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PPL-Catalyzed Enzymatic Asymmetrization of a 2-Substituted Prochiral 1,3-Diol With Remote Chiral Functionality: Improvements Toward Synthesis of the Eutomers of SCH 45012

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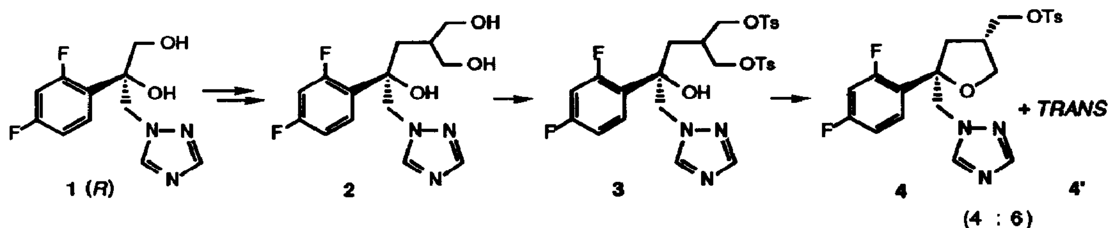
Abstract: Porcine pancreatic lipase (PPL) catalysis has been used to establish both stereocenters of the *cis*-(tetrahydrofuranylmethy) tosylate **4**. In addition to the enzymatic differentiation of the hydroxyl groups of the pro-chiral 1,3-diol segment of **2**, successful enzymatic resolution of the racemic diol **10** provided an alternate route to the important precursor **1**.

The [(*R*)-*cis*]-tosylate **4** is a key intermediate for the preparation of SCH 50001 and SCH 50002, antifungal compounds with therapeutic potential for oral treatment of systemic *Candida* and *Aspergillus* infections. These compounds are the active isomers (eutomers) of SCH 45012, a mixture of the four possible stereoisomers. The synthesis and antifungal activity of enantiomerically pure **4** from the chiral diol **1** (Scheme 1), its [(*S*)-*cis*]-enantiomer, and their corresponding *trans* stereoisomers is the subject of a separate communication[1]. In the initial stages, after the synthesis of diol **1**, a major synthetic inefficiency proved to be the base-induced cyclization of the ditosylate **3**. This cyclization was actually slightly diastereoselective for the less useful *trans* isomers under the best conditions, requiring cumbersome chromatographic separations.



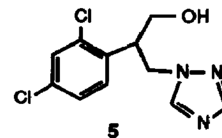
Scheme 1

Focusing specifically on the [(*R*)-*cis*]-series,[1] an obvious possible alternative was to first differentiate the two 1° hydroxyl groups of triol-**2** by taking advantage of the opportunity for chiral induction provided by the fixed stereocenter at the 3° alcohol. Several attempts at selective chemical functionalization were unsuccessful, probably due to conformational flexibility arising from the methylene spacer between the chiral hydoxyl center and the prochiral 1,3-diol segment where induction was needed.

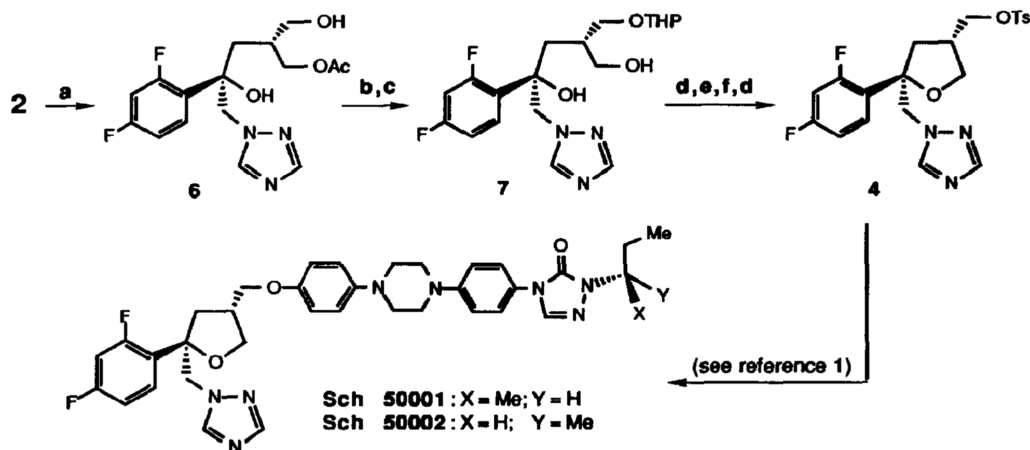
Recent successes of enzymatic reactions in organic solvents[2,3] and the refinements of theory and methods[4,5] offered a more promising alternative. This communication describes the lipase-mediated asymmetrization of the 1,3-diol segment of substrate **2** in methyl acetate as both solvent and acetyl transfer agent to produce a monoacetate **6** in high yield and excellent % ee.

