0273-2289/99/80/0205/$13.75

# Lipase-Catalyzed Synthesis of (*S*)-Naproxen Ester Prodrug by Transesterification in Organic Solvents

**Shau-Wei Tsai,* Chin-Shain Tsai, and Chun-Sheng Chang**

*Department of Chemical Engineering, National Cheng Kung University, Tainan, Taiwan 70101, Republic of China, E-mail: t62647@mail.ncku.edu.tw*

**Received August 13, 1998; Accepted December 3, 1998**

## Abstract

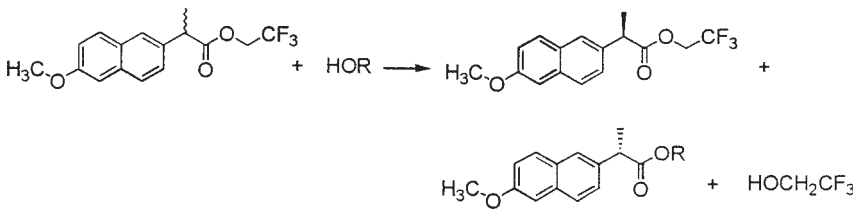
A lipase-catalyzed enantioselective transesterification process was developed for the synthesis of (*S*)-naproxen 2-*N*-morpholinoethyl ester prodrug from racemic 2,2,2-trifluoroethyl naproxen ester in organic solvents. By selecting isooctane and 37°C as the best solvent and temperature, the apparent fits of the initial conversion rates for transesterification and hydrolysis side reaction suggest a ping-pong Bi-Bi enzymatic mechanism with the alcohol as a competitive enzyme inhibitor. Improvements in the initial conversion rate and the productivity for the desired (*S*)-ester product were obtained after comparing with the result of an enantioselective esterification process. Studies of water content in isooctane and alcohol containing various *N*,*N*-dialkylamino groups on the enzyme activity and enantioselectivity, as well as the recovery of (*S*)-ester product by using extraction, were also reported.

**Index Entries:** Lipase; enantioselective transesterification; (*S*)-naproxen ester prodrugs.

## Introduction

Recently much effort has centered on the synthesis or resolution of optical active drugs and their intermediates by chemical or biochemical methods *(1,2)*. Profens (2-arylpropionic acids), an important group of nonsteroidal anti-inflammatory drugs (NSAIDs), have their pharmacological activity mainly on the (*S*)-enantiomer *(3)*. Therefore, considerable efforts are being made to synthesize (*S*)-profens, although most profens are still being used as racemates in therapeutics.

*Author to whom all correspondence and reprint requests should be addressed.



Scheme 1

Pharmacological studies of the acidic NSAIDs have shown that gastrointestinal side effects such as ulceration and hemorrhage constitute the most frequent adverse reactions *(4)*. The development of NSAID prodrugs that temporarily mask the NSAID acidic moiety has been proposed (5). The esterification of profens is an effective way to prepare the prodrug. However, a careful selection of the alcohol is needed, because a good hydrophilic-hydrophobic balance of the prodrug is essential to give an acceptable bioavailability *(4,6,7)*.

In previous reports, a facile enzymatic process was developed to directly synthesize (*S*)-naproxen (and [*S*]-ibuprofen) 2-*N*-morpholinoethyl ester prodrug from racemic naproxen (and ibuprofen), by using lipases as the biocatalyst in organic solvents *(8,9)*. Good enantioselectivity for racemic naproxen (and ibuprofen) in cyclohexane was obtained when using *Candida rugosa* lipase as the biocatalyst. Since naproxen solubility in cyclohexane (1.22 m*M* at 37°C) is very low, adding a surfactant to the reaction medium to enhance the solubility and hence the desired (*S*)-ester productivity was further proposed *(10)*. However, quite limited improvements were found when considering the separation and the enzyme inhibition caused by the surfactant. In the present article, as shown in Scheme 1, a lipase-catalyzed transesterification process to the enantioselective synthesis of ester prodrug of (*S*)-naproxen and 2-*N*-morpholinoethanol (or *N*,*N*-dialylamino alcohol) from racemic naproxen 2,2,2-trifluoroethyl ester in organic solvents is developed, in which a high substrate solubility and desired (*S*)-ester productivity in organic solvents is obtained.

## Materials and Methods

### *Materials*

Optical pure (*S*)-naproxen ([*S*])-2-[6-methoxyl-2-naphthyl]propionic acid) and lipase (triacylglycerol ester hydrolases, EC 3.1.1.3) from *C. rugosa* (type VII, 860 U/mg of solid) were purchased from Sigma (St. Louis, MO). Isooctane, cyclohexane, and benzene from Merck (Darmstadt, Germany), and dibutyl ether of guaranteed reagent (GR) grade from Fluka (Buchs, Switzerland) were dried over 4-Å molecular sieves and used without further purification. Other chemicals of analytical grade were commercially available as follows: thionyl chloride from Merck; 2-nitrotoluene from

Fluka; 2,2,2-trifluoroethanol and 2-*N*-morpholinoethanol from Aldrich (Milwaukee, WI); 2-dimethylaminoethanol, 2-(2-dimethylaminoethoxy) ethanol, 3-dimethylamino-1-propanol, 3-diethylamino-1-propanol, 1-dimethylamino-2-propanol, 1-diethylamino-2-propanol, 4-dimethylamino-1-butanol, and 6-dimethylamino-1-hexanol from Tokyo Kasei (Tokyo, Japan).

