cold, oxygen saturated Kreb's solution. The aortas were freed from adrentia and helically cut into a 5-mm large band. Two-centimeter strips of this band were suspended in 5-mL baths containing Kreb's solution at 37 °C; they were continually aerated with a mixture at 95% O_2/5% CO_2. A tension of 2 g was applied at the beginning and was adjusted several times during the 60–90-min equilibration period. The bath fluid was changed at intervals of 10–15 min. Concentrations indicated were always final concentrations in the tissue bath. Biological activities of the tissue strips in response to the applied peptides were recorded with force-displacement transducers (Grass FT 0.3) on a Grass polygraph Model 7 (Grass Co. Quincy, MA). The biological activities are expressed by the relative affinity (RA) compared to 1.

In Vivo Rat Blood Pressure. Male and female Wistar rats, weighing between 300 and 500 g, were used and purchased from Charles River Canada Inc., St-Constant, Quebec. Before the experiment, the animals were anesthetized with urethane (1.4 g/kg intraperitoneally), tracheotomized, and self-ventilated. The left jugular vein and the right carotid artery were carefully dissected. A catheter was implanted into each vessel leading to the body for drug injections (jugular vein) and direct blood pressure recording (carotid artery). The arterial blood pressure was monitored with arterial pressure transducers (Statham P 23Db) attached to the side arm of the carotid cannula.

The drugs were injected in volumes of 0.1 mL, followed by 0.2 mL of physiological saline (NaCl, 0.9%, w/v) in order to rinse the cannula.

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Synthesis of a Novel Class of Heteroaromatic Amino Acids and Their Use in the Preparation of Analogues of Luteinizing Hormone-Releasing Hormone[1]

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A novel class of heterocyclic aromatic amino acids based on the 3-(2-benzimidazolyl)alanine system has been generated by chiral synthesis from D- or L-aspartic acid. The use of variously substituted *o*-phenylenediamines for condensation with the β-carboxyl function of α-benzyl *N*-(benzyloxycarbonyl)-D-aspartate has led to a series of amino acids of graded hydrophobicity with a steric bulk similar to that of tryptophan. In a similar fashion, we have prepared 3-(2-benzothiazolyl)-D-alanine from *o*-aminothiophenol and 3-(2-benzoxazolyl)-D-alanine from *o*-aminophenol. Incorporation of these amino acids into the 6-position of luteinizing hormone-releasing hormone (LH-RH) led to a series of very potent agonist analogues (up to 160 times LH-RH potency), active in doses ranging from 0.1 to 0.5 μg by twice daily injection in a rat estrus cyclicity suppression assay designed to show the paradoxical antifertility effects of these compounds.

The preparation of unnatural amino acids with specially designed physical and chemical properties (e.g., basicity, lipophilicity, steric requirements, etc.) for incorporation into polypeptides is of increasing importance in peptide chemistry.[2-6] Such an approach can be useful in the investigation of specific sites in polypeptide hormones with binding and effector functions. Of particular importance for binding and effector functions are the indole[7] and imidazole[8] containing side chains of Trp and His.

The demonstration that the C-terminal carboxylic acid of peptides could be converted into benzimidazole functions[9] led us to use a similar route to convert the β-carboxylic acid function of Asp into a novel series of heteroaromatic amino acids with steric requirements similar to Trp (Scheme I). The use of N, O, and S as members of the heteroaromatic ring will allow the study of the effects of a range of basicities and lipophilicities with little change in steric requirements. In addition, the facile use of substituents on the aromatic portion of the side chain allows the study of a range of lipophilic and electronic effects.

An area of particular interest to us[6] has been the regulation of fertility using hydrophobic analogues of luteinizing hormone-releasing hormone (<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, LH-RH). Chronic administration of pharmacological doses of long lived LH-RH

Table I. Basicity of Side-Chain Functional Groups

compd	pK_a	method[a]
Dcb	3.4	A
Bia	4.7	A
Dmb	5.4	A
benzimidazole	5.5 (5.5)[b]	A
Tba	7.5	B

[a] The pK_a's were determined either spectrophotometrically in a series of graded aqueous buffers (method A) or by titration (method B) in aqueous medium (D. Nagami and T.-Y. Yang, unpublished results). For reference, the pK_a of His is 6.1.[36] The pK_a values were not determined for Nia (insoluble) or for the very weakly basic Boa and Bta. [b] Reference 18.

(1) Contribution no. 166 from the Institute of Bio-Organic Chemistry, Syntex Research. The unnatural amino acids have been given the following abbreviation: Bia, 3-(2-benzimidazolyl)-alanine (**3a**); Nia, 3-(2-naphthimidazolyl)alanine (**3b**); Dmb, 3-(5,6-dimethylbenzimidazol-2-yl)alanine (**3c**); Dcb, 3-(5,6-dichlorobenzimidazol-2-yl)alanine (**3d**); Bta, 3-(2-benzothiazolyl)alanine (**3e**); Boa, 3-(2-benzoxazolyl)alanine (**3f**); Tba, 3-(4,5,6,7-tetrahydrobenzimidazol-2-yl)alanine (**6**). The abbreviations for natural amino acids and protecting groups follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1971**, *247*, 977).

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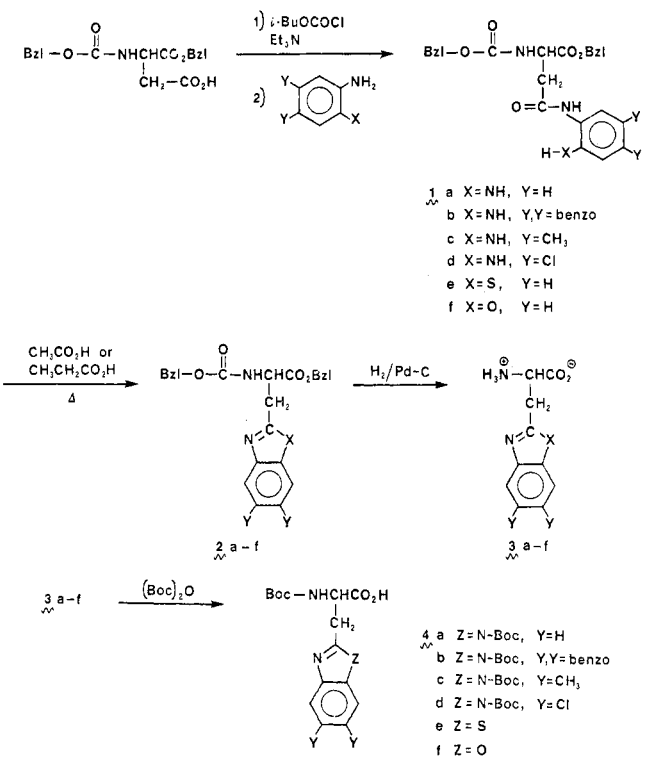
Table II. Amino Acid Intermediates

