ORGANIC LETTERS

2006
Vol. 8, No. 8
1653−1655

Efficient Chemoenzymatic Synthesis of Pelitrexol via Enzymic Differentiation of a Remote Stereocenter

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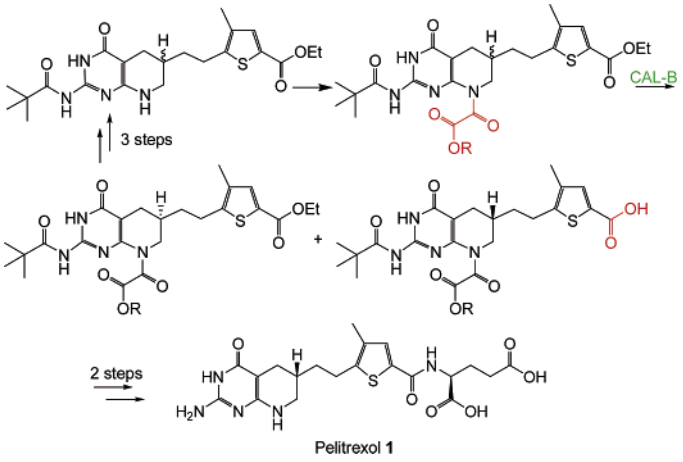
Received February 1, 2006

ABSTRACT



An efficient chemoenzymatic process is described for the synthesis of pelitrexol, a novel GARFT inhibitor. The remoteness of this molecule's stereocenter in the tetrahydropterin moiety from the terminal carbonyl group provided a significant challenge in synthesis. The introduction of an oxalamic ester adjacent to the stereocenter dramatically enhanced an enzyme's enantioselectivity for hydrolysis at the terminal ester, producing the desired *S*-acid with high optical purity and yield. The recycling of the "wrong" enantiomer is achieved via a dehydrogenation/hydrogenation strategy.

A novel investigational inhibitor of glycinamide ribonucleotide formyltransferase (GARFT), namely, pelitrexol,[1] has shown powerful antiangiogenesis activity.[2] This potent but complex molecule has presented a significant challenge in large-scale chiral synthesis for clinical trials. After a great deal of effort and investment, an asymmetric synthesis of pelitrexol was initially developed to support early clinical trials. This initial process involved a lengthy and inefficient 20-step linear synthesis with an overall yield of less than 2%.[2] This route suffered from low efficiency, low throughput, and exorbitant cost.

More recently, a shortened route has been developed originating from the precursor **2**, which increased efficiency through several convergent steps[3] (Scheme 1). However, this racemic route is heavily reliant on a chromatographic separation of two diastereomers of **4** in the penaultimate step, which suffers from several issues. One major drawback involved the wasted wrong diastereomer, which cannot be

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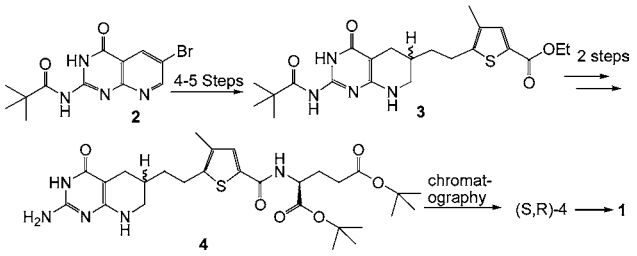
(1) Robert, F.; Garrett, C.; Dinwoodie, W. R.; Sullivan, D. M.; Bishop, M.; Amantea, M.; Zhang, M.; Reich, S. D. *J. Clin. Oncol. (Meeting Abstr.)* **2004**, *22*, 3075.

