Organic Process Research & Development **2007,** *11,* 903–906

# A Chemoenzymatic Synthesis of an Androgen Receptor Antagonist‡

Rajappa Vaidyanathan,* Lynsey Hesmondhalgh, and Shanghui Hu

*Chemical Research and Development, Pfizer Inc., Eastern Point Road, Groton, Connecticut 06340, U.S.A.*

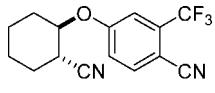
**Abstract:**

**A new scalable enzymatic resolution approach to both enantiomers of *trans*-2-hydroxycyclohexanecarbonitrile (9 and 11) was developed. Treatment of the racemic mixture (4) with succinic anhydride in the presence of Novozym 435 led to selective acylation of one enantiomer to the corresponding hemisuccinate, which was separated from the unreacted enantiomer by a simple basic extraction. This procedure produced the desired enantiomer in high ee, while obviating the need for chromatography or expensive catalysts and ligands. The application of this protocol to the large-scale synthesis of an androgen receptor antagonist (1) is described.**

## Introduction

Alopecia, or baldness, is a commonly observed trait that is not yet medically well understood or easily treated. Although the physiological mechanism by which balding occurs has not been unequivocally established, it is known that androgens are associated with this phenomenon. Compound **1** is an androgen receptor antagonist that was being developed for the treatment of both alopecia and excess sebum (oily skin).[1,2] Multiple kilograms of the active pharmaceutical ingredient (API) **1** were needed to support preclinical safety evaluation and early clinical studies. Therefore, the immediate aim of the project team was to develop a route that could be scaled up effectively to meet the API demand and possibly form the basis of the long-term manufacturing route for this compound.

## Early Synthetic Approaches



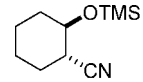

**1**

Scheme 1 shows the first-generation route for the synthesis of **1**. The racemic form of the API (**6**) was synthesized in three steps from cyclohexene oxide via a Lewis acid catalyzed ring opening with trimethylsilyl cyanide followed by hydrolysis of the trimethylsilyl group and subsequent O-arylation with **5** in the presence of lithium hydride. Racemate **6** was purified by chiral column chromatography to furnish the desired API **1**. This route was utilized to synthesize multiple grams of the target to satisfy initial bulk requests.

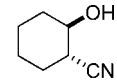
Several issues were foreseen if this chemistry were to be used for further scale-up. Lithium hydride is difficult to use on scale due to safety concerns around its reactivity and propensity to generate hydrogen gas. Although large-scale chiral column chromatography is possible, it is not preferred because of the cost of the stationary phase, high solvent usage, and loss of 50% of the material (the undesired enantiomer) in the last step. Furthermore, material isolated by chromatography is generally ultrapure and would likely not produce API with a purity profile representative of a future commercial process.

## Process Development Efforts

**Step 1.** Subsequently, several alternative routes were evaluated as part of early process research efforts.[3] Most asymmetric syntheses of TMS ether **8** and hydroxy nitrile **9** suffered from either insufficient ee's or scalability concerns.[4] Therefore, alternative routes to optically enriched hydroxy nitrile **9** were investigated.




**8**




**9**

An enzymatic resolution approach seemed to be an attractive way to achieve the desired optical purity for **1**. Typically, enzymatic acylations are performed using an acetate source such as vinyl acetate as the acylating agent, but the limitation of this methodology is that the unreacted alcohol and the product acetate are separable only by chromatography.[5] It was rationalized that the use of a cyclic anhydride such as succinic anhydride (**7**) as the acylating agent would result in the formation of the corresponding hemi-ester (Scheme 2).[6] This could then be separated from the unreacted alcohol through a basic extraction, thereby obviating the need for chromatography.

Several enzymes were screened for the acylation reaction of **4** with succinic anhydride. The best activity and selectivity were seen with *Candida antarctica* Lipase B (CaLB); hence, optimization efforts involved the use of immobilized CaLB in

* Author to whom correspondence should be addressed. E-mail: rajappa.vaidyanathan@pfizer.com.