compd	yield, %	mp, °C	$[\alpha]^{25}_D$, (*c*, MeOH), deg	formula	M_r[a]	anal.
2a	56	136-138	15.8 (1)	$C_{25}H_{23}N_3O_4$	429.48	C, H, N
2b	41	123-125	29.9 (0.7)	$C_{29}H_{25}N_3O_4$	479.54	C, H, N
2c	44	140-142	24.9 (1)	$C_{27}H_{27}N_3O_4$	457.54	C, H, N
2d	58	171-172	24.0 (1)	$C_{25}H_{21}N_3O_4Cl_2$	498.38	C, H, N
2e	69	74-75	33.9 (1)	$C_{25}H_{22}N_2O_4S$	446.53	C, H, N
2f	23	66-67	27.3 (1)	$C_{25}H_{22}N_2O_5$	430.47	C, H, N
3a	100	185-187	−23.3 (0.3, HOAc)	$C_{10}H_{11}N_3O_2 \cdot 1.5H_2O$	205.22	C, N; H[b]
3b	92	198-200 dec	−25.0 (0.5, HOAc)	$C_{14}H_{13}N_3O_2 \cdot HOAc \cdot H_2O$	255.28	C, H; N[c]
3c	94	198-200 dec	−24.3 (0.5, HOAc)	$C_{12}H_{15}N_3O_2 \cdot HOAc \cdot 0.3H_2O$	233.27	C, H; N[d]
3d	86	234-236 dec	−9.5 (0.5, HOAc)	$C_{10}H_9N_3O_2Cl_2$	274.12	C, H, N
3f	82	200-202 dec	18.6 (0.5, HOAc)	$C_{10}H_{10}N_2O_3 \cdot 0.5HOAc \cdot 0.5H_2O$	206.20	C, H, N
4a	75	160-162 dec	21.7 (1)	$C_{20}H_{27}N_3O_6$	405.46	C, H, N
4b	42	142-144 dec	20.2 (0.8)	$C_{24}H_{29}N_3O_6$	479.54	C, H, N
4c	63	120-122	21.5 (0.5)	$C_{22}H_{31}N_3O_6$	433.51	C, H, N
4d	60	110-112 dec	21.6 (1)	$C_{20}H_{25}N_3O_6Cl_2$	474.36	C, H, N
4e	59	148-150	8.4 (1)	$C_{15}H_{18}N_2O_4S$	322.39	C, H, N
4f	82	121-122	3.3 (0.5)	$C_{15}H_{18}N_2O_5$	306.33	C, H, N
5	78	182-185	13.3 (1, DMF)	$C_{48}H_{46}N_4O_8S_2 \cdot 0.5CH_2Cl_2$	871.04	C, H, N
6	98	134-140	−15.9 (0.6, HOAc)	$C_{10}H_{15}N_3O_2 \cdot HOAc \cdot 0.5H_2O$	209.25	C, H, N
7	62	164-170	18.7 (0.7)	$C_{20}H_{31}N_3O_6$	409.49	C, H, N

[a] Calculated for the unsolvated, free amino acid derivatives. [b] H: calcd, 6.07; found, 5.32. [c] N: calcd, 12.61; found, 12.10. [d] N: calcd, 14.06; found, 14.48.

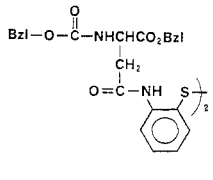
Scheme I



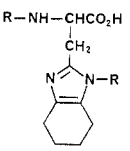
agonists leads to "paradoxical" antifertility[10] and antisteroidal[11] effects due to desensitization of target cells in the anterior pituitary[12] and gonads.[13] At the time when these studies were initiated, the most potent LH-RH agonist analogues contained D-Trp[14,15] at position 6, and our analysis of the structure–activity relationship for LH-RH analogues indicated a preference for lipophilic amino acids in this position. We therefore chose to examine the utility of these amino acids as hydrophobic analogues of Trp in a series of LH-RH analogues substituted at position 6 with the D isomers of benzimidazolyl-, benzothiazolyl- and benzoxazolylalanine residues as replacements for D-Trp.

Chemistry. The desired amino acids were prepared in optically pure form by chiral synthesis from either D- or L-aspartic acid (Scheme I). Mixed carbonic anhydride coupling[16] of α-benzyl *N*-(benzyloxycarbonyl)-D-asparate with the appropriate *o*-phenylenediamine, *o*-aminothiophenol, or *o*-aminophenol gave the crude intermediate amides (**1**), which were converted without purification to the desired heteroaromatic amino acids (**2**) by cyclization and dehydration in HOAc. While the cyclization step went well in HOAc for **1a–d**, the attempted cyclization to **2f** gave no product. Heating at a higher reflux temperature in propionic acid (~140 °C) was the best method of cyclization of **1f**. The preparation of **2e** proceeded more cleanly if the adduct **5** was formed from the disulfide of *o*-aminothiophenol. The dimeric intermediate (**5**) was then

