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Biocatalytic Resolution of Bis-tetrahydrofuran Alcohol

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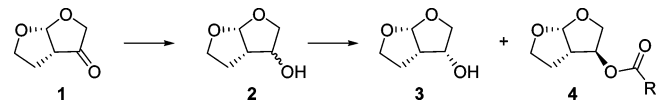
Abstract:

A simple and efficient process has been developed to effect the kinetic resolution of the racemic alcohol 2 using immobilized lipase to afford the desired optically pure (*R*)-bis-tetrahydrofuran (bis-THF) alcohol 3, to facilitate the rapid progression of a clinical candidate. Rapid optimization and development of reproducible and scalable processes are essential to meet aggressive timeframes for preclinical, safety, and early clinical drug development. Process parameters were initially scoped and optimized using a combination of a rational bioprocess screening design and parallel microscale empirical studies, specifically accounting for scale-up and downstream processing considerations. The choices of reaction solvent, acyl donor, and immobilized biocatalyst proved to be critical factors in the design of a conveniently scalable and enantioselective enzymatic resolution process. The improved process was initially validated on 3-g and then 90-g scale in simple impeller-stirred reactors, exhibiting excellent reproducibility. This methodology was successfully implemented on a multikilogram scale to give the target alcohol 3 with >99% ee.

Introduction

The synthetic intermediate (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-*b*]furan-3-ol, **3**, is a key component for the synthesis of various clinical HIV protease inhibitors (PIs) including darunavir, brecanavir, GS-9005, and SPI-256.[1−5] The bis-THF moiety has imbued these PIs with high potency and excellent resistance profiles. Not surprisingly, it has thus been the subject of numerous synthetic investigations.[1−4,6] The synthesis of the PIs requires the enantiomerically pure (3*R*)-form **3** of bis-THF-alcohol (Scheme 1). In general, achieving both the desired diastereoselectivity and enantioselectivity has remained a challenge, ultimately requiring additional steps and further enhancement of chiral purity. We present here some of our investigations aimed at improving the resolution step for the scaled-up synthesis of chiral alcohol **3**, to facilitate the rapid progression of SPI-256 as a clinical candidate.

Scheme 1



The racemic form of the alcohol (**2**) was derived by reduction of ketone **1**.[7] Lipase-catalyzed kinetic resolution promised to be the most effective and practical route for achieving high chiral purity of **3**. A published biocatalytic procedure[6] for enantioselective acylation of **2** with acetic anhydride catalyzed by *Pseudomonas* lipase in 1,2-dimethoxy ethane (DME) was found to result in relatively low ee values for **3**, modest volumetric productivity, and modest yield due to overacylation of the (*R*)-alcohol and challenging product isolation and purification in our hands. Scale-up processes of this general approach using lipases in DME have been reported but still suffer from challenges in control of water activity for hygroscopic DME, modest ee, product isolation issues, and practical process scale-up issues that ultimately necessitated the design of a continuous reactor.[4]

The goal of this work was to develop a more enantioselective, robust, and scalable biocatalytic process for kinetic resolution of **2**. An optimized process was developed to successfully resolve and conveniently isolate both enantiomers **3** and **4** on a multikilogram scale.

Results and Discussion

Optimization of Reaction Conditions. *Catalyst Evaluation.* Initially, several commercial immobilized *Pseudomonas* lipases from Amano were tested, including PS-C I, PS-C II, PS-D I, and AK-C I. The enzymes were immobilized on ceramic particles (code "C" in the enzyme designation) or diatomaceous earth (code "D"). The reactions were carried out in both DME

