Enzymatic Desymmetrization of Prochiral 2-Substituted-1,3-propanediols: A Practical Chemoenzymatic Synthesis of a Key Precursor of SCH51048, a Broad-Spectrum Orally Active Antifungal Agent

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Two examples of a practical enzymatic desymmetrization of a 2-substituted-1,3-propanediol and their application to the synthesis of SCH51048, a broad-spectrum orally active antifungal, are described. In each case, enzymatic catalysis under both hydrolytic and transesterification conditions is described. In the first example the key intermediate, the *R*,*S*-monoester of triol **6**, was obtained via Amano Lipase AK catalyzed hydrolysis of the dibutyrate **11b**, or Novo SP435 catalyzed acetylation of triol **6**. In the second example, desymmetrization of diol **13a** using Novo SP435 or of dibutyrate **13c** using Amano Lipase CE furnished the *S*-monoester (*S*)-**14b**,**c**, a key intermediate in a new efficient synthesis of SCH51048. Optimization of the Novo SP435 acetylation of diol **13a** and the scaleup of the reaction is also described.

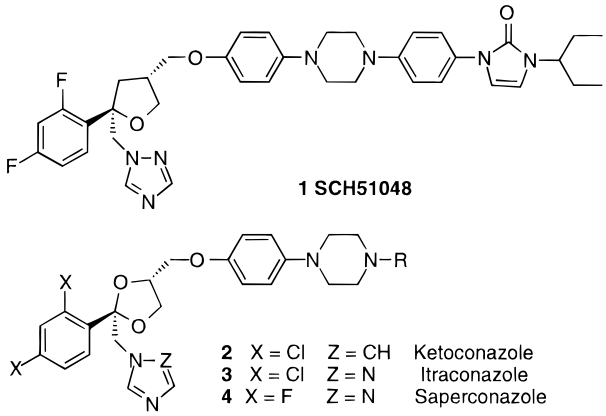
Introduction

SCH51048 (**1**) was identified as a potential antifungal agent with efficacy in the treatment of systemic *Candida* and *Aspergillus* infections in both normal and immunocompromised models.[1] In common with other azole antifungals (Chart 1), SCH51048 (**1**) contains the heterocyclic ring, a dihalogenobenzene ring and a rigid side chain, distributed around a central five-membered ring. Unlike Ketoconazole (**2**), Itraconazole (**3**) and Saperconazole (**4**), which contain a central 1,3-dioxolane ring, SCH51048 contains a central tetrahydrofuran ring which confers increased efficacy.[1e] Construction of this 2,2,4-trisubstituted ring with the required stereochemistry presented a synthetic challenge.

The initial approach[1c] introduced chirality (88−92% ee) via a Sharpless−Katsuki epoxidation[2] of the allyl alcohol **5**. A series of further reactions yielded the desired *R*-triol **6**, in which the enantiomeric excess had been enhanced to >98%. Tosylation of the two primary hydroxyls then gave ditosylate **7** (Scheme 1).

The tetrahydrofuran ring could now be formed via nucleophilic stereoselective displacement of one of the two hydroxyls by the tertiary hydroxyl under basic conditions (path a or b in Scheme 1). However, under all tested conditions, it was the undesired 2*R*,4*R*-trans isomer **8b** which predominated (60 trans:40 cis), and isolation of the desired 2*R*,4*S*-cis isomer **8a** required careful chromatography (25−30% yield from the triol **6**). Clearly the yield of the desired material could only be maximized by differentiation of the two primary hydroxyls prior to cyclization to the tetrahydrofuran ring. If the pro-*S* hydroxyl group of triol **6** could be blocked, tosylation and cyclization would then yield the required 2*R*,4*S*-cis isomer **8a**.[3] Since triol **6** is a 2-substituted-1,3-propanediol, enzymatic desymmetrization suggested itself as a convenient method to differentiate between the two primary alcohols.[4]

Chart 1



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(2) Katsuki, T.; Sharpless, K. B. *J. Am. Chem. Soc.* **1980**, *102*, 5974−5976.

(3) Blocking the pro-S hydroxyl of triol **6**, followed by tosylation, cyclization, deblocking, and tosylation would result in a five-step sequence to cis tosylate **8a**. Alternatively, blocking the pro-R hydroxyl would require a seven-step sequence to **8a** because of the extra steps to invert the 2*R* chiral center. Pro-R hydrolysis of the triol diester **10** or **11** would require six steps to **8a**.

(4) (a) Ramos Tombo, G. M.; Schar, H.-P.; Fernandez i Busquets, X.; Ghisalba, O. *Tetrahedron Lett.* **1986**, *27*, 5707−5710. (b) Boland, W.; Frössl, C.; Lorenz, M. *Synthesis* **1991**, 1049−1072. (c) Danieli, B.; Lesma, G.; Passerella, D.; Riva, S. In *Adv. Use Synthons Org. Chem.* **1993**, *1*, 143−219. (d) Schoffers, E.; Golebiowski, A.; Johnson, C. R. *Tetrahedron* **1996**, *52*, 3769−3826.

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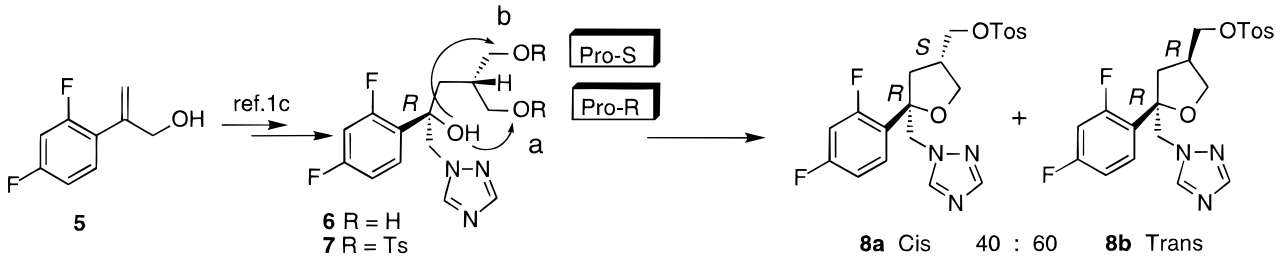
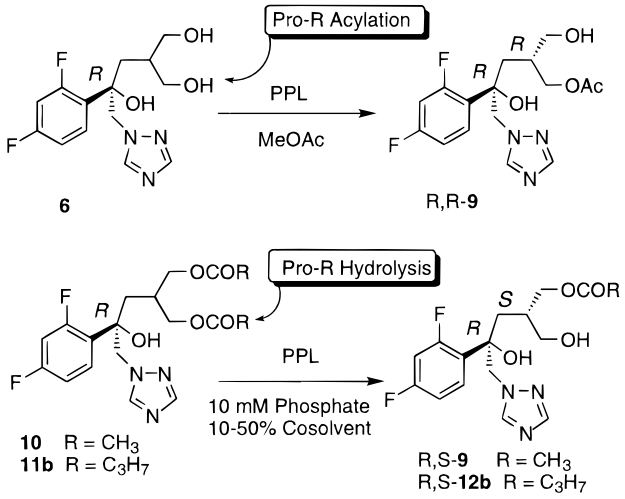
Scheme 1



Scheme 2



Results and Discussion

A. Desymmetrization of Triol 6. The enzymatic acetylation of triol **6** in neat methyl acteate using porcine pancreatic lipase (PPL Sigma Type II) had been previously reported to display pro-*R* selectivity to give (*R*,*R*)-**9** (Scheme 2), and six subsequent steps were required to arrive at the desired 2*R*,4*S*-cis tosylate **8a**.[1d,3] However, in our hands under the reported conditions, this acylation resulted in a mixture of diol, diacetate, and monoacetate with marginally useful diastereomeric excess (85−90% de).

Lipases generally display the same prochiral selectivity for the acylation of 2-substituted-1,3-propanediols and for the hydrolysis of the corresponding diesters. As a result, products of opposite absolute stereochemistry may be obtained using the same enzyme under either acylating or hydrolytic conditions (Scheme 2).[4b] Consequently, the PPL-catalyzed hydrolysis of the diesters **10** and **11b** was examined.[3] However the reaction showed poor selectivity. Extensive overhydrolysis to triol **6** occurred and the monoester **9** was formed in poor de. Furthermore prochiral selectivity varied with reaction conditions.[5]

A screen of our enzyme collection was undertaken to identify enzymes showing high pro-*S* selectivity for the acylation of triol **6**. Of the 86 commercially available enzyme preparations which were initially tested, 16 showed significant pro-*R* selectivity, using vinyl acetate (10 equiv) as acylating agent in EtOAc (Scheme 3). These gave the less desired monoacetate ((*R*,*R*-**9**), in some cases with moderate to high diastereomeric excess (Table 1). The 12 enzymes which showed the desired pro-*S* selectivity all suffered from low selectivity and/or rate of acylation. From these 12, Amano Lipase R (yielding the (*R*,*S*)-**9** isomer in 78% de at 20% conversion) was chosen for a study of different solvents and different acetylating agents, but no conditions were found to improve the diastereoselectivity.