(2) Jorgensen, E. C.; Windridge, G. C.; Hisch, K.-H.; Lee, T. C. In "Chemistry and Biology of Peptides"; Meienhofer, J., Ed.; Ann Arbor Science Publishers: Ann Arbor, MI, 1972; pp 513.
(3) Nestor, Jr., J. J.; Ferger, M. F.; du Vigneaud, V. *J. Med. Chem.* **1975**, *18*, 284.
(4) Rajh, H. M.; Mariman, E. C. M.; Tesser, G. I.; Nivard, R. J. F. *Int. J. Pept. Protein Res.* **1980**, *15*, 200.
(5) Fauchere, J. L.; Do, K. Q.; Tow, P. Y. C.; Hansch, C. *Experentia* **1980**, *36*, 1203.
(6) Nestor, Jr., J. J.; Ho, T. L.; Simpson, R. A.; Horner, B. L.; Jones, G. H.; McRae, G. I.; Vickery, B. H. *J. Med. Chem.* **1982**, *25*, 795.
(7) Prasad, K. U.; Roeske, R. W.; Weitl, F. L.; Vilchez-Martinez, J. A.; Schally, A. V. *J. Med. Chem.* **1976**, *19*, 492. Yabe, Y.; Miura, C.; Horikoshi, H.; Baba, Y. *Chem. Pharm. Bull.* **1976**, *24*, 3149.
(8) Hofmann, K.; Bohm, H. *J. Am. Chem. Soc.* **1966**, *88*, 5914.
(9) Maekawa, K.; Ohtani, J. *Agric. Biol. Chem.* **1977**, *41*, 811.
(10) Corbin, A.; Beattie, C. W. *Endocr. Res. Commun.* **1975**, *2*, 1.
(11) Vickery, B. H. in "Hormone Antagonists"; Agarwal, M. K. Ed.; de Gruyter: New York, 1982; p 623.
(12) Sandow, J.; von Rechenberg, W.; Gerzabek, G.; Stoll, W. *Fertil. Steril.* **1978**, *30*, 205.
(13) Hsueh, A. J. W.; Erickson, G. F. *Science* **1979**, *204*, 854.
(14) Vale, W.; Rivier, C.; Brown, M.; Ling, N.; Monahan, M.; Rivier, J. In "Clinical Endocrinology", 5th Suppl.; McIntyre, I.; Ed.; Blackwell Scientific Publishers: Oxford, England, 1976; p 2615.
(15) Coy, D. H.; Vilchez-Martinez, J. A.; Coy, E. J.; Schally, A. V. *J. Med. Chem.* **1976**, *19*, 423.
(16) Vaughn, Jr., J. R.; Osato, R. L. *J. Am. Chem. Soc.* **1952**, *74*, 676.



5



6 R = H
7 R = Boc

cleanly reduced with Zn/HOAc to the sulfhydryl intermediate **1e**, which spontaneously underwent cyclization to **2e** upon treatment with Et_3N in dioxane. Reduction[17] of **3a** with Adam's catalyst in 2 N HCl yielded **6**, which can be considered an analogue of His.

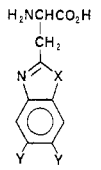
Since the pK_a of 2-alkylbenzimidazoles (~6)[18] is closer to that of 2-methylimidazole ($pK_a \approx 8$) rather than 3-methylindole ($pK_a \approx -5$), the reactivity of the side chain of benzimidazolylalanines was expected to be more similar to that of His rather than Trp. Previous studies[19] with His, as well as our preliminary synthetic studies with **4a**, indicated that the side chain must be protected during the coupling reaction. Since an unprotected His side chain does not prevent subsequent peptide couplings, we have chosen the simplicity of N^{α},N^{im}-bis(*tert*-butoxycarbonyl) (Boc) protection[20] for the benzimidazolylalanine type of amino acid. Careful control of the reaction conditions is necessary during the protection reaction due to the relatively slow reaction of the benzimidazole function with di-*tert*-butyl dicarbonate [$(Boc)_2O$] and the possibility of subsequent hydrolysis of the side-chain Boc group during workup. The benzothiazolyl- and benzoxazolylalanine side chains are very weakly basic and do not require protection.

All of the peptides were synthesized by the Merrifield solid-phase method.[21] Decapeptide analogues were synthesized on (benzhydrylamino)polystyrene–1% divinylbenzene resin.[22] Nonapeptide ethylamide analogues were synthesized on (chloromethyl)polystyrene–1% divinylbenzene resin and were removed from the resin by treatment with $EtNH_2$. N^{α}-Boc[23] protection was used on all amino acids except <Glu, which was unprotected. The side-chain protection for amino acids was as follows: Arg, Tos;[24] Tyr, 2,6-dichlorobenzyl;[25] Ser, Bzl; His, Tos.[26] Anhydrous liquid HF was used for the final deprotection.[27] The crude peptides were purified by preparative high performance liquid chromatography (prep-HPLC).[6]

Bioassay. The analogues were tested in an estrus suppression assay[6] designed to assess the paradoxical antifertility effect[10,11] of these agents. Adult female rats were injected (subcutaneously) twice daily for 14 days with a solution of test compound in 0.1% bovine serum albumin (BSA)–0.9% saline vehicle. The percent of rats showing complete suppression of estrus, determined by persistent diestrus from daily vaginal lavage (from day 4 onward) was plotted against log dose, and the ED_{50} for complete suppression of estrus was calculated (Table III).

Results and Discussion

The amino acids synthesized exhibit a range of hydrophobicities and basicities (see ref 1 for abbreviations). The heteroaromatic ring systems forming the side chains of the amino acids reported here may be grouped into the following three classes: (a) nonbasic, electron-rich aromatic rings (Boa and Bta), (b) weakly to moderately basic elec-



X = NH; Y = H — Bia
Y = CH_3 — Dmb
Y = Cl — Dcb
X = O; Y = H — Boa
X = S; Y = H — Bta
X,Y = Benzo — Nia

tron-rich aromatic rings (Bia, Dcb, Nia, and Dmb), and (c) the more basic, dialkylimidazole aromatic ring system (Tba). From the pK_a values determined for some of these compounds (Table I), only the side chains of Dmb and Tba should be appreciably protonated at physiological pH. The weakly basic, electron-rich character of the heteroaromatic ring systems of classes (a) and (b) makes them attractive candidates for replacement of Trp residues in polypeptides. The more strongly basic, imidazole character of Tba makes it a candidate for substitution as an analogue of His. It should be noted that the side chain of Tba is attached to the 2-position of the imidazole ring, rather than the 4-position as in His. The 5,6-dichloro-, 5,6-dimethyl-, and naphthimidazolylalanine side chains illustrate the utility of this approach for the investigation of electronic and hydrophobic effects in a structurally homologous series of analogues.

