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Synthesis of Cyclohexanone-Based Cathepsin B Inhibitors that Interact with Both the S and S' Binding Sites

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Abstract: Solution and solid phase methods are described for the synthesis of inhibitors of the cysteine protease cathepsin B. These inhibitors are based on a cyclohexanone pharmacophore and are designed to interact with both the S and S' subsites of the enzyme active site.

The cysteine proteases cathepsin B, cathepsin K, and the ICE-like proteases are involved in disease processes that include metastasis of cancer,[2] bone resorption in osteoporosis,[3] and the control of programmed cell death.[4] These proteases are important targets for the development of inhibitors, both as therapeutic agents and as tools that can help to clarify the biological function of the enzymes.[5] We recently reported a new class transition-state analog inhibitors for cysteine proteases that are based upon a 4-heterocyclohexanone pharmacophore.[6] These inhibitors react with the enzyme active site nucleophile to give a reversibly formed hemithioketal adduct.[7] The 4-heterocyclohexanone nucleus was derivatized on one side of the reactive ketone so that the inhibitors made contacts with only the S subsites of the enzyme active site. However, inhibitors that extend interactions into both the S and S' subsites may have increased potency and specificity when compared to their singly-sided counterparts.[8] In this paper we describe solution and solid phase methods for synthesizing inhibitors of cathepsin B that are designed to interact with both the S and S' subsites. Development of a solid phase protocol for synthesis makes possible the construction of a combinatorial library of protease inhibitors based upon the cyclohexanone pharmacophore.

Compound **16** (Scheme 2) was designed as an inhibitor for cathepsin B using a combination of molecular modeling studies[9] and data from an X-ray crystal structure of the enzyme with an epoxysuccinyl inhibitor irreversibly bound to the active site nucleophile.[10] The ornithine side chain at the P2 position of **16** is designed to form a salt bridge with Glu 245 at the base of the S2 binding pocket of the enzyme. Proline is meant to fit into the shallow S2' binding site, with the free C-terminal carboxylate of the inhibitor forming hydrogen bonds with His 110 and His 111 of the protease. The structure of inhibitor **16** is intended to mimic the backbone of a natural peptide substrate. However, modeling studies suggested that this compound may be slightly too short to interact optimally with the two His residues. Therefore we have also synthesized compound **17**, which is one methylene unit longer than **16**, in order to account for this possibility.

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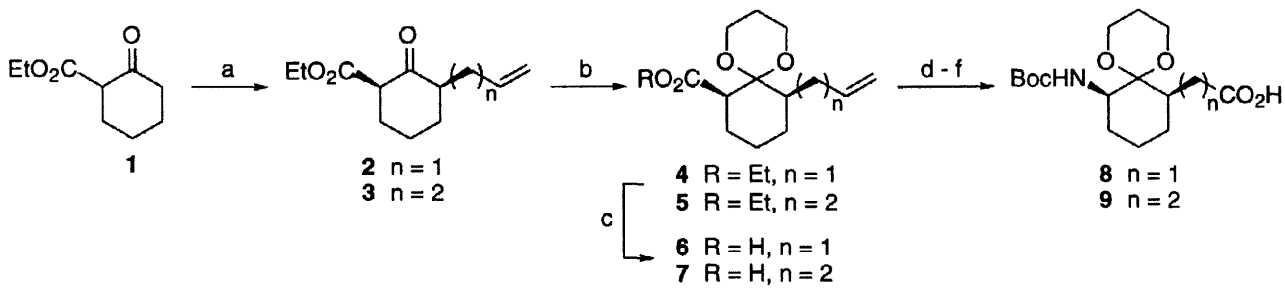
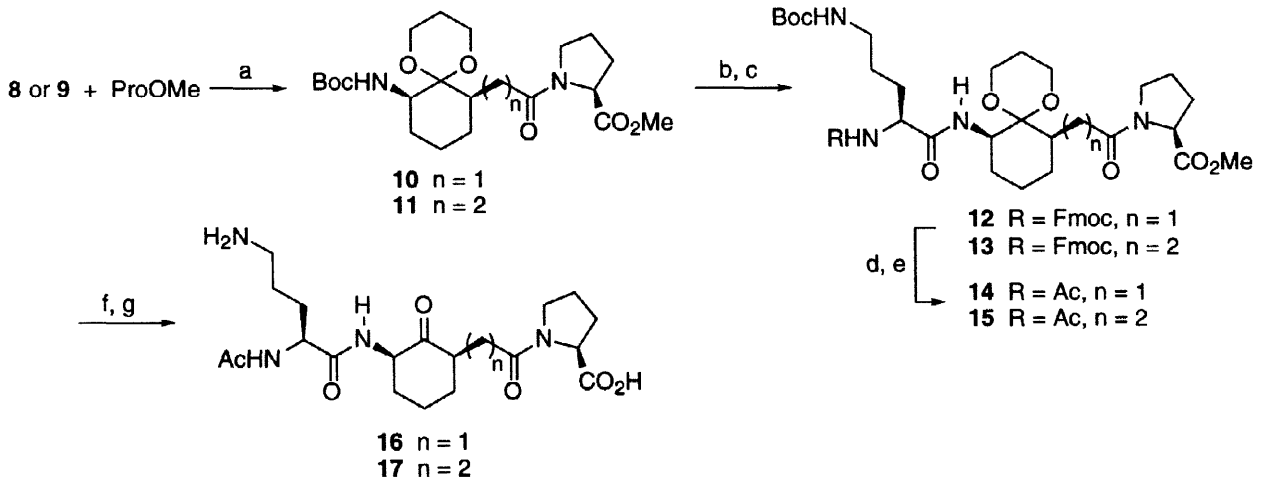
Synthesis of the cyclohexanone nucleus (Scheme 1) began with double deprotonation of ketoester **1**, followed by alkylation of the more reactive enolate with the appropriate bromoalkene to give compounds **2** and **3**.[11,12] Protection of the ketone with 1,3-propanediol and TMSCl,[13] followed by saponification of the ester gave carboxylic acids **6** and **7**. Reaction of the acids with diphenylphosphoryl azide in refluxing benzene induced the Curtius rearrangement.[14] The isocyanate product of these reactions was trapped with potassium *tert*-butoxide to yield the corresponding Boc protected amines. Finally, oxidative cleavage of the alkenes gave protected amino acids **8** and **9**. Analysis of the conformation of compound **7** by NMR studies using COSY and 1D-NOE experiments indicated that the carboxylic acid and butene substituents on the cyclohexanone ring were present in the thermodynamically favored *cis*-1,3 diequitorial orientation.



