Novel Inhibitors of Human Renin. Cyclic Peptides Based on the Tetrapeptide Sequence Glu-D-Phe-Lys-D-Trp

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

ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire SK10 4TG, U.K. *Received September 26, 1989*

Cyclic peptide inhibitors of human renin based on a linear peptide, Boc-D-Phe-Cys(Acm)-D-Trp-Leu-OMe (1), were prepared by solution-phase methods. Potent inhibitors were obtained in one series of compounds, Z-Glu-D-Phe-Lys-D-Trp-Leu-OMe (3), in which the D-phenylalanine residue was incorporated in a 15-membered ring structure. Any reductions or enlargements of the ring size led to inactive or less potent peptides. The most potent inhibitor of human renin, Me_3CCH_2-Glu-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$ (31) (IC_{50} 6.3×10^{-8} M), was obtained by changing N- and C-terminal parts of pentapeptide 3. It was about 650-fold more potent than linear tetrapeptide 1 and about 50-fold more potent than cyclic peptide 3. Compound 31 was also 112-fold more potent against human renin than against porcine renin.

Inhibitors of renin reported so far in the literature have been based on the angiotensinogen tetradecapeptide substrate, pepstatin or fragments of the pro-renin sequence. These have been obtained either by reducing the peptide bond between Leu^{10} and Leu^{11} to a $-CH_2NH-$ group or by substituting a leucine residue by a statine type of an amino acid residue.[1-6]

On the basis of the available structure–activity data on the substrate-based inhibitors mentioned above, it would appear that the enzyme can accommodate two hydrophobic residues in the P_1 and P_1' positions and these two residues may be either (a) two L-amino acid residues, e.g. Leu^{10}-Leu^{11} in the substrate or Phe-Phe in an inhibitor, or (b) one or both of these may be D-amino acid residues. The two α-C atoms bearing these hydrophobic side chains may be separated by an amide bond, a $-CH_2NH-$ group, or a $-CH(OH)CH_2-$ group. The distance between the two α-C atoms does not seem to be critical. This is probably due to the fact that the molecule is able to fold in such a manner that the hydrophobic residues can still interact with the enzyme.

We therefore tested a few analogues of the -D-Phe-Cys(Acm)-D-Trp- type (prepared for another research program) as inhibitors of renin on the basis of the above information. The compounds had two hydrophobic residues (D-Phe, D-Trp) and the distance between the α-C atoms of these was similar to that of the groups mentioned above. One of the analogues, Boc-D-Phe-Cys(Acm)-D-Trp-Leu-OMe (1), was found to inhibit human renin (IC_{50} = 40 μM). A number of analogues of this tetrapeptide were then prepared in the hope of improving the potency, but only a few analogues, e.g. Boc-D-Phe-Lys-D-Trp-Leu-OMe (2, $IC_{50} \approx 30$ μM), Boc-α-Me-D-Phe-Cys(Acm)-D-Trp-Leu-OMe (IC_{50} = 10 μM), and Boc-D-Phe-Cys(Acm)-D-Trp-Ile-OMe (IC_{50} = 6.5 μM), showed improved potency. Some of the pentapeptide derivatives, e.g. Boc-D-Phe-Cys(Acm)-D-Trp-Leu-Ser-OMe (IC_{50} = 3.2 μM), were also more potent.[7]

A number of cyclic peptide analogues were then prepared with the objective of finding more stable and potent analogues which may lead to useful antihypertensive drugs. These new results are reported here. In designing the cyclic analogues the following main findings from the earlier work were considered. (a) The D-Phe and the D-Trp residues of the tetrapeptide were very important for the biological activity, (b) the C-terminal Leu-OMe and the N-terminal Boc group could be replaced by various other groups, and (c) the Cys(Acm) residue could be replaced by a Lys or an Orn residue. On the basis of this information, we prepared cyclic peptides based around the tripeptide (D-Phe-Lys-D-Trp) unit which retains the more important features of 1 and 2 (D-Phe and D-Trp residues) and also contains a Lys residue which could be used for cyclization purposes. In one class of cyclic peptides, a glutamic acid residue was incorporated at the N-terminus to give compounds of the Z-Glu-D-Phe-Lys-D-Trp-Leu-OMe (3) type. In these analogues the D-Phe residue was part of a ring structure and, therefore, the conformational flexibility around this residue was much less compared to that of the D-Trp-Leu-OMe residue. In the other type of analogues, e.g. Z-D-Phe-Lys-D-Trp-Leu (35), the reverse was true; i.e. the D-Phe residue had more conformational flexibility than the D-Trp residue.

Some pentapeptide analogues of the type X-D-Phe-Cys(Acm)-D-Trp-Leu were also synthesized. These were based on the reported observations that the peptides with alternating D- and L-amino acid residues exist as hairpin-bend structures.[8] Such structures are likely to have the N- and the C-terminal ends of the peptides near to each other. Therefore, linking these two ends may hold the peptide into its biologically active conformation.

The analogues reported here are listed in Table I along with their renin inhibitory activities. The inhibitory potency of each analogue was determined with partially purified human renal renin and angiotensinogen purified from volunteer plasma (see the Experimental Section for details). The analogues were initially tested (twice) at a high concentration (100 μM) and, for compounds not fully active at this concentration, only the percent inhibition

(1) Kobubu, T.; Hiwada, K. *Drugs Today* **1987**, *23*, 101.
(2) Greenlee, W. J. *Pharmaceutical Res.* **1987**, *4*, 364.
(3) Wood, J. M.; Criscione, L.; de Gasparo, M.; Buhlmayer, P.; Rueger, H.; Stanton, J. L.; Jupp, R. A.; Kay, J. *J. Cardiovasc. Pharmacol.* **1989**, *14*, 221.
(4) Tree, M.; Szelke, M.; Leckie, B.; Atrash, B.; Donovan, B.; Hallett, A.; Jones, D. M.; Lever, A. F.; Morton, J. J.; Sueiras-Diaz, J.; Manhem, P.; Robertson, J. I.; Webb. D. *J. Cardiovasc. Pharmacol.* **1985**, *7* (suppl. 4), S49.
(5) Bock, M. G.; DiPardo, R. M.; Evans, B. E.; Freidinger, R. M.; Rittle, K. E.; Payne, L. S.; Boger, J.; Whitter, W. L.; LaMont, B. I.; Ulm, E. H.; Blaine, E. H.; Schorn, T. W.; Veber. D. F. *J. Med. Chem.* **1988**,, *31*, 1918.
(6) Smith, C. W.; Saneii, H. H.; Sawyer, T. K.; Pals, D. T.; Scahill, T. A.; Kamdar, B. V.; Lawson, J. A. *J. Med. Chem.* **1988**, *31*, 1377.
(7) Dutta, A. S.; Gormley, J. J.; McLachlan, P. F.; Major, J. S. *J. Chem. Res. (S)* **1990**, 2.
(8) Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C. *Biochem. Biophys. Res. Commun.* **1982**, *107*, 910.