The LH-RH analogues generated by incorporation of these novel amino acids exhibited a wide range of hydrophobicities, as measured by k' values from reversed-phase HPLC.[28] The potencies of these analogues varied from 0.3 to 1.6 (compared to [D-Trp^6,Pro^9-NHEt]LH-RH; potency = 1). The potencies for this series of analogues appear to reach a maximum for the compounds of intermediate hydrophobicity. It is interesting to note, however, that the most potent analogues in our series are less hydrophobic than [D-Trp^6,Pro^9-NHEt]LH-RH (k' = 1.29). In a quantitative structure–activity (QSAR) analysis of LH-RH potency data,[29] it was suggested that the potency of LH-RH analogues increased with increasing hydrophobicity in position 6 and that the peak of LH-RH activity would be found in compounds more hydrophobic than [D-Trp^6,Pro^9-NHEt]LH-RH (potency ~100 times LH-RH[6]). In our previous studies[6] with LH-RH analogues containing unnatural, very hydrophobic carbocyclic amino acids, we generated compounds more hydrophobic than those reported here. Within the previous series were compounds 200 times as potent as LH-RH.[6] In the previous very hydrophobic LHRH analogue series, the Pro-NHEt-containing analogues were less potent.[6] In the present more hydrophilic series, the increased hydrophobicity imparted by the Pro-NHEt substitution was beneficial (compare **9** and **14**, **8** and **12**).

In this series, the most potent analogues also contain the most basic amino acids (Tba and Dmb). Thus, Tba, which is ~50% protonated at physiological pH (pK_a = 7.5), is able to effectively replace the hydrophobic, uncharged Trp residue in [D-Trp^6,Pro^9-NHEt]LH-RH and yield a more

(17) Hartmann, M.; Pannizon, L. *Helv. Chim. Acta* **1938**, *21*, 1692.
(18) Albert, A. in "Heterocyclic Chemistry—An Introduction", 2nd ed.; Athlone Press: London, 1968; p 441.
(19) Schaich, E.; Fretzdorff, A. M.; Schneider, F. *Z. Physiol. Chem.* **1973**, *354*, 897.
(20) Fridkin, M.; Goren, H. *J. Can. J. Chem.* **1971**, *49*, 1578.
(21) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.
(22) Pietta, P. G.; Marshall, G. R. *Chem. Commun.* **1970**, 650.
(23) Carpino, L. A. *J. Am. Chem. Soc.* **1957**, *79*, 4427.
(24) Ramachadran, J.; Li, C. H. *J. Org. Chem.* **1962**, *27*, 4006.
(25) Yamashiro, D.; Li, C. H. *Int. J. Pept. Protein Res.* **1972**, *4*, 181.
(26) Fujii, T.; Sakakibara, S. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 3954.
(27) Sakakibara, S.; Shimonishi, Y. *Bull. Chem. Soc. Jpn.* **1965**, *38*, 1412.
(28) Rivier, J.; Brown, M.; Rivier, C.; Ling, N.; Vale, W. In "Peptides 1976"; Loffet, A., Ed.; Editions de l'Universite de Bruxelles: Brussels, 1976; pp 427–451.
(29) Nadasdi, L.; Medzihradszky, K. *Biochem. Biophys. Res. Commun.* **1981**, *99*, 451.

Table III. LH-RH Analogues

no.	compd[a]	$[\alpha]^{25}_D$ (c, HOAc), deg	formula[b]	M_r	HPLC: k'[c]	TLC,[d] R_f BAW	TLC,[d] R_f BEAW	estrus suppression: potency[e]
8	[D-Bia^6]LH-RH	−28.8 (0.5)	$C_{65}H_{85}N_{19}O_{15}$	1372.53	0.56	0.22	0.24	0.3
9	[D-Tba^6]LH-RH		$C_{66}H_{91}N_{19}O_{15}$	1390.61	0.66	0.28	0.47	0.6
10	[D-Boa^6]LH-RH	−29.6 (0.7)	$C_{65}H_{83}N_{18}O_{16}$	1372.51	0.71	0.30	0.55	1.2
11	[D-Bta^6]LH-RH	−35.5 (0.3)	$C_{65}H_{83}N_{18}O_{15}S$	1388.57	0.83	0.31	0.54	1.0
12	[D-Bia^6,Pro^9-NHEt]LH-RH	−27.7 (0.8)	$C_{65}H_{86}N_{18}O_{14}$	1343.53	0.96	0.32	0.50	1.0
13	[D-Dmb^6]LH-RH	−20.4 (0.5)	$C_{67}H_{89}N_{19}O_{15}$	1400.58	1.01	0.32	0.49	1.6 (1.2-2.2)[f]
14	[D-Tba^6,Pro^9-NHEt]LH-RH	−29.7 (0.4)	$C_{65}H_{90}N_{18}O_{14}$	1347.56	1.20	0.27	0.41	1.4
15	[D-Dcb^6]LH-RH	−28.5 (0.5)	$C_{65}H_{82}N_{19}O_{15}Cl_2$	1440.41	1.38	0.32	0.63	0.9 (0.6-1.3)[f]
16	[D-Nia^6,Pro^9-NHEt]LH-RH	−27.5 (0.3)	$C_{69}H_{88}N_{18}O_{14}$	1393.59	1.79	0.37	0.67	0.8

[a] Unnatural amino acid abbreviations are given in ref 1. Acceptable amino acid analyses were obtained for all LH-RH analogues (see Experimental Section). [b] Formulas are given for an assumed composition of the molecule as the acetate but the actual composition will contain various amounts of water and acetic acid. [c] k' = (retention volume − void volume)/void volume; conditions are given in Experimental Section: k' for [D-Trp^6,Pro^9-NHEt]LH-RH is 1.39. [d] BAW = 1-BuOH/HOAc/H_2O, 4:1:5 (upper phase); BEAW = 1-BuOH/EtOAc/HOAc/H_2O, 1:1:1:1. [e] Potency is calculated relative to [D-Trp^6,Pro^9-NHEt]LH-RH as standard in each assay (mean ED_{50} ± SE of the standard = 0.17 ± 0.02 μg). [f] Mean value (range) for three assays.

