Organic Process Research & Development **2004**, *8*, 22–27

Efficient Preparation of a Key Intermediate in the Synthesis of Roxifiban by Enzymatic Dynamic Kinetic Resolution on Large Scale¶

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Abstract:

Additional information is presented for the transformation of a kinetic resolution into a dynamic kinetic resolution of the isobutyl ester 5b to form the acid 2 in high yield and ee via the intermediacy of a thioester 6c, (Pesti, J. A.; Yin, J.; Zhang, L.-H; Anzalone, L. *J. Am. Chem. Soc.* 2001, *123*, 11075−11076.). The development of optimized reaction conditions for the preparation of 6c, its dynamic kinetic resolution to 2, and the scale-up of both reactions into a pilot plant are described.

Recent reports have identified roxifiban (**1**) as a potent and selective antagonist of the platelet glycoprotein IIb/IIIa receptor.[1] This isoxazoline derivative holds strong promise for the prevention and treatment of a wide variety of thrombotic diseases resulting from undesired platelet adhesion such as stroke, acute myocardial infarction, transient ischemic attack, and unstable angina. The efficacy of existing anti-platelet therapies is well established. However, none possesses all of the key attributes desired by clinicians, namely selectivity for the platelet, freedom from adverse effects, activity versus all agonists, rapid onset of action, and oral activity. The ability of roxifiban to bind to both activated and resting platelets is unlike other known IIb/IIIa antagonists. This reservoir of **1** residing on unactivated platelets is expected to result in increased anti-platelet activity and dampen the typical peak/valley pharmacokinetic profile seen for other therapies. This property, among others, encouraged us to develop a safe, efficient, and inexpensive large-scale process to prepare roxifiban to advance it into the clinic.[2,3] Herein we describe the development of a practical process for the preparation of the key intermediate **2**, including the transition from a kinetic to a dynamic kinetic resolution and the scale-up of this chemistry in the pilot plant.[4]

$CH_3CO_2H \bullet H_2N$ HN H $NHCO_2$n-Bu N O N O CO_2CH_3

1

NC N O O OH

2

Results and Discussion

Background. The chiral acid **2** has been previously resolved by chiral preparative HPLC, by fractional crystallization of its cinchonidine salts,[5] or via an enzyme-mediated kinetic resolution of **5a**, itself prepared by a highly efficient [3 + 2] cycloaddition (Scheme 1).[1] As the *n*-butyl butenoate **4a** was not readily available, we found a better alternative to be the isobutyl ester **4b** which is inexpensive and available in large quantities. This reaction regiospecifically produced **5b** and was smoothly scaled up in the pilot plant.[2,3]

Fortuitously, the conditions previously established for enzymatic resolution of the *n*-butyl ester **5a** were also effective for the *i*-butyl ester **5b**.[2,3,5] The *R*-enantiomer was selectively hydrolyzed, isolated, and purified after separation from the remaining *S*-ester by simple filtration, eventually on large scale in our pilot plant. The unreacted *S*-isomer was subsequently racemized and returned for another cycle of

¶ This work was conducted while the authors were part of the DuPont Pharmaceutical Company and its predecessor, the DuPont Merck Pharmaceutical Company.

