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SYNTHESIS OF NOVEL CYCLIC PROTEASE INHIBITORS USING GRUBBS OLEFIN METATHESIS

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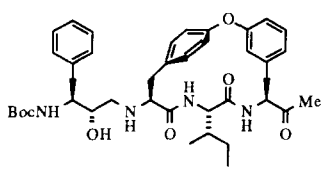
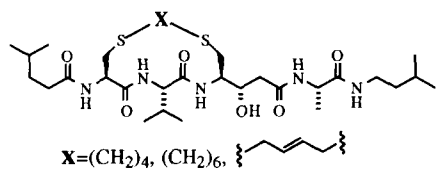
Abstract: The unusual amino acid bishomoallylglycine was synthesized and used to form cyclic P_3-P_1 tripeptide inhibitors via a Grubbs olefin metathesis method. These compounds show micro- to nanomolar inhibition of Rhizopus *chinensis* pepsin and represent a new class of simplified aspartic protease inhibitors lacking P' residues.

Conformational restriction has been applied to many molecules[1] including peptide hormones[2] and enzyme inhibitors[3] to characterize the biologically active conformation of these molecules and to obtain pharmacologically useful analogs.[4] One motif shown to stabilize enzyme-bound, extended β-structures is cyclization between the P_3 and P_1 sidechains.[5] Natural products, such as K13 and OF4949,[6] contain this feature and synthetic variants have been used to prepare inhibitors of aspartic proteases (e.g. **1**,[7] **2**,[8] **3**,[9] and **4**[10]).

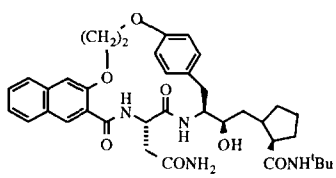
Scheme 1



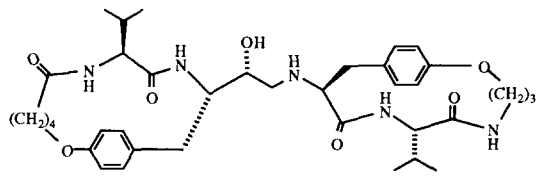
1



2



3



4

In the course of applying the de novo design computer program, GrowMol,[11] to generate novel templates for elaboration into novel inhibitors of aspartic proteases,[12] we required an efficient synthesis of cyclic peptides exemplified by structure **5** (Figure 1). We report here that **6** and **7** can be synthesized efficiently by use of the Grubbs's olefin metathesis to produce small, effective inhibitors of aspartic proteases.

The novel P_3-P_1 substituents were designed by use of the structure-generating program GrowMol. We used the high-resolution crystal structure of pepstatin bound to Rhizopus *chinensis* pepsin[13] for the structure-growing process. Pepstatin was simplified to the tripeptide shown in Figure 2 and growth was initiated at the beta carbon of the P_3 residue:

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Figure 1. *GrowMol generated structure 5 and the structurally simplified inhibitors 6 and 7.*

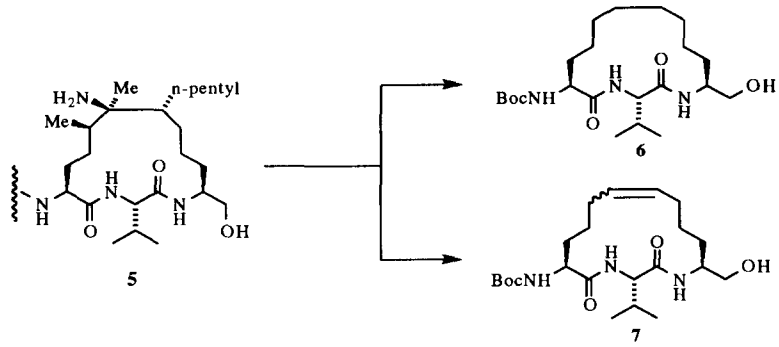
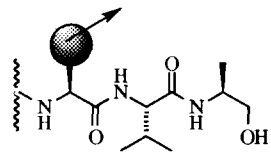