(2) For synthesis and activity of other GARFT inhibitors, see: (a) Shih, C.; Grindey, G. B.; Taylor, E. C.; Harrington, P. M. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 339. (b) Singh, S. K.; Singer, S. C.; Ferone, R.; Waters, K. A.; Mullin, R. J.; Hynes, J. B. *J. Med. Chem.* **1992**, *35*, 2002. (c) Charles, J.; Wilson, T. M.; Wendel, S. R.; Winningham, M. J.; Deeter, J. B. *J. Org. Chem.* **1994**, *59*, 7038. (d) Varney, M. D.; Romines, W. H. U.S. Patent 5646141, 1997.

(3) Dovalsantos, E.; Flahive, E. J.; Halden, B. J.; Mitchell, M. B.; Reinhard, W.; Notz, L. N.; O'Neill-Slawecki, S. A.; Tian, Q. U.S. Patent App. 0266796, 2004.

10.1021/ol0602755 CCC: $33.50
Published on Web 03/25/2006

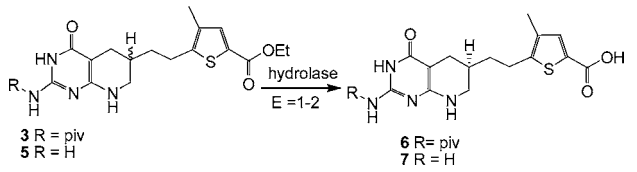
Scheme 1. Convergent Synthesis of Pelitrexol



recycled, resulting in a maximum of 50% yield. Additionally, the low solubility of both diastereomers in most column-compatible organic solvents requires hundreds of liters of organic solvent per kilogram of material in this resolution, which provided poor separation and a recovery of less than 25%, even after extensive optimization. Here, we report the development of a robust, efficient, and environmentally more friendly route to Pelitrexol, centered on the derivation of an early racemic precursor **3** with oxalamic esters to amplify the size difference between substituents for the subsequent enzymatic resolution.

Although a few examples have been reported for enzymatic resolution with a remote stereocenter as far as six or seven bonds away from the reaction center,[4] in practice, enzymatic or classical resolution involving a remote chiral center is still a daunting task. For example, screening the racemic precursors **3** and **5** against a comprehensive hydrolase library[5] showed that several enzymes, including *Candida antartica* Lipase B (CAL-B), hydrolyzed the terminal ester group with decent activity (>85% conversion after 5 h). Unfortunately, all showed relatively low enantioselectivity ($E = 1$ to 2) (Scheme 2). Optimization by adding cosolvents

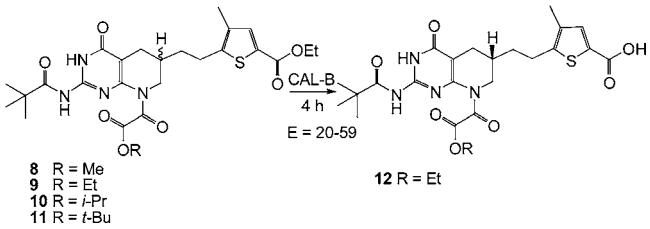
Scheme 2. Enzymatic Hydrolysis of Esters **3** and **5**



and other attempts provided only moderate gains with a final E value of <10, producing the undersired *R*-acid with low yields and moderate optical purity.

Alternatively, although enzymatic acylation of the piperidine nitrogen found only one carbon away from the chiral center might provide a higher enantioselectivity, in reality no enzymes were found with the necessary reactivity. Recently, we reported that a number of racemic secondary amines could be successfully resolved via protease-catalyzed enantioselective hydrolysis of an oxalamic ester derivative, yielding chiral oxalamic acids in high optical purities and good yields.[6] It was thus envisioned that the oxalamate derived esters **8**−**11** would be ideal for enzymatic hydrolysis. Here, the key requirement was that an enzyme must both regio- and enantioselectively hydrolyze the oxalamic ester adjacent to the chiral center instead of the remote terminal carboxylic ester. Surprisingly, screening of four common oxalamic ester derivatives **8**−**11** against the same enzyme library showed that the hydrolysis took place extensively on the terminal carboxylic ester, and no hydrolytic activity was identified on the newly formed oxalamic esters. More interestingly, several enzymes, including CAL-B, showed good to excellent enantioselectivity in the hydrolytic cleavage of the terminal ester group, resulting in the desired *S*-acids in high optical purities and good yields (>95% ee and 38−42% yield) (Scheme 3). In particular, the use of the