‡ Dedicated to the memory of Prof. G. Sundararajan, Department of Chemistry, Indian Institute of Technology, Madras.

(1) Hu, L-Y.; Lei, H.; Du, D. Y.; Lefker, B. A. U.S. Pat. Appl. Publ. (2005), US 2005182132 A1 20050818.

(2) Hu, L-Y.; Lefker, B. A.; Du, D. Y.; Smith, Y. D.; Lei, H.; Harter, W. G.; Downs, V. L.; Boys, M. L.; Iula, D. M. U.S. Patent US 2006/009427A1.

(3) Hu, L-Y.; Du, D.; Hoffman, J.; Smith, Y.; Huang, Y.; Kesten, S.; Harter, W.; Johnson, T. R.; Yue, W. S.; Li, J. J.; Barvian, N.; Mitchell, L.; Kostlan, C.; Fedij, V.; Krieger-Burke, T.; Samas, B.; Lei, H. J.; Lefker, B.; Carroll, M.; Dettling, D.; Yalamanchili, R.; Lapham, K.; Pocalyko, D.; Sliskovic, D.; Ciotti, S.; Stoller, B.; Welgus, W. Manuscript in preparation.

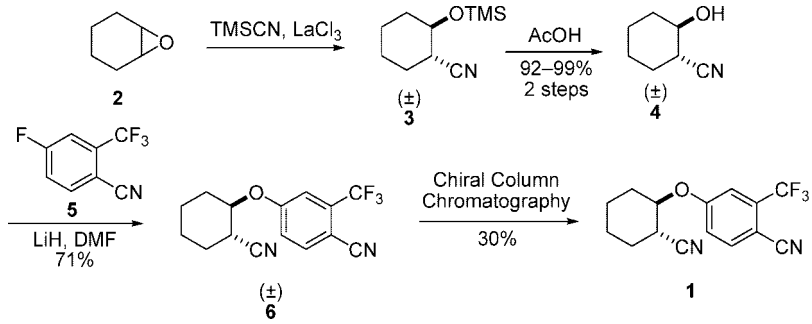
(4) Schaus, S. E.; Jacobsen, E. N. *Org. Lett.* **2000**, *2*, 1001–1004.

(5) Forro, E.; Gyogyszerkemiai, I; Szegedi, T.; Szeged, H. *Acta Pharm. Hung.* **2001**, *71*, 119–126.

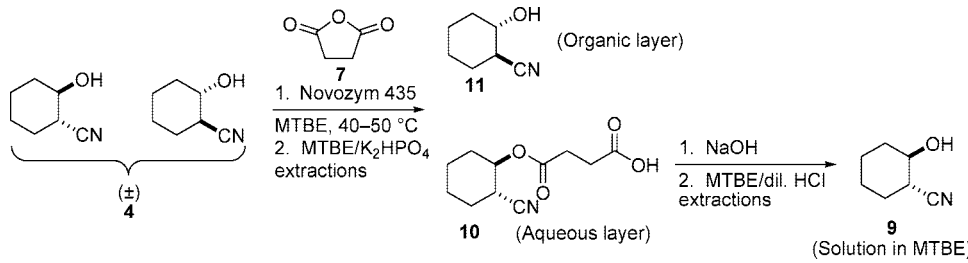
(6) Terao, Y.; Tsuji, K.; Murata, M.; Achiwa, K.; Nishio, T.; Watanabe, N.; Seto, K. *Chem. Pharm. Bull.* **1989**, *37*, 1653–1655.

