ratio of protease inhibition to cell culture potency are not fully understood and may be due to a combination of size, lipophilicity, and other factors. The IleAMBI-containing compounds consistently yielded the most potent viral inhibitors. Compound 18 (L-687,908) was found to be the most potent of all the analogues with an MIC of 12 nM. The compound exhibited an identical MIC when the assay was performed with the IIIb-unrelated RF isolate of HIV-1.

In addition, L-687,908 was shown to inhibit the HIV-1 protease mediated cleavage of the viral *gag* precursor protein, p55, to the mature p24 and p17 core and matrix proteins (Figure 1). Since the prevention of this virus maturation step leads to the production of noninfectious viral particles,[5] it is highly likely that the inhibition of viral infectivity mediated by L-687,908 is indeed due to its ability to inhibit the viral protease enzyme.

Analogues of L-364,505 are potent inhibitors of HIV-1 protease both in vitro and in cell culture. Modifications of this structure led to L-687,908 (18), which potently inhibited HIV-1 protease both in vitro and in cell culture. The structure–activity relationships discovered in this series should serve as a basis to design compounds which are intrinsically potent and exhibit enhanced oral bioavailability.

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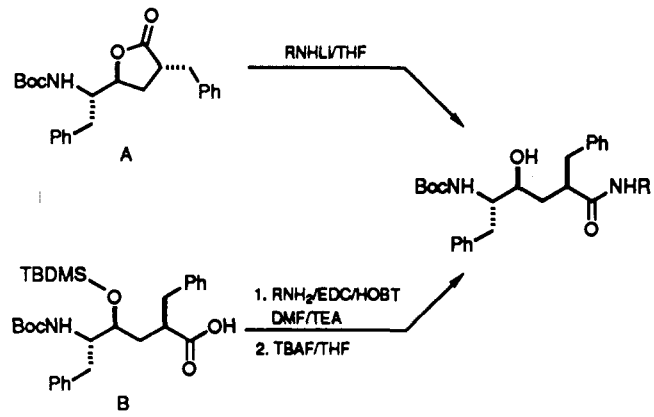
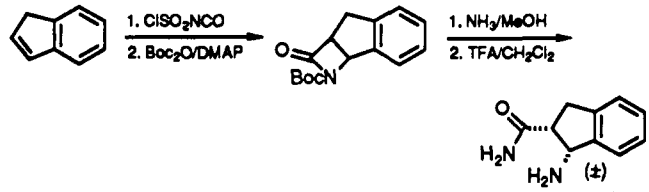
Benzocycloalkyl Amines as Novel C-Termini for HIV Protease Inhibitors

Soon after the discovery that the human immunodeficiency virus (HIV) was the causative agent of acquired immunodeficiency syndrome (AIDS), a number of biochemical pathways required for the replication of such retroviruses were proposed as potential therapeutic targets. A virally derived protease was subsequently identified as a key element required for the maturation and replication of HIV.[1] Sequence homology studies had indicated that this enzyme was closely related to known aspartic acid proteases, and was likely dimeric in its functional form.[2,3] As an initial step toward identifying inhibitors of this enzyme, a number of known inhibitors of aspartic acid proteases were tested against the HIV protease. This led to the synthesis of the series of inhibitors described in the preceding paper in this issue.[4] With the goal of a therapeutically useful HIV protease inhibitor in mind, several deficiencies of our lead series of compounds needed to be addressed. Vulnerability to degradative enzymes, rapid biliary clearance, and poor oral absorption were among the primary concerns for these peptide-based compounds.[5] A common strategy for approaching these issues has been to minimize peptide-like character without adversely affecting biological activity.

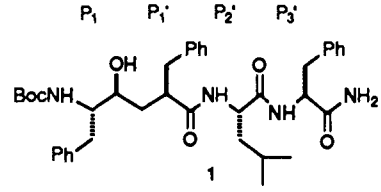
Scheme I



Scheme II



An early part of our effort therefore was centered on replacing the C-terminal dipeptide unit found in our lead inhibitor 1.[4] This approach had several potential benefits, in that it would eliminate one or more peptide amide bonds, reduce the molecular weight, and diminish the potential for recognition by degradative proteases.



A key discovery made during the process of modifying the C-terminus of 1 was that replacing the leucylphenylalanylamide dipeptide by a benzyl amide afforded an inhibitor which retained inhibitory activity against the HIV protease[4] (Table I). However, due to its relatively weak inhibition when compared to 1 (111 nM vs 0.5 nM, respectively), an effort was undertaken to prepare modified amides with improved activity against the enzyme. One approach toward this objective was to prepare conformationally restricted amides which might promote favorable nonbonded interactions within the enzyme active site. Initially we chose to investigate a series of amides derived from conformationally constrained benzylic amines exem-

(1) Kohl, N. E.; Emini, E. A.; Schleif, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, R. A. F.; Scolnick, E. M.; Sigal, I. S. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4686.
(2) (a) Ratner, L. *Nature* **1985**, *313*, 277. (b) Toh, H.; Ono, M.; Saigo, K.; Miyata, T. *Nature* **1985**, *315*, 691.
(3) Pearl, L.; Taylor, W. *Nature* **1987**, *329*, 351.
(4) Vacca et al., preceding paper in this issue.
(5) Plattner, J. J.; Norbeck, D. W. In *Drug Discovery Technologies*; Clark, C. R., Moos, W. H., Eds.; Ellis Horwood Limited: Chichester, 1990; pp 92-126.