### *Analysis*

High-performance liquid chromatography (HPLC) was used to monitor the transesterification and hydrolysis of racemic naproxen 2,2,2-trifluoroethyl ester with alcohol and water, respectively. The mobile phase was composed of *n*-hexane:isopropanol:acetic acid (97:3:1, v/v/v) at a flow rate of 1 mL/min. Ultraviolet detection at 270 nm was for quantification at 25°C. A chiral column (Chiral OD, Daicel, Japan) capable of separating the internal standard of 2-nitrotoluene and also (*R*)- and (*S*)-naproxen 2,2,2-trifluoroethyl esters, (*R*)- and (*S*)-naproxen, and (*R*)- and (*S*)-naproxen 2-*N*-morpholinoethyl esters with the retention time of 4.9, 6.3, 6.7, 16.3, 18.0, 36.1, and 52.4 min, respectively, was employed. This analytic condition was also used in the quantification of esterification reaction between racemic naproxen and 2-*N*-morpholinoethanol in isooctane.

### *Synthesis of Naproxen 2,2,2-Trifluoroethyl Ester*

Racemic naproxen was obtained by racemizing (*S*)-naproxen at 140°C in ethylene glycol with sodium hydroxide, as previously described *(11)*. By following a standard procedure *(12)*, the acid chloride of racemic naproxen was prepared from 3.5 g of the racemate reacted with 3.2 g of thionyl chloride in toluene (20 mL). After removing the solvent and excess thionyl chloride by evaporation, and then adding benzene (30 mL), pyridine (1.9 g), and 2,2,2-trifluoroethanol (2.7 g), the resultant solution was maintained at 85°C and with reflux for 4 h. Aqueous solution (200 mL) containing sodium hydroxide at pH 12.5 and deionized water (200 mL) was successively employed to extract the excessive alcohol, acid chloride, and pyridine. Then, magnesium sulfate was used to adsorb water in the organic phase and was removed by centrifuge. After evaporating the solvent by vacuum, racemic naproxen 2,2,2-trifluoroethyl ester of 74% yield was obtained.

### *Effects of Organic Solvent, Temperature, Water Content, and Alcohol Concentration*

Unless specified otherwise, 100 mg of lipase taken straight from the bottle was added to 20 mL of isooctane containing 2 m*M* racemic naproxen 2,2,2-trifluoroethyl ester and 15 m*M* 2-*N*-morpholinoethanol at 37°C. The resultant mixture was stirred with a magnetic stirrer, and samples were removed for HPLC analysis at different time intervals. Similar experiments were performed in which isooctane was replaced by cyclohexane or dibutyl ether. Experiments were also carried out to investigate the effect of tem-

perature on the enzyme activity and enantioselectivity in isooctane. Effects of water content on the enzyme performance were further studied, in which 28 m*M* to 167 m*M* of deionized water was added. The lipase lyophilized for 6 h was taken straight from the bottle and used as the biocatalyst for reaction.

Transesterification reactions were further performed to study the effect of 2-*N*-morpholinoethanol concentration, varied from 2 to 75 m*M*, on the enzyme activity. Esterification was also carried out in which 100 mg of lipase was added to 20 mL of isooctane containing 0.2 m*M* racemic naproxen and alcohol of different concentration at 37°C.

### *Effects of Alcohol Containing* N,N*-Dialkylamino Group*

Alcohol containing *N*,*N*-dialkylamino group was reported to have high potential in preparing ester prodrugs of carboxylic acid agents *(4,13)*. Therefore, the previously described lipase-catalyzed transesterification process was extended to the enantioselective synthesis of (*S*)-naproxen ester prodrug containing an *N*,*N*-dialkylaminoalcohol group.

### *Separation of Ester Product by Extraction*

To 20 mL of aqueous solution containing 1 *M* HCl were added 20 mL of the reaction medium (at 170 h) in which lipase particles had been removed by filtration using a Durapore HVLP membrane from Millipore (Bedford, MA). The mixture was violently stirred for 20 min at the ambient temperature and settled for phase separation. The superstratum was separated and sampled for HPLC analysis. An aqueous solution containing 6 *M* NaOH was then added in drops to neutralize the remaining aqueous phase to pH 7.0. After drying the aqueous solution by evaporation, the desired solid product was dissolved in 20 mL of isooctane for HPLC analysis.

## Model Development

### *Transesterification*

According to a previous analysis *(14)*, a ping-pong Bi-Bi mechanism with substrate inhibition has been utilized to describe the kinetic behavior of lipase-catalyzed transesterification in microaqueous organic solvents. Since the crude enzyme usually contains water available for reaction, the hydrolysis side reaction in transesterification reaction is not negligible. As shown in Eq. 1, the acyl donor (*S*)-naproxen 2,2,2-trifluoroethyl ester ($NTF_A$) at first liberates 2,2,2-trifluoroethanol (TF) in the acylation step. Then in the deacylation step (Eqs. 2 and 3), 2-*N*-morpholinoethanol (MO) competes with water (WA) in combining the enzyme-acyl complex $E^*NA_A$ to form (*S*)-naproxen 2-*N*-morpholinoethyl ester ($NME_A$) and (*S*)-naproxen ($NA_A$), respectively. Since 2,2,2-trifluoroethanol is a good leaving group, one may assume the acylation step, and hence transesterification and hydrolysis, to be irreversible. However, with the formation of (*S*)-naproxen in hydrolysis and the addition of excess 2-*N*-morpholinoethanol, a revers-

ible lipase-catalyzed esterification between $NA_A$ and MO may occur. This may decrease the acid concentration and enhances the desired (*S*)-ester production. Finally, equilibrium exists among the compounds of (*S*)-naproxen, water, (*S*)-naproxen 2-*N*-morpholinoethyl ester, and 2-*N*-morpholinoethanol. Equation 4 also assumes that the alcohol reversibly inhibits the enzyme. For the (*R*)-enantiomer, the subscript A is changed to B and a superscript of prime is added to the kinetic parameters in Eqs. 1–3.