Table IV. Amino Acid Analyses

		amino acid ratios found[a]										
no.	X	Glu	His	Trp	Ser	Tyr	X	Leu	Arg	Pro	Gly	NH_3
8	Bia	1.00	0.98	1.08	0.89	0.99	1.08	1.01	1.00	1.03	1.03	1.12
9	Tba	0.98	0.93	0.85	0.98	1.02	1.07	1.06	1.00	0.97	1.01	1.27
10	Boa	0.97	1.04	0.90	0.87	1.05	1.03[b]	0.97	0.92	0.88	1.05	1.61
11	Bta	1.03	0.99	0.99	0.81	0.98	c	1.04	0.94	1.05	1.02	2.01[c]
12	Bia	0.98	1.00	1.09[d]	0.87	0.99	0.83	1.03	1.00	0.94		
13	Dmb	0.99	0.97	0.95	1.01	1.09	e	1.00	0.99	0.88	1.05	0.88
14	Tba	1.00	0.98	1.10[d]	0.83	1.03	1.15	1.04	0.96	0.99		
15	Dcb	1.04	1.00	0.90	0.81	1.01	e	1.01	0.94	1.00	1.00	1.11
16	Nia	0.99	0.98	1.03[d]	0.96	1.06	e	1.07	0.93	0.91		

[a] Results are not corrected for the routinely seen 10–15% decomposition of Ser; consequently, Ser and NH_3 values may vary outside the usually accepted range (±0.10). [b] The value given is for Asp. Boa is hydrolyzed to Asp under the hydrolysis conditions. [c] Bta is completely decomposed to NH_3 under these hydrolysis conditions. [d] Trp and $EtNH_2$ do not separate under these analysis conditions. The color yield of $EtNH_2$ is 10% of that of Trp. [e] Dmb, Dcb, and Nia were not eluted from the amino acid analyzer under these conditions. Confirmation of their presence in the products is given in the Experimental Section.

potent analogue (**14**; ~140 times LH-RH). Although previous studies with basic amino acid substitutions have also yielded analogues more potent than LH-RH[30] (e.g., [D-Arg^6]LH-RH, 3.9 times LH-RH; [D-Lys^6]LH-RH, 3.8 times LH-RH), the combination of hydrophobicity and charge in the Tba residue may be responsible for the high potency imparted by this substitution. It is interesting that recent studies on a series of LHRH antagonists have shown that a D basic amino acid in position 6 can impart increased potency and prolonged biological activity.[31,32]

Experimental Section

General Methods. Melting points were obtained on a Thomas-Hoover apparatus, and optical rotations were measured on a Perkin-Elmer Model 141 polarimeter in a 1-dm microcell at 25 °C at the concentration indicated (w/v %). Thin-layer chromatography (TLC) was performed in a solvent vapor saturated chamber on 5 × 20 cm glass plates coated with a 250-μm layer of silica gel GF (Analtech) with the solvent systems in footnote *d* of Table III or solvent A (CH_2Cl_2/CH_3OH/HOAc, 9:1:1), B (CH_2Cl_2/Et_2O, 3:1), or C (CH_3CN/HOAc/H_2O, 8:1:1). The plates were visualized by UV irradiation and by chlorination (Cl_2), followed by 1% KI/starch spray. Silica gel column chromatography was performed on E. Merck silica gel 60 (70–230 mesh) with gravity flow.

Analytical HPLC was performed under isocratic conditions on a Chromatronix Model 3500 equipped with a 20-μL loop injector (Rheodyne) and an Altex Ultrasphere 5-μm C-18 reversed-phase column (4.6 × 250 mm). The eluent contained 40% CH_3CN (Burdick and Jackson, UV) and was 0.03 M in NH_4OAc (Tridom/Fluka, puriss) at pH 7. The column effluent was monitored at 214 nm with a Schoeffel SF770 spectroflow detector.

Amino Acid Analysis. Amino acid analyses were performed on a Beckman 119CL analyzer in the single column mode after 18–24-h hydrolysis in 4 N $MeSO_3H$–0.2% 3-(2-aminoethyl)indole reagent[33] (Pierce Chemical Co.). The buffer sequence pH 3.25 (50 min), pH 4.12 (67 min), pH 6.25 (100 min) was used. Satisfactory amino acid analyses (±10%) were obtained for compounds **8**–**16** (Table IV).

Certain of the most hydrophobic amino acids (Dmb, Dcb, and Nia) were not eluted from the amino acid analyzer under the conditions used. Both Boa and Bta were decomposed under the hydrolysis conditions used. The presence of Boa in **10** is confirmed by its hydrolysis back to Asp (Table IV). Bta decomposed to yield 1 equiv of NH_3 as the only product identifiable on the amino acid analyzer (Table IV). The presence of Dmb [λ_{max} 289 nm (ε 9295)], Dcb [λ_{max} 297 nm (ε 6773)], and Nia [λ_{max} 318 nm (ε 9354)] in the peptides can be confirmed by the UV spectra in MeOH of **13** [λ_{max} 289 nm (ε 13 417)], **15** [λ_{max} 298 (ε 9792)], and **16** [λ_{max} 318 nm (ε 7820)].

(30) Rivier, J.; Ling, N.; Monahan, M.; Rivier, C.; Brown, M.; Vale, W. in "Peptides: Chemistry, Structure and Biology"; Walter, R.; Meienhofer, J.; Eds.; Ann Arbor Science Publishers: Ann Arbor, MI, 1975; p 863.
(31) Coy, D. H.; Horvath, A.; Nekola, M. V.; Coy, E. J.; Erchegyi, J.; Schally, A. V. *Endocrinology* **1982**, *110*, 1445.
(32) Nestor, Jr., J. J.; Ho, T. L.; Tahilramani, R.; Horner, B. L.; Jones, G. H.; McRae, G. I.; Vickery, B. H. In "LHRH and its Analogs: Contraceptive and Therapeutic Applications"; Vickery, B. H.; Nestor, Jr., J. J.; Hafez, E. S. E.; Eds.; MTP Press: Lancaster, England, in press.
(33) Simpson, R. J.; Neuberger, M. R.; Lin, T. Y. *J. Biol. Chem.* **1976**, *251*, 1926.

General Synthetic Procedures. Benzyl *N*-(Benzyloxycarbonyl)-3-(2-benzimidazolyl)-D-alaninate (2a). A solution of α-benzyl *N*-(benzyloxycarbonyl)-D-aspartate[34] (2.0 g, 5.6 mmol) and Et_3N (0.57 g, 5.6 mmol) in dry THF (25 mL) was treated at −10 °C with isobutyl chloroformate (0.77 g, 5.6 mmol). After 10 min at −10 °C, a solution of *o*-phenylenediamine (0.67 g, 6.2 mmol) in THF (12 mL) was added. The reaction mixture was allowed to stir while slowly warming to room temperature (1 h).

