# Kinetic and Thermodynamic Investigation of Lipase-Catalyzed Hydrolysis of (*R*,*S*)-3-Phenylbutyl Azolides

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## Supplementary Information

### 1. EXPERIMENTAL SECTION

The synthesized substrates are confirmed from the retention time in HPLC analysis (Table S1) or $^1$H NMR spectra recorded at 500 MHz on Brucker spectrometer (Avance DRX 500) in DMSO-$d_6$ solution with TMS as an internal standard as follows:

(*R*,*S*)-3-Phenylbutyl 4-methylpyrazolide (**1**). $^1$HNMR (DMSO-$d_6$/TMS) δ: 1.21-1.28 (3H, q), 2.04 (3H, s), 3.28-3.46 (1H and 2H, m), 7.25-7.29 (5H, m), 7.71 (1H, s), 8.09 (1H, s). The abbreviations d, q, m and s are the peak multiplicities of doublet, quartet, multiplet and single, respectively.

(*R*,*S*)-3-Phenylbutyl 4-bromopyrazolide (**2**). $^1$HNMR (DMSO-$d_6$/TMS) δ: 1.27-1.29 (3H, d), 3.32-3.47 (1H and 2H, m), 7.25-7.30 (5H, m), 8.00 (1H, s), 8.62 (1H, s).

(*R*,*S*)-3-Phenylbutyl 4-nitropyrazolide (**3**). $^1$HNMR (DMSO-$d_6$/TMS) δ: 1.30-1.31 (3H, d), 3.35-3.56 (1H and 2H, m), 7.25-7.31 (5H, m), 8.57 (1H, s), 9.36 (1H, s).

(*R*,*S*)-3-Phenylbutyl 1,2,4-triazolide (**8**). $^1$HNMR (DMSO-$d_6$/TMS) δ: 1.30-1.31 (3H, d), 3.36-3.57 (1H and 2H, m), 7.26-7.35 (5H, m), 8.28 (1H, s), 9.27 (1H, s).

(*R*,*S*)-Methyl 3-phenylbutyrate (**9**). $^1$HNMR (DMSO-$d_6$/TMS) δ: 1.22-1.23 (3H, m), 2.61 (2H, d), 3.15-3.21 (1H, q), 3.54 (3H, s), 7.17-7.36 (5H, m).

(*R*,*S*)-3-(Boc-amino)-3-phenylpropionyl 4-methylpyrazolide (**10**). $^1$HNMR (DMSO-$d_6$/TMS) δ: 1.32 (9H, s), 2.03 (3H, s), 3.44-3.45 (2H, d), 5.01 (1H, s), 7.28-7.35 (5H, m), 7.51 (1H, s), 7.70 (1H, s), 8.10 (NH, s).

## 2. KINETIC ANALYSIS

At the initial stage, eq 1 for the fast-reacting substrate without and with adding the acid product is, respectively, reduced to

$$V_R = \frac{k_{2R}(S_R)_0(E_t)K_{mR}^{-1}}{1 + (S_R)_0[K_{mR}^{-1} + K_{mS}^{-1}]} \quad \text{(S1)}$$

$$V_R = \frac{k_{2R}(S_R)_0(E_t)K_{mR}^{-1}}{1 + (S_R)_0[K_{mR}^{-1} + K_{mS}^{-1}] + (I)K_I^{-1}} \quad \text{(S2)}$$

Moreover if the lipase has high (*R*)-enantioselectivity, the (*R*)-enantiomer will completely convert to the acid product (i.e. $(I) = (S_R)_0$) when estimating the initial rate $V_S$. Therefore, the initial rate equation for the slow-reacting substrate is expressed as

$$V_S = \frac{k_{2S}(S_S)_0(E_t)K_{mS}^{-1}}{1 + (S_S)_0[K_{mR}^{-1} + K_I^{-1}]} \quad \text{(S3)}$$

By using the data of Figure 2, $k_{2R}K_{mR}^{-1}$ and $[K_{mR}^{-1} + K_{mS}^{-1}]$ are first estimated from eq S1, and then $K_I$ from eq S2. Similarly, the kinetic constants $k_{2S}$ and $K_{mS}$ are estimated from eq S3, and then $k_{2R}$, $K_{mR}$, and enantiomeric ratio $E$ defined as $k_{2R}k_{2S}^{-1}K_{mR}^{-1}K_{mS}$. Therefore, the time-course variations of $(S_R)_0$ and $(S_S)_0$, and hence $X_R$ and $X_S$ (Figures 1 and S1) are predicted from solving eqs 1-3 by using a fourth-order Runge-Kutta numerical method.

