Pergamon

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Tetrahedron: *Asymmetry* 14 (2003) 1385–1391

TETRAHEDRON: *ASYMMETRY*

A scalable chemoenzymatic preparation of (*R*)-tetrahydrofuran-2-carboxylic acid

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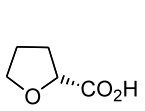
Received 19 February 2003; accepted 7 March 2003

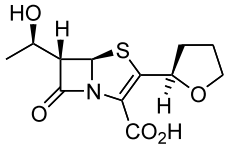
Abstract—To develop a practical scalable approach to (*R*)-tetrahydrofuran-2-carboxylic acid (THFC) **1**, a chiral building block for furopenem **2**, enantioselective hydrolysis of its esters is explored: When ethyl (±)-tetrahydrofuran-2-carboxylate **3d** (2 M, 288 g/L) is digested by an *Aspergillus melleus* protease {0.2% (w/v)} in a 1.5 M potassium phosphate buffer (pH 8) for 20 h, enantioselective hydrolysis proceeds with $E=60$ to give (*R*)-THFC **1** in 94.4% ee. On separation from the left-over antipodal ester (*S*)-**3d** by partition, (*R*)-THFC **1** is treated with *N*,*N*-dicyclohexylamine (DCHA) in methyl ethyl ketone/methanol (5:1) to precipitate the crystalline salt **4** that contains (*R*)-THFC **1** of >99% ee in 22% overall yield from (±)-**3d**.

1. Introduction

(*R*)-Tetrahydrofuran-2-carboxylic acid (THFC) **1** found its niche in the pharmaceutical industry in the mid-1980s when it was first incorporated into a penem skeleton[1] to give rise to furopenem **2**,[2] a clinically efficacious non-natural β-lactam antibiotic (Fig. 1). Such secure application notwithstanding, however, no preparative method to supply (*R*)-THFC **1** has since been reported other than resolution via diastereomeric salt formation.[3] Thus, as part of our process R & D program of chemoenzymatic synthesis,[4,5] kinetic resolution via enzyme-catalyzed enantioselective hydrolysis was explored to access (*R*)-THFC **1** on a practical scale.[6]



(*R*)-THFC **1**



Furopenem **2**

Figure 1. Structures of (*R*)-THFC **1** and furopenem **2**.

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2. Results and discussion

Enzyme-catalyzed enantioselective hydrolysis of the (*R*)-enantiomer of esters of (±)-THFC **1**, (±)-**3**, should have the following tactical benefits (Scheme 1): (1) facile separation of the digested (*R*)-acid **1** from the left-over neutral (*S*)-ester **3** by partition; (2) reuse of the (*S*)-ester **3** by base-induced racemization, which should be much simpler than that of its parent carboxylic acid (*S*)-THFC **1**;[7] (3) the moderate size and relatively high oxygen-content of (±)-**3** both favorable for the hydrolase catalysis taking place in an aqueous medium.

2.1. Enzyme screen

Keeping in mind such tactical benefits, we tested not less than a hundred commercially available hydrolase preparations for enantioselective hydrolysis of the methyl ester of (±)-THFC **1**, (±)-**3a**,[3a] which had been prepared by the conventional acid-catalyzed esterification method: (±)-THFC **1**, MeOH, H_2SO_4, PhMe; 73% yield. The enzyme screen {[(±)-**3a**] = 1 M, [enzyme] = 2% (w/v), potassium phosphate buffer (0.25 M, pH 7), room temperature, 16 h} nominated four hydrolases for further investigation: porcine pancreatic lipase (PPL), E[8] = 10; *Aspergillus melleus* protease, $E=22$; *A. oryzae* protease, $E=11$; and *Bacillus subtilis* protease, $E=9.2$ (Table 1).

