Access to Wieland−Miescher Ketone in an Enantiomerically Pure Form by a Kinetic Resolution with Yeast-Mediated Reduction†

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Both enantiomers of Wieland−Miescher ketone [3,4,8,8a-tetrahydro-8a-methyl-1,6(2*H*,7*H*)-naphthalenedione], in a highly enantiomerically enriched form, became readily available by a newly developed kinetic resolution with yeast-mediated reduction. From a screening of yeast strains, *Torulaspora delbrueckii* IFO 10921 was selected. The collected cells of this strain, obtained by an incubation in a glucose medium, smoothly reduced only the isolated carbonyl group of the (*S*)-enantiomer, while the (*R*)-enantiomer remained intact. Starting from both enantiomers (∼70% ee) prepared by an established proline-mediated asymmetric Robinson annulation, the reduction with *T. delbrueckii* gave the (*R*)-enantiomer (98% ee) and the corresponding alcohol (4a*S*,5*S*)-4,4a,5,6,7,8-hexahydro-5-hydroxy-4a-methyl-2(3*H*)-naphthalenone (94% ee, 94% de) in preparative scale in nearly quantitative yields. An approach for the asymmetric synthesis of the Wieland−Miescher ketone was also successful. 2-Methyl-2-(3-oxobutyl)-1,3-cyclohexanedione, the prochiral precursor, was reduced with this strain to give a cyclic acetal form of (2*S*,3*S*)-3-hydroxy-2-methyl-2-(3-oxobutyl)-cyclohexanone, in a stereomerically pure form.

Introduction

Enantiomerically enriched forms of Wieland−Miescher ketone **1**[1] [3,4,8,8a-tetrahydro-8a-methyl-1,6(2*H*,7*H*)-naphthalenedione] have so far been utilized as the starting materials for the syntheses of naturally occurring products, including terpenoids and steroids.[2] For the efficient preparation of the enantiomerically enriched form of **1**, a method of separation of one enantiomer by utilizing the formation of an inclusion complex in *trans*-4,5-bis(hydroxydiphenylmethyl)-2,2-dimethyl-1,3-dioxacyclopentane, a derivative of tartaric acid, has been developed.[3] On the other hand, much effort has been devoted to the asymmetric cyclization of its prochiral precursor, 2-methyl-2-(3-oxobutyl)-1,3-cyclohexanedione **2**, and excellent chemical (the use of proline−enamine)[4] and biochemical (the use of catalytic antibody)[5] procedures have been established. The former method could be performed in a >100 g scale in the laboratory, and the development of this procedure was, indeed, the reason this starting material has been so widely applied to natural product syntheses. While this proline−enamine cyclization is a very efficient way, there still remains a drawback to the method; that is, the enantiomeric excess (ee) of the product is only ∼70%. To obtain an enantiomerically pure sample from this material, an elegant procedure by a fractional crystallization has been established.[4,6] In this procedure, the contaminating undesired enantiomer can be removed through the preferential crystallization of its racemic form, which is, however, accompanied by the loss of an equal amount of the desired enantiomer (path A, Scheme 1). Moreover, the success of the fractional crystallization has been found to depend strongly upon the ee and the chemical purity of the partially enantiomerically enriched form of **1**. Here we propose an alternative procedure for the kinetic resolution of the partially enantiomerically enriched form of **1** using yeast-mediated reduction, which can convert the undesired enantiomer to the corresponding alcohol (path B, Scheme 1). This would provide an easier and more efficient procedure to approach a highly enantiomerically enriched form of **1**, as the newly formed product resulting from the yeast-mediated transformation can be separated from the unreacted starting material by a simple chromatographical analysis.

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(1) Wieland, P.; Miescher, K. *Helv. Chim. Acta* **1950**, *33*, 2215−2228.

