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Articles

Enzymatic Kinetic Resolution of Piperidine Atropisomers: Synthesis of a Key Intermediate of the Farnesyl Protein Transferase Inhibitor, SCH66336

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The resolution of secondary amines via enzyme-catalyzed acylation is a relatively rare process. The kinetic resolution of a series of intermediates of SCH66336 (**1**), by either enzymatic acylation of the pendant piperidine (**4**, **5**) or hydrolysis of the corresponding carbamate **3**, was investigated. In the case of **4**, the molecule exists as a pair of enantiomers due to atropisomerism about the exocyclic double bond. The enzymatic acylation of (±)-**4** was optimized in terms of acylating agent, solvent, and moisture content. The use of lipase, Toyobo LIP-300, and trifluoroethyl isobutyrate as acylating agent resulted in isobutyrylation of the (+)-enantiomer, which is easily separated from the unwanted (−)-**4**. Hydrolysis of the isobutyramide **6c** yielded the desired (+)-**4** in high enantiomeric excess. (−)-**4** may be recovered from the resolution step, racemized, and resubjected to enzymatic acylation to increase material throughput.

Introduction

Ras proteins play a major role in signal transduction from the cell surface to the nucleus. In normal signaling, the signal is terminated by the hydrolysis of bound GTP to GDP by the intrinsic GTPase activity of the normal Ras protein. In mutated Ras protein, the GPTase activity is impaired and the signal remains active, leading to unregulated cell proliferation. Point mutations in the *ras* genes that encode for the Ras proteins have been observed in ~30% of all human cancers, including 50% of colon cancers and 95% of pancreatic cancers.[1]

To exhibit biological activity, the Ras protein must undergo binding to the cell membrane, which is dependent on the following sequence of posttranslational events: (i) farnesylation of the cysteine residue near the carboxyl terminus, (ii) proteolysis of the three amino acids at the carboxyl terminus, and (iii) esterification of the new farnesylated cysteine carboxyl terminus.[2] Since farnesyl protein transferase (FPT) catalyzes the transfer of a farnesyl group from farnesyl diphosphate to the cysteine of Ras, it is an attractive target for disrupting the association of Ras to the cell membrane and thus

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(1) (a) Barbacid, M. *Annu. Rev. Biochem*. **1987**, *56*, 779−827. (b) Bos, J. L. *Cancer Res*. **1989**, *49*, 4682−4689. (c) Lowy, D. R.; Willumsen, B. M. *Annu. Rev. Biochem*. **1993**, *62*, 851−891.
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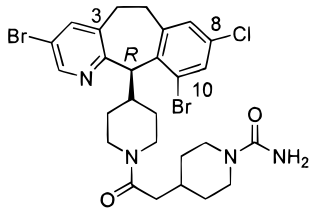
Chart 1



1 SCH66336

terminating the signal for cell proliferation.[3] However, since the cell also contains a number of geranylgeranylated proteins, farnesyl protein transfer inhibitors (FPTI) must be selective for FPT over geranylgeranyl protein tranferase-1 (GGPT-1).

SCH66336 (**1**) (Chart 1) has emerged as a novel, selective, nonpeptide, nonsulfhydryl farnesyl protein transferase inhibitor, showing activity in the nanomolar range.[4]

The synthesis of **1**, which is outlined in Scheme 1, begins with Loratadine (**2**),[5] which is regiospecifically brominated at positions 3 and 10 of the diarylcycloheptane portion of the molecule.[4] Acidic hydrolysis of the carbamate **3** followed by reduction of the exocyclic double bond,[6] produces racemic **5**, which is resolved by diastereomeric salt formation with *N*-Ac-L-phenylalanine followed by recrystallization from EtOH. After liberation of the free base, the desired (*R*)-(+)-**5** is obtained in 30−40% molar yield and 97−98% enantiomer excess. Subsequent coupling with the appropriate 4-substituted piperidine and several other steps complete the synthesis of **1**.

Results and Discussion

As a potential alternative to the chemical resolution of (±)-**5**, the enzymatic resolution of key chiral intermediates of SCH66336 was explored. The approaches considered were (a) microbial hydrolysis of the carbamate **3**, (b) enzymatic hydrolysis of the acetamide or phenylacetamide of **4**, (c) enzymatic acylation of (±)-**5**, and (d) enzymatic acylation of (±)-**4**. Of these four options, the most attractive are those that result in the resolution of derivatives of **3** or **4**. Although they do not contain a chiral center, both **3** and **4** exist as pairs of conformational enantiomers due to the atropisomerism[7] resulting from the reduced conformational mobility of the diaryl-[a,d]cycloheptane ring. The bromine substituent at the 10-position prevents flipping of the piperidinylidene ring from one face of the tricyclic system to the other.[8] Moreover, in the case of **4**, thermal equilibration of the separated atropisomers had been demonstrated. Therefore, resolution of either **3** or **4** offers the potential of recovering, racemizing, and recycling the unwanted enantiomer, thereby increasing the yield of a valuable intermediate.

The selective enzymatic acylation and deacylation of alcohols has become a powerful technique for the preparation of optically enriched compounds and is practiced both in laboratory- and large-scale production.[9,10] In contrast, the corresponding resolution of amines is much less developed. In particular, the selective enzymatic acylation/deacylation of cyclic secondary amines has only sporadically been reported,[11] even though such compounds (e.g., pyrrolidines, piperidines, piperazines) are common building blocks in pharmaceuticals.

The deacylation of carbamate (±)-**3** by microbial hydrolysis was initially evaluated (Scheme 2). Of the 373 cultures that were examined, none showed significant formation of deacylated product **4**, nor of residual nonracemic starting material **3**.

Similarly poor results were obtained for the hydrolysis of the amide derivatives (±)-**6a,b**. No product was detected when the acetamide (±)-**6a** or the phenylacetamide (±)-**6b** were incubated with commercially available hydrolytic enzymes. In light of these initial negative results, efforts were concentrated on the kinetic resolution of **4** and **5** via enzymatic acylation.

A. Enzymatic Resolution of (±)-4. *(a) Enzymatic Acetylation*. The enzymatic resolution of (±)-**4** was screened against 233 commercially available enzyme preparations under acylating conditions, using trifluoroethyl acetate (TFEOAc) as acylating agent (Scheme 3). Five enzymes produced enantiomerically enriched acetamide (**6a**) under these conditions, with the best results being observed with ChiroCLEC PC (*Pseudomonas ce-*

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(6) Complete details of the reduction of (±)-**5** will be presented elsewhere: Njoroge et al., manuscript in preparation.

(7) Since this behavior results from rotation about two bonds instead of one, it is not atropisomerism as strictly defined. Nevertheless, the more familiar term will be used: Oki, M. *Topics in Stereochemistry*; John Wiley and Sons: New York, 1983; Vol. 14, pp 1−81.

(8) Conformational diastereomers due to restricted piperidinylidene ring flipping have been described in similar tricyclic systems: Piwinski, J. J.; Wong, J. K.; Chan, T.-M.; Green, M. J.; Ganguly, A. K. *J. Org. Chem*. **1990**, *55*, 3341−3350.

