Synthesis and Characterization of Pseudopeptide Bradykinin B2 Receptor Antagonists Containing the 1,3,8-Triazaspiro[4.5]decan-4-one Ring System

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A series of pseudopeptides containing alkyl-, cycloalkyl-, aryl-, and aralkyl-substituted 1,3,8-triazaspiro[4.5]decan-4-one-3-acetic acids as amino acid surrogates to replace the Pro^2-Pro^3-Gly^4-Phe^5 section of the peptide bradykinin B2 receptor antagonist [Pro^3, Phe^5]HOE 140 (D-Arg^0-Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-D-Tic^7-Oic^8-Arg^9) were prepared. These psuedopeptides were examined *in vitro* for their B2 receptor affinities as well as for their ability to block bradykinin mediated actions *in vivo*. Two compounds in particular, NPC 18521 (**I**) and NPC 18688 (**V**) were quite potent in these latter assays, indicating that a significant portion of this prototypical second generation decapeptide antagonist can be replaced with a more compact nonpeptide molecule.

I. Introduction

Nearly all cells in most species express kinin receptors which mediate the diverse physiological and pathophysiological activities of bradykinin (Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9).[1] Kinin receptors are known to be G-protein coupled, and their activation leads to relaxation of venular smooth muscle and hypotension, increased vascular permeability, contraction of smooth muscle of the gut and airway leading to increased airway resistance, stimulation of sensory neurons, alteration of ion secretion of epithelial cells, production of nitric oxide, release of cytokines from leukocytes and the production of eicosanoids from various cell types.[2] Because of their broad spectrum of activity, kinins have been implicated in many disorders including pain, sepsis, asthma, rheumatoid arthritis, pancreatitis, and a wide variety of other inflammatory diseases. Moreover, a recent report demonstrated that bradykinin B2 receptors on the surface of human fibroblasts were upregulated 3-fold beyond normal in patients with Alzheimer's disease, implicating bradykinin as a participant in the peripheral inflammatory processes associated with that disease.[3]

In contrast to the adverse physiologies associated with bradykinin release, there is a growing body of literature which implicates bradykinin as a protective agent during periods of cardiac or renal stress.[4−6] In this regard there is substantial evidence that the cardioprotective effects afforded by angiotensin-converting enzyme (ACE) inhibitor treatment are a result of metabolically preserving bradykinin and are therefore mediated by bradykinin B2 (and possibly B1) receptors. These more recent results point to a possible therapeutic role for a kinin receptor agonist.

More than three decades have passed since the initial discovery of bradykinin, yet there has only recently been a single report of a nonpeptidic antagonist.[7] However, no nonpeptides have been described that are highly potent, competitive and selective for the receptor. In previous reports we have described the results of systematic investigations into the role of the amino acid sequence Pro^2-Pro^3-Gly^4-Phe^5 in the peptide antagonist [Pro^3, Phe^5]HOE-140[8,9] (D-Arg^0-Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-D-Tic^7-Oic^8-Arg^9 (K_i = 0.21 ± 0.02 nM) as it relates to the affinity for the bradykinin B2 receptor.[10] In these sequences, D-Tic represents the D stereoisomer of tetrahydroisoquinoline carboxylic acid and Oic corresponds to *S*,*S*,*S*-octahydroindolecarboxylic acid. It was discovered that this tetrapeptide segment could be replaced with simple nonpeptidic moieties while maintaining affinities (K_i) to the receptor in the range of 40−400 nM. Efforts to optimize these amino acid replacements led to the development of several heterocyclic tetrapeptide mimetics. Of those examined, one of the most interesting series was comprised of a 1,3,8-triazaspiro[4.5]decan-4-one-3-acetic acid motif. This spirocyclic ring system was chosen as a tetrapeptide (Pro^2-Pro^3-Gly^4-Phe^5) mimetic on the basis of comparative computer modeling and solution conformations of peptide agonists and antagonists containing the Pro-Pro-Gly-Phe domain.[11,12] Of particular use were comparisons made to a recently reported cyclic peptide kinin antagonist (D-Arg-Arg-Cys-Pro-Gly-Phe-Cys-D-Tic-Oic-Arg). The cyclic portion of the peptide encompassed only five residues, one of which was Pro and two of which were constrained by the requisite geometry of a disulfide bond. These features rendered the cyclic domain of the compound highly inflexible such that reasonable computational models could be constructed.[10] Ultimately, the spirocyclic mimetic reported herein was devised on the basis of its ability to mimic this cyclic domain of the peptide precursor. Shown in Figure 1 is the 1-phenyl-substituted spirocyclic mimetic comparatively overlayed with the cyclic pentamer domain of the previously reported cyclic decapeptide antagonist.