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Published on Web 12/24/2003
10.1021/op0300239 CCC: $27.50
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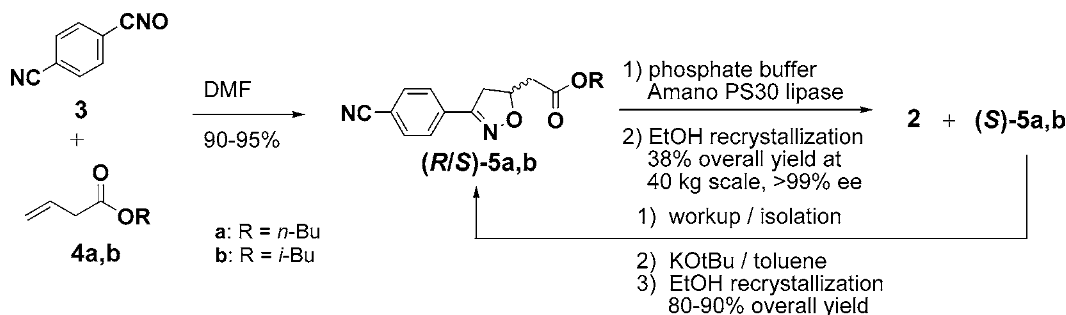
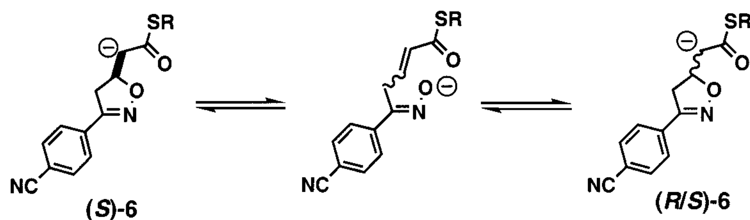
Scheme 1. **Current method for resolution of 2**[2]



Scheme 2. **Retro-Michael/Michael racemization mechanism of (*S*)-6 in the presence of base**[4]



resolution. While efficient and very amenable to further scale-up, this protocol of enantiomer separation, racemization, purification, and enzymatic resolution recycle was inconvenient and lengthy. We therefore decided to streamline the process by searching for a dynamic kinetic resolution.

While dynamic kinetic resolutions are not trivial to develop,[6] such a change would offer other advantages beyond eliminating the need to recycle (*S*)-**5b**. For most kinetic resolutions, the enantiomeric excess of the product (ee_p) begins to deteriorate as the conversion approaches 50%. This occurs since the ratio of the less-reactive to the more-reactive enantiomer will continue to increase until the advantage of the enzyme's discrimination between the enantiomers is overcome. At that point reaction products derived from the less-reactive isomer begin to predominate, and the ee_p deteriorates. Establishment of a dynamic kinetic resolution should maintain a high ee_p at all times[7] and avoid the degradation in ee_p experienced by dynamic kinetic resolutions at $\gtrsim$50% conversions.

Development of the Thioester Resolution to 2. All of our efforts to achieve the dynamic kinetic resolution of **5b** by manipulation of reaction conditions (higher pH) or the substrate (appending electron-withdrawing groups) in the presence of lipases were unsuccessful; 55% ee_p was not exceeded in any instance. Instead we decided to consider the use of thioesters, suggested by the publication of the first examples of the dynamic kinetic resolution of thioesters.[8] These reactions proceed by the exploitation of their ability to lower the pK_a of the adjacent protons, sometimes sufficiently so as to permit facile racemization. While these examples also required the presence of an α-thiophenyl substituent to sufficiently lower the pK_a,[9] such a corresponding change within our chemistry would have been unreasonable for a viable process. Instead, we have previously established that the racemization of **5b** can occur under relatively mild basic conditions.[2,10] This is due to a facile retro-Michael/Michael reaction equilibrium between the isoxazoline ring and derived acrylate.[4] We anticipated that the combination of this ring-cleavage equilibrium and the conversion of the ester to a thioester **6** would enable racemization to occur under conditions mild enough to also support enzymatic resolution (Scheme 2). This premise proved to be true.

A useful conversion of **5b** to **2** via thioesters would require several prerequisites: the discovery of an efficient thioester/enzyme couple, the establishment of thioester racemization conditions, optimization of the best combination of thioester/enzyme/racemization conditions, and ultimately an excellent preparation of the selected thioester to overcome the inherent disadvantage of an extra synthetic step between **5b** and **2**. Each of these components was studied individually to allow an orderly approach to developing an efficient enzymatic dynamic kinetic resolution.