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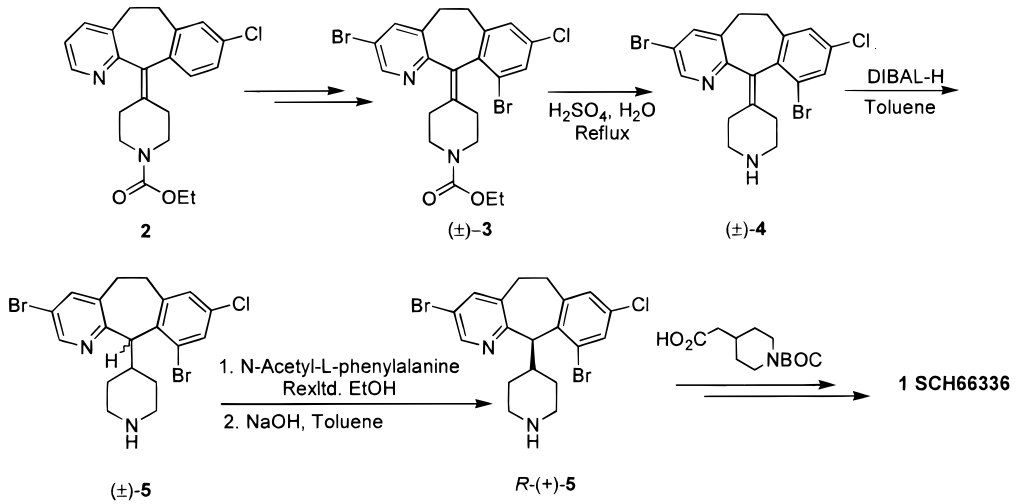
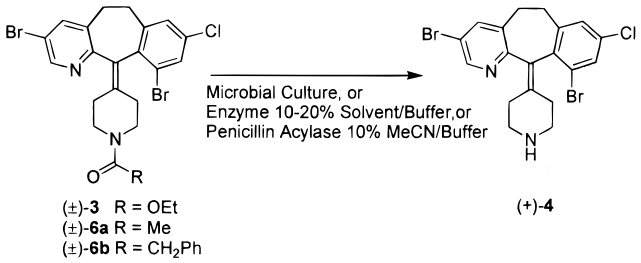
Scheme 1



Scheme 2



pacia) (enantiomer ratio,[12] $E = 8$) and Toyobo LIP-300 (*Pseudomonas aeruginosa*) ($E = 18$). All enzymes showed the same selectivity; the desired atropisomer (+)-**4**, which eventually produces (*R*)-(+)-**5** upon reduction, was acetylated. This resulted in a less convenient direct resolution, since an extra step was required to remove the acetamide moiety from **6a**. Since the enantiomeric excess (ee) of the product decreases with increasing conversion during a direct resolution, a very selective process (high *E* value) was required for such a resolution to be practical.

On the basis of catalyst cost, the acylation was further investigated using Toyobo LIP-300, and those factors known to affect enzyme selectivity and reactivity (e.g., solvent, temperature, acylating agent, moisture content, enzyme source) were examined.

From an examination of 12 common solvents, the best combination of reactivity, selectivity, and solubility was observed in TBME. Since the reactivity and selectivity of enzymatic acylations are in large part dictated by the stereo and electronic properties of the acylating agent, 26 acyl transfer agents were examined in TBME.

As previously observed for amine substrates, the use of vinyl acetate resulted in extensive decomposition of the starting material, whereas isopropenyl acetate, both neat and in solution, resulted in complete acetylation. Poor selectivity was also observed for ethyl trifluoroacetate, methyl methoxyacetate, neat methyl acetate and the trifluoroethyl esters of chloroacetic, dichloroacetic, and trifluoroacetic acids. The reaction with dibenzyl carbonate was sluggish, and trifluoroethyl benzyloxycarbonate (TFEOBOC) showed moderate selectivity ($E = 30$).[13]

Moderate to good enantioselectivity was observed for the trifluoroethyl esters of straight-chain acids (acetic, butyric, hexanoic, and lauric) ($E = 10-80$), and for triacetin and tributyrin ($E = 20-80$).

(b) Enzymatic Isobutyrylation. (i) Acylating Agent. To enhance the enantioselectivity of the reaction, bulkier acylating agents were examined. It was previously observed that increasing the steric bulk of the acyl group can result in a significant increase in enzyme selectivity.[14] Repeating the Toyobo LIP-300 catalyzed acylation with trifluoroethyl isobutyrate (TFEOiBu) in either TBME or MeCN gave dramatically different results. The reaction in MeCN was extremely slow compared to a similar reaction using TFEOAc as acylating agent. In contrast, the reaction with TFEOiBu in TBME showed excellent enantioselectivity, with the isobutyramide **6c** being formed with 98% ee at 37% conversion ($E > 100$). The reaction with trifluoroethyl benzoate also showed high selectivity ($E > 100$) but was slower. Trifluoroethyl dimethylbutyrate showed no sign of reaction under these conditions, and trifluoroethyl adamantanecarboxylate resulted in a low conversion to racemic product.

While more selective, the enzymatic isobutyrylation reaction was also considerably slower, requiring large loadings of catalyst to effect a useful conversion within 24 h.[15] However, more reactive isobutyrylating reagents showed poor results.[16] A comparison of three acylating agents under similar conditions confirmed that the

(12) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299.

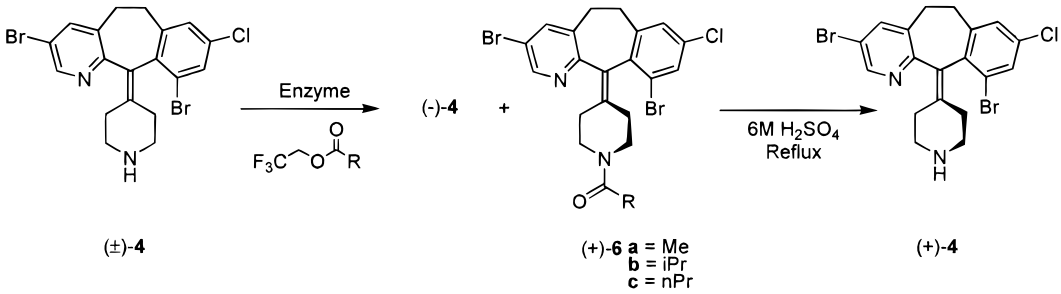
(13) The moderate selectivity of TFEOBOC suggested the enzymatic introduction of the Cbz group followed by catalytic hydrogenation to remove the Cbz group and reduce the olefin to yield (*R*)-(+)-**5** directly. However transfer hydrogenation of (±)-**4** with cyclohexane/10% Pd/C in refluxing EtOH resulted in complete removal of the 3-Br substituent, with the olefin remaining intact.

(14) Increasing the size of the acylating agent from vinyl acetate to isobutyric anhydride resulted in a dramatic increase in the selectivity of a Novozyme 435 catalyzed desymmetrization of a prochiral 1,3-diol. See: (a) Nielsen, C. M.; Sudhakar, A. U.S. Patent 5,756,830, 1998. (b) Morgan, B.; Dodds, D. R.; Zaks, A.; Andrews, D. R.; Klesse, R. *J. Org. Chem.* **1997**, *62*, 7736−7743.

(15) Toyobo LIP-300 is a lipoprotein lipase (*P. aeruginosa*) immobilized on Hyflo Supercel. The commercial catalyst contains ~1% enzyme. See: Nakatani, T.; Hiratake, J.; Yoshikawa, K.; Nishioka, T.; Oda, J. *Biosci. Biotech. Biochem.* **1992**, *56*, 1118−1123.