For a variety of reasons, it was not feasible to screen a wide array of enzyme/ solvent/ acyl ester combinations. Instead, based on the most closely related precedents which included the substrate **5**,[6] containing both dihalophenyl

and 1,2,4-triazole rings as does **1**, it was determined to examine only Sigma 3126 PPL[7] and to perhaps optimize the other variables. Compound **2** is among the more structurally complex synthetic organic molecules[8] to be subjected to lipase-catalyzed acylation that we are aware of. It not only presents significant steric bulk, it contains a distal triazole ring and a chiral hydroxyl group, both of which are capable of physically interacting with proteins and possibly affecting the enzyme catalytic reactivity. The effects of distal chiral centers on the stereoselectivity of lipase-catalyzed acylations has not been broadly studied.[8]



The first experiment, conducted in neat ethyl acetate, produced a 57% yield of a single monoacetate **6**[9] from **2** in 24 hours with no evidence of diacetate formation (Scheme 2). Proof of stereochemistry was accomplished by meticulous elaboration of **6** to **4** / **4'** in a manner to avoid the possibility of base-catalyzed trans-acetylation or diastereoselective reaction or separations. Comparison of pmr spectra and HPLC with authentic samples[1] of **4** and **4'** showed the product to be ≥99% [(*R*)-*cis*]-**4**.[10] Thus, the enzyme-catalyzed acetylation had occurred with excellent stereoselectivity for the pro-(*R*) hydroxyl group, ≥98% ee This was more than satisfactory to justify some optimization for a large-scale protocol.



Conditions: (a) PPL (1:1 w/w), EtOAc, r.t., 24 h (57%); (b) dihydropyran, *p*-TsOH, CH_2Cl_2 (98%); (c) KOH, THF-H_2O, 18 h (96%); (d) *p*-TsCl, N,N-dimethylaminopyridine, THF, 18 h (96%); (93%); (e) NaH, THF, 1 h (95%); (f)PTSA, MeOH, 24 h (94%).

Scheme 2

The reaction appeared to be slower than those of most other reports of PPL catalyzed acylation. This could be due to the effect of the steric bulk alone on the binding kinetics, or counterproductive conformational effects resulting from the interaction of the hydroxyl or triazole groups with the enzyme. Without further mechanistic investigation, variations were made in solvent (Table 1), and substrate (**2**) concentration (Table 2), while temperature had no noticeable effect between 15°C and 40°C. An increase of the PPL:**2** weight ratio to 3:1 at 0.05 M in neat methyl acetate then afforded >90% conversion[11] within 48 hours without undue mechanical difficulties. The use of limited amounts of methyl acetate, ethyl acetate, or isopropenyl acetate in $(i\text{-}Pr)_2O$ or $CHCl_3$ as bulk solvents produced only negligible amounts of product.

Table 1. Comparison of acyl ester / solvents. (0.2 M, 1:1 PPL:2, ambient temp.)

time (hr)	% conversion to mono-acetate			
	neat EtOAc	"wet" EtOAc	neat MeOAc	isopropenyl acetate/$CHCl_3$
1	2		4	
9	6		21	
24	18	0	53	0

Table 2. Comparison of diol 2 concentrations. (neat MeOAc,1:1 PPL:2, ambient temp.)

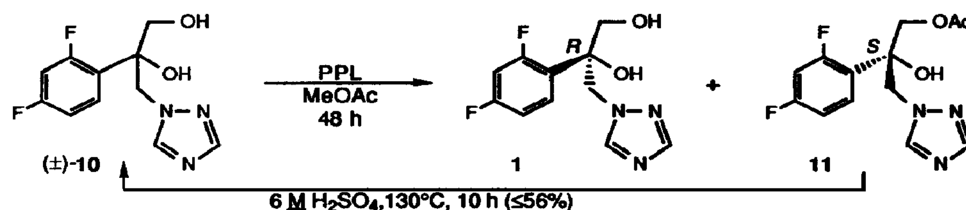
time (hr)	% conversion to mono-acetate			
	0.5 M	0.3 M	0.05 M	0.01 M
1	1	7	4	3
24	19	50	59	44
48	34	69	78	54

From this data, the following procedure was adopted:

Compound **2** (12 g) was dissolved in 0.77 L reagent grade methyl acetate, and the solution degassed by alternating vacuum and nitrogen. Sigma 3126 PPL[7] (36 g) was added, the mixture was mechanically stirred for 48 hours, and then it was suction-filtered to separate the enzyme material.[12] The filtrate was evaporated and the residue crystallized from 200 mL benzene to give 5.7 g (42%) isomerically pure **6**. Chromatography of the mother liquor and crystallization of the isolate gave an additional 4.8 g **6** (total 10.5 g, 77%, ≥96% ee).

The attempt at PPL-catalyzed hydrolysis of the di-primary acetate of **2** to afford the diastereomeric monoacetate complementary to **6** was unsuccessful, giving instead a random mixture of both monoacetates along with fully hydrolyzed **2**. This would have been the most desirable situation since this other monoacetate might have been derivatized and cyclized directly without additional protection–deprotection steps.

