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Enzymatic Optical Resolution of Dibenzoxepins and Its Application to an Optically Active Antiallergic Agent with Thromboxane A_2 Receptor Antagonistic Activity

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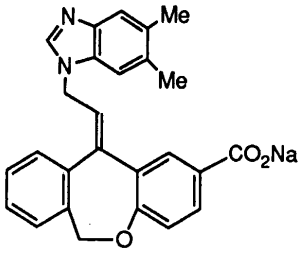
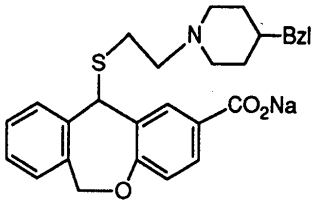
Methyl (±)-11-(2-hydroxyethyl)thio-6,11-dihydrodibenz[b,e]oxepin-2-carboxylate (5) was enantioselectively acylated with acetic anhydride in an organic medium by Lipase Amano P to give methyl (−)-11-(2-acetoxyethyl)thio-6,11-dihydrodibenz[b,e]oxepin-2-carboxylate (8), and the (+)-enantiomer by Lipase Sigma Type VII. Using Lipase Amano P, (+)- and (−)-(5) could be prepared with high optical purity (84–94% *e.e.*). These products were respectively converted to (+)- and (−)-KW-4099, which had antiallergic activity with complete retention of the optical purity.

Thromboxane A_2(TXA_2) has been considered as an important mediator in a variety of circulatory disorders, including angina pectoris, thrombosis, and asthma.[1–3] Several prostanoid and non-prostanoid drugs with TXA_2 antagonistic activity[4–7] have been reported. Recently, such dibenzoxepin derivatives as KW-3655 (**1**)[7] and KW-4099 (**2**)[8] have been found to be TXA_2 receptor antagonists, and **2** showed antagonistic activity toward both receptors of TXA_2 and histamine (H_1), so it was expected to be a new type of antiallergic agent.



KW-3635 (1)

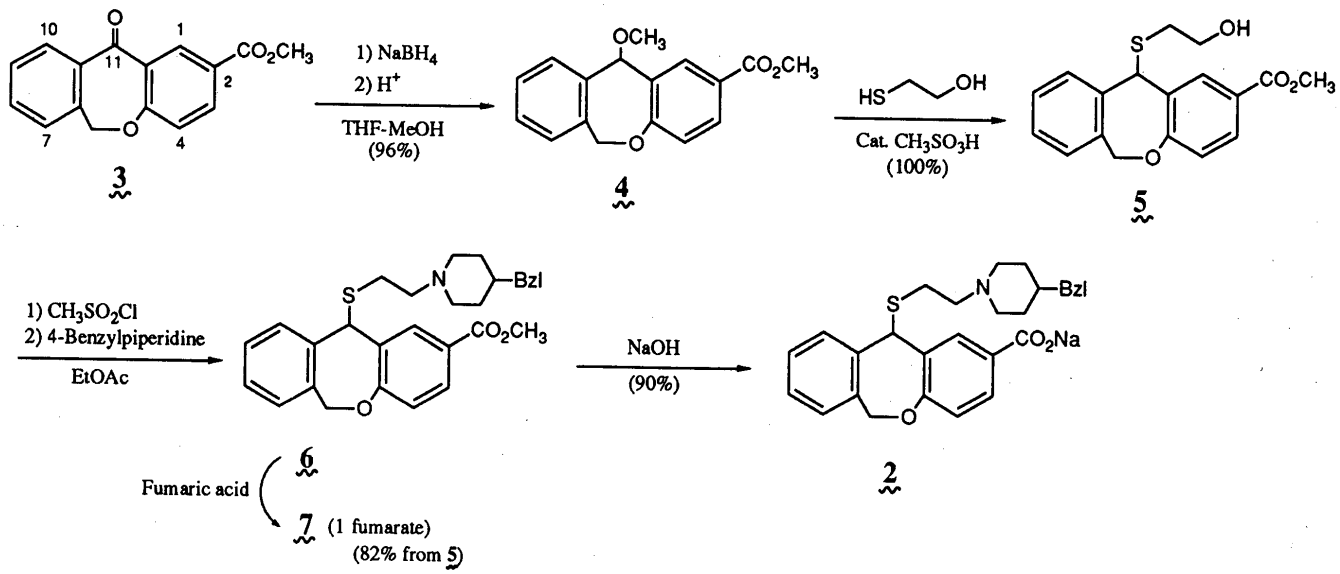


KW-4099 (2)