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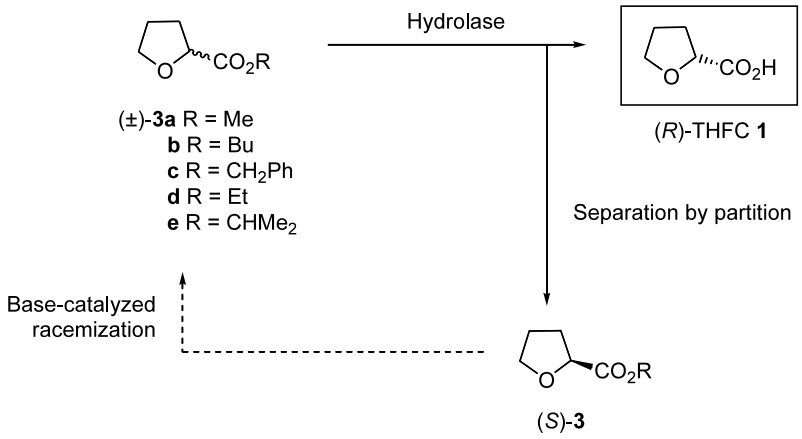
doi:10.1016/S0957-4166(03)00249-0



Scheme 1. Enzyme-catalyzed enantioselective hydrolysis of esters of (±)-THFC **1**, (±)-**3**, to (*R*)-**1**.

Table 1. Enzyme-catalyzed enantioselective hydrolysis of esters of (±)-THFC **1**, **3a**,**b**,**c** to (*R*)-**1**[a]

Substrate	Reaction profile[b]	Lipase PPL[c]	Protease of the microbial origin		
			A. melleus[d]	*A. oryzae*[e]	*B. subtilis*[f]
(±)-**3a**[g]	Ee (%) (*E*[h])	78.6 (10)	87.7 (22)	78.0 (11)	76.1 (9.2)
R = Me	Conversion (%)	21.1	29.8	25.5	23.4
(±)-**3b**[i]	Ee (%) (*E*[h])	60.9 (15)	85.8 (48)	85.8 (27)	80.1 (17)
R = Bu	Conversion (%)	61.0	52.6	44.9	42.9
(±)-**3c**[i]	Ee (%) (*E*[h])	71.9 (13)	79.2 (17)	78.7 (14)	82.9 (17)
R = CH_2Ph	Conversion (%)	49.6	45.5	39.0	35.4

[a] [Substrate] = 1 M, [enzyme] = 2% (w/v), 0.25 M potassium phosphate buffer (pH 7), room temperature.
[b] For the method to determine ee (%) and conversion (%) of the reaction, see Section 4.3.
[c] Porcine pancrease lipase available from Sigma.
[d] Available as Protease XP-488 from Nagase ChemteX Corporation; see Ref. 9.
[e] Available as Sumizyme from Shin Nihon Chemical Co., Ltd.
[f] Available as Bioprase from Nagase ChemteX Corporation; see Ref. 9.
[g] Reaction time: 16 h.
[h] For the definition of *E*, see Ref. 8.
[i] Reaction time: 72 h.

To identify the hydrolase of choice and gain quick insight into the approximate structure–selectivity relationship, the butyl and benzyl esters of (±)-THFC **1**, (±)-**3b** and (±)-**3c**, were prepared and subjected to hydrolysis catalyzed by each of the four enzymes (Table 1): [substrate] = 1.0 M, [enzyme] = 2% (w/v), potassium phosphate buffer (0.25 M, pH 7), room temperature, 72 h. Indeed, the *B. subtilis* protease showed an *E* value of 17, which is comparable to that of the *A. melleus* protease for the hydrolysis of (±)-**3c** (R = CH_2Ph). However, it was the *A. melleus* protease that showed a balanced combination of the enantioselectivity {ee (%) for (*R*)-**1** and *E*[8]} and kinetics {conversion (%)} of the reaction with all the three substrates, (±)-**3a**, (±)-**3b**, and (±)-**3c**: *E* = 22 at 29.8% conversion after 16 h for (±)-**3a**; *E* = 48 at 52.6% conversion after 72 h for (±)-**3b**; *E* = 17.0 at 45.5% conversion after 72 h for (±)-**3c**. Hence, the *A. melleus* protease[9] was nominated for further investigation into developing practical chemoenzymatic processes.

As regards the favorable structural traits with the alcoholic residues of (±)-**3**, the butyl ester of (±)-THFC, (±)-**3b**, was better accommodated by all the four hydrolases than the two other ester homologues, (±)-**3a** (R = Me) and (±)-**3c** (R = CH_2Ph), as can be deduced from Table 1. In fact, the alcoholic residue pertinent to the enzymatic hydrolysis should be larger than Me and smaller than Bu, in view of the fact that the conversion rate tended to be lower with increase in the size of the alcoholic residue: 29.8% conversion after 16 h versus 52.6% conversion after 72 h for the *A. melleus* protease-catalyzed hydrolysis of (±)-**3a** (R = Me) and (±)-**3b** (R = Bu), respectively.

