Structure-Activity Studies of a Novel Bicyclic Oxytocin Antagonist

D. David Smith,[†,§] Jirina Slaninova,[‡] and Victor J. Hruby*[,†]

Department of Chemistry, University of Arizona, Tucson, Arizona 85721, and Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 16610 Praha 6, Czechoslovakia. *Received August 23, 1991*

In this report, we describe structure-activity studies of the bicyclic oxytocin antagonist [Mpa[1],cyclo(Glu[4],Lys[8])]oxytocin. The monocylic analogue [dPen[1],Glu[4],Lys[8])]oxytocin was a weak oxytocin antagonist with a pA_2 value of 5.8 in the uterotonic assay. Bicyclization of this analogue yielded [dPen[1],cyclo(Glu[4],Lys[8])]oxytocin, a potent antagonist of oxytocin in the uterotonic assay (pA_2 8.74) with a potency 3 times greater than that of [Mpa[1],cyclo(Glu[4],Lys[8])]oxytocin. [dPen[1],cyclo(Glu[4],Lys[8])]oxytocin also was a weak antagonist in the pressor assay with a pA_2 of 6.3. To establish if the potent antagonistic effects of these bicyclic compounds was because of the lactam ring or merely the result of obtaining an optimal degree of lipophilicity of the side chains in positions 4 and 8, we synthesized a series of analogues containing neutral and/or charged groups on these side chains. Monocyclic derivatives of [Mpa[1],Gln[4],Lys(CHO)[8]]oxytocin were moderate to weak agonists of oxytocin all following classical structure-activity profiles of oxytocin. The monocyclic derivatives of [dPen[1],Gln[4],Lys(CHO)[8]]oxytocin were antagonists of oxytocin which was attributed to the dPen[1] substitution. However, the potency of all of these latter derivatives was at least 1 order of magnitude less than [dPen[1],cyclo(Glu[4],Lys[8])]oxytocin. These results suggest that the potent antagonistic properties of the bicyclic analogues [Mpa[1],cyclo(Glu[4],Lys[8])]oxytocin and [dPen[1],cyclo(Glu[4],Lys[8])]oxytocin can be attributed to the effect of the lactam bridge on the conformational flexibility and topographical properties of the analogues, rendering them more favorable for binding to the receptor in such a manner as to prevent transduction of a biological response.

Introduction

Oxytocin (H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2, OT, I)[1] is a neurohypophyseal hormone synthesized in the hypothalamus and transported to the posterior lobe of the pituitary along with its carrier proteins, the neurophysins, where it is stored. Upon release into the circulation it is generally accepted that oxytocin is responsible for the maintenance of labor and for milk ejection in mammals. Less clear is the exact role of oxytocin in the central nervous system where it has been shown to be involved in memory and learning processes, and grooming and sexual behaviors.[2]

Recently, we described the design, synthesis, and biological activity of a novel bicyclic analogue of oxytocin, [Mpa[1],cyclo(Glu[4],Lys[8])]oxytocin (IV), Figure 1.[3] Whereas the monocyclic precursor had very weak agonistic activity, upon lactam cyclization between the γ-carboxyl group of Glu[4] and the ϵ-amino group of Lys[8], the resulting bicyclic analogue was a potent antagonist with a pA_2 value of 8.2 in the uterine smooth muscle assay. We arrived at the bicyclic structure after careful examination of the X-ray crystal structure of deaminooxytocin[4,5] and consideration of the extensive structure-activity relationships of oxytocin.[2] The potent antagonistic activity of the bicyclic analogue was attributed to (i) a predominant conformation highly favorable for binding, and (ii) a structure with a high degree of rigidity which prevents transduction of a biological response. Evidence for rigidity within the molecule was obtained from preliminary NMR data.[3]

To obtain further evidence that the high antagonistic activity of IV was as a result of the rigid structure, and not merely the result of obtaining an optimal degree of lipophilicity of the side-chain residues in positions 4 and 8, we have synthesized a variety of analogues of IV containing neutral and/or charged side chains in positions 4 and 8 and determined their biological activities.

Classically, most oxytocin antagonists have a residue in position 1 with bulky alkyl groups on the β-carbon, such as in [Pen[1]]oxytocin (II) and related analogues.[5,7] The absence of an N-terminal α-amino group usually enhances the potency (compare [Pen[1]]OT, pA_2 = 6.86 to [dPen[1]]OT,

Table I. Analytical Characteristics of Oxytocin Analogues

peptide	TLC[a] R_f values A	B	C	D	HPLC[b] K' values	FAB-MS (M + H)[+] calcd	obsd
VI	0.24	0.60	0.59	0.83	8.17	1018	1018
VII	0.11	0.41	0.28	0.75	0.54	1007	1007
VIII	0.13	0.42	0.30	0.77	0.67	1035	1035
IX	0.17	0.51	0.53	0.82	1.00	1035	1035
X	0.20	0.53	0.54	0.83	1.14	1063	1063
XII	0.15	0.39	0.57	0.82	1.67	1036	1036
XIII	0.23	0.50	0.46	0.81	1.05	1036	1036
XIV	0.26	0.53	0.48	0.82	1.99	1064	1064

[a] Silica gel F 250-μm (Merck 5714) glass plates (5 × 20 cm) were used. The following solvent systems were used: (A) *n*-BuOH/AcOH/H_2O, 4/1/5 (v/v/v) (upper phase); (B) *n*-BuOH/AcOH/pyr/H_2O, 15/3/10/12 (v/v/v/v); (C) *n*-pentanol/pyr/H_2O, 7/7/6 (v/v/v); (D) EtOAc/pyr/HOAc/H_2O, 5/5/1/3 (v/v/v/v). [b] Vydac C_{18} column (25 cm × 4.6 mm), 0.1% aqueous TFA/CH_3CN 80/20, flow rate 1 mL/min, monitored at λ = 220 nm.