In this paper we fully describe the synthetic procedures required to prepare these novel pseudopeptide bradykinin B2 receptor antagonists. In addition, through the presentation of a series of differentially derivitized spirocyclic mimetics, a structure−activity relationship is described. Finally, *in vivo* activities for two of these novel kinin antagonists are presented.

II. Methods

The preparation of the 1,3,8-triazaspiro[4.5]decan-4-one-3-acetic acid structural fragment is outlined in Scheme 1.[15] 1-Benzyl-4-piperidone (**1**) was subjected to

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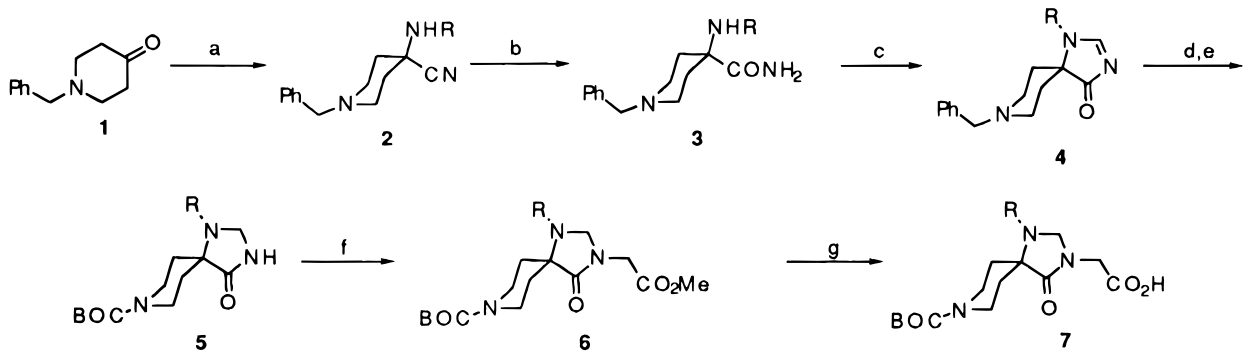
Scheme 1[a]



[a] Reagents: (a) RNH_2, KCN, H^+; (b) H_2SO_4; (c) $(EtO)_3CH$, H^+; (d) H_2, H^+, Pd/C; (e) $(BOC)_2O$; (f) NaH, $Br(CH_2)CO_2Me$; (g) Na_2CO_3.

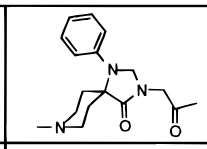
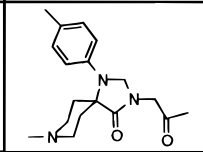
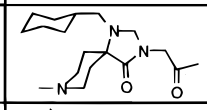
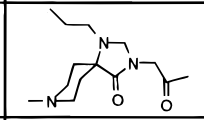
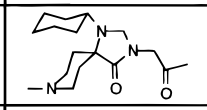
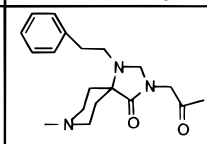
Strecker amino nitrile synthesis, followed by hydration to give the amino amides **3** possessing the various R substituents. In some cases (R = *n*-propyl and cyclohexyl) these amino amides were commercially available. The spirocyclic ring system was then prepared by heating **3** with triethyl orthoformate. Hydrogenation over palladium on carbon reduced the carbon nitrogen double bond with concomitant removal of the benzyl group. Protection of the secondary amine with a Boc group under standard conditions gave spirocyclic amide **5**. Abstraction of the NH proton with sodium hydride and alkylation of the resulting amide anion with methyl bromoacetate gave the ester **6**. Finally, standard saponification afforded the desired Boc-protected spirocyclic amino acid **7** (Scheme 1).

The individual spirocyclic structural fragments were incorporated into the pseudopeptides using standard coupling procedures (see the Experimental Section). Receptor binding assays were performed following known procedures and made use of membrane preparations from a CHO cell line, engineered to express human B2 receptors.

