lated from the extent of substrate cleavage as determined by HPLC.[8]

Entries 3–5 in the table illustrate the beneficial effect of constraining the benzylic and phenethyl amides as the corresponding 1- or 2-aminoindan analogues. In the case of the 1-aminoindan amides, both diastereomers (entries 4 and 5) showed very similar inhibitory activity. In addition, two other ring sizes were explored as illustrated by the 1-aminotetralin and 1-aminobenzocyclobutene amides (entries 6 and 7). Very little effect on the IC_{50} was observed for the various ring sizes. Because all of these 1-substituted cyclic benzylic amides were of comparable potency, which was still 1 order of magnitude less than our lead analogue 1, it was of interest to determine the effect of introducing substituents onto the aliphatic portion of these amides. As seen by entry 8, introduction of an alcohol group at the 2-position trans to the nitrogen on the indan ring resulted in a significant loss in activity. However, the corresponding cis alcohol configuration resulted in a considerable increase in inhibition. As shown in entries 9 and 10, the difference in activity for the two cis-substituted diastereomers was approximately 100-fold. Independent crystallography studies have demonstrated that the more active diastereomer possesses *S* stereochemistry at the 1-position of the aminohydroxyindan as indicated.[9]

A primary carboxamide group cis to the nitrogen in the 2-position (entry 11) afforded an inhibitor with significant activity, indicating that a hydroxyl group is not a strict requirement for potency. Additional substitution at the 3-position of the indan was also well tolerated, as exemplified by entries 12 and 13 which had potencies in the low nanomolar range.

The compounds shown in entires 9, 12, and 13 have been evaluated for their ability to inhibit the spread of HIV-1 in human T-lymphoid cell culture (as described in ref 4) with 100% minimal inhibitory concentrations of 400, 100, and 400 nM, respectively. These antiviral activities are substantially better than for compound 1 (6 μM) and compare favorably to the more potent analogues described in the preceding paper.[4]

In summary, the C-terminal dipeptide unit of our lead inhibitor 1 has been successfully replaced by a variety of substituted 1-aminobenzocycloalkanes. Introduction of a hydroxyl group cis to the amino functionality is particularly effective in providing protease inhibitors with potencies in the subnanomolar range having considerable antiviral activity. Previous structure–activity investigations[4] suggested that the P_2' carbonyl oxygen of 1 participated in a critical hydrogen bond. Subsequent crystallographic experiments on enzyme–inhibitor complexes[9] have confirmed that the 2-hydroxyindan group acts a P_2' carbonyl surrogate. A possible implication is that conformationally contrained β-hydroxyamides may be generally useful as amino acid replacements. Studies are in progress to investigate the in vivo behavior of some of these compounds and will be reported in due course.

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Design and Conformational Analysis of Several Highly Potent Bradykinin Receptor Antagonists

Bradykinin, a linear peptide hormone (Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9), has been implicated in a multitude of pathophysiological processes.[1,2] Of particular significance is its role as a potent pain-producing agent[2] and the more recent suggestion that it may be associated with the symptoms of the common cold.[3,4] Because of these physiological functions related to this neurotransmitter, a bradykinin receptor antagonist may have significant therapeutic value.

In the absence of a large number of known receptor antagonists upon which to base an SAR, the solution conformations of bradykinin, bradykinin fragments, and several related peptide analogues have been studied extensively in order to gain insight into a possible bioactive conformation. The spectroscopic methods used in these studies include CD,[5-7] ^{13}C and ^1H NMR,[6-11] and laser Raman spectroscopy.[6] In the earliest of these analyses, most of which were performed in an aqueous environment, the general conclusion was that bradykinin existed in many conformational states, none of which were preferred. More recently, bradykinin and a bradykinin receptor antagonist, NPC 567[12] (D-Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Phe^5-Ser^6-D-Phe^7-Phe^8-Arg^9), were examined by NMR at 500 MHz in

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(16) See Scheme II. Chromatographic separation (SiO_2) of the diastereomeric amides was carried out prior to desilylation.
(17) See Scheme III.
(18) See Scheme IV; the Cbz group was removed by catalytic hydrogenation using Pd/C after the desilylation.

