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SYNTHESIS AND ACTIVITY OF CONFORMATIONALLY-CONSTRAINED MACROCYCLIC NORSTATINE-BASED INHIBITORS OF HIV PROTEASE

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Abstract: Two diastereomers of a macrocyclic hydroxyamide (norstatine-based) peptide, having an 18-membered ring system, have been synthesized as HIV protease inhibitors. The (*R*)-diastereomer (IC_{50} 19 nM) was ~17-fold weaker than an acyclic analog, but had comparable or better antiviral activity, suggesting improved cell penetration properties and/or resistance to cellular enzymes for the macrocyclic inhibitor.

Introduction. HIV protease, the aspartic proteinase encoded by the Human Immunodeficiency Virus (HIV), has been identified as a promising target for the treatment of Acquired Immune Deficiency Syndrome (AIDS).[1] Accordingly, a great number of inhibitors have been developed for this enzyme, in particular, a variety of peptide-derived transition-state analog inhibitors.[2] Norstatine-based inhibitors of HIV protease, which bear a key hydroxyamide functionality, have been investigated in our[3] and other[4] research groups. Several examples have exhibited low or sub-nanomolar inhibitory activities, and potent antiviral activity.[3,4]

We recently described a series of macrocyclic peptide-based inhibitors of HIV protease which incorporate a *hydroxyethylamine* functionality as the transition-state mimetic group.[5] One potent example (IC_{50} = 1 nM) exhibited HIV antiviral activity (RF/MT-2 cell assay, EC_{50} 4 nM) which was equal to that of the Roche acyclic hydroxyethylamine inhibitor Ro31-8959 (Saquinavir),[6] which has recently been approved for marketing in the U.S. Another example of a macrocyclic HIV protease inhibitor, also linked from the P_1 to P_3 positions (terminology of Schechter and Berger[7]), has been described by Podlogar et al.[8] This derivative incorporates a *difluoroketone* group as the transition-state mimetic, and provides very effective inhibitory activity (K_i = 20 nM). Based on the potent activities achieved with our macrocyclic hydroxyethylamine inhibitors,[5] and the success of the linear norstatine-based inhibitors,[3,4] we have investigated the incorporation of the *hydroxyamide* (norstatine) transition-state analog group into our macrocyclic peptide template structure.

Results and Discussion. In our previously reported synthesis of macrocyclic hydroxyethylamines,[5] we generated the complete linear structure, and then performed the macrocyclization (85% yield) as the final step. In