Reagents and Conditions: a) LDA (2 equiv.), 3-bromo-1-propene or 4-bromo-1-butene (1 equiv.), **2**: 64%, **3**: 60%; b) 1,3-propanediol, TMSCl, **4**: 70%, **5**: 62%; c) NaOH, MeOH, **6**: 58%, **7**: 80%; d) $(C_6H_5O)_2PON_3$, benzene, reflux; e) t-BuOK, THF; f) $KMnO_4$, $NaIO_4$, **8**: 70%, **9**: 59% (3 steps). One of two enantiomers is shown.

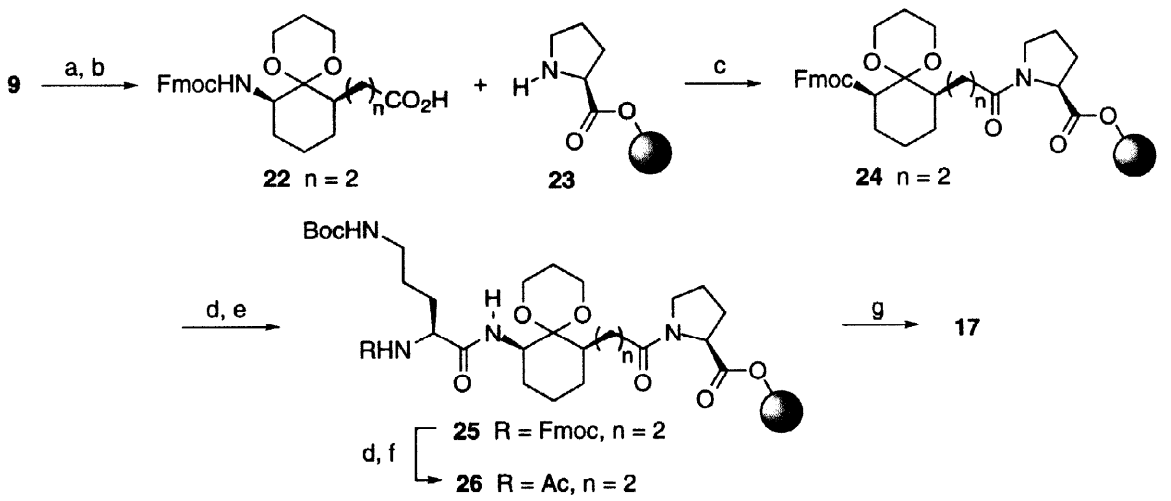
Scheme 1

The cyclohexanone nucleus was next coupled to proline methyl ester to give compounds **10** and **11** as mixtures of two diastereomers (Scheme 2). Removal of the Boc group followed by coupling to N-α-Fmoc-N-δ-Boc-Orn gave compounds **12** and **13**. The N-terminus was subsequently deprotected and capped with acetic anhydride to yield **14** and **15**. Finally the methyl ester was saponified, and the ketal and Boc protecting groups were removed by treatment with TFA in the presence of a small amount of water to yield inhibitors **16** and **17**.