The solvent was evaporated, and the residual brown solid was partitioned between EtOAc and H_2O. The EtOAc layer was washed with 5% $NaHCO_3$ (2 × 100 mL) and brine (1 × 100 mL) and dried over $MgSO_4$. The solution was filtered, the solvent was evaporated, and the residual solid (**1a**) was dissolved in glacial HOAc (100 mL). The solution was heated at 65 °C for 2 to 6 h. The solvent was evaporated to yield crude **2a** as a brown oil. The crude product was purified by chromatography on silica gel with a linear eluent gradient (CH_2Cl_2 to CH_2Cl_2/Et_2O, 1:1). The product fractions [R_f (A) 0.35] were pooled and concentrated, and the product was crystallized from EtOAc/hexane; yield 1.34 g (Table II).

3-(2-Benzimidazolyl)-D-alanine (3a). A mixture of **2a** (3.0 g, 7.0 mmol) and 10% Pd/C (0.4 g) in HOAc (70 mL) was treated with H_2 at atmospheric pressure for 3 h. The mixture was filtered through Celite, and the filtrate was concentrated in vacuo. Traces of HOAc were removed by coevaporation with dioxane (2 × 50 mL). The pure product was obtained by crystallization from CH_3OH/Et_2O: yield 1.45 g (Table II).

***N*-(*tert*-Butoxycarbonyl)-3-[*N*-(*tert*-butoxycarbonyl)-2-benzimidazolyl]-D-alanine (4a).** A cold (0 °C) solution of **3a** (0.41 g, 2 mmol) in a mixture of 1 N NaOH (6 mL) and dioxane (16 mL) was treated with $(Boc)_2O$[35] (2.52 g, 12 mmol) and MgO (0.32 g, 8 mmol). The mixture was stirred for 1 h at 0 °C and for 7 h at room temperature. The reaction mixture contained both N^{α}-Boc and N^{α},N^{im}-$(Boc)_2$ protected compounds. An additional portion of $(Boc)_2O$ (0.4 g, 2 mmol) was added, and the mixture was stirred overnight. A further portion of $(Boc)_2O$ (0.2 g, 1 mmol) was added, and the mixture was stirred for 2 h.

The reaction mixture was filtered and concentrated to dryness in vacuo (bath <30 °C), and the residue was taken up in H_2O (10 mL) and washed with Et_2O. The H_2O layer was brought to pH 2.5 with aqueous $NaHSO_4$, and the product was extracted with EtOAc. The EtOAc extract was dried ($MgSO_4$) and filtered, and the filtrate was concentrated to dryness. The crude product [R_f (A) 0.86] was crystallized from EtOAc/hexane, 0.61 g (Table II).

α-Benzyl *N*-(Benzyloxycarbonyl)-D-aspartate *o*-Mercaptoanilide, Disulfide Derivative (5). To a cold solution of o-aminophenyl disulfide (0.93 g, 3.75 mmol) in 75 mL of dry THF was added the freshly crystallized acid chloride of α-benzyl *N*-(benzyloxycarbonyl)-D-aspartate[34] (2.82 g, 7.5 mmol) and *i*-Pr_2EtN (1.3 mL, 1.75 g, 14 mmol). After the reaction had proceeded for 4 h at 4 °C, the solvent was evaporated at reduced pressure, and the residue was slurried in EtOH. The solid was filtered, washed well with EtOH and dried in vacuo to yield **5** as an off-white solid, suitable for most uses: yield 2.55 g (78%). An analytical sample was obtained by prep-TLC (CH_2Cl_2/Et_2O/acetone, 7:7:1) and reprecipitation from EtOAc/hexane (Table II).

Benzyl *N*-(Benzyloxycarbonyl)-3-(2-benzothiazolyl)-D-alaninate (2e). A sample of **5** (2.18 g, 2.5 mmol) in glacial HOAc (200 mL) was warmed to 50 °C. Zn powder (4.0 g, 0.06 mol) was added slowly while most of the disulfide dissolved. Three further additions of Zn powder (0.5 g each) at 30-min intervals were necessary to complete the reduction of **5**. The Zn was filtered, the solution was concentrated to dryness, and remaining traces of HOAc were removed by coevaporation with dioxane.

The residue was dissolved in dioxane (200 mL), and the pH was adjusted to 10 with Et_3N. The solution was stirred under N_2 at room temperature overnight. The reaction mixture was concentrated in vacuo to yield 3 g of crude **2e**. The product was purified by prep-TLC (CH_2Cl_2/Et_2O/acetone, 2:2:1), followed by crystallization from EtOAc/hexane, to yield 1.5 g of **2e** (Table II).

***N*-(*tert*-Butoxycarbonyl)-3-(2-benzothiazolyl)-D-alanine (4e).** A mixture of **2e** (1.25 g, 2.8 mmol) and 10% Pd/C (0.7 g) in glacial HOAc (100 mL) was treated with H_2 at ambient pressure for 2 h. Fresh catalyst (0.25 g) was added, and reduction continued for a total of 4 h. While removal of the benzyl ester was rapid and some of the desired **3e** was formed [R_f (C) 0.38], a substantial portion of the N^{α}-benzyloxycarbonyl-protected acid [R_f (C) 0.82] remained. Filtration of the catalyst and addition of fresh Pd/C (0.5 g) did not completely convert the product to **3e**.

The mixture was filtered, concentrated, and treated with 4 N HBr/HOAc (75 mL) for 1.5 h at 25 °C to remove the residual N^{α}-protecting group. The mixture was concentrated to dryness and purified by prep-TLC (solvent system C) to yield 0.9 g of **3e** as a light yellow solid.

A mixture of **3e** (0.9 g) and $(Boc)_2O$[35] (0.6 g, 2.75 mmol) in 50% dioxane/water (50 mL) was maintained at pH 9.5 with 0.5 M NaOH on a pH-stat. Additional portions of $(Boc)_2O$ (0.22 g, 1 mmol) were added at 0.5-h intervals until all of **3e** had been converted to **4e** [R_f (C) 0.86]. The mixture was concentrated to remove dioxane and washed with Et_2O, and the aqueous layer was acidified with $NaHSO_4$ (pH 2.5). The product was extracted with EtOAc (3 × 100 mL), the extract was dried ($MgSO_4$) and filtered, and the filtrate was concentrated to dryness. The residue was crystallized from EtOAc/hexane to yield **4e** as 0.54 g of white crystals (Table II).