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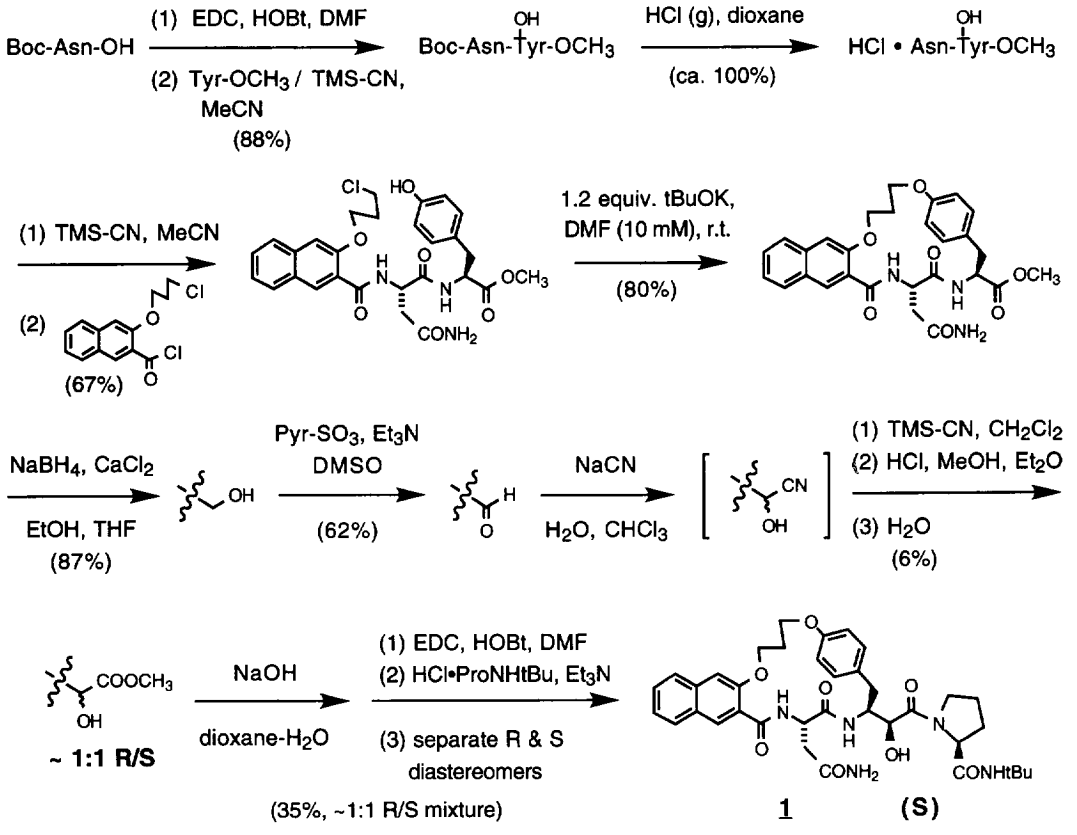
contrast, for the preparation of the target macrocyclic hydroxyamide inhibitor, we decided to first assemble the P_3-P_1 macrocycle, then generate the hydroxyamide functionality, and finally incorporate the P_1' group. After preparation of the requisite P_3 group,[9] the synthesis of the macrocycle template was achieved efficiently in four steps, with an overall yield of 47% (Scheme). Unfortunately, conversion of this macrocyclic methyl ester to the required α-hydroxy ester was problematic,[10] and the route which was successful in providing comparable amounts of both diastereomers proceeded in only low yield. However, pure samples of both the (*S*)-CHOH- (**1**) and (*R*)-CHOH- (**2**) diastereomers of final hydroxyamide product could be obtained, with stereochemical assignments being made by analogy with linear analogs.[11]

Scheme



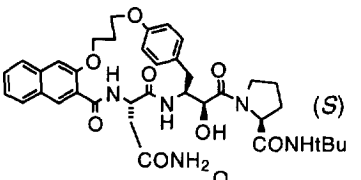
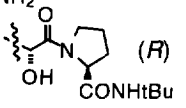
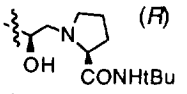
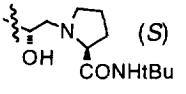
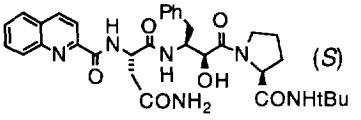
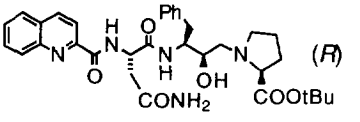
Enzyme inhibition studies were carried out with protease from the BRU (IIIB) strain of HIV-1 virus, as described previously.[3,5] HIV-Antiviral activity was determined in a cell assay which used MT-2 cells infected with virus strain HTLV-1 RF, and measured for the level of p24 core antigen.[5] The inhibitory activities for the two diastereomers of the macrocyclic hydroxyamide (**1**, **2**) are given in the Table, accompanied by data for related macrocyclic hydroxyethylamines, and related acyclic analogs. Structure/activity relationship observations are as

follows:

Effect of chirality at –CHOH–: Similar to the observations for our hydroxyethylamines **3** and **4**,[5] the macrocyclic hydroxyamide diastereomer with preferred chirality at –CHOH– (**1**, designated *S* for the hydroxyamide structure) provides ~200-fold greater HIV protease inhibition than the *R*-diastereomer **2**.