(16) Vinyl isobutyrate resulted in decomposition of starting material, whereas acetone oxime isobutyrate and isobutyric anhydride showed little or no selectivity at low temperature.

Scheme 3



(±)-**4** (+)-**6 a** = Me, **b** = iPr, **c** = nPr (+)-**4**

Table 1. Enzymatic Acylation of (±)-4[a]

run	R	time, h	ee_s, %	ee_p, %	conv., %	*E*
1	Me	2.75	81.1	89.3	47.6	44
2	*n*Pr	5	71.1	95.6	42.7	95
3	^{i}Pr	26	96.3	97.0	49.8	>200

[a] Conditions: (±)-**4**, 2.3 g; LIP-300, 5 g; acylating agent, 3 equiv; TBME, 46 mL; 200 rpm; room temperature.

reaction with TFEOiBu showed highest selectivity (Table 1). Reaction with TFEOAc was rapid but showed poor selectivity, whereas the reaction with TFEOBu was slower but more selective (E = 95). Although much slower, the reaction with TFEOiBu was clearly the most selective: when run under these conditions, the product was typically formed in ≥97% ee (E > 100) after 24 h. Following removal of the enzyme, the mixture of (+)-**6c** and (−)-**4** could conveniently be separated by exploiting the difference in pK_a between the pyridine and the piperidine. Extraction of the reaction mixture with 0.5 M H_2SO_4 selectively removed the more basic unreacted (−)-**4**. Subsequent extraction with 6 M H_2SO_4 isolated the product (+)-**6c** which was refluxed in the same acidic solution to remove the isobutyramide group and yield (+)-**4**. The recovered (−)-**4** could be racemized and subjected to further resolution (vide infra), and the acylation reaction solution after both acidic extractions could be washed, dried, and replenished with additional TFEOiBu for further reaction.

To confirm the improvement in enzyme selectivity, 17 enzyme preparations were reexamined using TFEOiBu as acylating agent. These included other preparations of *P. aeruginosa*, selected *Pseudomonas sp*. lipases, and those enzymes that had shown some selectivity in the initial screen using TFEOAc as acylating agent. The data in Table 2 confirm that the best results were achieved with Toyobo LIP-300. Essentially no reaction was observed for the enzymes from *C. antarctica*, *C. rugosa* and *Alcaligenes sp*. (entries 8−13). Boehringer-Mannheim Chirazyme L4, Lipase (*Pseudomonas sp*.), and Altus ChiroCLEC™ PC all showed low conversions and poor selectivities (entries 5−7). High selectivity was observed with Boehringer-Mannheim Chirazyme L6 (E = 110), but the reaction was slower. The most surprising result was the variation in the performance, both reactivity and selectivity, of the various Toyobo preparations (Table 2 and Figure 1).The crude lipase preparation, LPL-701, showed good selectivity (E ∼ 100) but only about 20% conversion after 24 h (run 4, Figure 1).[17] In contrast, the purified enzyme, LPL-311, showed poor selectivity and poor reactivity (run 2). However, when LPL-311 is immobilized on Hyflo Supercel, i.e., LIP-300/301, enhanced reactivity and selectivity were observed, and the reactions proceeded to ∼40% conversion with E > 100 (runs 1, 5). Similarly good results were obtained with LIP-321 (run 3), a recombinant form of the lipase, which is also purified and immobilized. Whether these results were due to differences in protein stability/conformation under immobilized versus nonimmobilized conditions, differences in enzyme preparation (e.g., "pH memory"),[18] or whether they were due to differences in moisture content of the various preparations (vide infra) was not established.

(ii) Moisture Content. It was quickly established that the degree of conversion of the enzymatic isobutyrylation was sensitive to the moisture content of the reaction: reactions carried out in the presence of 4 Å molecular sieves typically proceeded to ∼10% higher conversion after 24 h. No reaction was observed when the reaction was carried out in water-saturated TBME or in the presence of $Na_2SO_4/Na_2SO_4{\cdot}10H_2O$, a salt hydrate buffer reported to maintain the water activity (a_w) of solutions at 0.76.[19]

Toyobo LIP-300 gave better conversion if dried immediately before use, although the effect was somewhat batch dependent. Substrate solutions in TBME could be dried by azeotropic distillation, moisture contents <200 ppm being conveniently achieved in this fashion.[20] Figure 2 illustrates that addition of increasing amounts of water to such an azeotropically dried solution of (±)-**4** resulted in a corresponding decrease in conversion. Nonetheless, under these conditions, using 2:1 (w/w) enzyme/substrate, 37−48% conversion could be obtained in 24 h if the moisture content of the system was <800 ppm.

The inhibitory effect of high moisture levels could be attributed to hydrolysis of the trifluoroethyl isobutyrate; under standard conditions, the presence of 1% water in the bulk solvent resulted in 90% hydrolysis of the TFEOiBu within 4 h. The higher the moisture content of the system, the less acylating agent remains available and the reaction slows down. Hydrolysis of TFEOiBu releases isobutyric acid which can also inhibit the enzyme[21] or protonate the piperidine ring of (±)-**4** so that

(17) Sawa is a supplier of the Toyobo enzymes. LIP-300 and LIP-301 are the same enzyme; the different designations are for different pricing schedules.

(18) Zaks, A.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3192−3196.

(19) Halling, P. J. *Biotech. Tech*. **1992**, *6*, 271−276. Hogberg, H.-E.; Edlund, H.; Berglund, P.; Hedenstrom, E. *Tetrahedron: Asymm*. **1993**, *4*, 2123-2126.

(20) Azeotropic distillation of the substrate solution in the presence of the enzyme resulted in ∼10% lowered conversion, but without diminution of enzyme selectivity.

(21) For the effect of acids on the enantioselectivity of *C. cylindracea* catalyzed acylations with anhydride. See: Berger, B.; Rabiller, C. G.; Konigsberger, K.; Faber, K.; Griengl, H. *Tetrahedron: Asymm*. **1990**, *1*, 541−546.

Table 2. Isobutyrylation of (±)-4 with Various Enzymes in TBME[a]

run	vendor	enzyme	source	ee_s, %	ee_p, %	conv. , %	*E*
1	Toyobo	LIP-300	*P. aeruginosa*	72.4	98.7	42.3	>200
2	Toyobo	lipoprotein lipase (LPL-701)	*P. aeruginosa*	26.7	97.9	21.4	123
3	Toyobo	lipoprotein Lipase (LPL-311)	*P. aeruginosa*	5.9	92.0	6.0	25
4	Boehringer-Mannheim	Chirazyme L6	*Pseudomonas sp.*	26.0	97.7	21.0	110
5	Boehringer-Mannheim	Lipase	*Pseudomonas sp.*	51.7	79.1	39.6	14
6	Boehringer-Mannheim	Chirazyme L4	*Pseudomonas sp.*	9.2	91.0	9.2	23
7	Altus	ChiroClec PC	*Pseudomonas cepacia*	5.3	90.8	5.5	22
8	Boehringer-Mannheim	Chirazyme L3	*C. rugosa*	2.1	n/d	n/d	n/d
9	Amano	Lipase AY-30	*C. cylindracea*	1.7	n/d	n/d	n/d
10	Meito	Lipase MY	*C. cylindracea*	2.0	n/d	n/d	n/d
11	Meito	Lipase QLC	*Alcaligenes sp*	1.4	n/d	n/d	n/d
12	Meito	Lipase QLG	*Alcaligenes sp*	1.3	n/d	n/d	n/d
13	Novo	Novozyme 435	*C. antarctica, type B*	1.1	n/d	n/d	n/d

[a] Conditions: (±)-**4**, 25 mg, 50 mM; TFEOiBu, 5 equiv; enzyme, 6−27 mg; 4 Å sieves, 25−40 mg; TBME, 1.0 mL; 250 rpm; room temperature; 23.5 h.