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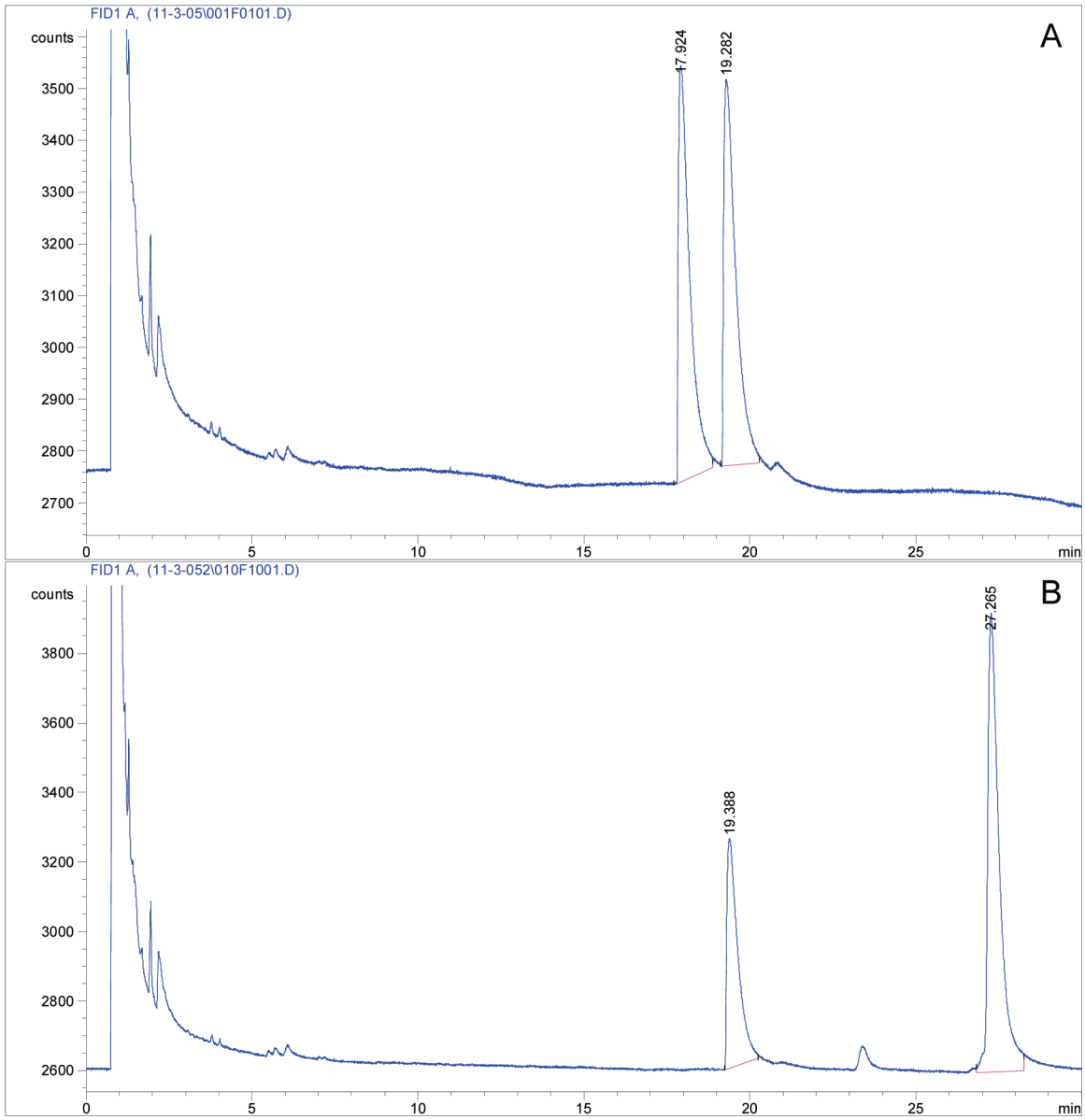


Figure 1. **GC analysis of enzymatic acylation of bis-THF alcohol in DME with vinyl butyrate using lipase PS-C I. (A) Starting racemic bis-THF alcohol; (B) reaction after 3 h. Retention times: (*S*)-bis-THF alcohol, 17.9 min; (R)-bis-THF alcohol, 19.3 min; (*S*)-bis-THF alcohol butyrate, 27.3 min.**

and methyl *tert*-butyl ether (MTBE) at 25 °C using vinyl butyrate as the acyl donor. Each reaction contained 26 g/L enzyme, 20 g/L racemic bis-THF alcohol, and 2-fold molar excess of acyl donor, in a total volume of 1 mL. The reactions were done in 2-mL screw-capped glass vials with agitation in an orbital shaker at 300 rpm.