10.1021/op700146c CCC: $37.00 
Published on Web 09/06/2007

***Scheme 1***




***Scheme 2***




the form of Novozym 435.[7] Preliminary results using Novozym 435 seemed promising, leading to selective conversion of the desired enantiomer **9** to the corresponding hemisuccinate **10** in >95% ee via reaction with succinic anhydride (Scheme 2). After a systematic study of the reaction parameters, the enzymatic reaction was optimized to routinely furnish product with optical purity of 94–95% ee at conversions of 47–49%. The reaction was typically complete within 30 h at ca. 40 °C in methyl *tert*-butyl ether. Early experiments also revealed that the enzyme could be reused, although a small extra charge of fresh enzyme in each subsequent batch made the reactions more reliable. The ability to reuse the enzyme could potentially reduce costs significantly in multibatch campaigns.

Further development of this route focused mainly on the work-up. The first step of the work-up involved removal of the immobilized enzyme by filtration of the reaction mixture. It was found that a significant portion of **10** was still bound to the immobilized enzyme cake after the filtration. Initially, a sodium bicarbonate/sodium chloride wash proved effective for removing most of the product from the filter cake. However, upon scale-up to 20 g in the laboratory, carbon dioxide gas evolution was observed (from reaction of bicarbonate with succinic anhydride/succinic acid) along with rapid expansion of the enzyme cake. This led to the quest for alternative bases to remove residual product from the enzyme cake. Several inorganic bases were evaluated, but issues with incomplete phase separations and aqueous solubility of the inorganic base itself were encountered. Ultimately, dipotassium phosphate ($K_2HPO_4$) emerged as the base of choice for the work-up.

Dipotassium phosphate showed good solubility in water. Thus, water volumes could be kept to approximately 10 mL per gram of **4** while still attaining a pH high enough to remove hemisuccinate **10** from the filter cake after a single wash. The organic and aqueous filtrates were combined, stirred, and separated. A series of MTBE/$K_2HPO_4$ washes led to the removal of unreacted enantiomer **11** from the product-containing aqueous phase. Addition of sodium hydroxide to the aqueous phase led to hydrolysis of **10** to the desired hydroxy nitrile enantiomer **9**, which was isolated as a solution in MTBE via an extractive work-up. In the pilot plant, this enzymatic resolution followed by hydrolysis of hemisuccinate **10** proceeded in excellent yield (104% of theory; ca. 6% of the undesired enantiomer) on a 22-kg scale (input of **4**).

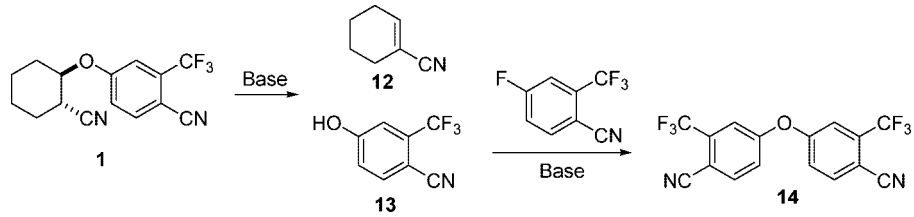
**Step 2.** The next step was to optimize the conditions for the final arylation step and subsequent product isolation. The initial conditions utilized for the arylation chemistry involved treatment of **4** or **9** with 1.5 equiv of aryl fluoride **5** in the presence of LiH in DMF at room temperature. Several issues had to be resolved prior to scaling up this chemistry in the pilot plant. The use of hydride bases such as LiH and NaH for this reaction on a large scale was undesirable as a result of not only hydrogen evolution concerns but also the bases' known incompatibility with DMF.[8] Furthermore, the reactions typically produced significant levels of two impurities (vide infra). These impurities, as well as the excess aryl fluoride were removable only by column chromatography. Therefore, non-chromatographic purification conditions needed to be established prior to scale-up.