$$E + NTF_A \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} ENTF_A \xrightarrow{k_2} E^*NA_A + TF \tag{1}$$

$$E^*NA_A + WA \underset{k_{-3}}{\overset{k_3}{\longleftrightarrow}} E^*NA_AWA \underset{k_{-4}}{\overset{k_4}{\longleftrightarrow}} E + NA_A \tag{2}$$

$$E^*NA_A + MO \underset{k_{-5}}{\overset{k_5}{\longleftrightarrow}} E^*NME_A \underset{k_{-6}}{\overset{k_6}{\longleftrightarrow}} E + NME_A \tag{3}$$

$$E + MO \underset{k_{-7}}{\overset{k_7}{\longleftrightarrow}} EMO \tag{4}$$

By assuming all enzyme complexes to be in a pseudo-steady state, complicated rate equations for $NTF_A$, $NA_A$, $NME_A$, $NTF_B$, $NA_B$, and $NME_B$ may be derived (not shown here). However, in the present article, only the rate equations at the initial stage are given in the following section.

## *Estimation of Parameters at Initial Stage in Transesterification*

At the initial stage, one may neglect the effect of products, i.e., $[NA_A] = [NA_B] = [NME_A] = [NME_B] = 0$, on the initial rates. If a high enantiomeric ratio of the enzymes (i.e., $k_2/K_{m1} >> k_2'/K_{m1}'$), $K_{m1} >> [NTF_A]$ and $K_{m1}' >> [NTF_B]$, are further assumed, the rate equations for $NTF_A$, $NA_A$, and $NME_A$ are expressed as follows:

$$V_{A,NTF} = k_2[NTF_A][E_t]/K_{m1}G \tag{5}$$

$$V_{A,NA} = (k_2[NTF_A][E_t]/K_{m1})/[1 + (k_6K_{m3}[MO]/k_4K_{m5}[WA])]G \tag{6}$$

$$V_{A,NME} = (k_2k_6K_{m3}[NTF_A][MO][E_t]/k_4K_{m1}K_{m5}[WA])/ [1 + (k_6K_{m3}[MO]/k_4K_{m5}[WA])]G \tag{7}$$

$$G = 1 + \{(k_2[NTF_A]/K_{m1})/[(k_4[WA]/K_{m3}) + (k_6[MO]/K_{m5})]\} [1 + ([MO]/K_{m5}) + ([WA]/K_{m3})] + ([MO]/K_{MO}) \tag{8}$$

Table 1
Definitions of Parameters in Eqs. 5–8[a]

| Parameter | Definition |
|---|---|
| $K_{m1}$ | $(k_{-1} + k_2)/k_1$ |
| $K_{m3}$ | $(k_{-3} + k_4)/k_3$ |
| $K_{m5}$ | $(k_{-5} + k_6)/k_5$ |
| $K_{m\mathrm{NA}}$ | $(k_{-3} + k_4)/k_{-4}$ |
| $K_{\mathrm{MO}}$ | $k_{-7}/k_7$ |

[a]All kinetic constants and parameters with the superscript of prime and the subscript of B in Eqs. 5–8 refer to (*R*)-enantiomer.

The kinetic parameters in Eqs. 5-8 are defined in Table 1. Similar equations for (*R*)-enantiomer can be derived except that the subscript A is changed to B and a superscript of prime is added to the kinetic parameters in Eqs. 5–8. Therefore, from Eq. 5 and the corresponding equation for (*R*)-ester substrate, one obtains the same equation previously derived by Chen et al. *(15)* to calculate the apparent enantiomeric ratio for the enzyme as

$$E^{**} = k_2K_{m1}'/k_2'K_{m1} = \ln[(1 - X_{\mathrm{NTF}})\,(1 - ee_s)]/\ln[(1 - X_{\mathrm{NTF}})\,(1 + ee_s)]$$

in which $X_{\mathrm{NTF}}$ and $ee_s$ represent conversion and enantiomeric excess for racemic naproxen 2,2,2-trifluoroethyl ester (NTF), respectively. Obviously, the E** value is not an accurate index for determining the enzyme enantioselectivity in transesterification when hydrolysis side reaction is not negligible.

The ratio of Eqs. 5 and 6 can be expressed as follows:

$$V_{\mathrm{A,NTF}}/V_{\mathrm{A,NME}} = 1 + (k_4K_{m5}[\mathrm{WA}]/k_6K_{m3}[\mathrm{MO}]) \tag{9}$$

Then, from the variation of this ratio with $[\mathrm{MO}]^{-1}$, one can determine the parameter of $k_4K_{m5}[\mathrm{WA}]/k_6K_{m3}$. The other parameters of $k_2/K_{m1}$, $[\mathrm{WA}]/K_{m3}$, $K_{m5}$, $K_{\mathrm{MO}}$, $k_6/K_{m5}$, and hence $k_4$ in Eqs. 5–8 can be determined from the variation of $V_{\mathrm{A,NTF}}$ and $V_{\mathrm{A,NA}}$ with the initial concentration of $[\mathrm{NTF_A}]$ and/or [MO]. Corresponding equations similar to Eqs. 5–8 can be used to find the parameters for (*R*)-enantiomer.