## 3. THERMODYNAMIC ANALYSIS

According to the transition theory with $k_{-1R} >> k_{2R}$ and $k_{-1S} >> k_{2S}$ for eqs 1 and 2 in the Michaelis-Menten kinetics, the logarithm of enantiomeric ratio can be expressed as $Rln(E) = -\Delta\Delta G/T = -\Delta\Delta H/T + \Delta\Delta S$. Therefore from the variation of $ln(E)$ with $T^{-1}$,

one may estimate –ΔΔH, -ΔΔS, and hence -ΔΔG at a specified temperature between the transition states of both enantiomers. The results of -ΔΔH and -ΔΔS along with others for Novozym 435-catalyzed resolution of (*R*,*S*)-azolides containing an α-chiral center are represented in Table S2.

## REFERENCES


(1) Wang, P. Y.; Chen, Y. J.; Wu, A. C,; Lin, Y. S.; Kao, M. F.; Chen, J. R.; Ciou, J. F.; Tsai, S. W. (*R*,*S*)-Azolides as novel substrates for lipase-catalyzed hydrolytic resolution in organic solvents. *Adv. Syn. Cat.* **2009**, *351*, 2333-2341.

(2) Wu, A. C.; Wang, P. Y.; Lin, Y. S.; Kao, M. F.; Chen, J. R.; Ciou, J. F.; Tsai, S. W. Improvements of enzyme activity and enantioselectivity in lipase-catalyzed alcoholysis of (*R*,*S*)-azolides. *J. Mol. Cat. B: Enzymatic* **2010**, *62*, 235-241.

(3) Ciou, J. F.; Wang, P. Y.; Wu, A. C.; Tsai, S. W. Lipase-catalyzed alcoholytic resolution of (R,S)-flurbiprofenyl azolides for preparation of (R)-NO-flurbiprofen ester prodrugs. *Proc. Biochem.* **2011**, *46*, 960-965.

(4) Lin, Y. S.; Wang, P. Y.; Wu, A. C.; Tsai, S. W. Lipase-catalyzed enantioselective resolution of (*R*,*S*)-*N*-2-methylalkanoyl-3-(2-pyridyl)pyrazoles in organic solvents. *J. Mol. Cat. B: Enzymatic*, **2011**, *68*, 245-249.

(5) Kao, M. F.; Lu, P. Y.; Kao, J. Y.; Wang, P. Y.; Wu, A. C.; Tsai, S. W. (*R*,*S*)-2-Chlorophenoxyl pyrazolides as extreme substrates for improving lipase-catalyzed hydrolytic resolution. Chiralty, **2011** (in press)

