stituent, the *R* diastereomer **14R** is more active than the *S* diastereomer **14S**, a result that is consistent with the data reported by Roberts et al.[8a] Surprisingly, when a P_4–P_3 unit is added to either **14R** or **14S**, both diastereomers **15S**, **15R** are equally effective against HIV-PR. These results clearly demonstrate that substituents in the P_4–P_3 and P_3' inhibitor subsites of HEA inhibitors influence the binding of HEA inhibitors to HIV-PR and can shift the preference from the *R* diastereomer in smaller inhibitors to the *S* diastereomer in longer inhibitors. Aromatic groups at P_3 and P_3' also are preferred in the *S* series as shown by comparing compounds **16S** and **17S** to **9S**. Detailed discussion of the structure–activity relationships for the *S* inhibitors will be reported separately.

Our results confirm the stereochemical preferences for the two HEA inhibitor series reported to date. The fact that the configuration of the hydroxyl group necessary for maximal inhibitory activity is dependent upon the peptide framework has profound implications when extrapolating structure–activity data from one HIV protease inhibitor series to another in the course of designing new inhibitors. The divergent hydroxyl group stereochemistry for the two closely related series of compounds is particularly surprising when compared to the preferred hydroxyl group configuration required to inhibit other aspartic proteinases (vide supra).[11,12] Our results require that the (*R*)-HEA inhibitors (e.g. **2**, **3**) bind to the protease in a new mode in order to maintain the hydrogen bonds between the inhibitor hydroxyl group and the enzyme carboxyl groups. X-ray crystallography of the corresponding enzyme–inhibitor complexes will eventually elucidate the molecular details of this new binding mode. However, our preliminary molecular modeling studies[27] indicate that the (*R*)-HEA-derived inhibitors (e.g. **3**) can bind to HIV-PR in a closely related fashion that utilizes similar interactions to stabilize the enzyme-inhibitor complex (Figure 1). When compared with the reported conformation for JG-365 bound to HIV-PR,[19,20] it is evident that the critical hydroxyl group in each inhibitor series can interact with the protease's catalytic carboxylic acid groups while maintaining the interactions to the Asn-Phe side chains, the hydrogen bonds from the Asn and Pro carbonyl groups to water-301, and the hydrogen bonds from water-301 to the enzyme. This hydrogen bonding pattern involving water-301 is present in all HIV-PR–inhibitor complexes reported to date.[9,17–20] To maintain these favorable binding interactions, the *R* diastereomer **3** must place the *tert*-butyl amide in the S_2' enzyme subsite rather than along the peptide backbone. In this new binding mode, the *tert*-butyl group in **3** replaces the isobutyl group of isoleucine in **1S**. The remaining binding interactions between HIV-PR and the inhibitors appear to be conserved. The decahydroisoquinoline (DIQ)[8a] ring system occupies the S_1' subsite, while only subtle differences in overall geometry of the protease itself are needed to accommodate either diastereomer.

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Supplementary Material Available: Experimental procedures for the synthesis of hydroxyethylamine derivatives **1S**, **1R**, **8**, **9S**, **9R**, **14R**, **14S**, **15R**, **15S** (20 pages) is provided. Ordering information is given on any current masthead page.

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L-687,908, a Potent Hydroxyethylene-Containing HIV Protease Inhibitor

The human immunodeficiency virus type 1 (HIV-1), a member of the Lentivirinae subfamily of retroviruses,[1] is the etiologic agent of the acquired immunodeficiency syndrome (AIDS).[2] During viral replication, the *gag*, *pol*, and *env* genes of HIV-1 are translated as precursor polyproteins that are proteolytically processed into the viral structural proteins and enzymes (protease, reverse transcriptase, and integrase).[3] The virus-encoded protease responsible for processing the *gag* and *pol* gene products is a member of the aspartyl protease family and exists as a symmetrical dimer. Each monomer contributes one of the two aspartic acid residues at the active site.[4] Inactivation of the protease by site-directed mutagenesis results in the production of noninfectious virions.[5] As a result, the protease is recognized as an attractive target for antiviral therapy.