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I. (D)Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Thi^5-Ser^6-(D)Tic^7-Tic^8-Arg^9

II. (D)Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Thi^5-Ser^6-(D)Tic^7-(D)Tic^8-Arg^9

III. (D)Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Thi^5-Ser^6-(D)Tic^7-Aoc^8-Arg^9

IV. (D)Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Thi^5-Cys^6-(D)Tic^7-Phe^8-Arg^9-Cys^{10}

V. (D)Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Thi^5-Cys^6-(D)Phe^7-Phe^8-Cys^{9}Arg^{10}

Figure 1. Peptides expected to adopt β-turn conformations at their C-terminus.

Table I. Pharmacological Data Measured for Peptides I–V and NPC 567

peptide	K_i, nM	pA_2
I	0.381 ± 0.042	9.1 ± 0.2
II	571 ± 49	5.6 ± 0.4
III	0.176 ± 0.025	9.4 ± 0.1
IV	inactive	
V	876 ± 185	5.4 ± 0.5
NPC 567	26.8 ± 1.24	8.2 ± 0.2

a 9:1 dioxane-d_6/H_2O solvent mixture as a mimic of the amphiphilic membrane-embedded bradykinin receptor environment.[13] Under these conditions a well-defined conformation characterized by a β-turn spanning the C-terminal residues Ser^6-Pro^7-Phe^8-Arg^9 in bradykinin as well as a similar turn structure spanning residues Ser^6-D-Phe^7-Phe^8-Arg^9 in NPC 567 was reported. Support for this conformation was described recently wherein bradykinin was examined by NMR in SDS micelles using two-dimensional experiments at 500 MHz.[14] In this work, the authors also described a β-turn-like structure spanning the residues Ser^6-Pro^7-Phe^8-Arg^9 in bradykinin.

Drawing on the reported spectroscopic data, and in particular the possible significance of β-turn structures[15] at the C-terminus of receptor-active compounds, we prepared[16] several types of peptides to challenge the hypothesis that this conformation is preferred in ligand binding to the bradykinin receptor. Listed in Figure 1 are the primary sequences of the five peptides chosen for the study. Although peptides I and III have been recently disclosed in a European patent application describing them as bradykinin antagonists,[17] the former was discovered

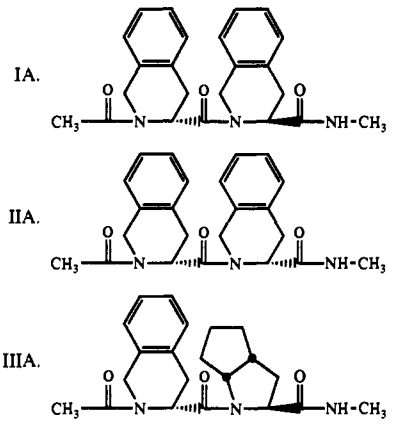

Figure 2. Model peptides used in conformational analysis. IA corresponds to *N*-acetyl-D-Tic-L-Tic-*N'*-methylamide, IIA corresponds to *N*-acetyl-D-Tic-D-Tic-*N'*-methylamide, and IIIA corresponds to *N*-acetyl-D-Tic-L-Aoc-*N'*-methylamide. For both IA and IIA either endo or exo boat conformation of the saturated six-membered ring were considered explicitly.

