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Asymmetric synthesis of (*R*)-(+)-etomoxir via enzymatic resolution

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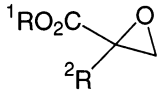
Abstract

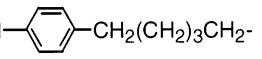
An asymmetric synthesis of (*R*)-(+)-etomoxir **3**, employing enzymatic resolution of ethyl 2-alkyl-2,3-dihydroxypropionate using Amano AK via transacylation is reported.

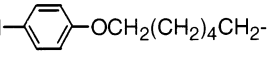
1. Introduction

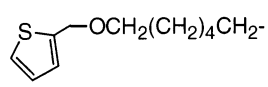
Substituted oxirane-2-carboxylates such as palmoxirate,[1] clomoxir,[2] etomoxir,[3] JG381[4] have been recognized as powerful hypoglycemic agents in animals including humans. Their hypoglycemic effect was associated with the irreversible inhibition of carnitine palmitoyl transferase I (CPT I), which is essential for the transportation of palmitoyl CoA into the mitochondria matrix side for its β-oxidation[5].

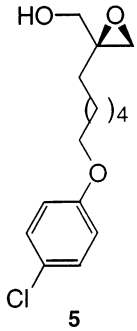


1 Palmoxirate R^1 = Me R^2 = $CH_3(CH_2)_{12}CH_2$-

2 Clomoxir R^1 = Et R^2 =  $CH_2(CH_2)_3CH_2$-

3 Etomoxir R^1 = Et R^2 =  $OCH_2(CH_2)_4CH_2$-

4 JG381 R^1 = Et R^2 =  $OCH_2(CH_2)_4CH_2$-



5

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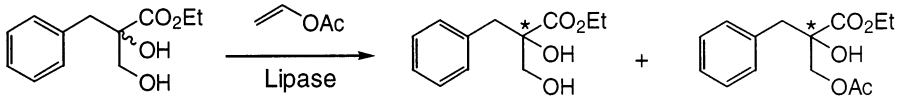
The further mechanistic investigation revealed that only the (*R*)-enantiomer of etomoxir shows biological activity by covalent bond formation between its CoA ester with CPT I.[5c] Because of the stereospecificity for hypoglycemic activity, several asymmetric synthetic methods of (*R*)-(+)-etomoxir have been reported using enzymatic resolution,[6] Sharpless epoxidation[7] and asymmetric bromolactonization[8] as key steps. Among them, Prasad's enzymatic resolution method is quite attractive for practical application. They reported the synthesis of **5** as an intermediate for (*R*)-(+)-etomoxir (92% ee), but the known process[7] for the conversion of **5** to (*R*)-(+)-etomoxir is relatively impractical (four steps, 23%). Herein, we present the highly enantioselective and practical synthesis of (*R*)-(+)-etomoxir via enzymatic resolution.

2. Results and discussion

First of all, ethyl 2-benzyl-2,3-dihydroxypropionate **6**[9] was chosen as the substrate to investigate the kinetic resolution with various lipases via transacylation[10] using vinylacetate. Transacylation was initiated by addition of various lipases and vinylacetate to **6** in methyl *tert*-butylether (MTBE) at room temperature.[11] After around 50% conversion of **6**, **6a** and **7** were isolated and the enantioselectivities were measured. The enantiomeric excess of **6** was determined by ^{1}H NMR analyses of the diasteromeric Mosher esters. In the case of **7**, the same method was employed after the hydrolysis of **7** under basic conditions. The absolute configurations were assigned by comparison with literature data.[9] As shown in Table 1, Amano AK gave the best enantioselectivity of both diol **6a** (*R*, 99% ee) and acetate **7** (*S*, 97% ee). Interestingly, the stereo-orientation was the reverse with Amano AY.

Table 1
Model study of enzymatic resolution with ethyl 2-benzyl-2,3-dihydroxypropionate **6**



6 **6a** **7**

No.	Lipase[a] (90 mg/mmol of substrate)	Temp (°C)	Solvent	Time (h)	Conv.[b] (%)	**6a**	**7**	*E* value
						%ee[c] (%*y*)	%ee[d] (%*y*)	
1	Amano PS	20	MTBE	4	53	96(*R*) (40)	85(*S*) (55)	48
2	Amano A	25	MTBE	24	38	40(*R*) (58)	64(*S*) (40)	7
3	Amano AK	20	MTBE	2	51	99(*R*) (42)	97(*S*) (55)	210
4	Amano AY	20	MTBE	24	36	43(*S*) (57)	76(*R*) (38)	11