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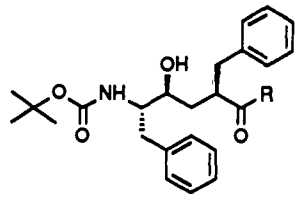
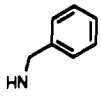
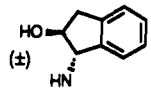
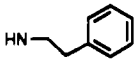
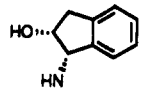
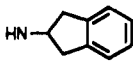
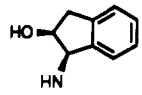
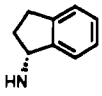
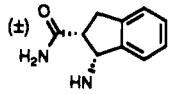
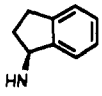
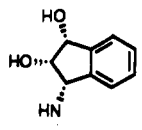
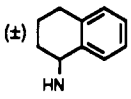
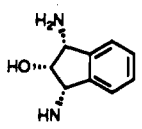
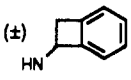
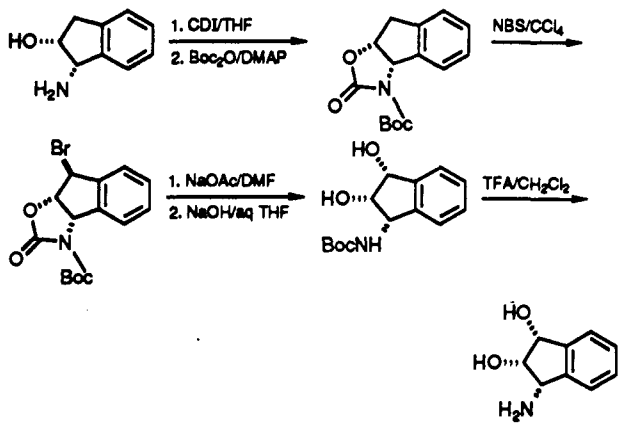
Table I. Structures and Inhibitory Activities of C-Terminal Amides

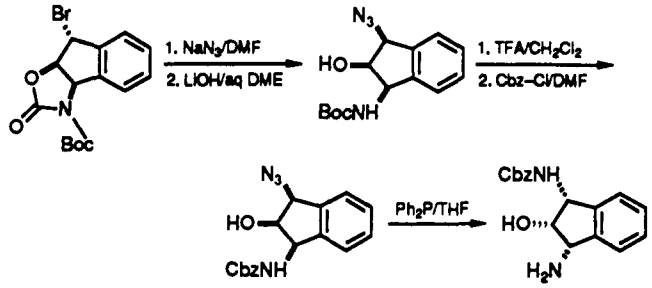


no.	R	ref	IC_{50}, nM	no.	R	ref	IC_{50}, nM
1		10	111	8		13	259
2		10	2800 ± 430	9		14, 11	0.3
3		10	130 ± 12	10		14, 11	229
4		10, 11	19	11		15, 16	1.8
5		10, 11	21	12		17	0.1
6		10	16	13		18	1.9
7		12	22				

Scheme III



Scheme IV



plified by the aminobenzocycloalkanes shown in the table.

The synthesis of these amides is depicted in Scheme I, and is closely related to a previously published route.[6] Two methods were used in their preparation, either a direct base-catalyzed amine opening of the previously described lactone A (Table I, entries 1–3) or an active ester coupling of the amine to the silyl-protected acid B.[6] Previously known amines not available commercially were prepared or resolved by the literature methods cited (see Schemes II–IV). In some cases, coupling of racemic amines to the silyl acid B resulted in mixtures of diastereomers which were separated by column chromatography. When these mixtures were not separable, they were desilylated with tetrabutylammonium fluoride[6] and tested as a mixture of two diastereomers. All final compounds were purified by silica gel chromatography using chloroform–methanol solvent mixtures and isolated by trituration with ethyl acetate–hexanes.[7]

Initial evaluation of these compounds as HIV protease inhibitors was carried out by using the HIV-1 enzyme substrate cleavage assay which has been described elsewhere.[8] Each compound was dissolved in DMSO and added to the enzyme mixture to provide the required concentrations of inhibitor. The IC_{50} values were calcu-

(6) Evans, B. E.; Rittle, K. E.; Homnick, C. F.; Springer, J. P.; Hirshfield, J.; Veber, D. F. *J. Org. Chem.* **1985**, *50*, 4615.

(7) All new compounds were characterized by 300-MHz ^{1}H NMR and homogeneity determined by RP-HPLC and combustion analysis (±0.4%).

(8) Heimbach, J. C.; Garsky, V. M.; Michelson, S. R.; Dixon, R. A. F.; Sigal, I. S.; Darke, P. L. *Biochem. Biophys. Res. Commun.* **1989**, *164*, 955.

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

(9) Private communication from Drs. J. P. Springer and W. J. Thompson.
(10) Aldrich Chemical Company, Milwaukee, WI 53233.
(11) Rittle, K. E.; Evans, B. E.; Bock, M. G.; DiPardo, R. M.; Whitter, W. L.; Homnick, C. F.; Veber, D. F.; Freidinger, R. M. *Tetrahedron Lett.* **1987**, *28*, 521.
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(15) Moriconi, E. J.; Mazzocchi, P. H. *J. Org. Chem.* **1966**, *31*, 1372.
(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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