 h. Insoluble material was removed by filtration, the filtrate was evaporated and the product was purified by silica gel chromatography using 2% methanol in chloroform as eluant: yield 2.75 g, (94%)) as an oil; R_{FQ} 0.20, R_{FH} 0.48, R_{FK} 0.75. The structure was confirmed by NMR.

Boc-D-Trp(*S*)-(−)-2-methylbutylamide. Boc-D-Trp (13.7 g, 45 mmol) and (*S*)-(−)-2-methylbutylamine (Aldrich; 4 g, 45 mmol) were coupled by using the DCCI–HOBt method with 4-(dimethylamino)pyridine (0.1 equiv). The product was purified by silica gel chromatography using chloroform as eluant and was obtained as a stiff oil (95%). A sample solidified on standing over several days: mp 196–200 °C dec.

Boc-Lys(Z)-D-Trp-(*S*)-(−)-2-methylbutylamide. Boc-Lys(Z)-OH (14.75 g, 38.7 mmol) and D-Trp-$NHCH_2CHMeEt$·HCl (12 g, 38.7 mmol) (obtained from the Boc compound by treatment with HCl–AcOH) were coupled by using the DCCI–HOBt method in the presence of triethylamine. The reaction mixture was filtered, the filtrate was evaporated, and the residue, in ethyl acetate, was washed successively with 1 N citric acid, water, 10% sodium bicarbonate, and water and dried ($MgSO_4$). The product was recrystallized from ethyl acetate–petroleum ether (60–80 °C) yield 14.9 g (60%); mp 165–166 °C. Anal. ($C_{35}H_{49}N_5O_6$) C, H, N.

Boc-Lys-D-Trp-(*S*)-(−)-2-methylbutylamide. Boc-Lys(Z)-D-Trp-$NHCH_2CHMeEt$ (3.2 g, 5 mmol) was hydrogenolyzed in ethanol–water over 10% Pd–C with ammonium formate (1 g). The reaction was complete within 15 min. The catalyst was removed by filtration and after evaporation of the solvents the product was obtained as a frothed up solid (96%): mp 114–118 °C. Anal. ($C_{27}H_{43}N_5O_4$) C, H, N.

Boc-Lys-($CO(CH_2)_3CO$-D-Phe-OMe)-D-Trp-(*S*)-(−)-2-methylbutylamide. A DCCI–HOBt coupling of $HO_2C(CH_2)_3CO$-D-Phe-OCH_3 (1.4 g, 4.8 mmol) and Boc-Lys-D-Trp-$NHCH_2CHMeEt$ formate (2.4 g, 4.8 mmol) in DMF (80 mL) with triethylamine (0.72 mL, 5 mmol), followed by purification using silica gel chromatography, gave the product: yield 2.68 g (73%); mp 177–179 °C. Anal. ($C_{42}H_{60}N_6O_8$) C, H, N.

Lys($CO(CH_2)_3CO$-D-Phe-OH)-D-Trp-(*S*)-(−)-2-methylbutylamide. The above product (2 g, 2.57 mmol) was first saponified with 4 N NaOH in methanol–water, and without further purification it was then treated with 3 N HCl–AcOH. The product was obtained as a solid by trituration with ether: yield 1.68 g (93%); mp 138–141 °C. Anal. ($C_{36}H_{51}N_6O_6$) C, H, N, Cl.

$CO(CH_2)_3CO$-D-Phe-Lys-D-Trp-$NHCH_2CHMeEt$ (5). The branched, linear compound Lys($CO(CH_2)_3CO$-D-Phe-OH)-D-Trp-$NHCH_2CHMeEt$·HCl (1.58 g, 2.25 mmol) was dissolved in DMF (400 mL), sodium bicarbonate (970 mg, 5 equiv) was added, and the reaction mixture was cooled in an ice bath. Diphenyl phosphorazidate (0.6 mL, 1.1 equiv) was added and the reaction mixture was stirred at 4 °C for 4 days. The salts were removed by filtration, and the filtrate was evaporated to leave a solid. This was redissolved in DMF, filtered, and chromatographed on a column of Sephadex LH-20 in DMF. Product-containing fractions were combined and evaporated. The product was collected in ether: yield 390 mg (27%); mp >250 °C; $[\alpha]^{24}_D$ +28.54° (*c* 1, DMF); MS *m/e* MH^+ 645. Anal. ($C_{36}H_{48}N_6O_5$) C, H, N.

Boc-D-Phe-Lys($CO(CH_2)_2COOH$)-D-Trp-isoamylamide. Succinic anhydride (1.0 g, 10 mmol) was added to a cooled (0 °C) and stirred solution of Boc-D-Phe-Lys-D-Trp-isoamylamide (acetate salt, 3.54 g, 5 mmol) and triethylamine (1.15 mL, 8 mmol) in DMF (20 mL). The reaction mixture was left overnight at room temperature. The solution was diluted with ethyl acetate (250 mL), washed, dried (as above) and evaporated to dryness. The residue was collected with ether and dried: yield 3.4 g (90.9%); mp 160–162 °c; MS *m/e* M + Na^+ 771. Anal. ($C_{40}H_{56}N_6O_8$) C, H, N.