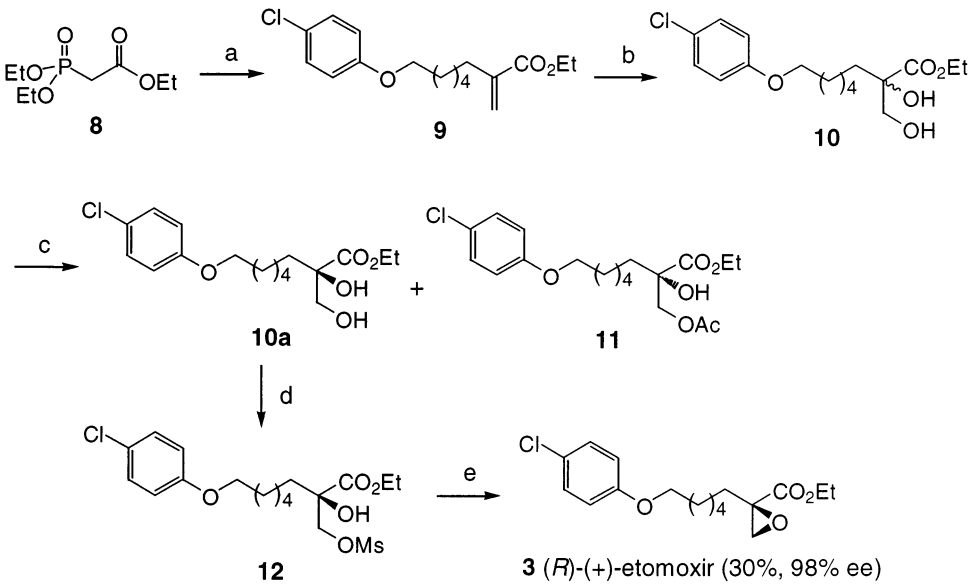
[a] The enzymes, *Pseudomonas cepacia* lipase (Amano PS), *Aspergillus niger* lipase (Amano A), *Pseudomonas fluorescens* lipase (Amano AK), and *Candida rugosa* lipase (Amano AY) were donated from Amano Co.

[b] The degree of conversion was determined by analysis of ^{1}H NMR of the mixture of **6a** and **7**.

[c] The %ee was determined by ^{1}H NMR spectrum with Mosher ester derivatives.

[d] The %ee was determined by ^{1}H NMR spectrum with Mosher ester derivatives after conversion of **7** to enantiomer of **6a** by hydrolysis.

The highest E value (210) shown for Amano AK was chosen to be applied to the preparation of (*R*)-etomoxir **3**. The substrate **10** for enzymatic resolution was prepared from triethyl phosphonoacetate in two steps (Scheme 1). The alkylation of **8** with 6-*p*-chlorophenoxyhexylbromide, followed by Wittig reaction with formaldehyde gave **9**. The dihydroxylation using NMO and OsO_4 provided diol **10**. Enzymatic resolution of **10** with Amano AK was optimized by using different solvents. As shown in Table 2, MTBE also showed the best selectivity [(*R*)-**10a**, 99% ee:*S*-**11**, 96% ee)]. The treatment of methansulfonylchloride with **10a** under basic conditions gave mesylate **12**, which was converted to (*R*)-etomoxir **3**, $[\alpha]_D^{20}$ +8.55 (*c* 0.75, $CHCl_3$), 98% ee (lit.[7] 8.56, *c* 1, $CHCl_3$, >98% ee) by epoxidation under basic conditions.



Scheme 1. Reagents and conditions: (a) (i) *p*-$ClPhOCH_2(CH_2)_4CH_2Br$, NaH, DME, reflux, 12 h; (ii) NaH, $(CH_2O)_n$, DME, rt, 12 h, 75%, (b) NMO, cat. OsO_4. *tert*-BuOH:H_2O (1:1), rt, 1 h, 96%, (c) lipase, MTBE, rt, 2 h, 44%, (d) methanesulfonylchloride, triethylamine, CH_2Cl_2, (e) anhydrous K_2CO_3, abs. EtOH, rt, 6 h, 95%

Table 2
Enzymatic resolution of **10** with Amano AK

No.	Lipase (90 mg/mmol of substrate)	Temp (°C)	Solvent	Time (h)	Conv.[a] (%)	**10a**	**11**	E value
						%ee[b] (%y)	%ee[c] (%y)	
1	Amano AK	20	DIPE	2	45	79 (*R*) (47)	95 (*S*) (50)	138
2	Amano AK	20	Benzene	6.5	45	80 (*R*) (42)	>99 (*S*) (50)	220
3	Amano AK	20	Toluene	7.5	33	48 (*R*) (60)	>99 (*S*) (35)	125
4	Amano AK	20	MTBE	2	51	98 (*R*) (44)	96 (*S*) (54)	211

[a] The degree of conversion was determined by analysis of ^1H NMR of the mixture of **10a** and **11**.

