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Studies on the lipase-catalyzed resolution of amino alcohols and its application to the total synthesis of the antituberculous drug ethambutol

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Synthesized tosylated aminobutanol was subjected to lipase-catalyzed esterification using enzymes from different sources (*Candida rugosa*, porcine pancreas, and wheat germ) to screen for the lipase that gives the best resolution. It was found that *C. rugosa* gave the best resolved S-(+)-tosylated alcohol needed to produce (S,S)-(+)-ethambutol, the active isomer against *Mycobacterium tuberculosis*. However, detosylation of this compound yielded no success. Due to this, other protecting groups were used. Among those tried were t-butyloxycarbonyl (BOC) and benzyloxycarbonyl (CBZ) groups. BOC-protected alcohol failed to undergo lipase-resolution while CBZ-protected alcohol underwent smooth resolution. Deprotection of CBZ-protected alcohol yielded the desired S-(+)-alcohol with a dismal 83% enantiomeric excess (%ee). A double resolution afforded to increase the %ee to >99%.

Keywords: enantiomeric excess; lipase-catalyzed resolution; ethambutol; protecting groups; *Mycobacterium tuberculosis*

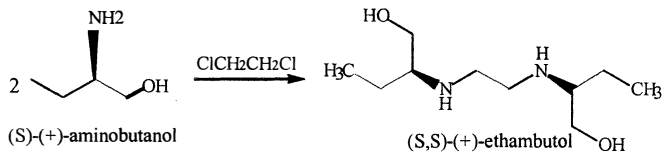
INTRODUCTION

Enantiomers of a drug have identical chemical properties except for their interactions toward optically active reagents [1–3]. The activity and toxicity of drugs are associated with their interaction with proteins, genetic material, and other biological molecules, all of which exhibit stereoisomerism. As a result, the enantiomers of drugs often have different therapeutic and toxicological profiles [2].

Most optically active compounds are obtained by separation of a racemic modification into enantiomers, a process called resolution. Most resolutions are accomplished through the use of reagents that are optically active; these reagents are generally obtained from natural sources since living organisms usually produce only one enantiomer of a pair [1].

The natural enantioselectivity of enzymes is gaining increasing popularity for the purpose of resolution. Lipases, the most widely used enzymes, are used in this particular study because they are easy to handle and are readily available [4]. Aqueous or organic solvents can be used as reaction medium, and the reaction conditions (reaction, temperature, and pH) are mild. Product isolation is also easy (filtration of enzyme followed by solvent extraction) and convenient, thus this enzyme is the choice whenever possible.

The chiral amino alcohol derived from the lipase-catalyzed process was used as a starting material for the total synthesis of the optically pure ethambutol (see scheme 1). This drug has been known for decades and is still currently being used in the chemotherapy of tuberculosis. The S-(+)-isomer will be used for the synthesis of (S,S)-(+)-ethambutol because of its activity against *M. tuberculosis* [5]. Tuberculosis is a widespread disease in the Philippines [6]. In fact, one-third of the world's



Scheme 1. Total synthesis of ethambutol

population is latently infected with the tubercle bacilli [7].It is important to be able to isolate the starting material in an enantiomerically pure form because of the harmful effect of its enantiomer. The enantiomer (R,R)-(-)-ethambutol was reported to purportedly cause blindness [8].

EXPERIMENTAL

General procedure. FTIR spectra were recorded using a BIORAD FTS-40A Spectrometer. Mass spectral analyses were carried out using a Finnigan MAT LCQ Mass Spectrometer. ^{13}C-NMR and ^{1}H-NMR were conducted using a JEOL Lambda 400 MHz High Resolution NMR spectrometer. Reactions were monitored by thin layer chromatography using pre-coated TLC polyester plates with fluorescent indicator where UV illumination served as indicator. Column chromatography was conducted with a mixed solvent (ethyl acetate-hexane) as the eluent. Optical activities of the resolved products were determined using a CD-ORD Jasco Spectrometer and a Atago polarimeter. (+/–)aminobutanol (Fluka), p-tosyl chloride (Fluka), triethylamine (Fluka), BOC anhydride (Aldrich) and CBZ chloride (Aldrich) were used without further purification. All solvents (JT Baker) were AR grade. The lipases used were all acquired from Sigma.

