Organic Process Research & Development

Article

pubs.acs.org/OPRD

Process for Producing Optically Active (*R*)-Tetrahydrothiophene-3-ol with High Optical Purity and High Purity: Bioconversion and Crystallization

Kaname Konuki,* Hazuki Nagai, Masashi Ito, and Tomohiro Sameshima

MicroBiopharm Japan Co., Ltd., 1808, Nakaizumi, Iwata, Shizuoka 438-0078, Japan

ABSTRACT: (*R*)-Tetrahydrothiophene-3-ol (**1**) is a key intermediate in the synthesis of penem-based antibiotics. However, it is a viscous liquid at room temperature, which makes it impossible to purify the (*R*)-isomer especially in the presence of the (*S*)-isomer. In this study, we successfully developed a process for producing (*R*)-alcohol **1** with high optical purity by combining bioconversion and crystallization. (*R*)-Alcohol **1** was prepared by enantioselective bioreduction which used tetrahydrothiophene-3-one (**2**) as the substrate, and the optical purity was 70−92% ee. The (*R*)-alcohol **1** liquid was collected from incubation solution and purified to furnish (*R*)-alcohol **1** with 98.7% ee by cooling crystallization from organic solvents. The scale-up using common crystallization process was difficult, but we developed a crystallization process that employed a jacketed pressure filtration vessel equipped with an agitator which can be operated under low temperature from crystallization to filtration. This led to the establishment of a process for producing (*R*)-alcohol **1** with high optical purity, and the validity of this process was proved by the scale-up test.

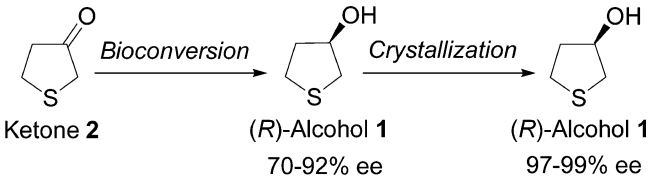
INTRODUCTION

(*R*)-Tetrahydrothiophene-3-ol (**1**) is employed as a key intermediate in the synthesis of penem-based antibiotics[1,2] and is a viscous liquid at room temperature. Some reported methods for generation of (*R*)-alcohol **1** are by synthesizing from L-aspartic acid,[2] by hydroboration of 2,3-dihydrothiophene,[3] by asymmetric hydrogenation using iridium catalysts[4] or transition metal complex catalysts,[5] by enzymatic resolutions[6] of esterified racemate **1**, and by biocatalytic reduction of tetrahydrothiophene-3-one (**2**)[7] which is also our method.[8] On the other hand, as general methods to improve the optical purity of compounds, crystallization separation by formation of diastereomeric salt, preferential crystallization by addition of seed crystals with high optical purity, and the chromatographic separation by optical isomer column are known. Crystallization separation is generally considered simple and less expensive, compared with chromatographic separation. However, (*R*)-alcohol **1** is liquid at room temperature, and there are no characteristic functional groups which can form diastereomeric salts.

Thus, although crystallization is generally an effective method of separation, it was considered that crystallization which improves the optical purity of (*R*)-alcohol **1** was impossible via the previous methods. Prior to this research, no crystallization technique which improved the optical purity of the (*R*)-alcohol **1** was reported.

Herein, we report bioconversion which carries out enantioselective reduction from ketone **2** to (*R*)-alcohol **1** and development of a crystallization process which leads (*R*)-alcohol **1** to high optical purity (Scheme 1). This crystallization has the novel and interesting feature that optical purity improves only by crystallizing without using seed crystals.

Scheme 1. Process for producing optically active (*R*)-tetrahydrothiophene-3-ol with high optical purity



RESULTS AND DISCUSSION

Bioconversion from Ketone 2 to (*R*)-Alcohol 1. In order to obtain the optically active (*R*)-alcohol **1**, we searched the microorganisms which could stereoselectively reduce the oxo group of ketone **2**. The microorganisms which mainly belong to *Penicillium*, *Aspergillus*, or *Streptomyces* showed high selectivity. In the shake-flask incubation examinations which use those microorganisms, the (*R*)-alcohol **1** with 70−92% ee was produced. For whole-cell reactions, several reductases which have low selectivity existed. The difference in the expression level of these enzymes led to (*R*)-alcohol **1** with 70−92% ee. The microorganisms which had a conversion ability of more than 90% ee at lab scale were used in the optimization experiments using 3-L jar fermenters. As a result of this optimization, the scale-up test (described later) using the 200-L jar fermenter was successful in preparing the (*R*)-alcohol **1** with 92% ee.