### *Estimation of Parameters in Esterification*

In an enantioselective esterification between racemic naproxen and 2-*N*-morpholinoethanol at the initial stage, the mechanism of Eqs. 2–4 for (*S*)-enantiomer and the alcohol is still valid. However, the reversible reactions of $E^*NA_A$ and WA as well as E and $NME_A$ are negligible. Thus, Eqs. 2 and 3 are rewritten as follows:

$$E + NA_A \underset{k_4}{\overset{k_{-4}}{\longleftrightarrow}} E^*NA_AWA \underset{}{\overset{k_{-3}}{\longleftrightarrow}} E^*NA_A + WA \tag{10}$$

$$E^*NA_A + MO \underset{k_{-5}}{\overset{k_5}{\longleftrightarrow}} E^*NME_A \underset{}{\overset{k_6}{\longleftrightarrow}} E + NME_A \quad (11)$$

By making similar assumptions as in transesterification, the initial rate for (*S*)-naproxen can be derived as follows *(16)*:

$$V_{A,NA} = (k_{-3}[NA_A][E_t]/K_{mNA})/ \\ [1 + ([MO]/K_{MO}) + (k_{-3}K_{m5}[NA_A]/k_6K_{mNA}[MO])] \quad (12)$$

From the variation of $V_{A,NA}$ with the initial concentration of [MO] and/or [$NA_A$], coupled with the data of $K_{MO}$ and $K_{m5}/k_6$ obtained in transesterification reaction, one can determine the parameter of $k_{-3}/K_{mNA}$.

## Results and Discussion

### *Selection of Organic Solvent in Transesterification*

Figure 1 illustrates effects of cyclohexane and isooctane on the time-course conversion of (*S*)-naproxen 2,2,2-trifluoroethyl ester ($X_{A,NTF}$), the time-course yield of (*S*)-naproxen 2-*N*-morpholinoethyl ester ($X_{A,NME}$), and variations of the enantiomeric excess for (*S*)-naproxen 2-*N*-morpholinoethyl ester ($ee_{p,NME}$) with $X_{A,NME}$. Almost similar performances of $X_{A,NTF}$ and $ee_{p,NME}$ in both cases are demonstrated. However, a higher yield of $X_{A,NME}$ at any specific time in isooctane is shown. Therefore, from the difference of $X_{A,NTF}$ and $X_{A,NME}$ in each case, a lower yield of (*S*)-naproxen ($X_{A,NA}$) owing to hydrolysis in isooctane is obtained. This is owing to the more hydrophilic of cyclohexane (log $P$ = 3.2, with $P$ as the partition coefficient of solvent between *n*-octanol and water) than isooctane (log $P$ = 4.5), in which the former contains a higher water activity for hydrolysis.

As described under Model Development, there are two possible routes leading to the desired (*S*)-ester product: (1) by irreversible transesterification (Eqs. 1 and 3), and (2) by irreversible hydrolysis (Eqs. 1 and 2) and then by reversible esterification (Eqs. 2 and 3). Therefore, the (*S*)-naproxen yield may first increase owing to the higher hydrolysis rate compared with the transesterification rate. The slow consumption of the enzyme-acyl complex by 2-*N*-morpholinoethanol and the depletion of the substrate to provide the enzyme-acyl complex would cause the reverse reaction in Eq. 2, which results in a decrease in (*S*)-naproxen concentration and a maximum of $X_{A,NA}$ in both cases (Fig. 1). Of course, an equilibrium among the acid, alcohol, water, and ester product will finally be reached. To obtain the highest $X_{A,NME}$ and/or $ee_{p,NME}$, studies on finding optimal conditions such as the best reaction time, water content, and substrate concentration are needed.

When dibutyl ether (log $P$ = 2.9) was used as the reaction medium, a very low yield of $X_{A,NME}$ = 0.07 at 170 h was obtained. Although dibutyl ether and cyclohexane have quite similar log $P$ values, water solubility is about 20 times higher in the former. Thus, water stripping should occur to a much

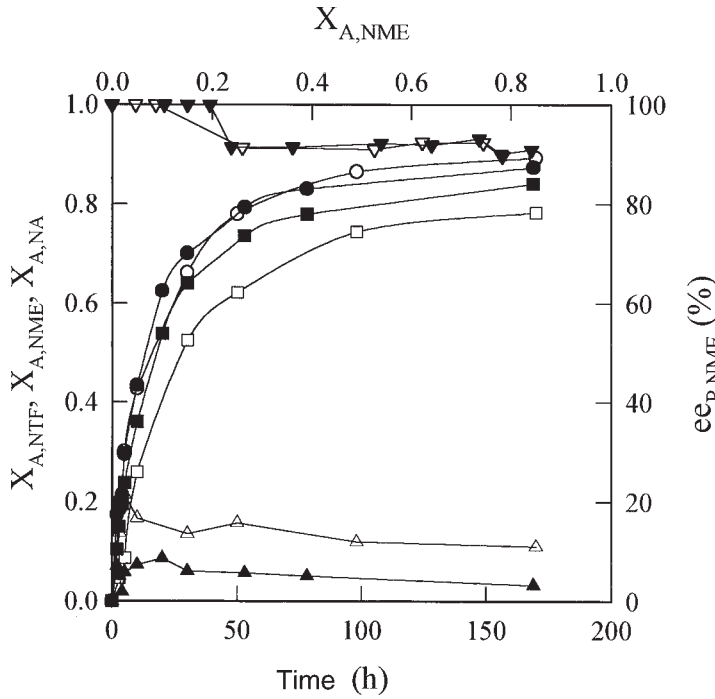



Fig. 1. Time-course conversion of $X_{A,NTF}$, time-course yields of $X_{A,NME}$ and $X_{A,NA}$, and variation of $ee_{p,NME}$ with $X_{A,NME}$ in organic solvents: $[NTF_A]_o = [NTF_B]_o = 1$ m*M*, [MO] = 15 m*M*, $[E_t]$ = 5 mg/mL and 37°C. For isooctane: (●), $X_{A,NTF}$; (■), $X_{A,NME}$; (▲), $X_{A,NA}$; (▼), $ee_{p,NME}$. For cyclohexane: (○), $X_{A,NTF}$; (□), $X_{A,NME}$; (△), $X_{A,NA}$; (▽), $ee_{p,NME}$.

higher extent in ether, meaning that the enzyme could be much less hydrated in ether and therefore much less active in the solvent, for both transesterification and hydrolysis. Based on the lipase performance and the (*S*)-naproxen yield from hydrolysis, isooctane was selected as the best solvent in the following experiment.