Reagents and Conditions: a) EDC, HOBt, **10**: 84%, **11**: 92%; b) TFA; c) N-α-Fmoc-N-δ-Boc-Orn, EDC, HOBt, **12**: 56%, **13**: 62% (2 steps); d) tris(2-aminoethyl)amine; e) Ac_2O, **14**: 51%, **15**: 71% (2 steps); f) LiOH; g) TFA, H_2O, **16**: 97%, **17**: 82% (2 steps). One of two diastereomers is shown.

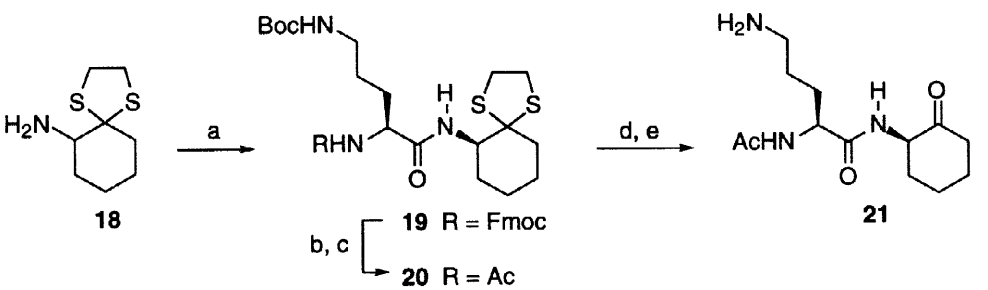
Scheme 2



Reagents and Conditions: a) TFA; b) N-(9-fluorenylmethoxycarbonyloxy)succinimide; c) HBTU, DIEA; d) piperidine; e) N-α-Fmoc-N-δ-Boc-Orn, HBTU, DIEA; f) Ac_2O; g) TFA, H_2O. One of two diastereomers is shown.

Scheme 3

We have also developed a solid phase protocol for synthesizing these cyclohexanone-based protease inhibitors. The protocol, which is outlined in Scheme 3, is analogous to the Fmoc strategy for synthesizing peptides on a solid support. This synthesis required a derivative of the cyclohexanone pharmacophore that had a free C-terminal carboxylate, an Fmoc group on the N-terminus, and a protecting group on the ketone that could be removed under mild conditions. Compound **22** fulfilled these requirements. Solid phase synthesis of inhibitor **17** was performed on Wang resin that was preloaded with Fmoc-Pro. Standard coupling and Fmoc deprotection procedures were employed.[15] The N-terminus was capped with acetic anhydride, and TFA was used to cleave compound **26** from the solid support and to remove the Boc group. The ketal protecting group was removed by adding H_2O (30% v/v) to the cleavage cocktail and stirring the solution overnight at room temperature. The crude material was isolated by lyophilization and purified by reverse phase HPLC to yield inhibitor **17** that was identical to material obtained from the solution phase synthesis.



Reagents and Conditions: a) N-α-Fmoc-N-δ-Boc-Orn, EDC, HOBt, 80%; b) tris(2-aminoethyl)amine; c) Ac_2O, 99% (2 steps); d) N-bromosuccinimide, H_2O; e) TFA, 80% (2 steps). One of two diastereomers is shown.

Scheme 4

In order to determine how much the Pro residue in **16** and **17** contributes to the potency of the inhibitors, we have synthesized control compound **21** which lacks any binding interactions with the S' subsites of the enzyme. The synthesis of **21** (Scheme 4) began with amine **18**,[16] and was similar to the synthesis of the N-

terminal portion of inhibitors **16** and **17**. The only difference was that the ketone was carried through the synthesis as a thioketal, which was deprotected at the end of the sequence using N-bromosuccinimide and H_2O.[17]

The inhibitors were assayed against cathepsin B using the methylcoumarylamide substrate Z-Arg-Arg-NMec.[18] The hydrolysis reactions were monitored by fluorescence spectroscopy using excitation and emission wavelengths of 350 and 440 nm respectively. Control compound **21** is a poor inhibitor of cathepsin B with an inhibition constant of 24 mM. Compounds **16** and **17** have K_i values of 6.6 and 6.1 mM, respectively.[19] These results demonstrate that the potency of cyclohexanone-based inhibitors can be improved significantly by building in functionality that interact with the S' binding sites. Although our design efforts have not yet yielded inhibitors with high potency against cathepsin B, this work has set the stage for the solid phase synthesis of a combinatorial library of inhibitors that are constructed around the 4-heterocyclohexanone pharmacophore.

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