METHODOLOGY

Synthesis of protected aminobutanol

N-tosyl-2-aminobutanol. To a solution containing 5.30 mL 2-aminobutanol and 100 mL CH_2Cl_2 was added 7.840 mL triethylamine. Then, 16.065 g of p-tosyl chloride was gradually added with continuous stirring. The mixture was then refluxed for 6 h. After reflux, the mixture was extracted with 1M KOH to remove any excess p-tosyl chloride present in the solution. The aqueous part collected was acidified with dilute HCl to pH 2–3.5 and was again extracted with ethyl acetate. The combined ethyl acetate extracts was dried with Na_2SO_4. Evaporation of the dried solution gave a white alum-like solid with a yield of 84.75%; mp 69–71°C; Rf = 0.27; m/z = 243.1; FTIR: 3492.85, 3172.51, 2960.24, 2902.35, 1922.03, 1813.97, 1700, 1605.55, 1462.75, 1316.09, 1134.69, 1080.66, 1007.33, 895.406, 822.075, 652.257, 567.347 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.8 (2H, d), 7.3 (2H, m), 5.17 (1H, s, exch), 3.52 (2H, m), 3.17 (1H, s, exch), 2.43 (1H, s), 2.42 (3H, s), 1.42 (2H, m), and 0.73 (3H, s); ^{13}C NMR: δ 143.51, 137.66, 129.71, 127.13, 64.4, 57.6, 24.73, 21.54, 10.15.

N-BOC-2-aminobutanol. To 4.71 mL (0.05 mol) of 2-aminobutanol in 200 mL dichloromethane was added 10 mL triethyl amine. To the resulting solution was added 0.06 mol BOC anhydride. The mixture was stirrred overnight at room temperature, after which all the solvents and triethylamine were removed by rotary evaporation. Further drying was done under high vacuum. The resulting product, which was pure as observed from its ^{1}H- NMR was used in the next step (resolution) as a crude. The reaction was quantitative. ^{1}H NMR ($CDCl_3$) δ0.94 (t, 3H), 1.44 (s, 9H), 1.52 (m, 2H), 2.88 (exch, 1H), 3.52–3.63 (m, 5H), 4.72 (exch, 1H). ^{13}C NMR ($CDCl_3$) δ 156.8, 79.7, 65.6, 54.4, 28.6, 24.7, 10.7.

N-CBZ-2-aminobutanol [9]. To 4.71 mL (0.05 mol) of 2-aminobutanol in 200 mL dichloromethane was added 10 mL triethylamine. To the resulting solution was added 10.2 g (0.06 mol) CBZ chloride dropwise using an addition funnel. The mixture was stirred at room temperature overnight. The mixture was diluted with 20% citric acid (50 mL), the layers were separated and dried over Na_2SO_4 and concentrated in vacuo. The crude oil was chromatographed on silica gel using 1:1 EtOAc/hexanes as eluent to afford 88% of pure N-CBZ-2-aminobutanol that was subjected to resolution. ^{1} H NMR ($CDCl_3$) δ 0.94 (t, 3H), 1.40–1.72 (m, 2H), 2.25 (exch, 1H), 3.54–3.78 (m, 3H), 4.94 (exch, 1H), 5.10 (s, 2H), 7.34 (m, 5H).

Lipase-catalyzed resolution (Typical procedure)

Esterification route in organic solvent. To a mixture of 2.0075 g of the protected alcohol dissolved in 80 mL ether was added 5 mL acetic anhydride. After dissolution, 2.26 g of lipase was added to the mixture and was stirred vigorously until 50% conversion of alcohol to ester was reached. The reaction was monitored by TLC using 40:60 ethyl acetate/hexanes as eluent. The reaction mixture was then washed with 10% $NaHCO_3$ to remove the side product HOAc. After washing, the filtrate was evaporated and a yellowish oily product was produced. This product was subjected to column chromatography to separate the resolved alcohol and ester formed from the reaction.