A variety of thioesters were quickly prepared by the conversion of racemic **2** to the acid chloride, followed by reaction with the copper salts of the requisite thiols.[11,12] (Table 1). We screened these thioesters against 21 enzymes (see Experimental Section for further details) and looked for combinations that produced both high conversion and ee_p.[13]

(6) (a) Ward, R. S. *Tetrahedron: Asymmetry* **1995**, *6*, 1475−1490. (b) Noyori, R.; Tokunaga, M.; Kitamure, M. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 36−56. (c) Faber, K. *Chem. Eur. J.* **2001**, *7*, 5004−5010. (d) Caddick, S.; Jenkins, K. *Chem. Soc. Rev.* **1996**, *25*, 447−456. (e) Gihani, M. T. E.; Williams, J. M. J. *Curr. Opin. Chem. Biol.* **1999**, *3*, 11−15.

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(9) The authors later considerably broadened the scope of the new reaction: Um, P.-J.; Drueckhammer, D. G. *J. Am. Chem. Soc.* **1998**, *120*, 5605−5610.

(10) See ref 4/Supporting Information for discussion of the mechanism of this racemization.

(11) Reissig, H.-U.; Scherer, B. *Tetrahedron Lett.* **1980**, *21*, 4259−4262.

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Table 1. **Thioesters prepared for initial screening**[a]

entry	thioester	compound	yield (%)
1	phenyl	**6a**	92
2	ethyl	**6b**	73
3	*n*-propyl	**6c**	86
4	*n*-butyl	**6d**	>99
5	*i*-propyl	**6e**	71[b]
6	*s*-butyl	**6f**	94
7	*tert*-butyl	**6g**	89
8	*i*-butyl	**6h**	91

[a] Reported as crude yields for 5−10-g scale experiments. [b] Prepared via the silylated mercaptan/$AlCl_3$ method.

While the mixture was basic (pH ≈ 9−10), we were not seeking to establish a racemization at this point but rather to identify a specific thioester/enzyme pair that would produce **2** in high ee_p. These preliminary screening experiments and several individual 1−2-g experiments produced two useful observations. Amano PS-30 lipase, the same enzyme that was optimal for the esters, was the only one that displayed acceptable enantioselectivity and reaction rate, at least for some thioesters. This was not unexpected as PS-30 had previously resolved two esters related to **6**. As regards the choice of thioester, only those with linear alkyl chains (ethyl, *n*-propyl, and *n*-butyl) were efficiently (1−2 days) hydrolyzed with acceptable enantioselectivity (>85% ee_p).[14] Remarkably, branching on the thioalkyl moiety (i.e., *s*-butyl, *i*-propyl, etc.) led to a significantly poorer conversion and ee_p. This is in contrast to the example of the *i*-butyl ester **5b** and indicated that the enzyme's active site is more restrictive of thioesters than oxoesters. Having identified three likely thioesters and an inexpensive enzyme, the focus shifted to the search for racemization conditions.

To quantitate the racemization rates, the chiral *n*-propyl thioester **6c** was prepared in the previously described manner but from resolved acid **2**. A heterogeneous mixture of this thioester, PS-30 lipase, and Triton X-100 in pH 9.25 phosphate buffer held at 40 °C for 4 days led to no racemization. More basic reaction conditions would have adversely affected the lipase;[15] therefore, we considered the addition of amines and/or toluene, which can significantly increase thioester racemization rates while retaining the activity of the enzyme.[8,16] The results of these experiments are listed in Table 2.

The following observations redirected our subsequent research:

(13) On the basis of our previous success with Amano lipase PS30 for the resolution of isoxazolines, newly prepared thioesters were typically tried first with PS30 in 1−2 g experiments rather than within the formal screen. Reaction for all experiments were followed by chiral LC which conveniently indicated both the extent of hydrolysis and the ee_p of the **2** produced. The majority of these screening experiments produced low conversion with poor enantioselectivity and therefore only the better results noted for each thioester are listed in the Experimental Section.

(14) We did not test the shortest "linear" alkyl thioester, methyl thioester, due to the problems of stench, the boiling point of methanethiol, and the poor resolution of the oxoester analogue.