First, it was important to understand the nature and origin of the impurities prior to fine-tuning the reaction conditions and work-up. Three impurities were identified from the reaction mixture by using GC/MS techniques. Their structures and plausible pathways for their formation are depicted in Scheme 3. The vinyl nitrile impurity (**12**) was the most abundant impurity referred to above and was formed to some extent (5–20% by GC area) in all reactions. It could arise by base-promoted decomposition of **1** as depicted in Scheme 3. This hypothesis was confirmed by stirring **1** with lithium hydroxide

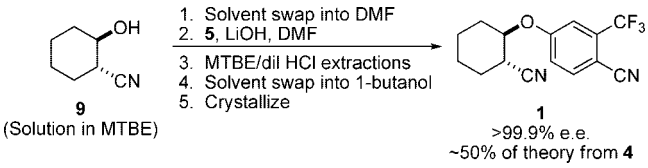
(7) Novozym 435 is purchased from Novozymes North America Inc, P.O. Box 576, 77 Perry Chapel Church Road, Franklinton, NC 27525.

(8) *Bretherick's Handbook of Reactive Chemical Hazards*, 5th ed.; Urban, P. G., Ed.; Butterworth-Heinemann: Oxford, 1995.

**Scheme 3**




**Scheme 4**




(1 equiv) in DMF (5 mL/g) at room temperature. After 48 h, 21% of **12** (relative to **1**) was detected by GC. This sample also contained significant amounts of phenol **13**. The other impurity, namely, biaryl ether **14**, was formed only when reaction mixtures were heated in an attempt to drive the arylation reaction to completion. As these impurities were all formed by base-promoted degradation of **1**, the excess base would have to be avoided.

An in-depth screening of solvents and bases was conducted in an effort to determine the optimum conditions for the arylation reaction. One interesting trend that emerged from this 70-reaction study was that lithium bases gave the highest conversions and best purity profiles. This is presumably because lithium alkoxides are more covalent in character than the other alkali metal alkoxides and consequently less basic and less capable of promoting base-induced decomposition. LiOH and LiH in DMF gave the best results with respect to reaction completion and impurity profile. The reaction profiles using LiOH were essentially the same as those using LiH. Therefore, it was decided to replace LiH with LiOH.[3] Most other bases either did not afford satisfactory conversion or led to significant product decomposition.

The stoichiometries of LiOH and aryl fluoride were investigated systematically in an effort to determine the right balance between reaction completion and impurity suppression. Ultimately, the best conditions were 1.1 equiv of LiOH and 1.5 equiv of **5** in DMF at room temperature. Under these conditions, the reactions proceeded to completion within 16 h. Although the same set of impurities was formed (albeit to a lesser extent) during the reaction as before, very little product degradation was observed even upon prolonged stirring at room temperature. An extractive work-up (MTBE/aq HCl) proved to be an excellent method for the removal of DMF. The organic phase was filtered (speck-freed), and the MTBE was replaced with 1-butanol. Subsequent cooling of the 1-butanol solution led to crystallization of the product (Scheme 4). Typical laboratory yields in this step were consistently around 60%. The crystallization parameters were not optimized further because of tight project timelines; however, it was gratifying to note that the crystallization using 1-butanol provided acceptable yields while obviating the need for chromatography.

The sequence described in Schemes 2 and 4 scaled up well in the pilot plant to produce over 10 kg of API. The enzymatic resolution followed by hydrolysis of the hemisuccinate proceeded in virtually quantitative yield (104% of theory; ca. 6% of the undesired enantiomer). The API was isolated in ca. 50% yield (>99.9% ee with a purity of over 99.2%). These numbers are significant in light of the fact that this was a "telescoped" process with no isolated intermediates or chromatographic purification. Future work will focus on optimizing the O-arylation chemistry and final isolation conditions.