$CO(CH_2)_2CO$-D-Phe-Lys-D-Trp-isoamylamide (6). Boc-D-Phe-Lys($CO(CH_2)_2COOH$)-D-Trp-$NH(CH_2)_2CHMe_2$ (3.3 g, 4.4 mmol) was treated with HCl–acetic acid to cleave the Boc group. The deprotected peptide was dissolved in DMF (750 mL) and the solution was cooled to 0 °C. $NaHCO_3$ (2.94 g, 35 mmol) was added followed by diphenyl phosphorazidate (1.37 g, 5 mmol) and the reaction mixture was stirred at 4 °C for 3 days. The DMF was evaporated off and the residue, after silica gel column chromatography gave the pure cyclic peptide: yield 900 mg (32.5%); mp 280 °C, dec, MS *m/e* MH^+ 631. Anal. ($C_{35}H_{46}N_6O_5$) C, H, N.

Boc-D-Phe-Lys($COCH_2OCH_2COOH$)-D-Trp-isoamylamide. A solution of Boc-D-Phe-Lys-D-Trp-isoamylamide (CH_3COOH salt, 3.54 g, 5 mmol) and triethylamine (1.15 mL, 8 mmol) in DMF (20 mL) was cooled in an ice bath, and diglycolic anhydride (0.7 g, 6 mmol) was added to it. After 2 h, DMF was evaporated off and the crude peptide was purified by silica gel column chromatography: yield 2.9 g (75.9%); mp 130–132 °C. Anal. ($C_{40}H_{56}N_6O_9$) C, H, N.

$COCH_2OCH_2CO$-D-Phe-Lys-D-Trp-isoamylamide (7). The above peptide (2.8 g, 3.66 mmol) was treated with a 2 N solution of HCl in acetic acid to cleave the *tert*-butoxycarbonyl group and was then cyclized as for compound 6. The crude cyclic peptide was purified by silica gel column chromatography: yield 430 mg (18.2%); mp >300 °C; MS *m/e* MH^+ 647. Anal. ($C_{35}H_{46}N_6O_6$) C, H, N.

Boc-D-Phe-Lys($COCH_2SCH_2COOH$)-D-Trp-isoamylamide. To a cooled (0 °C) solution of Boc-D-Phe-Lys-D-Trp-isoamylamide (acetate salt; 3.54 g, 5 mmol) and triethylamine (1.15 mL, 8 mmol) in DMF (20 mL) was added thiodiglycolic anhydride (0.79 g, 6 mmol). After 2 h DMF was removed by evaporation in vacuo and the crude peptide was purified by silica gel column chromatography: yield 3.1 g (79.4%); mp 168–170 °C; MS *m/e* M + Na^+ 803. Anal. ($C_{40}H_{56}N_6O_8S$) C, H, N.

$COCH_2SCH_2CO$-D-Phe-Lys-D-Trp-isoamylamide (8). The Boc group from the above peptide (3 g, 3.8 mmol) was cleaved by an HCl in acetic acid treatment and the resulting hydrochloride was dissolved in DMF and cyclized by the diphenyl phosphorazidate (1.1 g, 4 mmol) method. The pure peptide (8) was isolated and purified as for compound 7: yield 230 mg (9.2%); mp >280

(6) Dutta, A. S.; Furr, B. J. A.; Giles, M. B.; Valcaccia, B. *J. Med. Chem.* **1978**, *21*, 1018.

(7) Dutta, A. S.; Gormley, J. J.; Graham, A. S.; Briggs, I.; Growcott, J. W.; Jamieson, A. *J. Med. Chem.* **1986**, *29*, 1163 and 1171.

°C dec; MS *m/e* MH^+ 663. Anal. ($C_{35}H_{46}N_6O_5S$) C, H, N.

$HO_2CMe_2(CH_2)_2CO$-D-Phe-OMe. The reaction between 2,2-dimethylglutaric anhydride (1.7 g, 11 mmol) and D-Phe-OMe·HCl (2.16 g, 10 mmol) with triethylamine (15 mmol) was the same as for the glutaric anhydride reaction above: yield 3.0 g (93%) as a mobile oil. The structure was confirmed by ^{13}C NMR.

Boc-Lys($COCMe_2(CH_2)_2CO$-D-Phe-OMe)-D-Trp-$NHCH_2CHMeEt$. A DCCI–HOBt coupling of $HO_2CCMe_2(CH_2)_2CO$-D-Phe-OMe (2.0 g, 6.2 mmol) and Boc-Lys-D-Trp-$NHCH_2CHMeEt$ (2.5 g, 5 mmol) with 1 equiv of triethylamine gave the crude product. After acid/base washing of an ethyl acetate solution, followed by chromatography on silica gel using 2, 5, 6, and 10% methanol in chloroform as eluant, the product was obtained as a solid: yield 1.43 g (36%); mp 92–94 °C. Anal. ($C_{44}H_{64}N_6O_8$) C, H, N.

Lys($COCMe_2(CH_2)_2CO$-D-Phe-OH)-D-Trp-$NHCH_2CHMeEt$. The corresponding Boc-Lys($COCMe_2(CH_2)_2CO$-D-Phe-OMe)-D-Trp-$NHCH_2CHMeEt$ (1.40 g, 1.7 mmol) was treated with 4 N NaOH in aqueous methanol. The free acid obtained as an oil had R_{FQ} 0.10, R_{FH} 0.46, R_{FK} 0.75. Without further purification the Boc group was removed with 3 N HCl–AcOH. The solid was collected in ether: yield 1.0 g (79%); mp 111–113 °C. R_{FA} 0.61, R_{FB} 0.66, R_{FC} 0.26, R_{FK} 0.78; MS *m/e* MH^+ 691. Anal. ($C_{38}H_{54}N_6O_6$·HCl) C, H, N.