III. Results

A series of pseudopeptide bradykinin B2 receptor ligands were synthesized that contained differently substituted 1,3,8-triazaspiro[4.5]decan-4-one systems. These are shown in Table 1. All pseudopeptides were of the general sequence H-D-Arg-Arg-[1,3,8-triazaspiro-[4.5]decan-4-one]acetyl-Ser-D-Tic-Oic-Arg-OH, where the 1-position of the spirocyclic fragment is substituted variably to probe possible structure activity relationships. The isolated C-terminal tetrapeptide, Ser-D-Tic-Oic-Arg, contained within these pseudopeptides has been previously shown by NMR to exist nearly exclusively in a β-turn conformation in aqueous environment.[4] Given the structural rigidity which was described, the conformation reported previously was presumed to remain similar when incorporated into the current series, although definitive spectroscopic proof is still required. The N-terminus of each contained the dipeptide D-Arg-Arg, in accordance with the structure–activity relationship associated with previously reported first and second generation peptide antagonists.[10] This structure was derived by combining the known solution conformation of the C-terminal tetrapeptide Ser-D-Tic-Oic-Arg, with a model of the conformationally rigid spirocyclic mimetic described herein. The N-terminal D-Arg-Arg reflects an arbitrary conformation since there is no experimental evidence for any preferred state. The actual conformation(s) adopted by these new pseudopeptides remains to be eluciated, most likely *via* two-dimensional NMR experiments.

Table 1. Psuedopeptide Structures of the Sequence D-Arg-Arg-X-Ser-D-Tic-Oic-Arg with Corresponding Human B2 Kinin Receptor Affinity Data[a]

Peptide	Position X	K_i (nM)
I		7.7± 0.16 (3)
II		6.3 ± 0.04 (3)
III		6.8 ± 0.49 (3)
IV		27 ± 1.6 (3)
V		0.57 ± 0.08 (3)
VI		0.15 ± 0.02 (3)

[a] Affinity to human B2 receptor in CHO cell membranes determined by displacement of [^{3}H]NPC 17731.

Receptor binding assays indicate that, in this series of D-Arg-Arg-SPIROCYCLIC-Ser-D-Tic-Oic-Arg, all of the substitutions which were made at position 1 on the spirocyclic mimetic could be accommodated by the receptor binding site as is reflected in the measured affinities to the human B2 receptor (Table 1). Varying the size of the alkyl substituent at the 1-position of the spirocyclic mimetic revealed an interesting structure–activity relationship. The highest affinity compound, **VI** (K_i = 0.15 nM), also contained the largest substituent (phenethyl) at the 1-position of the mimetic. The lowest affinity compound, **IV** (K_i = 27 nM), corresponds to a structure with the smallest substituent (*n*-propyl) tested at the 1-position of the spirocyclic mimetic. This general observation was consitent throughout the series where the mimetics bearing large hydrophobic moieties at the 1-position had higher affinities to the B2 kinin receptor than did those with smaller substituents. Aromatic

Table 2. Pharmacological Profile for Pseudopeptides **I** and **V**

	ED50 (mg/kg^{-1})	
(bradykinin induced) assay	I	V
hypotension in rats[a]	400 (4)	600 (4)
hypotension in rabbits[b]	22.1 (4)	8.6 (4)

[a] BK challenge is 12 μg kg^{-1}, iv. [b] BK challenge is 1 μg kg^{-1}, iv.

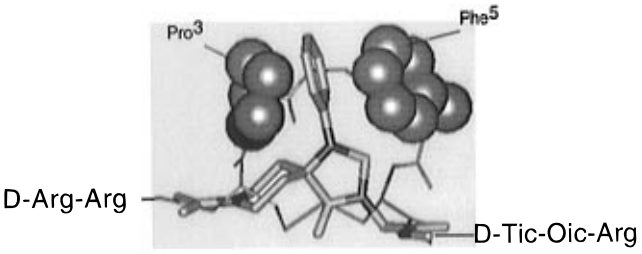


Figure 1. Stereoview of a putative model of compound I overlayed with the cyclic portion of a known cyclic peptide kinin antagonist, (D-Arg^0-Arg^1-Cys^2-Pro^3-Gly^4-Phe^5-Cys^6-D-Tic^7-Oic^8-Arg^9). The cyclization is between residues 2 and 6. Shown in dark gray are VDW radii of the Pro^3 and Phe^5 side chains in the cyclic peptide. The remainder of the cyclic portion of the peptide is shown as thin lines. The mimetic is drawn as thick lines without showing the chemical structure of the N-terminal D-Arg-Arg or the C-terminal Ser-D-Tic-Oic-Arg.

substituents showed similar pharmacology as nonaromatic substituents on the spirocyclic scaffold. One possible exception to this generalization can be found in a comparison of compounds **I** and **V**. Here, the only chemical difference is that **I** has a phenyl ring attached to the 1-position of the spirocyclic mimetic and **V** has cyclohexyl. The latter was approximately 10-fold more potent in the receptor binding assay than the former. The rank order potency of these two compounds was maintained *in vivo* in a model of bradykinin-induced hypotension in rabbits. However, in live animals, the ED_{50} values differed only by 2-fold. In a further study (bradykinin-induced hypotension performed in rats) these same two compounds (**I** and **V**) were effectively equipotent (Table 2).

