ORGANIC LETTERS

2001
Vol. 3, No. 23
3707−3709

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

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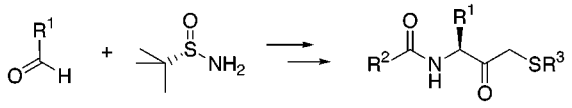
Received August 25, 2001

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 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]

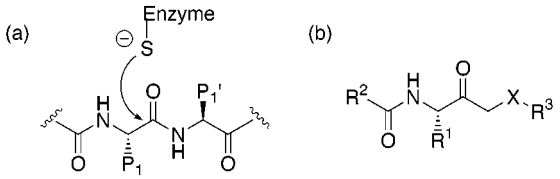


Figure 1. (a) Peptide proteolysis via cysteine protease activity. (b) Ketone-based cysteine protease inhibitors (X = S, O, C, NH, NR, O_2C).

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 P_1 side chain[8] is introduced as part of a

(1) (a) Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305−341. (b) Otto, H.-H.; Schirmeister, T. *Chem. Rev.* **1997**, *97*, 133−171.

(2) Suzuki, K.; Sorimachi, H. *FEBS Lett.* **1998**, *433*, 1−4.

(3) Bossard, M. J.; Tomaszek, T. A.; Thompson, S. K.; Amegadzie, B. Y.; Hanning, C. R.; Jones, C.; Kurdyla, J. T.; McNulty, D. E.; Drake, F. H.; Gowen, M.; Levy, M. A. *J. Biol. Chem.* **1996**, *271*, 12517−12524.

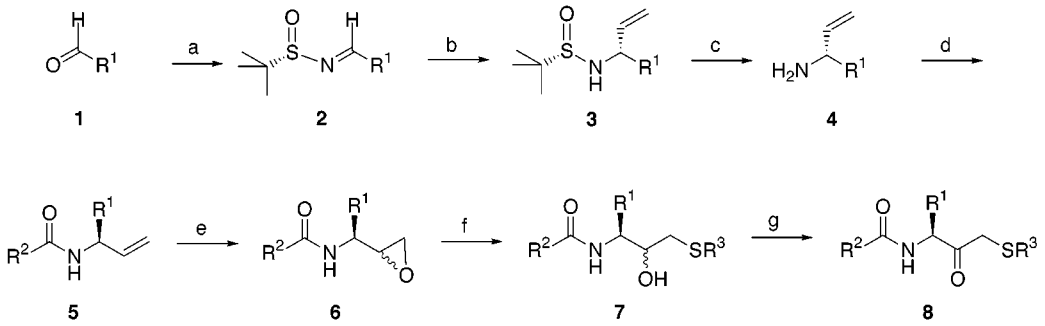
(4) Thornberry, N. A. *Chem. Biol.* **1998**, *5*, R97−R103.

(5) See also the Symposium-in Print on cysteine protease inhibitors featured in *Bioorg. Med. Chem.* **1999**, *7*, 571−644.

(6) (a) Marquis, R. W.; Ru, Y.; LoCastro, S. M.; Zeng, J.; Yamashita, D. S.; Oh, H.-J.; Erhard, K. F.; Davis, L. D.; Tomaszek, T. A.; Tew, D.; Salyers, K.; Proksch, J.; Ward, K.; Smith, B.; Levy, M.; Cummings, M. D.; Haltiwanger, R. C.; Trescher, G.; Wang, B.; Hemling, M. E.; Quinn, C. J.; Cheng, H.-Y.; Lin, F.; Smith, W. W.; Janson, C. A.; Zhao, B.; McQueney, M. S.; D′Alessio, K.; Lee, C.-P.; Marzulli, A.; Dodds, R. A.; Blake, S.; Hwang, S.-M.; James, I. E.; Gress, C. J.; Bradley, B. R.; Lark, M. W.; Gowen, M.; Veber, D. F. *J. Med. Chem.* **2001**, *44*, 1380−1395. (b) Mjalli, A. M.; Chapman, K. T.; Zhao, J. J.; Thornberry, N. A.; Peterson, E. P.; MacCoss, M. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1405−1408. (c) Krantz, A.; Copp, L. J.; Coles, P. J.; Smith, R. A.; Heard, S. B. *Biochemistry* **1991**, *30*, 4678−4687.