Isolation and Purification of the (*R*)-Alcohol 1 from Incubation Solution. Filtration which separates the mycelia cake by addition of a filter aid was performed, and the (*R*)-alcohol **1** was extracted from filtrate with ethyl acetate

Received: September 22, 2013

   dx.doi.org/10.1021/op4002627 | *Org. Process Res. Dev.* XXXX, XXX, XXX−XXX

(EtOAc). Extract solution was concentrated, and distillations of ketone **2** at 81−86 °C/20 mmHg and (*R*)-alcohol **1** at 102−104 °C/20 mmHg were carried out to separate the unreacted ketone **2** and the high-purity (*R*)-alcohol **1**. In scale-up examination, the emulsion formed at the time of solvent extraction was a problem. We hypothesized that the residual protein in the filtrate was the cause of emulsion, and thus ultrafiltration (UF) membrane filtration (molecular cutoff 13,000) was introduced in order to control emulsion for scale-up of the process. As a result, emulsion generation was reduced, and solvent extraction was performed without trouble.

Improvement in the Optical Purity by Crystallization of the (*R*)-Alcohol 1. Primary examination of crystallization was performed by using the (*R*)-alcohol **1** with 70.2% ee as the initial solution (Table 1). In order to develop a method that led to improvement in both low- and high-level optical purity samples, crystallization examination was performed using the samples of low optical purity. When the (*R*)-alcohol **1** was cooled slowly, it began to solidify at about 4 °C. Although the optical purity of crystals and mother liquor was investigated at this time, optical purity had not improved (70.2 → 71.1% ee). The addition of hexane, which is a poor solvent, had no influence on enantioselectivity (72.0% ee). However, when EtOAc which is a dissolution solvent was added, the optical purity of crystals improved to 88.3% ee. Since improvement in optical purity was observed by addition of the optimum dissolution solvent, crystallization was repeated twice on condition of EtOAc/hexane using resultant crystals (88.3% ee), and the optical purity of (*R*)-alcohol **1** was improved to 94.8% ee.

Table 1. Primary examination of crystallization

	optical purity[a] (% ee)	
crystallization conditions	crystals	mother liquor
[(*R*)-alcohol **1** as initial solution]	70.2	
only cooling	71.1	70.6
cooling + hexane	72.0	62.0
cooling + hexane + EtOAc	88.3	56.5
EtOAc/hexane under cooling (first)	92.1	69.4
EtOAc/hexane under cooling (second)	94.8	88.6

[a](*R*)-Alcohol **1**.

The (*R*)-alcohol **1** with 72.1% ee was used as the initial solution, secondary examination of crystallization was performed by the mixed system of dissolution solvents and hexane, and optical purity and yield were evaluated (Table 2). Crystallization temperature was changed, depending on the solubility in each dissolution solvent. Although crystallization was performed at −3 °C as for butyl acetate (BuOAc, entry 2) and toluene (entry 5), in other solvent systems, crystallization was performed at −15 °C. From the experimental results, the increase in yield led to decrease in optical purity and there were no effective solvent systems which could improve the yield and optical purity remarkably. However, the crystals obtained from acetone (entry 3) and 2-propanol (2-PrOH, entry 4) were needle-like, and their filterability was also better than for the other systems.

Table 2. Secondary examination of crystallization

				optical purity[a] (% ee)	
entry	mixed solvent system	*T* (°C)	yield (%)	crystals	mother liquor
[(*R*)-alcohol **1** as initial solution]			100	72.1	
1	EtOAc/hexane (1:2)	−15	78.3	77.8	49.5
2	BuOAc/hexane (1:2)	−3	59.9	82.1	58.0
3	acetone/hexane (1:2)	−15	39.6	85.8	63.1
4	2-PrOH/hexane (1:2)	−15	48.1	83.0	62.2
5	toluene/hexane (1:2)	−3	59.0	77.0	66.4

[a](*R*)-Alcohol **1**.

In consideration of scale-up, crystallization from the acetone/hexane system was chosen, and the optimization of the system was carried out.

The crystallization of (*R*)-alcohol **1** solution (83.5g) with 72.8% ee as the initial solution from the optimized acetone/hexane system was repeated three times (Figure 1). As a result, we succeeded in obtaining the (*R*)-alcohol **1** with 98.7% ee. These results showed that it was possible to obtain the (*R*)-alcohol **1** which has higher optical purity by repeating this crystallization.