Since a highly selective pro-*S* enzyme had not been identified, further work was undertaken with Amano Lipase AK, the most selective of the pro-*R* enzymes. Two approaches were possible.

(i) **Acylation**: Pro-*R* acetylation provided the monoacetate (*R*,*R*)-**9** in high de,[6] but a subsequent protection/deacylation sequence was required to invert the newly formed *R*-chiral center, resulting in a seven-step sequence from *R*-triol **6** to the cyclized cis tosylate **8a**.[3]

(ii) **Hydrolysis**: Since lipases generally display the same prochiral selectivity for acylation and hydrolysis reactions, Lipase AK catalyzed hydrolysis of the diacetate **10** would provide the desired monoacetate (*R*,*S*)-**9** in an overall six-step sequence from *R*-triol to cis tosylate **8a**.

Initial hydrolyses in phosphate buffer yielded monoacetate (*R*,*S*)-**9** with low de, so the effect of cosolvents, both miscible and immiscible, was examined. For miscible solvents the best de's were observed with 10−20% THF; when increased to 50% THF no reaction was observed. With 5% MeCN or 10% acetone the reaction was slower and less selective. Of the ether solvents, the best results were obtained with 20% Et_2O; 20% iPr_2O and 30% TBME resulted in product of low de.

The hydrolysis of a series of diesters was examined in 10−20%THF/50 mM KCl (pH 7.0) (Table 2). While the *R*,*S*-monoesters were formed with high de, the selectivity was poor and substantial amounts of overhydrolysis to triol occurred before complete consumption of starting material.

Nevertheless, hydrolysis of the dibutyrate **11a** was a convenient process, not only because of its high initial rate. Due to its low water solubility, the monobutyrate **12a** could be partially purified after the enzymatic step; aqueous extraction of the crude product preferentially removed the more water soluble triol **6** which was formed by over hydrolysis. Furthermore, the chemical acylation and enzymatic hydrolysis reactions could be carried out in a one-pot reaction. When butrylation of the triol **6** in

(5) The acylation of **6** and the hydrolysis of **10, 11b** varied with enzyme lot and with the nature of the acyl group. Using Sigma PPL Type II, lot no. 23H0294 was more reactive than lot no. 41H0954 under acylation conditions. With both enzyme samples butyrylation (10 equiv of trifluoroethyl butyrate in TBME; 2× enzyme) was faster than acetylation (neat MeOAc; 3× enzyme); however the *R*,*R*-monoester was formed in poor de under both conditions. Under hydrolytic conditions (10 mM phosphate buffer; pH 7; 10% THF, 10% MeCN or 50% TBME as cosolvent) lot no. 41H0954 was more reactive than lot no. 23H0294. Hydrolysis of the diacetate **10** yielded the expected R,S-monoacetate **9** with generally poor de. For hydrolysis of the dibutyrate **11b** under the same conditions, lot no. 23H0294 showed poor reactivity (10% conversion in 10%MeCN/buffer and 4% conversion in 50%TBME/buffer after 6h) but produced the expected R,S-monobutyrate **12b**. In contrast, lot no. 41H0954 produced the *R,R-monobutyrate* under all hydrolytic conditions tested, albeit in poor yield.

(6) After 2 h, a mixture of triol **6** (6.5 g), vinyl acetate (10 equivs), and lipase AK (6.6 g) in EtOAc (150 mL) showed a **6**:**9**:**10** ratio of 1:98:1. After workup (*R*,*R*)-**9** was obtained in 81% yield (97% de; $[\alpha]^{21}_D$ −17.7 (c 0.46, MeOH); lit.[1d] $[\alpha]^{21}_D$ −17.8 (c 1, MeOH))

Scheme 3

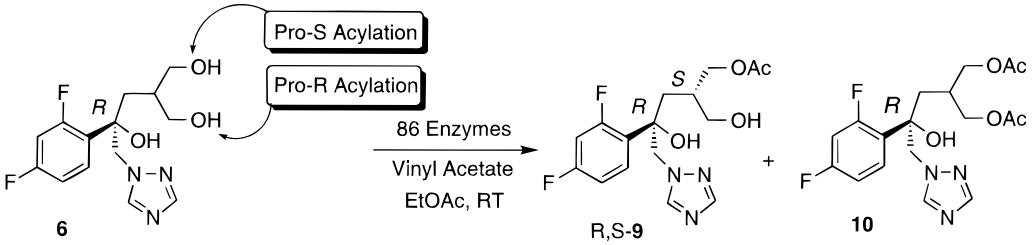
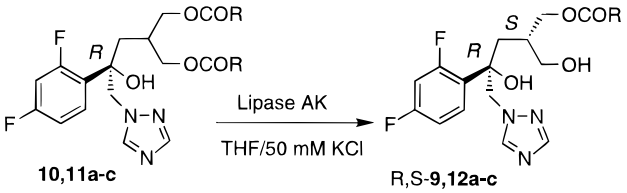


Table 1. Acetylation of Triol 6. Screen Results[a]

Enzyme	time, h	% triol **6**	% monoacetate **9**	% diacetate **10**	% de
Pro-*R* Acylating Enzymes					
Amano Lipase AK (*Pseudomonas sp.*)	21	0	92	8	98.1
Novo Lipozyme (*Mucor miehei*)	21	0	53	47	93.5
Amano Lipase PS-30 (*Pseudomonas fluorescens*)	21	0	76	24	92.4
Amano Lipase CES (*Pseudomonas sp.*)	21	1	97	2	92.1
Sigma Porcine Pancreatic Lipase Type II	21	0	98	2	85.7
Solvay Porcine Pancreatic Lipase	21	31	69	0	84.3
Biocatalysts *P. fluorescens*	19	1	94	5	84.0
Pro-*S* Acylating Enzymes					
Amano Lipase R (*Penicillium roquefortii*)	21	83	17	0	77.6
Interspex Bacterial Lipase/Esterase	48	55	39	5	73.5
Amano Newlase A (*Aspergillus. niger*)	49	39	58	3	72.1
Quest Acid Protease 200,000	49	78	21	0	56.7
Meito OF (*Candida cylindracea*)	21	41	56	3	55.5
Sigma Protease Type IV (*Streptomyces caespitosus*)	49	67	32	0	54.3
Amano Lipase G (*Penicillium camembertii*)	21	43	57	0	36.1
Biocatalysts *C. cylindracea*	21	82	18	0	33.8
Biocatalysts *P. ciclopium*	21	87	9	4	31.3
Enzeco Fungal Acid Protease	48	32	64	3	26.2
Sigma Protease Type XXIV	49	80	18	2	23.6
Solvay AFP 2000	48	2	75	23	21.5

[a] Conditions: triol **6** (0.11−0.16 mmol), vinyl acetate (7−43 equiv), EtOAc 1.0 mL, enzyme 8-180 mg, 250 rpm, rt.

Table 2. Hydrolysis of Diesters 10 and 11a−d with Lipase AK[a]



	R	cosolvent	time, h	% triol	% monoester	% diester	% de	initial rate μmol/h/mg enzyme
10	CH_3	10% THF	48	17	57	26	97.0	0.03
11a	$CH_2CH_2CH_3$	10% THF	3	5	57	38	96.7	0.46
11b	$CH(CH_3)_2$	20% THF	49	10	81	9	95.9	0.03
11c	$(CH_2)_4CH_3$	20% THF	6	16	64	20	>98	0.17

[a] Conditions: diester (60−100 mg); enzyme (60−100 mg)(except for **10,** 300 mg); 50 mM aqueous KCl; pH 7.0; rt.

THF was complete, the entire reaction mixture was diluted 10 fold with 50 mM KCl, the pH adjusted to 7.0, and the enzyme added. From this reaction mixture, the monobutyrate **12a** was obtained (Scheme 4), essentially free of triol, in good yield (64%) with 99% de.

