Communications to the Editor

Effect of Hydroxyl Group Configuration in Hydroxyethylamine Dipeptide Isosteres on HIV Protease Inhibition. Evidence for Multiple Binding Modes

Inhibition of HIV-1 protease (HIV-PR), the aspartic protease that cleaves specific amide bonds in precursor *gag-pol* proteins to form the mature proteins needed for production of infectious human immunodeficiency virus (HIV) particles,[1] is regarded as a promising approach for treating acquired immunodeficiency syndrome (AIDS) and related diseases. Tight-binding inhibitors of HIV-PR have been discovered quickly[2-10] because the principles for inhibiting this class of enzyme were known from earlier studies of inhibitors of renin and other aspartic proteinases.[11,12] One structural feature present in most tight-binding aspartic protease inhibitors is a critical hydroxyl group that hydrogen bonds to the catalytically active aspartic acid carboxyl groups in a mechanistically related fashion.[11,12] Structure–activity data indicate that an (*S*)-hydroxyl is preferred,[13] and to date, all crystal structures of chiral inhibitors bound to fungal aspartic proteinases[14-16] and to HIV-PR,[17-20] contain the *S* diastereomer (or its equivalent) in closely related conformations.[21,22]

We have described the synthesis and in vitro activity of a series of HIV protease inhibitors that contain hydroxyethylamines (HEA)[7,23] derived from Phe-Pro. The HEA unit was inserted as a mixture of epimeric alcohols into peptide sequences related to the p17/p24 cleavage site[24] of the *gag-pol* precursor protein to form a series of very potent HIV-PR inhibitors (e.g. **1RS**; JG-365).[7] The X-ray crystal structure[19,20] of JG-365 complexed to synthetic HIV protease revealed that the *S* diastereomer (**1S**) had selectively crystallized from the mixture, as expected if the *S* diastereomer were the more potent of the two diastereomers. However, a second series of HEA inhibitors of HIV-PR independently developed by Roberts et al. is more active against HIV-PR when the alcohol configuration is *R*, as shown in inhibitors **2** and **3**.[8a] These contrasting structural requirements suggested that either the weaker binding diastereomer of **1RS** had crystallized with HIV-PR or the two classes of inhibitors were binding to the protease in significantly different ways. To resolve this issue, we have carried out highly stereoselective syntheses of the HEA diastereomers **4** and **5** and incorporated these into the corresponding HIV protease inhibitors (Table I). The results confirm the preferred configuration of the hydroxyl group reported for each inhibitor series, and provide evidence for new binding interactions to the protease.

Pure (*S*)-HEA diastereomers were prepared by opening the corresponding *R*-epoxides **7a** (or **11**) with tripeptide

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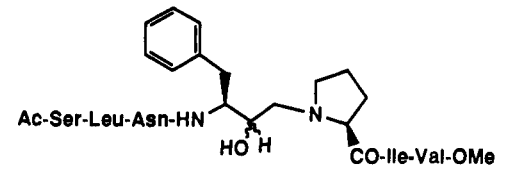
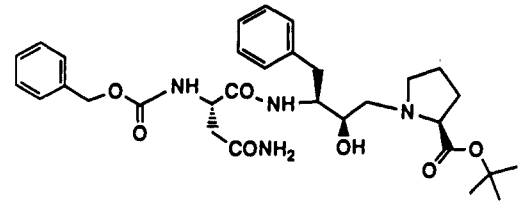


1RS; JG-365



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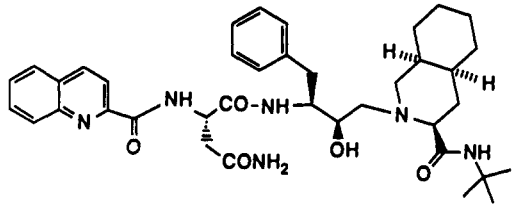

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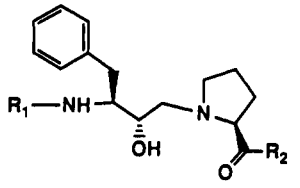