S-(+)-N-tosyl-2-aminobutyl acetate. FTIR: 3471.34, 3277.66, 3168.65, 2968.47, 2933.22, 2878.71, 1924.61, 1813.97, 1732.92, 1598.89, 1460.96, 1320.53, 1159.56, 1091.59, 815.461, 665.647, 551.011 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.78 (2H, d), 7.31 (2H, d), 4.82 (1H, broad d), 3.54 (2H, m), 3.17 (1H, broad sextet), 2.43 (3H, s), 2.07 (1H, exch), 1.46 (2H, m), 0.75 (3H, t); ^{13}C NMR ($CDCl_3$): δ 143.55, 137.62, 129.73, 127.14, 64.43, 57.15, 24.81, 21.56, 10.14.

(R)-(–)-N-tosyl-2-aminobutanol. FTIR: 3583.99, 3280.58, 2970.93, 2883.05, 2740.25, 1922.03, 1743.68, 1597.84, 814.356, 659.976, 479.743 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.76 (2H, d), 7.30 (2H, d), 4.97 (1H, broad d), 3.93 (2H, m), 3.39 (1H, broad sextet), 2.42 (3H, s), 1.93 (3H, s), 1.48 (2H, m), 0.83 (3H, t); ^{13}C NMR ($CDCl_3$): δ 170.88, 143.38, 138.15, 129.68, 127.01, 65.42, 54.25, 25.42, 21.51, 20.63, 9.95.

(S)-(+)-N-CBZ-2-aminobutanol. ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3H), 1.40-1.72 (m, 2H), 2.25 (exch, 1H), 3.54–3.78 (m, 3H), 4.94 (exch, 1H), 5.10 (s, 2H), 7.34 (m, 5H).

(R)-(-)-N-CBZ-2-aminobutyl acetate. ^{1}H NMR ($CDCl_3$): δ 0.95 (t, 3H), 1.53 (m, 2H), 2.03 (s, 3H), 3.83 (m, 1H), 4.09 (m, 2H), 4.79 (exch, 1H), 5.10 (s, 2H), 7.35 (m, 5H).

Hydrolysis in aqueous buffer for double-resolution. A buffer solution (0.1 M sodium phosphate, 60 mL) was added to 5.05 mmol of the ester in a round-bottom flask. The mixture was stirred fairly vigorously. Lipase (1.0 g) was added and the mixture was stirred at room temperature. The progress of the reaction was monitored by TLC and stopped when it has reached about 80% conversion.

Deprotection of N-CBZ-2-aminobutanol

N-CBZ-2-aminobutanol (0.01 g) was dissolved in 10 mL methanol. To this was added 10 mg 5% Pd on carbon. The flask was evacuated and filled with an atmosphere of hydrogen gas using a balloon attachment. The suspension was stirred at room temperature after which the catalyst was filtered off. Evaporation of the solvent afforded the pure 2-aminobutanol in a quantitative yield.

Standard procedure for the synthesis of ethambutol

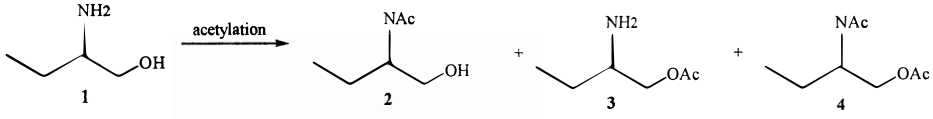
A 2:1 mole ratio of 2-aminobutanol and 1,2-dichloroethane was mixed and stirred into a round-bottom flask. The reaction was then slightly heated until the color of the mixture turned yellow. The precipitate was filtered and dried. Synthesis of racemic ethambutol gave white, needle-like solid after drying with a yield of 90%. m/z = 204.1; FTIR: 3261.28, 2971.82, 2929.37, 2856.03, 2821.3, 1566.96, 1458.89, 1358.55, 1211.19, 1146.27, 1065.99, 973.532, 887.31, 829.794, 644.19 cm^{-1}; ^{1}H NMR (MeOD): δ 4.82 (2 H, s), δ 3.61 (2 H, q), δ 3.43 (4 H, q), δ 2.78 (4 H, m), δ 2.53 (2 H m), δ 1.48 (4 H, m), δ 0.94 (6 H, t); ^{13}C NMR ($CDCl_3$): δ 63.68, δ 61.95, δ 47.07, δ 24.65, δ 10.68.