0022-2623/90/1833-2552$02.50/0

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

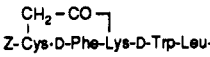
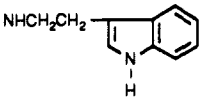
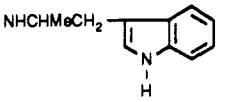
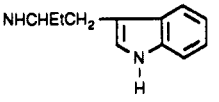
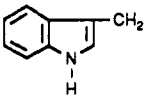
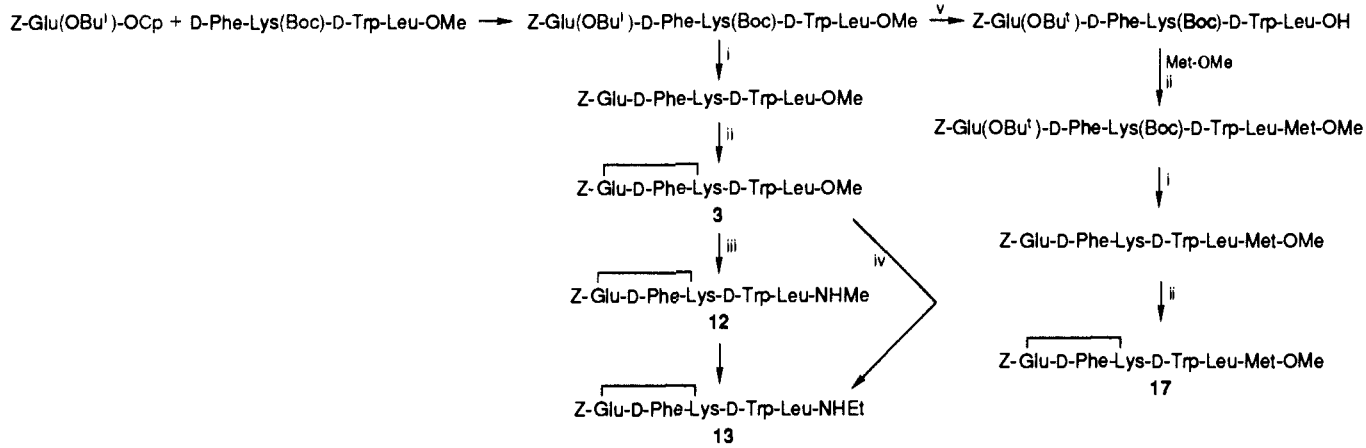
no.	compd	inhibition of human renin: IC_{50}, μM	inhibition of human renin: % inhibn at 100 μM
1	Boc-D-Phe-Cys(Acm)-D-Trp-Leu-OMe	40	
2	Z-D-Phe-Lys-D-Trp-Leu-OMe	31	
3	Z-Glu-D-Phe-Lys-D-Trp-Leu-OMe	3.2	
4	Z-Lys-D-Phe-Glu-D-Trp-Leu-OMe		inactive
5	Z-Asp-D-Phe-Lys-D-Trp-Leu-OMe		32, 45
6	Z-Glu-D-Phe-Orn-D-Trp-Leu-OMe		42, 56
7	Z-Asp-D-Phe-Orn-D-Trp-Leu-OMe		inactive
8	Boc-Cys-D-Phe-Cys-D-Trp-Leu-Met-OMe		22, 44
9			53
	Z-Glu-D-Phe-Lys-D-Trp-X		
10	X = OMe		69.2, 69.8
3	Leu-OMe	3.2	
11	Cha-OMe		67, 76
12	Leu-NHMe	0.34	
13	Leu-NHEt	0.85	
14	Ile-NHMe	0.81	
15	$NHCH_2CMe_3$	1.7	
16	$NHCH_2CH_2CHMe_2$	0.87	
17	Leu-Met-OMe	0.45	
18	Leu-D-Met-OMe	0.29	
19	Leu-D-Ala-OMe	0.32	
20	Leu-Ser-OMe	0.40	
21	Leu-Pro-OMe		76 at 10 μM
	Boc-Glu-D-Phe-Lys-X		
22	X = OMe		inactive
23	NH_2		inactive
24			79, 86
25			33, 37
26			41, 52
	X-Glu-D-Phe-Lys-D-Trp-$NHCH_2CH_2CHMe_2$		
27	X = H	0.71	
28	Boc	0.21	
16	Z	0.87	
29	$C_6H_5OCH_2CO$	0.43	
30	Me_2CH		93, 97
			30 at 1 μM
31	Me_3CCH_2	0.063	
32	$EtCHMeCH_2$	0.13	
33	Bzl	0.52	
34		1.9	
35	Z-D-Phe-Lys-D-Trp-Leu		inactive
36	D-Phe-Lys-D-Trp-Leu		inactive
	X-D-Phe-Cys(Acm)-D-Trp-Leu		
37	X = Gly		inactive
38	Sar		inactive
39	Pro		38, 52
40	Cys(Acm)		56, 70

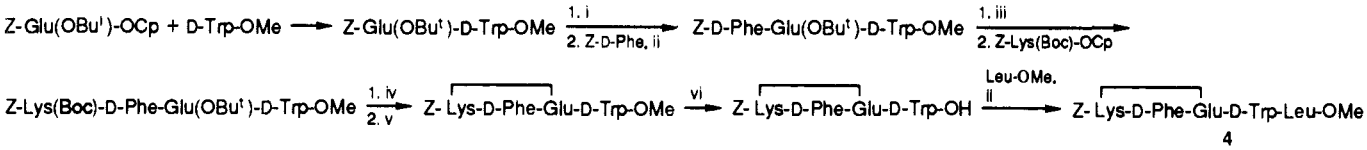
figure is recorded in Table I. Further testing (IC_{50} determinations) was only carried out on analogues which completely inhibited human renin at the high concentration and for these analogues the IC_{50} values are listed in Table I. Pepstatin was used as a reference compound in each assay. The standard deviation in the IC_{50} values for pepstatin (29.3 μM) was 10.5 μM in over 300 determinations, indicating an interassay coefficient of variation of

Scheme I. Syntheses of Compounds 3, 12, 13, and 17[a]



[a] Reagents: (i) HCl–acetic acid; (ii) DCCI–HOBt; (iii) CH_3NH_2; (iv) $C_2H_5NH_2$; (v) NaOH.

Scheme II. Synthesis of Compound 4[a]



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

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

no.	IC_{50}, μM		ratio of porcine/ human renin
	porcine	human renin	
16	5.8	0.87	6.6
19	1.1	0.32	3.4
20	1.1	0.40	2.7
27	46.0	0.71	64.7
28	8.5	0.21	40.4
29	8.2	0.43	19.2
31	7.1	0.063	112.6
33	8.2	0.52	15.7

35.7. 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

Analogues 3, 5–7, 12, 13, and 17 were prepared by the procedures outlined in Scheme I. Z-Glu(OBu^t)-OCp was reacted with D-Phe-Lys(Boc)-D-Trp-Leu-OMe to give the protected pentapeptide. The side chain protecting groups of this pentapeptide were cleaved and the peptide cyclized by the DCCI–HOBt method[9] to give Z-Glu-D-Phe-Lys-D-Trp-Leu-OMe (3). Compound 5, Z-Asp-D-Phe-Lys-D-Trp-Leu-OMe, was prepared in a similar way except that Z-Asp(OBu^t)-OCp was used in the first step in place of Z-Glu(OBu^t)-OCp. Compounds 6 and 7 were prepared by the above procedures from D-Phe-Orn(Boc)-D-Trp-Leu-OMe and Z-Glu(OBu^t)-OCp or Z-Asp(OBu^t)-OCp.

Compound 8, Boc-Cys-D-Phe-Cys-D-Trp-Leu-Met-OMe, was prepared from Boc-Cys(Acm)-D-Phe-Cys(Acm)-D-Trp-Leu-OMe. The methyl ester group of the linear pentapeptide was cleaved by saponification and the peptide was then coupled to Met-OMe by the DCCI–HOBt method. The resulting hexapeptide derivative was converted to 8 by an iodine in methanol treatment.[10]

Z-Cys(CH_2COOBu^t) required for the synthesis of compound 9 was prepared by the reaction of cysteine with *tert*-butyl bromoacetate followed by a reaction with benzyloxycarbonyl chloride. It was reacted with D-Phe-Lys(Boc)-D-Trp-Leu-OMe by the DCCI–HOBt method to give Z-Cys(CH_2COOBu^t)-D-Phe-Lys(Boc)-D-Trp-Leu-OMe. The pentapeptide derivative, after an HCl–acetic acid treatment, was converted to 9 by a cyclization reaction using diphenyl phosphorazidate.[11]

The synthetic route to compound 4 is shown in Scheme II.

Scheme III shows the syntheses of compounds 10, 11, 14–16, and 27–34. The cyclic tetrapeptide Z-Glu-D-Phe-Lys-D-Trp-OMe (10) was saponified to yield Z-Glu-D-Phe-Lys-D-Trp-OH. The tetrapeptide acid was then reacted with the required alkylamine, amino acid, or dipeptide derivative to give compounds 11 and 14–16. Compound 16 was hydrogenolyzed to give Glu-D-Phe-Lys-D-Trp-NH$(CH_2)_2CHMe_2$ (27), which on reaction with Boc-O-Boc or a chloroformate gave compounds 28 and 29. The other analogues (30–34) were obtained by the reductive alkylation of 27 with the required aldehyde or ketone and sodium cyanoborohydride.