Recently, peptidomimetic inhibitors have been reported that substitute the hydroxyethylene,[6] hydroxyethylamine,[7]

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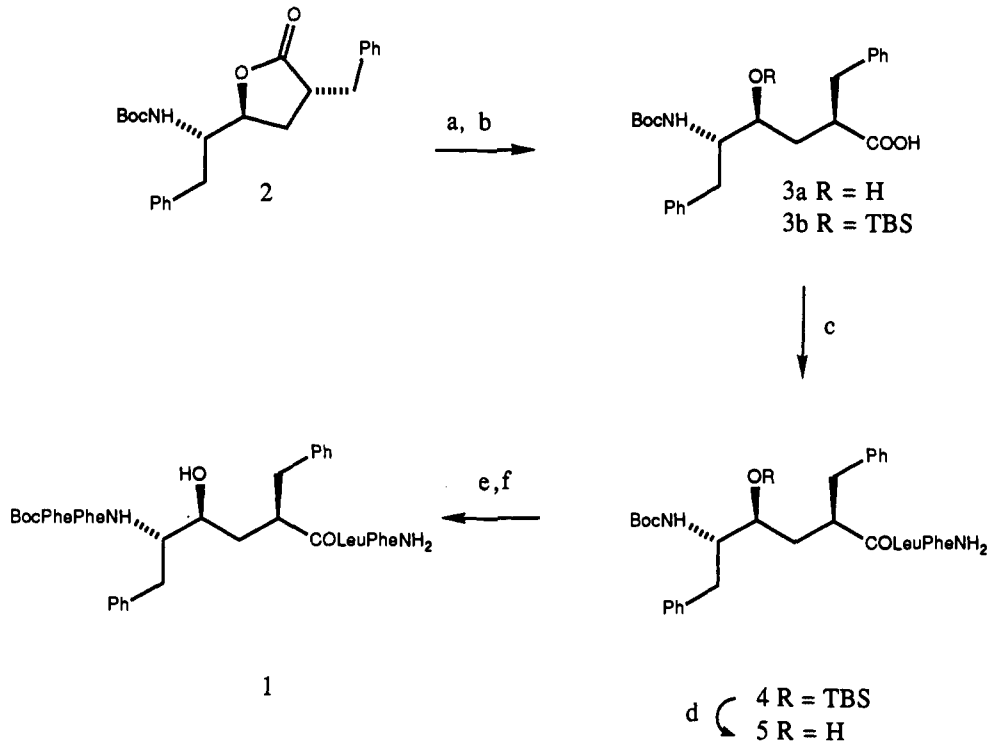
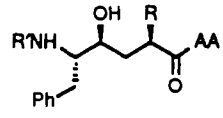
Scheme I[a]



[a] (a) 1 M LiOH/DME, room temperature, 3 h. (b) *tert*-butyldimethylsilyl chloride, imidazole, DMF, room temperature 18 h; MeOH, 1 h. (c) EDC, HOBt, H_2NLeuPheNH$_2$, DMF, pH = 8.0–9.0. (d) Tetrabutylammonium fluoride, THF, room temperature 18 h. (e) TFA, 0 °C, 30 min, CH_2Cl_2. (f) EDC, HOBt, BocPhePheOH, pH = 8.0–9.0, DMF, room temperature 18 h.

or phosphonic acid[8] dipeptide isosteres of P_1–P_1' for the scissile peptide bond found in substrates. Erickson et al.[9a] and Kempf[9b] also have described a class of novel inhibitors of HIV-1 protease. In addition, a non-peptidyl HIV-1 protease inhibitor was reported by DesJarlais.[10] In the course of screening a collection of renin inhibitors previously prepared in our laboratories, L-364,505[11] (1) was identified as a potent inhibitor of HIV-1 protease (IC_{50} = 1 nM vs HIV-1 protease, IC_{50} = 73 nM vs renin). Compound 1 is a seven amino acid analogue which contains the hydroxyethylene transition state[12] isostere and is of similar length[13] to that of reported minimum substrates. Binding to the protease by 1 is stereoselective in that potent inhibition of the enzyme requires the absolute configuration at the hydroxy (C-4) and P_1' (C-2) substituted carbons to

Table I. In Vitro and Cell Culture Potencies of Selected Inhibitors



entry	R′	R	AA	IC_{50},[a] nM	MIC,[b] μM
1	Boc-PhePhe	CH_2Ph	LeuPheNH$_2$	1.0	50
7	BocPhe	CH_2Ph	LeuPheNH$_2$	20.0	NT
5	Boc	CH_2Ph	LeuPheNH$_2$	0.6	6.0
8	tBuCH$_2$C(O)	CH_2Ph	LeuPheNH$_2$	6.0	NT
9	Boc	PPE[c]	LeuPheNH$_2$	0.15	6.0
10	Boc	CH_2Ph	LeuNH(CH$_2$)$_2$Ph	12.0	NT
11	Boc	CH_2Ph	LeuNHBn	1.4	3.0
12	Boc	CH_2Ph	LeuNH$_2$	12.0	6.0
14	Boc	CH_2Ph	NH(CH$_2$)$_2$CH(CH$_3$)$_2$	470	NT
13	Boc	CH_2Ph	NHBn	111	>50
15	Boc	CH_2Ph	IleNHBn	0.71	1.5
16	Boc	CH_2Ph	IleNHCH$_2$CH(OH)-CH$_2$OH	0.15	1.5
17	Boc	CH_2Ph	IleAMBI[d]	0.07	0.2
18	Boc	PPE	IleAMBI	0.03	0.012

