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Aspartic Protease Inhibitors Designed from Computer-Generated Templates Bind As Predicted

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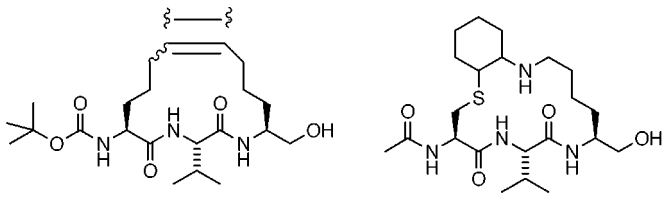
ABSTRACT



7ab **8ab**

Novel tripeptide-derived peptidomimetics 1, 7ab, and 8ab, inspired by templates generated by the structure-generating program GrowMol, were synthesized, shown to inhibit *Rhizopus chinensis* pepsin, and found by X-ray crystallography to bind to the enzyme in the GrowMol-predicted mode. Repetitive evaluation of the computer-generated templates for synthetic feasibility and optimal enzyme interactions led to the designed compounds.

The design of peptidomimetics,[1−3] highly modified peptides or small organic molecules that mimic the structure or action of natural peptides, increases in importance with each peptide sequence found in the human genome. Although peptides have important biological structures and functions, they are transformed into drugs with great difficulty, primarily because metabolic instability and poor penetration into membranes diminish in vivo bioavailability of larger peptides. New structures that mimic the topography of bioactive peptides are sought in the hope that the resulting compounds will prove more pharmaceutically useful.

In this and the two following Letters, we describe successful applications of a structure-generating program[4] to the design of novel peptidomimetics. Beginning with a well-characterized enzyme−ligand complex, novel structural features were designed, synthesized, and shown to inhibit the target enzymes and to bind these enzymes as predicted. Our results illustrate the usefulness of such computer programs in the creation of novel peptidomimetics.

Generation of Cyclic Inhibitor 1. The structure-generating process for inhibitor **1** followed the methods described by Bohacek and McMartin[5] for other enzyme systems; details are provided in the Supporting Information. The known 1.8 Å X-ray crystal structure of pepstatin bound to Rhizopus

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pepsin[6] was used as the starting point. Amino acid residues within 5−6 Å of the bound inhibitor set boundaries for the growth area. The types of atoms permitted in generated structures were set to maximize carbon−heteroatom and carbon−carbon pairings (Table 1, Supporting Information). Growth was started from the P_3 side chain of tripeptide fragment **2** of pepstatin (Figure 1) and limited to 30 heavy atoms.

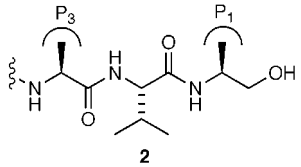

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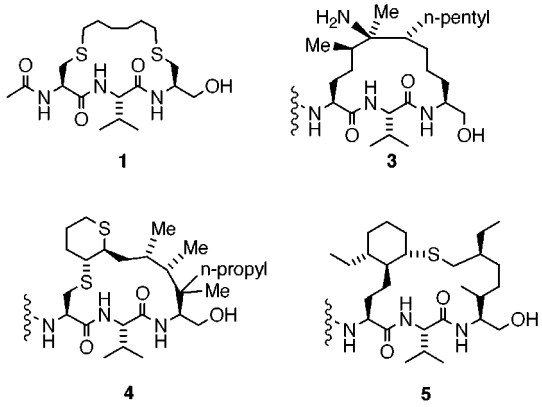
Figure 1. Fragment used for the growth of templates **5**, **7ab**, and **8ab**.

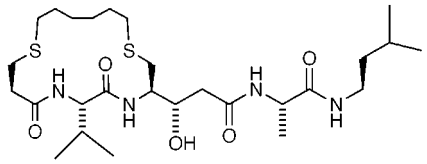
With these constraints, GrowMol generated over 20 000 structures.[7] These compounds were visually inspected and sorted to yield around 1000 cyclic templates; selected examples (structures **1**, **3**−**5**) are shown in Figure 2.



1 **3** **4** **5**

Figure 2. Examples of new templates generated by GrowMol.