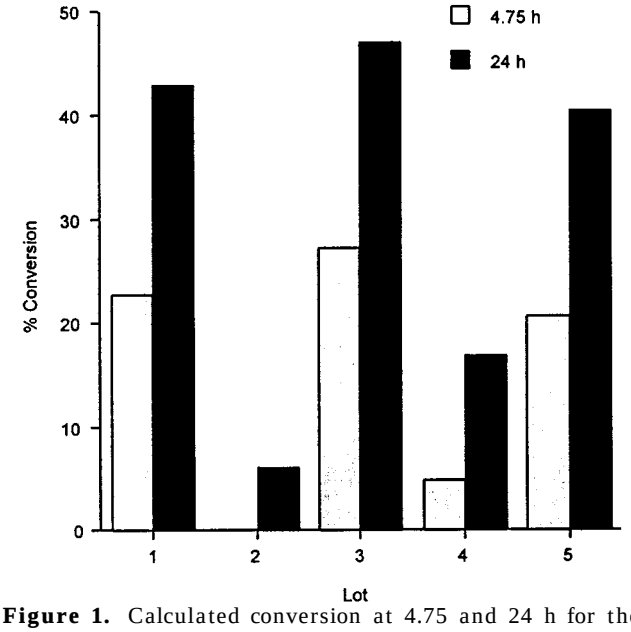


Figure 1. Calculated conversion at 4.75 and 24 h for the isobutyrylation of (±)-**4** using various *P. aeruginosa* preparations: **1**, LIP-300; **2**, LPL-311; **3**, LIP-321; **4**, LPL-701 (Sawa); **5**, LIP-301 (Sawa). Conditions: (±)-**4** (53 mg, 60 mM), enzyme (50 mg), TFEOiBu (5 equiv), TBME (2.0 mL), 4 Å sieves (47−68 mg), 250 rpm, room temperature.

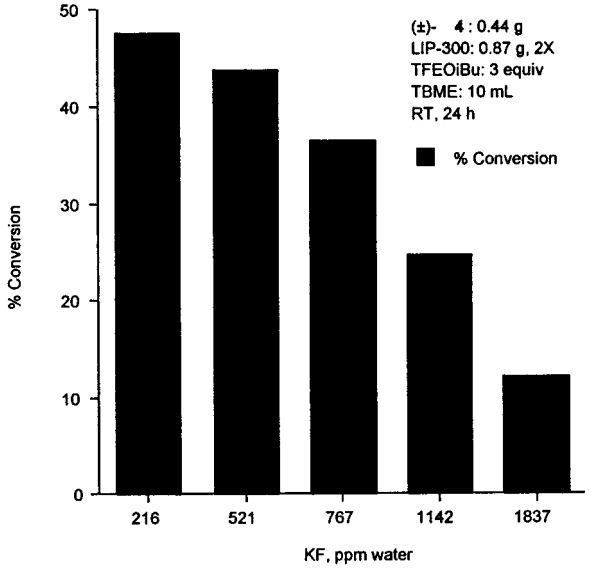


Figure 2. Effect of increasing moisture content on the enzymatic isobutyrylation of (±)-**4**.

it is unavailable for reaction.[22] In addition the hydrolysis of TFEOiBu releases trifluoroethanol, which is also capable of inhibiting the reaction. The effects of the concentration of TFEOiBu, isobutyric acid, and trifluoroethanol are shown in Figures 3−5.

As an alternative to drying the reaction mixture by azeotropic distillation or addition of molecular sieves, running the reaction in 10−20% Et_3N/TBME also resulted in higher conversions. Presumably the added base neutralized the isobutyric acid formed by adventitious hydrolysis and reversed any enzyme inhibition or substrate protonation. The beneficial effect of organic base on both the reactivity and selectivity of enzymatic acylations has been reported,[23] but in the present case, there was no further improvement in the selectivity of the reaction.

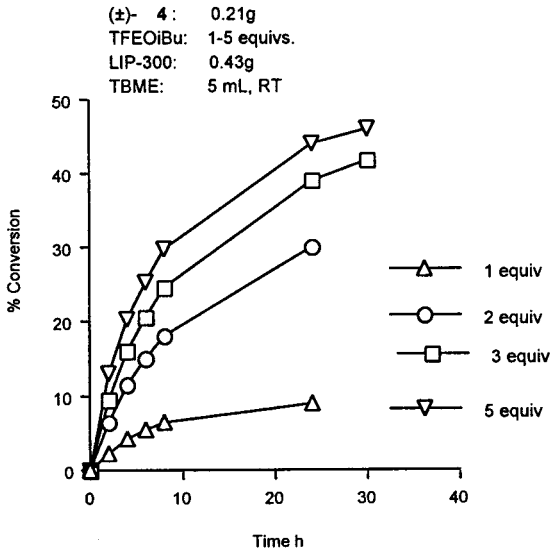


Figure 3. Effect of TFEOiBu concentration on the enzymatic isobutyrylation of (±)-**4**.

(iii) Enzyme Recovery and Reuse. The use of a bulky acylating agent to enhance the enantioselectivity of the enzyme resulted in a sluggish reaction which required 2:1 (w/w) catalyst/substrate to achieve ~40% reaction in 24 h. At such high catalyst loadings, recovery and reuse

(22) At the concentrations shown in Figure 5, to produce 0.5 equiv of isobutyric acid by hydrolysis of TFEOiBu would require 486 ppm water.

(23) (a) Rakels, J. L. L.; Straathof, A. J. J.; Heijnen, J. J. *Tetrahedron: Asymm.* **1994**, *5*, 93−100. (b) Boaz, N. W.; Zimmerman, R. L. *Tetrahedron: Asymm.* **1994**, *5*, 153−156. (c) Stead, P.; Marley, H.; Mahmoudian, M.; Webb, G.; Noble, D.; Ip, Y. T.; Piga, E.; Rossi, T.; Roberts, S.; Dawson, M. J. *Tetrahedron: Asymm.* **1996**, *7*, 2247−2250. (d) Parker, M.; Brown, S. A.; Robertson, L.; Turner, N. J. *Chem. Commun.* **1998**, 2247−2248.