RESULTS AND DISCUSSION

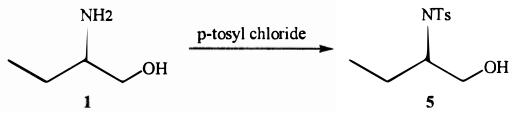
The original plan was to protect the amino functional group using acetyl chloride. The process however proved non-trivial and a mixture of different products was formed that were not easily separable by ordinary chromatography. The molar ratio of the component acetyl chloride and 2-aminobutanol **1** was strictly set to 1:1 but the reaction repeatedly gave **2**, **3**, and **4** in various ratios (see scheme 2). We reasoned it is the lesser steric crowding in the alcohol moiety as opposed to higher basicity and nucleophilicity of the amine group (supposed to afford solely 2) that resulted in a non-specific acetylation. We decided to differentiate between the alcohol and amine groups by the use of tosyl chloride as a solution to this problem. The amine part of the racemic starting material aminobutanol was then protected with tosyl group from p-tosyl chloride (see scheme 3). This was done to generate a single major product since the next step esterification would possibly yield two products, the monoacetylated and the diacetylated product. The N-tosyl-2-aminobutanol produced was subjected to lipase-cata-

Table 1. Results for lipase-catalyzed acetylation of N-tosyl-2-aminobutanol.

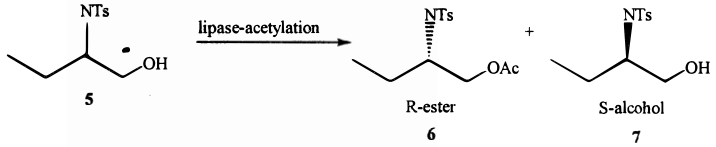
Lipase	*Combined ester and alcohol % yield*	*Duration of Incubation*
Candida rugosa	82.36%	5 h
Porcine pancreatic lipase (PPL)	91.64%	1½ h
Wheat germ	56.35%	2 weeks



Scheme 2. Acetylation of 2-amino-1-butanol



Scheme 3. Tosylation of 2-amino-1-butanol



Scheme 4. Lipase-catalyzed acetylation of N-tosyl-2-amino-1-butanol

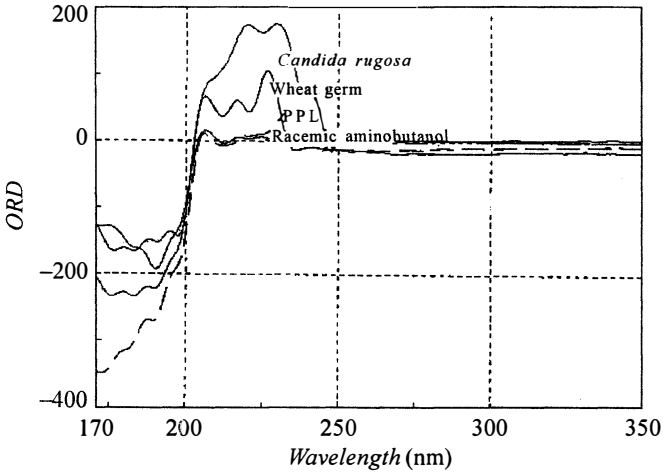


Fig. 1. Optical Rotatory Dispersion (ORD) spectrum of the resolved N-tosyl-2-aminobutanol for the three different lipases with the racemic N-tosyl 2-aminobutanol.

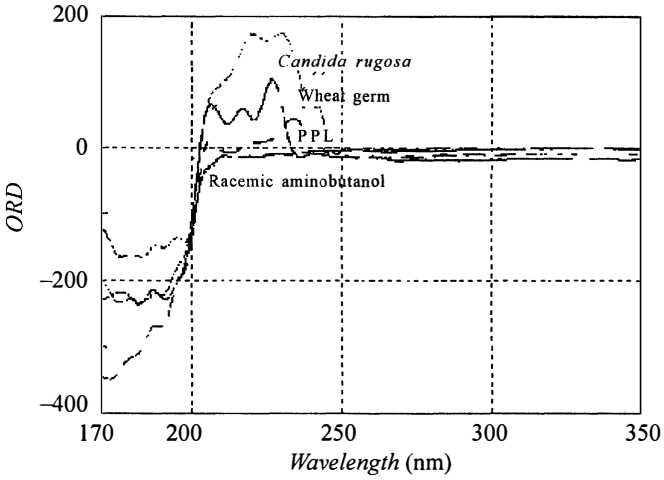


Fig. 2. Optical Rotatory Dispersion (ORD) spectrum of the resolved N-tosyl-2-aminobutanol for the three different lipases with the standard R-(–)-aminobutanol.