(iii) **Pro-*S* Acylation**: As the hydrolysis of the dibutyrate **11b** was being developed, an enzyme displaying useful levels of the desired pro-*S* selectivity was finally identified. Treatment of triol **6** with Novo SP435 in EtOAc or MeCN with vinyl acetate as acylating agent resulted in formation of (*R*,*S*)-**9** with 94−97% de (Table 3).

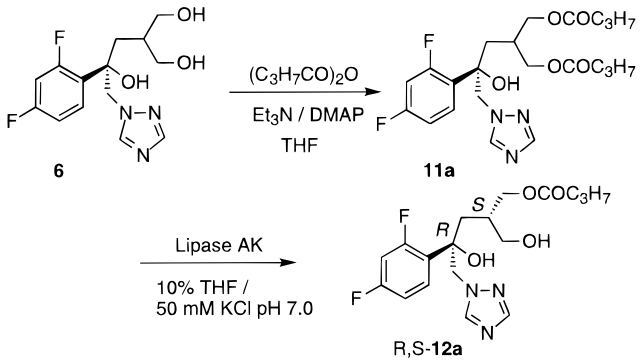
B. Improved Route to Tosylate 8a. Before either the acylation or hydrolysis routes to the key monoesters **9**, **12** could be exploited, a more efficient route to cis tosylate **8a** was identified.[1f] In this sequence (Scheme 5) the tetrahydrofuran ring is formed *via* an iodocyclization reaction from diol **13a**. A 2*S* chiral center resulting from a enzymatic desymmetrization of diol **13a** could be used to control the stereochemistry of the iodocyclization reaction, forming predominantly the cis iodide **15** and leading ultimately to the desired (*R*,*S*)-tosylate **8a**.[7]

From an initial screen of 53 hydrolases, 3 enzyme preparations were found to provide the monoacetate **14b** with high enantiomeric excess (Table 4). The screen was subsequently extended, and a total of 205 commercial enzyme preparations have been examined under similar conditions; only one other enzyme preparation, Chirazyme L-2 (Boehringer-Mannheim),[8] displayed high pro-*S* selectivity.

(7) Acylation of the pro-*S* hydroxyl of **13a** would result in a five-step sequence to cis tosylate **8a** (acylation, iodocyclization, triazole introduction, deacylation, and tosylation), while pro-*R* acylation would require two extra steps to invert the 2*R* chiral center. Pro-*R* hydrolysis of the diester **13a**,**b** would result in a six-step sequence to **8a**.

(8) Both Novo SP435 and Boehringer-Mannheim Chirazyme L-2 are immobilized forms of Lipase Type B from *Candida antarctica.*

Scheme 4



Scheme 5

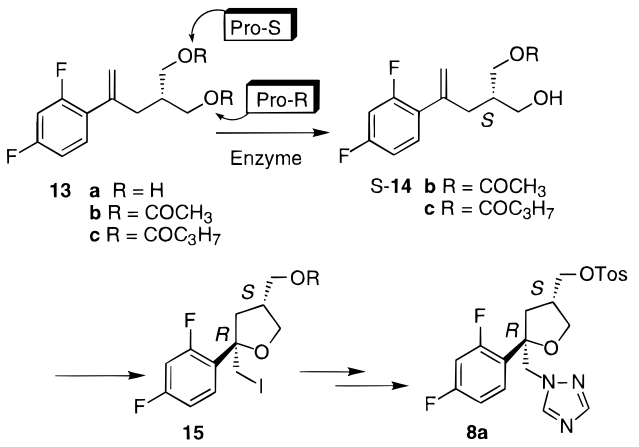
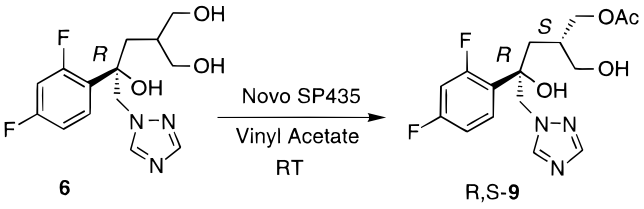


Table 3. Acetylation of Triol 6 with Novo SP435[a]



run	solvent	time, min	% triol **6**	% monoacetate **9**	% diacetate **10**	% de
1	EtOAc	75	5	83	12	94.0
2	MeCN	55	4	95	1	96.6

[a] Conditions: run 1: triol 64 mg, enzyme 15 mg, EtOAc1 mL, vinyl acetate 10 equiv. Run 2: triol 0.5 g, SP435 51 mg, MeCN 5 mL, vinyl acetate 2 equiv, rt.

Scheme 6

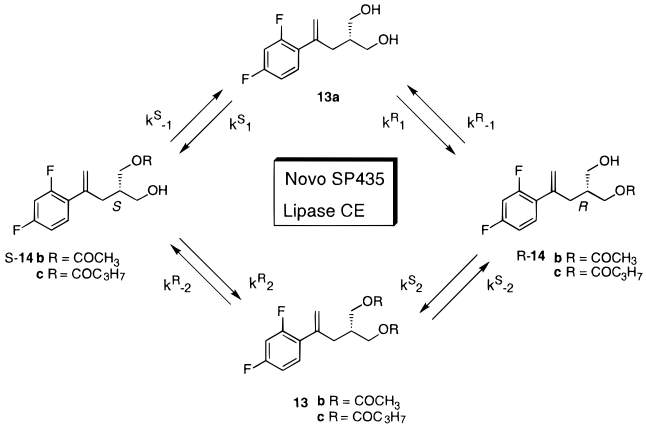


Table 4. Acetylation of Diol 13a: Initial Screen Results[a]

enzyme	time, min	% diol **13a**	% monoacetate **14b**	% diacetate **13b**	% ee	pref
Lipase SP435 (Novo)	60	0	83	17	97	pro-*S*
Lipase CE (*H. lanuginosa*) (Amano)	95	0	97	3	99	pro-*R*
Lipase *H. lanuginosa* (Biocatalysts Ltd.)	220	1	98	1	98	pro-*R*

[a] Conditions: diol 50 mg; enzyme 10−200 mg; vinyl acetate 10 equivs; toluene 1.0 mL; rt.

The availability of two enzymes with opposite prochiral selectivity allowed three approaches to the preparation of the desired *S*-monoester precursor.

(i) Pro-*R* acetylation of diol **13a** using Lipase CE gave the monoacetate (*R*)-**14b** with high chemical and optical yield. As the reaction profile indicated, $k^{R}_{1} >> k^{S}_{1}$ or k^{S}_{2} (Scheme 6). However, protection/deacylation was required to invert the chiral center, resulting in a seven-step sequence from diol **13a** to tosylate **8a**.[7] Since attempts to introduce a tetrahydropyranyl protecting group resulted in significant racemization, presumably via 1,3 acyl migration, this route was not pursued and was only used to prepare small quantites of (*R*)-**14b**.

(ii) Lipase CE catalyzed pro-*R* hydrolysis of the diester would yield the *S*-monoester ($k^{R}_{-2} >> k^{S}_{-2}$), for a six-step sequence from diol **13a** to tosylate **8a**.[7] While hydrolysis of the dibutyrate **13c** produced the monobutyrate (*S*)-**14c** with high enantiomeric excess, the chemoselectivity was poor, and the reaction mixture consisted of a mixture of diol **13a**, dibutyrate **13c**, as well as the desired monoester (*S*)-**14c**.

(iii) SP435 catalyzed pro-*S* acetylation of diol **13a** provided (*S*)-**14b** directly for a five-step route to tosylate **8a**.[7] Because the prochiral selectivity of this enzyme is lower than for Lipase CE ($k^{S}_{1}/k^{R}_{1} \approx 20$), optical purity was purchased at the cost of chemical yield, and a significant amount of the diacetate **13b** had to be formed before the ee of the remaining monoacetate **14b** reached useful levels (Scheme 6 and Figure 1).[9]

Nevertheless, because it provided the *S*-monoester directly under operationally simple conditions, the SP435 catalyzed acetylation of diol **13a** was selected for optimization.

Ironically, the first problem was not improvement, but reproduction of the favorable results from the initial screen. Diol **13a** is prepared by reduction of the corresponding 2-substituted diethyl malonate and samples from different sources ($LiAlH_4$ vs $NaBH_4$/LiCl reduction, chromatographically pure vs crude product) displayed different reaction rates and selectivities. The different reaction rates were attributed to moisture in the samples, since the rate of diol consumption could be decreased by addition of small amounts of water, while molecular sieves increased the rate.

More disturbing was the observation that, for certain samples, significant amounts of diol remained unconsumed, even though the reaction was run out to high diacetate formation.