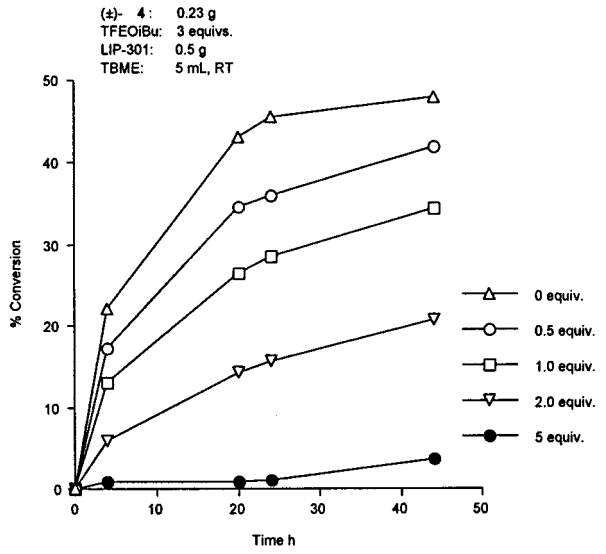


Figure 4. Effect of added isobutyric acid on the enzymatic isobutyrylation of (±)-**4**.

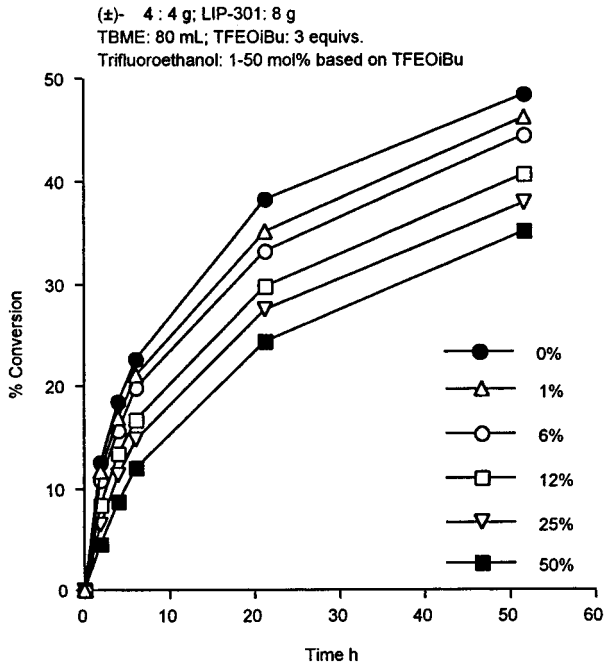


Figure 5. Effect of added trifluoroethanol on the enzymatic isobutyrylation of (±)-**4**.

of the enzyme becomes essential; column containment is a convenient way to reuse enzyme over a large number of cycles.

As shown in Figure 6, the enzyme performed consistently over 15 cycles, producing isobutyramide **6c** in 99% ee and 44−49% conversion after 24 h. The results indicated that the enzyme was stable when submerged in TBME for 3−4 weeks.

Despite the encouraging results of the packed enzyme column, due to accelerated project time-lines, the enzymatic step was scaled up to run in batch mode. A single 50 kg batch of the enzyme behaved as expected when used in the acylation of two 25 kg batches of (±)-**4** in quick succession. The enzyme was then recovered, stored as a TBME damp filter cake for ∼1 month, then finally dried, and stored at room temperature. When samples of this recovered enzyme were used in subsequent reac-

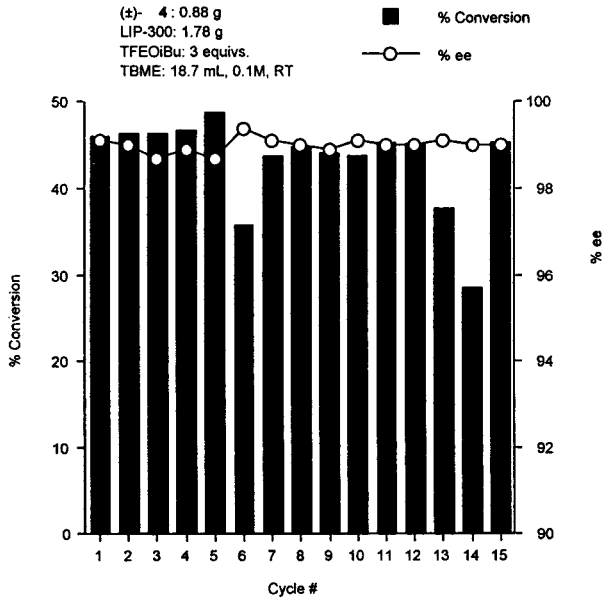


Figure 6. Reuse of Toyobo LIP-301 in a packed column (5.5 × 1.0 cm enzyme bed); flow rate 1.0 mL/min ((±)-**4** (0.87 g) in TBME (19 mL, 0.1 M)).

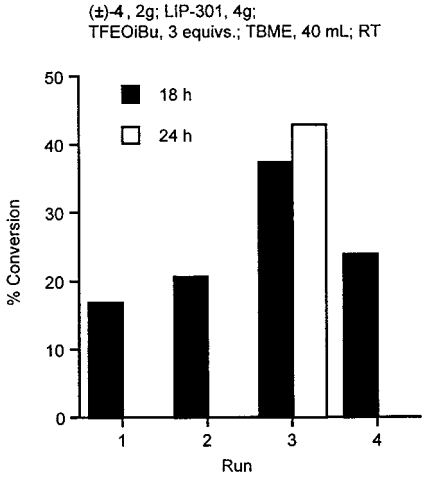


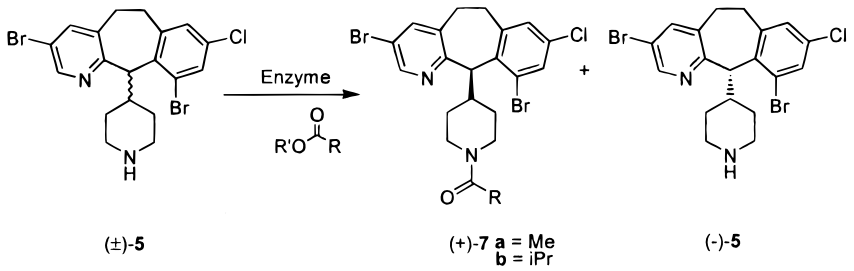
Figure 7. Performance of recovered enzyme after various treatments. (See text for details).

tions, poor reactivity was observed. Further drying of this enzyme did not restore activity, but complete activity could be restored by suspending the enzyme in damp TBME, followed by evaporating the solvent, and drying the enzyme under vacuum.

The behavior of recovered and reactivated enzyme is shown in Figure 7. Using the recovered enzyme without rehydration (run 1), or with only further drying (run 2), resulted in poor activity. Suspending the enzyme in TBME alone, followed by decanting the supernatant solvent, and evaporating and drying the residue, did not fully restore performance (run 4). Only when suspended in damp TBME, filtered, and subsequently dried (run 3) did the catalyst perform comparably to unused enzyme samples.

Our experience using Toyobo LIP-300 lipase, both fresh and recovered enzyme, emphasizes the importance of moisture content as a parameter in enzyme-catalyzed transesterifications. While multiple reuse was demonstrated for enzyme samples contained in a column, enzyme recovered from batch reactions showed a large

Scheme 4



decrease in activity but no major decrease in enantioselectivity. Since the reactivity could be restored by suspending the enzyme in damp TBME (0.5−7.5% water/enzyme v/w) and then redrying, it was concluded that the enzyme had become dehydrated. However, the mechanism of such dehydration remains obscure.

B. Racemization and Recycling. The perceived advantage for carrying out the enzymatic resolution of (±)-**4** rather than that of (±)-**5** (whether by enzymatic resolution or by diastereomeric salt formation), was the potential for racemizing the unwanted (−)-**4** and recycling the racemate through further resolutions, increasing the yield of SCH66336.