Chart

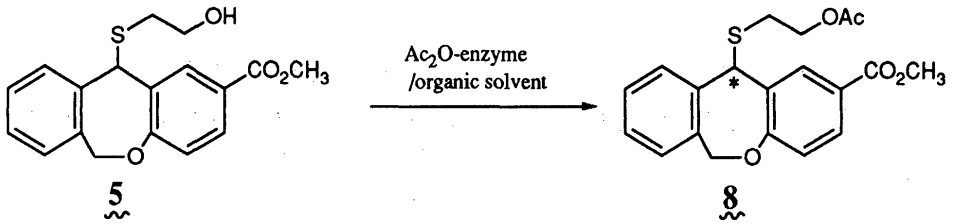
The synthetic route to KW-4099 is shown in Scheme 1.[9–12] We have already achieved a multi-kilogram-scale synthesis of KW-4099 by this route, and the optical resolution of ester **6** by using both atropisomers of 2,2′-(1,1′-binaphthyl)phosphoric acid (BNPPA) as a resolving agent.[10,11] By this method, the preparation of (+) and (−)-KW-4099 was accomplished on a gram scale. However, these reagents are very expensive and it is very difficult to obtain them in large quantities.

For further pharmacological studies on KW-4099, more efficient and inexpensive resolution methods were needed. Recently, the use of enzymes in organic chemistry, such as lipases, esterases and proteases, has become an effective method for preparing many optically active compounds. These enzymes are usually inexpensive and easy to obtain in large quantities. For these reasons, we studied the enzymatic preparation of optically active KW-4099. As far as we know, the enzymatic optical resolution of dibenzoxepin derivatives has not previously been reported. Stereoselective reduction of **3** by yeasts or other reductases



Scheme 1. Synthesis of KW-4099.

* Since this enzymatic reaction is reversible, enantioselective hydrolysis of (±)-**8** by these enzymes would also be possible. In fact, (±)-**8** in a mixture of acetone and 1 M-phosphate buffer (1 : 1) was hydrolyzed in the presence of Lipase Amano P at room temperature for 48 h, and (+)-**8** was recovered as the unreacted substrate with high optical purity. However, the yield was rather disappointing (4%, 95% *e.e.*).



5 8

Scheme 2. Enzymatic Acylation of **5** in Organic Media.

Table I. Result of Screening for the Enzymatic Acylation of **5**

Organic solvent / Enzyme	Ethyl acetate			Tetrahydrofuran/Diisopropyl ether (1/10)		
	Conversion rate (%)	*e.e.* of **8** (%)	*e.e.* of remaining **5** (%)	Conversion rate (%)	*e.e.* of **8** (%)	*e.e.* of remaining **5** (%)
Lipozyme (*Mucor miehei*)	30	3.4 (−)	3.8 (+)	NR		
Lipase B (*Pseudomonas fragi*)	11	0.7 (−)	0.7 (+)	NR		
Lipase Type VII (*Candida cylindracea*)	17	34.9 (+)	6.8 (−)	NR		
SP382 (*Candida* sp.)	73	0.9 (−)	0.7 (+)	NR		
Lipoprotein Lipase (*Pseudomonas fluorescens*)	57	7.8 (−)	11.6 (+)	12	42.6 (−)	4.8 (+)
Lipase Amano P (*Pseudomonas fluorescens*)	37	48.7 (−)	41.5 (+)	8	37.2 (−)	3.7 (+)

Reaction conditions; (±)-**5**, 20 mg/ml; Ac_2O, leq; enzyme, 20 mg/ml in an organic solvent; stirred at room temp. for 4 h. Conversion rate and optical purity were measured by the HPLC method (see Experimental section).

might be possible as one of the strategies for creating the chirality at C-11, but the subsequent S_N2 substitution at C-11 by such nucleophiles as methanol and mercaptoethanol is unfortunately considered to be much more troublesome because of the high cationic stability at C-11.[11] Therefore, we attempted the kinetic resolution of **5** by acylating with acetic anhydride, using commercially available enzymes in organic media (Scheme 2). As a result, Lipase Amano P and Lipase Type VII were the most effective for enantioselective acylation. Also, the resolved optically active alcohols could be converted to (+)- and (−)-KW-4099 with complete retention of their optical purities.