[b] The %ee and absolute configuration were determined by comparison of specific rotation of **3** with literature values.

[c] The %ee and absolute configuration were determined by specific rotation after conversion of **11** to the enantiomer of **10a** by hydrolysis.

3. Conclusion

In summary, highly enantioselective enzymatic resolution of ethyl 2-alkyl-2,3-dihydroxypropionate was developed by using Amano AK in MTBE. By this process, (*R*)-(+)-etomoxir could be prepared in 30% yield and 98% ee over five steps from triethyl phosphonoacetate. The high enantioselectivity could be applied to the synthesis of chiral 2-oxirane carboxylate derivatives (**1**, **2**, **4**). Also, as the enantioselectivity of asymmetric dihydroxylation of ethyl 2-alkylacrylate is generally relatively poor ($\cong$70% ee),[12] highly enantioselective chiral 2-alkyl-2,3-dihydroxypropionate derivatives prepared from this new method should be useful as chiral building blocks for asymmetric synthesis.

4. Experimental

4.1. General

Optical rotations were measured with a JASCO DIP-1000 digital polarimeter. Infrared spectra were taken on a Perkin–Elmer 1710 FT-IR spectrometer. Mass spectra were obtained on a VG Trio-2 GC-MS instrument; high resolution mass spectra were obtained on a HP 5890 Series II. ^{1}H and ^{13}C NMR spectra were measured with a JEOL JNM-LA 300, a JEOL JNM-GCX 400 or a Bruker AMX-500 spectrometer using TMS as the internal standard. All reactions were carried out under an argon atmosphere, using anhydrous solvents except for those involving hydrolysis. Most reagents were obtained from commercial suppliers and used without further purification unless noted. Tetrahydrofuran was distilled from Na^o and benzophenone.

4.2. Enzymatic resolution of ethyl 2,3-dihydroxy-2-benzyl propionate *6* with Amano AK via transacylation

To **6** (100 mg, 0.45 mmol) and Amano AK (45 mg) in MTBE was added vinyl acetate (116 mg, 1.35 mmol) and the reaction mixture was stirred at room temperature. The reaction was stopped at around 50% conversion of **6** and filtered with Celite in vacuo. After removal of solvent, the residue was dried in vacuo. After the determination of the degree of conversion by ^{1}H NMR, the residue was purified by column chromatography (ethyl acetate:*n*-hexane = 1:2) to give (*R*)-**6a** (42 mg, 42%) and (*S*)-**7** (61 mg, 55%). Compound (*R*)-**6a**: $[\alpha]_D^{20}$ −15.5 (*c* 0.85, $CHCl_3$), 99% ee (lit.[9] +10.43, *c* 0.86, $CHCl_3$, 76% ee, (*S*)-**6**); IR (neat) 3445, 1733 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31–7.17 (m, 5 H), 4.26–4.15 (m, 2 H), 3.92 (t, J = 11.09 Hz, 1 H), 3.73–3.67 (m, 1 H), 3.37 (s, 1 H), 3.00 (d, J = 13.65 Hz, 1 H), 2.90 (d, J = 13.65 Hz, 1 H), 2.19–2.14 (m, 1 H), 1.28 (t, J = 7.14 Hz, 3 H); MS (EI) *m*/*e* 225 [M$^+$+1]. Compound (*S*)-**7**: $[\alpha]_D^{20}$ +15.0 (*c* 0.94, $CHCl_3$); IR (neat) 3503, 1748 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.60–7.19 (m, 5 H), 4.39–4.09 (m, 4 H), 3.00 (q, J = 7.56 Hz, 2 H), 2.07 (s, 3 H), 1.26 (t, J = 7.07 Hz, 3 H); MS (EI) *m*/*e* 267 [M$^+$]. The hydrolysis of (*S*)-**7** in the ethanol solution of potassium carbonate gave (*S*)-**6a**: $[\alpha]_D^{20}$ +15.0 (*c* 0.65, $CHCl_3$), 97% ee (lit.[9] +10.43, *c* 0.86, $CHCl_3$, 76% ee, (*S*)-**6**).