Although spectroscopically unproven, this data might indicate that, when bound to the receptor, the 1-position of the 1,3,8-triazaspiro[4.5]decan-4-one mimetic is accommodated by some type of hydrophobic domain of the B2 kinin receptor. On the basis of the results presented herein, this domain is likely quite large and hydrophobic given that the sterically demanding phenethyl substitution could be bound readily and with high affinity. Given the overall structure of these new pseudopeptides, it seems highly probable that the substituent on the 1-position of the spirocyclic mimetic used interacts with the same hydrophobic site or sites on the receptor which binds Pro^3 and/or Phe^5 in the parent decapeptide antagonists. This hypothesis is depicted in Figure 1. Of course only detailed crytallographic analysis of an appropriate ligand−receptor complex could confirm this experimentally.

Antagonist character was confirmed by the assessment of the ability of these pseudopeptides to inhibit bradykinin induced hypotension in rats and rabbits. Compounds **I** and **V** were capable of dose dependently blocking bradykinin-induced hypotension in these species. As shown in Table 2, both compounds (**I** and **V**) block the hypotensive response of iv bradykinin. In these assays, it was demonstrated that the rabbit was considerably more sensitive to bradykinin than was the rat in that, to achieve a comparable hypotensive response in each species, 1 μg kg^{-1} bradykinin was needed in the rabbit while 12 μg kg^{-1} bradykinin was needed in the rat. In a related study published elsewhere, compounds **I** and **V** were also shown to possess antinociceptive activity in rodents.[14]

IV. Conclusions

In conclusion the 1,3,8-triazaspiro[4.5]decan-4-one ring system, differentially substituted with alkyl and aryl groups at position 1, can serve as a worthy surrogate for the Pro-Pro-Gly-Phe segment of peptide antagonists of the type D-Arg^0-Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-D-Tic^7-Oic^8-Arg^9. These new pseudopeptides bind to B2 kinin receptors with high affinity and inhibit bradykinin both *in vitro* and *in vivo* in several species. The 1-position of the mimetic should ideally be a large hydrophobic moiety such that, upon receptor complexation, an appropriate interaction with a hydrophobic site on the receptor can occur. We propose that this site is the same site which accommodates the phenylalanine side chain at position 5 and/or the side accommodating Pro^3 of known first and second generation decapeptide kinin antagonists.

Finally, these new pseudopeptides represent a significant reduction of the peptidic nature of their precursory second generation decapeptide antagonists. Although one ultimately hopes for the discovery of novel nonpeptidic compounds, pseudopeptides **I**−**VI** represent an important milestone. They reveal that a significant section of a known decapeptide antagonist can be replaced by a nonpeptide moiety while maintaining good biological activity *in vivo* and *in vitro*.

V. Experimental Section

A. General. Reagents and chemicals were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI) and used without purification. Chromatography refers to flash chromatography on silica gel. Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC-400 spectrometer. Combustion analyses were performed by Atlantic Microlabs, Inc., Norcross, GA.

All peptides were synthesized manually according to standard solid phase techniques, using *tert*-butyloxycarbonyl (Boc) amino acids. All amino acids were purchased from Bachem Bioscience Inc. (King of Prussia, PA). Boc-Oic-OH was synthesized by modifying previously reported procedures.[16] The unnatural amino acids used during this study were prepared according to procedures reported below. Boc-Arg-(Tos)-PAM resin (0.79 mmol/g) was purchased from Advanced ChemTech (Louisville, KY) and used for the syntheses. Syntheses were run at a 0.5 g resin-aa scale (0.4 mmol), and couplings were monitored using qualitative ninhydrin determination of free amine (Kaiser test). Amino acids were coupled using active esters of the Boc-protected amino acids, generated by treating individual amino acids with equimolar quantities of hydroxybenzotriazole hydrate and diisopropylcarbodiimide in methylene chloride:DMF (1:1) at 0 °C, prior to introduction into solid phase synthesis. Couplings were continued until no further free amine was detected using the Kaiser test, and in the event of incomplete coupling, amino acids were recoupled. Routine N-capping was performed by treating peptide-resins with acetic anhydride and 10% diisopropylethylamine (1:1) in DMF after every coupling to reduce the formation of deletion peptides. The finished peptidyl-resin was washed and dried to a constant weight and then treated with anhydrous hydrogen fluoride (in presence of anisole 10%) at 10 mL/g of peptidyl-resin for 1 h at 0 °C. After removal of the hydrogen fluoride, the resin peptide was washed extensively with anhydrous diethyl ether (3 × 20 mL) and then extracted with 15% aqueous acetic acid.