In this report, screening of the enzymes and the optimization of the enzymatic kinetic resolution of **5** are described.

Results and Discussion

Twenty enzymes, including lipases and proteases, were screened in order to examine their ability for catalyzing the enantioselective acylation of alcohol **5** in the presence of acetic anhydride as an acyl donor in an organic solvent. The results are summarized in Table I. Lipase Amano P of Amano Pharmaceuticals Co. from *Pseudomonas fluorescens* (*P. cepacia*) preferentially acylated the (−)-isomer, and Lipase Type VII of Sigma Co. from *Candida cylindracea* acylated the (+)-isomer, both in ethyl acetate. Further studies were done with these two enzymes.

Screening of the organic solvents was first tried. The lower the polarity of the solvent used, the better the enantioselectivity that was obtained. Finally, ethyl acetate and toluene containing 10% tetrahydrofuran were chosen because of their solubility for **5**. When this acylation was done at higher than 30°C, the enantioselectivity decreased, and therefore, the reaction had to be done at under 25°C. The concentration of the enzyme and substrate did not have a significant effect on either the reaction rate or the enantioselectivity. Concerning the concentration of acetic anhydride, the more acetic anhydride that was added, the higher the reaction rate that was obtained. Five eq. of acetic anhydride was used, because it was found that non-enzymatic acylation occurred when more than 10 eq. of acetic anhydride was added (data not shown).

Table II. Optimization of Enzymatic Acylation

	Amano P	Type VII
Solvent	Ethyl acetate	Toluene/ Tetrahydrofuran (10/1)
Substrate concentration (mg/ml)	20	20
Enzyme concentration (mg/ml)	20	20
Acetic anhydride (eq.)	5	3
Reaction temperature (°C)	5−room temp.	room temp.

The results of the optimization with these enzymes are summarized in Table II. Under these optimized conditions, the enantioselective acylation by each enzyme was monitored (Fig.). However, the optical purity of each acetates formed was not satisfactory. On the other hand, the optical purity of the alcohols increased as the conversion rate increased, especially in the case of Lipase Amano P. Finally, the (+)-alcohol could be recovered as the unreacted substrate (94% *e.e.*) after the acylation had been catalyzed by Lipase Amano P at room temperature for 47 h. To

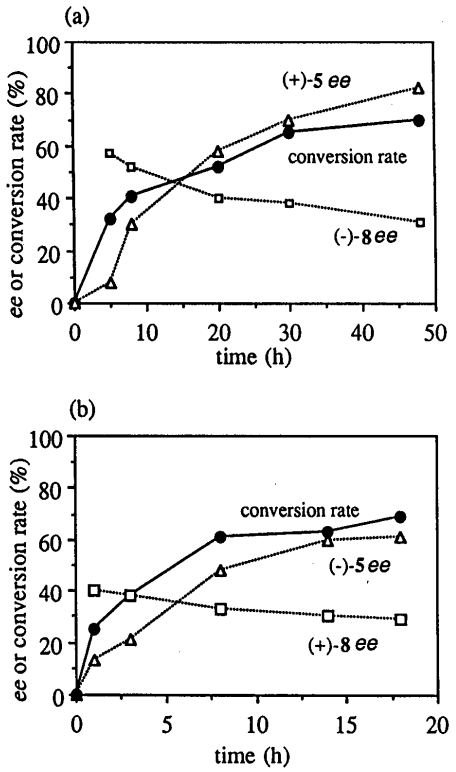


Fig. Time–Course for the Enzymatic Acylation of (±)-**5**

Enzyme: a) Lipase Amano P, b) Lipase Type VII. The other conditions are the same as those shown in Table II.