(15) Amano Enzyme U.S.A. Co., Ltd. Company enzyme catalog and technical information, 2001.

(16) (a) Chang, C.-S.; Tsai, S.-W.; Kuo, J. *Biotechnol. Bioeng.* **1999**, *64*, 120−126. (b) Chang, C.-S.; Tsai, S.-W. *Biochem. Eng. J.* **1999**, *3*, 239−242. (c) Lin, C.-N.; Tsai, S.-W. *Biotechnol. Bioeng.* **2000**, *69*, 31−38.

Table 2. **Racemization conditions for thioester 6c**[a]

entry	amine (equiv)	reaction period	% racemized[b] *(%)*
1	trioctylamine (0.2)	145 h	9
2	trioctylamine (0.2)[c]	8 h	49
3	trioctylamine (2.0)	161 h	15
4	triethylamine (2.0)	141 h	47
5	triethylamine (2.0)[c]	100 min	52
6	trimethylamine (0.2)	50 h	7
7	trimethylamine (1.0)	146 h	50
8	trimethylamine (2.0)	91 h	50
9	trimethylamine (2.0)[c]	24 h	49
10	trimethylamine (3.0)	83 h	50
11	none[d]	97 h	0

[a] Reaction conditions: 1.0−1.5 g **6c**, 0.2 N NaH_2PO_4, pH = 9.2, 40 °C, and Triton X-100. [b] A value of 50% represents one-half-life or 50% ee. [c] 10% v/v toluene added as well. [d] PS-30 lipase present as well.

1. The combination of toluene (10% v/v; 1.5 mL/g of thioester) with any amine dramatically increased the racemization rate.[17]

2. Trioctylamine was ineffectual unless toluene was also present.

3. The addition of ≥2 equiv of trimethylamine produced an acceptable, but not optimal, racemization rate without the necessity of toluene.

Of further significance was that in no instance was ester hydrolysis detected. This increased confidence that nonenzymatic hydrolysis would not degrade the ee_p once dynamic kinetic resolution conditions were established.

There was now present sufficient information from these individual studies to consider the combination of the best examples. A dynamic kinetic resolution should result by matching the established choices of enzyme (Amano PS-30), class of thioesters (C2−C4 linear chains), and racemization conditions (toluene, amine, phosphate buffer, surfactant). Key experiments are listed in Table 3.

An enantioselective dynamic kinetic resolution had occurred although the conversion was incomplete when toluene was present (entries 4 and 8). Despite the increase in the rate of racemization induced by toluene, the enzyme appeared to be significantly inhibited or inactivated by the solvent under these reaction conditions. Fortunately, our earlier investigations had established that trimethylamine alone would induce a sufficient but suboptimal racemization rate. Modifying the reaction mixture by introducing two equivalents of trimethylamine without the presence of toluene produced a >99% conversion to (*R*)-**2** with 97.6% ee_p after 22.1 h (entry 3). The "enantiomeric ratio" *E* of 74 determined for this reaction under nonracemizing conditions predicted a maximum ee_p of 97.3%,[4,18] in satisfyingly close agreement to that found above. The ethyl and *n*-butyl analogues (**6b** and **6d**, respectively) hydrolyzed nearly as stereoselectively (entries 6 and 7) but **6c** produced the best overall process in regards to workup, yield, purity, and safety. We believe these are the first examples wherein enzymatic resolution is coupled with a retro-Michael/ Michael equilibrium, a unique means to achieve a dynamic kinetic resolution.[4]

(17) The amine was still necessary. When the only additive was toluene and the pH was adjusted to ~9.5 with aqueous NaOH, no racemization was detected.

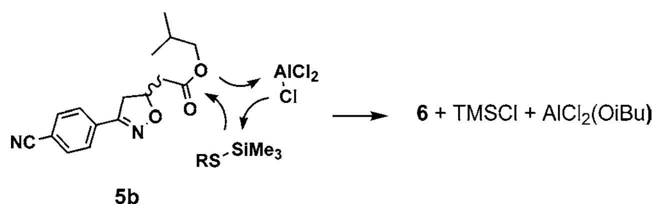
(18) Stecher, H.; Faber, K. *Synthesis* **1997**, 1−16.