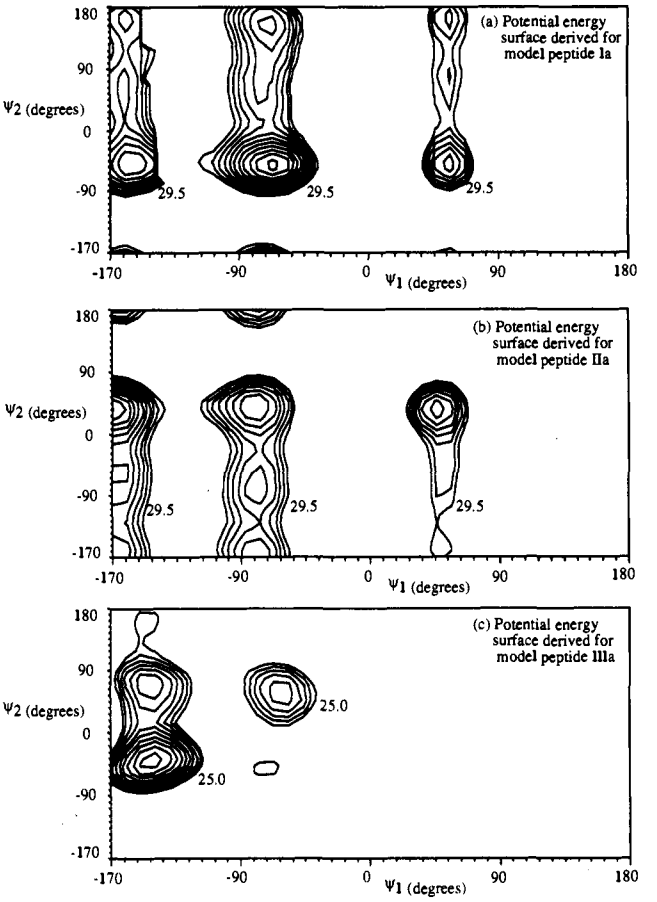

Figure 3. Potential energy contour plots corresponding to the three respective conformational searches done on model compounds (a) IA, (b) IIA, and (c) IIIA. Energy units are kilocalories/mole and the highest value contour intervals are shown on the plots. The contour interval is 0.5 kcal mol^{-1}.

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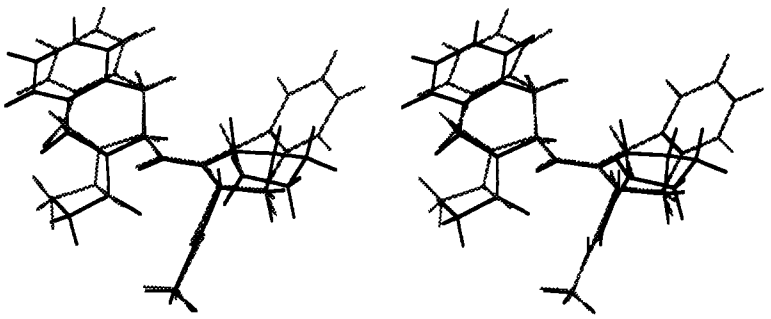

Figure 4. Representative conformations extracted from the potential energy wells corresponding to the preferred β-turn in model peptides IA (grey) and IIIA (black).

coincidentally and independently in our laboratories. Each was considered likely to stabilize a β-turn structure either indirectly through the incorporation of unnatural amino acids into the sequence (I–III), or directly via covalent cyclization (IV and V). Each peptide was tested for its ability to displace [^{3}H]bradykinin binding from guinea pig ileal membrane as described previously.[18] The receptor affinities of I–V and NPC 567 are presented in Table I as are their pA_2 values, the latter of which were determined in SV-T_2 fibroblasts by using bradykinin-stimulated prostaglandin synthesis as described elsewhere.[19] These cells express only B_2 bradykinin receptors.[20] At concentrations up to 10 μM, none of the compounds had any agonist activity in the cells (EC_{50} for bradykinin is 0.3 nM). Results of these binding studies indicate that compounds II, IV, and V are essentially inactive in binding. However, I and III are even more potent in binding than the reference antagonist NPC 567 and are true antagonists.