Table III. Effects of Various Acyl Donors Acylation by Lipase Amano P

Acyl donor	Conversion rate (%)	*e.e.* of remaining **5** (%)
$(CH_3CO)_2O$	72	80.3 (+)
$CH_3CO_2CH=CH_2$	54	53.6 (+)
$(CH_3CH_2CO)_2O$	75	71.8 (+)
$CH_3CH_2CO_2CH=CH_2$	53	44.6 (+)
$[CH_3(CH_2)_2CO]_2O$	66	52.8 (+)
$CH_3(CH_2)_2CO_2CH=CH_2$	51	39.4 (+)
$[CH_3(CH_2)_{10}CO]_2O$	88	79.5 (+)
$CH_3(CH_2)_{10}CO_2CH=CH_2$	53	42.2 (+)
$(PhCO)_2O$	16	7.3 (+)
$CH_3CH=CHCO_2CH=CH_2$	41	13.9 (+)
$PhCH=CHCO_2CH=CH_2$	68	7.1 (−)

3eq. of an acyl donor were added. The other conditions are the same as those shown in Fig. 1.

prepare the (−)-alcohol, (±)-**5** was treated with Lipase Amano P at 5°C for 6 h. The (−)-acetate thus obtained, after isolating by silica gel chromatography, was treated with sodium methoxide in MeOH to give the (−)-alcohol (57% *e.e.*). This alcohol enriched with the (−)-isomer was reacylated enzymatically under the same conditions to enhance the optical purity. This double enzymatic acylation and the subsequent base treatment afforded (−)-**5** with 84% *e.e.*

At this stage, both (+)- and (−)-**5** each with high optical purity could be prepared. Next, we screened a variety of other acid anhydrides or vinyl esters as the acyl donors in order to attain higher optical purity. The results are shown in Table III. No acyl donor superior to acetic anhydride were found, and in all cases, acetate **8** was formed as a by-product (2–30%). This was due to the mixed acid anhydride formed by enzymatic transesterification between the acyl donors and ethyl acetate (solvent). Acetic anhydride was thus the most suitable agent for this enzymatic acylation in ethyl acetate.

In summary, optical resolution of **5** by enzymes was achieved.* This is the first enzymatic optical resolution of dibenzoxepines. Especially in the case of **5**, the enzymatic method was very effective, since partial crystallization of the diastereomeric ester derivatives of **5** with optically active acids would seem to be rather difficult because of the great distance between the hydroxy group and the asymmetric center, C-11. These optically active alcohols were converted to optically active ester **6**. Hirayama has reported that the absolute configuration of the (+)-**6** ester was (*R*), and that of the (−)-isomer was (*S*).[13]

We are now investigating the effect of ester groups at C-2 on the enantioselectivity to attain higher optical purity. These optically active alcohols were finally converted to (+)- and (−)-KW-4099 with complete retention of their optical purity. Pharmacological studies on these isomers are now under investigation and will be reported in the near future.

Experimental

Infrared (IR) spectra were recorded by a Shimadzu IR-435 spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained with a Brucker AC-300 spectrometer, and signals are given in ppm using tetramethylsilane as an internal standard. The optical purity of alcohol **5** and acetate **8** was each measured by the HPLC method (column, CHIRALCEL OD 4.6 × 250 mm; mobile phase, *n*-hexane : iso-PrOH = 3 : 7; flow rate, 0.5 ml/min; temp., 20°C; detection, UV 254 nm). The optical purity of KW-4099 was also measured by the HPLC method (column, CHIRALCEL OD 4.6 × 250 mm; mobile phase, *n*-hexane : EtOH : AcOH = 7 : 3 : 0.01; flow rate, 0.5 ml/min; temp., 20°C; detection, UV 254 nm). Optical rotation values were measured with a Horiba SEPA-200 polarimeter.