$COCMe_2(CH_2)_2CO$-D-Phe-Lys-D-Trp-$NHCH_2CH(CH_3)C_2H_5$ (9). This was prepared by a diphenyl phosphorazidate cyclization of the linear, branched compound Lys($COCMe_2(CH_2)_2CO$-D-Phe-OH)-D-Trp-$NHCH_2CHMeEt$·HCl (850 mg, 1.17 mmol) as for compound **5** above. Purification by silica gel chromatography using chloroform and 1, 2, and 4% methanol in chloroform as eluants gave the product: yield 287 mg (37%); mp 241–244 °C; $[\alpha]^{24}_D$ +27.4° (*c* 1, DMF); MS *m/e* MH^+ 673. Anal. ($C_{38}H_{52}N_6O_5 \cdot {}^1/_2H_2O$) C, H, N.

Boc-Lys($COCH_2CMe_2CH_2CO$-D-Phe-OMe)-D-Trp-$NHCH_2CHMeEt$. The reaction of 3,3-dimethylglutaric anhydride (Aldrich; 1.42 g, 10 mmol) with D-PheOMe·HCl (2.16 g, 10 mmol), as described for the glutaric anhydride reaction above, and purification by silica gel chromatography provided $HO_2CCH_2CMe_2CH_2CO$-D-Phe-OMe as an oil: yield 3.1 g (96%). The structure was confirmed by NMR. A DCCI–HOBt coupling of $HO_2CCH_2CMe_2CH_2CO$-D-Phe-OMe (1.6 g, 5 mmol) and Boc-Lys-D-Trp-$NHCH_2CHMeEt$ (2.5 g, 5 mmol) with 1 equiv of triethylamine and purification by silica gel chromatography provided the product: yield 3.0 g (75%); mp 92–94 °C; R_{FP} 0.14, R_{FQ} 0.40, R_{FH} 0.63, R_{FK} 0.95. Anal. ($C_{44}H_{64}N_6O_8$) C, H, N.

Lys($COCH_2CMe_2CH_2CO$-D-Phe-OH)-D-Trp-$NHCH_2C$-HMeEt. The above Boc methyl ester (2.38 g, 2.96 mmol) was first saponified using 4 N NaOH in aqueous methanol, and after the usual workup, it was treated with 3 N HCl–AcOH to remove the Boc group. The product was obtained as a hygroscopic solid: yield 1.94 g (90%); mp 149–153 °C; R_{FA} 0.55, R_{FB} 0.63, R_{FC} 0.3, R_{FK} 0.8; MS *m/e* MH^+ 691. Anal. ($C_{38}H_{55}N_6O_6Cl \cdot H_2O$) C, H, N, Cl.

$COCH_2CMe_2CH_2CO$-D-Phe-Lys-D-Trp-$NHCH_2CHMeEt$ (10). The branched linear compound Lys($COCH_2CMe_2CH_2COPheOH$)-D-Trp-$NHCH_2CHMeEt$ (1.83 g, 2.5 mmol) was cyclized with diphenyl phosphorazidate as described previously. The product was purified by chromatography on silica gel using 2 and 3% methanol in chloroform followed by Sephadex LH-20 in DMF: yield 1.0 g (59%); mp 188–190 °C; MS *m/e* MH^+ 673. Anal. ($C_{38}H_{52}N_6O_5 \cdot {}^1/_2H_2O$) C, H, N.

Boc-Trp-isoamylamide. Boc-D-Trp (50 g, 164 mmol), isoamylamine (16 g, 184 mmol), and HOBt (22 g, 164 mmol) were dissolved in DMF (600 mL) and cooled to 4 °C. A solution of DCCI (37.2 g, 1.1 equiv) in DMF (50 mL) was added and the reaction mixture was left to stir at 4 °C for 2 days. Solids were removed by filtration and the residue after evaporation of the DMF was partitioned between ethyl acetate and water. The organic phase was washed with acid and base, dried ($MgSO_4$), and evaporated to a solid: yield 49 g (80%); mp 124–125 °C. Anal. ($C_{21}H_{31}N_3O_3$) C, H, N.

Boc-D-Phe-Lys($CO(CH_2)_2CMe_2CO_2H$)-OMe. Boc-D-Phe-Lys-OMe (4.54 g, 11.1 mmol, obtained by hydrogenolysis of Boc-D-Phe-Lys(Z)-OMe under standard conditions) was reacted with 2,2-dimethylglutaric anhydride (1.75 g, 12.3 mmol) and Et_3N (2.35 mL, 16.8 mmol) under conditions previously described for anhydride reactions. The crude product was purified by flash chromatography using 5% methanol in chloroform as eluant: yield 5.7 g (93%), glassy solid; R_{FP} 0.13, R_{FQ} 0.33, R_{FH} 0.58, R_{FK} 0.68; MS *m/e* MH^+ 550. Anal. ($C_{28}H_{43}N_3O_8 \cdot H_2O$) C, H, N.