(2) For recent examples from (*R*)-**1**, see the folllowing. (+)-Halenaquinol: Harada, N.; Sugioka, T.; Ando, Y.; Uda, H.; Kuriki, T. *J. Am. Chem. Soc.* **1988**, *110*, 8483−8487. (−)-Chaparrinone: Grieco, P. A.; Collins, J. L.; Moher, E. D.; Fleck, T. J.; Gross, R. S. *J. Am. Chem. Soc.* **1993**, *115*, 6078−6093. (+)-Grinderic acid: Paquette, L. A.; Wang, H.-L. *J. Org. Chem.* **1996**, *61*, 5352−5357. (−)-*O*-Methylshikoccin: Paquette, L. A.; Backhaus, D.; Braun, R.; Underiner, T. L.; Fuchs, K. *J. Am. Chem. Soc.* **1997**, *119*, 9662−9671. For recent examples from (*S*)-**1**, see the following. (+)-Paspalicine: Smith, A. B., III; Kingery-Wood, J.; Leenay, T. L.; Nolen, E. G.; Sunazuka, T. *J. Am. Chem. Soc.* **1992**, *114*, 1438−1449. (+)-Pyripyropene: A, Nagamitsu, T.; Sunazuka, T.; Obata, R.; Tomoda, H.; Tanaka, H.; Harigaya, Y.; Omura, S. *J. Org. Chem.* **1995**, *60*, 8126−8127. Paclitaxel: Danishefsky, S. J.; Masters, J. J.; Young, W. B.; Link, J. T.; Snyder, L. B.; Magee, T. V.; Jung, D. K.; Isaacs, R. C. A.; Bornmann, W. G.; Alaimo, C. A.; Coburn, C. A.; Di Grandi, M. J. *J. Am. Chem. Soc.* **1996**, *118*, 2843−2859. (+)-Avarol: An, J.; Wiemer, D. F. *J. Org. Chem.* **1996**, *61*, 8775−8779. (+)-Labd-8(17)-ene-3*β*,15-diol: Pemp, A.; Seifert, K. *Tetrahedron Lett.* **1997**, *38*, 2081−2084.

(3) Toda, F.; Tanaka, K. *Tetrahedron Lett.* **1988**, *29*, 551−554.

(4) Eder, U.; Sauer, G.; Wiechert, R. *Angew. Chem., Int. Ed. Engl.* **1971**, *10*, 496−497. Hajos, Z. G.; Parrish, D. R. *J. Org. Chem.* **1974**, *39*, 1615−1621. Gutzwiller, J.; Buchschacher, P.; Fürst, A. *Synthesis* **1977**, 167−168. Buchschacher, P.; Fürst, A. *Org. Synth.* **1985**, *63*, 37−43. Hajos, Z. G.; Parrish, D. R. *Organic. Syntheses;* Wiley & Sons: NewYork, 1990, VII, 368−373.

(5) Zhong, G.; Hoffmann, T.; Lerner, R. A.; Danishefsky, S.; Barbas, C. F., III. *J. Am. Chem. Soc.* **1997**, *119*, 8131−8132. Hoffmann, T.; Zhong, G.; List, B.; Shabat, D.; Anderson, J.; Gramatikova, S.; Lerner, R. A.; Barbas, C. F., III. *J. Am. Chem. Soc.* **1998**, *120*, 2768−2779.

(6) Harada, N.; Sugioka, T.; Uda, H.; Kuriki, T. *Synthesis* **1990**, 53−56.

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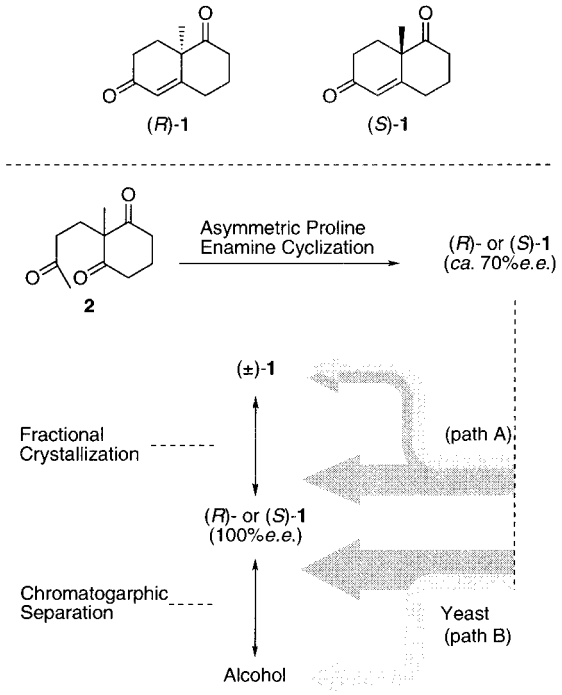
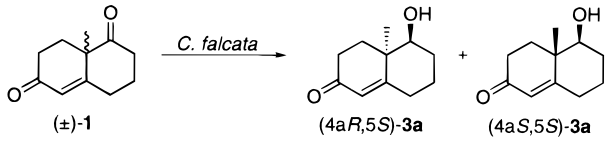
Scheme 1. Wieland−Miescher Ketone 1 and the Preparation of Its Enantiomerically Pure Forms



Scheme 2. Reduction of (±)−1 with *C. falcata*



Results and Discussion

Among the various microorganisms which have been sucessfully applied to the related octalones,[7] Prelog and Acklin reported a microbial approach to obtain an enantiomerically enriched form of **1** from (±)−**1**. They disclosed the use of *Curvularia falcata*, a fungus strain that reduced only the isolated carbonyl group of **1**.[8] In their report, the reduction proceeded in a highly enantiofacially selective manner, according to the so-called "Prelog rule". However, no difference between the rates of the two enantiomers of **1** was observed, which resulted in the formation of a mixture of an equal amount of the two diasteromeric alcohols (4a*S*,5*S*)-**3a** and (4a*R*,5*S*)-**3a** (Scheme 2).