4.3. Ethyl 2-hydroxy-2-benzyl-3-[α-methoxy-α-(trifluoromethyl)phenylacetoxy]-propanoate

To a tetrahydrofuran solution of (*R*)-**6a** (20 mg, 0.09 mmol) and dimethylaminopyridine (22 mg, 0.18 mmol) was added (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid chloride (MTPC) (46 mg, 0.18 mmol). After stirring the reaction solution for 30 min, tetrahydrofuran was removed in vacuo. The residue was purified by column chromatography (ethyl acetate: *n*-hexane = 1:10) to give MTPC derivative of (*R*)-**6a** as a colorless oil (25 mg): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.58–7.19 (m, 10 H), 4.55 (s, 2 H), 4.40–4.36 (q, 2 H, minor), 4.17–4.09 (q, 2 H, major), 3.52 (s, 3 H, minor), 3.50 (s, 3 H, major), 3.07 (q, 2 H), 1.21 (t, J = 7.2 Hz, 3 H, major), 1.15 (t, J = 7.1 Hz, 3 H, minor). The same procedure was employed for the preparation of MTPC derivatives of (*S*)-**6**.

4.4. Ethyl 2-[6-(p-chlorophenoxy)hexyl]acrylate **9**[7]

To a 1,2-dimethoxyethane suspension of 95% NaH (1.84 g, 46.09 mmol) was added a 1,2-dimethoxyethane solution of triethylphosphonoacetate (9.14 mL, 46.09 mmol) at room temperature. The reaction mixture was stirred for 1 h and then 6-(*p*-chlorophenoxy)-1-bromohexane (14.10 g, 46.09 mmol) was added to the reaction mixture. The reaction mixture was refluxed for 10 h. After the reaction mixture was cooled down to room temperature, 95% NaH (1.84 g, 46.09 mmol) was added at 0°C. The reaction mixture was warmed to room temperature and stirred for 1 h. The 1,2-dimethoxyethane solution of 95% paraformaldehyde (1.54 g, 51.17 mmol) was added at room temperature. The reaction mixture was stirred for 1 h. The excess solvent was removed in vacuo and the residue was diluted with ethyl acetate. The ethyl acetate solution was washed with water and brine, dried over anhydrous $MgSO_4$, the residue was purified by column chromatography (ethyl acetate:hexane = 1:20) to give **9**[7] as a colorless oil (11.33g, 75%): IR (neat) 1717 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.24–7.19 (m, 2 H), 6.84–6.78 (m, 2 H), 6.13 (s, 1 H), 5.51 (s, 1 H), 4.21 (q, J = 7.15 Hz, 2 H), 3.91 (t, J = 6.45 Hz, 2 H), 2.31 (t, J = 6.95 Hz, 2 H), 1.85–1.33 (m, 8 H), 1.28 (t, J = 6.83 Hz, 3 H); MS (EI) m/e 310 [M$^+$].

4.5. Ethyl 2,3-dihydroxy-2-[6-(p-chlorophenoxy)hexyl]propanoate **10**

To *tert*-butanol:H_2O (1:1, 50 mL) a mixture of NMO (9 mmol) was added 0.08 M OsO_4 in toluene (2 mL, 0.4 mmol) and **9** (2.59 g, 8 mmol). The reaction solution was stirred for 1 h at room temperature. The excess OsO_4 was quenched by addition of $NaHSO_3$ (1.7 g) and the *tert*-butanol was removed in vacuo. The residue was diluted with water (20 mL) and extracted with methylene chloride (20 mL×3). The combined methylene chloride was washed with water (10 mL) and brine (10 mL), was removed in vacuo and the residue was purified by column chromatography (methylene chloride:methanol = 20:1) to give **10** (2.65 g, 96%) as colorless oil: IR (neat) 3447, 1733 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.22 (d, J = 6.72 Hz, 2 H), 6.80 (d, J = 6.84 Hz, 2 H), 4.28 (m, 2 H), 3.90 (t, J = 6.45 Hz, 2 H), 3.78 (t, J = 10.47 Hz, 1 H), 3.62–3.57 (m, 1 H), 3.55 (s, 1 H), 2.17–2.13 (m, 1 H), 1.77–1.29 (m, 13 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.21, 157.63, 129.22, 125.30, 115.70, 78.49, 68.11, 67.89, 62.28, 34.76, 29.32, 29.00, 25.76, 22.90, 14.19; MS (EI) m/e 344 [M$^+$], HRMS (EI) calcd for $C_{17}H_{25}O_5^{35}Cl$ [M$^+$] 344.1319, found 344.1305.