The crude material, obtained from lyophilization, was purified by reverse phase high-performance liquid chromatography, using a linear gradient of 5−60% water/acetonitrile (both containing 0.2% acetic acid), on a C_{18} Vydac (22.5 mm i.d. × 25 cm, 10 μm, 300 Å) column, at room temperature. The homogeneous pooled pure fractions were lyophilized and the purity of the white fluffy powders determined by analytical reverse phase HPLC, using a linear gradient of 5−80% water/acetonitrile (both containing 0.1% TFA), on a C_{18} Vydac (4.6 mm i.d. × 25 cm, 10 μm, 300 Å) column, at room temperature, and fast atom bombardment mass spectroscopy.

Preparation of 8-Boc-1,3,8-triazaspiro[4.5]decan-4-one-3-acetic Acids (7). a. 1-Benzyl-4-cyano-4-[(2-phenylethyl)amino]piperidine (2f). To a stirred mixture of 1-benzyl-4-piperidone (**1**) (37.87 g, 200 mmol), 2-phenylethylamine (27.16 g, 224 mmol), and acetic acid (100 mL) at room temperature was added dropwise a solution of potassium cyanide (16.10 g, 247 mmol) in water (35 mL). After stirring at room temperature for 48 h, the reaction mixture was poured into a mixture of concentrated NH_4OH (200 mL) and crushed ice (200 g). The mixture was extracted with $CHCl_3$ (5 × 100 mL). The organic extracts were washed with saturated sodium chloride, dried over Na_2SO_4, and filtered, and the solvent was removed *in vacuo*. Recrystallization from diisopropyl ether gave 32.37 g (51%) of **2f** as white crystals: mp 65−68 °C; ^{1}H NMR ($CDCl_3$) δ 1.25 (br s, 1H), 1.73 (t, 2H, J = 13.5 Hz), 1.98 (d, 2H, J = 12.6 Hz), 2.34 (t, 2H, J = 11.0 Hz), 2.70−2.90 (m, 2H), 2.82 (t, 2H, J = 6.9 Hz), 3.01 (q, 2H, J = 6.9 Hz), 3.53 (s, 2H), 7.18−7.35 (m, 10H). Anal. ($C_{21}H_{25}N_3$) C, H, N.

b. 1-Benzyl-4-[(2-phenylethyl)amino]piperidine-4-carboxamide (3f). To 90% H_2SO_4 (200 mL) was added **2f** (32.00 g, 100 mmol) and the mixture stirred for 20 h. After this mixture was cautiously poured into a mixture of concentrated NH_4OH (750 mL) and crushed ice (750 g), the resulting alkaline mixture was extracted with $CHCl_3$ (5 × 100 mL). The organic extracts were washed with saturated sodium chloride, dried over Na_2SO_4, and filtered, and the solvent was removed *in vacuo*. Recrystallization from 2-propanol gave 27.03 g (80%) of **3f** as an off-white powder: mp 89−92 °C; ^{1}H NMR ($CDCl_3$) δ 1.57 (d, 2H, J = 11.3 Hz), 1.95−2.06 (m, 2H), 2.06−2.17 (m, 2H), 2.62−2.80 (m, 6H), 3.42 (s, 2H), 5.29 (s, 1H), 6.85 (s, 1H), 7.16−7.35 (m, 10H). Anal. ($C_{21}H_{27}N_3O$) C, H, N.