### *Effect of Water Content*

The water content (or more precisely the thermodynamic water activity) in nonaqueous solvents has a crucial role on the thermodynamic equilibrium and kinetics in enzymatic reactions *(17,18)*. Considering the results with and without hydrolysis side reaction in transesterification reaction, it may be possible to obtain a higher $ee_{p,NME}$ in the former. This is owing to two successive enantioselective reactions, i.e., hydrolysis and then esterification, in the synthesis of the desired (*S*)-ester product. Moreover, if both hydrolysis and esterification rates are higher than the transesterification rate, a higher productivity of the (*S*)-ester product at a desired product purity may be obtained. However, this idea was not fulfilled in our experiments.

Figure 2 demonstrates the effects of water content on the time-course yield of $X_{A,NME}$. In general, increasing the water content to 56 m*M* results in

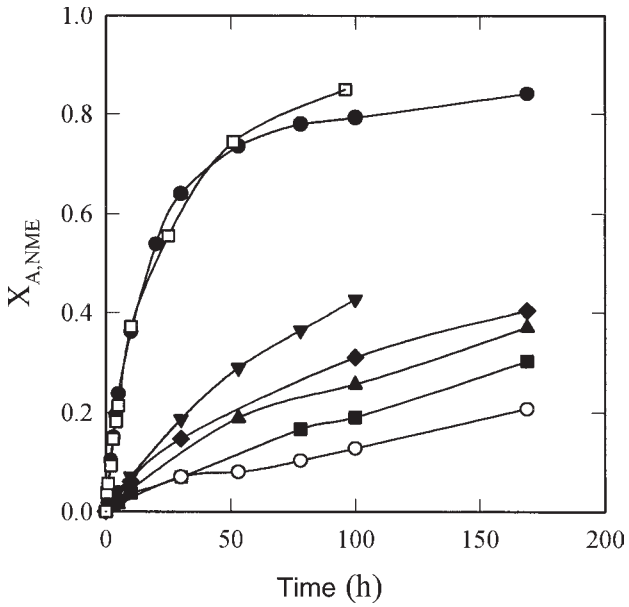



Fig. 2. Effects of water content on the yield of (*S*)-ester product in isooctane. Conditions are as in Fig. 1. (□), lyophilized for 6 h; (●), without adding water. Water added: (▼), 28 m*M*; (■), 56 m*M*; (▲), 83 m*M*; (◆), 111 m*M*; (○), 167 m*M*.

a decrease of $X_{A,ME}$ at any specific time. Since water solubility in isooctane is very small, the excess water will disperse as a new aqueous phase in isooctane when more than 28 m*M* water is added. This certainly causes an aggregation of enzyme particles and decreases the enzyme activity. Since it is quite difficult to control the state of enzyme aggregation when water is added, no generality for the time-course variation of $X_{A,NME}$ at the higher water content was found. Further studies on testing the enhancement of (*S*)-ester productivity and purity by using immobilized lipases to avoid enzyme aggregation is needed. Moreover, quite similar results were found when the crude lipase was used without adding water and was lyophilized, as shown in Fig. 2. This implies that the water adsorbed on the crude lipase is difficult to remove after 6 h of lyophilization. Based on the enzyme performance and the simplicity for enzyme preparation, the crude lipase without lyophilization is used in the following study.

## *Selection of Reaction Temperature*

Results similar to those in Fig. 1 for the time courses of $X_{A,NME}$ and $X_{A,NA}$ and the variation of $ee_{P,NME}$ with $X_{A,NME}$ (data not shown) were obtained by varying the reaction temperature from 30 to 60°C. As shown in Table 2, increasing temperature results in the enhancement of initial rates for both (*S*)-ester product and (*S*)-naproxen by-product. Obviously reaction temperature has more effects on the increase of hydrolysis rate, in which case the stripping of water molecules from the crude lipase by isooctane may

Table 2
Effect of Temperature on Initial Rates and Yields (at 50 h)
for (*S*)-Naproxen and (S)-Ester Product, and Enantiomeric Excess
for Ester Product in Transesterification[a]

| Temperature (°C) | $V_{A,NA} \times 10^2$ (m*M*/h) | $V_{A,NME} \times 10^2$ (m*M*/h) | $ee_{P,NME}$ (%) | $X_{A,NA}$ | $X_{A,NME}$ |
|---|---|---|---|---|---|
| 30 | 1.14 | 3.69 | 93.5 | 0.057 | 0.66 |
| 37 | 1.16 | 4.83 | 91.0[b] | 0.061[b] | 0.74[b] |
| 50 | 4.27 | 6.80 | 88.4 | 0.12 | 0.73 |
| 60 | 10.30 | 8.15 | 88.1 | 0.058 | 0.69 |

[a]Conditions as in Fig. 1.
[b]At 53 h.