**Table S1. HPLC analytical conditions for various substrates illustrated in Scheme 1.**

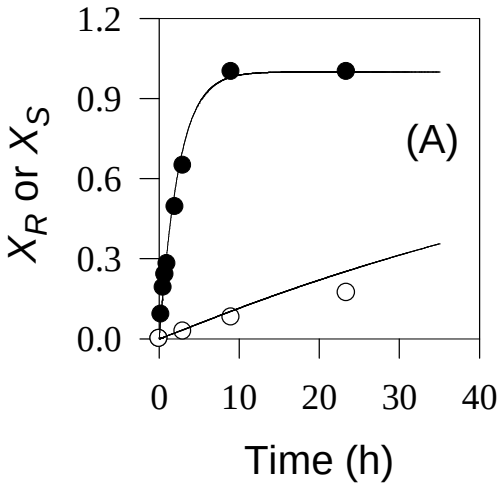
| Substrate | Column | UV (nm) | Flow rate (mL $min^{-1}$) | Internal standard[a] | Mobile phase[b] (HEX:IPA:AA, v/v) | Retention time (min) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Internal standard | (*R*)-substrate | (*S*)-substrate |
| **1** | OJ-H | 270 | 2.0 | A | 89.5:10:0.5 | 13.5, 14.7 | 4.7 | 8.0 |
| **2** | OJ | 270 | 2.0 | B | 88:12.0:0 | 3.0 | 4.5 | 5.2 |
| **3** | OJ-H | 270 | 1.5 | B | 85:15:0 | 3.2 | 8.9 | 9.8 |
| **4** | OJ-H | 270 | 2.0 | B | 95:5:0 | 2.7 | 3.6 | 4.1 |
| **5** | OJ-H | 270 | 2.0 | C | 90:10:0 | 2.0 | 5.3 | 6.4 |
| **6** | (*S*,*S*)-Whelk-O1 | 270 | 2.0 | C | 99:1:0 | 1.7 | 4.8 | 5.7 |
| **7** | OJ-H | 270 | 2.0 | C | 88:12:0 | 2.0 | 16.8 | 19.9 |
| **8** | OD-H | 220 | 1.0 | C | 50:50:0 | 3.6 | 11.1 | 6.4 |
| **9** | OJ-H | 220 | 2.0 | B | 95:5:0 | 3.4 | 4.4 | 3.2 |
| **10** | OJ-H | 270 | 2.0 | C | 90:10:0 | 2.0 | 5.1 | 7.9 |

[a] (A) (*R*,*S*)-Naproxen, (B) 2-nitrotoluene, (C) benzene. [b] IPA and AA as isopropyl alcohol and acetic acid, respectively.

**Table S2. -ΔΔH and -ΔΔS for Novozym 435-catalyzed kinetic resolution of (*R*,*S*)-azolides.**

| Substrates | -ΔΔH (kJ $mol^{-1}$) | -ΔΔS (J $mol^{-1}$ $K^{-1}$) | Reaction, solvent, stereo-preference |
|---|---|---|---|
| (*R*,*S*)-3-Phenylbutyl 4-methylpyrazolide | 20.39 | 37.97 | Hydrolysis, water-saturated CYC, R |
| (*R*,*S*)-3-Phenylbutyl 4-bromopyrazolide | 17.61 | 26.74 | Hydrolysis, water-saturated CYC, R |
| (*R*,*S*)-2-Phenylpropionyl 1,2,4-triazolide[1] | 19.39 | 23.71 | Hydrolysis, water-saturated MTBE, R |
| (*R*,*S*)-Naproxenyl 1,2,4-triazolide[2] | 17.09 | 15.45 | Alcoholysis by methanol, anhydrous MTBE, R |
| (*R*,*S*)-Flurbirpofenyl 1,2,4-triazolide[3] | 8.43 | -10.23 | Alcoholysis by methanol, anhydrous MTBE, R |
| (*R*,*S*)-Flurbirpofenyl 4-bromopyrazolide[3] | 7.54 | -20.38 | Alcoholysis by 2,3-dibromo-1-propanol, anhydrous MTBE, R |
| (*R*,*S*)-2-Methylheptyl 3-(2-pyridyl)pyrazolide[4] | 27.17 | 51.12 | Hydrolysis, water-saturated MTBE, R |
| (*R*,*S*)-2-Methylheptyl 3-(2-pyridyl)pyrazolide[4] | 12.35 | 5.43 | Hydrolysis, water-saturated IPE, R |
| (*R*,*S*)-2-Methylheptyl 3-(2-pyridyl)pyrazolide[4] | 28.45 | 51.46 | Alcoholysis by methanol by methanol, anhydrous MTBE, R |
| (*R*,*S*)-2-Methylheptyl 3-(2-pyridyl)pyrazolide[4] | 59.10 | 151.0 | Alcoholysis by methanol, anhydrous IPE, R |
| (*R*,*S*)-2-(2-Chlorophenoxy)propionyl 3-(2-pyridine)pyrazolide[5] | 22.89 | 40.89 | Hydrolysis, water-saturated MTBE, S |
| (*R*,*S*)-2-(3-Chlorophenoxy)propionyl 3-(2-pyridine)pyrazolide[5] | 23.92 | 47.94 | Hydrolysis, water-saturated MTBE, S |
| (*R*,*S*)-2-(4-Chlorophenoxy)propionyl 3-(2-pyridine)pyrazolide[5] | 9.93 | -13.70 | Hydrolysis, water-saturated MTBE, S |
| (*R*,*S*)-2-(2,4-Dichlorophenoxy)propionyl | 13.02 | -5.51 | Hydrolysis, water-saturated MTBE, S |