lyzed esterification (see scheme 4) using three different enzyme preparations (*C. rugosa*, porcine pancreatic lipase (PPL) and wheat germ). Of the three lipases, porcine pancreatic lipase gave the fastest 50–50% conversion. Esterification with porcine pancreatic lipase took only about an hour to catalyze the reaction. It was followed by the reaction with *C. rugosa* lipase then with wheat germ lipase as catalyst. The results are summarized in Table 1.

After the 50–50% conversion was reached, the lipase was filtered off and the reaction mixture was washed with 10% $NaHCO_3$ to neutralize the acetic acid that may be produced from the reaction. The reaction mixture was then evaporated through rotavap and a slightly yellowish viscous product was obtained.

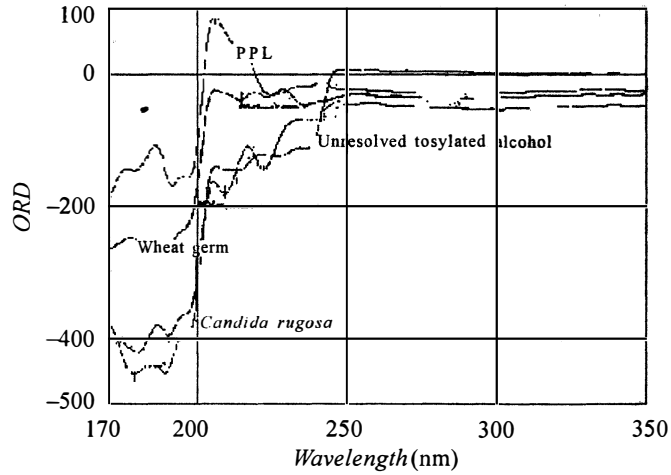


Fig. 3. Optical Rotatory Dispersion (ORD) spectrum of the resolved N-tosyl-2-aminobutyric acid for the three different lipases with the unresolved N-tosyl-2-aminobutanol.

The product obtained was subjected to column chromatography for complete separation of the two resolved products. The two pure products were obtained after column chromatography. The first eluted product (ester) was a yellowish oily liquid and the last eluted product (alcohol) was a white, "alum-like" solid. The optical activities of these two compounds were analyzed using ORD (see Figs. 1–5).

The following discussion and analyses of ORD spectra relate to determination of relative configurations. The assignments of absolute configurations for these compounds were based on actual comparison of values of optical rotations ($[a]_D$) using the sodium D-line of a polarimeter.

From the ORD spectra of resolved tosylated alcohol (Figs. 1 and 2), it can be seen that the curve for *C. rugosa* exhibits the (+)-ORD curve and the most resolved curve with respect to the standard R-(–)-aminobutanol.

On the other hand, Figs. 3 to 5 show the spectra for the tosylated esters after resolution. From Fig. 5, we can see that all the curves for the three lipases follows the L-designation (also designating as S configuration) comparing to the standard L-aminobutyric acid and in contrast with D-aminobutyric acid (Fig. 4). This L-designation also shows a (+)-ORD curve. This time, the curve for PPL shows the (+)-ORD curve and the least is the *C. rugosa*. With comparison to the unresolved starting material (Fig. 3), it also shows that reaction with PPL shows the best resolution for the esters.

