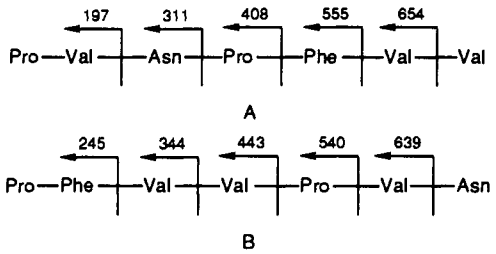
753.4299; amino acid analyses Asp, (or Asn), Phe, Pro, and Val in the ratio 1:1:2:3. The molecular formula for axinastatin 1 (3) was deduced from high-field (400 MHz) ^1H and ^{13}C NMR studies (see Table I of the supplementary material) in conjunction with the high-resolution FAB MS peak matching experiments just noted. Combined ^1H, ^1H COSY, ^1H, ^{13}C COSY, ^1H, ^1H relayed COSY,[9a] HMBC,[9b] and NOESY experiments confirmed the amino acid sequence and cyclic structure 3. The amino acid components and sequence of axinastatin 1 were confirmed as *cyclo*-(Asn-Pro-Phe-Val-Val-Pro-Val) by tandem (MS/MS) mass spectrometry.[10]

Protonation upon FAB results in ring opening of the cyclic peptide at an N-acyl bond to give a linear acylium ion.[10] The major fragmentation processes observed by tandem mass spectrometry involve losses of amino acid residues from the C terminus. Protonation is favored at proline, and with axinastatin 1 there are two possibilities. The FAB MS/MS spectrum of the [M + H] species contains two series (A and B) of ions resulting from protonation at the two proline units. All of the ions in both series



were observed. Additional supporting information for the sequence was obtained by MS/MS experiments on source-produced fragment ions to confirm the interrelationship of the fragment ions and by exact mass measurements on the fragment ions to verify elemental composition and correct assignment.

The absolute configuration of cycloheptapeptide 3 was ascertained by analyzing the acid hydrolysate *N*-pentafluoropropionyl-isopropyl ester[4] derivatives using chiral GC (Chirasil-Val III column). Each amino acid was found to have the L configuration. The disproportionatly high representation of L-Pro and L-Val in axinastatin 1 (3) and other strongly antineoplastic peptides[4] we have discovered in marine animals suggests that the presence of these amino acids may be an important structural requirement for controlling cell growth in peptide mediated systems.

The halichondrins proved to be remarkably potent against all of the 60 cell lines in the U.S. NCI's human tumor cell line in vitro screen,[11] yet with sufficient differences in relative sensitivity among the lines to yield a distinctive mean graph[12] profile. For halichondrin B and homohalichondrin B, the log molar GI_{50}'s for each line ranged from -8.10 to -9.70 and -8.05 to -10.08, respectively. The mean log molar GI_{50}'s were -8.95 and -8.99 for halichondrin B and homohalichondrin B, respectively. The characteristic mean graph "fingerprints" of the halichondrins were very similar; an analysis by the COMPARE pattern-recognition algorithm[13] showed their mean graph profiles to be most highly correlated to those produced by structurally unrelated, tubulin-binding standard agents[11] such as vincristine and taxol.

Discovery of the halichondrins in an *Axinella* sp., a sponge unrelated to their original source,[7] suggests that these exceptionally active[14] Porifera constituents may have a microorganism source. Either by exogenous and/or endogenous biosynthetic processes the marine porifera continue to be an especially fruitful source of potentially useful antineoplastic substances of novel structure.

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Supplementary Material Available: NMR spectra of axinastatin 1 and interpretation of tandem MS-MS spectra (28 pages). Ordering information is given on any current masthead page.