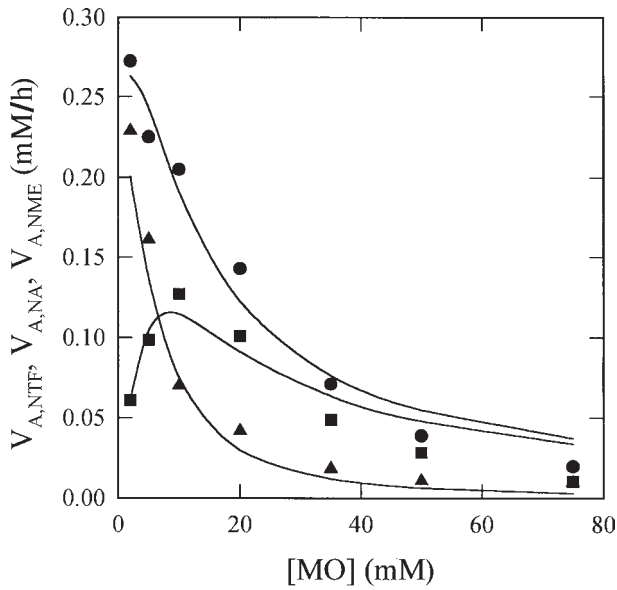



Fig. 3. Effects of 2-*N*-morpholinoethanol concentration on the initial rates of (*S*)-ester substrate, (*S*)-naproxen, and (*S*)-ester product in isooctane. Conditions are as in Fig. 1. (●), $V_{A,NTF}$, (▲), $V_{A,NA}$; (■), $V_{A,NME}$; (——), theoretical results.

play an important role. At the reaction time of about 50 h, a maximum of $X_{A,NME}$ at 37°C is shown in Table 2. This is attributed to the lower thermal stability of the lipase at the high temperature. Moreover, increasing temperature also results in the decrease of $ee_{P,NME}$ (Table 2), which indicates that the racemic temperature *(19)* of the lipase is more than 60°C. After considering enzyme enantioselectivity and thermal stability, we selected 37°C as the best temperature in the following experiment.

## *Effect of Alcohol Concentration*

Figure 3 illustrates the effect of alcohol concentration on the initial rates for the depletion of (*S*)-ester substrate and the formation of (*S*)-ester

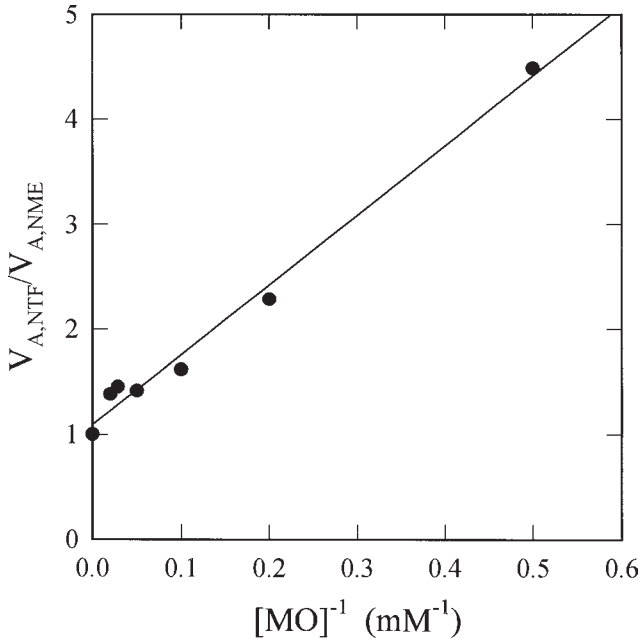



Fig. 4. Effects of $[MO]^{-1}$ on $V_{A,NTF}/V_{A,NME}$ in isooctane. Conditions are as in Fig. 1. (●), Experimental data; (——), theoretical results.

Table 3
Kinetic Parameters Obtained
from Data in Figs. 3 and 4, Coupled with Eqs. 5, 6, 8, and 12

| $k_2/K_{m1}$ (mL/h·mg) | $K_{m5}/k_6$ (h·mg/mL) | [WA]/$K_{m3}$ | $K_{MO}$ (m*M*) | $K_{m5}$ (m*M*) | $k_4$ (m*M*·mL/h·mg) | $k_{-3}/K_{mNA}$ (mL/h·mg) |
|---|---|---|---|---|---|---|
| 2.46 | 108 | 0.18 | 0.23 | 107 | 0.36 | 0.55 |

product and (*S*)-naproxen by-product. Because alcohol and water are both nucleophilic competitors, increasing the alcohol concentration will suppress hydrolysis, and hence increase transesterification rate. However, a maximum of $V_{A,ME}$ is shown at the alcohol concentration of about 10 m*M*. This implies that 2-*N*-morpholinoethanol might have acted as an enzyme inhibitor, which was previously found in the enantioselective esterification of racemic naproxen (or ibuprofen) with 2-*N*-morpholinoethanol in cyclohexane *(8,9)*.

By using the data of Fig. 4 and Eq. 9, the parameter of $k_4K_{m5}[WA]/k_6K_{m3}$ = 6.96 m*M* can be determined from the variation of $V_{A,TF}/V_{A,ME}$ with $[MO]^{-1}$ (*see* Fig. 4). With the results in Fig. 3 and Eqs. 5, 6, and 8, the remaining parameters can also be determined and tabulated in Table 3. Then, the theoretical rates can be calculated and represented in Fig. 3, in which quantitative agreements with the experimental data are illustrated.

