Organic Process Research & Development **2006**, *10,* 588–591

Development of a Scalable Process for a Key Intermediate of (*R*)-Metalaxyl by Enzymatic Kinetic Resolution†

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Abstract:

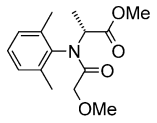
A scale-up study was carried out for the enzyme-catalyzed kinetic resolution of 2-methoxyethyl *N*-(2, 6)-dimethylphenyl)-alaninate *rac*-3 for the preparation of (*R*)-metalaxyl at 20-L scale. Immobilization of lipase PS on a polymeric support enabled the reuse of the enzyme. The unreacted enantiomer was racemized by means of acid/aldehyde catalyzed Schiff base intermediate formation. The combination of lipase PS-catalyzed hydrolytic kinetic resolution and racemization of the remaining ester gave a satisfactory reaction yield (> 80%) and high enantiomeric excess (96% ee) for one recycle.

Introduction

A large number of useful chiral chemicals contain at least one stereogenic center and require thought for setting or resolving the stereochemistry when their preparations are considered.[1] The importance of such enantiomerically pure compounds is expanding rapidly in pharmaceutical, agrochemical, and synthetic organic chemistry. In particular, the necessity to efficiently obtain chiral molecules has further increased due to the strategy of the "chiral switch", the need to convert marketed racemic pharmaceuticals and agrochemicals into their single active enantiomers for reduction of the environmental, legal, and medical burdens of undesired enantiomers.[2] Obviously, the need for process-viable means to resolve fine chemicals is increasing and deserve dissemination when successful examples exist.

Methyl (*R*)-*N*-(2, 6-dimethylphenyl)alaninate (**(*R*)-1**) is a common intermediate to chiral metalaxyl, benalaxyl, and furalaxyl.[3] (*R*)-Metalaxyl (**(*R*)-2**) is of particular importance as it is the primary ingredient of mefenoxam (Metalaxyl-M), an important fungicide.[4] The current means of its preparation results in 95.6% enantiomeric excess (ee) at multiton scale.[5] Its asymmetric synthesis using chiral catalysts is problematic for scale-up due to catalyst cost and availability. Due to the importance of metalaxyl, we felt it justified to further examine means to prepare this compound.

Our selection of the ester substrate and the enzyme for the improved preparation of (*R*)-metalaxyl has been reported.[6] In the current manuscript, we describe our work at increasing the scale of the hydrolytic kinetic resolution of 2-methoxyethyl *N*-(2, 6-dimethylphenyl)alaninate (***rac*-3**) to form the corresponding chiral acid **(*R*)-1** by the use of lipase PS. This research was transformed into a scalable process by a reusable immobilized enzyme and racemization of the unreacted substrate to establish a process that can be used for large-scale manufacture of this important intermediate.



(*R*)-**2** (Mefenoxam)

Results and Discussion

Synthesis of Racemic 2-Methoxyethyl *N*-(2, 6-Dimethylphenyl)alaninate (*rac*-3). Since methyl *N*-(2, 6-dimethylphenyl)alaninate (*rac*-**1**) is readily available, it would be attractive to retain this intermediate for the synthesis of the 2-methoxyethyl ester, an efficient substrate for lipase PS-catalyzed resolution but to shorten the route to it. The synthetic route to ***rac*-1** has been already established in the manufacture of racemic metalaxyl.[6,7] Thus, our first goal was to find a more efficient path to this 2-methoxyethyl ester. The procedure used for the small-scale synthesis of this ester as well as the various esters screened in the preliminary work required the acid and the corresponding acid chloride as intermediates. A direct transesterification would be an obvious improvement. For the preparation of the 2-methoxyethyl ester from methyl ester, we examined the reactions of ***rac*-1** with 2-methoxyethanol in the presence of DBU[8] and DMAP at 135−140 °C. While experiments using DMAP were incomplete, DBU permitted complete transesterifcation at 135−140 °C (97%) as long as 5% w/w of the base was charged initially. Our final conditions also increased the