All of the tested lipases showed high enantioselectivity, selectively acylating (*S*)-bis-THF alcohol to form ester **4**, and leaving behind the desired (*R*)-enantiomer **3** of bis-THF alcohol. However, the enzymes differed in their catalytic activity and convenience for use and reuse. The reaction rate was the highest with PS-C I, followed by PS-C II, PS-D I and AK-C I. Specifically, after 1 h incubation in MTBE, the reaction afforded alcohol **3** with >99% ee using PS-C I, 99% ee using PS-C II, 83% ee using PS-D I, and 75% ee using AK-C I. In all cases, the reaction gave alcohol **3** with >99% ee after 3 h. As an example, Figure 1 shows the reaction profile using lipase PS-C I after 3 h. No reaction took place in the absence of enzyme.

Selection of Acyl Donor and Reaction Solvent. The acyl donors vinyl acetate, vinyl butyrate, and isopropenyl acetate were selected for evaluation. Vinyl and isopropenyl esters typically offer better reaction control for enantioselective acylation, and longer-chain (butyl, isopropenyl) esters typically react more efficiently with better enantioselectivity using lipases. Isopropenyl esters also typically afford few side reactions and better biocatalyst recycle. Reactions were again done in two different solvents, DME and MTBE containing 15% v/v methylene chloride (MTBE/CH_2Cl_2). Lipase PS-C I was used in this set of experiments at a lower concentration of 7 g/L, to reduce the reaction rate and thus allow for a more precise comparison of the different conditions. Test reactions were carried out on a 1-mL scale at 25 °C and orbital shaking at 300 rpm, using 20 g/L racemic bis-THF alcohol and 2-fold molar excess of acyl donor.

All different acyl donor/solvent combinations were tested, and reactions were analyzed by chiral GC after 1 and 3 h.

Table 1. **Effect of solvent, acyl donor, and reaction time on enantioselective resolution of bis-THF alcohol catalyzed by lipase PS-C I**

	enantiomeric excess of (*R*)-bis-THF alcohol, %			
	DME		MTBE	
acyl donor	1 h	3 h	1 h	3 h
vinyl acetate	59	>99	85	>99
vinyl butyrate	44	98	91	>99
isopropenyl acetate	26	89	48	>99

Table 2. **Chromatographic properties of bis-THF alcohol and its esters**

compound	GC retention time, min	TLC R_f[a]
(*R*)-bis-THF alcohol	19.3	0.12
(*S*)-bis-THF alcohol	17.9	0.12
(*S*)-bis-THF alcohol acetate	11.5	0.29
(*S*)-bis-THF alcohol butyrate	27.3	0.38

[a] Silica gel, MTBE/*n*-hexane = 4:1 v/v.

Selected results of this study are presented in Table 1; Table 2 shows the comparison of chromatographic properties of the corresponding reactants and products that are important for purification assessment. In all cases the enzyme showed higher activity in MTBE/CH_2Cl_2 compared to DME (Table 1). Furthermore, the boiling point of MTBE (55 °C) is substantially lower than that of DME (85 °C), and therefore the postreaction removal of MTBE/CH_2Cl_2 is easier and more cost-effective and provides a higher recovered yield of the volatile alcohol product.[8] Using the reaction rate and the achieved alcohol ee as selection criteria, the best performing system was lipase PS-C I using vinyl butyrate or acetate as acyl donor. The use of vinyl butyrate offers easier extractive or chromatographic separation of the target (*R*)-bis-THF alcohol from (*S*)-bis-THF alcohol ester, as predicted on the basis of the chromatographic and physical characteristics of these compounds (Table 2), while vinyl acetate is a less costly reagent at scale.