2.2. Nomination of the ethyl ester of (±)-THFC

To corroborate the above-mentioned hypothesis, the ethyl, and isopropyl esters of (±)-THFC **1**, (±)-**3d**, and (±)-**3e** were prepared. They were then subjected to the *A. melleus* protease-catalyzed hydrolysis along with (±)-**3a**, (±)-**3b** where the substrate concentration was set at 2 M and the reaction period was limited to 2 h to identify the most suitable substrate for the enzymatic kinetic resolution in terms of space–time–yield or volume efficiency.[10] Each substrate (2 M) was treated with

the *A. melleus* protease {0.2% (w/v)} in 0.25 M potassium phosphate buffer (pH 7) at room temperature for 2 h, and the reaction was analyzed for the enantioselectivity and conversion rate as summarized in Table 2. Under such deliberately designed conditions, the butyl ester of (±)-THFC **1**, (±)-**3b**, provided the highest enantioselectivity: $E=62$, while, it underwent the hydrolysis only to a marginal degree: 0.3%. In contrast, however, the *A. melleus* protease-catalyzed hydrolysis proceeded in a more viable manner with the ethyl ester of (±)-THFC **1**, (±)-**3d**, to give (*R*)-THFC **1** of 92.9% ee with $E=29$ at 7.1% conversion. In the meantime, it turned out that the *A. melleus* protease was not able to accommodate the isopropyl ester of (±)-THFC **1**, (±)-**3e**, at all since no hydrolysis was observed under the above-specified conditions. In the event, the ethyl ester of (±)-THFC **1**, (±)-**3d**, was identified as the substrate of choice, and it was nominated for investigation into the optimum conditions.

Table 2. *A. melleus* protease-catalyzed enantioselective hydrolysis of esters of (±)-THFC **1**, (±)-**3a**,**b**,**c**,**d**,**e** to (*R*)-**1**[a]

Substrate	Ee (%)[b] for (*R*)-**1**	Conversion (%)[b]	*E*[c]
(±)-**3a** (R = Me)	85.5	28.1	18
(±)-**3b** (R = Bu)	96.8	0.3	62
(±)-**3c** (R = CH_2Ph)	90.3	0.2	18
(±)-**3d** (R = Et)	92.9	7.1	29
(±)-**3e** (R = *i*-Pr)	–[d]	–[d]	–[d]

[a] [Substrate] = 2 M, [*A. melleus* protease] = 0.2% (w/v), 0.25 M potassium phosphate buffer (pH 7), room temperature, 2 h.
[b] For the method to determine ee (%) and conversion (%) of the reaction, see Section 4.3.
[c] For the definition of *E*, see Ref. 8.
[d] No significant reaction took place under the conditions specified above.[a]

2.3. Optimization of the reaction parameters

Having chosen (±)-**3d** as the most proper substrate, we next directed our effort towards optimizing reaction parameters, such as buffer concentrations, pH, temperatures, and reaction period, to complement the enzymatic process.

2.3.1. Effect of concentrations of potassium phosphate buffer. To assess the effect of the potassium phosphate buffer concentration on the enantioselectivity, (±)-**3d** (2 M) was subjected to the hydrolysis catalyzed by the *A. melleus* protease {0.2% (w/v)} in the potassium phosphate buffer (pH 7) at higher concentrations than 0.25 M at room temperature for 2 h. As can be seen from Table 3, with increase in the concentration of potassium phosphate buffer from 0.25 to 1.0 M, the enantioselectivity increased from $E=29$ to $E=40$ with the conversion rate being doubled from 7.1 to 13.9%.

2.3.2. Effect of temperatures and pH. In expectation that the enzymatic hydrolysis would proceed with higher enantioselectivity at lower temperature, we attempted to decrease the reaction temperature from room temperature to 10°C. In parallel, we also varied pH from 7 to 9 to see how the enantioselectivity would be affected by such fluctuation in pH. As indicated in Table 4, when (±)-**3d** (2 M) was treated with the *A. melleus* protease {0.2% (w/v)} at pH 8 in a 1.0 M potassium phosphate buffer at 10°C for 4 h, the highest enantioselectivity and conversion was achieved: 94.5% ee for (*R*)-THFC **1** with $E=43$ at 17% conversion.