(1) Symbols and abbreviations are in accord with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1972**, *247*, 977). All optically active amino acids are of the *L* variety unless otherwise stated. Other abbreviatiokns include the following: N^{α}-Boc, N^{α}-*tert*-butyloxycarbonyl; DIC, diisopropylcarbodiimide; TLC, thin-layer chromatography; RP-HPLC, reversed-phase high performance liquid chromatography; FAB-MS, fast atom bombardment mass spectrometry; TFA, trifluoroacetic acid; HOBT, *N*-hydroxybenzotriazole; DMF, *N*,*N*-dimethylformamide; *p*-MBHA, *p*-methylbenzhydrylamine; DCM, dichloromethane; TEA, triethylamine; DPPA, diphenyl phosphorazidate; OT, oxytocin; Pen, penicillamine (β,β-dimethylcysteine); dPen, deaminopenicillamine, (β,β-dimethyl-β-mercaptopropionic acid); Mpa, β-mercaptopropionic acid (dCys).

(2) Hruby, V. J.; Chow, M. S.; Smith, D. D. Conformational and Structural Considerations in Oxytocin-Receptor Binding and Biological Activity. *Annu. Rev. Pharmacol. Toxicol.* **1990**, *30*, 501-534, and references therein.

(3) Hill, P. S.; Smith, D. D.; Slaninova, J.; Hruby, V. J. Bicyclization of a Weak Oxytocin Agonist Produces a Highly Potent Oxytocin Antagonist. *J. Am. Chem. Soc.* **1990**, *112*, 3110-3113.

(4) Wood, S. P.; Tickle, I. J.; Trehanne, A. M.; Pitts, J. E.; Mascarenkas, Y.; Li, J. Y.; Husain, J.; Cooper, S.; Blundell, T. L.; Hruby, V. J.; Buku, A.; Fishman, A. J.; Wyssbrod, H. R. Crystal Structure Analysis of Deamino-Oxytocin: Conformational Flexibility and Receptor Binding. *Science* **1986**, *232*, 633-636.

* Author to whom reprint requests and correspondence should be addressed at the University of Arizona.

[†] University of Arizona.

[‡] Czechoslovak Academy of Sciences.

[§] Present address: Department of Biomedical Sciences, Creighton University, 2500 California Street, Omaha, NE 68178.

0022-2623/92/1835-1558$03.00/0

Table II. Amino Acid Compositions

compd	Tyr	Ile	Glu	Asp	Cys	Pro	Lys	Gly
VI	1.07 (1)	0.93 (1)	1.05 (1)	0.97 (1)	0.93 (1)	1.10 (1)	1.07 (1)	1.02 (1)
VII	1.03 (1)	0.99 (1)	1.08 (1)	1.15 (1)	0.95 (1)	0.92 (1)	1.03 (1)	1.06 (1)
VIII	1.07 (1)	0.93 (1)	1.05 (1)	0.93 (1)	0.99 (1)	1.06 (1)	1.02 (1)	1.05 (1)
IX	1.03 (1)	0.93 (1)	1.07 (1)	0.96 (1)	0.91 (1)	0.94 (1)	1.04 (1)	1.07 (1)
X	1.09 (1)	0.97 (1)	1.07 (1)	0.91 (1)	0.98 (1)	0.96 (1)	1.03 (1)	1.06 (1)
XII	1.04 (1)	0.96 (1)	1.04 (1)	1.00 (1)	0.91 (1)	1.01 (1)	1.04 (1)	1.04 (1)
XIII	1.04 (1)	0.94 (1)	1.07 (1)	0.94 (1)	0.94 (1)	0.94 (1)	1.03 (1)	1.05 (1)
XIV	1.05 (1)	0.95 (1)	1.03 (1)	0.97 (1)	0.92 (1)	0.99 (1)	1.01 (1)	1.01 (1)

Table III. Biological Activities of the New Modified Analogues of Oxytocin

		biological activity[a]		
	compound	uterotonic in vitro (IU/mg)	pressor (IU/mg)	galactogogic in vivo (IU/mg)
I	oxytocin	450	3.1	450
II	[Pen^1]oxytocin	pA_2 = 6.86	0[b]	ND[b]
III	[Mpa^1]oxytocin	803	1.44	541
IV	[Mpa^1,cyclo(Glu^4,Lys^8)]oxytocin	pA_2 = 8.2	0.1	pA_2 = 6.0
V	[dPen^1]oxytocin	pA_2 = 6.94	pA_2 = 6.27	MC[c]
VI	[dPen^1,cyclo(Glu^4,Lys^8)]oxytocin	pA_2 = 8.74	pA_2 = 6.3	MC[c]
VII	[Mpa^1,Lys^8]oxytocin	80.2	368	272
VIII	[dPen^1,Lys^8]oxytocin	pA_2 = 7.78	pA_2 = 6.5	0[b]
IX	[Mpa^1,Lys(CHO)^8]oxytocin	173.2	101.6	517
X	[dPen^1,Lys(CHO)^8]oxytocin	pA_2 = 7.40	pA_2 = 6.2	1.1
XI	[Mpa^1,Glu^4,Lys^8]oxytocin	0.4	0.1	9.1
XII	[dPen^1,Glu^4,Lys^8]oxytocin	pA_2 = 5.8	0	<0.01
XIII	[Mpa^1,Glu^4,Lys(CHO)^8]oxytocin	0	0	8.0
XIV	[dPen^1,Glu^4,Lys(CHO)^8]oxytocin	pA_2 = 5.1	0	0

[a] All activities were determined in rat. [b] 0 means inactive up to dose 2×10^{-2} mp; ND = not determined. [c] MC = mixed character.

I	Oxytocin	H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2
II	[Pen^1]oxytocin	H-Pen-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2
III	[Mpa^1]oxytocin	Mpa-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2
IV	[Mpa^1,cyclo(Glu^4,Lys^8)]oxytocin	Mpa-Tyr-Ile-Glu-Asn-Cys-Pro-Lys-Gly-NH_2
V	[dPen^1]oxytocin	dPen-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2
VI	[dPen^1,cyclo(Glu^4,Lys^8)]oxytocin	dPen-Tyr-Ile-Glu-Asn-Cys-Pro-Lys-Gly-NH_2
VII	[Mpa^1,Lys^8]oxytocin	Mpa-Tyr-Ile-Gln-Asn-Cys-Pro-Lys-Gly-NH_2
VIII	[dPen^1,Lys^8]oxytocin	dPen-Tyr-Ile-Gln-Asn-Cys-Pro-Lys-Gly-NH_2
IX	[Mpa^1,Lys(CHO)^8]oxytocin	Mpa-Tyr-Ile-Gln-Asn-Cys-Pro-Lys(CHO)-Gly-NH_2
X	[dPen^1,Lys(CHO)^8]oxytocin	dPen-Tyr-Ile-Gln-Asn-Cys-Pro-Lys(CHO)-Gly-NH_2
XI	[Mpa^1,Glu^4,Lys^8]oxytocin	Mpa-Tyr-Ile-Glu-Asn-Cys-Pro-Lys-Gly-NH_2
XII	[dPen^1, Glu^4,Lys^8]oxytocin	dPen-Tyr-Ile-Glu-Asn-Cys-Pro-Lys-Gly-NH_2
XIII	[Mpa^1,Glu^4,Lys(CHO)^8]oxytocin	Mpa-Tyr-Ile-Glu-Asn-Cys-Pro-Lys(CHO)-Gly-NH_2
XIV	[dPen^1,Glu^4,Lys(CHO)^8]oxytocin	dPen-Tyr-Ile-Glu-Asn-Cys-Pro-Lys(CHO)-Gly-NH_2