[a] IC_{50} values were determined by using the protocol described by Heimbach et al.[16] [b] MIC = minimum concentration of compound required to prevent virus infection in cell culture (see text). [c] PPE = 3-phenylprop-2-ene. [d] AMBI = 2-(aminomethyl)benzimidazole. NT = not tested. MIC of AZT = 50 μM.

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be *S* and *R*, respectively.[14] Despite high intrinsic activity, compound **1** inhibits the spread of viral infection in H9 cell culture only at high micromolar concentrations. Modification of **1** resulted in a series of compounds which are devoid of renin activity and which are highly potent in preventing the spread of viral infection in cell culture.

The compound L-364,505 was synthesized by the route shown in Scheme I. Lactone **2**, prepared according to known methods,[11] was hydrolyzed to the hydroxy acid **3a** (R = H). In a one-pot sequence, both the hydroxy group and acid were converted to the *tert*-butyldimethylsilyl (TBS) ether and ester. The ester was removed by stirring the reaction with excess methanol for 1 h prior to workup. Acid **3b** was then coupled to leucylphenylalanylamide by using standard methods to afford amide **4**. Removal of the TBS group in **4** with tetrabutylammonium fluoride gave compound **5**. N-terminal modifications were accomplished by removing the BOC group of **5** and acylating the resulting amide **6**. All compounds in Table I were synthesized by this general route.[15]

Table I illustrates the enzyme-inhibitory properties of representative examples from each series of compounds synthesized. IC_{50} values were determined by using a peptide hydrolysis assay that employs an octapeptide substrate, H_2N-ValSerGlnAsn(β-naphthyl-Ala)ProIle-Val-OH (430 μM, K_m = 160 μM), enzyme at 0.030 nM, and HPLC quantitation.[16] Deleting one amino acid residue (-Phe-) from compound **1** to afford hexapeptide analogue **7** resulted in a 20-fold drop in potency. In contrast, pentapeptide analogue **5** was a more potent inhibitor of HIV protease than **1** and was inactive against renin.[17] Isosteric replacement of the BOC group in **5** with *tert*-butylacetyl (**8**) resulted in a 10-fold drop in potency. Carbamates were more potent than their amide counterparts in all cases examined.

A series of P_1' side chain modifications were explored, and *trans*-3-phenylprop-2-ene (PPE) provided the most potent inhibitors (**9**). Compounds containing this group, for example, were generally 2–4 times more potent than their benzyl counterparts. Elimination of the P_3' carboxamide (**5** vs **10**) led to a 20-fold loss in intrinsic potency, while shortening the chain by a methylene group (**10** vs **11**) regained most of the lost potency. Removing P_3' altogether (**10** vs **12**) resulted in a 20-fold loss in affinity. Removal of the P_2' leucine (**11** vs **13**) led to a further 10-fold loss in potency. Although compound **13** suffered from diminished activity, it served as a new lead for further development of smaller, more potent inhibitors.[18]

Small branched-chain amino acids were preferred in the P_2' position, perhaps as a result of stabilizing the extended conformation which has been observed for bound inhibitors. Optimization of P_3' was carried out with isoleucine occupying the P_2' site. Arylmethyl amides (e.g. **15**) were particularly effective at this position. The most potent compounds contained a hydrogen bond donor in the C-terminus where a P_3' amide carbonyl would normally be found (**16**), and the combination of a hydrogen-bond donor incorporated into an aryl group, as in (aminomethyl)-benzimidazole (AMBI), gave a very potent compound (**17**).