Although the overall yield of crystallization was 23%, the yield could be improved by recrystallization of recovered mother liquors.

In Figure 1, the precipitated crystals were filtered at room temperature. Since (*R*)-alcohol **1** was liquid at room temperature when scale-up of these operations is assumed, it was thought that the precipitated crystals would melt at the time of filtration. It was considered that crystals happen to melt with decreasing yield in the process of a slurry transfer. Therefore, we introduced into the crystallization process the pressure filtration vessel with a jacket and an agitator which can be operated under low temperature from crystallization to filtration. A stable scale-up method was accomplished by the introduction of this equipment.

Scale-up Test. The scale-up test using a 200-L jar fermenter was carried out. After the microorganism was proliferated in stages, ketone **2** was fed continuously, and bioconversion to the (*R*)-alcohol **1** was performed. The result of the incubation solution is shown in Table 3. It is possible to increase the production concentration of (*R*)-alcohol **1** by controlling proliferation of the microorganism.

The process for producing (*R*)-alcohol **1** with high optical purity and high purity was performed, using this incubation solution. An outline of the established process is shown in Figure 2.

The process from incubation solution to a concentrate was examined using the total volume of incubation solution, and subsequent processes (distillation, crystallization, redistillation) were examined using some concentrates. By distillation, a great portion of unreacted ketone **2** was removed, and the (*R*)-alcohol **1** (optical purity is 91.8% ee) of 92.5% purity was obtained. The crystallization which used a pressure filtration vessel with a jacket and an agitator was performed using this distillate (Figure 3). After crystallization, the equipment was pressurized, to perform filtration at the same temperature as crystallization. As a result, (*R*)-alcohol **1** with 97.0% ee was obtained with 63% yield. Moreover, the ratio of ketone **2** decreased to 0.8% and purity increased to 95.6%. The pressure filtration vessel with a jacket and an agitator ensured that the process was operated under low temperature which prevented crystal-melting from crystallization to filtration step. The (*R*)-alcohol **1** was redistilled to furnish the (*R*)-alcohol **1** of high purity (100%). It showed be noted that although the (*R*)-