Figure 1. Structures of oxytocin and oxytocin analogues examined in these studies.

pA_2 = 6.94) on the rat uterine assay.[6,7] The antagonism in these cases is attributed to an increased conformational rigidity of the peptide backbone in the 20-membered ring, and in some of the side-chain groups as a result of the conformational properties and the bulky substituents.[8] We, therefore, were interested in the effect on biological activity that would result from substitution of the β-Mpa^1(dCys) residue in our bicyclic analogue with a dPen. To complete the picture, we also have synthesized and examined all of the corresponding model monocyclic analogues and determined their biological activities.

Results and Discussion

Peptides were prepared by the solid-phase method of peptide synthesis[9] utilizing the *p*-methylbenzhydrylamine resin (p-MBHA-resin) and using standard methods developed in our laboratory.[10] Coupling of the N^{α}-Boc-amino acid derivatives to the growing peptide chain was accomplished using diisopropylcarbodiimide (DIC). For the coupling of N^{α}-Boc-asparagine and N^{α}-Boc-glutamine, hydroxybenzotriazole (HOBT) was added to the coupling mixture to prevent dehydration of the carboxamide side-chain moiety. The peptides were cleaved from the resin, oxidized to the disulfide compound, and purified using methods similar to those previously described.[3] The lactam ring of the bicyclic analogues was also formed using the optimized methods described in the previous paper.[3] The purity and structure of the peptides was assessed by TLC, FAB-MS, amino acid analysis, and analytical RP-HPLC. Analytical data are shown in Table I, and amino

(5) Hruby, V. J. Implications of the X-ray Structure of Deamino-Oxytocin to Agonist/Antagonist - Receptor Interactions. *Trends Pharm. Sci.* **1987**, *8*, 336–339.
(6) Lebl, M. Analogs with Inhibitory Properties. In *Handbook of Neurohypophyseal Hormone Analogues*; Jost, K., Lebl, M., Brtnik, F., Eds.; CRC Press: Boca Raton, FL, 1987; Vol. 2, Part 1, pp 17–74.
(7) Hruby, V. J.; Smith, C. W. Structure-Activity Relationships of Neurohypophyseal Peptides. In *The Peptides: Analysis, Synthesis, Biology*; Smith, C. W., Ed.; Academic Press: New York, 1987; Vol. 8, pp 77–207.
(8) Meraldi, J. P.; Hruby, V. J.; Brewster, A. I. R. Relative Conformational Rigidity in Oxytocin and [1-Penicillamine]-oxytocin: A Proposal for the Relationship of Conformational Flexibility to Peptide Hormone Agonism and Antagonism. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 1373–1377.
(9) Merrifield, R. B. Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide. *J. Am. Chem. Soc.* **1963**, *85*, 2149–2154.
(10) Hruby, V. J.; Upson, D. A.; Agarwal, N. S. Comparative Use of Benzhydrylamine and Chloromethylated Resins in Solid Phase Synthesis of Carboxamide Terminal Peptides: Synthesis of Oxytocin Derivatives. *J. Org. Chem.* **1977**, *42*, 3552–3557.

acid compositions are shown in Table II.

In order to make peptide VI (Figure 1), we needed to make deaminopenicillamine with its side-chain sulfhydryl group protected with the HF-labile *p*-methylbenzyl group. This was easily accomplished following essentially the same procedure as Schulz and du Vigneaud for the synthesis of *S*-benzyldeaminopenicillamine.[11] In the presence of piperidine at reflux for 13 h, 4-methylbenzyl mercaptan reacted with 3,3-dimethylacrylic acid through a Michael addition to form *S*-(4-methylbenzyl)deaminopenicillamine in 53% yield. This is a better yield than was obtained in the analogous benzyl derivative preparation and is probably due to the inductive effect of the para-substituted methyl group rendering the sulfur of the mercaptan more nucleophilic and therefore promoting the attack on the electron-deficient conjugated double bond. Recently in a communication, Yim et al.[12] and Stanfield and Hruby[13] have reported the synthesis of several β,β-dialkyl-β-mercaptopropionic acid derivatives (including *S*-(*p*-methylbenzyl)deaminopenicillamine) using a similar procedure.

The oxytocin analogues were examined, as in our previous studies, in two oxytocin assays, the in vitro uterotonic assay on isolated rat uterine strips and the in vivo galactogogic assay, and in a vasopressin assay, namely the rat pressor activity in vivo.[14] All biological activities are listed in Table III together with the activities of oxytocin, deaminooxytocin, a classical oxytocin agonist, and [dPen^1]-oxytocin and [Pen^1]oxytocin, classical oxytocin antagonists.

As may have been predicted, [dPen^1,Glu^4,Lys^8]oxytocin (XII) was found to be a weak antagonist in the uterotonic assay with a pA_2 value of 5.8 (Table III). This antagonism can be attributed to the dPen^1 substitution. However, XII is much less potent than the classical oxytocin antagonist [Pen^1]oxytocin (pA_2 = 6.86) or the slightly more potent deamino version, [dPen^1]oxytocin (pA_2 = 6.94).[6,7] Compound XII had no measurable activity in either the galactogogic assay or in the pressor assay. This very large drop in potency in these latter two assays is apparently due to the presence of a Glu residue in position 4, which has previously been shown[6,7] to greatly reduce the potency of analogues in both the oxytocin and vasopressin series (see also compound XI, Table III, in this series).