Table 4. Effect of pH on the *A. melleus* protease-catalyzed enantioselective hydrolysis of (±)-**3d** to (*R*)-**1**[a]

pH	Ee (%)[b] for (*R*)-**1**	Conversion (%)[b]	*E*[c]
7	94.4	14.2	40.7
8	94.5	17.0	42.8
9	94.4	17.7	42.4

[a] [(±)-**3d**] = 2 M, [*A. melleus* protease] = 0.2% (w/v), 1.0 M potassium phosphate buffer, 10°C, 4 h.
[b] For the method to determine ee (%), see Section 4.3.
[c] For the definition of *E*, see Ref. 8.

2.3.3. Optimal conditions. The enzymatic hydrolysis in question being a kinetic resolution, an effective yield of (*R*)-THFC **1** would remain at a less practical level unless the hydrolysis could be driven near the halfway point. Thus, we prolonged the reaction period to 20 h such that the conversion would exceed not less than 30% at pH 8 and 10°C. In addition, we dared to increase the concentration of the potassium phosphate buffer (pH 8) beyond 1.0 M up to 2.0 M to identify the optimal conditions for the scalable kinetic resolution. As can be seen from Table 5, the best results were obtained when (±)-**3d** (2.0 M) was exposed to the *A. melleus* protease {0.2% (w/v)} in a 1.5 M potassium

Table 3. Effect of the potassium phosphate buffer concentration on the *A. melleus* protease-catalyzed enantioselective hydrolysis of (±)-**3d**[a]

[Potassium phosphate buffer] (M)	Ee (%)[b] for (*R*)-**1**	Conversion[b] (%)	*E*[c]
0.25	92.9	7.1	29
0.5	94.0	12.1	37
1.0	94.3	13.9	40

[a] [(±)-**3d**] = 2 M, [*A. melleus* protease] = 0.2% (w/v), potassium phosphate buffer (pH 7), room temperature, 2 h.
[b] For the method to determine ee (%) and conversion (%), see Section 4.3.
[c] For the definition of *E*, see Ref. 8.

Table 5. Optimization of the *A. melleus* protease-catalyzed enantioselective hydrolysis of (±)-**3d** to (*R*)-**1**[a]

[Potassium phosphate buffer] (M)	Ee (%)[b] for (*R*)-**1**	Conversion (%)[b]	*E*[c]
1	94.1	32.7	52
1.5	94.4	36.5	60
2	91.7	31.1	35

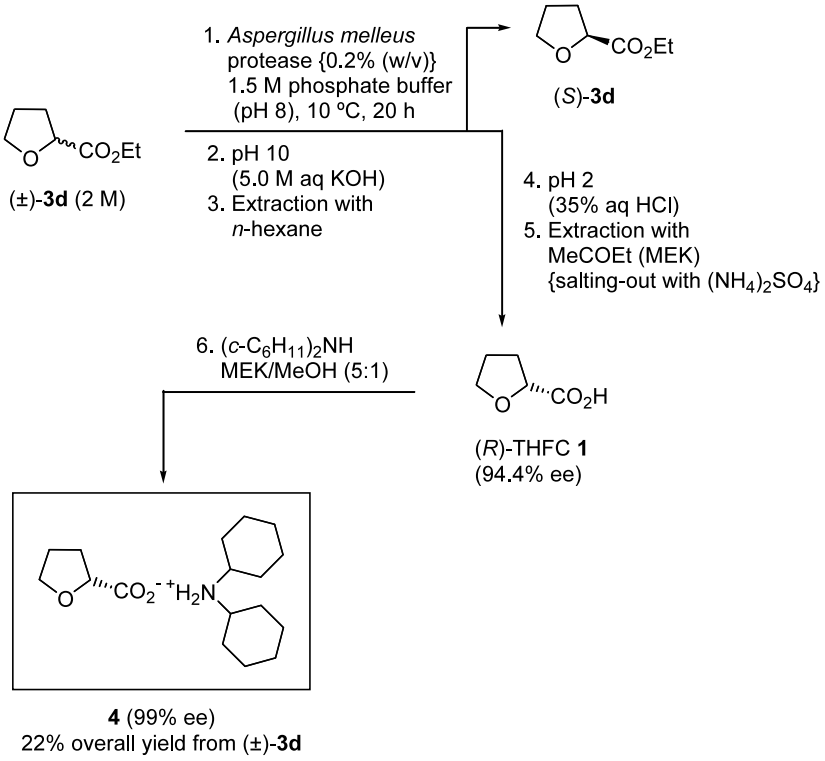
[a] [(±)-**3d**] = 2 M, [*A. melleus* protease] = 0.2% (w/v), potassium phosphate buffer, (pH 8), 10°C, 20 h.
[b] For the method to determine ee (%) and conversion (%) of the reaction, see Section 4.3.
[c] For the definition of *E*, see Ref. 8.