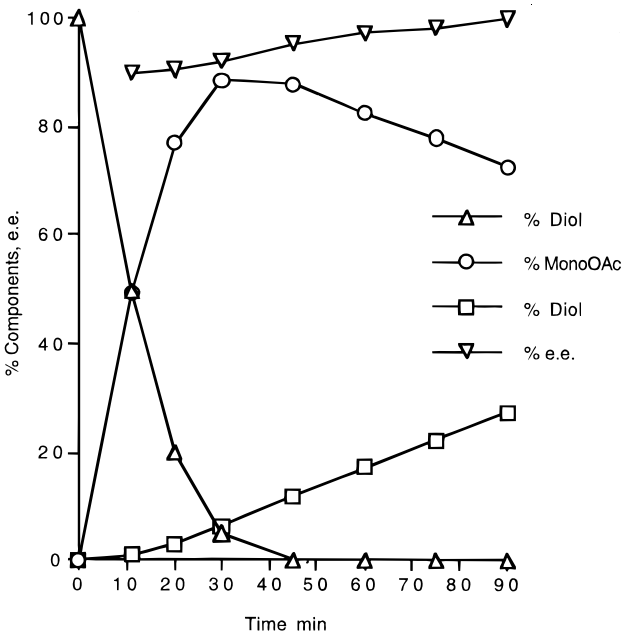


Figure 1. Acylation of **13a** with Novo SP435: Diol, 50 mg; SP435, 10 mg; vinyl acetate, 10 equiv; toluene, 1.0 mL; rt.

Table 5. Incomplete Enzymatic Acetylation[a]

run	solvent	vinyl acetate equiv	diol/enzyme ratio (w/w)	time (min)	product distribution (%) diol **13a**	monoacetate **14b**	diacetate **13b**
1	toluene	2.2	10	175	90	10	0
2	MeCN	2.2	10	105	93	7	0
3	MeCN	5.0	4.4	1080	46	38	17
4	MeCN	5.0	4.4	1170	35	32	33

[a] Conditions: diol, 0.5 g; SP435, 50 mg; solvent, 10 mL; 0 °C.

For one sample (Table 5, runs 1−3) the reaction was sluggish in both toluene and MeCN at 0 °C, with <10% reaction after 2−3 h. Despite increasing the amount of enzyme, acylating agent, and reaction time, 46% of the diol **13a** remained, even though 17% diacetate **13b** had been formed. Similar results were observed with a second sample (run 4). ^{1}H NMR (DMSO-d_6) examination of the crude reduction product revealed that a significant amount of diol **13a** was complexed as a borate ester (from $NaBH_4$ reduction of the malonate ester) and was unavailable for reaction under the reaction conditions.[10,11] (During HPLC analysis (5%EtOH/hexane) transesterification of the borate ester revealed the unreacted diol.) Refluxing the partially complexed diol with AcOH/MeOH, removal of trimethyl borate by distillation, and crystallization from toluene resulted in a product which underwent enzymatic acetylation reproducibly without incident.

With a secure supply of boron-free, crystallized diol **13a**, optimization of the reaction was examined. Since

Scheme 7

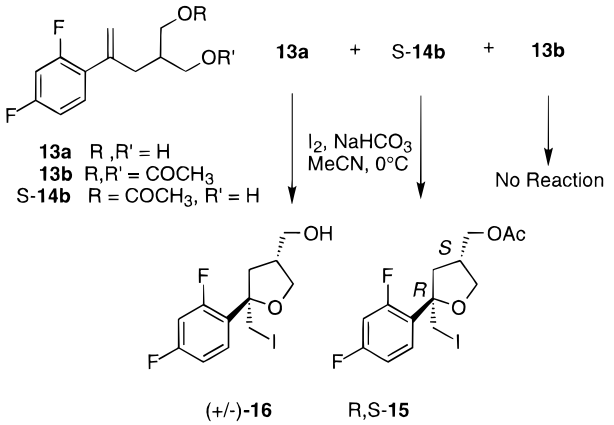


Table 6. Product Distribution and Enantiomeric Excess at >94% Diol Conversion in Various Solvents[a]

run	solvent	vinyl acetate equiv	diol/ enzyme ratio (w/w)	time (min)	product distribution (%) diol **13a**	mono-acetate **14b**	di-acetate **13b**	% ee (*S*)
1	iPr_2O	10.0	4.0	90	6	84	10	91
2	THF	10.0	4.0	120	2	77	22	90
3	dioxane	10.0	4.0	90	1	75	24	93
4	acetone	10.0	4.0	90	1	83	16	94
5	iPrOAc	2.0	9.0	260	1	78	21	97
6	tBME	2.0	9.0	226	3	75	22	94
7	MeCN	5.0	4.0	60	1	85	14	96
8	toluene	5.0	4.0	120	1	85	14	96

[a] Conditions: diol 50 mg; solvent 1.0 mL; 0 °C (except run 3, 20 °C).

the selectivity of SP435 is not absolute and the enantiomeric excess of the monoacetate (*S*)-**14b** increases over the course of the reaction, albeit at the expense of chemical yield, the question was what compromise should be struck between optical and chemical yield. Like the *S*-monoacetate **14b**, unreacted diol **13a** also undergoes iodocyclization to yield racemic material **16** (Scheme 7) which, if carried forward would dilute the optical purity of the key precursor, cis tosylate **8a**. The diester **13b** was shown not to react and may be recovered as the diol at a subsequent stage.

While minimization of both diol and diester would be desireable, it is more important that the amount of unreacted diol be minimized. At the outset it was not clear how much of the wrong enantiomer could be purged in the subsequent steps to SCH51048, so the following specification was applied: the acetylation would be monitored until the enantiomeric excess of the *S*-monoacetate **14b** was >95% with <2% diol **13a** remaining.

With this constraint the effect of temperature, solvent, acylating agent, and substrate and enzyme loading on the SP435 catalyzed acetylation of diol **13a** was examined. Decreasing the temperature to 0 °C slowed the reaction in toluene, but yielded monoacetate **13b** in higher optical purity.

For a series of solvents the reaction mixture compositions were compared when ~2% diol remained (Table 6).[12] The best results were obtained with toluene, MeCN, and

(9) Wang, Y.-F.; Chen, C.-S.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1984**, *106*, 3695−3696.

(10) While boronic acids are well known reversible inhibitors of serine proteases (Jones, J. B.; Martichonok, V. *J. Am. Chem. Soc.* **1996**, *118*, 950−958 and refs 14,15 therein), we are unaware of reports of borate esters as inhibitors. However, the fact that diacetate **13b** continued to be produced while diol **13a** apparently remained unconsumed, suggested that the borate ester does not inhibit Novo SP435.

(11) Subsequent acylations which were doped with increasing amounts of trimethyl borate resulted in a corresponding increase in the amount of unreacted diol. ^{1}H NMR and mass spectrometry confirmed the presence of the cyclic borate ester (see Supporting Information).

(12) Unlike Novo SP435, Lipase CE catalyzed acetylation showed a pronounced solvent dependence. From a comparison of 10 common solvents, the reaction was fastest in neat vinyl acetate, toluene, or tBME. As for SP435, the Lipase CE acetylation was very slow in isopropenyl acetate at room temperature, but unlike SP435 the reaction was also very slow in MeCN. Furthermore, excess vinyl acetate was required for reasonable reactivity; at 1.5−2.0 equiv of vinyl acetate the reaction was considerably slower.

iPrOAc all of which showed ee > 0.97 with <2% diol remaining. Acetone, tBME, and dioxane were less useful, and THF, iPr_2O, and *tert*-amyl alcohol (results not shown) gave poor selectivity. MeCN was selected as the reaction solvent since the subsequent iodocyclization step was also carried out in MeCN at 0 °C, and the prospect of simply removing the enzyme beads by filtration and carrying out the iodocyclization on the crude reaction mixture was especially attractive.