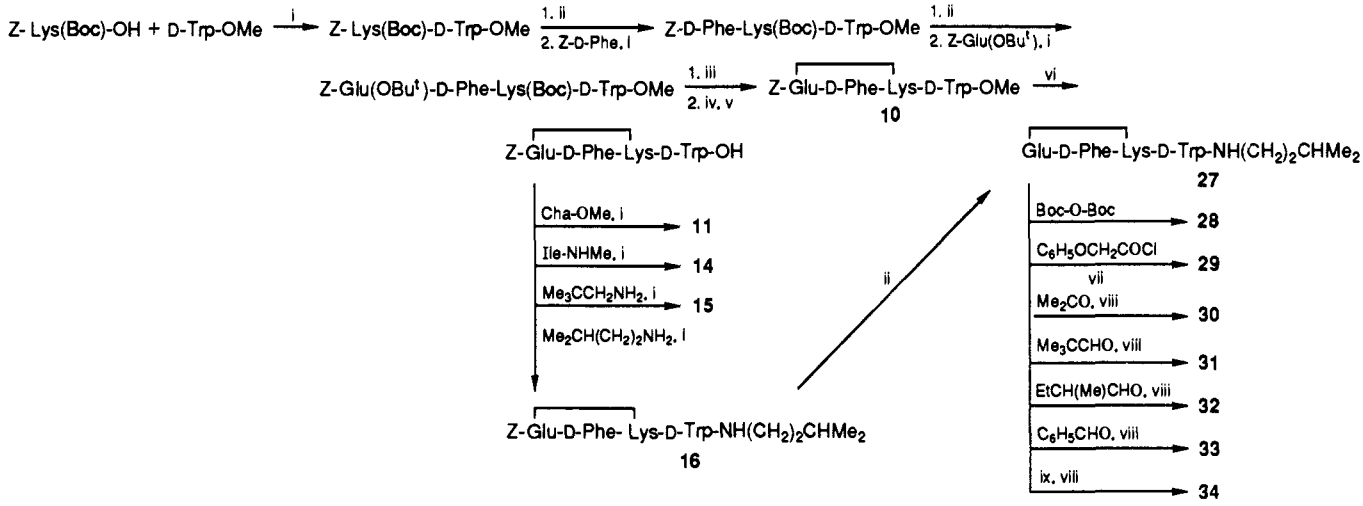
Compounds 18–21 were prepared from 3. The C-terminal methyl ester group of 3 was cleaved by saponification

(9) Konig, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.

(10) Kamber, B.; Hartmann, A.; Eisler, K.; Riniker, B.; Rink, H.; Sieber, P.; Rittel, W. *Helv. Chim. Acta* **1980**, *63*, 899.

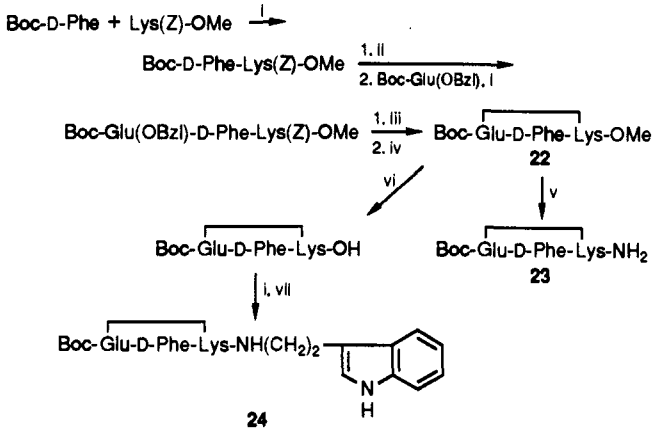
(11) Brady, S. F.; Freidinger, R. M.; Paleveda, W. J.; Colton, C. D.; Homnick, C. F.; Whitter, W. L.; Curley, P.; Nutt, R. F.; Veber, D. F. *J. Org. Chem.* **1987**, *52*, 764.

Scheme III. Syntheses of Compounds **10**, 11, 14–16, and **27–34**[a]



[a] Reagents: (i) DCCI-HOBt; (ii) 10% Pd-C, ammonium formate; (iii) HCl–acetic acid; (iv) $NaHCO_3$; (v) diphenyl phosphorazidate; (vi) NaOH; (vii) triethylamine; (viii) $NaBH_3CN$; (ix) indole-3-carboxyaldehyde.

Scheme IV. Syntheses of Compounds **22–24**[a]



[a] Reagents: (i) DCCI–HOBt; (ii) HCl–acetic acid; (iii) H_2, Pd–C; (iv) diphenyl phosphorazidate; (v) NH_3/methanol; (vi) NaOH; (vii) tryptamine.

and the resulting peptide was coupled to D-Met-OMe by the DCCI–HOBt method to give Z-Glu-D-Phe-Lys-D-Trp-Leu-D-Met-OMe (**18**). Similar reactions with D-Ala-OMe, Ser-OMe, or Pro-OMe gave Z-Glu-D-Phe-Lys-D-Trp-Leu-D-Ala-OMe (**19**), Z-Glu-D-Phe-Lys-D-Trp-Leu-Ser-OMe (**20**), and Z-Glu-D-Phe-Lys-D-Trp-Leu-Pro-OMe (**21**), respectively.

Analogues **22–26** were prepared by the route shown in Scheme IV. The DCCI–HOBt-mediated coupling of Boc-Glu-D-Phe-Lys-OH (obtained from **22**) with the required amine gave compounds **24–26**.

Cyclic peptide **35**, Z-D-Phe-Lys-D-Trp-Leu, was prepared from Z-D-Phe-Lys-D-Trp-Leu-OMe. After cleaving of the methyl ester group, the peptide was cyclized by the diphenyl phosphorazidate method to give **35**. Catalytic hydrogenolysis of **35** gave D-Phe-Lys-D-Trp-Leu (**36**).

Cyclic pentapeptide derivatives **37–40** were obtained by first deprotecting the C-terminal end of the protected linear peptide, followed by the deprotection of the N-terminal protecting group and cyclization of the resulting peptide. Cyclization in the case of compound **39** was achieved by the DCCI–HOBt method. All the others were cyclized by the diphenyl phosphorazidate method.

Results and Discussion

As mentioned above, three types of cyclic peptides were prepared. In the first series of analogues, e.g. Z-Glu-D-Phe-Lys-D-Trp-Leu-OMe (**3**), the cyclization was achieved by forming a ring between the side chains of the amino acid residues on either side of the D-Phe residue. In the second series, e.g. Z-D-Phe-Lys-D-Trp-Leu (**35**), the side-chain amino group of the Lys residue was reacted with the carboxyl group of the C-terminal leucine residue. Finally, a series of cyclic peptides, e.g. Gly-D-Phe-Cys(Acm)-D-Trp-Leu (**37**), in which the C-terminal carboxyl group was reacted with the N-terminal amino group, were prepared. Only the first series of analogues showed potent renin inhibitory activity. Of the other two series of analogues Z-D-Phe-Lys-D-Trp-Leu (**35**), D-Phe-Lys-D-Trp-Leu (**36**), and X-D-Phe-Cys(Acm)-D-Trp-Leu (X = Gly, Sar for **37**, **38**) were inactive and X-D-Phe-Cys(Acm)-D-Trp-Leu (X = Pro, Cys(Acm) for **39**, **40**) were very weak inhibitors of renin (38–70% inhibition at 100 μM).

On the basis of the above results, further work was restricted to the first series of analogues. In comparison to the linear tetrapeptides (**1** and **2**), the cyclic pentapeptide **3** was 10-fold more potent in inhibiting human renin. When the positions of the Glu and Lys residues were interchanged, the resulting analogue Z-Lys-D-Phe-Glu-D-Trp-Leu-OMe was inactive. These two analogues (**3** and **4**) have the same ring size and amino acid composition, but the position and the direction of the side chain amide bond is different. This difference probably results in significant conformational changes. Alterations of the ring size were then attempted in order to optimize the binding of the cyclic peptide **3** to renin. Analogues **5** and **6** obtained by replacing the Glu by Asp or the Lys by Orn, had one $-CH_2-$ group less in the ring compared to **3**. Both of these analogues (**5** and **6**) were at least 30-fold less potent than **3**. Compound **7**, with two $-CH_2-$ groups less in the ring, was inactive. Further reduction in the ring size also led to very poor inhibitors of renin. In one such poor inhibitor, Boc-Cys-D-Phe-Cys-D-Trp-Leu-Met-OMe (**8**), the ring was formed by a disulfide bridge instead of an amide bond.

Slight enlargement of the ring (9) also resulted in a significant reduction in potency.