$CO(CH_2)_2CMe_2CO$-D-Phe-Lys-OMe. The above Boc compound (5.5 g, 10 mmol) was deblocked with 3 N HCl–AcOH and the hydrochloride was precipitated by addition of ether: yield 4.59 g (94%). The linear peptide hydrochloride (4.45 g, 9.16 mmol) was cyclized by using the diphenyl phosphorazidate method described previously. The crude product was purified by silica gel chromatography using chloroform and 1 and 2% methanol in chloroform as eluants: yield 1.58 g (40%); mp 190–194 °C; MS *m/e* MH^+ 432. Anal. ($C_{23}H_{33}N_3O_5$) C, H, N.

$CO(CH_2)_2CMe_2CO$-D-Phe-Lys-OH. The cyclic peptide methyl ester (1.5 g, 3.47 mmol) from above, was saponified with 2 N NaOH in aqueous methanol. The organic solvent was evaporated, the aqueous solution was washed with ether and acidified with citric acid, and the product was extracted into ethyl acetate. This was dried ($MgSO_4$) and evaporated to a solid: yield 1.3 g (90%); mp 120–125 °C; R_{FK} 0.88, R_{FB} 0.57, R_{FC} 0.39. Anal. ($C_{22}H_{31}N_3O_5 \cdot H_2O$) C, H, N.

$CO(CH_2)_2CMe_2CO$-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$ (11). A DCCI–HOBt coupling of the cyclic acid $CO(CH_2)_2CMe_2CO$-D-Phe-Lys-OH (1.0 g, 2.39 mmol) and D-Trp-$NH(CH_2)_2CHMe_2$·HCl (825 mg, 2.66 mmol) with 1 equiv of triethylamine gave the crude product. This was partitioned between ethyl acetate and water and the organic phase washed in the standard way with acid and base. Purification by chromatography on silica gel using chloroform and 1, 2, and 4% methanol in chloroform gave the product: yield 320 g (20%); mp 230–233 °C; R_{FQ} 0.31, R_{FH} 0.63, R_{FK} 0.92; MS *m/e* MH^+ 673. Anal. ($C_{38}H_{52}N_6O_5 \cdot {}^1/_2H_2O$) C, H, N.

$HO_2CCH_2CHMeCH_2CO$-D-Phe-OMe. The reaction between 3-methylglutaric anhydride (Aldrich; 1.5 g, 11 mmol) and D-Phe-OMe·HCl (2.16 g, 10 mmol) and Et_3N (2.1 mL, 15 mmol) as described for the reaction with glutaric anhydride above gave the product as an oil which slowly crystallized on standing: yield 3.0 g (97%; mp 83–87 °C. The structure was confirmed by NMR. (MS *m/e* MH^+ 308, $C_{16}H_{21}NO_5$ requires MH^+ 308.)

Boc-Lys($COCH_2CHMeCH_2CO$-D-Phe-OMe)-D-Trp-$NHCH_2CHMeEt$. Boc-Lys-D-Trp-$NHCH_2CHMeEt$ (2.5 g, 5 mmol) and the above acid $HO_2CCH_2CHMeCH_2CO$-D-Phe-OMe (1.6 g, 5.5 mmol) were coupled via the DCCI–HOBt method. The product was purified by silica gel chromatography using chloroform and 1, 2, and 5% methanol in chloroform as eluants: yield 1.02 g (26%): mp 181–184 °C; MS *m/e* MH^+ 791. Anal. ($C_{43}H_{62}N_6O_8$) C, H, N.

Lys($COCH_2CHMeCH_2CO$-D-Phe-OH)-D-Trp-$NHCH_2C$-HMeEt·HCl. The above compound (860 mg, 1.08 mmol) was first saponified with 4 N NaOH in aqueous methanol and then treated with 3 N HCl–AcOH to remove the Boc group. The solid was collected in ether: yield 737 mg (95%); mp 158–162 °C dec; R_{FA} 0.47, R_{FB} 0.60, R_{FC} 0.30. (MS *m/e* MH^+ 677. $C_{37}H_{52}N_6O_6$·HCl requires MH^+ 677.)

$COCH_2CHMeCH_2CO$-D-Phe-Lys-D-Trp-$NHCH_2CHMeEt$ (12). The branched linear peptide Lys($COCH_2CHMeCH_2CO$-D-Phe-OH)-D-Trp-$NHCH_2CHMeEt$·HCl (815 mg, 1.14 mmol) was cyclized by the diphenyl phosphorazidate method as previously described. The crude product was partitioned between ethyl acetate and water and washed with acid and base in the standard way. Chromatography on Sephadex LH-20 in DMF followed by silica gel in chloroform and 3% methanol in chloroform gave the product: yield 150 mg (20%); mp >250 °C; $[\alpha]^{24}_D$ +39.88° (*c* 1, DMF); MS *m/e* MH^+ 659. Anal. ($C_{37}H_{50}N_6O_5$) C, H, N.

$HO_2CCH(Ph)(CH_2)_2CO$-D-Phe-OMe. The reaction between 2-phenylglutaric anhydride (Aldrich; 2.9 g, 15 mmol) and D-Phe-OMe·HCl (2.16 g, 10 mmol) with Et_3N (2.8 mL, 20 mmol) in DMF (30 mL) required overnight stirring for completion. The product was obtained after purification by silica gel chromatography using chloroform and 2% methanol in chloroform as eluants: yield 3.4 g (97%). Anal. ($C_{21}H_{23}NO_5$) C, H, N.