Bicyclization of monocyclic compound XII produced [dPen^1,cyclo(Glu^4,Lys^8)]oxytocin (VI), which proved to be a highly potent antagonist in the uterotonic assay with a pA_2 value of 8.74. Hence this compound is 3 times more potent than the previously reported bicyclic antagonist [Mpa^1,cyclo(Glu^4,Lys^8)]oxytocin (IV). Again no activity was seen in the galactogogic assay. However, VI was a weak antagonist in the pressor assay with a pA_2 value of 6.5. Thus VI has about a 400-fold selectivity for the uterine receptor over the pressor receptor. Furthermore, comparison of the bicyclic analogues IV with VI (Table II) indicates that substitution of dPen for Mpa^1 leads to a change in interaction of the analogue with the pressor receptor (from a very weak agonist to a weak antagonist). On the basis of previous conformational studies on [Pen^1]-substituted analogues of oxytocin[15] it is reasonable to assume that the penicillamine substitution will introduce more rigidity into the peptide backbone. Thus the increased antagonistic potency of compound VI over compound IV in the uterotonic assay may be attributed to compound VI being a more rigid molecule, quite likely existing predominantly in a conformation favorable for binding to the receptor in its antagonist (nonproductive) conformation.

Compounds VII and IX are full oxytocin agonists in all assays used, with only a small drop in potency over oxytocin. This is in accord with previous structure–activity studies. The presence of a positive charge at position 8 generally results in only a modest decrease in potency of an oxytocin agonist analogue at the oxytocin receptor, while in the case of antagonists it generally leads to more potent binding. This is further corroborated by the fact that arginine-vasopressin and lysine-vasopressin also are full agonists in these assays.[7] However, introduction of a negative charge at position 4 as in compounds XI and XIII results in a drastic reduction in potency and a complete loss of activity, respectively (Table III). Past structure–activity studies also have shown that substitution of a Glu residue for Gln in position 4 results in a drop in potency ([Glu^4]oxytocin has 1.5 IU/mg and [Mpa^1,Glu^4]oxytocin has 13.3 IU/mg of activity in the rat uterotonic assay, see Table III). Thus, the topography and conformation of compound IV, which results in its possessing antagonistic activity, is a direct result of its bicyclic structure and not because of the presence of neutral polar side chains in positions 4 and/or 8 of deaminooxytocin. The monocylic derivatives of compound IV possess no antagonistic activity and all follow classical structure–bioactivity profiles of oxytocin and deaminooxytocin.

Predictably, all of the monocyclic derivatives of the bicyclic analogue VI (compounds VIII, X, XII, and XIV) are antagonists of oxytocin (Table III). This is undoubtedly a result of the [dPen^1] substitution which has been shown to confer antagonistic activity on neurohypophyseal hormones. The introduction of Lys into position 8 of [dPen^1]oxytocin either with its positively charged side chain as in compound VIII, or with its blocked, neutral, polar side chain as in compound X, results in approximately a 10-fold increase in antagonist potency in the uterine assay.

Both analogues have similar antagonistic potencies in the pressor assay, values that are comparable to those for [dPen^1]oxytocin. Compound VIII, which contains a Lys^8 residue, does not appear to have a greater affinity for the pressor assay over compound X or [dPen^1]oxytocin. This is surprising since VIII may be viewed as much a lysine–vasopressin analogue as an oxytocin analogue.

When considering the potencies of Glu^4,Lys^8- or Lys(CHO)^8-substituted compounds XII and XIV, we find that once again a negative charge on the side chain of residue 4 results in a drastic loss in potency. In this case, compounds XII and XIV are antagonists with very weak po-

(11) Schulz, H.; du Vigneaud, V. Synthesis of 1-L-Penicillamine-Oxytocin, 1-D-Pennicillamine-Oxytocin and 1-Deaminopenicillamine-Oxytocin, Potent Inhibitors of the Oxytocic Response of Oxytocin. *J. Med. Chem.* **1966**, *9*, 647–650.

(12) Yim, N. C. F.; Moore, M. L.; Huffman, W. F.; Bryan, H. G.; Chang, H. L.; Kinter, L. B.; Edwards, R.; Stassen, F. L.; Schmidt, D.; Heckman, G. Potent Antagonists of Vasopressin Antidiuretic Activity That Lack the β,β-Cyclopentamethylene-β-mercaptopropionic Acid Substitution at Position 1. *J. Med. Chem.* **1986**, *29*, 2425–2426.

(13) Stanfield, C. F.; Hruby, V. J. The Michael Addition of Sulfur Anions to β,β-Substituted N$^{\alpha}$-Formyl-α,β-Dehydroamino Acid Esters. *Synth. Commun.* **1988**, *18*, 531–543.

(14) Slaninova, J. Fundamental Biological Evaluation. In *Handbook of Neurohypophyseal Hormone Analogues*; Lebl, M., Jost, K., Brtnik, F., Eds.; CRC Press: Boca Raton, FL, 1987; Vol. 1, Part 2, pp 83–107.

(15) For a review, see Hruby, V. J.; Lebl, M. Conformational Properties of Neurohypophyseal Hormone Analogs in Solution as Determined by NMR and Laser Raman Spectroscopies. In *Handbook of Neurohypophyseal Hormones*; Jost, K., Lebl, M., Brtnik, F., Eds.; CRC Press: Boca Raton, FL, 1987, Vol. 1, Part 1, pp 105–155.

tencies (pA_2 values of 5.8 and 5.1, respectively), the drop in potency being greater than 10-fold relative to [dPen^1]oxytocin. Analogous to their deaminoxytocin derivatives VI and VII, compounds XII and XIV had virtually no activity in the galactogogic and pressor assays. From these results we can conclude that the potent antagonistic activity of compound VI can be attributed to the bicyclic nature of the compound and the effect that this has on the resulting conformation and topography.

Clearly the differences in potencies and activities between bicyclic compounds IV and VI and their corresponding linear derivatives suggest that covalently linking the side chains of residues 4 and 8 has an important effect on the overall shape of the molecules, resulting in a very favorable ability to bind to the uterotonic receptor, but a loss in the ability of the compound to transduce the signal necessary for inducing agonist activity. This effect on the shape cannot be achieved through weaker hydrophobic interactions or salt bridges as might be envisaged in the monocyclic derivatives.