dx.doi.org/10.1021/op4002627 | Org. Process Res. Dev. XXXX, XXX, XXX−XXX

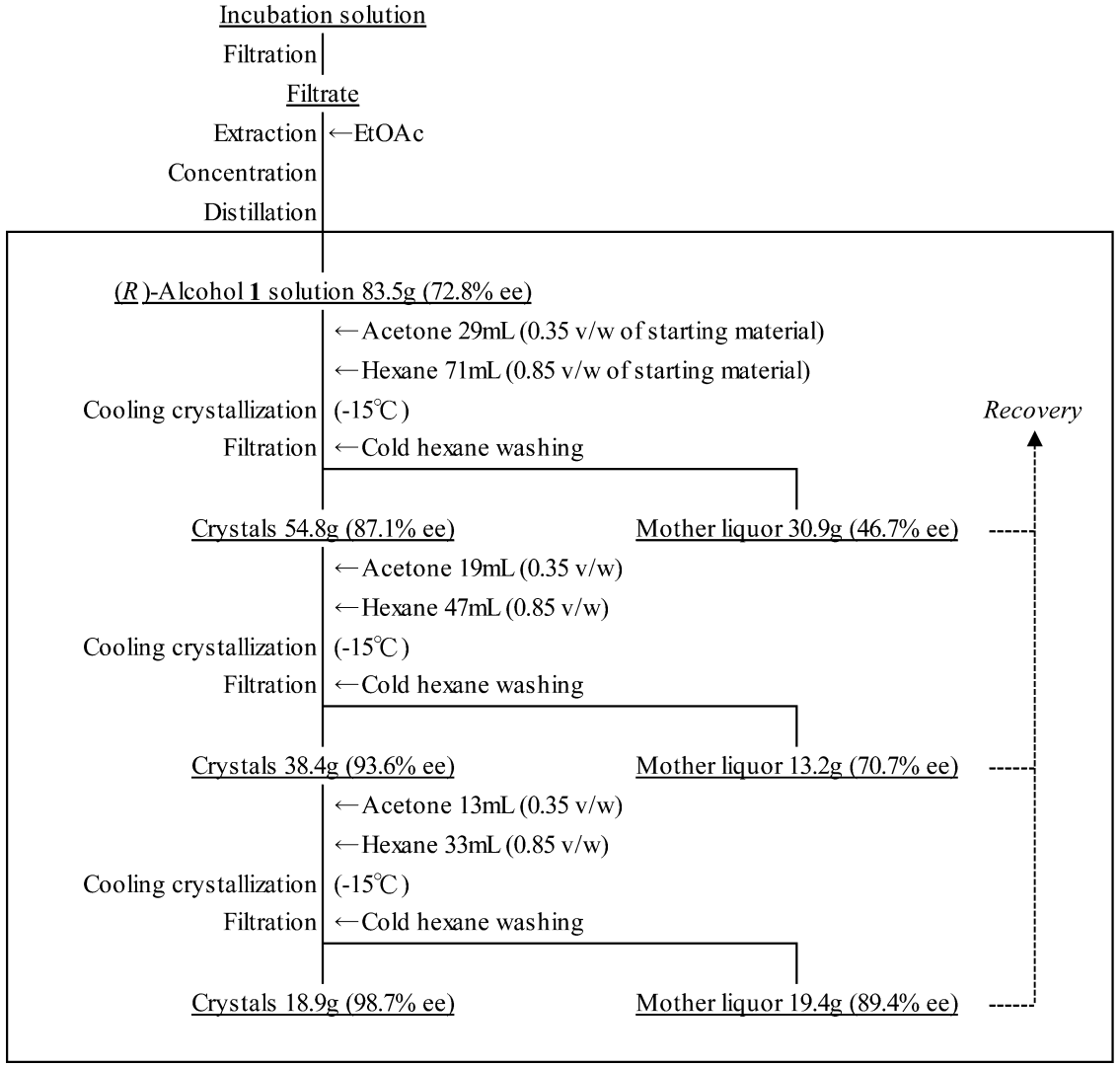


Figure 1. Crystallization flow for obtaining (*R*)-alcohol **1** with high optical purity.

Table 3. Scale-up test using a 200-L jar fermenter

volume of incubation solution	130 L
pH	6.0
concentration[a]	17.1 g/L
production[a]	2.2 kg
conversion[b]	87.8%
optical purity[a]	92.2% ee

[a](*R*)-Alcohol **1**. [b]Molar yield.

alcohol **1** with low optical purity needed carrying out the crystallization thrice to give the desired optical purity, the use of (*R*)-alcohol **1** with 92% ee required crystallization only once to achieve the same results.

In subsequent scale-up examination, RO (Reverse Osmosis) membrane concentration (99% of NaCl-rejection rate) was introduced after UF membrane filtration. By RO membrane separation, there was almost no loss of (*R*)-alcohol **1**. By RO membrane concentration, solution containing (*R*)-alcohol **1** was concentrated and the volume of solution was decreased. As a result, EtOAc used for extraction was reduced thus enabling cost reduction.

■ CONCLUSIONS

The process of producing (*R*)-alcohol **1** with the high optical purity which combined bioconversion and crystallization was developed. This entailed crystallization of (*R*)-alcohol **1** (72.8% ee) from acetone/hexane at low temperature and subsequent recrystallization to improve the optical purity of (*R*)-alcohol **1** to about 99% ee. Furthermore, we developed a scale-up method in consideration of feasible equipment that offered a practical and stable process of producing (*R*)-alcohol **1**.

■ EXPERIMENTAL SECTION

Analytical Method. Concentration and optical purity was determined by HPLC system with a UV detector. HPLC condition: Chiralpak AS-H column (4.6 mm ×250 mm; Daicel Corporation) kept at 30 °C, elution is hexane/isopropanol (96/4) at a flow rate of 1 mL/min and detection at 210 nm.

Bioconversion from Ketone 2 to (*R*)-Alcohol 1. Twenty-five milliliters of FI medium (potato starch 20 g/L, glucose 10 g/L, soy flour 20 g/L, KH_2PO_4 1 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L) was added to 250 mL conical flasks, and autoclave heating was carried out at 121 °C for 20 min. The microorganism was inoculated into these flasks, and shaking cultivation was carried out at 25 °C for 72 h. Ketone **2** (30−50 mg) as a substrate was

dx.doi.org/10.1021/op4002627 | Org. Process Res. Dev. XXXX, XXX, XXX−XXX

Incubation solution
| Filtration which separates the mycelia cake
Filtrate
| Filtration by UF membrane
| (Molecular cutoff 13,000)
UF filtrate
| Extraction by EtOAc
Extraction solution
| Concentration
Concentrate
| Distillation for the improvement in purity
| ((*R*)-Alcohol **1**: 102-104℃/20mmHg)
Distillate
| Crystallization to improve optical purity
| (Acetone/hexane system)
Crystals
| Redistillation
(*R*)-Alcohol **1** with high optical purity and high purity