3-(2-pyridine)pyrazolide[5]


1.2
0.9
0.6
0.3
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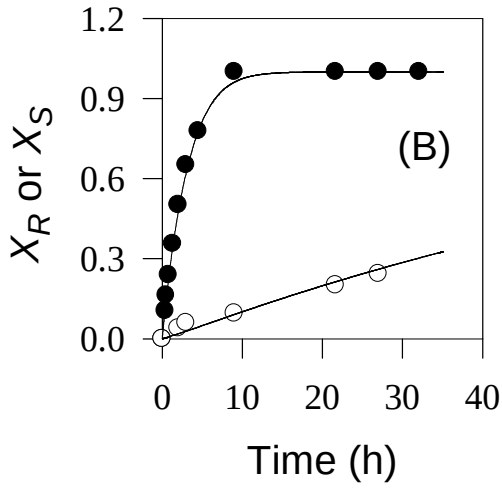
$X_R$ or $X_S$
(A)
0
10
20
30
40
Time (h)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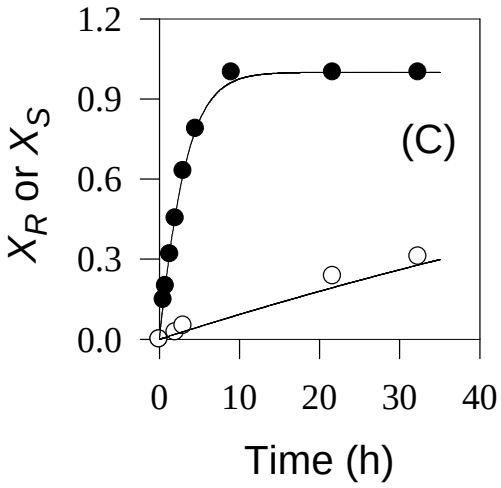
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$X_R$ or $X_S$
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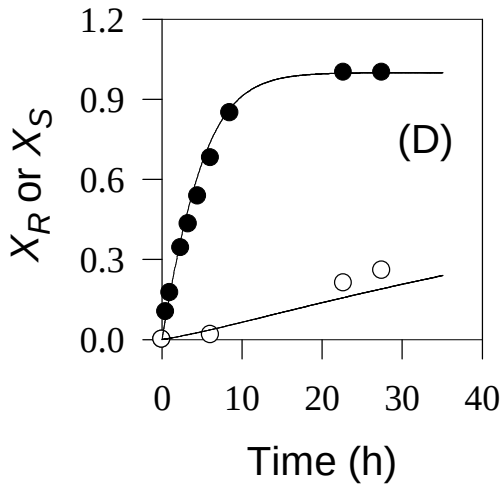
Time (h)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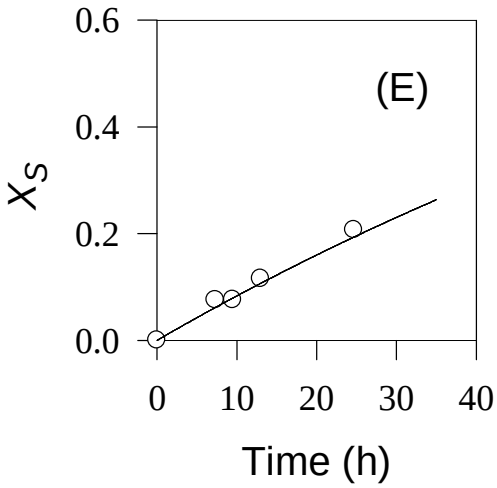
0.6
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0.2
0.0
$X_S$
(E)
0
10
20
30
40
Time (h)

**Figure S1.** Time-course conversions $X_R$ (●) and $X_S$ (○) at 25 $^{o}$C in water-saturated CYC containing 40 mg mL$^{-1}$ of Novozym 435 and (A) 15 mM, (B) 30 mM, (C) 60 mM, and (D) 90 mM of **1**, or those of $X_S$ (○) for (E) 75 mM of (*S*)-**1**; theoretical predictions via eqs 1-3 (—).