Using analytical HPLC we have been able to quantitatively assess the lipophilic nature of each compound using reversed-phase C18 chromatography. The K' values of the monocyclic analogues were between 0.5 and 2.00, with the dPen^1-substituted analogues having slightly larger values than the analogous Mpa-substituted compounds as predicted (e.g., compare compound IX, $K' = 1.00$, to compound X, $K' = 1.14$, Table I). When these values are compared with the K' value of 8.17 for the bicyclic analogue VI, it is clear that the corresponding dPen^1 monocyclic compounds are much less lipophilic. This increase in hydrophobicity of the bicyclic compound may be partly responsible for the enhanced binding of the analogue to the oxytocic receptor.

From structure–activity studies it has been clearly shown that oxytocin antagonists bind in a different manner than agonists when they interact with the uterine receptor. To date we do not know how the antagonist IV interacts with the uterine receptor, but the fact that substitution of Mpa^1 for dPen has an additive effect on the potency of the antagonism might suggest that compound IV interacts with the uterine receptor in a similar fashion to classical oxytocin antagonists such as [dPen^1]oxytocin. Detailed conformational analyses of compounds IV and VI are currently being performed by using 2D NMR techniques.

Experimental Section

General Methods. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 983 instrument. NMR spectra were obtained on a Bruker WM250 machine operating at 250 MHz for ^{1}H and 62.9 MHz for ^{13}C. Elemental analyses were performed by Desert Analytics (Tucson, AZ). Amino acid analysis was performed on a Beckman 7300 machine after acid hydrolysis in sealed tubes with 4 M methanesulfonic acid at 110 °C for 48 h. Fast atom bombardment mass spectra (FAB–MS) determinations were performed by the Midwest Center for Mass Spectrometry (Lincoln, NE). Standard solid-phase peptide synthesis methodology[10,16] was used to make peptides VI–XIV with a Vega (Tucson, AZ) Model 250 or 1000 peptide synthesizer. Amino acid derivatives were either purchased from Bachem (Torrence, CA) or prepared according to literature procedures.[16] All of the peptides were made on a *p*-methylbenzhydrylamine (MBHA) resin which was obtained from Bachem (Torrence, CA) (0.55 mequiv/g of resin). Either the carbodiimide method of activation by using a 2.5-fold excess of amino acid and diisopropylcarbodiimide (DIC), or for N^α-Boc-Asn and N^α-Boc-Gln, a 3-molar excess of the hydroxybenzotriazole active ester were used for coupling reactions. Completion was monitored by the ninhydrin[17] or the chloranil[18] tests. Purity of the final product was assessed by thin-layer chromatography (TLC) in four different solvents, reversed-phase high-performance liquid chromatography (RP-HPLC), fast atom bombardment mass spectrometry (FAB-MS), and amino acid analysis. In all cases when the amino acid composition of the peptides are reported, values in brackets refer to the number of residues of a given type in a molecule. Cysteine was determined as cysteic acid.

***S*-(4-Methylbenzyl)deaminopenicillamine [dPen(*S*-4-MeBzl)].** A mixture of 3,3-dimethylacrylic acid (8 g, 80 mmol), 4-methylbenzyl mercaptan (11.04 g, 80 mmol), and piperidine (12 mL, 121 mmol) were refluxed for 13 h. Upon cooling, the reaction mixture was acidified with concentrated HCl and extracted with ether (3 × 100 mL). The combined ethereal extracts were washed with saturated aqueous sodium hydrogen carbonate ($NaHCO_3$) solution (3 × 100 mL), and the combined alkaline washings were acidified to pH 2 with concentrated HCl before being washed with ether (3 × 50 mL). The combined ether extracts were dried over anhydrous sodium sulfate (Na_2SO_4), and after removal of the drying agent the solvent was evaporated under reduced pressure. The resultant yellow oil (which solidified on standing) was distilled under reduced pressure to yield the desired product (bp 155–160 °C, 0.5 mmHg) as a pale yellow viscous liquid containing trace amounts of the mercaptan. The product was dissolved in saturated aqueous $NaHCO_3$ (100 mL) and washed with ether (3 × 50 mL) to remove the mercaptan. The aqueous solution was acidified with concentrated HCl, and the product was extracted into ether (3 × 50 mL). The combined ether extracts were dried over anhydrous Na_2SO_4 and filtered, and the solvent was removed under reduced pressure from the filtrate to yield a colorless oil which was further dried over sodium hydroxide pellets under reduced pressure for 2 days to give the desired product as a white crystalline material: 10.13 g (53%); mp 58–60 °C; IR (Nujol) 3400–2500 (br, OH), 1701 (s, C=O), 1410, 1233 (m, C–O str and O–H bending), 824 (m, aromatic C–H) cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.48 (s, 6 H, β-CH_3's), 2.31 (s, 3 H, *p*-CH_3), 2.65 (s, 2 H, SCH_2), 3.81 (s, 2 H, CH_2CO_2H), 7.22 (2 d, 4 H, p-substd aromatic Hs), 10.59 (s, 1 H, CO_2H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 21.0 (*p*-CH_3), 28.6 (β-CH_3's), 33.0 (α-C), 43.8 (β-C), 46.9 (Bzl CH_2), 128.9, 129.2, 134.4, 136.6 (aromatic C's), 176.7 ppm (C=O). Anal. ($C_{13}H_{18}SO_2$) C: calcd, 65.51; found, 65.44; H: calcd, 7.61; found, 7.73.

