

Parallel Solution-Phase Synthesis of Mechanism-Based Cysteine Protease Inhibitors

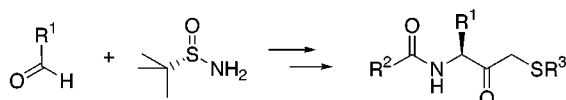
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ABSTRACT



A seven-step parallel solution-phase synthesis has been developed for access to ketone-containing mechanism-based cysteine protease inhibitors. The use of liquid–liquid extractions, volatile or solid-supported reagents, and resin-bound scavengers eliminates the need for intermediate column chromatographic purification during this synthesis sequence.

Cysteine proteases are important pharmaceutical targets because of their role in the pathogenesis of many diseases.¹ Characterized by a conserved cysteine residue in the active site, this class of proteases includes the calpains,² which have been implicated in neurodegenerative disorders, cathepsin K,³ which has been linked to osteoporosis, and the caspase family of proteases,⁴ which are involved in programmed cell death.

Cysteine proteases catalyze the hydrolysis of amide bonds in peptides and proteins through nucleophilic attack of the

active site cysteine residue upon the amide carbonyl (Figure 1a). A common feature of virtually all cysteine protease

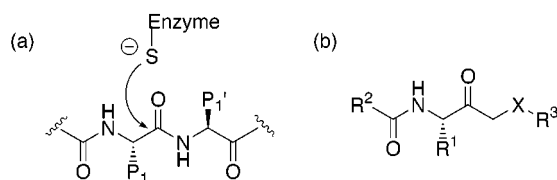


Figure 1. (a) Peptide proteolysis via cysteine protease activity. (b) Ketone-based cysteine protease inhibitors (X = S, O, C, NH, NR, O₂C).

inhibitors is an electrophilic functionality that can react with the nucleophilic cysteine residue.^{1,5} The display of functionality about the electrophilic ketone carbonyl has proven to be a particularly powerful strategy for accessing both reversible and irreversible inhibitors.⁶

We have previously reported a strategy for the solid-phase synthesis of ketone-containing mechanism-based cysteine protease inhibitors (Figure 1b) that allows for display of diverse functionality on both sides of the ketone carbonyl.⁷ In this method, the P₁ side chain⁸ is introduced as part of a

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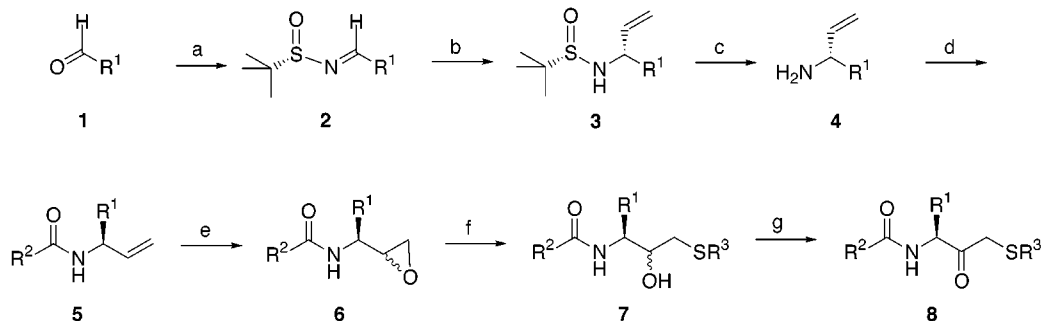
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Scheme 1^a

^a (a) i. (*S*)-*tert*-butanesulfinamide, Ti(OEt)₄, CH₂Cl₂; ii. 2:1 sand/Na₂SO₄·10H₂O; (b) i. vinyl Grignard/Et₂O, CH₂Cl₂; ii. aqueous workup; (c) i. 4 N HCl/dioxane, MeOH; ii. 1 M KOH; iii. sulfonic acid resin, CH₂Cl₂; iv. saturated NH₃/MeOH; (d) acyl transfer resin (preloaded with R²CO₂H), CH₂Cl₂; (e) DMDO, acetone; (f) i. HSR³, P-TBD, THF, reflux, 6–12 h; ii. isocyanate resin, CH₂Cl₂, reflux, 12 h; (g) i. Dess–Martin periodinane, CH₂Cl₂; ii. thiosulfate resin, tertiary amine resin.

chloromethyl ketone scaffold, which is prepared from the corresponding amino acid. For this reason, the P₁ side chains that can readily be introduced are limited by the commercial availability of the appropriate amino acids.

Herein we report an alternative route to access ketone-based inhibitors that enables the introduction of diverse hydrocarbon functionality at the P₁ position using readily available aldehyde inputs (Scheme 1). First, the asymmetric synthesis of allylic amines **4** is accomplished using the *tert*-butanesulfinamide chiral auxiliary.⁹ The allylic amines **4** are then further functionalized to provide the desired ketones **8**. The key feature of the synthesis sequence is the complete reliance on volatile or solid-supported reagents and support-bound scavengers,¹⁰ thereby eliminating the need for intermediate column chromatographic purification during the synthesis sequence.

The synthesis of the allylic amine **4** proceeds through the initial condensation of *tert*-butanesulfinamide and aldehyde **1** in the presence of Ti(OEt)₄ which serves as both a Lewis acid and water scavenger. Titanium adducts are removed by incubation of the reaction mixture with a finely crushed mixture of 2:1 sand/Na₂SO₄·10H₂O in methylene chloride. Simple filtration and solvent removal leads to the sulfinimine product **2**, which is used immediately in the next step.