A range of acylating agents was surveyed, either neat, or in toluene or MeCN solution. The best results were obtained with vinyl acetate or acetic anhydride. Since vinyl acetate releases acetaldehyde during transesterification, there was concern whether the recovery and reuse of the enzyme would be compromised.[13] Isopropenyl acetate, which releases acetone upon transesterification, was examined under similar conditions, but was less reactive. When alkyl acetates (Me, Et, iPr) were used as both acylating agent and solvent, the reactions reached equilibrium before diol consumption was complete, and the monoacetate **13b** was produced with low ee. With MeOAc in the presence of molecular sieves adsorption of the released MeOH shifted the equilibrium toward the diacetate, and the monoacetate was formed in high ee. None of the trifluoroethyl esters which were tested were superior to vinyl acetate. Under similar conditions, both trifluoroethyl acetate and butyrate were slower than vinyl acetate and less readily available. It was hoped that the use of a bulky acylating agent would decrease diester formation and increase the selectivity of the initial acylating event. Using trifluoroethyl isobutyrate did suppress diester formation, but the monoester was formed with low ee; even after extended reaction and formation of diisobutyrate, the ee remained low. With trifluoroethyl 2-methylbutyrate, diester formation was completely suppressed, but the monoester was also formed in very low ee. Vinyl butyrate was less selective than, and offered no advantage over, vinyl acetate. Acetic anhydride was similar to vinyl acetate in both reactivity and selectivity, but presented some disadvantages. The effect of released acetic acid and unconsumed anhydride on the optical purity of the monoacetate was a concern, because of the potential for 1,3 acyl migration under acidic conditions or nonenzymatic acetylation. For batches which must be stored after removal of the enzyme, an aqueous extraction would be necessary to completely remove acid and anhydride before the reaction mixture could be used in the subsequent base-catalyzed iodocyclization. For operational simplicity, vinyl acetate was retained as the acylating agent of choice.

Although ~20% overreaction to the diester was required to furnish monoester of acceptable enantiomeric excess, the reaction could be carried out with as little as 1.25 equiv of vinyl acetate. However, 1.4−2.0 equiv were generally used to ensure low levels of diol. Diol concentration was increased from 0.2 M in early reactions to 0.9 M (200 g/L) in MeCN, without any loss of performance. Enzyme loading was decreased to 5% (w/w) and could be decreased further to 1% with a corresponding 5-fold increase in reaction time.

To demonstrate enzyme recovery and reuse, a 50 mg sample of SP435 was carried through 10 reaction cycles over a period of 14 days. After 10 cycles the enzyme sample still produced monoacetate of acceptable quality

(13) Weber, H. K.; Stecher, H.; Faber, K. *Biotechnol. Lett.* **1995**, *17*, 803−808.

Scheme 8

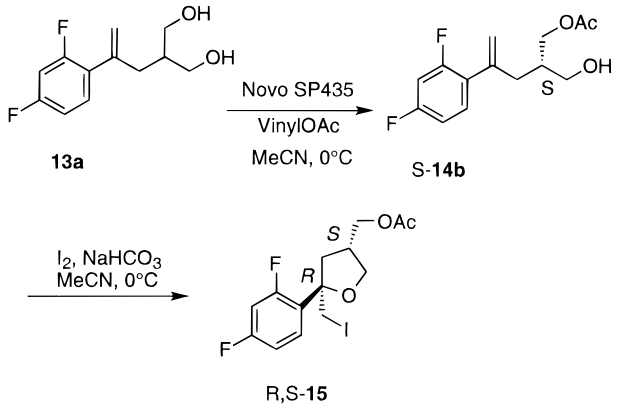


Table 7. Scaleup of Enzymatic Acetylation

run	diol **13a**, kg	% diol **13a**	% monoacetate S-**14b**	% diacetate **13b**	% ee
1	9	2	81	17	97[a]
2	33	<1	74	26	99[b]

[a] ee of (*S*)-**14b**. [b] ee of (*R*,*S*)-**15b**.

(97% ee), but the rate of the reaction had slowed down considerably. This decrease in rate was probably due more to mechanical losses of enzyme during recycling than to inactivation of the enzyme.

The conditions transferred to pilot plant for scaleup were as follows: 20% diol **13a** solution i.e., 200 g/L; 5% Novo SP435 loading (w/w); 2.0 equiv of vinyl acetate; industrial grade MeCN; 0 °C reaction temperature; monoacetate specification: (*S*)-**14b** > 95% ee; <2% diol **13a** remaining. A representative result is shown in Table 7 (run 1). The specifications for the enzymatic acylation were subsequently tightened to produce monoacetate (*S*)-**14b** of 98−99% ee with <1% unreacted diol remaining. This could be achieved using the above conditions but running the acetylation out to ~30% diacetate formation (Table 7, run 2).

Conclusion

Optically enriched 2,2,4-trisubstituted tetrahydrofuran (*R*,*S*)-**15b** is now prepared by an asymmetric synthesis, the key steps of which are an enzymatic desymmetrization of a 2-substituted-1,3-propanediol and a subsequent, efficient iodocyclization. The acetylation of diol **13a**, catalyzed by Novo SP435, has shown itself to be a robust, reproducible and economical step in the synthesis of SCH51048. Further efforts to improve the prochiral selectivity of this enzymatic conversion are in progress and will be reported in due course.

Experimental Section

General. HPLC was carried out on a Waters 715 Ultra Wisp. For triol **6** and related compounds the instrument was equipped with a Zorbax Rx-C18 (50% MeOH/50 mM phosphate buffer (pH 5.7) or 30% MeCN/H_2O; 1 mL/min; 210 or 254 nm; rt), a Novapak C18 column (Waters)(50% MeOH/20 mM phosphate buffer (pH 7); 1 mL/min; 210 or 254 nm; rt), or a YMC-Pack ODS-A column (40−50% MeCN/H_2O; 1 mL/min; 215 nm; rt). Under all conditions the order of elution was triol, (*R*,*R*)-monoester, (*R*,*S*)-monoester, and diester. For diol **13a** and related compounds the HPLC was equipped with a Chiralpak AS column (Chiral Technologies Inc.) (5% EtOH/hexane; 1.0 mL/min; 233 nm; rt). Optical rotations were determined on a Perkin-Elmer 243 B Polarimeter. Flash chromatography was carried out with Sorbisil C60 (40/60A) or Selecto 32-63. All chemicals and enzymes were used as received. Amano Lipase AK and Amano Lipase CE (Amano

International Enzyme Co.) are prepared by fermentation of a selected strain of *Pseudomonas* sp. and *Humicola lanuginosa*, respectively. Novo SP435 (Novozyme 435 from Novo Nordisk) is a lipase (Lipase Type B) whose gene coding has been transferred from a selected strain of *Candida antarctica* to a host organism, *Aspergillus oryzae*. The enzyme produced by the host organism is immobilized on a macroporous acrylic resin.

(2′*R*)-2-[2′-(2″,4″-Difluorophenyl)-2′-hydroxy-3′-(1″,2″,4″-triazolyl)propyl]-1,3-diacetoxypropane (10). A solution of Et_3N (10.0 mL, 71.7 mmol) in THF (10 mL) was added dropwise over a period of 10 min to a solution of triol **6**[1d] (6.06 g, 19.34 mmol) and acetic anhydride (4.5 mL, 47.7 mmol) in THF (100 mL). After stirring for 24 h at room temperature, most of the THF was removed, and the residue was dissolved in EtOAc (100 mL), washed with saturated $NaHCO_3$ (2 × 50 mL), water (100 mL), and saturated NaCl (50 mL), dried ($MgSO_4$), filtered, and evaporated to obtain a yellow viscous oil (6.43 g, 83.6%): $[\alpha]^{25}_D = -21.1°$ (*c* 2.142, MeOH); IR (neat on KBr) 3430 (b), 3120, 2959, 1739 (C═O), 1617, 1501, 1241, 1139, 1039, 967 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.78 (dd, 1H, J = 14 Hz and 7 Hz), 1.98 (s, 3H), 2.03 (s, 3H), 1.9−2.1 (m, 1H), 2.18 (dd, 1H, J = 11 Hz and 3 Hz), 3.81 (m, 2H), 4.08 (dd, 1H, J = 11 Hz and 7 Hz), 4.17 (dd, 1H, J = 11 Hz and 5 Hz), 4.49 (d, 1H, J = 14 Hz), 4.70 (d, 1H, J = 14 Hz), 5.05 (bs, 1H; D_2O exch), 6.80 (M, 2H), 7.51 (m, 1H), 7.82 (s, 1H), 7.93 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.71, 20.86, 33.38, 35.30 (d, $^3J_{C,F}$ = 4 Hz), 57.52 (d, $^4J_{C,F}$ = 5 Hz), 64.54, 64.71, 75.55 (d, $^4J_{C,F}$ = 5 Hz), 104.14 (t, $^2J_{C,F}$ = 26 Hz), 111.74 (dd, $^2J_{C,F}$ = 21 Hz, $^4J_{C,F}$ = 3 Hz), 124.29 (dd, $^2J_{C,F}$ = 13 Hz, $^4J_{C,F}$ = 4 Hz), 130.17 (dd, $^3J_{C,F}$ = 6 Hz and 9 Hz), 144.10, 151.79, 158.07 (dd, $^1J_{C,F}$ = 248 Hz, $^3J_{C,F}$ = 13 Hz), 162.72 (dd, $^1J_{C,F}$ =250 Hz, $^3J_{C,F}$ = 13 Hz), 170.83, 171.18; MS (CI + CH_4)(*m*/*z*) 398 (M + 1)(100%), 380 (14), 338 (27), 255 (5). Anal. Calcd for $C_{18}H_{21}F_2N_3O_5$: C, 54.40; H, 5.33; N, 10.57. Found: C, 54.60; H, 5.66; N, 10.48.

