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Enzyme-Catalyzed Asymmetric Deacylation for the Preparation of Lasofoxifene (CP-336156), a Selective Estrogen Receptor Modulator

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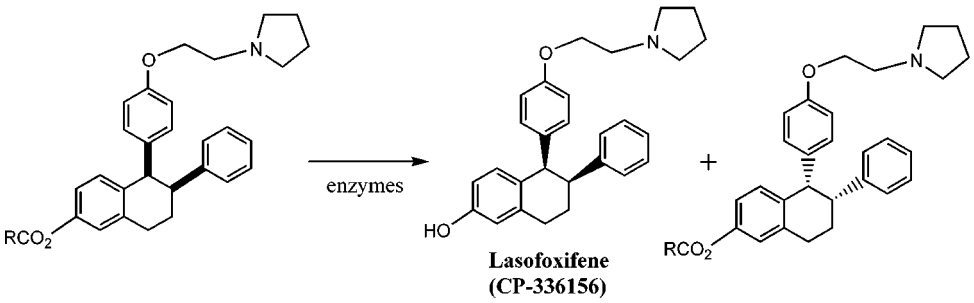
ABSTRACT



A potent and selective estrogen receptor modulator (SERM), Lasofoxifene (CP-336156), was prepared by an enzyme-catalyzed asymmetric deacylation with high optical purity and excellent yield even though the hydrolytic site is remote from the chiral centers.

Estrogen replacement therapy (ERT) has been widely used in postmenopausal women for osteoporosis treatment to prevent bone loss.[1a] In addition, ERT has other beneficial effects including decreases in the risk of coronary heart disease[1b] as well as improvements in short-term memory[1c] and cognitive function.[1d] However, significant and adverse side effects [2] that accompany this therapy include uterine bleeding, fluid retention, and increased risk of endometrial and breast cancer. Therefore, research efforts have focused on identifying a selective estrogen receptor modulator (SERM)[3] that possesses the desired attributes while minimizing negative side effects and associated risks of ERT.

Indeed, the first generation of recently discovered SERMs, such as raloxifene and droloxifene, have proven to be promising therapeutic agents.[3] These findings have provoked a flurry of activity among industrial and academic researchers. Lasofoxifene, CP-336156, a potent, nonsteroidal, newer generation of SERM, was identified recently at Pfizer.[4−6] This compound has improved oral bioavailability and is as efficacious as estrogen at preventing bone loss and lowering serum cholesterol in rats.[6] Furthermore, estrogen-like adverse

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(4) Cameron, K. O.; Jardine, P. A.; Rosati, R. L. Patents EP 802910, JP 98503215, US 5552412, and WO 9621656.

(5) For chemical resolution, see: Chiu, C. K.; Meltz, M. Patent WO 9716434.