10.1021/ol0166496 CCC: $20.00
Published on Web 10/19/2001

Scheme 1[a]



[a] (a) i. (S)-*tert*-butanesulfinamide, $Ti(OEt)_4$, CH_2Cl_2; ii. 2:1 sand/Na_2SO_4·$10H_2O$; (b) i. vinyl Grignard/Et_2O, CH_2Cl_2; ii. aqueous workup; (c) i. 4 N HCl/dioxane, MeOH; ii. 1 M KOH; iii. sulfonic acid resin, CH_2Cl_2; iv. saturated NH_3/MeOH; (d) acyl transfer resin (preloaded with R^2CO_2H), CH_2Cl_2; (e) DMDO, acetone; (f) i. HSR^3, P-TBD, THF, reflux, 6−12 h; ii. isocyanate resin, CH_2Cl_2, reflux, 12 h; (g) i. Dess−Martin periodinane, CH_2Cl_2; ii. thiosulfate resin, tertiary amine resin.

chloromethyl ketone scaffold, which is prepared from the corresponding amino acid. For this reason, the P_1 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 P_1 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/Na_2SO_4·$10H_2O$ 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 sulfinamide **3**. Quenching with a solution of aqueous ammonium chloride and brine followed by extraction of the reaction mixture removes the excess Grignard reagent. Sulfinamide **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]

Sulfinamide **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 ^{1}H 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 ^{1}H 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 class[13] 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 ^{1}H NMR, and these impurities are carried into the final step. A high-loading tertiary amine base (PS-DIEA, Argonaut) and Amberlyst

(7) Lee, A.; Huang, L.; Ellman, J. A. *J. Am. Chem. Soc.* **1999**, *121*, 9907−9914.

(8) Nomenclature for the substrate amino acid preference is P_n, ..., P_2, P_1, P_1', P_2',..., P_m'. Amide bond hydrolysis occurs between P_1 and P_1' (see Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1968**, *27*, 157−162).

(9) (a) Liu, G.; Cogan, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1997**, *119*, 9913−9914. (b) Cogan, D. A.; Liu, G.; Kim, K.; Backes, B. J.; Ellman, J. A. *J. Am. Chem. Soc.* **1998**, *120*, 8011−8019.

(10) For a recent review on solid-supported reagents and scavengers, see: Ley, S. V.; Baxendale, I. R.; Ream, R. N.; Jackson, P. S.; Leach, A. G.; Longbottom, D. A.; Nesi, M.; Scott, J. S.; Storer, R. I.; Taylor, S. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3815−4195.

(11) Cohen, B. J.; Karoly-Hafeli, H.; Patchornik, A. *J. Org. Chem.* **1984**, *49*, 922−924.

(12) Rao, A. S.; Paknikar, S. K.; Kirtane, J. G. *Tetrahedron* **1983**, *39*, 2323−2367.

(13) Huang, L.; Lee, A.; Ellman, J. A. Submitted.

(14) (a) Iijima, K.; Fukuda, W.; Tomoi, M. *J. Macromol. Sci., Pure Appl. Chem.* **1992**, *A29*, 249. (b) Xu, W.; Mohan, R.; Morrissey, M. M. *Tetrahedron Lett.* **1997**, *38*, 7337−7340.

(15) Booth, R. J.; Hodges, J. C. *J. Am. Chem. Soc.* **1997**, *119*, 4882−4886.

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	$CH_2CH_2C_6H_5$	100	93	$CH_2CH_2CO_2CH_2CH_3$	30
2				$CH_2CH_2CH_3$	80	100	$CH_2CH_2C_6H_5$	60
3				C_6H_5	85	100	$CH_2CH_2C_6H_4$-*p*-*t*-Bu	33
4	$CH_2CH(CH_2)_5$	88:12	50	$CH_2CH_2C_6H_5$	100	100	$CH_2CH_2CO_2CH_2CH_3$	56
5				C_6H_5	100	100	$CH_2CH_2C_6H_5$	33
6	$CH_2CH_2C_6H_5$	94:6	50	$CH_2CH_2C_6H_5$	100	100	$CH_2CH_2CO_2CH_2CH_3$	42
7				$CH_2CH_2CH_3$	100	100	$CH_2CH_2C_6H_4$-*p*-*t*-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 thiosulfate 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]

(16) "Wet" methylene chloride is known to accelerate the Dess−Martin oxidation reaction (see: Meyer, S. D.; Schreiber, S. L. *J. Org. Chem.* **1994**, *59*, 7549−7552).

(17) (a) Parlow, J. J.; Case, B. L.; South, M. S. *Tetrahedron* **1999**, 6785−6796. (b) South, M. S.; Dice, T. A.; Parlow, J. J. *Biotechnol. Bioeng.* **2000**, *71*, 51−57.

(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 P_1 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 P_1 position, demonstrating the viability of the method.

Acknowledgment. This work was supported by the NIH (R01GM54051). The Center for New Directions in Organic Synthesis is supported by Bristol-Myers Squibb as Sponsoring Member. A.L. gratefully acknowledges BMS for a graduate fellowship.

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.

OL0166496