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Novel Binding Mode of Highly Potent HIV-Proteinase Inhibitors Incorporating the (*R*)-Hydroxyethylamine Isostere

The recent communication from Professor Rich and his colleagues[1] describing their model of the binding of a hydroxyethylamine containing inhibitor (Ro 31-8959, compound 6, Table I) to the active site of HIV-1 proteinase

(1) Rich, D. H.; Sun, Q-C.; Prasad, J. V. N. V.; Pathiasseril, A.; Toth, M. V.; Marshall, G. R.; Clare, M.; Mueller, R. A.; Houseman, K. Effect of Hydroxyl Group Configuration in Hydroxyethylamine Dipeptide Isosteres on HIV Protease Inhibition. Evidence for Multiple Binding Modes. *J. Med. Chem.* **1991**, *34*, 1222-1225.

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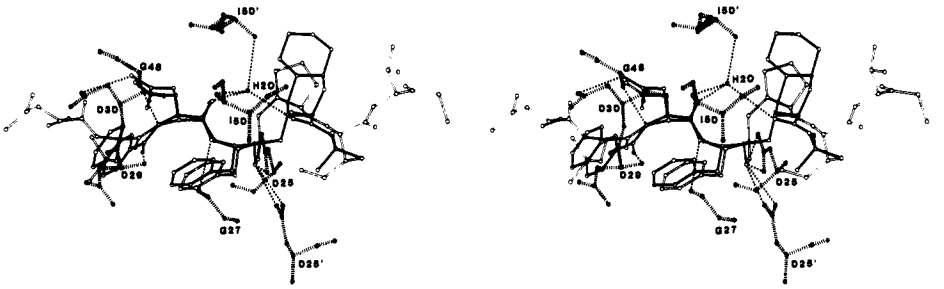

Figure 1. Stereodrawing showing superposition of the bound conformations of compound **6** (full bonds) and compound **8** (open bonds) in the active site of HIV-PR. The amino acid residues interacting with compound **6** are represented as dashed bonds. Putative hydrogen bonds are also represented.

(HIV-PR) has prompted us to report our own findings in this area. The therapeutic potential of inhibitors of HIV-PR has been the subject of much recent interest.[2] Incorporation of an hydroxyethylamine transition state mimetic has led to very potent inhibitors of HIV-PR.[3,4] Our own studies in this area have resulted in compound **6**, which has recently entered phase 2 clinical trials. Interestingly, in this series of inhibitors, the stereochemical requirement at the carbon atom bearing the hydroxyl group appears to be dependent both upon the length of the inhibitor and upon the nature of individual residues. This observation is in agreement with earlier findings in the field of renin inhibitors,[5-8] but is in sharp contrast to statine type inhibitors or to inhibitors based on the hydroxyethylene isostere, where there is a marked preference for the *S* stereochemistry at the hydroxyl group.

Hydroxyethylamine inhibitors of HIV-PR that have a prolyl residue at the S_1' subsite, but lack a P_3' residue, show moderate potency (compounds **1-3**) and a slight preference

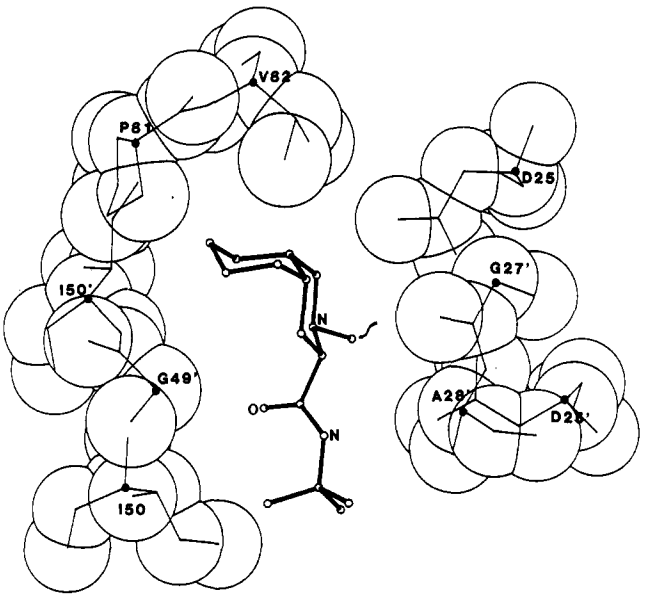

Figure 2. Crystal structure of the HIV-PR–compound **6** complex showing the observed locations of residues forming the S_1' subsite (van der Waals spheres), and with the $-CH_2$–DIQ–NH^{t}Bu group of compound **6** in ball and stick representation.