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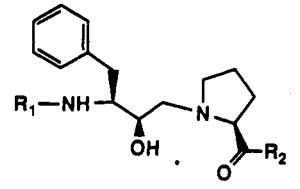

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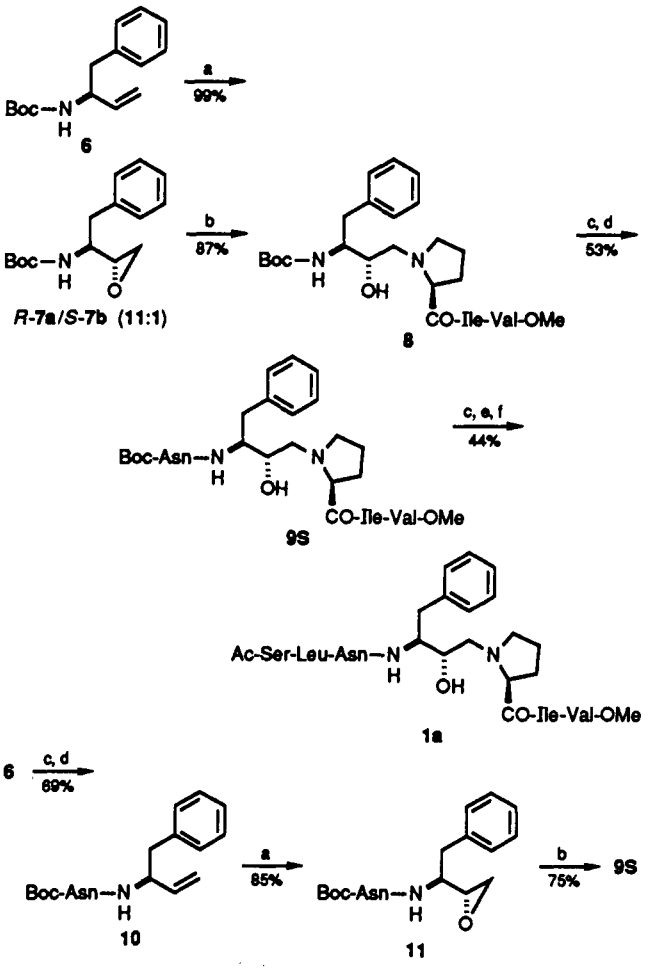
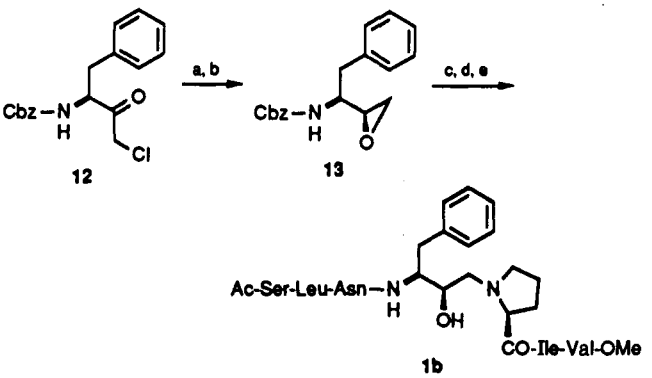
in the presence of triethylamine (Scheme I). The *R*-epoxide **7a** was prepared via stereoselective epoxidation of allylic amine **6** with *m*-chloroperbenzoic acid (mCPBA) according to the procedure described by Luly et al.[25] The obtained mixture of diastereomers **7a,b** was separated by flash chromatography and the stereochemistry assigned by comparison with the reported NMR data.[29] The *R*-epoxide **7a** was allowed to react with H-Pro-Ile-Val-OMe in refluxing methanol to give high yields of the protected HEA derivative **8** and the related derivatives **1S**, **9S**, **14S–17S**. This route avoids the intermediacy of the cor-

Scheme I[a]



[a] Reaction conditions: (a) mCPBA, methylene chloride, 0 °C; separated by silica gel chromatography; (b) HCl·H-Pro-Ile-Val-OMe, Et_3N, MeOH reflux; (c) 4 N HCl in methanol, 0 °C to room temperature, 1 h; (d) Boc-Asn-OH, EDCl, HOBt, NMM; (e) Ac-Ser(OBn)-Leu-OH, EDCl, HOBT, NMM; (f) $Pd(OH)_2$, hydrogen, $AcOH/H_2O$ 9:1, room temperature, 4 h.