Process Scoping. An optimization study for enzymatic resolution of bis-THF alcohol was initially carried out using immobilized lipase PS-C I and vinyl butyrate in MTBE/CH_2Cl_2. A broad range of reaction conditions was tested, as summarized in Table 3. The optimization study was done in 1 mL of reaction volume using shaking at 300 rpm. The reaction temperature was maintained at 25 °C. Reaction rates under essentially all conditions were typically very high (even at high alcohol concentrations full conversions in many cases were achieved after approximately 3 h), and therefore heating the reaction was not necessary.

Increasing enzyme concentrations resulted in higher reaction rates but also caused the undesirable overacylation of the target (*R*-)bis-THF alcohol (Table 3), especially at longer reaction times and higher starting concentrations of bis-THF alcohol. This could make the control of the reaction outcome at scale more difficult, and therefore moderate enzyme concentrations were preferable. The data in Table 3 were obtained using a 2-fold molar excess of vinyl butyrate over bis-THF alcohol. It was found that changing the molar excess to 0.75-fold did not noticeably affect reaction rates, yields, and observed ee values but offered improved workup and lower reagent costs.

While the commercial lipase PS-C I was determined to be an adequate catalyst for the process on the basis of results of initial enzyme testing, a more cost-effective catalyst was desired for large-scale production of (*R*-)bis-THF alcohol. Lipase PS-30, a commercial enzyme from the same microbial source as that for lipase PS-C I (*Pseudomonas cepacia*), was immobilized on Kieselgur Celite-521 using an internally developed protocol (see Experimental Section for details) and compared under conditions selected for lipase PS-C I: 100 g/L bis-THF alcohol in MTBE/CH_2Cl_2 using vinyl butyrate as acyl donor (0.75-fold molar excess over racemic bis-THF alcohol), 25 g/L enzyme, reaction temperature 25 °C. It was found that the immobilized PS-30 lipase afforded a full conversion of (*S*)-bis-THF alcohol, resulting in >99% ee of (*R*)-bis-THF alcohol after 3 h (Table 4). Thus, the immobilized PS-30 enzyme showed enantioselectivity and overall product yield similar to those observed with lipase PS-C I. On the basis of these parameters, immobilized PS-30 was chosen in all subsequent scale-up optimization reactions as a cost-effective alternative to the commercial immobilized lipase. Similarly, on the basis of cost considerations and easier downstream processing, vinyl acetate was chosen instead of vinyl butyrate for the large-scale resolution of bis-THF alcohol. Both acyl donors showed similar performance during the scoping studies (Table 1).

Scale-Up of Optimized Enzymatic Process. In the initial scale-up experiments two identical reactions were set up, differing only in impeller stirring speed (165 and 350 rpm, with the catalyst fully suspended at both stirring speeds), in order to verify the reaction performance upon scale-up and to explore the effect of stirring speed in larger reactors. The reaction was carried out on a 3-g scale of bis-THF alcohol in 30 mL of MTBE/CH_2Cl_2, 0.75-fold molar excess vinyl acetate, and 20 g/L immobilized PS-30 enzyme in a jacketed vessel at 25 °C (total reaction volume 30 mL). Both reactions were complete in 4 h with no overacylation, giving the desired (*R*)-alcohol with >99% ee by chiral GC analysis. Thus, the reaction was not diffusion-controlled, and the reaction rate and yield were not affected by the stirring speed, as long as the catalyst remained fully suspended in solution.

After completion of the reaction, the enzyme was separated by filtration, and the solvent and excess acyl donor were removed by evaporation under reduced pressure. This resulted in a clean mixture of the desired (*R*)-enantiomer of bis-THF-alcohol and the acetic acid ester of the (*S*)-enantiomer, ready for chromatographic separation. No decomposition or reduction in ee of the alcohol was observed after the workup of the reaction mixture.