Scheme 3. CAL-B Catalyzed Enantioselective Resolution of Oxalamic Esters **8**−**11**



inexpensive lipase CAL-B, available in crude solution, lyophilized, or immobilized forms, showed surprisingly high enantioselectivity, even under the suboptimal screening conditions. Both activity and selectivity varied substantially for the enzyme with small changes in oxalamic ester groups from methyl to *tert*-butyl, despite its distance from the reaction center. Among the oxalamic esters **8**−**11**, the ethyl-ester derivative **9** gave the highest selectivity ($E = 45-59$) and good activity with CAL-B, thus becoming the focus of the subsequent process optimization.

Clearly, introducing an oxalamic ester group on the piperidine nitrogen amplifies the chirality of this stereocenter, resulting in a dramatic enhancement on enzyme differentiation of this six-bond-distant remote chiral center. Although other more common protecting groups such as acyl, Boc, or benzyl may also affect the stereodifferentiation of the molecule, the oxalamic ester appears to be ideal because of the high enantioselectivity it provides as well as its ease of formation and subsequent removal. Moreover, the presence of the oxalamic ester moiety dramatically increased the solubility of the molecule in most organic solvents when compared to the unfunctionalized precursor **3**. This increase in solubility allowed for a much higher substrate concentration and vastly improved overall throughput.

(4) For resolving a remote stereocenter by enzymes, see: (a) Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis*; Wiley-VCH: Weinheim, 1999; pp 114−115 and references therein. (b) Hedenström, E.; Nguyen, B.; Silks, L. A., III. *Tetrahedron: Asymmetry* **2002**, *13*, 835. (c) Riva, S.; Danieli, B. *Tetrahedron* **2004**, *60*, 741.

(5) For screening methodology and discussion, see: Yazbeck, D.; Tao, J.; Martinez, C.; Kline, B.; Hu, S. *Adv. Synth. Catal.* **2003**, *345*, 524.

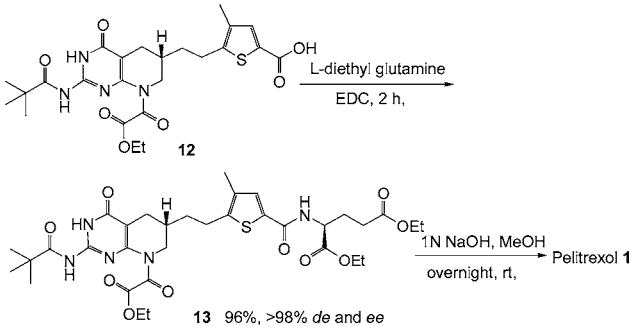
(6) Hu, S.; Tat, D.; Martinez, C. A.; Yazbeck, D. R.; Tao, J. *Org. Lett.* **2005**, *7*, 4329.

This serendipitous discovery was followed by extensive optimization of the enzymatic reaction. Manipulation of reaction pH led to a general increase in selectivity as pH was lowered. However, a dramatic decline in activity was observed below a pH of 3.0. The optimal pH for the reaction was between 4.0 and 5.0. Higher pH's, from 5.5 to 8.0, promoted extensive chemical cleavage of the oxalamic group from the nitrogen, suggesting the high lability of the leaving group.

Cosolvent optimization was also a valuable tool in the reaction's optimization, generating by far the largest gains in selectivity and kinetics. Screening of 10 cosolvents in various amounts, including both water miscible and immiscible choices, indicated that 25−40% DMF as a cosolvent produced more favorable enantioselectivity and reaction rates. The enantiomeric excess of the reaction in 30% DMF reached 95% at 45% conversion with an *E* value of up to 59 (Scheme 3).