Many computer-generated structures were very complex or structurally remote from typical peptide-derived peptidomimetics. Therefore, we decided to use a stepwise approach in which we would test the computer predictions, with each successive synthetic target becoming more complex or less precedented. Cyclic inhibitor **1** illustrates a common motif found in these computer-generated structures. This 17-membered ring system is isosteric with that found in the P_3−P_1 cyclic inhibitor **6**, a nanomolar inhibitor of pepsin (Figure 3).[8] A cocrystal structure of inhibitor **6** bound to *Rhizopus*



6

Figure 3. A low nanomolar inhibitor of *R. chinensis* protease related to macrocycle **1**.

chinensis protease[9] was obtained and solved to 1.8 Å (Figure 4, blue). The conformation of **6** bound to *R. chinensis*

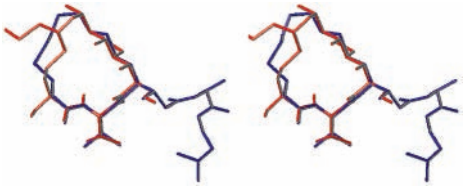
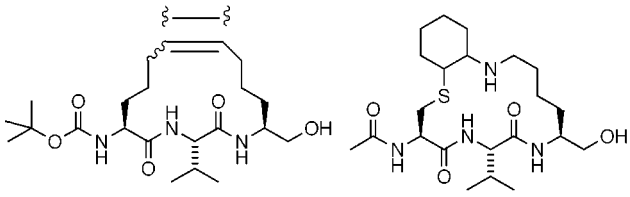


Figure 4. Enzyme-bound conformations of inhibitor **6** (blue) in the active site of *R. chinensis* aspartic protease, compared with a GrowMol minimized structure related to **1** (red).

protease is very similar to that of the GrowMol-generated structures related to **1**, except for small changes induced in the loop conformation by the extra carbon atoms that cause branching,[10] and corroborates a simple computer prediction with respect to both inhibitor structure and enzyme-bound conformation. A smaller carbon ring system was suggested from the family of computer-generated structures related to **3**. Simplification of **3** led to the 15-membered ring system inhibitors **7ab** (Figure 5), which were synthesized and shown to be μM inhibitors.[11]



7ab **8ab**

Figure 5. Novel simplified structures that were synthesized and evaluated for enzymatic inhibition.

Design of Novel Bicyclic Inhibitors. These two successes encouraged us to evaluate a more demanding computer-generated structure. Figure 2 shows representative examples of computer-generated bicyclic motifs that were evaluated

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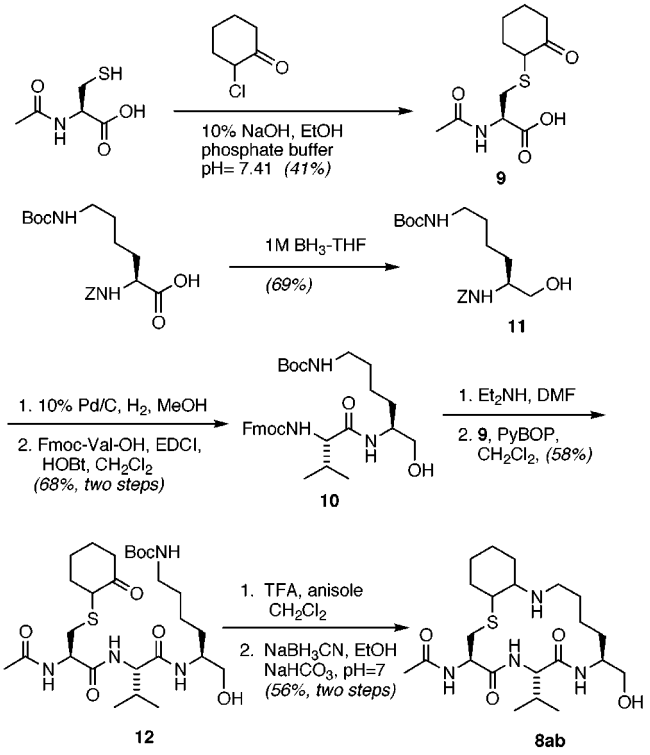
and simplified to create the target inhibitors **8ab** in Figure 5. Compounds **4** and **5** contain 15- and 17-membered rings, respectively. The 16-membered ring of **8ab** reflects a compromise in the two computer-generated templates. In addition, **8ab** also permits use of lysine to place an amine in the P_1 side chain. Electrostatic interactions with the ϵ-nitrogen of lysine with enzymatic residue Asp79 are known to enhance binding to fungal aspartic proteases.[12] To better understand the possible bound conformation of this newly designed compound, we minimized it inside the Rhizopus active site using the same minimization program (QXP[13]) that GrowMol uses in its original calculations. In this way we found the conformation that GrowMol would predict if it had grown this molecule itself.