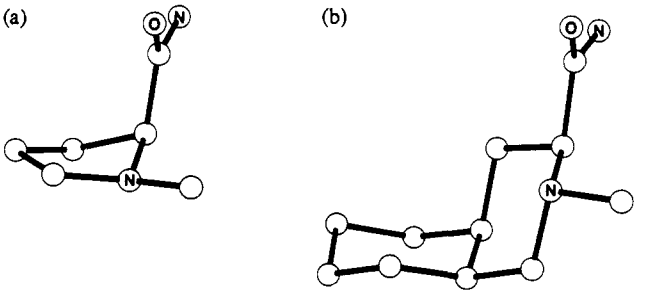

Figure 3. Crystal conformation of the P_1' residues (a) $-CH_2$–Pro–NH– and (b) $-CH_2$–DIQ–NH– showing the opposite *R* and *S* stereochemistries of the basic nitrogen atoms.

for the *R* diastereomer (compounds **2** vs **3**). However, when the prolyl residue is replaced by the [(4a*S*,8a*S*)-decahydroisoquinolin-3(*S*)-yl]carbonyloxy (DIQ) group, potency is markedly increased and a clear preference for the *R* diastereomer emerges (compounds **4** vs **5** and **6** vs **7**). Inhibitors with the prolyl residue at P_1' and extended as far as P_3' also show excellent potency but, in contrast to the shorter inhibitors, exhibit a clear preference for the *S* diastereomer (compounds **8** vs **9** and **10** vs **11**). These results led us to hypothesize that inhibitors containing the DIQ group must bind somewhat differently than inhibitors containing a prolyl residue at the P_1' position. Molecular modeling studies[9] suggested that the mode of binding

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Table I

no.	stereochem at -CH(OH)-	structure[a]	IC_{50} (nM)[b]
1	*R* and *S*	Ac-Ser-Leu-Asn-Phe-Ψ[CH(OH)CH_2N]Pro-Ile-OMe	420[3]
2	*R*	Z-Asn-Phe-Ψ[CH(OH)CH_2N]Pro-O^{t}Bu	140[2]
3	*S*	Z-Asn-Phe-Ψ[CH(OH)CH_2N]Pro-O^{t}Bu	300[2]
4	*R*	Z-Asn-Phe-Ψ[CH(OH)CH_2N]DIQ-NH^{t}Bu	<2.7[e]
5	*S*	Z-Asn-Phe-Ψ[CH(OH)CH_2N]DIQ-NH^{t}Bu	≫100[d]
6	*R* (Ro 31-8959)	QC-Asn-Phe-Ψ[CH(OH)CH_2N]DIQ-NH^{t}Bu	<0.4[e,ref2] (K_i 0.12)
7	*S*	QC-Asn-Phe-Ψ[CH(OH)CH_2N]DIQ-NH^{t}Bu	>100[d]
8	*R* and *S* (JG-365)	Ac-Ser-Leu-Asn-Phe-Ψ[CH(OH)CH_2N]Pro-Ile-Val-OMe	0.6[c]
9	*S*	Ac-Ser-Leu-Asn-Phe-Ψ[CH(OH)CH_2N]Pro-Ile-Val-OMe	0.24[c]
10	*R*	Z-Asn-Phe-Ψ[CH(OH)CH_2N]Pro-Ile-Val-OMe	≫100[d]
11	*S*	Z-Asn-Phe-Ψ[CH(OH)CH_2N]Pro-Ile-Val-OMe	13
12	*R*	Z-Asn-Phe-Ψ[CH(OH)CH_2N]DIQ-Ile-Val-OMe	≫100[d]
13	*S*	Z-Asn-Phe-Ψ[CH(OH)CH_2N]DIQ-Ile-Val-OMe	≫100[d]