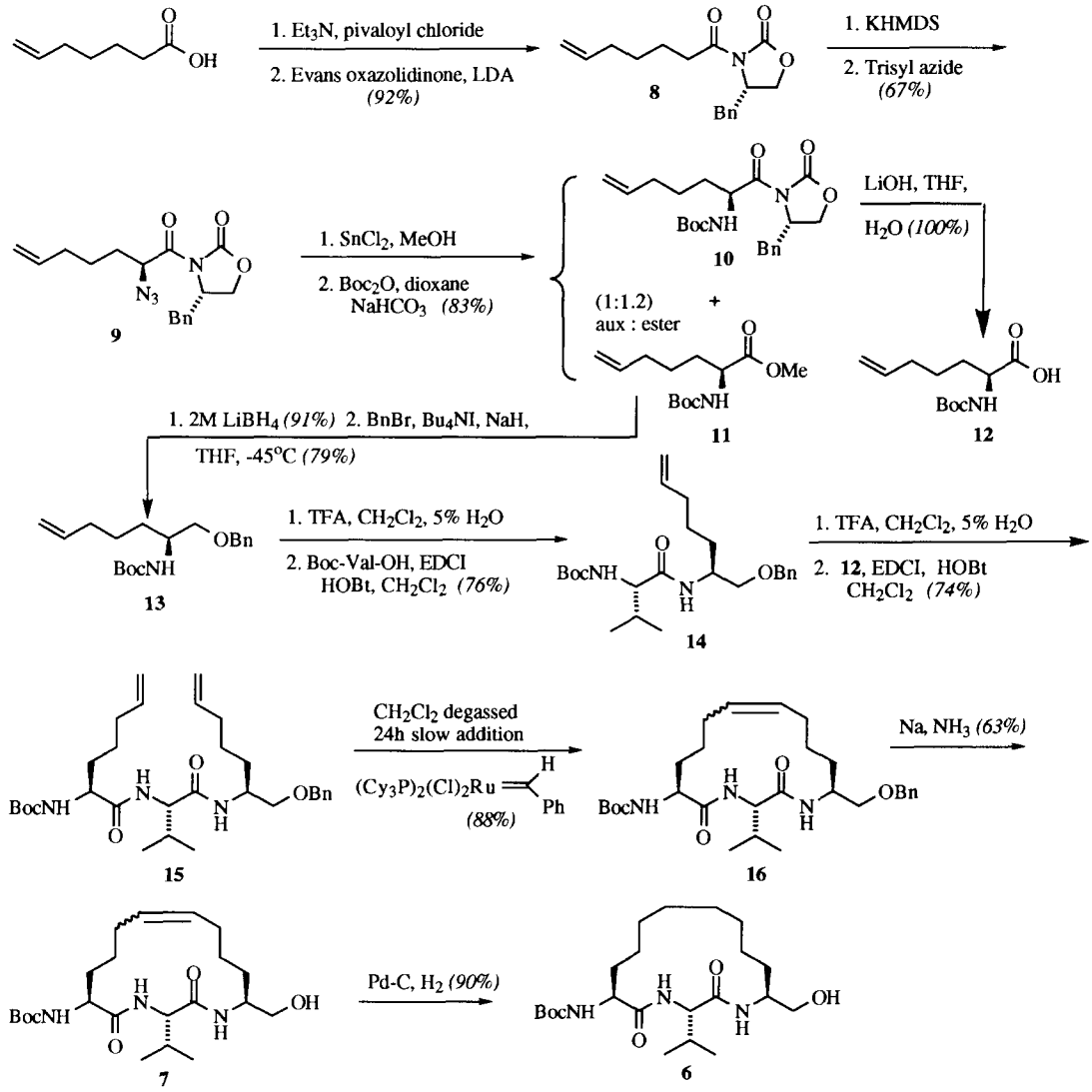
Figure 2. *The tripeptide fragment showing the growth point for GrowMol with an arrow indicating the desired direction of growth to give cyclic P_3-P_1 compounds.*



Several thousand structures were generated and visually inspected. One template, **5**, was simplified to give compounds **6** and **7** which were then synthesized. Compounds **6** and **7** represent two core variations of the template GrowMol generated. These structures are carbon analogs of the aspartic protease inhibitors developed by Szewczuk and Rich[8] (**2**; Scheme 1).

The synthesis (Scheme 2) began by using Evans'[14] methodology to give the unusual amino acid bishomoallylglycine. Acylation of Evans oxazolidinone with 6-heptenoic acid gave adduct **8**. Treatment of the potassium enolate of **8** gave the corresponding azide **9**. Reduction of the azide with $SnCl_2$ followed by protection with Boc anhydride gave a 1:1 ratio of **10** and ester **11**; the latter was formed by methanolysis of the acyl oxazolidinone in the presence of stannate ion. Saponification of **10** gave the desired Boc protected bishomoallylglycine **12**[15] in 100% yield. Ester **11** was reduced to the alcohol, which was protected with benzyl bromide to give compound **13** in 75% yield over two steps. Amino ether **13** was then treated with TFA/CH_2Cl_2/H_2O to remove the Boc group. It was found that addition of 5% H_2O to the normal deprotection conditions (TFA, CH_2Cl_2) prevented double bond isomerization that occurred under the anhydrous acidic conditions. Subsequent coupling to Boc protected valine gave dipeptide **14** in 93% overall yield. Deprotection of **14** and coupling to acid **12** afforded tripeptide **15**. Grubbs Ru alkylidene catalyst[16] was then used to effect the cyclization of the P_3-P_1 alkenes. Deprotection of the benzyl group of **16**[17] under dissolving metal conditions gave target **7** in 63% yield. Target **6** was then reached by simple palladium catalyzed hydrogenation of **7**.[18] Compounds **6** and **7** were assayed for inhibition of Rhizopus *chinensis* pepsin using a fluorescence assay.[19] The K_is of inhibitors **6** and **7** were 1.31 μM and 336 nM, respectively. These inhibitors also have the unique feature that they contain no P' residues; most tight binding aspartic protease inhibitors contain residues that span both the

Scheme 2



P and P' sites (**1**, **2**, **3**, **4**; Scheme 1). Inhibitors **6** and **7** show that cyclization between P_3 and P_1 reduce the need for the P' site residues. Further development of unique linkers through de novo design programs such as GrowMol may lead to other unusual constrained peptides with good inhibitory properties.

Pioneering work by Grubbs has shown that olefin metathesis can produce mimics of beta turns[20] and non-conformationally constrained dipeptide macrocycles through allyl protected tyrosines.[21] This is the first example of a flexible, non-conformationally constrained tripeptide that had been cyclized using Grubbs methodology. The methods reported here expand the work on olefin metathesis with peptides and will be useful for the synthesis of other cyclized tripeptides.

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