In the remaining analogues (10–34), the cyclic part of compound 3, Glu-D-Phe-Lys, was retained and the rest was modified systematically in the hope of improving the renin inhibitory potency. The following conclusions can be drawn from the results.

a. The insertion of another amino acid residue, e.g. Met (17), D-Met (18), D-Ala (19), and Ser (20), between the leucine residue and the methyl ester group resulted in analogues which were about 10-fold more potent than 3. Analogue with a Pro residue in this position (21) was some what less potent than 3.

b. The beneficial effect of the C-terminal methyl or ethylamide groups was observed in the pentapeptide series of analogues. Z-Glu-D-Phe-Lys-D-Trp-Leu-NHMe (12) and Z-Glu-D-Phe-Lys-D-Trp-Leu-NHEt (13) were 5–10-fold more potent than the C-terminal methyl ester analogue 3. The leucine residue in the pentapeptide series of analogues could also be replaced by an Ile residue (14) without much loss in potency. Cyclohexylalanine-containing analogue 11 was much less potent.

c. Elimination of the Leu-OMe group gave much less potent tetrapeptide analogues. Z-Glu-D-Phe-Lys-D-Trp-OMe (10) was about 25-fold less potent than 3. When an alkylamide group was substituted in place of the Leu-OMe group, the resulting tetrapeptide amides (15, 16) were somewhat more potent than 3. The most potent tetrapeptide analogue, Z-Glu-D-Phe-Lys-D-Trp-NH-$(CH_2)_2CHMe_2$ (16), was about 4-fold more potent than 3.

d. Further reduction in the size of pentapeptide derivative 3 to tripeptide derivatives did not lead to potent analogues. The most potent tripeptide derivative, Boc-Glu-D-Phe-Lys-NH$(CH_2)_2$-indol-3-yl (24), was 25-fold less potent than 3. Other indole-substituted amide analogues (25, 26) were poor inhibitors (at least 100-fold less potent than 3) of renin. Boc-Glu-D-Phe-Lys-OMe (22) and Boc-Glu-D-Phe-Lys-NH_2 (23) were also inactive.

e. Since Z-Glu-D-Phe-Lys-D-Trp-NH$(CH_2)_2CHMe_2$ (16) was about 4-fold more potent than 3, N-terminal Z group replacements were carried out on this analogue. Removal of the benzyloxycarbonyl group did not lead to any change in potency. Glu-D-Phe-Lys-D-Trp-NH$(CH_2)_2CHMe_2$ (27) was equipotent to 16. Boc-Glu-D-Phe-Lys-D-Trp-NH-$(CH_2)_2CHMe_2$ (28) was about 4-fold more potent than 16 and 27. The phenoxyacetyl analogue 29 was also more potent (2-fold) than 16 and 27.

Potent inhibitors of renin were also obtained by replacing the N-terminal Z or Boc groups by *N*-alkyl or *N*-aryl groups. Three such analogues (31–33) were more potent than 16, but two other analogues (30 and 34) were somewhat less potent than 16. Of the three more potent compounds, Me_3CCH_2-Glu-D-Phe-Lys-D-Trp-NH-$(CH_2)_2CHMe_2$ (31) was the most potent renin inhibitor to originate from this series of cyclic peptides.

Some of the analogues were also tested against procine renin, and a comparison of the inhibitory potencies against porcine and human renins is shown in Table II. The C-terminal residues of the cyclic peptides do not appear to differentiate between the two renins. Z-Glu-D-Phe-Lys-D-Trp analogues with an NH$(CH_2)_2CHMe_2$ (16), Leu-D-Ala-OMe (19), or Leu-Ser-OMe (20) group at the C-terminus were only about 3–6-fold more potent against human renin. In contrast, Glu-D-Phe-Lys-D-Trp-NH-$(CH_2)_2CHMe_2$ analogues modified at the N-terminus (27–29, 31, 33) were much more selective against human renin. The most potent analogue of the series (31) was also the most selective analogue against human renin.

Conclusions

Potent inhibitors of renin so far reported in the literature have been based on the renin substrate angiotensinogen. The octapeptide His-Pro-Phe-His-Leu-Leu-Val-Tyr, containing the Leu-Leu bond which is cleaved by renin, has been modified in a number of ways. The most potent inhibitors (in vitro $IC_{50} = 10^{-7}$–10^{-9} M against renin) have been obtained either by reducing the peptide bond (Leu^{10}-Leu^{11}) to a $-CH(OH)CH_2-$ or $-CH_2NH-$ group or by substituting a leucine residue by a statine residue (present in pepstatin—a naturally occurring inhibitor of renin).[1-6] These inhibitors bind in the same binding site which is occupied by the substrate in the normal renin–angiotensinogen interaction.

We have reported here a novel series of renin inhibitors which do not have any relationship with the existing substrate-based inhibitors. These inhibitors have been based on the linear tetrapeptides, e.g., Boc-D-Phe-Cys-(Acm)-D-Trp-Leu-OMe (1) and Z-D-Phe-Lys-D-Trp-Leu-OMe (2). The renin inhibitory activity of these peptides was significantly enhanced in a series of cyclic peptides. One of these compounds, Z-Glu-D-Phe-Lys-D-Trp-Leu-OMe (3), was at least 10-fold more potent than 1 or 2. Such enhancement of potency indicates that in the cyclic peptide the binding interactions of the linear peptide have been optimized. However, the possibility that some of the potency increase may be related to increased binding interactions of the Glu residue cannot be excluded at this stage. In this series of compounds the ring size appears to be very important. Any reduction or enlargement of the ring leads to at least 100-fold reduction in potency. The position of the amide bond between the Glu and Lys residues is also very critical. Z-Lys-D-Phe-Glu-D-Trp-Leu-OMe (4), which has the same size ring as 3, was inactive. This may be due to the change in the position and direction of this amide bond, which in turn disrupts some hydrogen bonding interactions with renin, or the change may also lead to a different overall conformation. Such reduction in potency by changing the direction of the amide bond and the ring size was earlier reported by us and others in different series of LHRH antagonists.[12,13]

The binding requirements for the N- and C-terminal regions of the cyclic peptides to renin appear to be relatively flexible. The Leu-OMe residue could be replaced by a 3-methylbutylamide group and the resulting compound 16 was about 4-fold more potent than 3. Additional amino acids could also be incorporated at this end to improve potency. Some of the hexapeptide derivatives, e.g. 17–20, were more potent than 3. Similarly, the N-terminal Z group could also be replaced by various other groups. One of these replacements gave the most potent analogue of the series, Me_3C-CH_2-Glu-D-Phe-Lys-D-Trp-NH-$(CH_2)_2CHMe_2$ (31), which was about 650-fold more potent than 1 and about 50-fold more potent than 3.

(12) Dutta, A. S.; Gormley, J. J.; McLachlan, P. F.; Woodburn, J. R. *Biochem. Biophys. Res. Commun.* **1989**, *159*, 1114.
(13) Rivier, J.; Kupryszewski, G.; Varga, J.; Porter, J.; Rivier, C.; Perrin, M.; Hagler, A.; Struthers, S.; Corrigan, A.; Vale, W. *J. Med. Chem.* **1988**, *31*, 677.

Attempts to gain more information about the overall conformation of this series of peptides are ongoing. Some of this work is reported in the next paper.

Experimental Section

Details of the various coupling and deblocking procedures have only been given in one case. In the other examples the methods have only been mentioned by name. The TLC systems, spray reagents, and other general consideration have been described earlier.[14] The syntheses of Z-D-Phe-Lys(Boc)-D-Trp-Leu-OMe, Z-D-Phe-Lys-D-Trp-Leu-OMe, Boc-Cys(Acm)-D-Phe-Cys-(Acm)-D-Trp-Leu-OMe, Boc-Gly-D-Phe-Cys(Acm)-D-Trp-Leu-OMe, Boc-Sar-D-Phe-Cys(Acm)-D-Trp-Leu-OMe, and D-Phe-Cys(Acm)-D-Trp-Leu-OMe, used in the preparation of some cyclic peptides, were described in an earlier paper.[7] The standard workup procedure for the protected peptides involved washing the ethyl acetate solutions of these peptides with 20% aqueous citric acid, saturated aqueous $NaHCO_3$, and water. The ethyl acetate solutions were dried (Na_2SO_4) and evaporated to dryness in vacuo below 40 °C. Symbols and abbreviations used follow the IUPAC–IUB recommendations. Amino acid analyses (AAA) were performed after a 16–24-h acid hydrolysis (6 N HCl + 1% phenol). Since tryptophan is partly destroyed under these conditions, its presence was confirmed in all cases, but the figures are not recorded in the Experimental Section. MH^+ figures were obtained by FAB-MS.

Z-Glu(OBu^t)-D-Phe-Lys(Boc)-D-Trp-Leu-OMe. Z-D-Phe-Lys(Boc)-D-Trp-Leu-OMe (38.5 g, 45.7 mmol) was dissolved in ethanol (500 mL) and hydrogenolyzed over 5% Pd–C (5 g) for 5 h. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue in DMF (100 mL) was reacted with Z-Glu(OBu^t)-OCp (25.8 g, 50 mmol) for 16 h. Standard workup procedure gave a gelatinous solid which was precipitated twice from methanol–water to give the pure peptide as a white solid (38.2 g, 81.3%), mp 201–202 °C; AAA Glu 1.03, Leu 0.95, Phe 1.01, Lys 1.04. Anal. ($C_{55}H_{75}N_7O_{12}$) C, H, N.