These results of the ORD spectra showed that the resolution using *C. rugosa* gave the best starting material S-(+)-alcohol for the synthesis of the active (+)-(S,S)-ethambutol. However, PPL can also be used if the tosylated ester would be used as the starting material (hydrolyze to convert to alcohol) to syn-

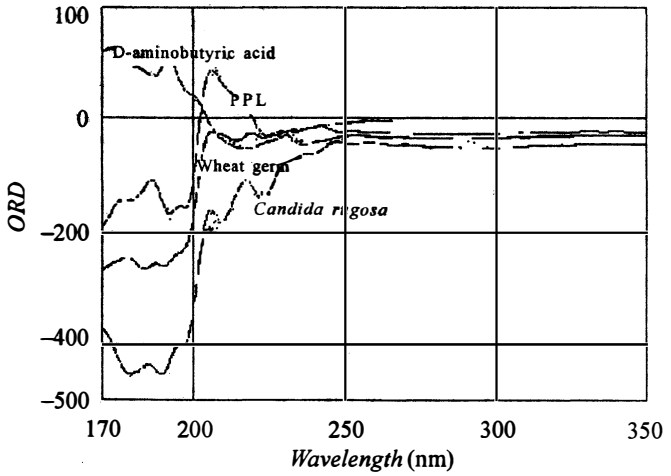


Fig. 4. Optical Rotatory Dispersion (ORD) spectrum of the resolved N-tosyl-2-aminobutyric acid for the three different lipases with D-aminobutyric acid.

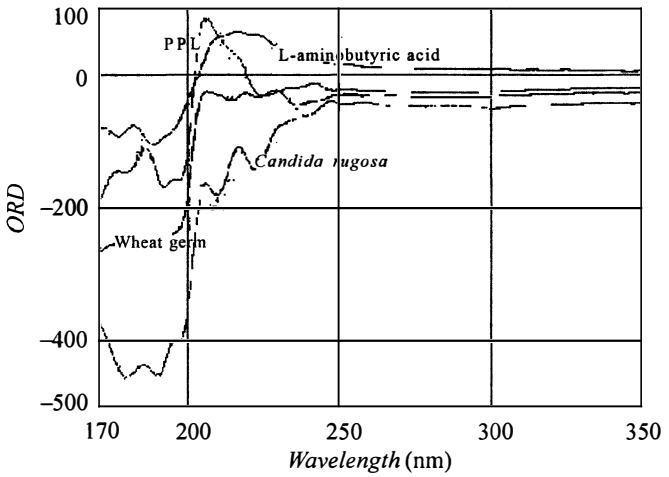
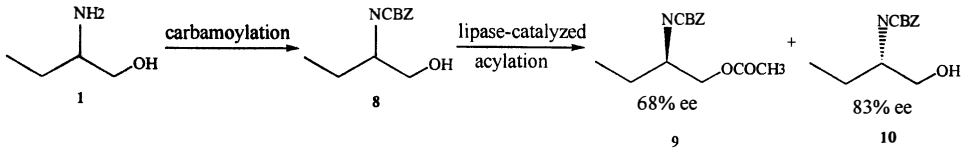
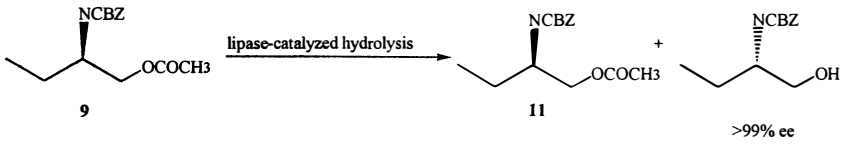


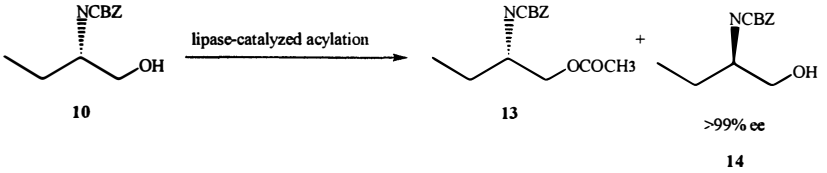
Fig. 5. Optical Rotatory Dispersion (ORD) spectrum of the resolved N-tosyl-2-aminobutyric acid for the three different lipases with L-aminobutyric acid.



Scheme 5. Lipase-catalyzed resolution of N-CBZ-2-amino-1-butanol



Scheme 6. Lipase-catalyzed hydrolysis of N-CBZ-2-amino-1-butanol for double resolution[12]



Scheme 7. Lipase-catalyzed acylation of N-CBZ-2-aminobutanol for double resolution

thesize the optically-active ethambutol. We based all our interpretations on the assumption that a similar ORD (which is to be expected) will result for two compounds if they have the same structure and the same absolute configurations.