## Experimental Section

**(1*S*,2*R*)-2-Hydroxycyclohexanecarbonitrile 9.** To a 1200-L glass-lined tank were charged racemic 2-hydroxycyclohexanecarbonitrile **4** (22.4 kg, 179 mol), succinic anhydride (14.3 kg, 143 mol), Novozym 435 (22.4 kg), and methyl *tert*-butyl ether (224 L). The reaction mixture was stirred at 50 °C for 24 h. GC analysis of the reaction mixture indicated reaction completion at this time. The reaction mixture was cooled and filtered to remove the enzyme. The enzyme cake was washed with 45 L of MTBE followed by 40% aqueous $K_2HPO_4$ (2 × 157 L). The filtrates were combined, and the aqueous phase was tested to ensure that the pH was above pH 8.0. The mixture was stirred for 15 min and allowed to settle. Three distinct phases were observed at this stage: an upper organic phase, a lower aqueous phase, and a middle oily yellow phase. The lower and middle phases were removed and washed with 157 L of MTBE. The combined MTBE layers were washed with 40% aqueous $K_2HPO_4$ solution (32 L), and the aqueous layer was combined with the lower and middle phases from the previous extraction. At this point, the unreacted enantiomer **11** was in the MTBE layer, and hemisuccinate **10** was in the combined aqueous and middle layers.

To the combined middle and aqueous layers was added 50% aqueous NaOH (71.1 kg), and the mixture was stirred at ambient temperature for 12 h to effect hydrolysis of **10** to **9**. When the hydrolysis was deemed complete by HPLC, MTBE (113 L) was added, and the reaction mixture was cooled to 15 °C. Concentrated HCl was slowly added to the reaction mixture until the pH reached 7.0–7.5 (ca. 77 kg of HCl was required). The mixture was stirred and allowed to settle. The aqueous phase was removed and washed with MTBE (112 L). The organic layers were combined and concentrated to ca. 50 L. Quantitative HPLC analysis of the solution indicated 11.6 kg of **9** (104% of theory) containing 5.7% of the wrong enantiomer. In other words, the enzymatic resolution and hydrolysis reactions had proceeded in 98.3% yield. A portion of the MTBE solution (38.9 kg; equivalent to 8.5 kg of **9**) was taken on to the next step.

**4-[(1*R*,2*S*)-2-Cyanocyclohexyloxy]-2-(trifluoromethyl)benzonitrile 1.** DMF (25 L) was added to the solution of **9** in MTBE from the previous step, and the mixture was concentrated

in vacuo to a volume of ca. 25 L. To this was added a solution of 4-fluoro-2-(trifluoromethyl)benzonitrile (20.5 kg, 108 moles) in DMF (13.5 L), followed by solid LiOH (5 × 385 g; 79.2 mol total). The reaction mixture was allowed to stir at ambient temperature. When the reaction was deemed complete by GC (ca. 30 h), the mixture was cooled to 15 °C and slowly transferred to a tank containing a cold solution made up of 12.4 kg of concentrated HCl, 50 L of $H_2O$, and 30 L of MTBE. The contents of the tank were stirred and allowed to settle. The aqueous phase was removed and the organic phase was washed with saturated NaCl solution(46 L). The organic phase was concentrated in vacuo to a volume of 20 L and diluted with 1-butanol (20 L). The distillation was continued until all the MTBE was removed. The contents of the tank were heated to 70–80 °C and filtered through a 0.6 $\mu$m filter. The filtrate was cooled to ambient temperature and stirred for 12 h. The solids that had precipitated were collected by filtration and washed with 10 L of 1-butanol. The cake was dried at 45–55 °C to afford 10.2 kg of **1** (52%).


## Acknowledgment

Yanqiao Xiang, Pete Zakarias, Scott Zugal and Zach Piller provided analytical support, and their prompt turnaround on assays is greatly appreciated. We thank Stephen Kesten and Lain-Yen Hu for providing background information on their work on the chiral column chromatography route and early process research efforts. We thank Sean Kelly for his support in screening enzymes for the acylation reaction and Neal Sach for screening conditions for the arylation reaction. Tim Puls and John Burt are acknowledged for their timely help. A special word of thanks to Thomas Runge for his support and guidance during the entire course of this work.


## Supporting Information Available

NMR spectra of **10**, **9**, and **1**. This information is available free of charge via the Internet at http://pubs.acs.org.


Received for review June 25, 2007.

OP700146C