Z-Glu-D-Phe-Lys-D-Trp-Leu-OMe. The above protected pentapeptide (5.8 g, 5.65 mmol) was dissolved in glacial acetic acid (15 mL) and 3 N HCl–acetic acid (10 mL, 30 mmol) was added to it. After 1 h at room temperature excess ether was added and the hydrochloride was collected, washed with ether, and dried. Purification by silica gel column chromatography using 10 and 15% methanol in chloroform gave the product as a white powder (3.5 g, 69.0%): mp 224–225 °C; AAA Glu 1.03, Leu 0.99, Phe 0.97, Lys 1.01. Anal. ($C_{46}H_{60}N_7O_{10}Cl$) C, H, N.

Z-Glu-D-Phe-Lys-D-Trp-Leu-OMe (3). Dicyclohexylcarbodiimide (1.03 g, 5 mmol) was added to a cooled (0 °C) and stirred solution of the above linear peptide hydrochloride (3.0 g, 3.3 mmol), 1-hydroxybenzotriazole (1.35 g, 10 mmol), and triethylamine (0.49 mL, 3.4 mmol) in DMF (1 L) and the reaction mixture was stirred at 4 °C for 48 h. Some starting material was still present after this time. Additional quantities of DCCI and HOBt (5 mmol of each) were added, and the stirring was continued at room temperature for 3 days. The solvent was then removed and the residue was worked up in a standard manner. The crude product (3.6 g) was loaded onto a silica column and the column was eluted with 2 and 3% methanol in chloroform to give the pure cyclic peptide (1.5 g, 53.5%): mp 125–127 °C; $[\alpha]^{21}_D$ +39.6° (*c* 2.5, MeOH); MS *m/e* MH^+ 852; AAA Glu 1.04, Leu 1.02, Phe 1.0, Lys 1.01. Anal. ($C_{46}H_{57}N_7O_9$) C, H, N.

Z-D-Phe-Glu(OBu^t)-D-Trp-OMe. A solution of Z-Glu-(OBu^t)-OCp (15.4 g, 30 mmol), D-Trp-OMe·HCl (7.6 g, 30 mmol), and triethylamine (4.3 mL, 30 mmol) in DMF (50 mL) was left overnight at room temperature. Ethyl acetate (750 mL) was added and the reaction mixture was worked up to give Z-Glu(OBu^t)-D-Trp-OMe as an oil. The Z group of the dipeptide derivative (16.1 g, 30 mmol) was cleaved by catalytic hydrogenolysis over 5% Pd–C (2 g). The resulting peptide was coupled to Z-D-Phe (8.97 g, 30 mmol) by the DCCI–HOBt method. The crude product was precipitated twice from ethyl acetate–petroleum ether (60–80 °C) to give the tripeptide derivative (14 g, 74.3%): mp 170–171 °C; MS *m/e* MH^+ 628.

Z-Lys(Boc)-D-Phe-Glu(OBu^t)-D-Trp-OMe. 10% Pd–C (3 g) in water (5 mL) was added to a solution of Z-D-Phe-Glu-(OBu^t)-D-Trp-OMe (12.56 g, 20 mmol) in methanol (500 mL) followed by ammonium formate (0.5 g). After 1 h at room temperature TLC showed some starting material to be present. The catalyst was removed by filtration and the above process was repeated once more. After 1 h the catalyst was removed and the filtrate was evaporated to dryness. The residue was taken up in ethyl acetate, washed with water, dried (Na_2SO_4), and evaporated to leave an oil which was dissolved in DMF (100 mL) and reacted with Z-Lys(Boc)-OCp (11.2 g, 20 mmol) for 48 h. The usual workup procedure followed by silica gel column chromatography using chloroform and 1, 2, and 3% methanol in chloroform as eluants gave the tetrapeptide derivative (12.6 g, 69.0%): mp 184–185 °C; $[\alpha]^{25}_D$ −3.74° (*c* 3.07, DMF); R_{FE} 0.75, R_{FF} 0.84, R_{FH} 0.51, R_{FP} 0.23, R_{FQ} 0.45; MS *m/e* MH^+ 913.

Z-Lys-D-Phe-Glu-D-Trp-OMe. Z-Lys(Boc)-D-Phe-Glu-(OBu^t)-D-Trp-OMe (12 g, 13.1 mmol) was treated with 3 M HCl in acetic acid (25 mL) for 30 min. The hydrochloride (9.6 g, 12.1 mmol) was dissolved in DMF (1500 mL) and then cyclized by the diphenylphosphoryl azide method. Purification by silica gel column chromatography using 5, 7.5, 10, 15, 20, and 25% acetone in chloroform as eluant gave the cyclic peptide (2.6 g, 29.1%): mp 134–135 °C; $[\alpha]^{25}_D$ +71.52° (*c* 2.29, DMF); AAA Glu 1.03, Phe 1.0, Lys 1.02; MS *m/e* MH^+ 740. Anal. ($C_{40}H_{46}N_6O_8 \cdot H_2O$) C, H, N.

Z-Lys-D-Phe-Glu-D-Trp-Leu-OMe (4). Z-Lys-D-Phe-Glu-D-Trp-OMe (2.46 g, 3.3 mmol) was saponified in aqueous methanol with 2 M NaOH (2 mL) to give Z-Lys-D-Phe-Glu-D-Trp-OH. A part of it (1.2 g, 1.65 mmol) was coupled to Leu-OMe (1.8 mmol) by the DCCI–HOBt method and the crude product was purified by the silica gel column chromatography using 1, 2, and 3% methanol in chloroform as eluant (0.95 g, 69.8%): mp 124–126 °C, $[\alpha]^{25}_D$ +47.29° (*c*, 2.1, DMF); AAA Glu 1.02, Leu 1.0, Phe 1.01, Lys 1.02; MS *m/e* MH^+ 853. Anal. ($C_{46}H_{57}N_7O_9 \cdot H_2O$) C, H, N.

Z-Asp(OBu^t)-D-Phe-Lys(Boc)-D-Trp-Leu-OMe. A solution of Z-Asp(OBu^t)-OSu (2.52 g, 6 mmol) and D-Phe-Lys(Boc)-D-Trp-Leu-OMe (6 mmol) in DMF (20 mL) was left at room temperature for 48 h. The crude product obtained after the normal workup was purified by silica gel column chromatography using 2.5% methanol in chloroform as eluant: yield 5.1 g (84%); mp 146–147 °C; AAA Asp 1.01, Leu 0.96, Phe 1.0, Lys 1.03. Anal. ($C_{54}H_{73}N_7O_{12}$) C, H, N.

Z-Asp-D-Phe-Lys-D-Trp-Leu-OMe (5). Z-Asp-D-Phe-Lys-D-Trp-Leu-OMe·HCl (2.6 g, 3 mmol), prepared by the HCl–acetic acid treatment of the above protected derivative, was dissolved in DMF (300 mL) and to the cooled solution (0 °C) was added $NaHCO_3$ (3 g) followed by diphenyl phosphorazidate (1.2 mL, 5.5 mmol). The reaction mixture was stirred at 4 °C for 3 days and then worked up by the procedure described for **3** to give the cyclic peptide (1.6 g, 63.7%): mp 118–120 °C; $[\alpha]^{25}_D$ +31.7° (*c* 2.68, DMF); AAA Asp 0.96, Leu 1.0, Phe 1.03, Lys 0.97; MS *m/e* MH^+ 838.7. Anal. ($C_{45}H_{55}N_7O_9 \cdot H_2O$) C, H, N.

Z-Glu(OBu^t)-D-Phe-Orn(Boc)-D-Trp-Leu-OMe. This was prepared by coupling Z-Glu(OBu^t)-OCp (2.42 g, 5 mmol) and D-Phe-Orn(Boc)-D-Trp-Leu-OMe (5 mmol) by the procedure described above for the corresponding lysyl analogue: yield 76%; mp 220–221 °C; $[\alpha]^{25}_D$ +7.13° (*c* 2.4, DMF); AAA Glu 1.02, Phe 1.0, Orn 1.04, Leu 0.96. Anal. ($C_{54}H_{73}N_7O_{12}$) C, H, N.

Z-Glu-D-Phe-Orn-D-Trp-Leu-OMe (6). The above protected peptide was deblocked and cyclized by the procedure described above for **3**. Purification by silica gel column chromatography using chloroform and 1, 2, and 3% methanol in chloroform as eluants gave the cyclic peptide (1.1 g, 53.6%): mp 212–213 °C; $[\alpha]^{25}_D$ +73.31° (*c* 2.05, DMF); MS *m/e* MH^+ 838. Anal. ($C_{45}H_{55}N_7O_9 \cdot 1.5\ H_2O$) C, H, N.