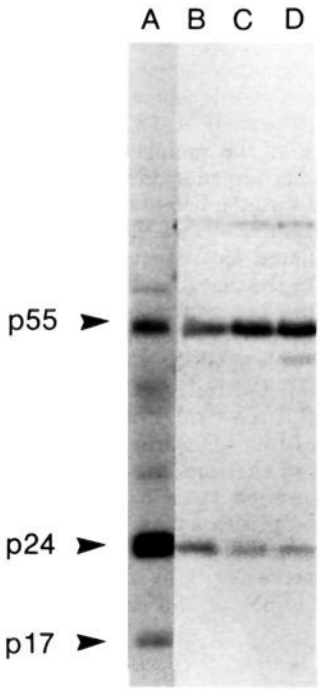

Figure 1. Inhibition of HIV-1 gag precursor, p55, processing mediated by L-687,908. H9 human T-lymphoid cells, persistently infected with the HIV-1 IIIb isolate, were washed and resuspended in culture medium containing several concentrations of the test compound. The cells were washed 24 h later and were again resuspended in medium containing the appropriate concentration of compound. After an additional 24 h, the cells were harvested, washed, and disrupted in detergent containing buffer. Following separation by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, the proteins were transferred onto nitrocellulose paper and the viral proteins were visualized by probing with virus antibody-containing human sera as described in ref 5. The infected cell cultures were either untreated (lane A) or treated with 12.0 μM (lane B), 3.0 μM (lane C), or 0.7 μM (lane D) L-687,908.

Finally, combining the potency-enhanced styryl group with compound **17** gave L-687,908 (compound **18**), which is one of the most potent inhibitors tested to date.

The compounds also were tested for their ability to inhibit HIV-1 spread in cell culture. The results are included in Table I. H9 human T-lymphoid cells were infected with the virus (IIIb isolate), in the absence of test compound, at a multiplicity of infection of 1.0%. Test compound at various concentrations was added 1 day following infection. The compound was initially dissolved in dimethyl sulfoxide and then serially diluted in culture medium (RPMI-1640 containing 10% fetal bovine serum). The medium, containing fresh compound, was replenished every 2–3 days. The cells were harvested after 14 days, and the extent of virus infection was assessed by specific immunofluorescence using anti-HIV-1 human serum. The lowest concentration of test compound required to totally prevent the spread of virus beyond the initially infected cells was defined as the compound's MIC (minimal inhibitory concentration). In this assay, AZT has an MIC of 50 μM after the 14-day period. AZT, unlike the protease inhibitors, is particularly ineffective when tested for such an extended period of time. The original lead compound **1**, which is analogous to a heptapeptide, was weakly active in this assay. However, the pentapeptide analogue **5** was significantly more potent, though its IC_{50} was similar to that of compound **1**. This may reflect the ability of compound **5** to penetrate cells more readily because of fewer amino acid residues. However, the factors which affect the

(14) Darke, P., unpublished results. The 2*R*,4*S*, 2*R*,4*R*, and 2*S*,4*R* isomers of **1** all had IC_{50}'s > 1000 nM.

(15) All new compounds gave satisfactory combustion analysis, ^1H NMR, mass spectra, and/or HPLC purity.

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(17) Siegl, P. K. IC_{50} of **5** vs purified plasma renin at pH = 7.4 > 10 000.

(18) See following paper in this issue.

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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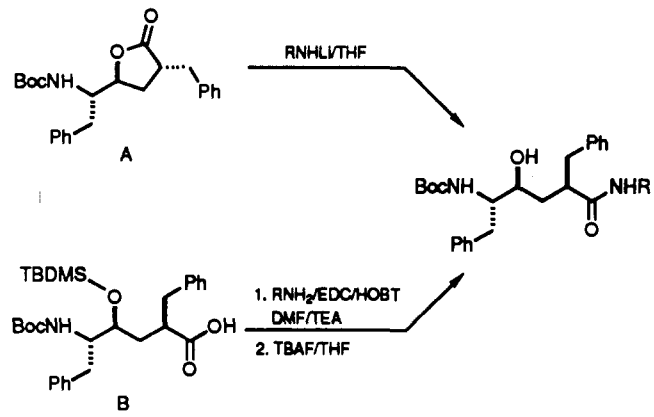
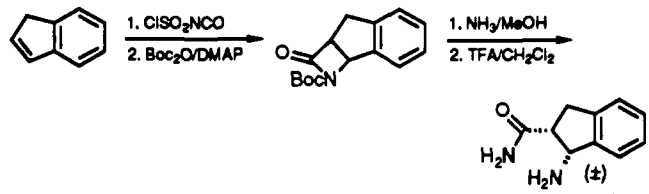
Benzocycloalkyl Amines as Novel C-Termini for HIV Protease Inhibitors

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.

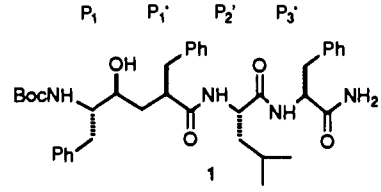
Scheme I



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-

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