This approach also led to great efficiency in the resulting downstream chemistry. Following the enzymatic resolution, the desired *S*-acid precipitated from the reaction mixture under the reaction conditions. Crystallization from a mixture of heptane/ethyl acetate provided the *S*-acid **12** with >98% ee and >38% isolated yield (Scheme 3). This intermediate was then coupled with the diethylglutamate to produce the desired product in high yields and optical purity (>96% yield and >98% de and ee, Scheme 4). Replacing the costly

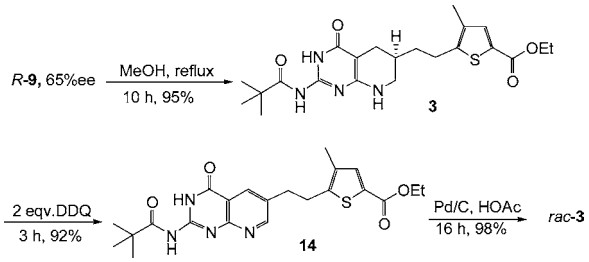
Scheme 4. EDC Coupling of (*S*)-Acid **12** and the Cleavage of Triprotecting Groups toward the Targeted Product **1**



L-glutamate-di-*tert*-butyl used in the old route (Scheme 1) with L-glutamate-di-OEt resulted in an equally robust reaction at one-sixth the original cost. Further optimization of this step gives 98% conversion by HPLC, resulting in actual recovered yields of about 95%, with >96% purity. Finally, the desired end product was obtained by a one-pot triple deprotection of the oxalamic ester, the piv, and the ethyl ester groups in the presence of NaOH. As a result, the Pelitrexol API (active pharmaceutical ingredient) is produced in 10−15% overall yield with the desired chemical and optical purities (>98% de and ee, and 98% chemical purity).

An added advantage of the new process resulting from performing the key resolution at an earlier stage in the reaction sequence is that only half the amount of L-glutamate-di-OEt is needed for coupling in comparison to the earlier route (Scheme 1), increasing the efficiency of the synthesis and decreasing its cost. However, the greatest increase in atom economy comes from the ability to recycle the wrong enantiomer into the final product. Following the enzymatic resolution and subsequent extraction, the wrong enantiomer is recovered from the aqueous layer. After removal of the oxalamic group in refluxed methanol, the undesired enantiomer is readily oxidized to **14** by DDQ. Subsequent hydrogenation with Pd yielded the racemic mixture **3** which was recoverd in up to 80% overall yield. In this manner, overall yields of the desired acid were increased from 38% to greater than 52% after only one round of recycling the undesired enantiomer, significantly improving the efficiency and throughput of the entire synthesis (Scheme 5).

Scheme 5. Racemization of an Undesired (*R*)-Enantiomer via Dehydrogenation/Hydrogenation



The success of this method confirms the value of creative substrate modification as an important tool in enzymatic resolution, especially in cases where remote stereocenters are present. The addition of a labile oxalamic ester protecting group has had a profound effect on the stereodifferentiation of the enzymatic resolution of precursors to the pelitrexol drug substance, which contains a remote chiral center over six bonds away from the reaction center. Following this initial discovery, standard optimization techniques led to a robust enzymatic resolution from what was initially a very unselective and unpromising reaction. Much attention in current biocatalysis research has been given to the power of directed evolution and other forms of protein modification to magnify an enzyme's selectivity toward a particular substrate. However, it is important to remember that substrate modification and clever chemistry such as this serendipitous discovery still remain as invaluable and far less expensive tools for chemoenzymatic synthesis. Using these tools, an efficient, robust, and environmentally friendly route was developed for the large-scale production of pelitrexol.

Acknowledgment. We thank Wolfgang Notz for technical assistance, Xiaobing Xiong for chiral method development, and Carlos Martinez, Van Martin, and Kim Albizati for general discussions.

Supporting Information Available: Experimental procedures, characterization data, and representative ^{1}H and ^{13}C spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0602755