(−)-**4** could be rapidly racemized in refluxing phenyl ether (260 °C), and the (+)-**4** isolated from a subsequent enzymatic resolution displayed an impurity profile which was essentially indistinguishable from that of the initial racemic material. However, the longer heating and cooling cycle required when carrying out the racemization on large scale resulted in unacceptable levels of several impurities that could not easily be removed subsequently.

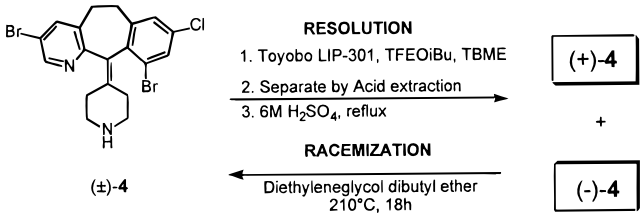
The racemization was therefore studied in a set of seven high-boiling solvents,[24] the best results being obtained in refluxing di(ethyleneglycol) dibutyl ether (DEGDBE). Moreover, impurity levels could be controlled by carrying out the reaction at lower temperature; the racemization was complete after heating in DEGDBE (5:1 v/w) at 210 °C for 12−18 h. Although the levels of some impurities increased under these conditions, several were completely removed during the subsequent enzymatic resolution and the others were reduced to acceptable levels by existing downstream processing. Multiple racemizations and resolutions of the same sample of (−)-**4** have been demonstrated. A sample of (±)-**4** was subjected to three rounds of enzymatic resolution, using the same sample of enzyme, with the recovered (−)-**4** subjected to two rounds of racemizations, resulting in a 65% overall yield of (+)-**4**, with an acceptable impurity profile (Table 3).

C. Enzymatic Acylation of (±)−5. Our first approach to the enzymatic kinetic resolution of racemic SCH66336 intermediates had been the acylation of (±)-**5** (Scheme 4). From an initial screen of 236 enzyme preparations, three enzymes showed enhanced reactivity.[25] Two of these, Toyobo LIP-300 (*P. aeruginosa*) and ChiroCLEC PC (*P. cepacia*), acylated the desired (*R*)-(+) enantiomer, resulting in a less convenient direct resolution; the third enzyme, Novozyme 435 *(Candida antarctica*) acylated the undesired (*S*)-(−) enantiomer, resulting in a potentially more useful subtractive resolution. However, Novozyme 435 showed poor selectivity ($E < 25$) when tested with a series of short-chain acylating agents (alkyl or trifluoroethyl esters including TFEOiBu, or anhydrides) either neat or in TBME, or when examined in 10 common solvents with TFEOAc as acylating agent. Similarly, poor enantioselectivity ($E < 20$) was displayed by ChiroCLEC PC using TFEOAc as acylating agent in eight common solvents.

Mindful of the enhancement of reactivity and selectivity in the resolution of (±)-**4** when using dried enzyme and a bulky acylating agent, the acylation of (±)-**5** was reexamined using trifluoroethyl isobutyrate (TFEOiBu) and freshly dried Toyobo LIP-300. The results of reactions in four solvents at two concentrations are shown in Table 4. In THF and toluene under dilute conditions, the extent of reaction was negligible (runs 4, 6), whereas it was slower but more selective in MeCN at higher dilution (runs 7, 8). Excellent selectivity ($E > 100$) and reactivity were observed in TBME under both conditions (runs 1, 2).

The enzymatic resolution of (±)-**5** on a 1 g scale was demonstrated using the same conditions (dried enzyme, azeotropically dried solvent) as used successfully for (±)-

Table 3. Enzymatic Resolution and Recycling of (±)-4



racemization	(±)-**4** yield, %	resolution	(+)-**4** yield, %	ee, %
		1st res.	42.2	95.6
1st racem.	72.0			
		2nd res.	41.3	98.0
2nd racem.	81.5			
		3rd res.	39.8	98.7
		overall	65	

Table 4. Acylation of (±)-5 Using LIP-300 in Various Solvents[a]

run	solvent	(±)-**5**, mg/mL	ee_s, %	ee_p, %	conv., %	*E*
1	TBME	25	81.8	97.1	45.7	>100
2		5	83.5	96.7	46.3	>100
3	THF	50	47.4	88.9	34.8	27
4		5	4.4	98.0	4.3	n/d
5	toluene	50	20.2	96.2	17.3	63
6		5	0.4	97.9	0.4	n/d
7	MeCN	25	23.6	93.2	20.2	36
8		5	12.3	97.2	11.3	81

[a] Conditions: (±)-**5**, 50 mg; TFEOiBu, 5 equiv; LIP-300, 50 mg; room temperature; 250 rpm; 42 h.

(24) A 5:1 solvent/(−)-**4** mixture was maintained at reflux, or else heated to 240 °C over a period of 2.5 h, maintained at 240 °C for 2 h, then cooled, and subjected to a standard workup. Solvents (bp, °C) were Decalin (189−191), diethylbenzene (180−182), di(ethyleneglycol) dibutyl ether (256), heptamethylnonane (240), hexadecane (287), *N*-methylacetamide (204−206), phenyl ether (259).

(25) Conditions: (±)-**5**, 5 mg; isopropenyl acetate, 10 equiv; EtOAc or TBME, 2 mL; room temperature; 250 rpm.

4, and proceeded with high enantioselectivity (ee_s, 98.5%; ee_p, 94.3%; *c*, 51.1%; *E* >100). Again, by choosing the appropriate acylating agent, a highly selective acylation could be effected. However, since there was no possibility for recovery and racemization of the unwanted enantiomer, this procedure was not pursued.

Conclusion

A highly selective enzymatic kinetic resolution of 4-substituted piperidines was developed by a careful choice of reaction conditions. In particular, the choice of the bulky trifluoroethyl isobutyrate as acylating agent resulted in formation of the isobutyramide product in high enantiomeric excess and allowed its facile separation from the unreacted starting material by a simple acid extraction. In the case of the compound (±)-**4** which exists as a pair of conformational enantiomers, the unreacted (−)-enantiomer could be recovered, racemized thermally, and resubmitted to enzymatic acylation. The enzyme could be recovered and reused but was sensitive to the water content of the system. While fresh enzyme had to be dried before initial use, the recovered enzyme required rehydration and redrying for optimum activity. This enzymatic step was successfully integrated into the synthesis of a novel farnesyl protein transferase inhibitor, SCH66336.

Experimental Section

General. HPLC was carried out on a Waters 715 Ultra Wisp. Chiral HPLC was carried out on a Chiralpak AD column (0.46 × 25 cm) (Chiral Technologies, Inc.) (20% ^{i}PrOH/heptane/0.2% DEA; 1.0 mL/min; 240 nm; 30 °C for **4** and **6c** and 15% EtOH/heptane/0.5% DEA/1.0 mL/min; 240 nm; 30 °C for **6a**). Retention times were as follows: for **4** (5.80, 17.53 min), **6c** (4.95, 10.08 min), **6a** (9.26, 10.92 min). Nonchiral reverse-phase HPLC was carried out on a Symmetry C-18 column (0.46 × 25 cm) (Waters) (33% MeCN/50 mM phosphate buffer (pH 3); 1.5 mL/min; 240 nm; room temperature). 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 were used as received from commercial sources; trifluoroethyl esters were prepared from trifluoroethanol and the corresponding acid chloride or anhydride under standard conditions. Trifluoroethyl isobutyrate was obtained from ChemoDynamics Inc., Sayreville, NJ. With the exception of Toyobo LIP-300/301, all enzymes were used as received from suppliers; Toyobo LIP-300/301 was typically dried under vacuum or under a N_2 stream before use.