Z-Lys(Boc)-D-Trp-isoamylamide. Z-Lys(Boc)-OH (17.9 g, 46.9 mmol) was coupled to D-Trp-NH$(CH_2)_2CHMe_2$·HCl (14.5 g, 46.9 mmol) in DMF (250 mL) by the DCCI–HOBt method in presence of 1 equiv of Et_3N. The reaction mixture was filtered and the DMF removed by evaporation. The crude product was partitioned between ethyl acetate and water and the organic phase was washed with acid and base in the usual way, dried ($MgSO_4$), and evaporated: yield 23.6 g (76%); R_{FP} 0.28, R_{FQ} 0.60, R_{FK} 0.76. Anal. ($C_{35}H_{49}N_5O_6$) C, H, N.

Z-Lys-D-Trp-3-methylbutylamide·HCl. The above Boc compound (6.5 g, 10.2 mmol) was deblocked with 3 N HCl–AcOH. The product was obtained as a solid by ether trituration: yield 5.5 g (96%); mp 182–184 °C. Anal. ($C_{30}H_{41}N_6O_4Cl$) C, H, N, Cl.

Z-Lys(COCHPh$(CH_2)_2$CO-D-Phe-OMe)-D-Trp-NH-$(CH_2)_2CHMe_2$. Z-Lys-D-Trp-NH$(CH_2)_2CHMe_2$ (4.6 g, 8.04 mmol) and $HO_2CCHPh(CH_2)_2$CO-D-Phe-OMe (2.88 g, 8.04 mmol) were coupled by using the DCCI–HOBt method with 1 equiv of Et_3N. The reaction mixture was filtered and the DMF was removed by evaporation to leave a residue which was redissolved in DMF and filtered into dilute aqueous sodium bicarbonate with stirring and ice-bath cooling. The precipitated solid was collected by filtration and washed thoroughly with water. Purification by silica gel chromatography using chloroform and 2, 4, and 5% methanol in chloroform gave the product: yield 4.40 g (62%); mp 165–167 °C; MS m/e MH^+ 888. Anal. ($C_{51}H_{62}N_6O_8$) C, H, N.

Lys(COCHPh$(CH_2)_2$CO-D-Phe-OH)-D-Trp-NH-$(CH_2)_2CHMe_2$. The above Z, methyl ester (4.2 g, 4.7 mmol) was saponified with 2 N NaOH and then hydrogenolyzed over 5% Pd–C in aqueous ethanol: yield 3.18 g (91%); mp 135–138 °C; R_{FA} 0.68, R_{FB} 0.67, R_{FK} 0.88. Anal. ($C_{42}H_{54}N_6O_6$) C, H, N.

COCHPh$(CH_2)_2$CO-D-Phe-Lys-D-Trp-NH$(CH_2)_2CHMe_2$ (13). The above branched, linear peptide (3.08 g, 4.17 mmol) was cyclized by using the diphenyl phosphorazidate method, in DMF (350 mL). The reaction mixture was filtered and the DMF was removed by evaporation. The solid was extracted with the ethyl acetate (insoluble material was removed by filtration), the solvent was evaporated, and the crude product was purified by silica gel chromatography using chloroform and 1 and 2% methanol in chloroform as eluants. The product was obtained by precipitating a DMF solution into water: yield 517 mg (17%); mp 255–257 °C dec; $[\alpha]^{24}_D$ +23.71° (c 1, DMF); MS m/e MH^+ 721. Anal. ($C_{42}H_{52}N_6O_5·H_2O$) C, H, N.

Z-D-Phe-Lys(CO$(CH_2)_2$CHPhCO$_2$H)-D-Trp-NH-$(CH_2)_2CHMe_2$. The reaction between 2-phenylglutaric anhydride (Aldrich; 0.63 g, 3.3 mmol) and Z-D-Phe-Lys-D-Trp-NH-$(CH_2)_2CHMe_2$·HCl (2.0 g, 2.78 mmol) with Et_3N (0.58 mL, 4.14 mmol) in DMF was essentially as described for the reaction of glutaric anhydride with phenylalanine methyl ester. The solvent was removed by evaporation and ethyl acetate was added to the residue. Solid separated out which was filtered and washed with ethyl acetate, then suspended in water, filtered, and washed with water. The product was dried in a vacuum desicator over P_2O_5: yield 1.7 g (70%); mp 139–141 °C; R_{FH} 0.53, R_{FK} 0.83; MS m/e MH^+ 873. Anal. ($C_{50}H_{60}N_6O_8$) C, H, N.