Table 3. **Enzymatic dynamic kinetic resolution optimization experiments**[a]

entry	thioester	amine (equiv)	reaction period (h)	conversion (%)	ee_p (*R*)
1	**6c**	trimethylamine (0.2)	65	61	96.5
2	**6c**	trimethylamine (1.0)	48	>99	95.6
3	**6c**	trimethylamine (2.0)	22	>99	97.6
4	**6c**	trimethylamine (2.0)[b]	70	94	96.8
5	**6c**	trimethylamine (3.0)	20	>99	97.3
6	**6b**	trimethylamine (2.0)	16	>99	96.3
7	**6d**	trimethylamine (2.0)	48	>99	97.3
8	**6c**	trioctylamine (0.2)[b]	168	87	97.2
9	**6c**	triethylamine (1.1)	66	>99	96.2
10	**6c**	none	7	23	96.5

[a] Reaction conditions: 1−2 g **6c**, 40 °C, phosphate buffer, Triton X-100, and Amano PS30 charged at 0.1 the weight of ester. The pH was maintained at 9.3−9.5 by 5 N NaOH added via a pH stat device. [b] Reaction medium also contained 10% v/v toluene.

Scheme 3. **Direct conversion of 5b to thioesters**[19]



Only one serious hurdle remained. A good preparation of the thioester was required to make commercial use of this new dynamic kinetic resolution. The procedure used to prepare thioesters during the screening process presented a hazardous and lengthy path for a viable process. We have previously reported an efficient means for converting **5b** directly to a variety of thioesters[19] by the aluminum chloride-mediated reaction of silylated mercaptans with esters (Scheme 3).[20] While the preparation of the thioester in this manner formally adds an extra step, the synthesis of **5b** had been well-established in the pilot plant, and it would be attractive to retain this intermediate in our new route. The combination of a high-yield process to convert **5b** directly to **6c** and the subsequent thioester dynamic kinetic resolution was a reasonable alternative to the longer ester kinetic resolution/unreacted ester recovery/racemization/recycle route and would allow the luxury of the additional thioester preparation step.

Our main objective during the process optimization phase for preparing **5b** was to discover a substitute for aluminum chloride so as to eliminate the need to handle a fuming and hazardous solid. We considered several likely Lewis acids that are liquids (boron tribromide and boron trifluoride complexed with various ethers) without success. The butyl ether complex of boron trifluoride produced the best results (6% solution yield after 6 days), which was obviously not competitive with our existing conditions. Instead, our engineers developed a safe means to handle the water-reactive aluminum chloride (described below), and the chemistry was maintained unchanged.

We explored any large-scale effects within this step by increasing the charge of **5b** 10-fold to 2 kg in our kilolab. This experiment proceeded nearly flawlessly in 93% crystallized yield (99.0 wt %). Our only change was the addition of salt to the third wash since the low acid content led to emulsions otherwise. These results were encouraging and permitted further scale-up.

This chemistry was adapted to the pilot plant with the precaution of using a bleach and sodium hydroxide-filled vessel as a thiol scrubber, followed by the combustion of any remaining volatiles in the thermal oxidizer. While we had planned to use *n*-hexyllithium to deprotonate the thiol, a sudden shortfall of the bulk supply forced our reliance upon *n*-butyllithium instead. As butane was now produced as the conjugate acid of the deprotonation of propanethiol, a concern was now the presence of the low-boiling (bp = −0.5 °C) hydrocarbon in solution. This would complicate attaining toluene reflux (111 °C) later and was also a safety concern. The butane was stripped from the solution of the lithium salt by sparging with nitrogen at 30 °C for 6 h. The volatilized hydrocarbon was directed to the thermal oxidizer, and its heat of combustion provided an unconventional means to measure the endpoint of the sparge. After adding back 20 kg (∼6% of total weight) of heptane to make up for the solvent loss to the sparge, the solution of lithium silylsulfide was ready to be reacted with **5a**.