[a] Abbreviations: Z, benzyloxycarbonyl; QC, quinolin-2-ylcarbonyl; DIQ, [(4a*S*,8a*S*)-decahydroisoquinolin-3(*S*)-yl]carbonyloxy. [b] Values determined using colorimetric assay procedure.[16] [c] K_i values.[9] [d] Not tested at higher concentration. [e] Values affected by mutual depletion.

adopted by inhibitors containing the DIQ group might preclude extension to the C-terminus. This was later confirmed experimentally by compounds **12** and **13** which are not well accommodated by the enzyme irrespective of the stereochemistry at the hydroxyl group. The model of the binding of compound **6** to HIV-PR which has recently been published[1] is essentially in agreement with our own modeling studies, and we now report that this proposed mode of binding of compound **6** to HIV-PR has been confirmed by an X-ray crystallographic study of the enzyme–inhibitor complex.[10]

The X-ray crystal structure of HIV-PR complexed with compound **6** (resolution 2.3 Å, residual 16%) shows that the inhibitor binds in an extended conformation forming a characteristic set of hydrogen bonds with the enzyme (Figure 1). Except for the tips of the flap regions, the 2-fold symmetry of the enzyme is largely preserved, allowing the S and the S′ subsites to be essentially equivalent. In the X-ray structure of the HIV-PR–compound **6** complex, the (*R*)-hydroxyl group is located between the catalytic aspartic acids as is the (*S*)-hydroxyl group of compound **8** (JG-365).[11] Consequently the adjacent methylene groups in the two inhibitors fit into the active site in a completely different arrangement. The DIQ group occupies almost the entire S_1' subsite, making good hydrophobic contacts with both the flap regions and the body of the enzyme (Figure 2). The *tert*-butyl amide group fits tightly into the S_2' subsite with the methyl groups completely buried. The carbonyl of the DIQ group can still bind to the water molecule connecting the inhibitor with the flap regions, but the adjacent nitrogen atom occupies a location distinct from that observed in compound **8** (displacement ~1.8 Å). Extension of the inhibitor into the S_3' subsite is therefore no longer possible (Figure 1 and Table I). It should be noted that the *S*,*S*,*S* stereochemistry of the DIQ ring is optimal for good inhibitory activity. Also the nitrogen atom of the DIQ in compound **6** and that of the proline ring in compound **8** have opposite configurations (Figure 3). Thus the two inhibitors follow different paths in the active site depending critically on the nature of the P_1' residue and on extension beyond the S_2' subsite. The backbone of P_1 to P_3 residues in compound **6** has the same conformation as that in other HIV-PR inhibitor complexes.[11-15] The Asn and Phe side chains make similar contacts with the enzyme as in compound **8**. The quinaldyl group of compound **6** fits tightly into the S_3 subsite, increasing the potency of this inhibitor.

Hydroxyethylamine inhibitors incorporating the (*R*)-hydroxyl group thus represent a new class of small and highly potent inhibitors of HIV proteinase. The binding of the P_1–P_3 residues is similar to that of previously described inhibitors, but binding at the S_1' and S_2' subsites follows a different pattern and precludes extension of these inhibitors beyond the S_2' subsite.

Acknowledgment. We would like to thank Mrs. Anne Broadhurst for measuring the IC_{50} values of our inhibitors.

Supplementary Material Available: Experimental procedures for the synthesis of hydroxyethylamine derivatives **4**, **5**, **6**, **7**, **10**, **11**, **12**, and **13** are provided (13 pages). Ordering information is given on any current masthead page.

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