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Published on Web 02/04/2006

10.1021/op050166q CCC: $33.50

Table 1. **Racemization of (*S*)-3/(*R*)-3 (9/1)**

reaction entry	catalyst system	solvent (pressure)	time (h)	*R*/*S* ratio (chiral HPLC)
1	butyraldehyde (0.1 equiv) acetic acid (0.1 equiv)	toluene	24	60/40
2	butyraldehyde (0.5 equiv) benzoic acid (0.3 equiv)	toluene (1.5 bar)	6	50/50
3	butyraldehyde (0.1 equiv) acetic acid (0.1 equiv)	toluene (1.5 bar)	6	40/60

charge ratio of the 2-methoxyethanol/**1** to 1.37. We achieved a near quantitative yield of of ***rac*-3** from of ***rac*-1** at kilogram scale without problems.

Racemization of the Unreacted *S* Ester. As kinetic resolutions are limited by definition to a maximum of a 50% yield, a method of recovery or in situ conversion of the remaining (*S*)-ester was required for a commercial preparation to be viable. Fortunately, there are a wide variety of established means for this.[9,10] Dynamic kinetic resolution would have been ideal; however, attempts using the *n*-butanethioester as substrate produced poor enantioselectivity (45% ee in 3 h, (*R*)-selective). Further improvement in ee could not be achieved with various alkyl thioesters as reported in the literature.[11] As a result, we moved onto examining racemization conditions, both basic and acidic, to permit a recycle of the less reactive enantiomer. These experiments (Table 1) were performed using enzymatically prepared and enriched ***S*-3** (*S*/*R* = 90/10). Past experience using ***S*-1** has established that either no or insignificant racemization was detected in either toluene or methanol with the use of various bases or sulfuric acid. This suggested that the pK_a of the adjacent methine for **3** and **1** would be insufficient to permit racemization; not surprising, considering that it is an amino acid and the electronic influence of the amine would be counterproductive in this case. A popular means to circumvent such limitations is to convert the amine into either a Schiff base, hydantoin, or an oxazolone.[12,13] We found that the use of butyraldehyde, catalyzed by either acetic or benzoic acid to form the aldimine, was successful for racemization. We were not able to use these conditions directly upon the completed reaction mixture in an effort to avoid the need to work up the resolution, as the enzyme was subsequently nonreactive. Instead, the mixture was worked up, and the unreacted ester was racemized. Curiously, the application of slight pressure (1.5 bar) increased the rate of the racemization. After purification by distillation, the racemized ester was returned for another cycle of resolution. The efficient recovery and a single recycling of the unreacted ester effectively increased the yield of (*R*)-acid from racemic methoxyethyl ester up to 80% (Scheme 1).

Development of Enzymatic Reaction Conditions for Large Scale. Our studies at optimizing the enzymatic resolution revealed that exclusively aqueous environments were sufficient; there was no need for an organic cosolvent, which simplified logistics and waste treatment. Racemic **3** was suspended in pH 7 buffer (maintained by 5 N aqueous NaOH addition/pH stat) and stirred at room temperature with the enzyme. Upon 45% completion, the unreacted ester **(*S*)-3** was extracted into toluene. The aqueous reaction mixture was acidified to pH 2−3 with hydrochloric acid, followed by extraction of **(*R*)-4** into toluene. However, these extractions were plagued by emulsions, complicating the phase separation. Lipase PS contains insoluble components such as Celite and active proteins, which could act as surfactants to induce incomplete separations.

We were able to solve this problem by the simple elimination of the buffer and pH control itself! The use of buffers or a pH stat became extraneous since, when the hydrolysis was conducted in water, the hydrolyzed acid precipitated as small particles. These could be easily extracted into solvent after the enzymatic kinetic resolution was complete (at ∼50% conversion).