Scheme II[a]



[a] Reaction conditions: (a) sodium borohydride, methanol; separation of diastereomers by column chromatography; (b) alcoholic KOH, room temperature, 1 h; (c) HCl·H-Pro-Ile-Val-OMe, Et_3N, MeOH, reflux; (d) $Pd(OH)_2$, methanol, *p*-toluenesulfonic acid, room temperature, 2 h; (e) steps d to f as in Scheme I.

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(27) Briefly, the (*R*)-hydroxyl diastereomer of JG-365 was constructed, and the single bonds were activated by using TWIST in SYBYL (Tripos Assoc.). Multiple conformations of proline were evaluated by replacing proline with *N*-methylalanine. By suitable rotations, it was possible to identify several conformations that satisfied the modeling criteria (vide supra). Each potential conformation was minimized (AMBER) and then minimized within the active site of HIV-protease.[19,20] Related model building and transformations led to the model for the HIV-Pr–3 complex. Details of the modeling studies for all analogues will be reported separately. Vara Prasad, J. V. N.; Pathiasseril, A.; Rich, D. H.; Clare, M. Unpublished results.

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Table I. HEA Inhibitors and Their IC_{50} Values Tested against HIV Protease in Vitro

no.	structure	IC_{50},[a] nM
1RS	Ac-Ser-Leu-Asn-Phe-HEA(*RS*)-Pro-Lle-Val-OMe[b]	9 (K_i = 0.6 nM)[c]
1S	Ac-Ser-Leu-Asn-Phe-HEA(*S*)-Pro-Ile-Val-OMe	3.4 (K_i = 0.24 nM)[c]
1R	Ac-Ser-Leu-Asn-Phe-HEA(*R*)-Pro-Ile-Val-OMe	65 (K_i = 20 nM)[c]
9S	Boc-Asn-Phe-HEA(*S*)-Pro-Ile-Val-OMe	16
9R	Boc-Asn-Phe-HEA(*R*)-Pro-Ile-Val-OMe	850
14S	Cbz-Asn-Phe-HEA(*S*)-Pro-O^{t}Bu	450 [300][d]
14R	Cbz-Asn-Phe-HEA(*R*)-Pro-O^{t}Bu	51 [140][d]
15S	Ac-Ser-Leu-Asn-Phe-HEA(*S*)-Pro-O^{t}Bu	14
15R	Ac-Ser-Leu-Asn-Phe-HEA(*R*)-Pro-O^{t}Bu	14
16S	Cbz-Asn-Phe-HEA(*S*)-Pro-Ile-Phe-OMe	4
17S	Qua-Asn-Phe-HEA(*S*)-Pro-Ile-Phe-OMe[e]	2.3

[a] Average of two independent determinations at six concentrations of inhibitor. [b] Value obtained for the mixture of *RS* diastereomers. [c] K_i independently determined. [d] Values in square brackets are the IC_{50} values reported by the Roche group.[8] [e] Qua, quinoline-2-carboxylic acid. See ref 8 for details.