Methyl (±)-11-(2-hydroxyethyl)thio-6,11-dihydrodibenz[b,e]oxepin-2-carboxylate (**5**). Under an N_2 atmosphere, $NaBH_4$ (0.7 g, 18.6 mmol) was added to a stirred suspension of **3** (10.0 g, 37.3 mmol) in 30 ml of tetrahydrofuran. MeOH (45 ml) was then gradually added at less than 10°C. After the addition, the mixture was stirred at the same temperature for 1 h, the pH was adjusted to 1 with 6 N HCl, and the resulting solution was stirred at 35°C for 2 h. The solution was cooled to room temperature and adjusted to pH 7 with 10 N NaOH. After removing half of the solvent by evaporating, 30 ml of water was added for crystallization. Ester **4** was obtained as colorless crystals (96%). 2-Mercaptoethanol (2.65 ml, 37.7 mmol) and methanesulfonic acid (0.22 ml, 3.59 mmol) were added to a solution of **4** (35.9 mmol) in methylene chloride (40 ml) at room temperature. The mixture was heated under reflux for 5 h, before adding triethylamine (0.51 ml, 3.59 mmol). The solvent was removed by evaporating, before adding MeOH (60 ml) for crystallization to afford **5** as colorless crystals (11.9 g, 100% from **4**). *Anal.* Calcd. for $C_{18}H_{18}O_4S$: C, 65.43; H, 5.49%. Found: C, 65.39; H, 5.50%. IR ν_{max} (KBr) cm^{-1}: 3400, 2950, 1750, 1685, 1610. NMR ($CDCl_3$) δ_H: 2.32 (1H, br.), 2.58–2.77 (2H, m), 3.66–3.77 (2H, m), 3.90 (3H, m), 4.94 (1H, d, J = 13.0 Hz), 5.13 (1H, s), 6.45 (1H, d, J = 13.0 Hz), 6.89 (1H, d, J = 8.0 Hz), 7.22–7.35 (4H, m), 7.83 (1H, d, J = 8.0 Hz), 8.01 (1H, s).

Methyl (+)-11-(2-hydroxyethyl)thio-6,11-dihydrodibenz[b,e]oxepin-2-carboxylate ((+)-**5**). To a solution of (±)-**5** (10 g, 30.3 mmol) in ethyl acetate (500 ml), 10 g of Lipase Amano P was added at room temperature, before adding acetic anhydride (15.5 g, 151.8 mmol). The mixture was stirred at room temperature for 47 h, and then the enzyme was removed by filtration. The filtrate was washed with a sat. $NaHCO_3$ aqueous solution and dried over anhydrous $MgSO_4$. The solution was concentrated and purified by SiO_2 chromatography (*n*-hexane : ethyl acetate = 2 : 1) to recover (+)-**5** as the unreacted substrate (2.13 g, 21.3%). *Anal.* Calcd. for $C_{18}H_{18}O_4S$: C, 65.43; H, 5.49%. Found: C, 65.28; H, 5.63%. Optical

purity, 94% *e.e.* $[\alpha]_D^{20}$ +199.3° (c=2, $CHCl_3$).

Methyl (−)-11-(2-hydroxyethyl)thio-6,11-dihydrodibenz[b,e]oxepin-2-carboxylate ((−)-**5**). To a solution of (±)-**5** (10 g, 30.3 mmol) in ethyl acetate (500 ml), 10 g of Lipase Amano P was added at 5°C, before adding acetic anhydride (15.5 g, 151.8 mmol). The mixture was stirred for 8 h at 5°C, and then the enzyme was removed by filtration. The filtrate was washed with a sat. $NaHCO_3$ aqueous solution, dried over anhydrous $MgSO_4$, concentrated, and purified by SiO_2 chromatography to afford 5.38 g (47.7%) of methyl (−)-11-(2-acetoxyethyl)thio-6,11-dihydrodibenz[n,e]oxepin-2-carboxylate, (−)-**8**. *Anal.* Calcd. for $C_{20}H_{20}O_5S$; C, 64.50; H, 5.41%. Found: C, 64.25; H, 5.50%. Optical purity, 67% *e.e.* $[\alpha]_D^{20}$ −95.8° (c=2, $CHCl_3$). NMR ($CDCl_3$) δ_H: 2.09 (3H, s), 2.59–2.74 (2H, m), 3.89 (3H, m), 4.12–4.17 (3H, m), 4.91 (1H, d, J=12.8 Hz), 5.10 (1H, s), 6.41 (1H, d, J=12.8 Hz), 6.87 (1H, d, J=8.5 Hz), 7.24–7.33 (4H, m), 7.81 (1H, d, J=12.8 Hz). This acetate was dissolved in MeOH (50 ml) and then treated with 1.5 eq. of sodium methoxide for 0.5 h at 5°C. After crystallization by addition water, (−)-**5** was produced in a quantitative yield from (−)-**8** (4.77 g, 67% *e.e.*). This alcohol was acylated again under the same conditions as those just described to give (−)-**5** as colorless crystals (3.02 g, overall 30.2%). *Anal.* Calcd. for $C_{18}H_{18}O_4S$: C, 65.43; H, 5.49%. Found: C, 65.40; H, 5.65%. Optical purity, 84% *e.e.* $[\alpha]_D^{20}$ −189.7° (c=2, $CHCl_3$).