Excess amounts of vinyl Grignard in diethyl ether are used for 1,2-addition to sulfinimine **2** to provide sulfonamide **3**. Quenching with a solution of aqueous ammonium chloride and brine followed by extraction of the reaction mixture removes the excess Grignard reagent. Sulfonamide **3** is

obtained in diastereomer ratios that range from 88:12 to 94:6 (Table 1). As previously observed for Grignard additions to sulfinyl imines, ether is important for high diastereoselectivities.⁹

Sulfonamide **3** is cleaved under acidic methanol conditions, and the resultant ammonium salt is treated with aqueous potassium hydroxide. The free amine **4** is scavenged onto sulfonic acid resin. Rinsing of the resin removes Grignard reaction side products that are not captured by the resin. Elution with ammonia and methanol provides amine **4** in approximately 50% yield for the three steps as calculated by ¹H NMR analysis using *p*-xylene calibration (Table 1).

Amide **5** is obtained in 80–100% yield by reaction of allylic amine **4** with a carboxylic acid preloaded onto hydroxynitrobenzophenone resin.¹¹ Subsequent epoxidation is carried out with dimethyldioxirane. Excesses of this volatile reagent and the acetone byproduct are removed under reduced pressure to afford high yields of epoxide **6** (Table 1) in excellent purity as determined by ¹H NMR analysis.

Epoxide **6** can be opened with a variety of nucleophiles.¹² For our inhibitor development efforts, we are most interested in the mercaptomethyl ketone inhibitor class¹³ and therefore have focused on thiol nucleophiles. Thiol addition to the epoxide is accomplished using a support-bound guanidine base (P-TBD)¹⁴ in refluxing THF. The excess thiol is removed by subsequent scavenging with a resin-bound isocyanate.¹⁵ Filtration of the resins and solvent removal affords the alcohol **7**. Unfortunately, introduction of impurities occurs during this step as observed by ¹H NMR, and these impurities are carried into the final step. A high-loading tertiary amine base (PS-DIEA, Argonaut) and Amberlyst

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Table 1. Yields of Intermediates (Scheme 1)

entry	R ¹	3 , dr	4 (%)	R ²	5 (%)	6 (%)	R ³	8 (%)
1	CH(CH ₂) ₅	88:12	51	CH ₂ CH ₂ C ₆ H ₅	100	93	CH ₂ CH ₂ CO ₂ CH ₂ CH ₃	30
2				CH ₂ CH ₂ CH ₃	80	100	CH ₂ CH ₂ C ₆ H ₅	60
3				C ₆ H ₅	85	100	CH ₂ CH ₂ C ₆ H ₄ - <i>p</i> - <i>t</i> -Bu	33
4	CH ₂ CH(CH ₂) ₅	88:12	50	CH ₂ CH ₂ C ₆ H ₅	100	100	CH ₂ CH ₂ CO ₂ CH ₂ CH ₃	56
5				C ₆ H ₅	100	100	CH ₂ CH ₂ C ₆ H ₅	33
6	CH ₂ CH ₂ C ₆ H ₅	94:6	50	CH ₂ CH ₂ C ₆ H ₅	100	100	CH ₂ CH ₂ CO ₂ CH ₂ CH ₃	42
7				CH ₂ CH ₂ CH ₃	100	100	CH ₂ CH ₂ C ₆ H ₄ - <i>p</i> - <i>t</i> -Bu	31

^a Yield was based on *p*-xylene calibration in NMR analysis. ^b Yield over two steps (ring opening and oxidation) was calculated on the basis of isolated pure product.

A-21 ion-exchange resin (Aldrich) gave lower yields, while the polymer-bound BEMP (Aldrich) and macroporous tetraalkylammonium carbonate (Argonaut) gave comparable yields and purity to that observed with P-TBD. In addition, the presence of diastereomers complicates the NMR analysis of the alcohol product, preventing accurate yield determinations for this step by *p*-xylene calibration.

Oxidation of alcohol **7** to the desired mercaptomethyl ketone product **8** is accomplished using Dess–Martin periodinane in reagent grade methylene chloride.¹⁶ The oxidant and byproducts are then scavenged using resin-bound thio-sulfate and tertiary amine resin.¹⁷ Column chromatography of the final product **8** furnishes pure ketone in moderate yield (30–60%) over the final two steps (Table 1). Chiral HPLC analyses of the products indicate that no epimerization occurs during the synthesis sequence within the limits of detection.¹⁸

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(18) See entries 1, 2, and 4 in the Supporting Information for chiral HPLC analysis data.

A parallel synthesis strategy has been developed in the solution phase for access to mechanism-based cysteine protease inhibitors incorporating P₁ diversity from readily available aldehydes. The sequence combines the use of liquid–liquid extractions, volatile or solid-supported reagents, and support-bound scavengers to eliminate the need for intermediate column chromatographic purification. Seven mercaptomethyl ketones **8** have been synthesized using this seven-step sequence to incorporate both branched and unbranched nonproteinogenic side chains at the P₁ position, demonstrating the viability of the method.

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Supporting Information Available: Experimental procedures for compounds **4–8** and characterization for compounds **8**, entries 1–7. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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