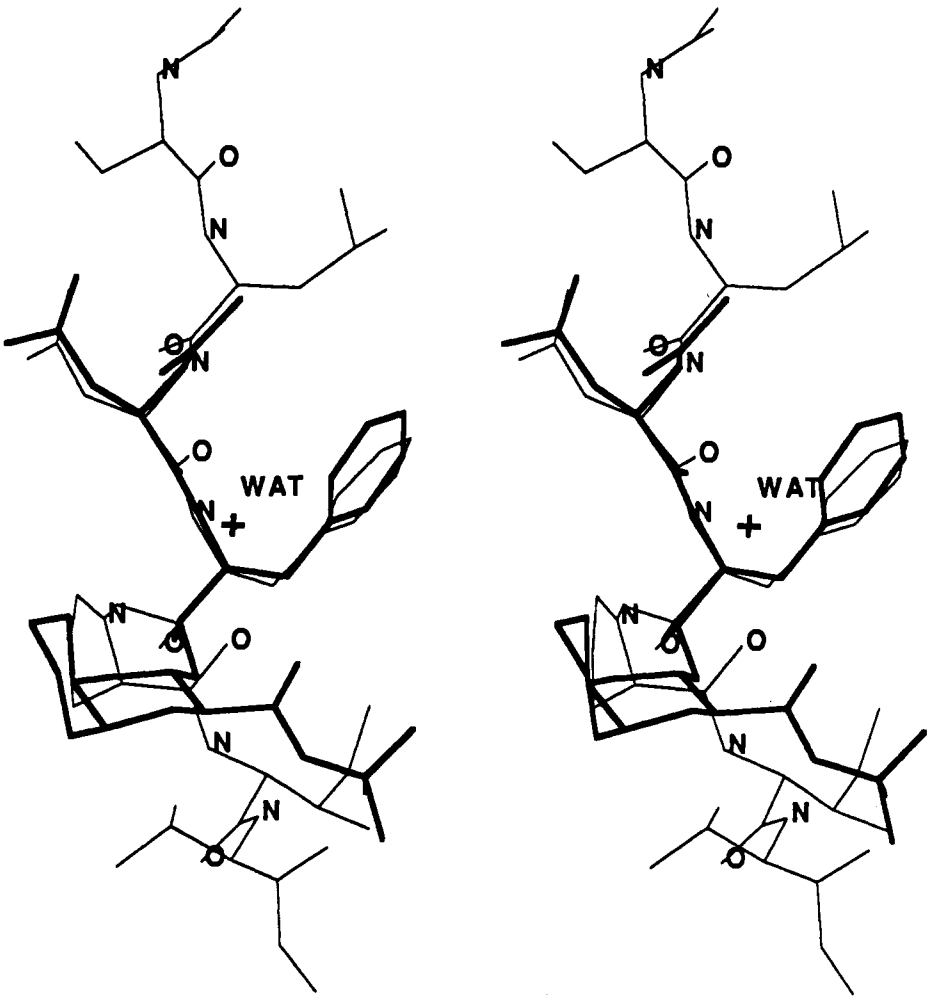

Figure 1. Relaxed stereo representation of the proposed conformation of the decahydroisoquinoline inhibitor[8a] **3** superimposed on the crystal structure of JG-365 bound to HIV-PR.[19,20] Bold lines show backbone atoms of inhibitor **3**; light lines show JG-365 with protons attached. The cross (WAT) indicates the position of the oxygen atom in water-301.[19] For clarity, the N-terminal portion of inhibitor **3** is not shown.

responding ketomethylamine, which is susceptible to epimerization at the carbon α to the ketone.[7] Interestingly, the stereoselectivity of the epoxidation was greatly improved (ds >99%) when the reaction with mCPBA was carried out at room temperature with the protected dipeptide olefin **10** to give the corresponding dipeptidyl epoxide **11**.

The *S*-epoxide **13** used to prepare the *R* diastereomers **1R**, **9R**, **14R**, **15R** (Table I) was prepared via reduction of the chloromethyl ketone **12** with sodium borohydride, followed by treating the diastereomerically pure hydroxy chloride with alcoholic potassium hydroxide (Scheme II) as described by Handa et al.[8b] JG-365 (**1RS**) was synthesized as previously described.[7,28]

IC_{50} values for inhibition of HIV protease were determined for each of the analogues in Table I by using the previously described methods.[7,26] K_i values were determined for compounds **1RS**, **1S**, **1R**. The results establish that the *S* diastereomer **1S** is about 80-fold more potent than the *R* diastereomer **1R**, and thus that the more potent diastereomer in the mixture **1RS** is the one that bound to the protease in the X-ray crystal structure solved by Swain et al.[19,20] Similarly, compound **9S** is more active than **9R**. In contrast, when inhibitors lack the P_3' sub-

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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