To quantify the conformational impact of the C-terminal substituents within these peptides, a systematic 10° grid search[21] was performed on three model compounds IA, IIA, and IIIA, shown in Figure 2. Contour plots representing all energy minima corresponding to no more than 5 kcal above the global minimum were plotted for each model peptide with a 0.5 kcal mol^{-1} contour interval. Generally, the ψ_1, ψ_2 (where ψ_i corresponds to the backbone dihedral angle for residue i defined by the four adjacent amino acid backbone atoms N_i-C^α_i-C_i-N_{i+1}) dihedral angle values corresponding to the local minima were the same regardless of the Tic residue being in the endo or exo boat form. Hence, all contour plots used in this report were derived from the endo conformation of the Tic residue, but for each local minimum it was assumed that either an endo or exo boat Tic conformation would be possible. Shown in Figure 3 are the potential energy contour plots corresponding to the three respective conformational searches done on the model compounds IA, IIA, and IIIA.

There are three local minima located on the contour plot corresponding to compound IIIA. The global energy minimum is centered about $\psi_1 = -140°$, $\psi_2 = -54.5°$ and is ca. 2 kcal lower in energy than either of the other two minima. This conformational class, which corresponds to a β-turn structure, would be expected to dominate the overall population of unique conformations in solution. Clearly this conformation state is driven by the bulky amino acid side chains severely restricting the angles ψ_1 and ψ_2. A representative conformation from within this local energy well is shown in Figure 4.

The contour plot corresponding to compound IA contains four broad zones representing local energy minima. In this case the global minimum is centered about $\psi_1 = -60°$, $\psi_2 = -40$. The same minimum ($\psi_1 = -140°$, $\psi_2 = 54.5°$) as observed in IIIA (β-turn) is also present here; however, the overall population of that state is likely diminished somewhat since its corresponding potential energy is ca. 1.5 kcal higher in energy than the global minimum. Again, the allowed conformational states for this peptide are driven by the bulky amino acid side chains severely restricting the angles ψ_1 and ψ_2. A representative conformation from within this energy well is shown in Figure 4.

The four local minima represented in the contour plot of IIA are located at positions that correspond to the same values of ψ_1 in IA; however, the ψ_2 values are inverted from those observed in IA. Of particular significance is the absence of the β-turn structure (minimum about $\psi_1 = -140°$, $\psi_2 = -54.5°$), as observed in the other two model systems. This phenomenon is dictated by the amino acids containing the adjacent D–D stereochemical configuration. This implies that the preferred β-turn conformations in IA and IIIA are unlikely geometric states for IIA.

On the basis of empirical energy calculations, the cyclic peptides (IV and V) likely adopt a *cis*-amide geometry either between residues 7 and 8 or residues 6 and 7. Energy-minimized models for these peptides containing all possible combinations of *cis*- and *trans*-amide bonds in β-turn-like states within the cyclic portion of the peptides have been studied. This work does not represent a complete systematic examination of all possible conformational states; however, for those conformations examined explicitly, the differences in the energy terms are slight.

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Hence, although a type II' β-turn state might be possible, a *cis*-amide bond linking residues 7 and 8 would best be classified as a type VIa β-turn. A turn of this nature would orient the lipophilic side chains of residues 7 and 8 into portions of space quite distinct from the orientation corresponding to the type II' turn preferred in compounds I and III, which have high affinities for bradykinin receptors. Additionally, the NMR experiments run in a lipophilic environment suggest that all amide bonds likely remain trans in both agonists and antagonists. Hence the inactivity of IV and V is not surprising. Another factor which might contribute to the lack of potency of these peptides is the additional steric space occupied by the two sulfur atoms contained in the disulfide bond. This additional steric volume might not be easily accommodated at bradykinin receptors.

In summary, five peptides assumed to have an inherently stabilized β-turn geometry at their C-terminus were designed and prepared. That two of these five peptides (I and III) are highly potent bradykinin receptor antagonists suggests that the solution conformations discovered for both bradykinin and NPC 567 in a lipophilic environment (either dioxane–water or SDS micelles) must closely approximate the bioactive conformations. Furthermore, these NMR studies, followed by the prudent design of potent compounds containing conformational constraints, demonstrate another in a growing number of examples of truly rational molecular design.