The resolved compounds (tosylated alcohol and tosylated ester) were subjected to detosylation to give the desired starting materials for ethambutol. Three different procedures were tried and these were deprotection by the use of a) $NaNH_2$, b) glacial $HOAc/H_2SO_4$, and c) glacial HOAc/HBr. However, none of these three gave appreciable results. It was that at this point that we decided to proceed with the use of easily removable N-protecting groups, namely BOC and CBZ. Since we already assessed that *C. rugosa* is the best-resolving lipase among the three lipases, it was the one we used for the resolution of BOC-protected and CBZ-protected alcohol. BOC-protected aminobutanol failed to undergo lipase-catalyzed resolution while CBZ-protected aminobutanol underwent smooth resolution (see scheme 5).

When the esterification of CBZ-protected aminobutanol was carried out, the resolution resulted in an ester **9** of 68% enantiomeric excess (%ee) based on optical rotatory measurements. The theoretical value was 30°C (c 1.0 $CHCl_3$). The remaining unreacted alcohol **10** was obtained in 83% ee. Both the undesired ester and desired alcohol were subjected to a second round

of lipase resolution to enrich enantiopurity to >99% ee each. In addition, via double-resolution, we were able to prepare the undesired optical isomer R-(–)-alcohol in >99% ee. This isomer might find applications in the enantiospecific synthesis of other natural products. The ester was hydrolyzed in phosphate buffer while the alcohol was esterified in the presence of acetic anhydride (see scheme 6 and 7).

Deprotection of **12** gave the needed starting material S-(+)-alcohol for the total synthesis of (S,S)-(+)-ethambutol. Even though we had the desired S-(+)-alcohol in hand, there was no sufficient time to carry out the desired transformation of **7** to (S,S)-(+)-ethambutol. The desired transformation of (S)-(+)-alcohol to (S,S)-(+)-ethambutol has been published in the literature (see scheme 1 for representation) [5, 6]. Furthermore, we have already synthesized the racemic ethambutol as a standard using (+/–)-2-amino-1-butanol as starting material and the data gave appreciable results.

CONCLUSION

The process of double diastereoselection or resolution using the enzyme lipase from *C. rugosa* was successfully performed for the preparation of optically pure (S)-(+)-aminobutanol needed for the enantiospecific synthesis of (S,S)-(+)-ethambutol. The method developed in the present study represents an economical and general route to the preparation of optically pure amino alcohols needed for asymmetric synthesis of natural products.

The conversion of S-(+)-aminobutanol to (S,S)-(+)-ethambutol will be performed as described in the literature. Several lipases isolated from indigenous sources will be applied to the same process and %ee of the products resulting from resolution with those lipases will be compared with commercially available lipases employed in the present study.

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REFERENCES

1. Fessenden, R. J. and Fessenden, J. S. *Organic Chemistry*, 5th Ed., p. 167. (Brooks/Cole Pub. Co., California, USA, 1994).

2. http://www.ejb.org/content/vol3/issue3/full/3/bip

3. Morrison, R. T. and Boyd, R. N. *Organic Chemistry*, 6th Ed., p. 136. (Prentice-Hall International, Inc., Englewood Cliffs, New Jersey, 1992).

4. Rubin, B. and Dennis, E. A. (Eds.). *Lipases, Part B: Enzyme Characterization and Utilization.* (Academic Press, San Diego, CA, 1997).

5. Wilkinson, R. G. et al. *J. Am. Chem. Soc.* 83, 2212 (1961).

6. Tupasi, et al. *International Journ. of Tuberculosis and Lung Disease.* 6, 471 (1999).

7. Barry, C. E., Slayden, R. A., Sampson, A. E., and Lee, R. E. *Biochemical Pharmacology.* 59, 221 (1997).

8. Trost, B. M., Bunt, R. C., Lemoine, R. C., and Calkins, T. L. *J. Am. Chem. Soc.* 122, 5968 (2000).

9. Fernandez, S., Brieva, R., Rebolledo, F., and Gotor, V. *J. Chem. Soc. Perkin Trans.* 1, 2885 (1992).