N^α-Boc-Gly-MBHA Resin. MBHA resin (10 mmol, 11.36 g) was neutralized with a 10% solution of diisopropylethylamine (DIEA) in dichloromethane (DCM) (2 × 2 min) and washed with DCM (5 × 1 min) before being mixed with N^α-Boc-Gly (4.4 g, 25 mmol) and DIC (3.9 g, 25 mmol) in DCM (50 mL) for 1 h after which the ninhydrin result was negative. The resin was washed subsequently with DCM (2 × 1 min), ethanol (2 × 1 min), DCM (2 × 1 min), ethanol (2 × 1 min), and DCM (5 × 1 min) and dried under reduced pressure. Amino acid analysis of an aliquot of resin showed a substitution of 0.5 mmol of glycine per gram of resin.

dPen-Tyr-Ile-Glu-Asn-Cys-Pro-Lys-Gly-NH$_2$ (XII). The title compound was prepared using methods similar to those previously reported[10] with some modifications. The N^α-Boc group was removed from N^α-Boc-Gly-MBHA resin (1.0 mmol) by treatment with 50% trifluoroacetic acid (TFA) in DCM for 2 and 20 min. N^α-Boc-Lys(N^ϵ-2,4-Cl_2Z) (2.5-fold excess) was coupled to the resin using the carbodiimide method. This process was repeated for the stepwise addition of N^α-Boc-Pro, N^α-Boc-Cys(*S*-4-MeBzl), N^α-Boc-Asn, N^α-Boc-Glu(*O*-Bzl), N^α-Boc-Ile, N^α-Boc-Tyr(*O*-2,6-Cl_2-Bzl), and dPen(*S*-4-MeBzl). N^α-Boc-Asn and N^α-Boc-Gln were coupled as their hydroxybenzotriazole active esters to avoid nitrile formation. After the last residue was coupled

(16) Stewart, J. M.; Young, J. D. *Solid Phase Peptide Synthesis*, 2nd ed.; Pierce Chemical Co.: Rockford, IL, 1984.

(17) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. Color Test for Detection of Free Terminal Amino Groups in the Solid-Phase Synthesis of Peptides, *Anal. Biochem.* **1970**, *34*, 595–598.

(18) Christensen, T. A Chloranil Color Test for Monitoring Coupling Completeness in Solid Phase Peptide Synthesis. In *Peptides, Structures and Biological Function*; Gross, E., Meinenhofer, J., Eds.; Pierce Chemical Co.: Rockford, IL, 1979; pp 385–388.

to the growing peptide chain, the resulting dPen(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2Bzl)-Ile-Glu(*O*-Bzl)-Asn-Cys(*S*-4-MeBzl)-Pro-Lys-(N^{ϵ}-2,4-Cl_2Z)-Gly-MBHA resin was dried under reduced pressure.

The petpide resin was treated with anhydrous hydrogen fluoride (HF) (20 mL) in the presence of *p*-thiocresol (2 g) at 0 °C for 1 hr to simultaneously cleave the peptide from the resin and its side-chain protecting groups. After evaporation of the HF, the peptide resin mixture was washed with degassed ethyl ether (2 × 50 mL) to remove excess scavenger and extracted with 5% aqueous acetic acid (100 mL) and glacial acetic acid (50 mL). The two acid extracts were lyophilized separately to yield a fluffy off-white powder.

The linear peptide was suspended in water (2 L), and the pH was adjusted to 8.5 with aqueous ammonia before a 0.01 M $K_3Fe(CN)_6$ solution was added dropwise, until the yellow color persisted for 30 min. The solution was stirred for another 30 min to ensure complete cyclization, before terminating the reaction by lowering the pH to 4.5 with glacial acetic acid. Amberlite IRA68 (Cl^- form) (30-mL settled volume) was added, stirring was continued for 1 h to remove the excess ferro- and ferricyanide ions, the mixture was filtered, and the solution was concentrated under reduced pressure and lyophilized. The product was purified by (i) gel filtration chromatography on a Sephadex G-15 column (100 × 2.1 cm) with 50% aqueous AcOH (v/v), (ii) partition chromatography on a Sephadex G-25 (block polymerizate) column (70 × 2.7 cm) using *n*-BuOH/EtOH/H_2O (with the H_2O containing 3.5% acetic acid and 1.5% pyridine) 4/1/5 (v/v/v) (R_f = 0.48), and (iii) gel filtration chromatography on a Sephadex G-15 column (100 × 2.1 cm) with 0.2 M aqueous AcOH to give the product as a white fluffy powder, 280 mg (28%). The TLC, analytical HPLC, and FAB-MS data are given in Table I. Amino acid analysis data are given in Table II.

dPen-Tyr-Ile-Glu-Asn-Cys-Pro-Lys-Gly-NH_2 (VI). dPen-Tyr-Ile-Glu-Asn-Cys-Pro-Lys-Gly-NH_2·HOAc (XII) (100 mg, 0.1 mmol) was dissolved in 5% aqueous HCl (2 mL) and applied to a (diethylamino)ethylcellulose column (Cl^- form) (1 × 10 cm) previously equilibrated with 5% aqueous HCl. The column was eluted with 5% aqueous HCl, and the eluant was continuously monitored at 280 nm. Fractions corresponding to the single large peak were pooled and lyophilized to yield the hydrochloride salt of XII, which was used immediately in the next step.

XII·HCl was dissolved in DMF (10 mL) and cooled to −25 °C in an EtOH–dry ice bath. With stirring, triethylamine (TEA) was added until the pH was 7.2, as judged by narrow-range moist pHydrion paper, followed by a solution of diphenyl phosphorazidate (DPPA) (26 μL, 0.12 mmol) in DMF (100 μL). The mixture was stirred for 1 h at −25 °C before being left to stand for 2 days at −25 °C and 4 days at 4 °C with periodic addition of TEA to maintain the pH in the range of 7.0–7.5. TLC (solvent A) and the ninhydrin test showed the reaction to be complete.

After water (1 mL) and mixed-bed ion-exchange resin (AG501-X8) (1-mL settled volume) were added, the mixture was stirred for 6 h. The resin was filtered off and washed with DMF (2 mL), and the combined filtrate and washings were concentrated under reduced pressure. The residue was taken up into 50% aqueous AcOH and applied to a gel filtration column (100 × 2.1 cm) of Sephadex G-15. The column was eluted with 50% aqueous AcOH at a flow rate of 11 mL/h. Fractions of 3.7 mL were monitored by the Folin–Lowrey method to reveal the product in test tubes 61–73. These were pooled and lyophilized to yield the product as a fluffy white powder (50 mg, 50%). TLC and analytical HPLC showed the compound to be greater than 98% pure. A sample (20 mg) was further purified by semipreparative C18 reversed-phase HPLC by using a gradient of 10–40% acetonitrile in 0.1% aqueous TFA over 30 min. The eluant was monitored at 220 nm, and the product was obtained as its trifluoroacetate salt (11 mg, 55% recovery). The TLC, analytical HPLC, and FAB-MS data are given in Table I. Amino acid analysis data are given in Table II.