phosphate buffer (pH 8) at 10°C for 20 h: 94.4% ee for (*R*)-THFC **1** with $E = 60$ at 36.5% conversion.

Under the ultimate conditions, the ethyl ester of (±)-THFC **1** {(±)-**3d**, 2 M, 288 g/L} was treated with the *A. melleus* protease {0.2% (w/v), 2 g/L} in 1.5 M potassium phosphate buffer (pH 8.0) at 10°C for 20 h (Scheme 2). The spent mixture was basified to pH 10 with 5.0 M aqueous KOH, and extracted with *n*-hexane to recover the left-over (*S*)-ester **3d**. The aqueous layer was salted out with ammonium sulfate and then acidified to pH 2 with 35% aqueous HCl. Extraction with methyl ethyl ketone (MEK) recovered the digested acid (*R*)-**1**, which was otherwise difficult to extract into the organic phase in an acceptable yield due to its high water-solubility. The MEK solution was then analyzed by chiral HPLC [Chiralpak WH (Daicel), 2 mM aqueous $CuSO_4$] for the content of (*R*)-THFC **1** and its enantiomeric purity: 36.5% yield and 94.4% ee with $E = 60$.

2.4. Down-stream processing via crystalline salt formation

Its enantiomeric purity being still less than satisfactory, (*R*)-THFC **1** thus obtained was combined with an achiral organic base to form a crystalline salt in the expectation that its fractional crystallization would increase the enantiomeric purity of (*R*)-**1** to >99% ee. After intensive experimentation, such down-stream processing could be established in a telescoped manner as follows (Scheme 2): The MEK solution of (*R*)-**1** (94.4% ee) was concentrated in vacuo until the volume diminished to 16% of its original volume. To the residue was added MeOH to adjust the MEK/MeOH ratio to 5:1. *N*,*N*-Dicyclohexylamine (DCHA, 1.0 equiv.)[11] was then added to the solution to precipitate crystals of the DCHA salt of (*R*)-THFC **1**, **4**, in 22% overall yield from (±)-**3d** with (*R*)-**1** of 99.1% ee contained in it as confirmed by the chiral HPLC analysis mentioned above; on liberation from **4**,[3,7] (*R*)-**1** showed the spectral and polarimetric data that corroborated its chemical and stereochemical integrity.



Scheme 2. Scalable preparation of (*R*)-THFC **1** by the *A. melleus* protease-catalyzed enantioselective hydrolysis in combination with crystalline salt-formation.

3. Conclusion

(*R*)-THFC **1** of >99% ee could be obtained successfully via kinetic resolution featuring the commercially available *A. melleus* protease-catalyzed enantioselective hydrolysis of the ethyl ester of (±)-THFC **1**, (±)-**3d**, for the first time. The points deserving comment from a practical viewpoint can be itemized as follows: (1) enzymatic hydrolysis at as high a concentration of (±)-**3d** as 2 M (288 g/L); (2) high enantioselectivity {94.4% ee for (*R*)-**1**, $E=60$ at 36.5% conversion} achieved under the controlled conditions of as high a concentration of the potassium phosphate buffer (pH 8) as 1.5 M and a relatively low reaction temperature of 10°C; (3) through processes to precipitate crystalline DCHA salt of (*R*)-THFC **1**, **4**, in 22% overall yield from (±)-**3d** with increase in the enantiomeric purity of (*R*)-**1** beyond 99% ee.

4. Experimental

4.1. General

Melting points were measured on an Electrothermal 1A8104 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 400 MHz on a Varian UNITY-400 spectrometer in a $CDCl_3$ solution with tetramethylsilane as an internal standard. FT-IR spectra were recorded on a Nicolet Avatar 360 FT-IR spectrometer. Mass spectra were recorded on a Hewlett Packard 5890 series II (GC)-HP5971A (MS). Elemental analyses were performed on an Elementar vario EL analyzer. Optical rotations were measured on a Horiba SEPA-200 polarimeter.