Effect of hydroxyamide vs. hydroxyethylamine functionality: For *acyclic* derivatives having the same peptide frame, and with Proline at P_1', hydroxyamides have been found to be >10-fold more potent as HIV protease inhibitors than the corresponding hydroxyethylamines.[3] This trend is also observed for the *macrocyclic* systems, in that the hydroxyamides **1** and **2** are ~20 times more potent than the hydroxyethylamine analogs **3** and **4**, respectively, which lack only the carbonyl functionality at the transition-state mimetic group.

Table

			HIV Protease IC_{50} (nM)	HIV Cell Assay EC_{50} (nM)
Hydroxyamides	**1**	 (*S*)	19.	76.
	2	 (*R*)	3,800.	—
Hydroxyethyl-amines (ref. 5)	**3**	 (*R*)	400.	—
	4	 (*S*)	90,000.	—
Related Acyclic Analogs (ref. 3 & 6)	**5**	 (*S*)	1.1	145.
	6	 (*R*)	23.	—

Effect of the macrocycle structure on HIV protease inhibition: In the case of our hydroxyethylamines,[5] the conformationally-constrained macrocyclic inhibitors were generally less effective as HIV protease inhibitors than related acyclic analogs. Similarly, the macrocyclic hydroxyamide **1** is observed to be ~17-fold less effective as an HIV protease inhibitor than the related acyclic analog **5**.

Effect of the macrocycle structure on HIV antiviral activity: In our study of a set of 15 hydroxyethylamines,[5] the ratio of inhibitory activities in the cell assay vs. enzyme assay (i.e., EC_{50}/IC_{50}) was generally superior for the macrocyclic inhibitors, as compared to the ratio observed for acyclic analogs. Similarly, *the*

EC_{50}/IC_{50} ratio for **1** *is ca. 30-fold better than that for* **5**. That is, although **1** provides ~17-fold less HIV protease inhibition than **5**, it is comparable to or better than **5** in the HIV cell assay. These observations, in both this and our previous[5] studies, suggest that our macrocyclic inhibitors may have improved cell permeability and/or resistance to cellular enzymes, relative to their acyclic counterparts.

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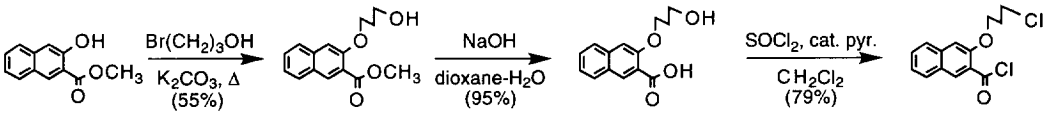
9. The following scheme provided the P_3 acyl chloride:



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11. Diastereomeric hydroxyamides **1** and **2** were separated and purified by preparative silica gel thin layer chromatography. Assignments of configuration for these hydroxyamides are based on NMR characteristics and relative polarities, according to assignments given to acyclic derivatives.[3,10] The final products had similar, appropriate 300 MHz ^{1}H NMR, ^{13}C NMR, and FAB-MS characteristics, and were >96% pure, based on reverse phase HPLC (0.05 M aqueous NH_4OAc/acetonitrile gradient). **1**: more polar diastereomer, TLC R_f = 0.17 (10% MeOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$) includes 4.1 ppm (m, C*H*(OH)CO). **2**: less polar diastereomer, TLC R_f = 0.24 (10% MeOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$) includes 4.2 ppm (m, C*H*(OH)CO). Abbreviations: Bn = benzyl; NMM = *N*-methyl-morpholine; EDC = *N*-ethyl-*N'*-dimethylaminopropyl-carbodiimide hydrochloride; HOBt = 1-hydroxybenzotriazole.

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