Z-Asp(OBu^t)-D-Phe-Orn(Boc)-D-Trp-Leu-OMe. This was prepared by the method described above for Z-Asp(OBu^t)-D-Phe-Lys(Boc)-D-Trp-Leu-OMe by the reaction of Z-Asp-(OBu^t)-OSu (2.1 g, 5 mmol) and D-Phe-Orn(Boc)-D-Trp-Leu-OMe (5.0 mmol): yield 4.2 g (84%); mp 125–127 °C; AAA Asp 1.02, Leu 0.98, Phe 1.0, Orn 1.03. Anal. ($C_{53}H_{71}N_7O_{12}$) C, H, N.

Z-Asp-D-Phe-Orn-D-Trp-Leu-OMe (7). The above protected

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

peptide was treated with HCl–acetic acid to give Z-Asp-D-Phe-Orn-D-Trp-Leu-OMe·HCl, which was cyclized by the DCCI–HOBt method described above for **6**. The crude product was first chromatographed on a silica column using chloroform and 1, 2, and 2.5% methanol in chloroform as eluant and then on a second column using 5, 10, 15, 20 and 25% acetone in chloroform as an eluant: yield 26.3%; mp 109–111 °C; AAA Asp 0.98, Phe 1.02, Orn 0.95, Leu 1.0; $C_{44}H_{53}N_7O_9$ requires MH^+ 824, found MH^+ 824.

Boc-Cys(Acm)-D-Phe-Cys(Acm)-D-Trp-Leu-Met-OMe. Boc-Cys(Acm)-D-Phe-Cys(Acm)-D-Trp-Leu-OMe (14.5 g, 15.6 mmol) was saponified and then coupled to Met-OMe by the DCCI–HOBt method by using the procedure described for Z-Glu(OBu^t)-D-Phe-Lys(Boc)-D-Trp-Leu-Met-OMe. The crude product was purified as **6**: yield 12.3 g (74.5%); foam; $[\alpha]^{21}_D$ −61.27° (*c* 2.7, MeOH); AAA Cys 2.06, Met 0.96, Leu 0.98, Phe 1.04; MS *m/e* MH^+ 1057. Anal. ($C_{49}H_{71}N_9O_{11}S_3$) C, H, N, S.

Boc-Cys-D-Phe-Cys-D-Trp-Leu-Met-OMe (8). A solution of Boc-Cys(Acm)-D-Phe-Cys(Acm)-D-Trp-Leu-Met-OMe (2.1 g, 2 mmol) in DMF (200 mL) was added to a stirred solution of iodine (6.08 g, 24 mmol) in methanol (2 L). After 6 min, the solution was cooled (~5 °C) and a 1 M solution of sodium thiosulfate was added slowly until a clear solution was obtained. The solution was concentrated to a small volume (~10 mL) and then partitioned between ethyl acetate and water. The ethyl acetate layer was washed with a 0.1 M solution of sodium thiosulfate and water, dried (Na_2SO_4), and evaporated to leave a yellow oil which was purified as **6** (250 mg, 13.7%): mp 215–217 °C; MS *m/e* MH^+ 914; AAA Cys 2.03, Phe, 1.01, Leu 0.98, Met 0.97.

Z-Cys(CH_2COOBu^t)-D-Phe-Lys(Boc)-D-Trp-Leu-OMe. Z-Cys(CH_2COOBu^t) (10 mmol) was reacted with D-Phe-Lys-(Boc)-D-Trp-Leu-OMe (7.07, 10 mmol) by the DCCI–HOBt method. The crude product was precipitated twice from ethyl acetate–petroleum ether (60–80 °C) to give the pentapeptide methyl ester (7.2 g, 68%): mp 154–155 °C; $[\alpha]^{25}_D$ −4.39° (*c* 2.82, DMF). Anal. ($C_{55}H_{75}N_7O_{12}S$) C, H, N.

—CH_2CO—

Z-Cys-D-Phe-Lys-D-Trp-Leu-OMe (9). The above protected peptide (7.0 g, 6.6 mmol) was treated with 2.5 N HCl in acetic acid (40 mL) for 30 min at room temperature. The crude product, after silica gel column chromatography using 5, 10, and 12.5% methanol in chloroform as eluants, gave Z-Cys(CH_2COOH)-D-Phe-Lys-D-Trp-Leu-OMe (4.1 g, 66.2%); MS *m/e* MH^+ 902.

It was dissolved in DMF (1200 mL) and cyclized by the diphenyl phosphorazidate method. Purification by silica gel column chromatography using 1 and 2.5% methanol in chloroform as eluant gave **9**: yield 26%; mp 197–198 °C; MS *m/e* MH^+ 884°, AAA Leu 1.0, Phe 1.02, Lys 1.03. Anal. ($C_{46}H_{57}N_7O_9S$) C, H, N, S.

Z-D-Phe-Lys(Boc)-D-Trp-OMe. Z-Lys(Boc) (38 g, 100 mmol) was coupled to D-Trp-OMe (100 mmol) by the DCCI–HOBt method to give the dipeptide derivative as an oil. The crude product was dissolved in methanol (150 mL) and the Z group was cleaved with 10% Pd–C (5 g) and ammonium formate (18.9 g, 300 mmol). Lys(Boc)-D-Trp-OMe thus obtained was coupled to Z-D-Phe (29.9 g, 100 mmol) by the DCCI–HOBt method. The tripeptide derivative was purified by precipitation from ethyl acetate–petroleum ether (60–80 °C) and methanol–water (30.5 g, 41.9%): mp 168–169 °C; R_{FE} 0.59, R_{FP} 0.74. Anal. ($C_{40}H_{49}N_5O_8$) C, H, N.

Z-Glu(OBu^t)-D-Phe-Lys(Boc)-D-Trp-OMe. The procedures used for the cleavage of the benzyloxycarbonyl group from Z-D-Phe-Lys(Boc)-D-Trp-OMe (30 g, 41.2 mmol) and coupling with Z-Glu(OBu^t) (15.1 g, 45 mmol) were similar to the ones described above for the synthesis of Z-D-Phe-Lys(Boc)-D-Trp-OMe: yield 17 g, (45.2%); mp 172–173 °C; R_{FE} 0.53, R_{FH} 0.69, R_{FP} 0.23, R_{FQ} 0.53. Anal. ($C_{49}H_{65}N_6O_{11}$) C, H, N.

Z-Glu-D-Phe-Lys-D-Trp-OMe (10). A solution of the above tetrapeptide derivative (16 g, 17.5 mmol) in acetic acid (20 mL) was treated with 1 N HCl in acetic acid (80 mL) for 1 h to give, Z-Glu-D-Phe-Lys-D-Trp-OMe·HCl (12.6 g, 90.7%), which was dissolved in DMF (3 L) and cyclized with $NaHCO_3$ (12.6 g, 150 mmol) and diphenyl phosphorazidate (4.6 mL, 20 mmol). Purification by the method used for **6** gave the product (7.0 g, 60.3%): mp 119–121 °C; $[\alpha]^{25}_D$ +35.12° (*c* 2.9, DMF); MS *m/e* MH^+ 739. Anal. ($C_{40}H_{46}N_6O_8$) C, H, N.

Z-Glu-D-Phe-Lys-D-Trp-Cha-OMe (11). Z-Glu-D-Phe-Lys-D-Trp-OH (0.72 g, 1 mmol) was coupled to cyclohexylalanine methyl ester (1.5 mmol) by the DCCI–HOBt method. Purification by silica gel column chromatography using 1.5 and 2.5% methanol in chloroform as eluants gave the product (600 mg, 67.2%): mp 125–127 °C; $[\alpha]^{25}_D$ +35° (*c* 2.72, DMF): MS *m/e* MH^+ 892. Anal. ($C_{49}H_{61}N_7O_9$) C, H, N.

Z-Glu-D-Phe-Lys-D-Trp-Leu-NHMe (12). Methyl ester **3** (550 mg, 0.64 mmol) was dissolved in methanol (10 mL) and treated with a 30% solution of methylamine in methanol (10 mL) for 24 h at room temperature. The solvent was removed and the residue, after silica gel column chromatography using 2, 4, and 5% methanol in chloroform as eluants, gave the methylamide (480 mg, 89.5%): mp 205–206 °C; $[\alpha]^{25}_D$ +39.47° (*c* 2.1, DMF); AAA Glu 1.03, Leu 0.98, Phe 1.0, Lys 1.0; MS *m/e* MH^+ 839. Anal. ($C_{45}H_{58}N_8O_8$) C, H, N.