Figure 2. Outline of the process for preparation of (*R*)-alcohol **1** with high optical purity and high purity.

added to this incubation solution, and the transformation reaction was performed by shaking cultivation at 25 °C for 24 h. Moreover, the incubation examination using 3-L jar fermenter was also carried out. FI medium (1.5 L) was added to 3-L jar fermenters, and autoclave heating was carried out at 121 °C for 20 min. The microorganism was inoculated into these 3-L jar fermenters, and aerated and agitated culture was carried out at 25 °C for 72 h. Ketone **2** (4 mL) as a substrate was added to this incubation solution, and the transformation reaction was performed by aerated and agitated culture at 20−23 °C for 24 h. The concentration of the generated (*R*)-alcohol **1** was determined by HPLC analysis.

Crystallization for Obtaining the (*R*)-Alcohol 1 with High Optical Purity. Acetone (29 mL) and hexane (71 mL) were added to (*R*)-alcohol **1** (83.5 g) with 72.8% ee, and this mixture was agitated and cooled to −15 °C. The precipitated crystals were filtered and washed with cold hexane, and 54.8 g of (*R*)-alcohol **1** with 87.1% ee was obtained as a result. Acetone (19 mL) and hexane (47 mL) were added to (*R*)-alcohol **1** (54.8 g), and this mixture was agitated and cooled to −15 °C. The precipitated crystals were filtered and washed with cold hexane, and 38.4 g of (*R*)-alcohol **1** with 93.6% ee was obtained as a result. Acetone (13 mL) and hexane (33 mL) were added to (*R*)-alcohol **1** (38.4 g), and this mixture was agitated and cooled again to −15 °C. The precipitated crystals were filtered and washed with cold hexane to afford 18.9 g of (*R*)-alcohol **1** with 98.7% ee.

Scale-up Test. Twenty-five milliliters of FI medium was added to a 250-mL conical flask, and autoclave heating was carried out at 121 °C for 20 min. The microorganism was inoculated into this flask as first seed and shaking cultivation was carried out at 25 °C for 72 h. FI medium (1.5 L) was added to a 3-L jar fermenter, and autoclave heating was carried out at 121 °C for 20 min. The flask which is the first seed was inoculated into this 3-L jar fermenter, and aerated and agitated culture was performed as the second seed at conditions of 25 °C, 500 rpm, and 0.5 vvm for 72 h. One hundred liters of medium (potato starch 20 g/L, glucose 22 g/L, soy flour 40 g/L, KH_2PO_4 2 g/L, $MgSO_4 \cdot 7H_2O$ 1 g/L) was added to a 200-L jar fermenter, and autoclaved at 121 °C for 20 min. The 3-L jar fermenter which is the second seed was inoculated into this 200-L jar fermenter. The aeration and agitation of the culture was performed as the main culture under conditions of 25 °C, 140 rpm, and 1 vvm for 72 h. Ketone **2** (3.6 kg) as a substrate was continuously added to this incubation solution, and the transformation reaction was performed in the aerated and agitated culture under conditions of 23 °C and pH control for 46 h. Moreover, in the transformation reaction, glucose and yeast extract were added if needed. Finally, the incubation solution shown in Table 3 was obtained.

The obtained incubation solution (130 L) was acidified and heated at 80 °C for 1 h. Filtration which separated the mycelia cake by addition of filter aid was performed, and the filtrate was further filtered with UF membrane filtration (molecular cutoff 13,000). (*R*)-Alcohol **1** was extracted from the filtrate (180 L) with EtOAc; concentration of EtOAc was performed, and 2.5 kg of concentrates (amount of (*R*)-alcohol **1** is 1.6 kg) was

	(*R*)-Alcohol **1**				Ketone **2**
	Purity	Amount	Yield	Optical purity	Ratio (2/1+2)
Distillate 621g	92.5%	575g	100%	91.8% ee	4.1%
←Hexane 489mL (0.85 v/w of (*R*)-alcohol **1**)					
Agitation ←Acetone 172mL (0.3 v/w of (*R*)-alcohol **1**)					
Cooling crystallization (-18℃)					
Filtration ←Cold hexane 300mL washing					
Crystals 380g	95.6%	363g	63%	97.0% ee	0.8%
Mother liquor 240g	77.7%	186g	32%	83.3% ee	8.0%

Figure 3. Crystallization flow which used a pressure filtration vessel with a jacket and an agitator.

dx.doi.org/10.1021/op4002627 | *Org. Process Res. Dev.* XXXX, XXX, XXX−XXX

obtained. Distillation (ketone **2**: 81−86 °C/20 mmHg, (*R*)-alcohol **1**: 102−104 °C/20 mmHg) was performed using some concentrates, and crystallization was performed using the 621 g of distillate (amount of (*R*)-alcohol **1** is 575 g).