Mpa-Tyr-Ile-Gln-Asn-Cys-Pro-Lys-Gly-NH_2 (VII). The protected peptide resin N^{α}-Boc-Tyr(*O*-2,6-Cl_2Bzl)-Ile-Gln-Asn-Cys(*S*-4-MeBzl)-Pro-Lys(N^{ϵ}-2,4-Cl_2Z)-Gly-MBHA resin (VIIa) was obtained by sequential coupling and deprotection of N^{α}-Boc-Lys(N^{ϵ}-2,4-Cl_2Z), N^{α}-Boc-Pro, N^{α}-Boc-Cys(*S*-4-MeBzl), N^{α}-Boc-Asn, N^{α}-Boc-Gln, N^{α}-Boc-Ile, and N^{α}-Boc-Tyr(*O*-2,6-Cl_2Bzl) to (H)-Gly-MBHA resin. The peptide resin was halved and to one portion Mpa(*S*-4-MeBzl) was coupled.

The peptide was freed from the resin and side-chain protecting groups, and cyclized following the same procedure as for XII. The crude peptide was purified by gel filtration on Sephadex G-15 with 50% aqueous AcOH, followed by preparative C18 reversed-phase HPLC by using a gradient of 10–40% acetonitrile over 30 min. The eluant was continuously monitored at 220 nm. The yield of peptide was 27%. The TLC, analytical HPLC, and FAB-MS data are given in Table I. Amino acid analysis data are given in Table II.

dPen-Tyr-Ile-Gln-Asn-Cys-Pro-Lys-Gly-NH_2 (VIII). The peptide resin of the title compound was made by coupling dPen(*S*-4-MeBzl) to the second portion of peptide resin VIIa. Workup was as for VII to give the desired peptide VIII. The yield was 21%. The TLC, analytical HPLC, and FAB-MS data are given in Table I. Amino acid analysis data are given in Table II.

Mpa-Tyr-Ile-Gln-Asn-Cys-Pro-Lys(CHO)-Gly-NH_2 (IX). N^{α}-Boc-Lys(CHO), N^{α}-Boc-Pro, N^{α}-Boc-Cys(*S*-4-MeBzl), and N^{α}-Boc-Asn were coupled as above to (H)-Gly-MBHA resin (2 mM) to give the peptide resin N^{α}-Boc-Asn-Cys(*S*-4-MeBzl)-Pro-Lys(CHO)-Gly-MBHA resin (IXa). This was divided into two equal portions (IXa) and to one was coupled N^{α}-Boc-Gln, N^{α}-Boc-Ile, and N^{α}-Boc-Tyr(*O*-2,6-Cl_2Bzl) to give the peptide resin N^{α}-Boc-Tyr(*O*-2,6-Cl_2Bzl)-Ile-Gln-Asn-Cys(*S*-4-MeBzl)-Pro-Lys(CHO)-Gly-MBHA resin (IXb). The peptide resin was halved and to one portion was coupled Mpa(*S*-4-MeBzl) to yield the title protected resin Mpa(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2Bzl)-Ile-Gln-Asn-Cys(*S*-4-MeBzl)-Pro-Lys(CHO)-Gly-MBHA resin (IXc).

IXc was worked up as for VII to yield the title peptide IX in an overall yield of 10%. The TLC, analytical HPLC, and FAB-MS data are given in Table I. Amino acid analysis data are given in Table II.

dPen-Tyr-Ile-Gln-Asn-Cys-Pro-Lys(CHO)-Gly-NH_2 (X). To the second portion of peptide resin IXb was coupled dPen(*S*-4-MeBzl) to give protected peptide resin dPen(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2Bzl)-Ile-Gln-Asn-Cys(*S*-4-MeBzl)-Pro-Lys(CHO)-Gly-MBHA resin (Xa).

Xa was worked up following the same procedure for VII to give the title peptide X in an overall yield of 33%. The TLC, analytical HPLC, and FAB-MS data are given in Table I. Amino acid analysis data are given in Table II.

Mpa-Tyr-Ile-Glu-Asn-Cys-Pro-Lys(CHO)-Gly-NH_2 (XIII). N^{α}-Boc-Glu(*O*-Bzl), N^{α}-Boc-Ile, and N^{α}-Boc-Tyr(*O*-2,6-Cl_2Bzl) were coupled as before to the second portion of peptide resin IXa to yield N^{α}-Boc-Tyr(*O*-2,6-Cl_2Bzl)-Ile-Glu(*O*-Bzl)-Asn-Cys(*S*-4-MeBzl)-Pro-Lys(CHO)-Gly-MBHA resin (XIIIa). This peptide resin was dried and divided into two equal portions to one of which was coupled Mpa(*S*-4-MeBzl) to give Mpa(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2Bzl)-Ile-Glu(*O*-Bzl)-Asn-Cys(*S*-4-MeBzl)-Pro-Lys(CHO)-Gly-MBHA resin (XIIIb).

Workup was as for VII to give XIII in an overall yield of 12%. The TLC, analytical HPLC, and FAB-MS data are given in Table I. Amino acid analysis data are given in Table II.

dPen-Tyr-Ile-Glu-Asn-Cys-Pro-Lys(CHO)-Gly-NH_2 (XIV). The title peptide resin, dPen(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2Bzl)-Ile-Glu(*O*-Bzl)-Asn-Cys(*S*-4-MeBzl)-Pro-Lys(CHO)-Gly-MBHA resin was prepared by coupling dPen(*S*-4-MeBzl) as before to peptide resin XIIIa.

Workup was as for VII to give the title peptide XIV in an overall yield of 26%. The TLC, analytical HPLC, and FAB-MS data are given in Table I. Amino acid analysis data are given in Table II.

Biological Assay Methods. Wistar rats were used in all experiments. Rat uterotonic test in vitro was performed according to Holton[19] in Munsick's solution.[20] The rats were estrogenized

(19) Holton, P. A. Modification of the Method of Dale and Laidlaw for Standardization of Posterior Pituitary Extract. *Br. J. Pharmacol. Chemother.* **1948**, *3*, 328–334.