4.2. Preparation of the esters of (±)-THFC 1, (±)-3a, (±)-3b, (±)-3c, (±)-3d, and (±)-3e

4.2.1. Methyl (±)-tetrahydrofuran-2-carboxylate 3a. To a stirred solution of (±)-THFC **1** (34.8 g, 300 mmol) and MeOH (48.1 g) in PhMe (100 mL) was added concentrated H_2SO_4 (15.0 g) at room temperature. The solution was stirred with heating at 75–77°C for 6 h, and then allowed to cool to room temperature. Saturated aqueous solution of $(NH_4)_2SO_4$ (40 mL) was added, and the layers were separated. The aqueous layer was extracted with PhMe (10 mL×3). The combined PhMe layer and extracts were washed with saturated aqueous solution of $NaHCO_3$ (10 mL×1) and saturated aqueous solution of $(NH_4)_2SO_4$ (10 mL×1). The PhMe solution was dried (Na_2SO_4), and concentrated in vacuo. The oily residue was distilled in vacuo to give (±)-**3a** (28.6 g, 73.1%): bp 61–62°C/4.5 mmHg {lit.:[3b] bp 80°C/1.9 kPa for (*R*)-**3a**}; ^{1}H NMR δ 1.90–2.06 (m, 3H), 2.27 (m, 1H), 3.74 (s, 3H), 3.92 (m, 1H), 4.02 (m, 1H), 4.48 (dd, $J=5.2$, 8.4 Hz, 1H); MS (70 eV) m/z 130 (M^+).

4.2.2. Butyl (±)-tetrahydrofuran-2-carboxylate 3b. To a stirred solution of (±)-**1** (23.2 g, 200 mmol) in BuOH (104 g, 1.40 mol) was added concentrated H_2SO_4 (1.00 g, 10.2 mmol) at room temperature. The solution was stirred and heated at 105–120°C for 2 h during which H_2O (2 mL) was distilled out. The reaction mixture was allowed to cool to room temperature, and solid Na_2CO_3 (2 g) and H_2O (5 mL) were added. The mixture was cooled to 10–15°C, washed with saturated aqueous solution of NaCl (10 mL×2), dried (Na_2SO_4), and concentrated in vacuo to give an opaque oily residue (35.6 g), which was dissolved in AcOEt (20 mL). The solution was washed with water (10 mL), dried (Na_2SO_4), and concentrated in vacuo to give a transparent oily residue (33.2 g). This was distilled in vacuo to give (±)-**3b** (29.7 g, 86.3%): bp 97–100°C/5 mmHg; ^{1}H NMR δ 0.93 (t, $J=5.6$ Hz, 3H), 1.37 (m, 1H), 1.64 (m, 1H), 1.90–2.02 (m, 3H), 3.92 (m, 1H), 4.02 (m, 1H), 4.14 (t, $J=5.6$ Hz, 2H), 4.45 (dd, $J=4.8$, 8.0 Hz, 1H); MS (70 eV) m/z 172 (M^+).

4.2.3. Benzyl (±)-tetrahydrofuran-2-carboxylate 3c. To a stirred solution of (±)-**1** (6.10 g, 52.5 mmol) in PhMe (20 mL) was added Et_3N (5.31 g, 52.5 mmol) followed by benzyl chloride (6.33 g, 50.0 mmol). The homogeneous mixture was stirred and heated at 75–79°C for 2.75 h. The reaction mixture was allowed to cool to room temperature. The mixture was washed with water (20 mL×3), dried (Na_2SO_4), and concentrated in vacuo to give an oily residue (8.32 g). This was distilled in vacuo to give (±)-**3c** (5.78 g, 56.1%): bp 135–136°C/0.3 mmHg; ^{1}H NMR δ 1.90–2.04 (m, 3H), 2.24 (m, 1H), 3.93 (m, 1H), 4.03 (m, 1H), 4.52 (dd, $J=5.2$, 8.0 Hz, 1H), 5.18 (s, 1H), 7.35 (s, 5H); MS (70 eV) m/z 206 (M^+).