Having verified the reaction conditions on a 3-g scale, the reaction was further scaled up to a 90 g scale, using the following protocol. To 90 g (0.692 mols) of bis-THF alcohol in a 1-L medium bottle were added 47.9 mL (44.74 g, 0.520 mols) of vinyl acetate (0.75-fold molar excess with respect to racemic alcohol) and methylene chloride (112.5 mL). The mixture was swirled until a homogeneous solution was obtained. MTBE (637.5 mL) was added with mixing to obtain a

(8) A significant problem encountered when acetic anhydride was used in preliminary studies was the difficulty in its removal due to the relatively high boiling point.

Table 3. **Optimization of resolution of bis-THF alcohol catalyzed by lipase PS-C I**

		reaction time 1 h			reaction time 3 h			reaction time 6 h		
[bis-THF alcohol]g/L	[lipase]g/L	conv. of (*S*)-isomer %	ee %	overacylation of (*R*)-isomer %	conv. of (*S*)-isomer %	ee %	overacylation of (*R*)-isomer %	conv. of (*S*)-isomer %	ee %	overacylation of (*R*)-isomer %
20	7	89	81	0	100	100	0	nd[a]	nd[a]	nd[a]
50	25	100	100	0	100	100	1.5	100	100	<2
50	100	100	100	0	100	100	2.6	100	100	7.1
100	25	100	100	0	100	100	2.2	100	100	1.8
100	100	100	100	1.7	100	100	1.9	100	100	10.1
100	200	100	100	<2	100	100	7.2	100	100	15.6

[a] Not determined.

Table 4. **Comparison of lipase PS-C I and immobilized lipase PS-30 in enantioselective resolution of bis-THF alcohol**

	after 1.5 h reaction		after 3 h reaction	
enzyme	yield, %	ee, %[a]	yield, %	ee, %[a]
lipase PS-30	93	>99	100	>99
lipase PS-C I	100	>99	100	>99

[a] Enantiomeric excess of (*R*)-bis-THF alcohol.

homogeneous solution, and the reaction mixture was slowly poured onto the immobilized PS-30 lipase enzyme (18 g, 20 g/L) in a 2-L jacketed glass vessel maintained at 25 °C by a circulating water bath. The reaction was mixed with the use of an overhead stirrer assembly for 4 h at 350 rpm, which was sufficient to keep the enzyme fully suspended. The enzyme was removed by filtration, and the reaction mixture was analyzed by chiral GC, affording the desired (*R*)-alcohol with >99% ee (Figure 2). The reaction was repeated twice under the same conditions, giving identical results.

Reaction conditions identified and verified in the preliminary scale-up experiments were applied to perform the lipase-catalyzed resolution of racemic bis-THF alcohol on a multikilogram scale. A total of 10 kg of **3** was produced in several reaction batches, as described in the Experimental Section, after purification by flash chromatography. The purity of the final product, as determined by chiral GC analysis, was 99.7%, with ee > 99%.

In order to avoid the laborious and time-consuming chromatography step in the purification of **3**, an alternative highly efficient purification strategy was developed on the basis of chemical protection/deprotection of (*R*)-bis-THF alcohol. In this process, the crude mixture of reaction products **3** and **4** was dissolved in acetonitrile and treated with disuccinimidyl carbonate (DSC), resulting in the formation of a DSC derivative **5** (Scheme 2). The latter could be easily separated from the acetate **4** by sequential trituration with ethyl acetate and MTBE. It could then be used directly for further chemistry or subjected to alkaline hydrolysis to remove the DSC protection (see Experimental Section for details). The purity of the final product **3**, as determined by chiral GC analysis, was 99.5%, with ee > 99%, comparable to the purity obtained after purification with column chromatography.

Experimental Section

Chemicals. Lipase PS-30 and immobilized lipases PS-C I (1638 U/g), PS-C II (1090 U/g), PS-D I (899 U/g), and AK-C (597 U/g) were received from Amano Enzyme U.S.A. (Elgine, IL). Immobilized lipase PS-30 (1425 U/g) and racemic bis-THF alcohol were prepared at Indus Biosciences (lot # IB/TSR/049/071). All solvents and acyl donors were purchased from Aldrich (Milwaukee, WI) and were of the highest grade available.