To allow safe handling of the reactive aluminum chloride, our engineers designed a solids addition device which attached to a reactor port and allowed charging of a preweighed quantity of aluminum chloride into an inerted chamber. When appropriate, internal fins were actuated and the solids dropped into the reaction mixture. This worked very well with no significant solids adhesion within the device. Subsequent chemistry proceeded exactly as at smaller scale and we prepared 45 kg of thioester **6c** (98.6 wt %) in 91% yield in our initial batch.

Scale-up of the enzymatic step was even more facile. Lab-scale work (up to 183 g of **5b**) defined the basic parameters of the work, as described above, and was multiplied 10-fold when introduced into the kilolab (1.860 kg). The results were excellent (1.309 kg of **2**, 99.6 wt %, ≥99.5% ee, 88.7% yield). Only two points were important for further scale-up. The time needed to attain reaction completion (34 h; >99 LC area %) was not comparable to that of laboratory experiments (20 h). Perhaps differences in stirring efficiency, particle size, vessel size, and other physical factors rendered mixing less efficient in the kilolab and resulted in a slower conversion. Second, foaming occurred in the receiver during

(19) Pesti, J. A.; Yin, J.; Chung, J. C. *Synth. Commun.* **1999**, *2*, 3811−3820.
(20) Mukaiyama, T.; Takeda, T.; Atsumi, K. *Chemistry Lett.* **1974**, 187−188.

the filtration of the reaction mass. Foaming could be controlled by various means (greater receiver volume, reduced vacuum, pressure applied to the top of the cake in place of vacuum below the cake, etc.). The lack of adverse observations now permitted an attempt at our final objective: further scale-up into the pilot plant.

The thioester (44.9 kg) was converted into **2**, again with careful attention to capturing the volatile thiol produced during the hydrolysis. As regards design, the plant arrangement differed from the kilolab preparations only in that pH was not automatically controlled. A mixture of sodium phosphate, trimethylamine, surfactant, **6c**, and Amano PS-30 lipase were reacted until HPLC analysis indicated sufficient conversion (96%). This was conducted in a manner similar to that of the kinetic resolution process to produce 28.4 kg of **2** (>99.9 wt %) in 80.4% overall yield. While the ee_p was only 94.4% at the end of the reaction, the acid precipitation of crude **2** raised it to 98.6%, and subsequent recrystallization purified it to >99.9%. The multikilogram preparation ran as planned, and no changes were required. This high yield and well-functioning process leading to a pure product fulfilled our goal of transforming the kinetic resolution of **5b** into a dynamic kinetic resolution and successfully demonstrating this chemistry in the pilot plant.

While the thioester process to **2** is certainly more interesting chemistry when compared to the kinetic resolution, it can be difficult to decide between these two established routes. It is not unreasonable to argue that the need to prepare **6c** and handle malodorous reagents outweighs the benefits of the subsequent dynamic kinetic resolution. The preference expressed by potential contractors between the kinetic and dynamic resolutions should be noted. Their judgment was that the higher conversion and overall yield of the new process outweighed the disadvantages. Obviously, the selection between the two will be driven by the experience and equipment available to the chemist, and either route may be superior under different circumstances. In any event, the dynamic kinetic resolution does supply a new option for the preparation of the key roxifiban intermediate **2**.

Conclusions

The existing kinetic resolution of the isobutyl ester **5b** to form the acid **2** has been transformed into a dynamic kinetic resolution via the intermediacy of the thioester **6c**. Optimized reaction conditions for this dynamic kinetic resolution were determined by a series of screening trials examining the interactions of enyzmes and thioesters, racemization conditions of the thioester **6c**, and finally by dynamic kinetic resolution experiments derived from the previous two screens. The preparation of **6c** was improved by synthesizing it directly from **5b** with silylated propanethiol and aluminum chloride and by developing means to conduct this chemistry on large scale safely. The subsequent dynamic kinetic resolution scaled up to the pilot plant facilely and proceeded at large scale in a well-behaved manner. This new chemistry avoids the inconvenient recycling of the unreacted enantiomer required by the kinetic resolution by instead introducing an extra step to prepare the thioester and provides additional possibilities for the preparation of roxifiban.