c. 1-(2-Phenylethyl)-8-benzyl-1,3,8-triazaspiro[4.5]dec-2-en-4-one (4f). A mixture of **3f** (11.80 g, 34.97 mmol), triethyl orthoformate (23 mL, excess), acetic acid (5 mL), and toluene (75 mL) was refluxed for 20 h. The mixture was cooled to room temperature and diluted with water (100 mL) and $CHCl_3$ (100 mL). The mixture was made basic with aqueous sodium hydroxide, the layers were separated, and the aqueous layer was extracted with $CHCl_3$ (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4 and filtered, and the solvent was removed *in vacuo*. Chromatography (1−15% MeOH/CH_2Cl_2, gradient) gave 8.79 g (73%) of **4f** as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.57 (d, 2H, J = 12.5 Hz), 1.90−2.22 (m, 2H), 2.79 (br s, 2H), 3.07 (br s, 2H), 3.61 (t, 2H, J = 7.1 Hz), 3.68 (br s, 2H), 7.15 (d, 2H, J = 6.9 Hz), 7.20−7.42 (m, 8H), 7.80 (s, 1H). Anal. ($C_{22}H_{25}N_3O\cdot 0.5H_2O$) C, H, N.

d. 1-(2-Phenylethyl)-8-Boc-1,3,8-triazaspiro[4.5]decan-4-one (5f). A mixture of **4f** (8.75 g, 25.22 mmol), MeOH (150 mL), and concentrated HCl (30 mL) was hydrogenated at 45 psi of H_2 in the presence of 5% Pd/C (10 g) for 4 days. The catalyst was filtered off and the MeOH removed *in vacuo*. The remaining acidic aqueous solution was made basic by the addition of solid Na_2CO_3. This alkaline mixture was diluted with 1,4-dioxane (150 mL) and cooled to 0 °C, and di-*tert*-butyl dicarbonate (10.90 g, 50.0 mmol) was added. After stirring at room temperature for 20 h, the reaction mixture was diluted with water (250 mL) and extracted with ether (5 × 100 mL). The organic extracts were dried over Na_2SO_4 and filtered, and the solvent was removed *in vacuo*. Chromatography (1−5% MeOH/CH_2Cl_2, gradient) gave 8.47 g (93%) of **5f** as white crystals: mp 138−140 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.53−1.70 (m, 4H), 2.68−2.84 (m, 4H), 3.30−3.55 (m, 2H), 3.78−4.05 (m, 2H), 4.21 (s, 2H), 6.68 (br s, 1H), 7.10−7.34 (m, 5H). Anal. ($C_{20}H_{29}N_3O_3$) C, H, N.

e. 1-(2-Phenylethyl)-8-Boc-1,3,8-triazaspiro[4.5]decan-4-one-3-acetic Acid Methyl Ester (6f). Sodium hydride (60 wt %, 0.54 g, 14.42 mmol) was washed with hexane (3 × 5 mL), suspended in DMF (20 mL), and cooled to 0 °C. A solution of **5f** (4.20 g, 11.68 mmol) in DMF (20 mL) was added dropwise. After the mixture was stirred at 0 °C for 30 min, methyl bromoacetate (2.24 g, 14.42 mmol) was added and the mixture stirred at room temperature 3 h. After diluting with water, the mixture was extracted with EtOAc (5 × 50 mL). The organic extracts were washed with water (3 × 50 mL) and saturated aqueous sodium chloride and dried over Na_2SO_4. After filtration and removal of the solvent *in vacuo*, the residue was chromatographed (10−50% EtOAc/hexane, gradient) to give 4.42 g (88%) of **6f** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.52−1.75 (m, 4H), 2.68−2.85 (m, 4H), 3.35−3.55 (m, 2H), 3.76 (s, 3H), 3.80−4.04 (m, 2H), 4.10 (s, 2H), 4.28 (s, 2H), 7.16−7.35 (m, 5H). Anal. ($C_{23}H_{33}N_3O_5$) C, H, N.

f. 1-(2-Phenylethyl)-8-Boc-1,3,8-triazaspiro[4.5]decan-4-one-3-acetic Acid (7f). A mixture of **6f** (4.27 g, 9.78 mmol), Na_2CO_3 (4.50 g, excess), MeOH (75 mL), and water (25 mL) was refluxed for 3 h. After the MeOH was removed *in vacuo*, the resulting aqueous solution was cooled to 0 °C and carefully acidified (pH 4) with concentrated HCl. The product was extracted with EtOAc (3 × 100 mL); these organic extracts were washed with saturated aqueous sodium chloride, dried over Na_2SO_4, and filtered; and the solvent was removed *in vacuo* to give 3.80 g (93%) of **7f** as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.64−1.80 (m, 2H), 1.80−1.92 (m, 2H), 2.82−2.96 (m, 4H), 3.27−3.62 (m, 2H), 3.80−4.22 (m, 6H), 4.46 (br s, 2H), 7.15−7.35 (m, 5H). Anal. ($C_{22}H_{31}N_3O_5\cdot 0.75H_2O$) C, H, N.