(2*R*,2′*R*)-2-[2′-(2″,4″-Difluorophenyl)-2′-hydroxy-3′-(1″,2″,4″-triazolyl)propyl]-1-acetoxy-3-hydroxypropane ((*R*,*R*)-9). A mixture of triol **6** (2.05 g, 6.54 mmol), vinyl acetate (3.0 mL, 32.5 mmol), and Amano AK (2.0 g) was stirred in EtOAc (50 mL) at room temperature. After 190 min the reaction was filtered, the enzyme cake washed with EtOAc (10 mL), and the combined filtrate evaporated (<30 °C). The residue was purified by column chromatography, eluting with 0−5% MeOH/CH_2Cl_2. The required fractions were pooled and evaporated to obtain a foam (2.25 g, 96.6%; de 0.933: $[\alpha]^{25}_D$ = −16.6° (*c* 1.47, MeOH); IR (neat on NaCl): 3289 (b), 3129, 2959, 1734 (C═O), 1617, 1500, 1420, 1368, 1271, 1139, 1090, 1038, 968, 852, 679 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.91 (m,2H), 2.06 (s, 3H), 2.10 (m, 1H), 2.95 (bs, 1H; D_2O exch), 3.38 (m, 2H), 4.13 (m, 2H), 4.50 (d, 1H, J = 14 Hz), 4.72 (d, 1H, J = 14 Hz), 5.30 (s, 1H; D_2O exch), 6.79 (m, 2H), 7.49, (m, 1H), 7.80 (s, 1H), 7.94 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.91, 36.51 (2C), 57.32 (d, $^4J_{C,F}$ = 5 Hz), 62.95, 64.95, 75.29 (d, $^4J_{C,F}$ = 5 Hz), 104.21 (t, $^2J_{C,F}$ = 27 Hz), 111.66 (dd, $^2J_{C,F}$ = 20 Hz, $^4J_{C,F}$ = 3 Hz), 124.98 (dd, $^2J_{C,F}$ = 13 Hz, $^4J_{C,F}$ = 3 Hz), 129.91 (dd, $^3J_{C,F}$ = 6 Hz and 9 Hz), 144.07, 151.62, 158.46 (dd, $^1J_{C,F}$ = 258 Hz, $^3J_{C,F}$ = 12 Hz), 162.66 (dd, $^1J_{C,F}$ = 250 Hz, $^3J_{C,F}$ = 12 Hz), 171.69; MS (CI + CH_4)(*m*/*z*): 356 (M + 1)(100), 338 (37), 296 (20), 213 (8). Anal. Calcd for $C_{16}H_{19}F_2N_3O_4$: C, 54.08; H, 5.39; N, 11.83. Found: C, 53.75; H, 5.69; N, 11.54.

(2*S*,2′*R*)-2-[2′-(2″,4″-Difluorophenyl)-2′-hydroxy-3′-(1″,2″,4″-triazolyl)propyl]-1-acetoxy-3-hydroxypropane ((*R*,*S*)-9). A mixture of triol **6** (2.0 g, 6.38 mmol), vinyl acetate (1.4 mL, 13.06 mmol), and Novo SP435 (0.20 g) in MeCN (20 mL) was stirred at room temperature with continuous HPLC monitoring. After 85 min the reaction mixture was filtered, the enzyme beads washed with EtOAc (10 mL), and the filtrate evaporated (<30 °C)(de 0.964). The crude product was purified column chromatography, eluting with 0−6% MeOH/CH_2Cl_2. The required fractions were pooled and evaporated to obtain a foam (2.04 g, 89.9%; de 0.966): $[\alpha]^{25}_D$ = −49.19° (*c* 1.118, MeOH); IR (neat on KBr) 3214 (b), 2930, 1739 (C═O), 1616, 1498, 1419, 1367,1245, 1137, 1041, 966, 853, 679 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.70 (m,2H), 1.99 (s, 3H), 2.49 (dd, 1H J = 2 Hz and 12 Hz), 3.34 (dd, 1H, J = 10 Hz and 9 Hz), 3.64 (dd, 1H, J = 10 Hz and 3 Hz), 3.80 (m 2H,), 4.50 (d, 1H, J = 14 Hz), 4.57 (d, 1H, J = 14 Hz), 6.83 (m, 2H), 7.54, (m, 1H), 7.75 (s, 1H), 8.14 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.76, 36.25, 37.92 (d, $^3J_{C,F}$ = 4 Hz), 58.48 (d, $^4J_{C,F}$ = 4 Hz), 63.12, 65.39, 74.28 (d, $^4J_{C,F}$ = 5 Hz), 104.18 (t, $^2J_{C,F}$ = 27 Hz), 111.49 (dd, $^2J_{C,F}$ = 20 Hz, $^4J_{C,F}$ = 3Hz), 125.14 (dd, $^2J_{C,F}$ = 14 Hz, $^4J_{C,F}$ = 4 Hz), 130.52 (dd, $^3J_{C,F}$ = 6 Hz and 9 Hz), 144.39, 150.48, 158.66 (dd $^1J_{C,F}$ = 247 Hz, $^3J_{C,F}$ = 12 Hz), 162.68 (dd $^1J_{C,F}$ = 250 Hz, $^3J_{C,F}$ = 12 Hz), 171.11; MS (CI + CH_4)(*m*/*z*) 356 (M + 1)(100%), 338 (25), 296 (16), 213 (3). Anal. Calcd for $C_{16}H_{19}F_2N_3O_4$: C, 54.08; H, 5.39; N, 11.83. Found: C, 54.14; H, 5.68; N, 11.64.

(2′*R*)-2-[2′-(2″,4″-Difluorophenyl)-2′-hydroxy-3′-(1″,2″,4″-triazolyl)propyl]-1,3-dibutyroxypropane (11b). A mixture of triol **6** (5.99 g, 19.1 mmol), butyric anhydride (8.0 mL, 48.9 mmol), and DMAP (0.13 g, 1.1 mmol) in THF (100 mL) was stirred at room temperature for 2.5 h. Most of the THF was then removed in vacuo, and the residue was diluted with EtOAc (100 mL), washed with saturated $NaHCO_3$ (3 × 50 mL), water (100 mL), and saturated NaCl (50 mL), dried ($MgSO_4$), filtered, and evaporated. The crude product was dried in a vacuum oven at 80 °C overnight to yield a viscous yellow liquid (8.16 g, 94.1%): $[\alpha]^{25}_D = -20.1°$ (*c* 2.060, MeOH); IR (neat on NaCl): 3429 (bw), 3268 (bw), 3126 (bw), 2966, 2877, 1738 (C═O), 1617, 1500, 1459, 1420, 1273, 1179, 1139, 1092, 967, 851, 679 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, 3H, J = 7.4 Hz), 0.92 (t, 3H, J = 7.4 Hz), 1.58 (sext, 2H, J = 7.4 Hz), 1.62 (sext, 2H, J = 7.4 Hz), 1.76 (dd, 1H, J = 14.3 Hz and 7.3 Hz), 1.95 (sept, 1H), 2.14 (dd, 1H, J = 14.4 Hz and 4.0 Hz), 2.19 (t, 2H, J = 7.4 Hz), 2.26 (t, 2H, J = 7.4 Hz), 3.81 (m, 2H), 4.12 (m, 2H), 4.47 (d, 1H, J = 13.8 Hz), 4.69 (d, 1H, J = 13.8 Hz), 5.00 (bs, 1H, D_2O exch), 6.79 (m, 2H), 7.48, (m, 1H), 7.83 (s, 1H), 7.93 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.63, 18.27, 18.37, 33.55, 35.40 (d, $^3J_{C,F}$ = 4 Hz), 35.94, 36.07, 57.44 (d, $^4J_{C,F}$ = 5 Hz), 64.33, 64.43, 75.69 (d, $^4J_{C,F}$ = 5 Hz), 104.13 (dd, $^2J_{C,F}$ = 26 Hz and 28 Hz), 111.76 (dd, $^2J_{C,F}$ = 21 Hz, $^4J_{C,F}$ = 3 Hz), 124.28 (dd, $^2J_{C,F}$ = 13 Hz, $^4J_{C,F}$ = 4 Hz), 130.16 (dd, $^3J_{C,F}$ = 6 Hz and 9 Hz), 143.98, 151.88, 158.31 (dd, $^1J_{C,F}$ = 246 Hz, $^3J_{C,F}$ = 12 Hz), 162.71 (dd, $^1J_{C,F}$ = 251 Hz, $^3J_{C,F}$ = 13 Hz), 173.33, 173.72; MS(CI + CH_4)(*m*/*z*) 454 (M + 1)(30%), 394 (11), 366 (86), 283 (13), 89 (100). Anal. Calcd for $C_{22}H_{29}F_2N_3O_5$: C, 58.27; H, 6.45; N, 9.27. Found: C, 58.42; H, 6.85; N, 9.11.