4.6. Enzymatic resolution of **10** with Amano AK via transacylation

To **10** (100 mg, 0.29 mmol) and Amano AK (30 mg/mmol of diol) in MTBE was added vinyl acetate (75 mg, 0.87 mmol)) and the reaction mixture was stirred at room temperature. The reaction was stopped at around 50% conversion of **10** and filtered with Celite in vacuo. After removal of solvent, the residue was dried in vacuo. After the determination of the degree of conversion by ^{1}H NMR, the residue was purified by column chromatography (ethyl acetate:*n*-hexane = 1:2) to give (*R*)-**10a** (44%) and (*S*)-**11** (54%). Compound (*R*)-**10a**: $[\alpha]_D^{20}$ +6.05 (*c* 0.95, $CHCl_3$). The other spectral data were exactly same as **10**. Compound (*S*)-**11**: $[\alpha]_D^{20}$ +4.4 (*c* 0.75, $CHCl_3$); IR (neat) 1748 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.26–7.20 (m, 2 H), 6.82–6.79 (m, 2 H), 4.31–4.13 (m, 4 H), 3.90 (t, J = 6.45 Hz, 2 H), 3.41 (s, 1 H), 2.06 (s, 3 H), 1.80–1.15 (m, 13 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.13, 170.47, 157.65, 129.21, 125.29, 115.71, 76.35, 68.91, 68.09, 62.27, 34.95, 29.24, 28.98, 25.75, 22.80, 20.64, 14.16; MS (EI) *m*/*e* 386 [M$^+$], HRMS (EI) calcd for $C_{19}H_{27}O_6^{35}Cl$ [M$^+$] 386.1496, found 386.1426. The hydrolysis of (*S*)-**11** in the ethanol solution of potassium carbonate gave (*S*)-**10a**: $[\alpha]_D^{20}$ −5.95 (*c* 0.74, $CHCl_3$).

4.7. (R)-(+)-Etomoxir **3**

To an anhydrous tetrahydrofurane solution of **10a** (33 mg, 0.10 mmol) and triethylamine (15 mg, 0.15 mmol) was added methansulfonyl chloride (17 mg, 0.15 mmol) and the reaction solution was stirred for 0.5 h at 0°C. After removal of the solvent in vacuo, the residue was diluted with ethyl acetate (50 mL) and washed with water (10 mL) and brine (10 mL). The organic solution was filtered, dried over anhydrous $MgSO_4$ and evaporated to give almost pure **12** (38 mg). The crude **12** was used without further purification. To the anhydrous ethanol solution of **12** was added potassium carbonate (20 mg, 0.15 mmol) and the reaction mixture was stirred for 6 h at room temperature. After the removal of ethanol, the residue was purified by column chromatography (ethyl acetate:*n*-hexane = 1:20) to give (*R*)-(+)-etomoxir **3** as a colorless oil (28 mg, 95% yield): $[\alpha]_D^{20}$ +8.55 (*c* 0.75, $CHCl_3$), 98% ee (lit.[7] +8.56, *c* 1, $CHCl_3$, >98% ee); IR (neat) 2960, 1740, 1500, 1250 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.21 (d, J = 8.8 Hz, 2 H), 6.80 (d, J = 8.8 Hz, 2 H), 4.21 (dq, J = 7.2, 3.6 Hz, 2 H), 3.89 (t, J = 6.8 Hz, 2 H), 3.02 (d, J = 5.86 Hz, 1 H), 2.78 (d, J = 5.86 Hz, 1 H), 2.13–2.04 (m, 1 H), 1.78–1.73 (m, 2 H), 1.69–1.62 (m, 1H), 1.54–1.33 (m, 6 H), 1.28 (t, J = 7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.46, 157.67, 129.25, 125.29, 115.71, 68.13, 61.44, 57.02, 51.88, 31.16, 29.21, 29.01, 25.80, 24.70, 14.11; MS (EI) *m*/*e* 326 [M$^+$], HRMS (EI) calcd for $C_{17}H_{23}O_4^{35}Cl$ [M$^+$] 326.1285, found 326.1282.

Acknowledgements

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11. The general reaction procedure for enzymatic resolution was as follows: to the mixture of the diol and enzyme (90 mg/mmol of diol) in MTBE was added vinyl acetate (3.0 eq. of diol) and it was stirred at room temperature. The reaction was stopped at around 50% conversion of diol and filtered with Celite in vacuo. After removal of solvent, the residue was dried in vacuo. After the determination of the degree of conversion by ^{1}H NMR, the residue was purified by column chromatography (ethyl acetate:*n*-hexane = 1:2) to give diol and acetate.
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