Another potential means to avoid the emulsion problem would be the immobilization of the lipase.[14] Accurel is a hydrophobic polypropylene powder shown to be an effective support for immobilized lipases in organic media.[15] Our first attempt to follow this specific example failed as the heterogeneous nature of the reaction mixture of oily ester and water was not compatible with the packed bed. Thus, we surveyed various supports, both organic and inorganic. Five different immobilized enzyme preparations were submitted to the resolution conditions. If we found that the first resolution was acceptable and reasonably complete (≥48%), the same support was recycled to determine the robustness of the activity. Sepabead EC-butyl (Resindion, Italy) at 25 °C produced the best results and put down 46.6% reaction completion at 98.5% ee.[16,17] The expense and opportunity of the preparation of a supported catalyst also suggested we

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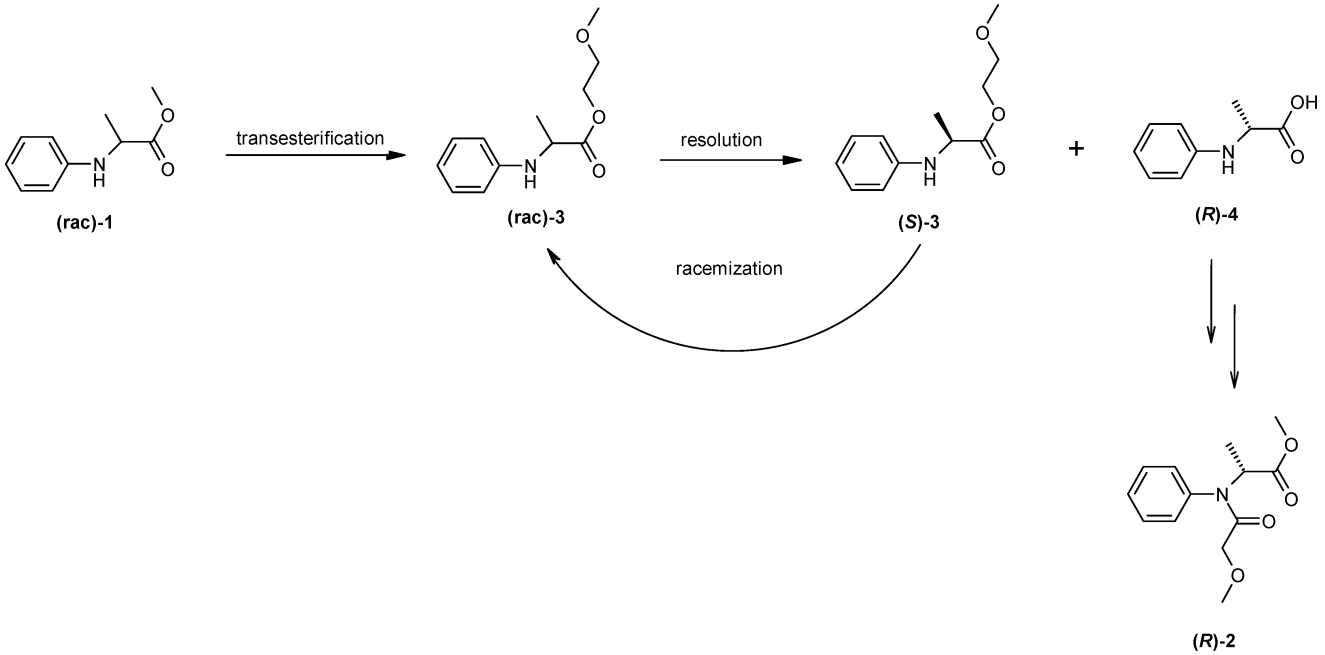
Scheme 1. **Lipase-catalyzed hydrolysis of racemic ester coupled with racemization**



examine the possibility of recycling the enzyme system. As a further test, it was submitted to 20 cycles, and we were happy to note that the extent of reaction remained constant. (Figure 1.)

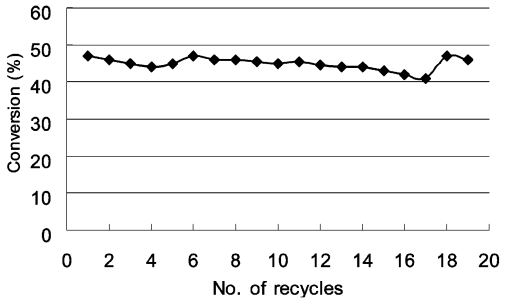


Figure 1. **Reuse of lipase PS on Sepabead EC-butyl and extent of conversion.**

Finally, a set of experiments were conducted to determine the optimum reaction concentration as a compromise between volume efficiency and reaction completion (Table 2). On the basis of these results, we decided to target a concentration of 400 g/L of **3** for scale-up, as this value permitted >35% conversion for at least three cycles. It appeared to be a reasonable compromise between volume efficiency and reactivity.