Benzyl *N*-(Benzyloxycarbonyl)-3-(2-benzoxazolyl)-D-alaninate (2f). A solution of α-benzyl *N*-(benzyloxycarbonyl)-D-aspartate (7.14 g, 20 mmol) and Et_3N (2.6 mL, 20 mmol) in dry THF (70 mL) was treated with isobutyl chloroformate (2.7 mL, 20 mmol) at −20 °C. After 30 min, a solution of *o*-hydroxyaniline (2.4 g, 22 mmol) in THF (50 mL) was added. The reaction mixture was stirred while slowly warming to room temperature (1 h). The solution was partitioned between 5% $NaHCO_3$ and EtOAc. The organic layer was washed with 5% $NaHSO_4$, H_2O, 5% $NaHCO_3$, H_2O, and brine. The EtOAc layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated to 8.5 g of yellow oil. Purification of the oil by silica gel chromatography was achieved by elution with a linear gradient from CH_2Cl_2 to CH_2Cl_2/Et_2O (2:1). The purified intermediate **1f** was obtained as 6 g of white solid.

Cyclization to **2f** was achieved by refluxing the intermediate **1f** in propionic acid for 4 h. The solution was concentrated in vacuo, and the residual oil was subjected to silica gel chromatography with a linear gradient from $CHCl_2$ to $CHCl_2/Et_2O$ (3:1). The product [R_f (B) 0.70] was obtained as 1.8 g of white solid (Table II).

3-(2-Benzoxazolyl)-D-alanine (3f). A mixture of **2f** (1.6 g, 4.1 mmol) and 400 mg of 10% Pd/C in HOAc (20 mL) was treated with H_2 for 1 h at atmospheric pressure. The catalyst was filtered through Celite, and the filtrate was concentrated in vacuo to yield **3f** as 0.7 g of off-white solid (Table II).

***N*-(*tert*-Butoxycarbonyl)-3-(2-benzoxazolyl)-D-alanine (4f).** A solution of **3f** (0.41 g, 2 mmol) in a mixture of 1 N NaOH (2 mL) and dioxane (8 mL) was treated with MgO (0.08 g, 2 mmol) and $(Boc)_2O$ (0.48 g, 2.2 mmol) for 4.5 h. The reaction mixture was filtered, the filtrate was concentrated to dryness, and the residue was taken up in H_2O. The aqueous layer was washed with Et_2O, acidified with $NaHSO_4$ (pH 2.5), and extracted with EtOAc. The organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated. The residue was crystallized from EtOAc/hexane to yield 0.5 g of white solid (Table II).

3-(4,5,6,7-Tetrahydrobenzimidazol-2-yl)-D-alanine (6). A mixture of **3a** (1.17 g, 5.74 mmol) and PtO_2 (0.5 g) in 120 mL of 2 N HCl was treated with H_2 at ambient pressure for 6 h. An additional portion of PtO_2 (0.1 g) was added, and hydrogenation continued for a total 24 h. The catalyst was filtered on Celite, and the filtrate was concentrated to 1.3 g of yellow oil. The oil was loaded on a cation-exchange resin (AG 50, H^+ form), washed with 300 mL of H_2O, and eluted with a gradient from H_2O to 1 N NH_4OH. The product-containing fractions were pooled and concentrated to give 1.1 g (98%) of **6** as a white solid (Table II).

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(34) Bergmann, M.; Zervas, L.; Salzmann, L. *Chem. Ber.* **1975**, *66*, 1298.
(35) Tarbell, D. S.; Yamamoto, Y.; Pope, B. M. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 730.
(36) Deutsch, A.; Eggleton, P. *Biochem. J.* **1938**, *32*, 209.

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Registry No. **1a**, 88549-09-1; **1b**, 88549-10-4; **1c**, 88549-11-5; **1d**, 88549-12-6; **1f**, 88562-94-1; **2a**, 81440-37-1; **2b**, 88549-13-7; **2c**, 88549-14-8; **2d**, 88549-15-9; **2e**, 81440-40-6; **2f**, 88549-16-0; **3a**, 81440-45-1; **3b**·HOAc, 88549-18-2; **3c**·HOAc, 88549-20-6; **3d**, 88549-21-7; **3e**, 81440-41-7; **3f**·$^1/_2$HOAc, 88549-23-9; **4a**, 81440-44-0; **4b**, 88549-24-0; **4c**, 88562-95-2; **4d**, 88562-96-3; **4e**, 81440-42-8; **4f**, 88562-97-4; **5**, 81440-38-2; **6**·HOAc, 88549-25-1; **7**, 81440-47-3; **8**, 88562-98-5; **9**, 88562-99-6; **10**, 88563-00-2; **11**, 88563-01-3; **12**, 88563-02-4; **13**, 88563-03-5; **14**, 88563-04-6; **15**, 88563-05-7; **16**, 88563-06-8; *o*-phenylenediamine, 95-54-5; 2,3-naphthalenediamine, 771-97-1; 4,5-dimethyl-1,2-benzenediamine, 3171-45-7; 4,5-dichloro-1,2-benzenediamine, 5348-42-5; *o*-hydroxyaniline, 95-55-6; acid chloride of α-benzyl *N*-(benzyloxycarbonyl)-D-aspartate, 75626-73-2; α-benzyl *N*-(benzyloxycarbonyl-D-asparate, 81440-35-9; *o*-aminophenyl disulfide, 1141-88-4.

Tri- and Tetrapeptide Analogues of Kinins as Potential Renal Vasodilators

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Tri- and tetrapeptide analogues were synthesized and evaluated as renal vasodilators. These peptides were prepared by standard coupling reactions, which also provided good yields with hindered α-methyl amino acid derivatives. Preliminary evidence of renal vasodilator activity was determined in anesthetized dogs by measuring the effects on renal blood flow and calculating the accompanying changes in renal vascular resistance. The most potent compounds contained, in their basic structure, the L-prolyl-DL-α-methylphenylalanyl-L-arginine and L-prolyl-DL-α-methylphenylalanylglycyl-L-proline arrays. Substitution on the N-terminal proline with 4-phenylbutyryl and 4-(4-hydroxyphenyl)butyryl side chains produced enhanced renal vasodilator activity and, in certain cases, selectivity for the renal vasculature.

