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E-1,2-Dichlorovinyl ethers as irreversible protease inhibitors

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Abstract—The synthesis of a novel motif for threonine protease inhibition is described. The desired *E*-1,2-dichlorovinyl ethers are obtained from alcohols and trichloroethylene as single diastereomers. Aqueous treatment at pH 11 unmasks the hidden α-chloroacetate, which is required for the reaction with the active site of the protease.

Cellular processes depend on the delicate balance of protein synthesis and degradation. Therefore cells feature two major pathways for protein degradation: proteolytic enzymes within the lysosome and the proteolytic core of the ubiquitin proteasome. The disregulation of protein half-life via disturbed destruction is common to many pathological processes. The selective inhibition of the multi-catalytic proteasome subunits is thus an attractive target for drug development in oncology and Alzheimer's disease.[1] For these therapeutic areas we investigate novel inhibitors of threonine proteases featuring reactive moieties, which bias their inhibition toward threonine over serine proteases. Several inhibitors of threonine proteases are known, both selective and unselective (Fig. 1).[2] Currently the most prominent threonine protease inhibitor: bortezomib **2**, is approved for the treatment of multiple myeloma by the FDA.[3]

We take the unspecific serine protease inhibitor **1** and its mode of action as our lead and intend to improve the selective inhibition of chymotrypsin-like activity of the 20S proteasome by reducing the inherent overkill. This over-activation derives from the dichlorovinyl ester, which reacts readily with all sorts of nucleophiles such as cysteine, serine and eventually threonine. The removal of the acyl function will significantly reduce the unspecific hydrolysis by ubiquitous nucleophiles and results in a reasonably stable dichlorovinyl ether. This ether tolerates acidic environment, but hydrolyses readily at pH 11 to be converted into an α-chloroacetate, which in turn reacts with nucleophiles. This dual reactivity, which is delivered in a cascade reaction, fulfils the specific requirements of an N-terminal threonine protease inhibitor. The general hydrolysis of an amide bond by a threonine protease is depicted in Scheme 1. Structural analysis of the proteasome β-subunits revealed that the 2°-hydroxyls of the N-terminal threonines serve as acyl

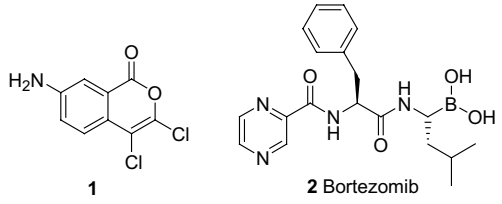
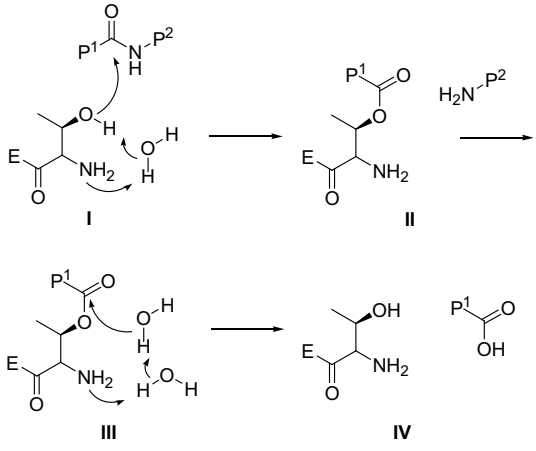


Figure. 1. Serine and threonine protease inhibitors.



Scheme 1. Hydrolysis by threonine proteases.

Keywords: Enol ether; Protease.
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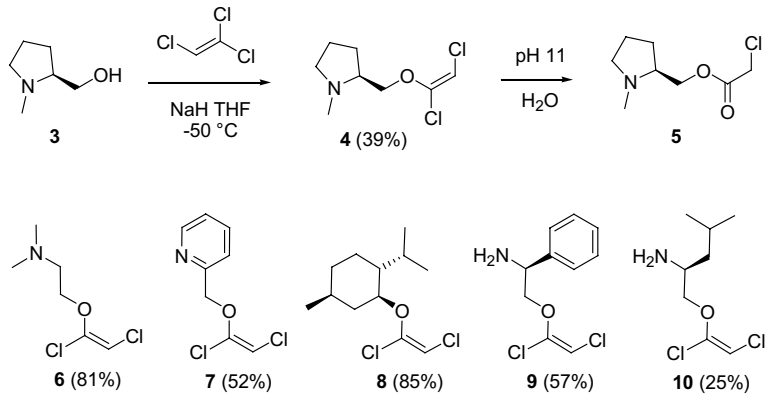
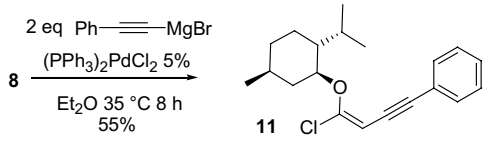