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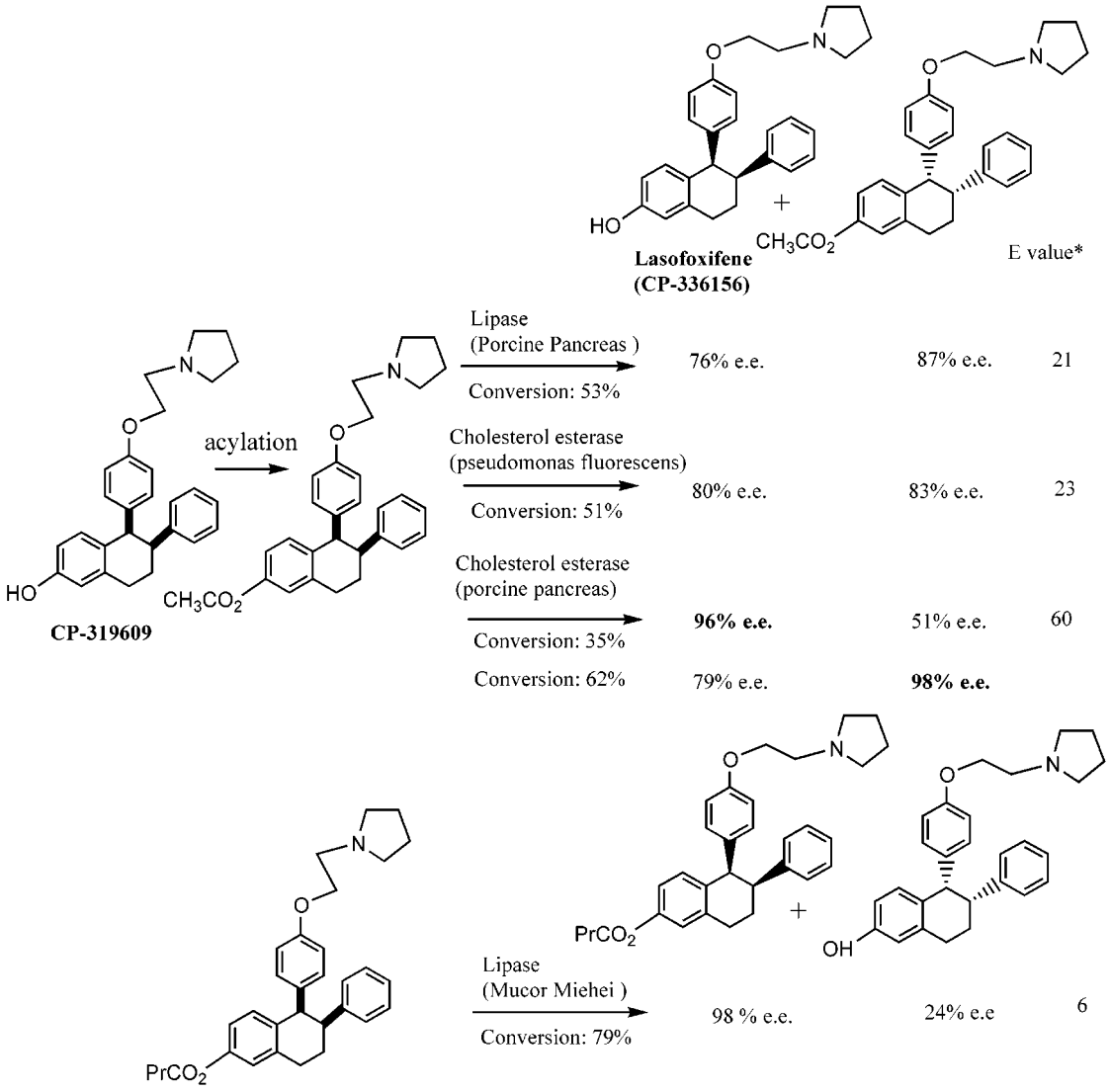
Scheme 1. Enzymatic Resolution of CP-319609



* E is the ratio of the specificity constants of the two enantiomers, see reference 9

effects on uterine and breast tissue were not observed. It is interesting to note that CP-336156 was significantly more potent than its dextrorotatory enantiomer (11.3 nM vs 270 nM) in an estrogen receptor binding assay.[6] In addition, CP-336156 also showed better oral bioavailability than its enantiomer (62% vs 29%).[6] Therefore, an efficient method for preparing optically pure CP-336156 is needed in order to supply this compound for further evaluation.

Recently, hydrolytic enzymes have been used as tools for enantiomeric resolution, more frequently as the demand for pure enantiomer in pharmaceuticals and agrochemicals[7] has increased. Also, mild and environmentally friendly reaction conditions, as well as recoverable and inexpensive biocatalysts used in the reactions, have made enzymatic resolution an attractive alternative to other traditional chemical resolution methods. Here we report an efficient preparation of Lasofoxifene with enzyme-catalyzed asymmetric deacylation even though the chiral centers are not adjacent to the site where enzymatic deacylation takes place.

The racemic free phenol, CP-319609, was converted to the corresponding acetate or butyrate. Then, these two esters were screened against a variety of hydrolytic enzymes, such as lipases and esterases, for enzymatic resolution in pH 7, 0.1 M phosphate buffer.[8] After the preliminary and analytical screens, the most promising acetate was selected for further studies and subjected to gram-scale reactions because the acetate consistently provided better resolution results when esterases or lipases were used as catalysts.