The renal vasodilator activity of the selective peripheral dopamine agonist 6-chloro-7,8-dihydroxy-1-(4-hydroxyphenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SK&F 82526) was described in an earlier report.[1] This compound is being evaluated clinically and should prove to be an important agent in furthering our understanding of the importance of renal blood flow (RBF) in essential hypertension.[2] Another goal of our research effort was the discovery of other selective renal vasodilators not having a dopaminergic mechanism or component of vasodilatation. Compounds as diverse as acetylcholine, histamine, prostaglandins, captopril, isoproterenol, and theophylline are reported to be vasodilatory.[3] Peptides exemplified by bradykinin, eledoisin, substance P and secretin produce vasodilatation.[3i,4] The vasodilator peptides seemed to offer viable opportunities for development of novel and specific vasodilators. Bradykinin, eledoisin, and substance P have as a common feature a phenylalanine as the fifth amino acid from the carboxy terminus, which might suggest possible structural importance to overall activity, perhaps by way of binding and/or recognition. When bradykinin, i.e., Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, was tested in our anesthetized dog protocol used to screen for intrinsic renal vasodilator activity, it exhibited, at an intravenous (iv) infusion dose of 0.1 to 30 μg/(kg min), an average maximum decrease in renal vascular resistance (RVR) of 67%. Although bradykinin showed a dose-related decrease in RVR resulting in enhanced RBF, bradykinin produced nonselective vasodilatation and a substantial decrease in systemic arterial blood pressure—a typical kinin response. A carboxy-terminal pentapeptide fragment of bradykinin, Phe-Ser-Pro-Phe-Arg (**36**), was marginally effective in the dog protocol as a renal vasodilator but showed a cardiovascular profile different from that of bradykinin (see Structure–Activity Relationships). Encouraged by the biological difference of the pentapeptide fragment and the renal vasodilator activity displayed by a terminal tripeptide fragment analogue of Pro-Phe-Arg, i.e., C_6H_5CO-Pro-Phe-Arg-NHC_6H_4-NO_2-*p*, a compound used to assay for kallikrein activity, it was decided to focus on the structure–activity relationships (SAR) of bradykinin fragments

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(1) Weinstock, J.; Wilson, J. W.; Ladd, D. L.; Brush, C. K.; Pfeiffer, F. R.; Kuo, G. Y.; Holden, K. G.; Yim, N. C. F.; Hahn, R. A.; Wardell, Jr., J. R.; Tobia, A. J.; Setler, P. E.; Sarau, H. M.; Ridley, P. A. *J. Med. Chem.* **1980**, *23*, 973.
(2) (a) Ackerman, D. M.; Weinstock, J.; Wiebelhaus, V. D.; Berkowitz, B. *Drug Dev. Res.* **1982**, *2*, 283. (b) Ackerman, D. M.; Blumberg, A. L.; McCafferty, J. P., Sherman, S. S.; Weinstock, J.; Kaiser, C.; Berkowitz, B. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1983**, *42*, 186. (c) Coleman, T. G.; Guyton, A. C.; Young, D. B.; DeClue, J. W.; Norman, R. A., Jr.; Manning, Jr., R. D. *Clin. Exp. Pharmacol. Physiol.* **1975**, *2*, 571.
(3) (a) Itskovitz, H. D.; Campbell, W. B. *Proc. Soc. Exp. Biol. Med.* **1976**, *153*, 161. (b) McGiff, J. C. *Clin. Sci.* **1980**, *59*, 105s. (c) Antonaccio, M. J.; Cushman, D. W. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1981**, *40*, 2275. (d) McGiff, J. C.; Wang, P. Y-K; *ACS Symp. Ser.* 1978, *83*, p 1. (e) Levinsky, N. G. *Circ. Res.* **1979**, *44*, 441. (f) Carriere, S. *Can. J. Physiol. Pharmacol.* **1975**, *53*, 1. (g) Zimmerman, B. G.; Wong, P. C.; Kounenis, G. K.; Kraft, E. J. *Am. J. Physiol.* **1982**, 243. *Heart Circ. Physiol.* **1982**, *12*, H277, and references cited therein. (h) Nasjletti, A.; Colina-Chouriv, J.; McGiff, J. C. *Acta Physiol. Latinoam.* **1974**, *24*, 587. (i) Fadem, S. Z.; Hernandez-Llamas, G.; Patak, R. V.; Rosenblatt, S. G.; Lifschitz, M. D.; Stein, J. H. *J. Clin. Invest.* **1982**, *69*, 604. (j) Williams, G. H.; Hollenberg, N. K. *N. Engl. J. Med.* **1977**, *297*, 184. (i) Nasjletti, A.; Colina-Chourio, J.; McGiff, J. C. *Circ. Res.* **1975**, *37*, 59. (j) Mills, I. M. *Nephron* **1979**, *23*, 61. (k) Flamenbaum, W.; Cagnon, J.; Ramwell, P. *Am. J. Physiol.* **1979**, *237*, F433. (l) Bicking, J. B.; Bock, M. G.; Cragoe, E. J.; DiPardo, R. M.; Gould, N. P.; Holtz, W. J.; Lee, T.-J.; Robb, C. M.; Smith, R. L.; Springer, J. P.; Blaine, E. H. *J. Med. Chem.* **1983**, *26*, 342.
(4) (a) Erdos, E. G.; Back, N.; Sicuteri, F. Eds. "Hypotensive Peptides"; Springer-Verlag: Berlin, 1966. (b) Tenner, Jr., T. E.; Yang, J. K.; Schimizu, M.; Pang, P. K. T. *Peptides* **1980**, *1*, 285. (c) McGiff, J. C. *Med. Clin. North Am.* **1968**, *52*, 263. (d) Barraclough, M. A.; Mills, I. M. *Clin. Sci.* **1965**, 28, 69. (e) McGiff, J. C.; Itskovitz, H. D.; Terragno, N. A. *Clin. Sci. Mol. Med.* **1975**, *49*, 125.

0022-2623/84/1827-0325$01.50/0