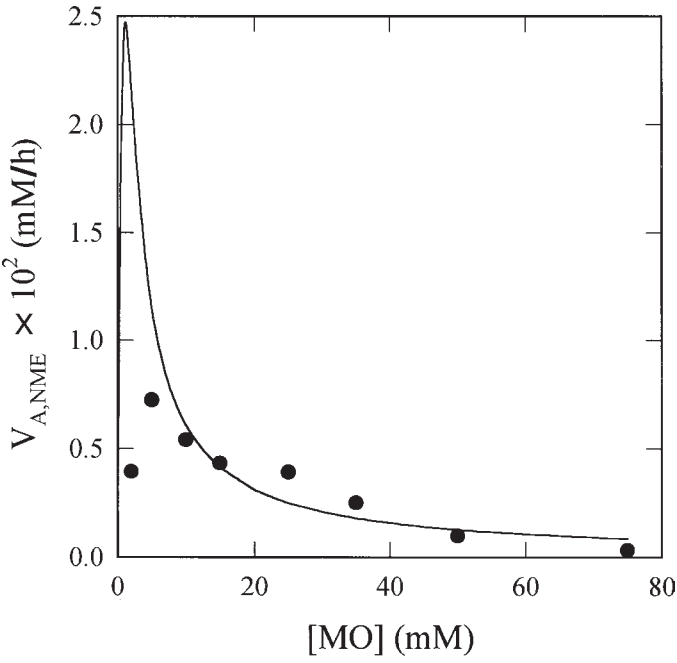



Fig. 5. Effects of 2-*N*-morpholinoethanol concentration on the initial rate of (*S*)-ester product in enantioselective esterification of racemic naproxen in isooctane: $[NA_A]_o = [NA_B]_o$ = 0.1 m*M*, $[E_t]$ = 5 mg/mL and 37°C. (●), Experimental data; (——), theoretical results.

## *Comparison of Transesterification with Esterification*

Figure 5 illustrates the effect of alcohol concentration on the initial esterification rate of (*S*)-ester product in isooctane, in which 0.2 m*M* racemic naproxen was used. A maximum at the alcohol concentration of about 5 m*M* was found. This behavior has been reported when Lipase MY from Meito Sangyo (Nagoya, Japan) was used as the biocatalyst in cyclohexane *(8)*. Comparing the results of Figs. 3 and 5, an order-of-magnitude enhancement of the initial rate for the desired (*S*)-ester is obtained. This is attributed to the higher substrate concentration that has been employed in transesterification. Since the saturated solubility of racemic naproxen 2,2,2-trifluoroethyl ester (2.6 *M*) in isooctane is about 1130-fold that of racemic naproxen (0.23 m*M*) at 30°C, the benefit of using transesterification to improve the substrate solubility, and then the desired (*S*)-ester productivity, is evident.

By using the kinetic parameters obtained in transesterification, and the data in Fig. 5 and Eq. 12, the kinetic parameter of $k_{-3}/K_{mNA}$ was determined and tabulated in Table 3. As illustrated (Fig. 5), deviations between the experimental and theoretical results for [MO] < 5 m*M* are shown. This implies that more experiments with [MO] < 5 m*M* should be conducted to support the proposed model.

Table 4
Effect of Alcohol Containing *N*,*N*-Dialkylamino Group
in Enantioselective Transesterification
of Racemic Naproxen 2,2,2-Trifloroethyl Ester in Isooctane[a]

| | $X_{TF}$ (20 h) (%) | $ee_P$ (%) | $X_{TF}$ (96 h) (%) | $ee_P$ (%) | E** |
|---|---|---|---|---|---|
| 2-Dimethylaminoethanol $[(CH_3)_2N(CH_2)_2OH]$ | 20.7 | 84.5 | 35.4 | 81.5 | 15 |
| 3-Dimethylamino-1-propanol $[(CH_3)_2N(CH_3)_3OH]$ | 22.3 | 72.2 | 30.9 | 70.1 | 8 |
| 1-Dimethylamino-2-propanol $[(CH_3)_2NCH_2CHOHCH_3]$ | 21.0 | 90.0 | 28.3 | 90.0 | 27 |
| 2-(2-Dimethylaminoethoxy)ethanol $[(CH_3)_2N(CH_2)_2O(CH_2)_2OH]$ | 10.1 | 75.2 | 20.5 | 61.9 | 6 |
| 4-Dimethylamino-1-butanol $[(CH_3)_2N(CH_2)_4OH]$ | 18.2 | 84.3 | 31.4 | 80.4 | 14 |
| 6-Dimethylamino-1-hexanol $[(CH_3)_2N(CH_2)_6OH]$ | 9.3 | 80.0 | 17.4 | 79.6 | 10 |
| 3-Diethylamino-1-propanol $[(C_2H_5)_2N(CH_2)_3OH]$ | 28.2 | 96.0 | 37.6 | 94.0 | 61 |
| 1-Diethylamino-2-propanol $[(C_2H_5)_2NCH_2CHOHCH_3]$ | 29.2 | 87.7 | 38.9 | 86.4 | 23 |

[a]$[NTF_A]_o = [NTF_B]_o$ = 1 m*M*, [MO] = 15 m*M*, $[E_t]$ = 5 mg/mL and 37°C.

## *Effects of Alcohol Containing* N,N*-Dialkylamino Group*

Table 4 demonstrates the effect of alcohol containing *N*,*N*-dialkylamino group on the enantioselective synthesis of (*S*)-naproxen ester prodrug by utilizing lipase-catalyzed transesterification. For primary *N*,*N*-dimethylaminoalcohol, the carbon chain length has little effect on the enzyme enantioselectivity. However, when 6-dimethylamino-1-hexanol or 2-(2-dimethylaminoethoxy)ethanol is used, the enzyme activity greatly decreases. In general, the apparent enantiomeric ratio is low (E** < 15), which implies that they are not good acyl acceptors in the enantioselective synthesis of (*S*)-naproxen ester prodrug. When a secondary alcohol such as 1-dimethylamino-2-propanol is used, the apparent enantiomeric ratio is improved, yet with little decrease in enzyme activity. A great improvement of enzyme enantioselectivity and activity was found when a bulky diethyl group in the amino moiety of the alcohol was used. Therefore, except for 2-*N*-morpholinoethanol, 3-diethylamino-1-propanol is also a good candidate in the enantioselective synthesis of the desired (*S*)-naproxen ester prodrug.