Z-Glu-D-Phe-Lys-D-Trp-Leu-NHEt (13). This was prepared from **3** and ethylamine by the procedure described above for methylamide analogue **12**: yield 69.8% mp 209–210 °C; $[\alpha]^{25}_D$ +40.4° (*c* 1.78, DMF); MS *m/e* MH^+ 853; AAA Glu 1.03, Leu 0.97, Phe 1.0, Lys 1.03. Anal. ($C_{46}H_{60}N_8O_8$) C, H, N.

Z-Glu-D-Phe-Lys-D-Trp-Ile-NHMe (14). Z-Glu-D-Phe-Lys-D-Trp-OH (0.36 g, 0.5 mmol, obtained by the saponification of the methyl ester **10**) was coupled with Ile-$NHCH_3$ (0.6 mmol) by the DCCI–HOBt method. The crude product was purified by silica gel column chromatography using 2.5 and 4% methanol in chloroform as eluants (340 mg, 80%): mp 227–228 °C, $[\alpha]^{25}_D$ +48.84° (*c* 2.76, DMF); MS *m/e* MH^+ 851. Anal. ($C_{46}H_{58}N_8O_8$) C, H, N.

Z-Glu-D-Phe-Lys-D-Trp-$NHCH_2CMe_3$ (15). Z-Glu-D-Phe-Lys-D-Trp-OH (0.72 g, 1 mmol) was coupled to 2,2-dimethylpropylamine (1.5 mmol) by the DCCI–HOBt method. The crude product was purified as compound **11** (610 mg, 75.5%): mp 143–145 °C; $[\alpha]^{25}_D$ +48.47° (*c* 2.76, DMF); MS *m/e* MH^+ 794. Anal. ($C_{44}H_{55}N_7O_7$) C, H, N.

Z-Glu-D-Phe-Lys-D-Trp-$NH(CH_2)_2CHMe_2$ (16). This was prepared and purified as the above 2,2-dimethylpropylamide **15**: yield 78.8%; mp 205–206 °C; $[\alpha]^{25}_D$ +44.5° (*c* 1.91, DMF); MS *m/e* MH^+ 794. Anal. ($C_{44}H_{55}N_7O_7$) C, H, N.

Z-Glu(OBu^t)-D-Phe-Lys(Boc)-D-Trp-Leu-Met-OMe. Z-Glu(OBu^t)-D-Phe-Lys(Boc)-D-Trp-Leu-OH (27 g, 26.6 mmol), obtained by the saponification of Z-Glu(OBu^t)-D-Phe-Lys-(Boc)-D-Trp-Leu-OMe (32 g, 31.1 mol) with aqueous NaOH (2 M, 20 mL, 40 mmol), was dissolved in DMF (250 mL) together with Met-OMe·HCl (6.98 g, 35 mmol), HOBt (3.78 g, 28 mmol), and triethylamine (5.05 mL, 35 mmol). The solution was cooled to 0 °C and DCCI (6.18 g, 30 mmol) was added. After overnight stirring at 4 °C the gelatinous reaction mixture was warmed to 50 °C and the dicyclohexylurea was filtered off. The filtrate was evaporated to dryness and the solid was precipitated from methanol–water, collected, washed with aqueous methanol, and dried (22 g, 71.3%): mp 228–229 °C, AAA Glu 1.03, Met 0.96, Leu 0.98, Phe 1.01, Lys 1.03. Anal. ($C_{60}H_{84}N_8O_{13}S$) C, H, N.

Z-Glu-D-Phe-Lys-D-Trp-Leu-Met-OMe·HCl. The above protected hexapeptide derivative (21 g, 18.1 mmol) was dissolved in acetic acid (30 mL) and treated with 3 N HCl in acetic acid (32.8 mL, 100 mmol) for 1 h. The product was purified by silica gel column chromatography using a mixture of chloroform–methanol–water (85:15:0.1) as eluant (6.6 g, 36.3%): mp 225–226 °C; $[\alpha]^{25}_D$ −46.04° (*c* 2.48, MeOH); AAA Glu 1.03, Met 0.95, Leu 0.96, Phe 0.97, Lys 1.02. Anal. ($C_{51}H_{69}N_8O_{11}SCl$) C, H, N.

Z-Glu-D-Phe-Lys-D-Trp-Leu-Met-OMe (17). Z-Glu-D-Phe-Lys-D-Trp-Leu-Met-OMe·HCl (6.6 g, 6.36 mmol) was cyclized by the procedure described above for compound **1**: yield 2.8 g (44.8%); mp 188–190 °C; AAA Glu 1.02, Met 0.96, Leu 1.0, Phe 1.02, Lys 0.98; MS *m/e* MH^+ 983. Anal. ($C_{51}H_{66}N_8O_{10}S$) C, H, N, S.

Z-Glu-D-Phe-Lys-D-Trp-Leu-D-Met-OMe (18). Z-Glu-D-Phe-Lys-D-Trp-Leu-OH (0.83 g, 1 mmol) was coupled to D-Met-OMe (300 mg, 1.5 mmol) by the DCCI–HOBt method. Silica gel column chromatography of the crude peptide gave the pure cyclic peptide (530 mg, 54.1%): mp 130–131 °C; $[\alpha]^{25}_D$ +44.36°

(c 1.9, DMF); MS m/e MH+ 983. Anal. ($C_{51}H_{66}N_8O_{10}S$) C, H, N.

Z-Glu-D-Phe-Lys-D-Trp-Leu-Ala-OMe (19). This was prepared as compound 18 from Z-Glu-D-Phe-Lys-D-Trp-Leu-OH and D-Ala-OMe: yield 48.7%; mp 133–135 °C; $[\alpha]^{25}_D$ +38.22° (c 2.2, DMF), MS m/e MH+ 923. Anal. ($C_{49}H_{62}N_8O_{10}$) C, H, N.

Z-Glu-D-Phe-Lys-D-Trp-Leu-Ser-OMe (20). This was prepared from Z-Glu-D-Phe-Lys-D-Trp-Leu-OH and Ser-OMe by the method described for compound 18: yield 31.9%; mp 151–153 °C; $[\alpha]^{25}_D$ +28.0° (c 1.8, DMF); MS m/e MH+ 939. Anal. ($C_{49}H_{62}N_8O_{11}\cdot H_2O$) C, H, N.

Z-Glu-D-Phe-Lys-D-Trp-Leu-Pro-OMe (21). This was synthesized from Z-Glu-D-Phe-Lys-D-Trp-Leu-OH and Pro-OMe by the procedure described above for compound 18: yield 45.3%; mp 140–142 °C; $[\alpha]^{25}_D$ +15.5° (c 2.45, DMF); MS m/e MH+ 949. Anal. ($C_{51}H_{64}N_8O_{10}\cdot H_2O$) C, H, N.

Boc-D-Phe-Lys(Z)-OMe. A DCCI–HOBt-mediated coupling of Boc-D-Phe (18.55 g, 70 mmol) and Lys(Z)-OMe (20.6 g, 70 mmol) followed by a precipitation from ethyl acetate–petroleum ether (60–80 °C) gave the product (32 g, 84.4%): mp 114–115 °C; $[\alpha]^{25}_D$ −5.19° (c 3.12, DMF); MS m/e MH+ 542. Anal. ($C_{29}H_{39}N_3O_7$) C, H, N.

Boc-Glu(OBzl)-D-Phe-Lys(Z)-OMe. Boc-Glu(OBzl) (23.6 g, 70 mmol) was coupled to D-Phe-Lys(Z)-OMe·HCl (36.19 g, 70 mmol; prepared from the above Boc derivative by an HCl in ethyl acetate treatment) in the presence of triethylamine (10.1 mL, 70 mmol) by the DCCI–HOBt method. Purification by silica gel column chromatography using 1% methanol in chloroform as eluant gave the product (51 g, 95.7%): mp 87–88 °C; MS m/e MH+ 761. Anal. ($C_{41}H_{52}N_4O_{10}$) C, H, N.

Boc-Glu-D-Phe-Lys-OMe (22). The above protected peptide (60 g, 78.9 mmol) was dissolved in ethanol (500 mL) and DMF (100 mL) and hydrogenolyzed over 5% Pd–C (5 g) for 24 h. Boc-Glu-D-Phe-Lys-OMe thus obtained was cyclized in DMF by the diphenyl phosphorazidate method. Purification by silica gel column chromatography using chloroform and 1% methanol in chloroform as eluants gave the cyclic tripeptide derivative (18.8 g, 45.9%): mp 185–187 °C; MS m/e MH+ 519. Anal. ($C_{26}H_{38}N_4O_7$) C, H, N.