(6) (a) Rosati, R. L.; Jardine, P. A.; Cameron, K. O.; Thompson, D. D.; Ke, H. Z.; Toler, S. M.; Brown, T. A.; Pan, L. C.; Eddinghaus, C. F.; Reinhold: A. R.; Elliott, N. C.; Newhouse, B. N.; Tjoa, C. M.; Sweetnam, P. M.; Cole, M. J.; Arriola, M. W.; Gauthier, J. W.; Crawford, D. T.; Nickerson, D. F.; Pirie, C. M.; Qi, H.; Simmons, H. A.; Tkalcevic, G. T. *J. Med. Chem.* **1998**, *41*, 2928−2931. (b) Ke, Hua Zhu; Paralkar, Vishwas M.; Grasser, William A.; Crawford, D. Todd; Qi, Hong; Simmons, Hollis A.; Pirie, Christine M.; Chidsey-Frink, Kristin L.; Owen, Thomas A.; Smock, Steven L.; Chen, Hong Ka; Jee, Webster S. S.; Cameron, Kimberly O.; Rosati, Robert L.; Brown, Thomas A.; Dasilva-Jardine, Paul; Thompson, David D. *Endocrinology* **1998**, *139*, 2068−2076.

However, due to a limited solubility of the acetate, adequate stirring was necessary to obtain consistent results and good yield. On the basis of the reaction rate and the desired enantiomeric selectivity, we narrowed down the choice of enzymes for gram-scale evaluation to cholesterol esterases from either pseudomonas fluorescens or porcine pancreas and lipase from porcine pancreas. All these enzymes hydrolyzed preferentially the 1*R*,2*S* enantiomer; therefore, CP-336156 can be isolated immediately from these enzymatic resolutions without further deacylation. In the large-scale enzymatic resolution, cholesterol esterase from porcine pancreas yielded encouraging and consistent results (Scheme 1). In this large scale esterase-catalyzed kinetic resolution, a good *E* value (the ratio of the specificity constants of the two enantiomers),[9] 60, was obtained. When the reaction was stopped at 35% conversion (70% theoretical yield), CP-336156 was obtained in 96% ee.[10] On the other hand, the acylated enantiomer of CP-336156 was obtained in 98% ee[10] and 76% theoretical yield when the reaction was stopped at 62% conversion. The excellent results are interesting because the site of enzyme-catalyzed hydrolysis is remote from the chiral centers; nevertheless, good yield and excellent ee values still can be achieved. Also of note was that the lipase from *Mucor miehei* preferred an enantiomer with 1*S*,2*R* configuration as the hydrolyzing substrate[10,11] when butyrate was used for the enzymatic resolution (Scheme 1); this observation was contrary to the majority of enzymes that we tested. The optical purity of CP-336156 obtained from the enzymatic resolution can be further improved to greater than 99% ee by recrystallization from 95:5 mixture of absolute ethanol/water solution. The absolute configuration of CP-336156 was determined through an X-ray diffraction study[11] of the corresponding hydrochloride salt and was found to correspond to an estradiol-like absolute configuration at C-1.

Investigations into various reaction parameters,[7] such as solvent and temperature effects, and into lipase-catalyzed irreversible acylation of the parent phenol (in order to improve the reaction yield and selectivity) were futile.

In summary, even though the chiral centers are remote from the center of enzymatic reaction,[12] Lasofoxifene was still efficiently prepared in good yield and excellent ee.

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(8) The representative experimental procedure for enzymatic resolution: substrate (1 mg) and a enzyme (1 mg) were added to 1 mL of pH 7 phosphate buffer and stirred under room temperature. The reaction was monitored on a chiral HPLC column for reaction conversion and the ee of product and substrate[10] without isolation. The enzyme was added accordingly if the reaction was slow. Typically, the enzymatic reactions were stopped at 30−70% conversions; then enzymes that gave good *E* values[9] were picked up for further evaluation. For large-scale reaction, the same process was adopted except reactions were done in 0.1 M concentration and ethyl acetate was added to extract compounds from aqueous solution. Product was separated from substrate with silica gel column chromatography (3% MeOH, 0.5% NH_4OH in CH_3Cl). Subsequently, the ee was determined on the isolated product and starting material again.

(9) *E* is the ration of specificity constants of two enantiomers, see: Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc*. **1982**, *104*, 7294.

(10) For ee determination: Chiralcel OD column; mobile phase ethanol: hexane = 95:5; flow rate 1 mL/min; wavelength 215 nm; rt; retention times for CP-336156 and its enantiomer are 23 and 31 min; retention times of the methyl acetates are 10 and 14 min.

(11) For determining the absolute configuration of CP-336156, see ref 6.

(12) For another example of enzymatic resolution in which the site of enzymatic hydrolysis is remote from the chiral center, see: Horiuchi, S.; Takikawa, H.; Mori, K. *Bioorg. Med. Chem.* **1999**, *7*, 723−726.