Scheme 2. Synthesis of dichlorovinyl ethers.

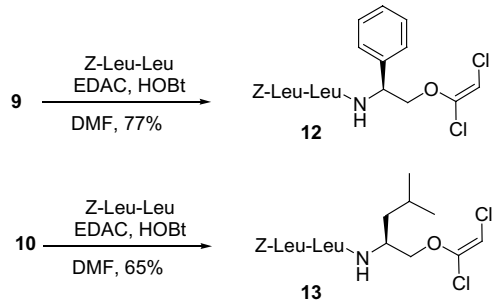
carriers. This difference from cysteine and serine proteases, which utilize less hindered 1° nucleophiles of an internal amino acid, holds potential for selective, mechanism-based inhibitors. The importance of the free amino terminus is apparent in the states **I** and **III**, where the amine directs a deprotonation cascade. These deprotonations result in hydrolysis of the amide and finally regenerate the catalytic site. Inhibitors addressing the active site may interact with the hydroxyl, the free amine or both. We have chosen compact, robust 2°-alcohols and aminoalcohols to explore the accessibility and stability of the required dichlorovinyl ethers. As it turned out, treatment of the alcohols with sodium hydride and trichloroethylene[4] in dry THF at −50 °C and slow warming to room temperature provided the desired ethers in moderate to excellent yields (Scheme 2).[5]

All compounds were obtained as single diastereomers, which were assigned the *E*-stereochemistry, based on the known menthyl ester **8**.[6] Further derivatization was accomplished by a palladium-mediated cross-coupling reaction, which resulted in the selective arylation of the 2-position (**11**, Scheme 3) by a magnesium acetylide species. All other methods and conditions for the cross-coupling (Stephens-Castro, Corey-House, Sonogashira) provided only trace amounts of the desired product. The initial work-up procedure involved extraction with sodium hydrogen carbonate solution and led to significant hydrolysis resulting in an α-chloroacyl ester. This reactivity suggests these derivatives as potential threonine protease inhibitors.

The introduction of amino acid sequences provides protease specificity and is accomplished by standard peptide synthesis in solution (Scheme 4). Condensation of the Z-protected Leucine–Leucine sequence is achieved using EDAC (1-ethyl-3-(3′-dimethylamino-



Scheme 3. Synthesis of an enyne chlorovinyl ether.



Scheme 4. Synthesis of tripeptide mimetics.

propyl)carbodiimide) and HOBt (*N*-hydroxybenzotriazole·H_2O) in DMF.

The resulting peptide mimetics are insensitive to air or moisture at neutral or slightly acid pH. However, exposure to strong nucleophiles or pH >11 leads to rapid hydrolysis. The inhibition of the β-subunits of the proteasome by the compounds **12**, **13** and close analogues thereof is subject to ongoing investigations.

Acknowledgements

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References and notes

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5. A suspension of NaH (48 mg, 2.0 mmol) in dry THF (2 mL) was treated with (*S*)-(−)-1-methyl-2-pyrrolidinemethanol (115 mg, 1.0 mmol) and stirred at −15 °C for 30 min. The mixture was cooled to −50 °C prior to the addition of

trichloroethylene (1.23 mmol, 110 μL) in THF (2 mL). The mixture was warmed to +8 °C within 2 h and quenched with 2 mL sat. NH_4Cl solution and 6 mL Et_2O. The organic phase was washed with 2 mL H_2O, dried (Na_2SO_4) and concentrated. The crude oil was purified by filtration through Alox 90-II using $CHCl_3$/EtOH 100:1 R_f: 0.88 (Alox 60-N-E) to give the ether as a colorless oil (82.3 mg, 39%).

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