CO$(CH_2)_2$CHPhCO-D-Phe-Lys-D-Trp-NH$(CH_2)_2CHMe_2$ (14). The above Z compound (1.5 g, 1.72 mmol) was hydrogenolyzed using ammonium formate (1.2 g) over 10% Pd–C in aqueous ethanol: yield 1.2 g (95%); mp 118–122°C; MS m/e MH^+ 739; R_{FA} 0.70, R_{FB} 0.68, R_{FC} 0.45, R_{FK} 0.80. The linear peptide (1.0 g, 1.35 mmol) was cyclized by using the diphenyl phosphorazidate method. The crude product was dissolved in DMF and purified by chromatography on Sephadex LH-20. Fractions taken from the column were checked by TLC and mass spectrometry. In this way it was possible to identify the required product and the cyclic urea among other byproducts. A final purification using chloroform and 1 and 2% methanol in chloroform as eluants on a silica gel column gave the product: yield 50 mg (5%); mp 128–130 °C; $[\alpha]^{24}_D$ +28.65° (c 1, DMF); MS m/e MH^+ 721. Anal. ($C_{42}H_{52}N_6O_5$) C, H, N.

Boc-D-Phe-Lys(COCH$_2$-Phe-OBzl)-D-Trp-NH-$(CH_2)_2CHMe_2$. *tert*-Butyl bromoacetate (32.4 mL, 200 mmol), in THF (50 mL), was added to a solution of Phe-OBzl (76.5 g, 300 mmol) and triethylamine (28.1 mL, 200 mmol) in THF (200 mL). The reaction mixture was left stirring for 4 days and was then concentrated in vacuo. The residue, after normal workup, was purifid by silica gel chromatography to leave an oil which was homogeneous on TLC: yield 68.5 g (92.8%); MS m/e MH^+ 370. The oil (30 g, 81 mmol) was dissolved in 2 N HCl in ethyl acetate (50 mL) and the solution was left at room temperature for 2 h. The ethyl acetate was evaporated in vacuo and the product was used in the next step without purification.

$HOOCCH_2$Phe-OBzl·HCl (4.3 g, 13.4 mmol) and Boc-D-Phe-Lys-D-Trp-NH$(CH_2)_2CHMe_2$ (13.4 mmol) were coupled by the DCCI–HOBt method. Purification by silica gel column chromatography gave the pure peptide: yield 3.6 g (28.6%); mp 88–90 °C; $[\alpha]^{25}_D$ +13.0° (c 2.73, DMF). (MS m/e MH^+ 944. $C_{54}H_{69}N_7O_8$ requires MH^+ 944.)

COCH$_2$NHCH(CH$_2$Ph)CO-D-Phe-Lys-D-Trp-NH-$(CH_2)_2CHMe_2$ (15). The above linear peptide (3.6 g, 3.8 mmol) was first hydrogenolyzed over 5% Pd–C to cleave the benzyl ester group and then it was treated with HCl in acetic acid to cleave the Boc group. The free peptide was then dissolved in DMF (1500 mL) and the solution was cooled to 4 °C. $NaHCO_3$ (2.2 g, 26.6 mmol) and diphenyl phosphorazidate (0.9 mL, 4.18 mmol) were added, and the reaction mixture was stirred at 4 °C for 3 days. After normal workup the crude peptide was purified by silica gel column chromatography: yield 700 mg (25.1%); mp 267–268 °C, $[\alpha]^{25}_D$ + 54.7° (c 3.08, DMF). (MS m/e MH^+ 736. $C_{42}H_{53}N_7O_5$ requires MH^+ 736.)

COCH$_2$NH-D-CH(CH$_2$Ph)CO-D-Phe-Lys-D-Trp-NHCH$_2$CH$_2$CHMe$_2$ (16). The synthesis of this compound was achieved by the method described above for compound **17** except that D-Phe-OBzl was used in the synthesis in place of Phe-OBzl: yield 15.8% mp 134–136 °C; $[\alpha]^{25}_D$ +24.7° (c 1.53 in DMF); MS m/e MH^+ 736. Anal. ($C_{42}H_{53}N_7O_5$) C, H, N.

BzlOOC-L-CH(CH$_2$Ph)NHCH$_2$CO-D-Phe-Lys(Z)-D-Trp-NHCH$_2$CH$_2$CHMe$_2$. $HOOCCH_2$-Phe-OBzl·HCl (7.1 g, 20.3 mmol) was coupled to D-Phe-Lys(Z)-D-Trp-$NHCH_2CH_2CHMe_2$·HCl (14.4 g, 20.3 mmol) in presence of triethylamine (5.8 mL, 41 mmol) by the DCCI–HOBt method. The crude product was purified by silica gel chromatography: yield 4.8 g (24.2%); mp 146–147 °C. (MS m/e MH^+ 978. $C_{57}H_{67}N_7O_8$ requires MH^+ 978.)

Phe-CH$_2$CO-D-Phe-Lys-D-Trp-NH(CH$_2$)$_2$CHMe$_2$ (17). The above linear peptide (4.3 g, 4.4 mmol) was hydrogenolyzed with 5% Pd–C as a catalyst to yield HOOCCH(CH_2Ph)-$NHCH_2CO$-D-Phe-Lys-D-Trp-NH$(CH_2)_2CHMe_2$: mp 140–142 °C, $[\alpha]^{25}_D$ +48.76° (c 1.05, DMF); MS m/e MH^+ 754. It was cyclized by the diphenyl phosphorazidate method. Normal workup procedure followed by silica gel column chromatography using chloroform and 2% methanol in chloroform as eluants gave pure cyclic peptide **17**: yield 325 mg (10%); mp 290–291 °C; $[\alpha]^{25}_D$ +32.5° (c 2.2, DMF); MS m/e MH^+ 736. Anal. ($C_{42}H_{53}N_7O_5$) C, H, N.