(2*S*,2′*R*)-2-[2′-(2″,4″-Difluorophenyl)-2′-hydroxy-3′-(1″,2″,4″-triazolyl)propyl]-1-butyroxy-3-hydroxypropane ((*R*,*S*)-12b). A solution of dibutyrate **11b** (2.0 g, 4.41 mmol) in THF (3 mL) was added to 50 mM KCl (20 mL) in a RB flask equipped with a magnetic stirrer and a pH electrode and NaOH delivery tube connected to a pH stat. The mixture was vigorously stirred and adjusted to pH 6.0. Amano AK (0.40 g) was added and the pH maintained at 6.0 by constant addition of 1 M NaOH. The reaction was stopped after 25 h when 4.17 mL of 1 M NaOH (94.6% theoretical) had been added. The reaction mixture was extracted with toluene (200 mL) which was then washed with water (2 × 50 mL), saturated $NaHCO_3$ (2 × 50 mL), and saturated NaCl (50 mL), dried ($MgSO_4$), filtered, and evaporated (<30 °C). The crude product (1.16 g) was purified by column chromatography, eluting with 0−5% MeOH/CH_2Cl_2. The relevant fractions were pooled and evaporated a colorless oil (1.00 g, 59.2%; de > 0.99): $[\alpha]^{25}_D$ = −53.50° (*c* 1.013, MeOH); IR (neat on KBr): 3233 (b), 2965, 2878, 1734 (C═O), 1617, 1496, 1419, 1271, 1181, 1137, 966, 851, 679 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, 3H, J = 7.4 Hz), 1.60 (sext, 2H, J = 7.4 Hz), 1.71 (m, 2H), 2.22 (t, 2H, J = 7.4 Hz), 2.43 (m, 1H, J = 12.0 Hz and 2.2 Hz), 3.39 (dd, 1H, J = 10.5 Hz and 7.3 Hz), 3.64 (dd, 1H, J = 10.4 Hz and 3.6 Hz), 3.81 (m, 2H, J = 5.4 Hz), 4.54 (s, 2H), 6.1 (bs, 1H, D_2O exch), 6.81 (m, 2H), 7.55, (m, 1H), 7.79 (s, 1H), 8.08 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.66, 18.30, 35.97, 36.35, 37.61 (d, $^3J_{C,F}$ = 4 Hz), 58.25 (d, $^4J_{C,F}$ = 4 Hz), 63.41, 65.18, 74.71 (d, $^4J_{C,F}$ = 5 Hz), 104.22 (dd, $^2J_{C,F}$ = 26 Hz), 111.59 (dd, $^2J_{C,F}$ = 21 Hz, $^4J_{C,F}$ = 3 Hz), 124.28 (dd, $^2J_{C,F}$ = 13 Hz, $^4J_{C,F}$ = 4 Hz), 130.43 (dd, $^3J_{C,F}$ = 6 Hz and 9 Hz), 144.31, 150.94, 158.48 (dd, $^1J_{C,F}$ = 247 Hz, $^3J_{C,F}$ = 12 Hz), 162.72 (dd, $^1J_{C,F}$ =250 Hz, $^3J_{C,F}$ = 13 Hz), 173.62; MS(CI + CH_4)(*m*/*z*) 384 (M + 1)(100%), 366 (26), 296 (28), 213 (9), 89 (18). Anal. Calcd

for $C_{18}H_{23}F_2N_3O_4$: C,56.39; H, 6.05; N, 10.96. Found: C, 56.27; H, 6.20; N, 10.80.

(2*S*,2′*R*)-2-[2′-(2″,4″-Difluorophenyl)-2′-hydroxy-3′-(1″,2″,4″-triazolyl)propyl]-1-butyroxy-3-hydroxypropane ((*R*,*S*)-12b). A solution of triol **6** (5.0 g, 16.0 mmol), butyric anhydride (5.3 g, 33.6 mmol), and DMAP (0.14 g, 1.2 mmol) in THF (25 mL) was stirred at room temperature for 1.75 h, by which time TLC (5% MeOH/CH_2Cl_2) indicated complete formation of the dibutyrate **11b**. The reaction mixture was diluted with 50 mM KCl (250 mL) and the reaction flask equipped with a pH electrode and delivery tube connected to a pH stat. With vigorous stirring the pH was adjusted to and maintained at pH 7.0 by delivery of 1 M NaOH. When pH had stabilized Amano AK (1.0 g) was added and the mixture stirred at room temperature. The reaction was terminated after 19.5 h when 17.4 mL of 1 M NaOH had been delivered. The reaction mixture was extracted with toluene (2 × 100 mL), and the combined organic extracts were washed with water (4 × 100 mL) and saturated NaCl (50 mL), dried ($MgSO_4$), filtered, and evaporated to get a viscous oil (3.94 g, 64.3%; de 0.994): $[\alpha]^{25}_D = -51.1°$ (*c* 1.272, MeOH); IR (neat on KBr): 3239 (b), 2966, 2878, 1735 (C═O), 1616, 1498, 1419, 1271, 1181, 1135, 1092, 968, 852, 680 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, 3H, $J = 7.4$ Hz), 1.62 (sext, 2H, $J = 7.4$ Hz), 1.70 (m, 2H), 2.22 (t, 2H, $J = 7.4$ Hz), 2.44 (m, 1H, $J = 12.2$ Hz and 2.2 Hz), 3.38 (dd, 1H, $J = 10.5$ Hz and 7.6 Hz), 3.64 (dd, 1H, $J = 10.3$ Hz and 3.7 Hz), 3.82 (m, 2H, $J = 5.6$ Hz), 4.54 (AB quartet, 2H), 6.2 (bs, 1H, D_2O exch), 6.81 (m, 2H), 7.55, (m, 1H), 7.78 (s, 1H), 8.09 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.67, 18.31, 35.98, 36.36, 37.70 (d, $^3J_{C,F} = 4$ Hz), 58.32 (d, $^4J_{C,F} = 4$ Hz), 63.39, 65.20, 74.65 (d, $^4J_{C,F} = 5$ Hz), 104.23 (t, $^2J_{C,F} = 26$ Hz), 111.58 (dd, $^2J_{C,F} = 21$ Hz, $^4J_{C,F} = 3$ Hz), 124.95 (dd, $^2J_{C,F} = 13$ Hz, $^4J_{C,F} = 4$ Hz), 130.46 (dd, $^3J_{C,F} = 6$ Hz and 9 Hz), 144.32, 150.85, 158.59 (dd, $^1J_{C,F} = 247$ Hz, $^3J_{C,F} = 12$ Hz), 162.72 (dd, $^1J_{C,F} = 250$ Hz, $^3J_{C,F} = 12$ Hz), 173.62; MS(FAB)(*m*/*z*) 454 (DiOBu, M + 1)(7%), 384 (M + 1)(100%), 366 (17), 296 (9), 224 (6), 213 (5). Anal. Calcd for $C_{18}H_{23}F_2N_3O_4$: C, 56.39; H, 6.05; N, 10.96. Found: C, 56.42; H, 6.60; N, 10.63.