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Book Reviews

The Biology and Chemistry of Polyamines. Edited by Sara H. Goldemberg and Israel D. Algranati. Oxford University Press, New York. 1989. xiii + 244 pp. 15.5 × 23 cm. ISBN 0-19-963147-6. $60.00.

This volume contains the proceedings of an Argentine–Japanese joint seminar held in Buenos Aires in April 1989 and represents Symposium 184 of the International Union of Biochemistry (IUB). It is as well volume 12 of the ICSU Symposium Series.

The book is divided into six sections (24 chapters) which focus on (1) the modulation of metabolism and synthesis of macromolecules by polyamines (three chapters), (2) enzyme systems involved in polyamine metabolism (eight chapters), (3 and 4) polyamine structure and function in microorganisms and viruses (six chapters), (5) polyamines as effectors of mammalian cell growth and differentiation (six chapters), and (6) strategies for polyamine chemical synthesis (one chapter). The volume is prefaced with a comprehensive synopsis of the meeting and is concluded with a brief subject index.

As a written record of a scientific meeting, the value of this book mirrors the quality of participant selection by the meeting organizing committee. The individuals that were selected to actively participate at this symposium are interested in a broad range of topics related to polyamine biology and chemistry. The chapters that these investigators contributed are, in general, well written and clearly illustrated. Most authors placed their research in clear scientific and historic context with concise and well-referenced introductions. The delay in publication of this volume is unfortunate and stunts its utility. Nonetheless, the editors have brought together a notable temporal landmark for this area of research.

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Hormones. From Molecules to Disease. Edited by Etienne-Emile Baulieu and Paul A. Kelly. Chapman and Hall, New York. 1990. viii + 697 pp. 21.5 × 28 cm. ISBN 0-412-02791-7. $62.50.

Striking advances in the field of endocrinology have been achieved in the last decade. New hormones, hormone-like molecules, receptors, and mechanisms of action continue to be elucidated. Techniques in molecular biology have enabled the determination of detailed gene structures of many compounds involved in hormonal systems. Marked homology has been noted between oncogene products and various components of the endocrine network. This observation, in turn, has led to the suggestion that deregulation of hormonal function is involved in the initiation and/or development of cancer. Also, it is now known that the central nervous system is both a target for and site of formation of many hormones and that these substances, neurotransmitters, growth factors, and immunopeptides all act via similar mechanisms. The last decade has also witnessed the recognition of calcium, phospholipid derivatives, and protein kinases, in addition to cAMP, as second messengers in the action of hormones. These discoveries have had broad therapeutic implications, such as new strategies for fertility control, management of diabetes, and treatments for cancer, cardiovascular disorders, and mental dysfunctions.

The objective of this book is to present this diverse and rapidly changing field in a clear and precise fashion. This is accomplished by contributions from the world's leading specialists who review the major hormones and hormonal functions in 14 chapters. Related topics are treated in an innovative series of short, single-subject essays inserted between the chapters. A very adequate subject index is included.

Medicinal chemists, endocrinologists, and scientists from various other disciplines in the health sciences will find this clearly written book a valuable resource for review or entry into fascinating new research areas.

Staff

Goodman and Gilman's The Pharmacological Basis of Therapeutics. Eighth Edition. Edited by Alfred G. Gilman, Theodore W. Rall, Alan S. Nies, and Palmer Taylor. Pergamon Press, New York. 1990. xvi + 1811 pp. 18.5 × 26 cm. ISBN 0-08-040296-8. $79.50.

Some believe that medicinal chemists know more pharmacology than pharmacologists know chemistry. If so, one reason may be that for 50 years chemists have had access to *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. In large measure, the popularity of this text derives from its appeal to scientists in fields ranging from chemistry to physiology, biochemistry, and clinical medicine. The eclectic nature of pharmacology lends itself to this type of coverage and, over the years, the editors of this text have set the standard for defining ther-