BzlOOC-D-CH(CH$_2$Ph)NHCH$_2$CO-D-Phe-Lys(Z)-D-Trp-NH(CH$_2$)$_2$CHMe$_2$. The synthetic route used for this compound was similar to that described above for the corresponding L-phenylalanine-containing analogue: yield 25.5%; mp 145–146 °C. (MH^+ 978. $C_{57}H_{67}N_7O_8$ requires MH^+ 978.)

D-Phe-CH$_2$CO-D-Phe-Lys-D-Trp-NH-(CH$_2$)$_2$CHMe$_2$ (18). Compound **18** was prepared from HOOCCH(CH_2Ph)-$NHCH_2CO$-D-Phe-Lys-D-Trp-NH$(CH_2)_2CHMe_2$ (3.01 g, 4 mmol) by the procedure described above for compound **17**; yield 5.6%; mp 287–288 °C; $[\alpha]^{25}_D$ +33.65° (c 1.6, DMF): MS m/e MH^+ 736. Anal. ($C_{42}H_{53}N_7O_5$) C, H, N.

***N*-(Carboxymethyl)-L-isoleucine Benzyl Ester Hydrochloride.** A solution of *tert*-butyl bromoacetate (21.4 g, 110 mmol) in THF (100 mL) was added dropwise over 45 min to a solution of Ile-OBzl·TosOH (39.35 g, 100 mmol) and Et_3N (28 mL, 200 mmol) in THF (500 mL). After stirring at room temperature for 6 days, the reaction mixture was filtered and the filtrate was evaporated. The residue, in ethyl acetate, was washed several times with a saturated NaCl solution, dried ($MgSO_4$), and evaporated. Purification by silica gel chromatography using chloroform as eluant gave $Bu^tO_2CCH_2$-Leu-OBzl·TosOH as a mobile liquid: yield 24 g (72%); R_{FP} 0.60, R_{FQ} 0.65. This product (24 g, 72 mmol) was treated with 3 N HCl–EtOAc for 1 h and the

N-carboxymethyl derivative was obtained as an oil by evaporation of the ethyl acetate and triturating with ether: yield 18.5 g (82%); R_{FQ} 0.23, R_{FH} 0.39, R_{FK} 0.71.

Z-D-Phe-Lys($COCH_2$-Ile-OBzl)-D-Trp-$NH(CH_2)_2CHMe_2$. Z-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$·HCl (2.5 g, 3.48 mmol) was coupled to HO_2CCH_2-Ile-OBzl·HCl (1.63 g, 5.08 mmol) in DMF using the DCCI–HOBt method in the presence of Et_3N (1.21 mL, 8.64 mmol). After the normal workup procedure the crude product was purified by silica gel chromatography using chloroform and 2, 4, and 10% methanol in chloroform as eluant: yield 2.54 g (77%); mp 126–128 °C; R_{FQ} 0.50, R_{FH} 0.71, R_{FK} 0.94; MS *m/e* MH^+ 944. Anal. ($C_{54}H_{69}N_7O_8$) C, H, N.

D-Phe-Lys($COCH_2$-Ile-OH)-D-Trp-$NH(CH_2)_2CHMe_2$. The above Z, benzyl ester (2.3 g, 2.4 mmol) was hydrogenolyzed over 10% Pd–C in aqueous ethanol: yield 1.7 g (97%); mp 132–136 °C; MS *m/e* MH^+ 720. Anal. ($C_{39}H_{57}N_7O_6$) C, H, N.

$COCH_2$-Ile-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$ (19). The above product (1.65 g, 2.3 mmol) was cyclized by the diphenyl phosphorazidate method. The crude product was chromatographed on a column of Sephadex LH-20 in DMF. Product-containing fractions (identified by mass spectrometry) were combined and purified further by silica gel chromatography using chloroform and 2, 4, 6, and 10% methanol in chloroform as eluants. The product was obtained as a freeze-dried solid from aqueous *tert*-butanyl alcohol: yield 170 mg (10%); $[\alpha]^{24}_D$ +51.5% (*c* 1, DMF; MS *m/e* MH^+ 702. Anal. ($C_{39}H_{55}N_7O_5·H_2O$) C, H, N.

BzlO$_2$C-L-CH(CHMeEt)NHCH$_2$CO-D-Phe-Lys(Z)-D-Trp-$NH(CH_2)_2CHMe_2$. D-Phe-Lys(Z)-D-Trp-$NH(CH_2)_2CHMe_2$·HCl (1.4 g, 1.95 mmol) was reacted with HO_2CCH_2-Ile-OBzl·HCl (923 mg, 2.92 mmol) by the DCCI–HOBt method in presence of Et_3N (0.69 mL, 2.5 equiv). After the standard workup procedure, the crude product was purified by silica gel chromatography using chloroform and 1, 2, and 3% methanol in chloroform as eluants: yield 1.59 g (86%); mp 164–166 °C; MS *m/e* MH^+ 944. Anal. ($C_{54}H_{69}N_7O_8$) C, H, N.

HO$_2$CCH(CHMeEt)NHCH$_2$CO-D-Phe-Lys-D-Trp-NH-$(CH_2)_2CHMe_2$. The above Z, benzyl ester compound (1.45 g, 1.5 mmol) was hydrogenolyzed over 10% Pd–C in aqueous ethanol. The product was obtained as a solid by ether trituration: yield 1.06 g (96%); mp 146–150 °C; MS *m/e* MH^+ 720. Anal. ($C_{39}H_{57}N_7O_6·{}^1/_2H_2O$) C, H, N.