Experimental Section

General.[21] Large-scale reactions were conducted in 200- and 300-gal glass-lined reactors and were vented through a scrubber containing 50 kg of 5−10% bleach, 65 kg of 30% sodium hydroxide, 8 kg of 2-propanol, and 25 kg of water. The gas stream was further directed to a thermal oxidizer.

[3-(4-Cyanophenyl)-4,5-dihydroisoxazol-5-yl]thioacetic Acid, *S*-Isobutyl Ester (6c). To a solution of THF (189 kg) and 1-propanethiol (24.5 kg, 322 mol) was charged a solution of 2.5 M *n*-butyllithium in hexane (90.6 L, 226.5 mol) over 130 min at <15 °C. Chlorotrimethylsilane (37.5 kg, 345 mol) was charged over 62 min at 0−15 °C. Nitrogen was sparged through the solution as the reaction temperature was raised to 30 °C for 6 h to remove dissolved butane. Heptane (20 kg) was charged to make up for the volume loss that occurred during the stripping operation, and the solution was cooled to 20 °C. The slurry was filtered through a Dacron bag, and cartridge filters in series into a solution of isobutyl 2-[3-(4-cyanophenyl)-4,5-dihydro-5-isoxazolyl]-acetate (**5b**) (50.0 kg, 96.5 wt %, 168.5 mol) followed by a mixture of THF (22 kg) and heptane (17 kg). After 10 min, the solution was cooled to ≤10 °C. Aluminum chloride (30.0 kg, 225 mol) was charged through a solids charging adapter over 35 min at ≤20 °C, followed by heptane (5 kg), and the slurry was heated to 65 °C over 75 min. After an hour at 65 °C, LC indicated an **6c**:**5b** area ratio of 97.03:0.20. After 110 min, the reaction was cooled to ≤20 °C. Water (213 L) and toluene (114 kg) were charged over 1 h at <30 °C. The phases were separated, and the organic layer was washed with water (2 × 100 L). A final wash was mixed with sodium chloride (14.1 kg) to aid the separation. The organic phase was reduced in volume by vacuum distillation at 40−45 °C and 50−75 mmHg vacuum. After 308 kg had been collected, heptane (256 kg) was charged at 35−40 °C over 68 min to crystallize **6c**. The slurry was cooled to 0−5 °C over 3 h and held 9 h. The crystals were filtered, and the cake was washed with 0−5 °C heptane (2 × 51 kg). The crystals were dried at 50 mmHg and 45 °C for 40 h to yield 44.9 kg (98.6 wt %, 91% yield). This material was identical to that previously reported.[19]

(*R*)-2-[3-(4-Cyanophenyl)-4,5-dihydroisoxazol-5-yl]acetic Acid (2). A solution of water (653 L), sodium phosphate monobasic monohydrate (67.5 kg), and aqueous 24% trimethylamine solution (73.5 kg, 0.297 kmol) was treated with sodium hydroxide solution (33%, 24.4 kg, 0.201 kmol) at <30 °C to raise the pH to 9.09. Triton X-100 (3.7 kg), **6c** (44.9 kg, 0.156 kmol) and lipase Amano PS-30 (4.5 kg) were charged sequentially, and the resulting mixture was heated to 40 °C over 1 h. The mixture was stirred at 40−43 °C, and the pH of the mixture was adjusted every 2−6 h to 9.2−9.5 by adding 33% NaOH solution (~3 kg each time; total of 21 kg charged). After 41 h, HPLC indicated an area ratio of **2**:**6c** of 96.1:3.7. The reaction mixture was cooled to

(21) HPLC methods are described in ref 4 and the corresponding Supporting Information, and in ref 19.