Enzymatic Isobutyrylation of (±)-4. (±)-**4** (2.34 g, 5.0 mmol) was dissolved in TBME (75 mL) and filtered, and the filtrate was dried by azeotropic distillation, removing 30 mL of solvent (KF 169 ppm). Toyobo LIP-300 (5 g) was added, and the mixture was stirred for a further 0.5 h (KF 283 ppm). TFEOiBu (2.4 mL, 14.9 mmol) was then added, and the mixture shaken at room temperature at 200 rpm. After 26 h, the reaction was filtered, and the enzyme cake washed with TBME (20 mL). The combined filtrate was extracted three times with 0.5 M H_2SO_4. The organic layer was then extracted twice with 6 M H_2SO_4. The combined 6 M H_2SO_4 extracts were added slowly to a mixture of 50% NaOH (9 mL) and ice (25 g). The solid which precipitated, (+)-**6c**, was filtered, washed with water, and dried under vacuum (1.15 g, 42.6%; 97.0% ee). $[\alpha]_D^{25} = +176.53$ (*c* 1.03, MeOH). IR (KBr pellet): 1625 (C═O) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09−1.15 (m, 6H), 2.00−2.12 (m, 1H), 2.24−2.44 (m, 2H), 2.59,2.64 (2m, 1H), 2.75 (dt, 1H, *J* = 14.0, 4.4, 4.4 Hz), 2.74−2.84 (m, 1H), 2.87 (bt, 1H), 3.20−3.36 (m, 3H), 3.42 (td, 1H, *J* = 13.6, 13.6, 4.4 Hz), 3.76 (m, 1H), 4.05 (m, 1H), 7.20 (d, 1H), 7.47 (d, 1H, *J* = 2.0 Hz), 7.52 (bs, 1H), 8.51 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) (signals doubled due to rotamers): δ 19.27, 19.38, 29.80, 30.00, 30.64, 30.79, 31.13, 31.68, 32.16, 41.90, 42.35, 45.16, 45.58, 118.88, 123.24, 126.85, 129.96, 130.10, 130.14, 130.60, 133.56, 134.30, 134.43, 139.32, 139.49, 140.81, 141.70, 141.83, 147.54, 147.65, 151.96, 152.10, 175.25, 175.30. MS (CI+CH4) (*m*/*z*): 539 (M+1, 100). HRMS (FAB) (*m*/*z*) Calcd for $C_{23}H_{23}N_2OBrCl^{81}Br$: 539.9924. Found: 539.9917. Anal. Calcd for $C_{23}H_{23}N_2Br_2ClO\cdot0.5\ H_2O$: C, 50.44; H, 4.42; N, 5.11; Br, 29.18; Cl, 6.47. Found: C, 50.46; H, 4.58; N, 5.10; Br, 27.17; Cl, 6.70.

The combined 0.5 M H_2SO_4 extracts were added slowly to a mixture of 50% NaOH (2 mL) and water (18 mL), and the precipitated solid, (−)-**4**, was filtered, washed with water, and dried under vacuum (1.00 g, 43.3%; 96.3% ee). $[\alpha]_D^{25} = -181.84$ (*c* 1.02, MeOH).

Enzymatic Butyrylation of (±)-4. Toyobo LIP-300 (5 g) and TFEOBu (2.4 mL, 15.8 mmol) were added to an azeotropically dried stock solution of (±)-**4** (2.5 g, 5.3 mmol) in TBME (50 mL), and the mixture was shaken at 200 rpm at room temperature for 5 h. The reaction was submitted to the same workup as described above to yield (+)-**6d** (1.18 g, 41.3%; 95.6% ee). $[\alpha]_D^{25} = +173.13$ (*c* 1.06, MeOH). IR (KBr pellet): 1640 (C═O), cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95, 0.96 (2t, 3H, *J* = 7.4 Hz), 1.65 (m, 2H), 2.07 (m, 2H), 2.24−2.42 (m, 1H), 2.59, 2.69 (2m, 1H), 2.75 (dt, 1H, *J* = 13.6, 4.0, 4.0 Hz), 2.87 (bt, 2H), 3.20−3.36 (m, 3H), 3.41 (td, 1H, *J* = 13.6, 13.6, 4.4 Hz), 3.72 (m, 1H), 4.03 (bsex, 1H), 7.20 (d, 1H, *J* = 1.6 Hz), 7.40 (d, 1H, *J* = 2.0 Hz), 7.54 (bd, 1H), 8.54 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) (signals doubled due to rotamers): δ 13.97, 18.76, 29.88, 30.72, 31.16, 31.60, 32.24, 35.28, 41.72, 42.16, 45.47, 45.88, 119.07, 123.37, 123.43, 126.95, 130.26, 130.35, 133.73, 133.80, 134.55, 134.77, 139.24, 139.40, 139.99, 140.16, 141.33, 141.54, 141.66, 141.84, 147.34, 147.56, 151.77, 151.81, 171.46, 171.53. MS (CI+CH4) (*m*/*z*): 539 (M+1) (100). Anal. Calcd for $C_{23}H_{25}N_2Br_2ClO\cdot1\ H_2O$: C, 49.62; H, 4.53; N, 5.03; Br, 28.70; Cl, 6.37. Found: C, 49.72; H, 4.17; N, 4.79; Br, 28.34; Cl, 6.24. (−)-**4** was obtained as described above (1.35 g, 54.0%; 71.1% ee). $[\alpha]_D^{25} = -128.18$ (*c* 1.04, MeOH)

Enzymatic Acetylation of (±)-4. Toyobo LIP-300 (4.6 g) and TFEOAc (1.7 mL, 14.5 mmol) were added to an azeotropically dried stock solution of (±)-**4** (2.3 g, 5.3 mmol) in TBME (46 mL), and the mixture was shaken at 200 rpm at room temperature for 2.75 h. The reaction was submitted to the same workup as described above to yield (+)-**6a** (1.0 g, 40.3%; 89.3% ee). $[\alpha]_D^{25} = +140.82$ (*c* 1.02, MeOH). IR (KBr pellet): 1639 (C═O), cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.0−2.1 (m, 1H), 2.09, 2.11 (2s, 3H), 2.29 (m, 1H), 2.37 (m, 1H), 2.59, 2.66 (2m, 1H), 2.75 (dt, 1H, *J* = 12.0, 4.4, 4.4 Hz), 2.87 (bt, 1H, *J* = 16 Hz), 3.22−3.36 (m, 4H), 3.42 (td, 1H, *J* = 13.8, 13.8, 4.4 Hz), 3.37 (bsept, 1H), 3.40 (bsept, 1H), 7.20 (t 1H *J* = 1.6 Hz), 7.48 (d, 1H *J* = 2.0 Hz), 7.52 (bs, 1H), 8.5 (t, 1H, *J* = 2.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) (signals doubled due to rotamers): δ 21.46, 29.72, 30.49 30.58, 31.22, 31.44, 32.23, 32.27, 41.63, 42.05, 46.24, 46.66, 118.97, 119.03, 123.28, 123.32, 126.94, 130.22, 130.30, 130.89, 133.66, 133.70, 134.34, 134.48, 139.26, 139.30, 139.37, 139.49, 140.76, 140.89, 141.72, 141.88, 147.67, 147.80, 152.01, 152.18, 168.84, 168.91. MS (CI+CH_4)(*m*/*z*): 511 (M+1) (100). Anal. Calcd for $C_{21}H_{19}N_2Br_2ClO\cdot1\ H_2O$: C, 47.71; H, 4.00; N, 5.30; Br, 30.23; Cl, 6.71. Found: C, 47.96; H, 3.57; N, 5.09; Br, 29.89; Cl, 6.56. (−)-**4** was obtained as described above (1.32 g, 57.9%. 81.1% ee). $[\alpha]_D^{25} = -124.50$ (*c* 1.00, MeOH).