The synthesis of inhibitors **8ab** is shown in Scheme 1.

Scheme 1



The unusual amino acid *N*-acetyl-3-[2-(oxocyclohexyl)thio]-alanine **9** was synthesized in a moderate yield of 41% following the method of Field.[14] The synthesis of dipeptide **10** began with a borane reduction of Cbz-Lys(Boc)-OH to give alcohol **11**. Deprotection of the Cbz group with Pd/C in MeOH was followed by coupling to Fmoc-protected valine to give the desired dipeptide in 68% overall yield. The final coupling between **9** and **10** proceeded best using PyBOP in CH_2Cl_2, giving a 58% yield of tripeptide **12**. Deprotection of the Boc group followed by reductive amination produced the final products **8ab** as a mixture of diastereomers at the cyclohexyl ring in 56% overall yield. The diastereomers were separated by HPLC and assayed separately. Inhibition constants for competitive inhibition were determined using a fluorometric assay.[7] Inhibitors showed micromolar to high nanomolar inhibition of *R. chinensis* pepsin (**8a**, 7.44 μM; **8b**, 517 nM) which is impressive since these inhibitors span only the P-sites of the active site cleft. Most aspartic protease inhibitors must span both the P- and P′-sites to achieve strong inhibition.[15]

Inhibitor **8a** (K_i = 517 nM) also was cocrystallized with Rhizopus pepsin and shown to bind in a conformation closely related to that predicted by GrowMol (Figure 6). The

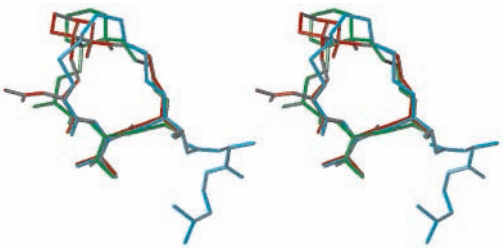


Figure 6. Enzyme-bound conformations of inhibitor and **8b** (red) in the active site of *R. chinensis* aspartic protease, compared with the original GrowMol minimized structure of **8b** (green). Inhibitor **6** (blue) is included for comparison.

predicted conformation (green) is compared with the enzyme-bound conformation of **8a** (red). There are small differences between these two, but it should be noted that the introduction of the lysine nitrogen alters both the electrostatics and geometry of the flap region of the enzyme so that exact overlay of connecting scaffolds is not expected.

The basic strategy described here is to generate a large number of potential inhibitor structures and then use structural novelty and synthetic feasibility to select targets. Careful examination of each target in the active site of the enzyme allowed us to identify groups that were unlikely to contribute to binding and so to delete them to simplify the synthesis. Even then, vast numbers of potential targets were possible and so before engaging in the synthesis of the more complex and synthetically demanding targets, we tested the program for feasibility in this enzyme system. GrowMol's templates with appropriate user modification have led to low molecular weight rigidified inhibitors of Rhizopus pepsin. The cyclohexyl group was an effective linker that would not have been an obvious choice based on the narrow, tailored

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binding site of Rhizopus pepsin. This helps to validate such programs as GrowMol as useful tools for chemists to generate novel templates that may lead to completely new classes of inhibitors.

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Supporting Information Available: The structure-generating process for inhibitor **1**, Table 1, and full experimental details and characterization data for compounds **8ab**−**12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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