## *Separation of Ester Product by Extraction*

Since the substrate and the product in transesterification are both ester, the present enzymatic process is competitive if they can be easily separated.

By following the method proposed by Palomer et al. *(20)*, an aqueous solution containing 1 *N* HCl was used to extract the product from the reaction medium with $X_{A,NME} = 0.9$ and $ee_{p,NME} = 0.9$. Both (*R*)- and (*S*)-ester products were completely extracted into the aqueous phase by using HPLC analysis for the remaining organic phase. Then, the resultant aqueous phase was neutralized and dried, and isooctane was added to dissolve the solid containing the desired product. HPLC analysis indicated that only 40% of (*S*)-ester product with a trace of (*S*)-naproxen and (*S*)-ester substrate was obtained. This implies that part of the product embedded in solid NaCl salt cannot dissolve into the solvent. Therefore, further studies on improving the recovery of ester product and racemizing the undesired (*R*)-ester substrate are needed.

## Conclusion

A lipase-catalyzed transesterification process was developed for the enantioselective synthesis of (*S*)-naproxen 2-*N*-morpholinoethyl ester prodrug from racemic naproxen 2,2,2-trifluoroethyl ester in organic solvents. By considering the enzyme enantioselectivity and activity, and the extent of hydrolysis side reaction, isooctane was selected as the best reaction medium. The addition of water in isooctane results in the formation of a new aqueous phase that causes an aggregation of enzyme particles, and hence decreases the enzyme activity. By further comparing the simplicity in enzyme preparation and the enzyme performance, the crude lipase without adding water or lyophilizing was selected as the best biocatalyst in all experiments. Increasing temperature may enhance the initial rates for transesterification and hydrolysis side reaction, while decreasing enzyme enantioselectivity. Therefore, the best temperature of 37°C was selected for our reaction.

The apparent fit of the initial rates in transesterification supports the ping-pong Bi-Bi mechanism with 2-*N*-morpholinoethanol as a competitive inhibitor to the lipase. A higher initial rate, and hence the productivity, for the desired (*S*)-ester prodrug was obtained by utilizing the transesterification process after comparing with the esterification process. A screen of alcohol containing *N*,*N*-dialkylamino group indicates that 3-diethylamino-1-propanol is also a good acyl acceptor for the synthesis of (*S*)-naproxen ester prodrug. A preliminary study on the product extraction indicates that the ester product can be completely recovered from the reaction medium.

## Acknowledgment

Financial support from the Chinese National Science Council (Grant No. NSC-86-2214-E006-004) is appreciated.

## References

1. Federsel, H. J. (1993), *CHEMTECH* **12,** 24–33.
2. Margolin, A. J. (1993), *Enzyme Microb. Technol.* **15,** 266–280.

3. Hutt, A. J. and Caldwell, J. (1984), *Clin. Pharmacokinet.* **9,** 371–373.
4. Shanbhag, V. R., Crider, A. M., Gokhale, R., Harpalani, A., and Dick, R. M. (1992), *J. Pharm. Sci.* **81,** 149–154.
5. Bundgaard, H. (1985), *Design of Prodrugs*, Elsevier, Amsterdam.
6. Nielsen, N. N. and Bundgaard, H. (1988), *J. Pharm. Sci*. **77,** 285–298.
7. Tammara, V. K., Narurkar, M. M., Crider, A. M., and Khan, A. M. (1993), *Pharm. Res.* **10,** 1191–1199.
8. Chang, C. S. and Tsai, S. W. (1997), *Enzyme Microb. Technol.* **20,** 635–639.
9. Tsai, S. W., Lin, J. J., Chang C. S., and Chen, J. P. (1997), *Biotechnol. Prog.* **13,** 82–88.
10. Chang, C. S. and Tsai, S. W. (1997), *Appl. Biochem. Biotechnol., Part A: Enzyme Eng. Biotechnol.* **68,** 135–142.
11. Tsai, S. W. and Wei, H. J. (1993), *J. Liq. Chromatogr.* **16,** 2993–3001.
12. Allenmark, S. and Ohlsson, A. (1992), *Chirality* **4,** 98–102.
13. Nielsen, N. M. and Bundgaard, H. (1987), *Int. J. Pharm.* **39,** 75–85.
14. Rizzi, M. P., Stylos, P., and Reuss, M. (1992), *Enzyme Microb. Technol*. **14,** 709–714.
15. Chen, C. S., Fujimoto, Y., Girdaukas, G., and Sih, C. J. (1982), *J. Am. Chem. Soc.* **104,** 7294–7299.
16. Tsai, S. W. and Wei, H. J. (1994), *Biocatalysis* **11,** 33–45.
17. Dordick, J. S. (1990), in *Applied Biocatalysis*, vol. 1, Blanch, H. W. and Clark, D. S., eds., Marcel Dekker, New York, pp. 1–52.
18. Halling, P. (1994), *Enzyme Microb. Technol*. **16,** 178–206.
19. Phillips, R. S. (1992), *Enzyme Microb. Technol.* **14,** 417–419.
20. Palomer, A., Cabre, M., Ginesta, J., Mauleon, D., and Carganico, G. (1993), *Chirality* **5,** 320–328.