Table 2. **Extent of reaction completion versus substrate concentration for repeated (rac)-3 resolution**

	concentration (g/L)					
recycle	200	300	400	600	800	1000
1	48.0%	46.2%	40.8%	36.6%	34.0%	36.8%
2	46.0%	45.8%	38.6%	33.8%	32.5%	32.3%
3	45.3%	44.9%	35.7%	34.1%	32.3%	29.6%
4	44.5%	44.3%	34.2%	35.2%	30.3%	26.8%
5	–	44.7%	35.9%	33.8%	30.5%	30.4%
6	46.2%	46.0%	33.8%	35.9%	29.7%	29.8%
7	46.6%	44.8%	32.7%	31.8%	28.9%	27.5%

The final scale-up of the enzymatic resolutions to 20 L was uneventful. The degradation of the enzyme was negligible, and at workup, simple filtration sufficed to remove the beads. The only drawback was that the ee of **(*R*)-4** dropped to 96% ee. We believe this was due to the changes in the dynamics of agitation that often occur during the introduction of heterogeneous reactions into larger reactors. However, this material could still be converted successfully into (*R*)-metalaxyl as previously defined, completing the development of an alternate commercial process for this fungicide.[6]

Summary

An efficient preparation of the fungicide intermediate **(*R*)-3** has been described using lipase PS immobilized on a Sepabead EC-butyl support, enabling the reuse of the enzyme >20 times and avoiding inconvenient workup conditions. Once the enzymatic kinetic resolution was complete and the resolved product removed, an efficient racemization of the remaining ester was achieved through the acid-catalyzed butyraldehyde Schiff base intermediate. A simple, economic, and environmentally acceptable process for the preparation of chiral methyl (*R*)-*N*-(2,6-dimethylphenyl)alaninate, a key intermediate to (*R*)-metalaxyl, has been achieved.

Experimental Section

General. Enzymatic optimization work was performed in 0.1 M phosphate buffer (1.0 mL, pH 7.0) at 50 mg of substrate at 30 °C. Reverse-phase HPLC was carried out on a C18 Capcell Pak column for the substrate conversion (250 × 4.6 mm, acetonitrile/water/trifluoroacetic acid = 70/30/0.1) and chiral HPLC on a Chiracel OD column (250 × 4.6 mm, *n*-hexane/*i*-PrOH/trifluoroacetic acid = 95/5/0.1 for acid, *n*-hexane/*i*-PrOH = 100/1 for esters and *n*-hexane/*i*-

PrOH = 30/70 for Mefenoxam) at UV 230 nm. The conversion can be calculated from the equation $c = ee_s/(ee_s + ee_p)$, where ee_s and ee_p represent the enantiomeric excess of starting ester and acid product, respectively.[18] GC analyses were performed on a chromatograph equipped with an FID detector using a capillary column AT-5 (Altech). The oven temperature was programmed as follows; at 100 °C/5 min, ramping 10 °C/min up to 250 °C, at 250 °C/5 min. Helium was used as a carrier gas. Accurel EP 100 (polypropylene powder) was a product of Membrana GmbH (Obernburg, Germany). TN-M and TN-A are inorganic silica supports derivatized with methacryloxy and amino groups, respectively and provided by Toyo Denka Kogyo (Koichi, Japan). Sepabeads EC-butyl (methacrylate resin) was obtained from Resindion-Mitsubishi Chemical Co. (Milan, Italy).[16] HP2MG Diaion (acrylate resin) was purchased from Mitsubishi Chemical Co. (Japan). Other chemicals of analytical grade were commercially available and used without further purification. Racemic **1** and racemic esters of (*R,S*)-*N*-(2,6-dimethylphenyl)alaninate were prepared as described in the literature from (*R,S*)-*N*-(2, 6-dimethylphenyl)alaninate after hydrolysis of racemic **1** via the acid chloride.[6]

The methyl ester **(*R*)-1** and metalaxyl **(*R*)-2** were prepared essentially as previously described, however at larger scale. Their spectral data are published elsewhere.[6]