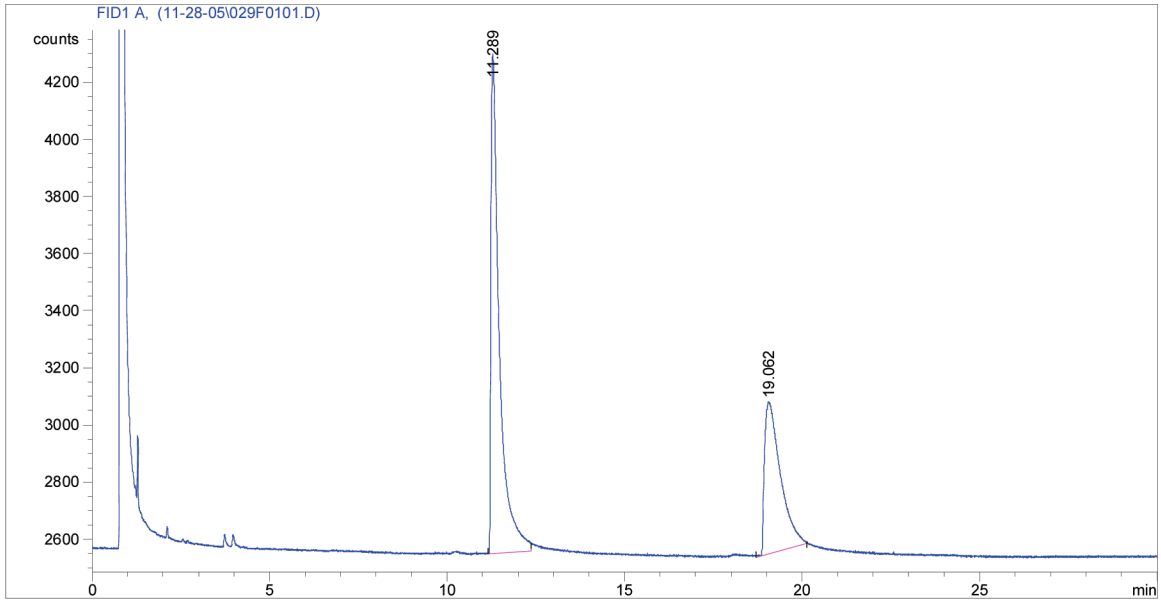
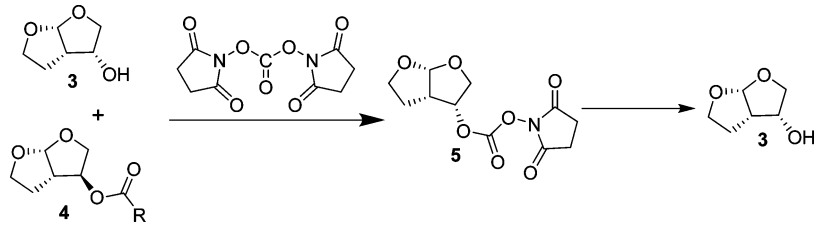


Figure 2. **GC analysis of enzymatic acylation of bis-THF alcohol with vinyl acetate on a 90-g scale. Retention times: (*R*)-bis-THF alcohol, 19.1 min; (*S*)-bis-THF alcohol acetate, 11.3 min.**

Scheme 2



Enzyme Immobilization. A suspension of Kieselgur Celite-521 (3 kg) was prepared in 30 L of 30 mM potassium phosphate buffer pH 7.0. The slurry was filtered and Celite was washed with 3 × 3 L of the buffer. After drying, the neutralized Celite was mixed with a suspension of 750 g of lipase PS-30 in 10 L of buffer. The resulting slurry was spread on plastic dishes and allowed to air-dry for 4 days.