The initial utility of PPL catalyzed acylation in this overall scheme evolved from the structural similarity between **5** and the racemic 1° alcohol **10**.[14] Compound **5** had been acetylated with a crude Sigma PPL giving 70% conversion but only 53% ee,[6,13] which was raised to 95% conversion and 99% ee by first precipitating the crude enzyme onto Celite. When **10** was acetylated using the same procedure given above for **2**, with no pre-treatment of the PPL, the (*S*)-acetate **11**[15] was produced leaving the (*R*)-diol **1**[1] which was readily isolated by precipitation from CH_2Cl_2 and crystallization from CH_3CN in 66% conversion (33% yield) and ≥98% ee (Scheme 3). Simultaneous hydrolysis and racemization of **10** could be accomplished with aqueous sulfuric acid[16] to regenerate **10** for recycling in 45-56% yield, the balance going to oligomeric tars. This approach complemented the reported[1] chiral epoxide based synthesis of **1**.



Scheme 3

We have demonstrated separate applications of stereoselective PPL-catalyzed transesterification in organic solvent at two stages of a multi-step synthesis to obtain the potentially useful pharmaceutical intermediate **4** having two chiral centers, from achiral starting material and without traditional chiral reagent or chiral auxiliary based chemical reactions. For the first center, racemic 1° alcohol **10** was enzymatically resolved to afford the chiral product **1** in respectable total 50% yield after one recycle. For the second center, the prochiral 1,3-diol **2** was asymmetrized through

highly stereoselective enzymatic acetylation in 77% isolated yield and ≥96% ee to give useful absolute stereochemistry. These examples may contribute to the increasing acceptance of enzyme-catalyzed transformations to solve traditional organic synthetic problems, and help to better define the scope of PPL-catalyzed transesterification.

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6. Bianchi, D.; Cesti, P.; Spezia, S.; Garavaglia, C.; Mirenna, L. *J. Agric. Food Chem.* **1991**, *39*, 197-201.
7. Sigma Chemical Co., St. Louis, MO 63178-9916, USA. This product, available inexpensively in bulk, is a crude preparation known to contain some amounts of esterases[3a] and is largely "inert" material. However, batch-to-batch reactivity has been quite consistent in our experience. When an attempt was made to obtain purified PPL[3a] (triacylglycerol acyl hydrolase, EC 3.1.1.3) from this crude product, the subsequent acylations were unsatisfactory, hence the importance of batch-to-batch consistency.
8. For examples in carbohydrates, see: Colombo, D.; Ronchetti, A.; Scala, A.; Taino, I.M.; Toma, L. *Bioorg. Med. Chem.* **1993**, *1*, 375-380.
9. This and subsequent compounds were examined for chemical homogeneity >98% by normal phase LC, and chiral composition where applicable by HPLC on a chiralcel® OD column with an appropriate EtOH-hexane eluant. They were characterized by consistent mass spectra and pmr spectra, and comparison to authentic samples[1] where available. Selective analytical data are given.
   6: $[\alpha]_D^{21}$ -17.8° (c=1, MeOH); mp 98-102°C; H^1 NMR ($CDCl_3 + D_2O$, 200 MHz) δ 7.91 (s,1H), 7.82 (s,1H), 7.48 (m,1H), 6.79 (m,2H), 4.77 (d,1H), 4.55 (d,1H), 4.14 (m,2H), 3.40 (m,2H), 2.10 (m,1H), 2.08 (s,3H), 1.89 (m,2H).
   7: H^1 NMR ($CDCl_3 + D_2O$, 200 MHz) δ 8.11 (d,1H), 7.79 (s,1H), 7.45 (m,1H), 6.78 (m,2H), 4.51 (d,1H), 4.41 (m,1H), 4.14 (m,2H), 3.69 (m,2H), 3.48 (m,3H), 3.13 (m,1H), 2.40 (m,1H), 1.9-1.4 (m,8H).
10. [(*R*)-*cis*]-4: $[\alpha]_D^{21}$ -39° (c=1, $CHCl_3$). Lit.[1] $[\alpha]_D^{21}$ -39.6° (c=1, $CHCl_3$).
11. Under these conditions, some batches produced the other diastereomer, calculated by HPLC at no more than 2% of total monoacetate.
12. The recovered enzyme could be dried and re-used without noticeable loss of stereoselectivity, but subsequent reactions were slower. No effort was made to optimize recycle.
13. At 0.2M substrate in ethyl acetate for 15 hours in a 2:1 weight ratio.
14. Saksena, A.K.; Cooper, A.B.; Guzik, H.; Girijavallabhan, V.M.; Ganguly, A.K. U.S. Patent 4 379 752, **1988**.
15. 11: $[\alpha]_D^{21}$ -58.7° (c=1.1, $CHCl_3$); H^1 NMR ($CDCl_3$, 200 MHz) δ 8.11(s,1H), 7.86 (s,1H), 5.05 (br s,1H), 4.80 (d,1H), 4.69 (d,1H), 4.42 (ABq,2H), 2.05 (s,3H).
16. A more efficient but laborious multi-step alternative was also used: hydrolysis of the acetate (NaOH; quant.), 1° tosylation (TsCl, DMAP, CH_2Cl_2; ~95%), cyclization to epoxide (NaH, DMF, r.t., 0.5 hr.; ~95%), and hydrolytic epoxide cleavage (9:1 $HCOOH$-H_2O, reflux, 18 hr.; 75%).

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