Sodium (+)-11-[2-(4-benzyl-1-piperidinyl)ethyl]thio-6,11-dihydrodibenz[b,e]oxepin-2-carboxylate ((+)-**2**). Alcohol (+)-**5** (3.3 g, 10 mmol, 87% *e.e.*) was dissolved in pyridine (10 ml) and cooled to 5°C. Methane sulfonylchloride (1.72 g, 15 mmol) was added in portions at 5°C, and the mixture was stirred for 2 h while cooling. Ethyl acetate (20 ml) and then water (10 ml) were added, and the mixture was adjusted to pH 3 with 6 N HCl while vigorously stirring. The organic layer was washed with a sat. $NaHCO_3$ aqueous solution, and 4-benzylpiperidine (3.51 g, 20 mmol) was added, before the mixture was stirred under reflux for 2 h. After cooling, the reaction mixture was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was dissolved in EtOH (23 ml) and heated to 50°C. Fumaric acid (1.28 g, 11 mmol) was then added at 50°C, and the mixture was gradually cooled to afford methyl (+)-11-[2-(4-benzyl-1-piperidinyl)ethyl]thio-6,11-dihydrodibenz[b,e]oxepin-2-carboxylate 1-fumarate, (+)-**7** (5.29 g, 88% *e.e.*). The product was extracted with ethyl acetate (50 ml) and 5% aqueous ammonia (50 ml), and the organic layer was dried over anhydrous $MgSO_4$. The solvent was removed by evaporating, and the residue was dissolved in a mixture of MeOH (110 ml), water (83 ml) and 10 N NaOH (1.76 ml), before being refluxed for 2 h. Half of the solvent was then removed by evaporating, and 115 ml of water was added. The pH was adjusted to 6 by using 6 N HCl. The precipitated free acid was collected by filtering, dissolved in a mixture of iso-PrOH (41.7 ml), water (4.2 ml) and 10 N NaOH (1.3 ml) while heating at 60°C, and the solution was then cooled. The sodium salt, (+)-**2**, was obtained as colorless crystals (2.7 g, overall 59.4%). IR ν_{max} (KBr) cm^{-1}: 3410, 2950, 1615, 1590, 1550. NMR (DMSO-d_6) δ_H: 1.12–2.73 (15H, m), 4.96 (1H, d, J=13.0 Hz), 5.29 (1H, s), 6.20 (1H, d, J=13.0 Hz), 6.69 (1H, d, J=8.0 Hz), 7.15–7.41 (9H, m), 7.68 (1H, d, J=8.0 Hz), 7.89 (1H, s). Optical purity, 87% *e.e.* $[\alpha]_D^{20}$ +81.9° (c=1, MeOH).

Sodium (−)-11-[2-(4-benzyl-1-piperidinyl)ethyl]thio-6,11-dihydrodibenz[b,e]oxepin-2-carboxylate ((−)-**2**). This compound was prepared from (−)-**5** (80% *e.e.*) by the same method as that employed for the synthesis of (+)-**2**. Optical purity, 81% *e.e.* $[\alpha]_D^{20}$ −77.6° (c=1, MeOH).

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