1-Phenyl-8-Boc-1,3,8-triazaspiro[4.5]decan-4-one-3-acetic acid (7a): mp 200−201 °C; ^{1}H NMR ($CDCl_3$) δ 1.50 (s, 9H), 1.63−1.83 (m, 2H), 2.38−2.56 (m, 2H), 3.43−3.70 (m, 2H), 3.90−4.15 (m, 2H), 4.23 (s, 2H), 4.79 (s, 2H), 6.68−7.05 (m, 3H), 7.20−7.35 (m, 2H). Anal. ($C_{20}H_{27}N_3O_5$) C, H, N.

1-(4-Methylphenyl)-8-Boc-1,3,8-triazaspiro[4.5]decan-4-one-3-acetic acid (7b): mp 173−175 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.64−1.84 (m, 2H), 2.20−2.40 (m, 5H), 3.42−3.60 (m, 2H), 3.88−4.10 (m, 2H), 4.20 (s, 2H), 4.75 (s, 2H), 6.78 (d, 2H, J = 11.4 Hz), 7.08 (d, 2H, J = 11.4 Hz). Anal. ($C_{21}H_{29}N_3O_5$) C, H, N.

1-(Cyclohexylmethyl)-8-Boc-1,3,8-triazaspiro[4.5]decan-4-one-3-acetic acid (7c): mp 148−149 °C; ^{1}H NMR ($CDCl_3$) δ 0.75−0.90 (m, 2H), 1.10−1.30 (m, 4H), 1.50 (s, 9H), 1.60−1.80 (m, 8H), 2.30 (d, 2H, J = 7.2 Hz), 3.40−3.50 (m, 2H), 3.80−4.00 (m, 2H), 4.05−4.15 (m, 2H), 4.20 (s, 2H). Anal. ($C_{21}H_{35}N_3O_5$) C, H, N.

1-*n*-Propyl-8-Boc-1,3,8-triazaspiro[4.5]decan-4-one-3-acetic acid (7d): mp 180−183 °C dec; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3H, J = 7.1 Hz), 1.46 (s, 9H), 1.50−1.90 (m, 6H), 2.55 (t, 2H, J = 6.5 Hz), 3.30−3.67 (m, 2H), 3.95−4.20 (m, 2H), 4.08 (br d, 2H, J = 7.2 Hz), 4.31 (brs, 2H). Anal. ($C_{17}H_{29}N_3O_5$) C, H, N.

1-Cyclohexyl-8-Boc-1,3,8-triazaspiro[4.5]decan-4-one-3-acetic acid (7e): mp 172−173 °C; ^{1}H NMR ($CDCl_3$) δ 1.20−1.42 (m, 8H), 1.47 (s, 9H), 1.58−1.85 (m, 6H), 2.64−2.74 (m, 1H), 3.42−3.70 (m, 2H), 3.70−3.95 (m, 2H), 3.95−4.15 (m, 2H), 4.39 (s, 2H). Anal. ($C_{20}H_{33}N_3O_5$) C, H, N.

B. Computational Procedures. All modeling work was done using the programs CHARMm and Quanta[13] on Silicon Graphics computers. Energy minimizations were terminated when the drop in energy after 50 steps of minimization was less than 0.2 kcal mol^{-1}. The spirocyclic mimetic has no rotatable bonds of structural consequence; hence no conformational search was performed. The structure was optimized using second-derivative minimization techniques. The comparative modeling entailed achieving an optimal end to end distance wherein the N- and C-terminal heteroatoms of the spirocyclic mimetic could closely match the corresponding heteroatoms of Pro[2] and Phe[5]. Moreover, the hydrophobic side chains of Phe[5] and Pro[3] were accounted for in the mimetic by a bulky substituent at the 1-position of the spirocycle. These features are represented in Figure 1 where the thick bonds correspond to the mimetic, the thin bonds are of the cyclic peptide (D-Arg-Arg-Cys-Pro-Gly-Phe-Cys-D-Tic-Oic-Arg), and the Pro[3], Phe[5] hydrophobic domain is shown as dark gray spheres.