Transesterification To Prepare 2-Methoxyethyl (*R,S*)-*N*-(2,6-Dimethylphenyl)alaninate (*rac*)-3. To a solution of 2-methoxyethanol (1036.35 g, 13.6 mol) and *rac*-**1** (1035.35 g, 5.0 mol) was charged 51.81 g of DBU over 30 min at <15 °C. The reaction temperature was raised to 135 °C for 1 h, and the reaction mixture was stirred for 5 h. After addition of additional 380 g of 2-methoxyethanol (0.5 mol), the mixture was maintained at 135 °C for 7 h. The organic phase was reduced in volume by vacuum distillation at 135 °C and 50−75 mmHg vacuum. The mixture was cooled to 25 °C. Two liters of toluene and 1 L of water were charged, and the mixture was stirred for 30 min at 25 °C. The layers were separated, and the organic layer was dried over anhydrous $MgSO_4$. The organic layer was filtered and concentrated in vacuo to produce 1210 g of *rac*-**3** (yield 96.3%, GC purity 98.5%).

Racemization to Prepare 2-Methoxyethyl (*R,S*)-*N*-(2,6-Dimethylphenyl)alaninate (*rac*)-3. To a mixture of *n*-butyraldehyde (8.61 g, 0.12 mol) and benzoic acid (5.84 g, 0.05 mol) was added a solution of 2-methoxyethyl *N*-(2,6-dimethylphenyl)alaninate **(*S*)-3** (30.0 g, 0.12 mol) in toluene (100 mL). The mixture was refluxed for 15 h under N_2. The mixture was cooled to 20 °C and washed with 5% Na_2CO_3 (3 × 100 mL) and H_2O (10 mL). The layers were separated, and the organic layer was dried over anhydrous $MgSO_4$. The organic layer was filtered and concentrated in vacuo. The residue was distilled (1 Torr) at 110 °C to give a pale-yellow oil (26.8 g, 89% yield, 97.3% purity, *R*/*S* = 48.3/51.7).

Immobilization of Lipase PS on Sepabead EC-Butyl. Lipase PS (5 g) was dissolved into 100 mL of 0.1 M phosphate buffer (pH 7.0) and filtered to remove the Celite. Sepabead EC-butyl (10 g) was added, and the mixture was stirred for 12 h. After filtration, 100 mL of 0.1 M phosphate buffer (pH 7.0) containing 100 uL of 25% glutaraldehyde solution was added and the mixture stirred. After washing with 50 mL of 0.1 M phosphate buffer (pH 7.0), the immobilized enzyme was ready for use.

Large-Scale Synthesis of (*R*)-2. A mixture of immobilized lipase PS on Sepabead EC-butyl (960 g) and **(*rac*)-3** (4 kg) was stirred in 16 L of 0.1 M phosphate buffer (1 mM $CaCl_2$, pH 7.0) for 24−41 h at 25−30 °C. The reaction was repeated five times with the recovered enzyme preparation. The reaction mixtures were combined (average 43.5% conversion), 40 L of toluene was added, and the mixture was stirred at 200 rpm with the addition of 6.1 kg of 30% NaOH solution over 2 h at <15 °C.

The organic layer was separated, and the aqueous layer was extracted twice with toluene (25 and 18 L). The combined organic extracts were kept for subsequent epimerization. Dichloromethane (40 kg) was added to the aqueous layer (80 kg), and the mixture was stirred at 130 rpm with the addition of 7.5 kg of concentrated hydrochloric acid over 2 h at <10 °C. The organic layer was separated, and the aqueous layer was extracted with dichloromethane (40 kg). The combined organic extracts were concentrated in vacuo, 36 L of *n*-hexane was added and the mixture stirred at 120 rpm to crystallize out a white solid. After filtration and drying, 5.3 kg of **(*R*)-4** (34% yield, 96.0% ee) was obtained. From the toluene layer 11 kg of unreacted **(*S*)-3** was obtained.

Acknowledgment

We thank Dr. G. H. Joe for his continued interest and support in this work and Dr. J. H. Chung and Mr. B. W. Seo for experimental help and discussion.

Received for review September 8, 2005.

OP050166Q

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