4.2.4. Ethyl (±)-tetrahydrofuran-2-carboxylate 3d. To a stirred solution of (±)-**1** (46.5 g, 400 mmol) and EtOH (92.1 g, 1.97 mol) in PhMe (100 mL) was added concentrated H_2SO_4 (12.0 g, 122 mmol) at room temperature. The mixture was stirred and heated at a gentle reflux for 4.5 h. It was allowed to cool to room temperature, and concentrated H_2SO_4 (8.0 g, 82 mmol) was added. The mixture was heated with stirring, and distilled under atmospheric pressure until the distillate amounted to 67 g. The residue was allowed to cool to room temperature, and diluted with PhMe (30 mL). The mixture was washed with saturated aqueous solution of $(NH_4)_2SO_4$ (20 mL×1) and H_2O (20 mL). The combined aqueous layer was extracted with PhMe (20 mL×2). The combined PhMe layer and extracts were washed with saturated aqueous solution of $(NH_4)_2SO_4$ (20 mL×1), and saturated aqueous solution of $NaHCO_3$ (10 mL×1), dried (Na_2SO_4), and concentrated in vacuo to give an oily residue (50.9 g). This was distilled in vacuo to give (±)-**3d** (44.9 g, 76.2%): bp 68–70°C/3.0 mmHg; ^{1}H NMR δ 1.23 (t, $J=5.6$ Hz, 3H), 1.91–2.05 (m, 3H), 2.25 (m, 1H), 3.91 (m, 1H), 4.03 (m, 1H), 4.20 (q, $J=5.6$, 2H), 4.45 (dd, $J=5.2$, 8.4 Hz, 1H); MS (70 eV) m/z 145 (M^++1).

4.2.5. Isopropyl (±)-tetrahydrofuran-2-carboxylate 3e. To a stirred solution of (±)-**1** (11.6 g, 100 mmol) in *i*-PrOH (30.1 g, 500 mmol) was added concentrated H_2SO_4 (1.00 g, 10.2 mmol). The solution was stirred and heated at reflux for 5.5 h, and allowed to cool to room temperature. Concentrated H_2SO_4 (2.00 g, 20.4

mmol) was added, and the mixture was heated with stirring at reflux for 2 h and 10 min. The mixture was allowed to cool to room temperature, and subjected to the same extractive workup as described in Section 4.2.2 to give crude (±)-**3e** (11.1 g, 70.4%): ^{1}H NMR δ 1.24 (d, $J=6.0$ Hz, 3H), 1.26 (d, $J=6.0$ Hz, 3H), 1.90–2.05 (m, 3H), 2.23 (m, 1H), 3.92 (m, 1H), 4.02 (m, 1H), 4.41 (dd, $J=5.2$, 8.4 Hz, 1H), 5.05 (m, 1H); MS (70 eV) m/z 158 (M^+). This was employed in the enzymatic reaction without further purification.

4.3. Screening of hydrolases and reaction parameters

A 2 mL eppendorf® microtube was charged with an appropriate amount of (±)-**3**, a hydrolase, and potassium phosphate buffer to set up a reaction specific to the experimental purpose. The tube was agitated for a specific period. The progress of the reaction was monitored and the enantioselectivity assessed by the following procedures: From the reaction mixture was taken a portion (0.2 mL), to which were added a 2.5 M aqueous solution of H_2SO_4 (0.1 mL), $(NH_4)_2SO_4$ (0.1 g), and *i*-PrOH (1 mL). The mixture was agitated, and centrifuged to separate layers. From the upper layer was taken a portion (10 μL), which was subjected to twofold chiral HPLC analyses, one designed to quantify the enantiomeric composition of **3** (Section 4.3.1) and the other designed to quantify that of **1** (Section 4.3.2).

4.3.1. Determination of the enantiomeric composition of 3. Column: Chiralcel OD (Daicel), 0.46 cmϕ×25 cm; elution: *n*-hexane/*i*-PrOH (99.5:0.5), 1.5 mL/min; detection: UV at 220 nm; (*R*)-**3a**, $t_R=17.4$ min; (*S*)-**3a**, $t_R=9.5$ min; (*R*)-**3b**, $t_R=13.4$ min; (*S*)-**3b**, $t_R=8.0$ min; (*R*)-**3c**, $t_R=35.7$ min; (*S*)-**3c**, $t_R=24.7$ min; (*R*)-**3d**, $t_R=14.2$ min; (*S*)-**3d**, $t_R=7.7$ min; (*R*)-**3e**, $t_R=13.2$ min; (*S*)-**3e**, $t_R=7.8$ min.