Large-Scale Resolution of Racemic Bis-THF Alcohol. A solution of bis-THF alcohol (4.0 kg, 30.74 mols) in dichloromethane (4 L) and MTBE (25 L) was placed in a 50-L reactor, followed by vinyl acetate (1.984 kg, 23.05 mols). The mixture was stirred for 15 min at ambient temperature. Immobilized lipase PS-30 (0.70 kg, 20 g/L) was then added, and the resulting suspension was stirred at 400 rpm at room temperature. Samples of the reaction mixture were periodically withdrawn and analyzed by chiral GC to monitor the reaction progress. Once the ee of the target alcohol **3** exceeded 99% (∼5 h reaction time), the enzyme was removed by filtration.

Purification of (*R*)-Bis-THF Alcohol Using Column Chromatography. The filtrate obtained after the biocatalytic reaction was concentrated under reduced pressure. The resulting mixture was separated by column chromatography (100−200 mesh silica gel, 10 kg), eluting with a step gradient of ethyl acetate in hexane (from 5 vol % to 30 vol % ethyl acetate in 5% steps) at 10 L/h flow rate. Fractions containing **3** were pooled and evaporated under reduced pressure to remove the solvent. The target chiral alcohol **3** was isolated in the amount of 1.23 kg (30% isolated yield) with purity of 99.7% as determined by chiral GC. ^{1}HNMR ($CDCl_3$, 200 MHz): δ 5.72 (d, 1H), 4.38−4.56 (q, 1H), 3.87−4.12 (m, 3H), 3.67−3.75 (m, 2H), 2.78−2.92 (m,1H), 2.28−2.34 (m,1H), 1.78−1.93 (m, 1H).

Purification of (*R*)-Bis-THF Alcohol Using Chemical Protection/Deprotection. The biocatalytic resolution reaction was carried out as described above on a 10-g scale. The filtrate obtained after the biocatalytic reaction was concentrated under reduced pressure. The crude product mixture (10.8 g) was dissolved in acetonitrile (60 mL) and treated with disuccinimidyl carbonate (1.5 equiv) in the presence of triethylamine (2 equiv) at room temperature for 24 h. Acetonitrile was then removed under reduced pressure, and the solids were dissolved in ethyl acetate (100 mL). The organic solution was washed with water (20 mL) and saturated NaCl solution (20 mL). The organic layer was separated and evaporated under reduced pressure. Obtained solids (12.6 g) were triturated in MTBE (20 mL) at 0 °C for 30 min. The solvent was removed by filtration, and the solid residue was washed with chilled hexane (5 mL) to produce 5.1 g of DSC derivative **5** (melting point 118−120 °C). The DSC derivative (0.5 g) was dissolved in THF (5 mL) and the solution stirred at 30 °C for 10 min, followed by the addition of 3 mL of 20% aqueous NaOH solution and stirring for 30 min at 30 °C. As a result of this treatment a biphasic mixture was formed, which was separated into aqueous and organic layers. The aqueous layer was extracted with ethyl acetate (5 mL). The organic extract was dried over sodium sulfate and concentrated under reduced pressure to give pure alcohol **3** (56% isolated yield). The purity of the final product **3**, as determined by chiral GC analysis, was 99.5%, with ee > 99%.

Chiral GC Analysis. Prior to chiral GC analysis, reaction samples were filtered to remove immobilized enzyme, and the filtrate was directly injected into a gas chromatograph. GC analysis was carried out on an Hewlett-Packard 5890 gas chromatograph with a flame ionization detector using a Chiraldex β-cyclodextrin trifluoroacetyl (BTA) capillary column (20 m × 0.25 mm) supplied by Astec (Whippany, NJ). The chromatography was conducted under isothermal conditions at oven temperature 155 °C, inlet temperature 200 °C, and detector temperature 250 °C. The flow rate of the carrier gas (helium) was 2.0 mL/min.

Conclusion

Bis-THF alcohol **3** has proven to be a critical intermediate in the synthesis of many of the most potent and effective HIV PIs described in the past decade. In this work we show that by making suitable adjustments to the originally described enzymatic resolution procedure this key intermediate can be prepared in high yield and purity on large scale.

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