Ile-CH_2CO-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$ (20). The above linear peptide (950 mg, 1.32 mmol) was cyclized by using the diphenyl phosphorazidate method. Purification of the crude product by chromatography first on Sephadex LH-20 in DMF, then on silica gel using chloroform and 2, 3, 4, and 5% methanol in chloroform as eluants gave the required product: yield 85 mg (9%); mp >250 °C; MS *m/e* MH^+ 702. Anal. ($C_{39}H_{55}N_7O_5$) C, H, N.

***N*-(Carboxymethyl)-D-Leu-OBzl·HCl.** Bu^{t}O$_2$CCH$_2$-D-Leu-OBzl was prepared as for the isoleucine analogue above. It was obtained as an oil after silica gel chromatography (yield 35%; MS *m/e* MH^+ 336). The diester derivative (5.2 g, 15.5 mmol) was treated with 3 N HCl–EtOAc. The residue from evaporation of the solvent was triturated several times with ether to give the product as a solid: yield 4.65 g (95%); mp 133–135 °C; MS *m/e* MH^+ 280. Anal. ($C_{15}H_{22}NO_4Cl$) C, H, N, Cl.

Z-D-Phe-Lys($COCH_2$-D-Leu-OBzl)-D-Trp-$NH(CH_2)_2CHMe_2$. Z-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$·HCl (2.5 g, 3.48 mmol) and HO_2CCH_2-D-Leu-OBzl·HCl (1.6 g, 5.06 mmol) were coupled by the DCCI–HOBt method in presence of Et_3N. The product was purified by silica gel chromatography using chloroform and 2 and 5% methanol in chloroform as eluants: yield 2.87 g (87%); mp 127–129 °C; MS *m/e* MH^+ 944. Anal. ($C_{54}H_{69}N_7O_8$) C, H, N.

D-Phe-Lys($COCH_2$-D-Leu-OH)-D-Trp-$NH(CH_2)_2CHMe_2$·HCl. The above Z compound (2.7 g, 2.9 mmol) was hydrogenolyzed over 10% Pd–C in aqueous ethanol. The product came out of solution as the reaction progressed. The addition of 1 N HCl (2.8 mL, 1 equiv) took the product back into solution. The catalyst was removed by filtration and on evaporation of the solvent the product was obtained as a solid: yield 2.0 g (92%); mp 178–181 °C; MS *m/e* MH^+ 720. Anal. ($C_{39}H_{57}N_7O_6$·HCl) C, H, N, Cl.

$COCH_2$-D-Leu-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$ (21). The above branched, linear peptide (2.12 g, 2.80 mmol) was cyclized by using the diphenyl phosphorazidate method. A portion of the crude product was purified by chromatography first on Sephadex LH-20 in DMF, then on silica gel. The product was obtained as a freeze-dried powder from aqueous *tert*-butanyl alcohol: yield 123 mg (6%): mp 142–146 °C; MS *m/e* MH^+ 702. Anal. ($C_{39}H_{55}N_7O_5·H_2O$) C, H, N.

PhCH$_2$O$_2$C-D-CH(CH$_2$CHMe$_2$)NHCH$_2$CO-D-Phe-Lys(Z)-D-Trp-$NH(CH_2)_2CHMe_2$. D-Phe-Lys(Z)-D-Trp-$NH(CH_2)_2CHMe_2$·HCl (1.4 g, 1.95 mmol) and HO_2CCH_2-D-Leu-OBzl·HCl (923 mg, 2.93 mmol) were coupled by using the DCCI–HOBt method in presence of Et_3N (0.69 mL, 2.5 equiv). The crude product was purified by silica gel chromatography using chloroform and 2 and 5% methanol in chloroform as eluants: yield 1.72 g (93%); mp 182–184 °C. (MS *m/e* MH^+ 944. $C_{54}H_{69}N_7O_8$ requires MH^+ 944.)

HO$_2$C-D-CH(CH$_2$CHMe$_2$)NHCH$_2$CO-D-Phe-Lys-D-Trp-NH-$(CH_2)_2CHMe_2$·HCl. The above Z, benzyl ester (1.6 g, 1.69 mmol) was hydrogenolyzed over 10% Pd–C in aqueous ethanol. One equivalent of 1 N HCl (1.7 mL) was added before the catalyst was removed. On evaporation of the solvent, the product was obtained as a solid: yield 1.17 g (91%); mp 200–202 °C; MS *m/e* MH^+ 720. Anal. ($C_{39}H_{58}N_7O_6Cl$) C, H, N, Cl.

D-Leu-CH_2CO-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$ (22). The linear peptide (1.05 g, 1.39 mmol) was cyclized by the diphenyl phosphorazidate method in DMF (250 mL). The crude product was purified by chromatography on Sephadex LH-20 in DMF, then on Sephadex G-25 in 50% AcOH and finally on silica gel using chloroform and 2, 4, and 5% methanol in chloroform: yield 60 mg (60%); mp 240–245 °C; MS *m/e* MH^+ 702. Anal. ($C_{39}H_{55}N_7O_5$) C, H, N.