(2*R*)-2-[2′-(2″,4″-Difluorophenyl)-2′-propenyl]-1-acetoxy-3-hydroxypropane ((*R*)-14b). Lipase CE (2.0 g) was added to a solution of diol **13a** (10.0 g, 43.8 mmol) and vinyl acetate (8.0 mL, 86.8 mmol) in HPLC grade toluene (200 mL). The mixture was stirred at room temperature with periodic HPLC monitoring. After 26 h the reaction mixture was filtered through a Celite pad which was washed with tBME (20 mL). The filtrate was evaporated (<30 °C) and purified by column chromatography, eluting with 10−35% EtOAc/hexane. Pooling the relevant fractions yielded the diacetate **13b** (0.66 g), a mixture of mono- and diacetate (0.89 g), and pure monoacetate (*R*)-**14b** as a viscous oil (10.37 g, 87.6%; ee 0.970): $[\alpha]^{24}_D = +13.9°$ (*c*, 1.085, EtOH); IR (neat on KBr): 3446 (b), 3082, 2951, 1737 (C═O), 1619, 1502, 1257, 1140, 1085, 1040, 970, 851, 610 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.81 (m, 1H), 1.95 (bs, 1H), 2.06 (s, 3H), 2.54 (sept, 2H), 3.50 (dd, 1H, $J = 11.3$ Hz and 6.2 Hz), 3.57 (dd, 1H, $J = 11.3$ Hz and 4.5 Hz), 4.07 (dd, 1H, $J = 11.3$ Hz and 6.4 Hz), 4.17 (1H, $J = 11.3$ Hz and 4.5 Hz), 5.22 (s, 1H), 5.28 (d, 1H, $J = 1.2$ Hz), 6.77−6.88 (m, 2H), 7.24 (dt, 1H, $^4J_{H,F} = 8.5$ Hz and $J_{H,H} = 6.5$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.88, 35.04 (d, $^4J_{H,F} = 3$ Hz), 38.68, 62.10, 63.89, 104.23 (t, $^3J_{C,F} = 26$ Hz), 111.36 (dd, $^2J_{C,F} = 21$ Hz, $^4J_{C,F} = 3$ Hz), 118.48, 125.06, 130.71 (dd, $^3J_{C,F} = 6$ Hz and 9Hz), 141.33, 159.87 (dd, $^1J_{C,F} = 237$ Hz, $^3J_{C,F} = 11$ Hz), 162. 28 (dd, $^1J_{C,F} = 237$ Hz, $^3J_{C,F} = 11$ Hz), 171.68; MS (CI + CH_4)(*m*/*z*): 271 (M + 1)(7%), 253 (22), 211 (32), 193 (100), 167 (14). Anal. Calcd for $C_{14}H_{16}F_2O_3$: C, 62.21; H, 5.97; F, 14.06. Found: C, 62.02; H, 5.85; F, 13.96.

(2*S*)-2-[2′-(2″,4″-Difluorophenyl)-2′-propenyl]-1-butyroxy-3-hydroxypropane ((*S*)-14c). A mixture of dibutyrate **13c** (1.01, 2.74 mmol) in 0.1 M phosphate buffer (pH 7.8) (10 mL) was stirred in a three-neck flask equipped with a pH electrode and a NaOH delivery line connected to pHstat. Amano CE (1.0 g) was added and the mixture rapidly stirred, maintaining pH 7.0 by automatic addition of 1 M NaOH. The reaction was terminated after 18.25 h when 3.62 mL of NaOH had been added. The reaction mixture was extracted with hexanes (2 × 25 mL) and 40% MeOH/water (3 × 25 mL). HPLC of the hexanes layer at this stage showed complete removal of diol **13a**. The hexanes layer was dried ($MgSO_4$), filtered, and evaporated (<30 °C) to obtain a colorless oil (0.43 g, 52.4%; ee 0.966): $[\alpha]^{24}_D = -13.6°$ (*c*, 1.437, EtOH); IR (neat on KBr): 3479 (b), 3082, 2965, 2875, 1735 (C═O), 1618, 1502, 1419, 1266, 1182, 1086, 970, 851 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.94 (t, 3H, $J = 7.4$ Hz), 1.64 (sext, 2H, $J = 7.4$ Hz), 1.80 (m, 1H), 2.29 (t, 2H, $J = 7.4$ Hz), 2.2−2.4 (bm, 1H, exch D_2O), 2.54 (sept, 2H), 3.46 (dd, 1H, $J = 11.3$ Hz and 6.3 Hz), 3.54 (dd, 1H, $J = 11.3$ Hz and 4.5 Hz), 4.08 (dd, 1H, $J = 11.2$ Hz and 6.1 Hz), 4.16 (dd, 1H, $J = 11.2$ Hz and 4.6 Hz), 5.12 (s, 1H), 5.27 (d, 1H, $J = 1.1$ Hz), 6.76−6.88 (m, 2H), 7.24 (dt, 1H, $^4J_{H,F} = 8.5$ Hz and $J_{H,H} = 6.5$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.69, 18.46, 35.05 (d, $^4J_{H,F} = 3$ Hz), 36.15, 38.79, 62.06, 63.63, 104.20 (t, $^3J_{H,F} = 26$ Hz), 111.34 (dd, $^2J_{H,F} = 21$ Hz, $^4J_{H,F} = 4$ Hz), 118.43, 125.05 ($^2J_{H,F} = 14$ Hz, $^4J_{H,F} = 4$ Hz), 130.75 (dd, $^3J_{H,F} = 9$ Hz and 6 Hz), 141.37, 159.88 (dd, $^1J_{H,F} = 248$ Hz, $^3J_{H,F} = 10$ Hz), 162.27 (dd, $^1J_{H,F} = 249$ Hz, $^3J_{H,F} = 12$ Hz), 174.34.); MS (CI + CH_4)(*m*/*z*): 299 (M + 1)(2%), 281 (58), 211 (44), 193 (100), 167 (17). Anal. Calcd for $C_{16}H_{20}F_2O_3$: C, 64.42; H, 6.76; F, 12.74. Found: C, 64.47; H, 7.07; F, 12.73.

(2*S*)-2-[2′-(2″,4″Difluorophenyl)-2′-propenyl]-1-acetoxy-3-hydroxypropane ((*S*)-14b). A mixture of diol **13a** (5.01 g, 21.95 mmol) and Novo SP435 (0.26 g) in tank car grade MeCN (25 mL) was cooled in an ice bath for 15 min. Added vinyl acetate (4.0 mL, 43.4 mmol) and stirred at 0 °C for 6 h. The reaction mixture was filtered, the beads were washed with EtOAc (30 mL), and the combined organics were evaporated (<30 °C). The residue was purified by column chromatography, eluting with 10−50% EtOAc/hexanes. Pooling the relevant fractions yielded the monoacetate (*S*)-**14b** (4.23 g, 71.3%; ee 0.982): $[\alpha]^{23}_D = -13.9°$ (*c*, 1.688, EtOH); IR (neat on KBr): 3453 (b), 3082, 2949, 2899, 1738 (C═O), 1618, 1502, 1246, 1140, 1085, 1040, 970, 851, 610 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.80 (m, 1H), 2.04 (s, 3H), 2.54 (m, 3H; D_2O exch sept, 2H), 3.48 (dd, 1H, $J = 11.2$ Hz and 6.1 Hz), 3.55 (dd, 1H, $J = 11.2$ Hz and 4.6 Hz), 4.07 (dd, 1H, $J = 11.2$ Hz and 6.3 Hz), 4.13 (1H, $J = 11.2$ Hz and 4.7 Hz), 5.21 (s, 1H), 5.28 (s, 1H), 6.77−6.88 (m, 2H), 7.24 (dt, 1H, $^4J_{H,F} = 8.4$ Hz and $J_{H,H} = 6.4$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.84, 35.07 (d, $^4J_{H,F} = 3$ Hz), 38.66, 61.99, 63.96, 104.20 (t, $^3J_{C,F} = 26$ Hz), 111.35 (dd, $^2J_{C,F} = 21$ Hz, $^4J_{C,F} = 3$ Hz), 118.42, 125.08 (dd, $^2J_{H,F} = 14$ Hz, $^4J_{H,F} = 3$ Hz), 130.78 (dd, $^3J_{C,F} = 6$ Hz and 9 Hz), 141.39, 159.90 (dd, $^1J_{C,F} = 253$ Hz, $^3J_{C,F} = 15$ Hz), 162. 19 (dd, $^1J_{C,F} = 249$ Hz, $^3J_{C,F} = 12$ Hz), 171.74; MS (CI + CH_4)(*m*/*z*): 271 (M + 1)(2%), 253 (16), 211 (41), 193 (100), 167 (22). Anal. Calcd for $C_{14}H_{16}F_2O_3$: C, 62.21; H, 5.97; F, 14.06. Found: C, 61.95; H, 6.31; F, 13.87.

Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds (*R*,*R*)-**9**, (*R*,*S*)-**9**, (*R*)-**10**, (*R*)-**11b**, (*R*,*S*)-**12b**, (*R*)-**14b**, (*S*)-**14c** and (*S*)-**14b**, and ^1H NMR spectra for the borate ester of **13a** (12 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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