24-48 h before the experiments. Cumulative dose-response curves were constructed. The antagonists were applied to the bath 1 min before the construction of the oxytocin dose-response curve was started. The galactogogic test was performed on ethanol-anesthetized lactating rats 5-15 days after delivery.[21,22] Synthetic oxytocin was used as standard in these assays. Pressor activity was determined on pithed rat preparation[23] using synthetic arginine-vasopressin as a standard. Detailed descriptions of the tests are given in ref 14.

Acknowledgment. This work was supported by a grant from the U.S. Public Health Service DK 17420 and the National Science Foundation. The Midwest Center for Mass Spectrometry, Lincoln, NE, a National Science Foundation Regional Instrumentation Facility, (Grant No. CHE 8211164) is acknowledged for performing many of the FAB-MS reported here.

(20) Munsick, R. A. Effect of Magnesium Ion on the Response of the Rat Uterus to Neurohypophysial Hormones and Analogues. *Endocrinology* **1960**, *66*, 451-457.
(21) Bisset, G. W.; Clark, B. J.; Haldar, J.; Harris, M.; Lewis, G. P., Rocha e Silva, M. The Assay of Milk-Ejecting Activity in the Lactating Rat. *Br. J. Pharmacol. Chemother.* **1967**, *31*, 537-549.
(22) Barth, T.; Jost, K.; Rychlik, I. Milk-ejecting and Uterotonic Activities of Oxytocin Analogues in Rats. *Endocrinol. Exp.* **1975**, *9*, 35-42.
(23) Frejci, I.; Kupkova, B.; Vavra, I. The Effect of Some 2-0 Alkyl Tyrosine Analogues of Oxytocin and Lysine Vasopressin on the Blood Pressure of Rat, Rabbit and Cat, *Br. J. Pharmacol. Chemother.* **1967**, *30*, 497-505.

A New Class of Bradykinin Antagonists: Synthesis and in Vitro Activity of Bissuccinimidoalkane Peptide Dimers[1,2]

John C. Cheronis,* Eric T. Whalley, Khe T. Nguyen,† Shad R. Eubanks, Lisa G. Allen, Matthew J. Duggan, Sharon D. Loy, Kathryn A. Bonham, and James K. Blodgett

Cortech, Inc., 6840 North Broadway, Denver, Colorado 80221. *Received October 28, 1991*

A systematic study on the dimerization of the bradykinin (BK) antagonist D-Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Phe^5-Ser^6-D-Phe^7-Leu^8-Arg^9 has been performed. The first part of this study involved compounds wherein dimerization was carried out by sequentially replacing each amino acid with cysteine and cross-linking with bismaleimidohexane. The second part of this study utilized a series of bissuccinimidoalkane dimers wherein the intervening methylene chain was varied systematically from $n = 2$ to $n = 12$ while the point of dimerization was held constant at position 6. The biological activities of these dimers were then evaluated on BK-induced smooth muscle contraction in two different isolated tissue preparations: guinea pig ileum (GPI) and rat uterus (RU). Several of the dimeric BK antagonists displayed remarkable activities and long durations of action. In addition, dimerization at position 4, 7, 8, or 9 produced dimeric analogues with markedly reduced potency. Rank order of antagonist potency as a function of dimerization position is as follows: rat uterus, 6 > 5 > 0 > 2 > 1 > 3 ≫ 4, 7, 8, 9; guinea pig ileum, 6 > 5 > 3 > 2 > 1 > 0 ≫ 4, 7, 8, 9. Evaluation of the linker length as represented by the number of methylene units indicated an optimal distance between the two monomeric peptides of six to eight methylene moieties. These studies also revealed that the carbon-chain length significantly affected the duration of action in vitro and resulted in partial agonism effects when $n > 8$. The optimum activity in vitro was achieved with dimerization at position 6 and $n = 6$ (designated herein as compound **25**; alternatively, CP-0127). Similar effects in potency were also seen when the monomeric antagonist D-Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Phe^5-Ser^6-D-Phe^7-Phe^8-Arg^9 (NPC-567) was dimerized using similar chemistry. These results suggest that the development of BK antagonists of significant therapeutic potential may be possible using a dimerization strategy that can overcome the heretofore limiting problems of potency and in vivo duration of action found with many of the BK antagonists in the literature.

Introduction

The design and synthesis of potent, stable, and specific bradykinin antagonists has long been considered a desirable goal in medicinal chemistry. Compounds with these characteristics could be important in the treatment of such diverse disorders as septic shock, asthma, and rhinitis.[3,4] Up until now, however, most antagonists have been plagued with the dual problem of relatively low potency and poor in vivo stability. These problems have been solved recently by an approach involving the introduction of the conformationally constrained amino acid analogues 1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid (D-Tic) and/or (7*S*,8*S*)-*endo-cis*-octahydroindole-2-carboxylic acid (Oic) in the carboxy-terminal region of decapeptide inhibitors, which improves potency and confers metabolic stability on these compounds.[5-7] This study describes an alternative approach which appears to have produced similar results. Described herein are two series of compounds that were produced using a standardized and

* Author for correspondence.
† Present address: Amylin Corp., 9373 Towne Centre Dr., Suite 250, San Diego, CA 92121.

(1) Abbreviations follow the IUPAC-IUB Joint Commission on Biochemical Nomenclature for amino acids and peptides: *Eur. J. Biochem.* **1984**, *158*, 9-31. Additional abbreviations used are as follows: Boc, *tert*-butyloxycarbonyl; DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea; DIPEA, diisopropylethylamine; DMF, dimethylformamide; HOBt, 1-hydroxybenzotriazole; pA_2, -log molar concentration of antagonist in the presence of which twice the concentration of agonist is required to produce the same response as in the absence of antagonist; Pam, (phenylacetamido)methyl; PBS, phosphate-buffered saline (0.15 M NaCl, 0.01 M phosphate); Succ-Cys, SuccinylCysteine; TFA, trifluoroacetic acid; THF, tetrahydrofuran.
(2) Preliminary accounts of this work have been presented at the Twelfth American Peptide Symposium in Boston, MA, June 12-16, 1991, Abstract No. 454, and at the International Kinin Conference in Munich, Germany, September 18-13, 1991.
(3) Farmer, S. G.; Burch, R. M. Pharmacology of Bradykinin Receptors. In *Bradykinin Antagonists: Basic and Clinical Research*; Burch, R. M., Ed.; Marcel Dekker, Inc.: New York, 1991; pp 1-32.

0022-2623/92/1835-1563$03.00/0