10 °C, Celite 545 (24.1 kg) was charged and the mixture was filtered through more filter aid slurried in water. A total of 180 L of water was used to wash through the remaining product solution. The filtrate was cooled to 10 °C, isopropyl acetate (20 kg) was added, and the mixture was acidified to pH 3.09 by the addition of 85% phosphoric acid (55.1 kg) over 1.75 h at <10 °C. The mixture was cooled to 5 °C, and the solids were collected by filtration. The cake was washed with 5 °C water (3 × 45 L) and then slurried with absolute ethanol (188 kg) at 0−5 °C. The wet solids were then recrystallized from ethanol (523 kg) by heating to reflux over ~1 h to dissolve all the solids. The mixture was then cooled to 5 °C over 4.5 h and held at 0−5 °C for 2 h. The resulting slurry was filtered, washed with ethanol (76 kg), and dried at 50 °C at 50 mmHg for 24 h to yield 28.4 kg (>99.9 wt %, >99.9 ee %, 80.4% yield) of **2**. This material was identical to that previously reported.[2,5]

Enzyme Screening Experiments.[13] The typical experiment consisted of a mixture of 20−25 mg of thioester, 10−20 mg of lipase, 3−4 drops of Triton X-100, and 2−3 mL of phosphate buffer, pH 8, maintained at 40−45 °C for 18−24 h while either shaken or stirred by a Teflon stir bar in a capped vial. The following Amano enzymes were screened against the thioesters listed in Table 1: lipase AP12 (*Aspergillus niger*), lipase AY30 (*Candida rugosa*), lipase G (*Penicillilum camembertii*), lipase GC20 (*Geotricum candidum*), lipase L10 (*C. lipolytica*), lipase AK (*Pseudomonas fluorescens*), lipase FAP (*Rhizopus oryzae*), lipase N (*R. niveus*), lipase PS30 and PS (*Pseudomonas cepacia*), lipase AY (*C. rugosa*), lipase D (*R. delemar*), lipase MAP10 (*Mucor* sp.), lipase CES (*P. burkholderia*), and lipase R10 (*P. roqueforti*). These lipases are classified as triacylglycerol acylhydrolases, (EC 3.1.1.3). Screened as well were the Amano proteases S (*Bacillus stearothermophilus*)) and N (*B. subtilis*), both classified as EC 3.4.24.28. The following Sigma enzymes were screened: acylase I (*Aspergillus* spp., EC 3.5.1.14), lipase II PPL (porcine pancreas, EC 3.1.1.3), protease XXIII (*A*. oryzae), and PLE (porcine liver esterase, C 3.1.1.1). The better results for each thioester follow: **6a**: no specificity >40% ee_p noted for any enzyme. **6b**: Amano PS30 (~99% ee_p after workup at ~92% conversion). **6c**: Amano PS30 (~97% ee_p after workup at ~85% conversion), Amano CES (74% ee_p at 79% conversion). **6d**: Amano PS30 (87% ee_p at ~60% conversion; Amano AK (95% ee_p at ~47% conversion). **6e**: Amano PS30 (31% ee_p at ~12% conversion). **6f**: Amano PS30 (68% ee_p at ~3% conversion). **6g**: Amano R10 (59% ee_p at ~15% conversion). **6h**: Amano AY (60% ee_p at ~1.5% conversion).

Acknowledgment

We thank the following colleagues for their contributions in the development of this chemistry: Robert Wethman, Dennis Potter, Bill Boucher, Walt Conway, Joseph Renai, Dawn Blackburn, Elton Watson, Fred Maccherone, and Jeff Koskey. We are grateful for insightful suggestions in the preparation of the manuscript from Robert Discordia, David Kronenthal, Edward Delaney, Robert DiCosimo, and Ambarish Singh.

Received for review May 16, 2003.

OP0300239