Distillate ((*R*)-alcohol **1** in 91.8% ee), hexane (489 mL), and acetone (172 mL) were added to the pressure filtration vessel with a jacket and an agitator, and this mixture was agitated and cooled to −18 °C. The precipitated crystals were filtered and washed with cold hexane (300 mL, 1/2 volume of (*R*)-alcohol **1**) to furnish 363g of (*R*)-alcohol **1** with 97.0% ee. Redistillation was performed using some crystals (104 g), and 92 g of colorless and transparent high-purity (100%) (*R*)-alcohol **1** without detection of ketone **2** was obtained.

AUTHOR INFORMATION

Corresponding Author

* E-mail: konuki-k@microbiopharm.com

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr. George Wanyoike and Professor Izumi Hirasawa (Department of Applied Chemistry, Graduate School of Advanced Science and Engineering, Waseda University) for the valuable advice.

REFERENCES

(1) Volkmann, R. A. *Diastereomeric 5R,6S-6-(1R-Hydroxyethly)-2-(cis-1-oxo-3-thiolanylthio)-2-penem-3-carboxylic Acids*. WO/1988/08845, 1988.

(2) Volkmann, R. A.; Kelbaugh, P. R.; Nason, D. M.; Jasys, V. J. *J. Org. Chem.* **1992**, *57*, 4352.

(3) Brown, H. C.; Prasad, J. V. N. V. *J. Am. Chem. Soc.* **1986**, *108*, 2049.

(4) (a) Taketomi, T.; Akutagawa, S.; Kumobayashi, H.; Takaya, H. Mashima, K. *Iridium-Optically Active Phosphine Complex and Process for Producing Optically Active Alcohols Using the Same*. U.S. Patent 5,159,093, 1992. (b) Zhang, X.; Taketomi, T.; Yoshizumi, T.; Kumobayashi, H.; Akutagawa, S.; Mashima, K.; Takaya, H. *J. Am. Chem. Soc.* **1993**, *115*, 3318.

(5) Sayo, N.; Kumobayashi, H. *2,2′-Bis(diphenylphosphino)-5,5′,6,6′,7,7′,8,8′-octahydro-1,1′-binaphthyl and Transition Metal Complex Containing the Same as Ligand*. U.S. Patent 5,206,399, 1993.

(6) Honig, H.; Shi, N.; Polanz, G. *Biocatalysis* **1994**, *9*, 61.

(7) (a) Liang, J.; Jenne, S.; Mundorff, E.; Voladri, R.; Lalonde, J.; Huisman, G. *Improved Ketoreductase Polypeptides for the Stereoselective Production of (R)-3-Hydroxythiolane*. WO/2009/029554 A2, 2009. (b) Huisman, G. W.; Liang, J.; Krebber, A. *Curr. Opin. Chem. Biol.* **2010**, *14*, 122. (c) Liang, J.; Mundorff, E.; Voladri, R.; Jenne, S.; Gilson, L.; Conway, A.; Krebber, A.; Wong, J.; Huisman, G.; Truesdell, S.; Lalonde, J. *Org. Process Res. Dev.* **2010**, *14*, 188.

(8) (a) Nagai, H.; Konuki, K.; Ito, S.; Sameshima, T. *Process for Producing Optically Active Tetrahydrothiophene Derivative and Method of Crystallizing Optically Active Tetrahydrothiophen-3-ol*. WO/2005/054491 A1, 2005. (b) Nagai, H.; Konuki, K.; Ito, S.; Sameshima, T. EP1712633 A1, 2006. (c) Nagai, H.; Konuki, K.; Ito, S.; Sameshima, T. *Method for Manufacturing Optically Active Tetrahydrothiophene Derivative and Method for Crystallization of Optically Active Tetrahydrothiophene-3-ol*. US2008/0050787 A1, 2008.