4.3.2. Determination of the enantiomeric composition of 1. Column: Chiralpak WH (Daicel), 0.46 cmϕ×25 cm; elution: 2 mM aqueous solution of $CuSO_4$, 1 mL/min; detection: UV at 254 nm; (*R*)-**1**, $t_R=15.0$ min; (*S*)-**1**, $t_R=13.4$ min.

4.4. (*R*)-Tetrahydrofuran-2-carboxylic acid (THFC) 1

To a 1.5 M potassium phosphate buffer solution (pH 8, 68.5 mL) was added (±)-**3d** (19.7 g, 137 mmol) followed by an *A. melleus* protease (137 mg). The mixture was stirred at 10°C for 20 h. The pH of the mixture was adjusted to 10.0 with 5 M aqueous solution of KOH. The mixture was extracted with *n*-hexane (30 mL×3). To the aqueous layer were added $(NH_4)_2SO_4$ (10.3 g) and 35% aqueous solution of HCl to adjust its pH to 2. The aqueous mixture was extracted with MEK (50 mL×5). The combined MEK extracts were analyzed for the content of (*R*)-**1** and its enantiomeric purity under the conditions described in Section 4.3.2: 36.5% yield and 94.4% ee.

4.5. *N*,*N*-Dicyclohexylammonium (*R*)-tetrahydrofuran-2-carboxylate 4

The MEK solution of (*R*)-**1** obtained in Section 4.4 was concentrated in vacuo until the volume was reduced to 35 mL (16% of the original volume). To the residue was added MeOH (7 mL) at room temperature. *N*,*N*-Dicyclohexylamine (DCHA, 9.06 g, 50 mmol) was added dropwise at room temperature. The stirred mixture was heated to reflux, and allowed to cool to 5°C. Precipitated solids were collected by filtration, and dried in vacuo at an oven temperature of 50°C to give **4** (8.95 g) in 22% overall yield from (±)-**3d**: mp 164.4–165.5°C; $[\alpha]_D^{20}$ +17.6 (*c* 1.0, MeOH); IR ν_{max} (KBr) 3427, 2931, 2808, 2521, 2420, 1581, 1460, 1416, 1311, 1068 cm^{-1}; ^{1}H NMR δ 1.14–1.27 (m, 6H), 1.43–1.52 (m, 3H), 1.64 (m, 2H), 1.74–2.04 (m, 14H), 2.23 (m, 1H), 3.84 (m, 1H), 3.96 (m, 1H), 4.26 (t, $J=6.4$ Hz, 1H), 7.0 (br s, 2H). Anal. found: C, 68.0; H, 10.4, N, 4.6. calcd for $C_{17}H_{31}NO_3\cdot 0.2\ CH_4O$ (methanol): C, 67.99; H, 10.55, N, 4.61. To a portion (50 mg) of the salt were added H_2O (0.5 mL) and $(NH_4)_2SO_4$ (0.1 g). The pH of the mixture was adjusted to 2 with 35% aqueous solution of HCl. The mixture was extracted with MEK (1 mL×1), and 10 μL of the MEK solution was injected to the chromatograph running under the conditions described in Section 4.3.2 to determine the enantiomeric purity of (*R*)-**1** contained in **4** to be 99.1% ee. From a portion of **4** was liberated free (*R*)-**1** according to the usual procedures[3,7] to confirm its chemical and stereochemical integrity: $[\alpha]_D^{20}$ +30.0 (*c* 0.34, $CHCl_3$) {lit:[3a] $[\alpha]_D$ +30.4 ($CHCl_3$)}; ν_{max} (KBr) 2984, 1743, 1448, 1402, 1351, 1203, 1180, 1080, 1038, 928, 804 cm^{-1}; ^{1}H NMR δ 1.97 (m, 2H), 2.10 (m, 1H), 2.32 (m, 1H), 3.96 (m, 1H), 4.05 (m, 1H), 4.52 (dd, $J=5.2$, 8.8 Hz, 1H), 10.24 (br. s, 1H).

Acknowledgements

M.I. thanks Mr. Masafumi Moriwaki, Director of Research and Development Center, Nagase & Co., Ltd., for his consistent encouragement throughout this project.

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