Enzymatic Isobutyrylation of (±)-5. A mixture of (±)-**5** (1.01 g, 2.2 mmol) in TBME (100 mL) was dried by azeotropic distillation, reducing the volume to 50 mL. This solution was transferred to a flask containing Toyobo LIP-300 (2 g) which had been held under a stream of N_2 for 2 h. After being shaken for 0.5 h, TFEOiBu (1.0 mL, 6.2 mmol) was added, and the reaction was shaken at 200 rpm at room temperature for 20 h. The reaction mixture was filtered, the enzyme cake was washed with CH_2Cl_2, and the filtrate was evaporated to dryness. The products were separated by silica gel chromatography, eluting with 5−20% MeOH/CH_2Cl_2/0.2% Et_3N and fractions of 20−50 mL were collected. Fractions 3−5 were combined and evaporated to yield the isobutyramide (+)-**7b** as a

white foam (0.49 g, 42.2%; 94.3% ee). $[\alpha]_D^{25} = +70.20$ (*c* 1.04, MeOH). IR (KBr pellet): 1636 (C=O) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.06−1.14 (m, 6H), 1.28−1.60 (m, 4H), 2.38 (m, 1H), 2.70−3.20 (m, 4H), 3.26 (dt, 1H, J = 17.6, 4.4 Hz), 3.63 (bt, 1H, J = 13.8 Hz), 3.90 (bt, 1H, J = 13.0 Hz), 4.61 (bd, 1H, J = 12.8 Hz), 4.89 (dd, 1H, J = 10.4 Hz), 7.14 (bs, 1H,), 7.49 (d, 1H, J = 2.0 Hz), 7.54 (bs, 1H), 8.44 (d, 1H, J = 2.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) (signals doubled due to rotamers): δ, 19.18, 19.53, 29.93, 30.05, 31.04, 31.30, 31.70, 32.02, 32.25, 41.73, 41.89, 41.98, 42.23, 45.31, 45.46, 57.96, 58.08, 118.77, 127.04, 127.15, 129.03, 130.99, 133.02, 135.01, 135.17, 136.98, 137.34, 141.21, 141.36, 142.44, 142.61, 147.21, 147.32, 154.69, 154.85, 175.06. MS (FAB-MS) (*m*/*z*): 541.0 (M+1). Anal. Calcd for $C_{23}H_{25}N_2Br_2ClO$: C, 51.09; H, 4.66; N, 5.18; Br, 29.55; Cl, 6.56. Found: C, 50.96; H, 4.71; N, 5.17; Br, 29.15; Cl, 6.92. Fractions 18−23 were combined and evaporated to yield unreacted starting material (−)-**5** as a pale yellow powder (0.36 g, 36.0%; 98.5% ee). $[\alpha]_D^{25} = -48.8$ (*c* 1.01, MeOH).

A sample of (+)-**7b** (0.37 g, 0.68 mmol) was refluxed in 6 M H_2SO_4 (5 mL) for 3.5 h, by which time HPLC indicated complete hydrolysis. The cooled solution was diluted with water (25 mL) and extracted with EtOAc (2 × 25 mL). The aqueous layer was made basic with 50% NaOH and extracted with EtOAc (25 mL), and the EtOAc layer was washed (2 × 25 mL of water), dried (Na_2SO_4), filtered, and evaporated (0.25 g, 79.4%; 93.9% ee). $[\alpha]_D^{25} = +49.41$ (*c* 1.02, MeOH). IR (KBr pellet): 3500 (b), 3333, 2946, 2910, 2815, 2737, 1436, cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.30 (td, 2H, J = 9, 3.6 Hz), 1.38−1.54 (m, 2H), 1.66 (bs, 1H), 2.27 (m, 1H), 2.45 (m, 2H), 2.79 (dt, 1H, J = 14.8, 4.4, 4.4 Hz), 2.97 (ddd, 1H, J = 17.4, 13.2, 4.4 Hz), 3.05 (m, 2H, J = 12.8 Hz), 3.27 (dt, 1H, J = 13.6, 4.4, 4.4 Hz), 3.64 (td, 1H, J = 17.2, 13.9, 4.4 Hz), 4.90 (d, 1H, J = 10.4 Hz), 7.12 (d, 1H, J = 2.0 Hz), 7.48 (d, 1H, J = 2.0 Hz), 7.52 (d, 1H, J = 2.4 Hz), 8.44 (d, 1H, J = 2.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 32.97, 33.28, 34.29, 43.37, 47.89, 48.11, 60.01, 119.72, 128.31, 130.00, 131.98, 133.81, 136.05, 138.65, 142.28, 143.55, 148.23, 156.40. MS (ESI) (*m*/*z*): 471.1 (M+1). Anal. Calcd for $C_{19}H_{19}N_2Br_2Cl$: C, 48.49; H, 4.07; N, 5.95; Br, 33.96; Cl, 7.53. Found: C, 48.54; H, 4.24; N, 5.78; Br, 33.79; Cl, 7.40.

Racemization of (−)-4. A mixture of (−)-**4** (100 g, 0.21 mole) (83.0% ee. $[\alpha]_D^{25} = -159.4$ (*c* 1.01, MeOH)) and diethyleneglycol dibutyl ether (500 mL) was heated at 210 °C under N_2 for 18 h, by which time racemization was complete. After being cooled to room temperature, the dark solution was diluted with EtOAc (2 L). The reaction flask was rinsed with 0.5 M H_2SO_4 and EtOAc which were combined with the diluted reaction mixture. The mixture was stirred for 0.5 h, and the aqueous acidic layer was separated. The organic layer was further extracted with two portions of 0.5 M H_2SO_4. Decolorizing charcoal (50 g) was added to the combined acidic extracts which were then refluxed for 1 h. After being cooled, the solution was filtered through Celite which was rinsed with 0.5 M H_2SO_4. The filtrate was added slowly to an ice cold mixture of 50% NaOH (100 mL) and water (750 mL), and the precipitated solid was collected, washed until the effluent was neutral, and dried under vacuum at 60 °C to yield a light yellow solid (75.8 g, 75.8%). Anal. Calcd for $C_{19}H_{17}N_2Br_2Cl \cdot 0.5\ H_2O$: C, 47.78; H, 3.80; N, 5.87. Found: C, 47.87; H, 3.63; N, 5.73.

Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds (+)-**4**, (+)-**6a**, (+)-**6b**, (+)-**6c,** and (+)-**7b**. This material is available free of charge via the Internet at http://pub.acs.org.

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