Boc-Glu-D-Phe-Lys-NH_2 (23). Ammonia gas was bubbled through a solution of the above methyl ester 22 (1.03 g, 2 mmol) in methanol (10 mL) for 2 h. After 48 h at room temperature the solvent was evaporated and the residue was collected with ether and dried (0.8 g, 79.5%): mp 160–161 °C; MS m/e MH+ 504. Anal. ($C_{25}H_{37}N_5O_6$) C, H, N.

Boc-Glu-D-Phe-Lys-*N*-[2-(3-indolyl)ethyl]amide (24). Boc-Glu-D-Phe-Lys-OH (504 mg, 1 mmol) obtained by the saponification of the methyl ester (22) was coupled to tryptamine (160 mg, 1 mmol) by the DCCI–HOBt method. The crude product after purification as for compound 11 gave the amide (460 mg, 71.1%): mp 140–142 °C; MS m/e MH+ 647. Anal. ($C_{35}H_{46}N_6O_6$) C, H, N.

Boc-Glu-D-Phe-Lys-*N*-[2-(3-indolyl)-1-methylethyl]amide (25). Prepared by coupling Boc-Glu-D-Phe-Lys-OH (504 mg, 1 mmol) to 2-(3-indolyl)-1-methylethylamine (174 mg, 1 mmol) by the DCCI–HOBt method and was purified by silica gel column chromatography using 1.5, 2.5, and 4% methanol in chloroform as eluants (600 mg, 90.7%) mp 150–153 °C; MS m/e MH+ 661. Anal. ($C_{36}H_{48}N_6O_6$) C, H, N.

Boc-Glu-D-Phe-Lys-*N*-[1-ethyl-2-(3-indolyl)ethyl]amide (26). The methods used for its preparation from Boc-Glu-D-Phe-Lys-OH (504 mg, 1 mmol) and 1-ethyl-2-(3-indolyl)ethylamine (188 mg, 1 mmol) and purification were similar to those of the above compound 25: yield 260 mg (38.5%); mp 161–163 °C; MS m/e MH+ 675. Anal. ($C_{37}H_{50}N_6O_6$) C, H, N.

Glu-D-Phe-Lys-D-Trp-NH(CH_2)$_2$CHMe$_2$ (27). The benzyloxycarbonyl group from 16 (2.5 g, 3.15 mmol) was removed by catalytic hydrogenolysis (5% Pd–C, 0.5 g) and the product was converted to the hydrochloride (2.13 g, 97.2%): mp 175–177 °C; $[\alpha]^{25}_D$ +37.58° (c 2.78, DMF); R_{FA} 0.53, R_{FB} 0.68, R_{FC} 0.43, R_{FF} 0.55, R_{FH} 0.35, R_{FQ} 0.12; MS m/e MH+ 660 ($C_{36}H_{49}N_7O_5$ requires MH+ 660).

Boc-Glu-D-Phe-Lys-D-Trp-NH(CH_2)$_2$CHMe$_2$ (28). This was prepared by reacting compound 27 with di-*tert*-butyl carbonate: yield 55.4%; mp 215–218 °C; MS m/e MH+ 760. Anal. ($C_{41}H_{57}N_7O_7$) C, H, N.

***N*-(Phenoxyacetyl)-Glu-D-Phe-Lys-D-Trp-NH(CH_2)$_2$CHMe$_2$ (29).** A solution of 27 (348 mg, 0.5 mmol) and triethylamine (0.145 mL, 1 mmol) was cooled in an ice bath and phenoxyacetyl chloride (0.085 mL, 0.5 mmol) was added. After 16 h at room temperature the solvent was removed and the residue was purified by silica gel column chromatography using 1, 2, and 3% methanol in chloroform as eluants (320 mg, 80.6%): mp 239–240 °C; MS m/e MH+ 794. Anal. ($C_{44}H_{55}N_7O_7\cdot{}^1/_2H_2O$) C, H, N.

Me_2CH-Glu-D-Phe-Lys-D-Trp-NH(CH_2)$_2$CHMe$_2$·HCl (30). A solution of 27 (696 mg, 1 mmol) in acetone (2 mL) and methanol (2 mL) was reacted with sodium cyanoborohydride (4 mmol) overnight at room temperature. The solvent was evaporated and the crude product was purified by silica gel column chromatography using 2, 3, 4, and 5% methanol in chloroform as eluants. It was converted to a hydrochloride, washed with ether, and dried (340 mg, 46.0%): mp 175–178 °C; MS m/e MH+ 702. Anal. ($C_{39}H_{55}N_7O_5\cdot HCl$) C, H, N.

Me_3C-CH_2-Glu-D-Phe-Lys-D-Trp-NH(CH_2)$_2$CHMe$_2$ (31). This was prepared from 27 (750 mg, 1.14 mmol) and trimethylacetaldehyde (0.26 mL, 2.4 mmol) by the procedure described above for compound 30 (205 mg, 24.6%): mp 190–192 °C; $[\alpha]^{25}_D$ −28.49° (c 3.06 in DMF); MS m/e MH+ 730 ($C_{41}H_{59}N_7O_5$ requires MH+ 730).

Et-CH(Me)-CH_2-Glu-D-Phe-Lys-D-Trp-NH(CH_2)$_2$CHMe$_2$ (32). This was prepared as the above isopropyl analogue 30 from 27 and 2-methylbutyraldehyde: yield 51.2%; mp 219–220 °C; $[\alpha]^{25}_D$ +50.6° (c 1.49, DMF); MS m/e MH+ 730 ($C_{41}H_{59}N_7O_5$ requires MH+ 730).

$C_6H_5CH_2$-Glu-D-Phe-Lys-D-Trp-NH(CH_2)$_2$CHMe$_2$·HCl (33). This was prepared from benzaldehyde and 27 by the procedure described above for isopropyl analogue 30: yield 33.0%; mp 182–185 °C; MS m/e MH+ 750. Anal. ($C_{43}H_{55}N_7O_5\cdot HCl$) C, H, N.

***N*-(3-Indolylmethyl)-Glu-D-Phe-Lys-D-Trp-NH(CH_2)$_2$CHMe$_2$ (34).** This was prepared as compound 30 from indole-3-carboxaldehyde and 27: yield 35.2%; mp 181–182 °C; $[\alpha]^{25}_D$ +44.4° (c 3.65, DMF); MS m/e MH+ 789 ($C_{45}H_{56}N_8O_5$ requires MH+ 789).

Z-D-Phe-Lys-D-Trp-Leu (35). Z-D-Phe-Lys-D-Trp-Leu-OMe (3.88 g, 5 mmol) was saponified with 2 N NaOH (6 mL, 12 mmol) for 1 h at room temperature to give Z-D-Phe-Lys-D-Trp-Leu-OH, which was then cyclized by the diphenyl phosphorazidate method to give the cyclic peptide (1.04 g, 29.3%): mp 292–293 °C; $[\alpha]^{25}_D$ −42.0° (c 1.65, DMF); MS m/e MH+ 709. Anal. ($C_{40}H_{48}N_6O_6$) C, H, N.

D-Phe-Lys-D-Trp-Leu (36). The benzyloxycarbonyl group from the above cyclic peptide 35 (760 mg, 1.07 mmol) was removed by catalytic hydrogenolysis over 5% Pd–C. The product was collected, washed with ether, and dried (350 mg, 57.0%): mp 293–296 °C: MS m/e MH+ 575. Anal. ($C_{32}H_{42}N_6O_4$) C, H, N.

Gly-D-Phe-Cys(Acm)-D-Trp-Leu (37). Boc-Gly-D-Phe-Cys(Acm)-D-Trp-Leu-OMe (2.4 g, 2.96 mmol) was treated first with aqueous sodium hydroxide and then with HCl in acetic acid to give Gly-D-Phe-Cys(Acm)-D-Trp-Leu. The pentapeptide was then cyclized by the diphenyl phosphorazidate method to give the cyclic pentapeptide: yield 300 mg (15%); mp 245 °C dec; AAA Gly 1.0, Leu 1.02, Phe 1.01, Cys 1.03; MS m/e MH+ 678 ($C_{34}H_{43}N_7O_6S$ requires MH+ 678).

Sar-D-Phe-Cys(Acm)-D-Trp-Leu (38). This was prepared from Boc-Sar-D-Phe-Cys(Acm)-D-Trp-Leu-OMe (2.5 g, 3.04 mmol) by the procedure described for compound 37: yield 25%; mp 267–268 °C; MS m/e MH+ 692 ($C_{35}H_{45}N_7O_6S$ requires MH+ 692).

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