C. Pharmacology. 1. Receptor Binding Assay. CHO/K cells (ATCC, Rockville, MD) were transfected with plasmid DNA containing the human bradykinin B2 receptor using Transfectase Reagent (Life Technologies, Gaithersburg, MD). Membranes were prepared from the transfected cells, which had been grown to confluency in Ham's F12 medium containing 10% fetal bovine serum and 500 μg/mL G418 (Life Technologies). Monolayers were washed one with Ca^{2+}- and Mg^{2+}-free Dulbecco's phosphate-buffered saline (DPBS).

Cells were scraped in DPBS and centrifuged at 500*g* for 10 min at 4 °C. Pellets were resuspended in 25 mM *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES) and 1 mM 1,10-phenanthroline pH 6.8 buffer and then homogenized using a Brinkmann Instrument Polytron (Westbury, NY) at setting 5 for 10 s. Membranes were isolated by centrifugation at 48000*g* for 10 min at 4 °C. Pellets were resuspended in TES buffer with 140 μg/mL bacitracin and 0.1% BSA, flash frozen in liquid nitrogen, and stored at −80 °C. Competition binding assays were carried out in a 3 mL volume using [^{3}H]NPC-17731 (Du Pont NEN, Boston, MA) at final concentrations of 15 pM. Nonspecific binding was determined in the presence of 1 μM bradykinin and represented less than 10% of the total binding. After a 90 min incubation at 25 °C, the assay was terminated by rapid vacuum filtration through Whatman GF/B filters (Maidstone, U.K.) presoaked in 0.2% polyethylenimine for 3 h followed by three washes with Tris (50 mM, pH 7.4, 0 °C). Radioactivity was counted with a Beckman scintillation counter (Beckman Instruments, Fullerton, CA).

2. Functional Assay. a. *In Vivo* Inhibition of Bradykinin-Induced Hypotension in Rabbits. Animals were maintained at a constant temperature of 20−22 °C on a 12 h light/dark cycle prior to experimentation. Food and water were provided *ad libitum*. Male New Zealand white rabbits weighing 1.5−2.0 kg were administered acepromozine (1 mg kg^{-1}, im) 30 min prior to anesthetizing with sodium pentobarbital (15−30 mg kg^{-1}, iv). Animals were placed on a temperature control unit (Narco Bio-Systems, Houston, TX). Mean systemic arterial blood pressure (MABP) was measured continuously using carotid catheter (PE-50 polyethylene tubing) filled with heparanized saline (20 units mL^{-1}). The catheter was connected to a physiological pressure transducer (P-50, Gould Statham). The jugular vein was cannulated (PE-10 polyethylene tubing) for the administration of bradykinin (BK) and bradykinin antagonist (BKA). A tracheotomy was performed, but animals were allowed to breath spontaneously. The data was recorded on a physiograph (Gould 4 channel, Model 30V8404).

After surgery, each animal was allowed a period of stabilization (15 min), after which three bolus doses of BK (1 μg kg^{-1}, iv) were administered at intervals of 5 min and the effect on MABP (Δ mmHg) measured. After 15 min the BKA was administered in increasing concentrations diluted in saline (0.03−1000 μg kg^{-1}, iv) followed by a bolus of BK (1 μg kg^{-1}, iv) 5 min after each dose of antagonist (the 5 min period allows for time to observe agonist effects). The decrease in MABP (mmHg) was calculated following each bolus of BK. The animal was then allowed to stabilize for 10 min before the next dose of antagonist was given. Each animal served as its own control.

Animals were individually assessed for percent inhibition of BK-induced hypotension and then averaged at each dose as mean ± sem ED_{50}'s were individually calculated using Probit analysis.

b. Inhibition of Bradykinin-Induced Hypotension in Rats. Anesthetized Sprague−Dawley rats were cannulated *via* the right carotid artery (for measurement of mean arterial blood pressure (MABP)) and the jugular vein (for administration of bradykinin and/or test agent). The airway was kept open *via* tracheotomy. Animals were stabilized for 30 min, after which hypotension was induced by bradykinin (12 μg kg^{-1}, iv). The baseline hypotensive effect was confirmed by two additional injections of bradykinin given at 5 min intervals after the first injection. Test agents to be evaluated for antagonist activity were administered 10−15 min (return to baseline MABP) after the third injection of bradykinin, followed by an additional bradykinin injection given 5 min later. MABP was measured to determine if the test compounds would prevent bradykinin-induced hypotension. Successive increasing doses of test compounds were administered to generate cumulative dose response.

Supporting Information Available: Analytical data for pseudopeptides (Table 3) (1 page). Ordering information can be found on any current masthead page.

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