In this context, our first attempt was a screening of microoganisms from stock cultures and soil samples, which can preferentially reduce one enantiomer to another, by applying (±)-**1**[10] as the substrate. Fortunately, two different types of microorganisms, *Torulaspora delbrueckii* IFO 10921 and *Candida melibiosica* IAM 4488 were found to be potent candidates. In the following section, we describe the stereochemical course of the reduction mediated by these microorganisms and the incubation conditions in detail.

Scheme 3. Yeast-mediated Reduction of (±)−1 with *T. delbrueckii* IFO 10921 and *C. melibiosica* IAM 4488

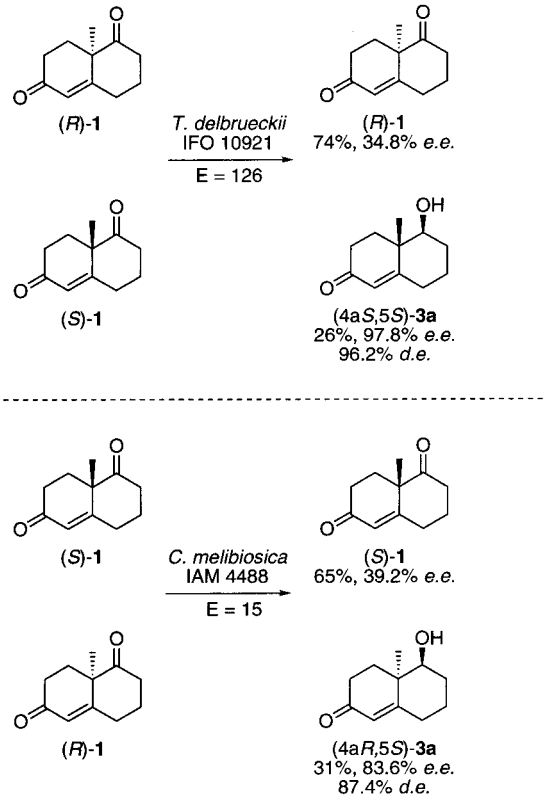


Table 1. Incubation Conditions for the Reduction of Racemic Wieland−Miescher Ketone (1)

entry	substrate concn (%)	wet cells (mg/mL)	glucose (%)	time (h)	convn[a] (%)	E^b
1	0.2	160	0.4	0.5	38.3	87
2	1.0	160	0.4	1.5	34.9	20
3	1.0	80	0.4	2.5	26.7	18
4	1.0	20	0.4	6.0	23.5	16
5	1.0	60	2.0	2.0	26.3	126

[a] Determined by ^{1}H NMR analysis (270 MHz) of the crude extract. [b] *E* was calculated on the basis of the ee of recovered **1** and conversion.[12]

***T. delbrueckii* IFO 10921-Mediated Preferential Reduction of the (*S*)-Enantiomer.** *T. delbrueckii* IFO 10921[11] preferentially reduced the isolated carbonyl group of (*S*)-**1** to give (4a*S*,5*S*)-**3a**, along with the unreacted (*R*)-**1** as shown in Scheme 3. The incubation conditions were examined as shown in Table 1. The collected cells of this strain, obtained by incubation in a conventional glucose medium (5% glucose), were resuspended (6% w/v). The reduction proceeded smoothly at

(7) Inayama, S.; Shimizu, N.; Ohkura, T.; Akita, H.; Oishi, T.; Iitaka, Y. *Chem. Pharm. Bull.* **1986**, *33*, 2660−2663. Shimizu, N.; Ohkura, T.; Akita, H.; Oishi, T.; Iitaka, Y.; Inayama, S. *Chem. Pharm. Bull.* **1987**, *35*, 429−432. Inayama, S.; Shimizu, N.; Ohkura, T.; Akita, H.; Oishi, T.; Iitaka, Y. *Chem. Pharm. Bull.* **1989**, *37*, 712−717. Shimizu, N.; Ohkura, T.; Akita, H.; Oishi, T.; Iitaka, Y.; Inayama, S. *Chem. Pharm. Bull.* **1989**, *37*, 1023−1027. Shimizu, N.; Ohkura, T.; Akita, H.; Oishi, T.; Iitaka, Y.; Inayama, S. *Chem. Pharm. Bull.* **1989**, *37*, 2561−2563. Shimizu, N.; Ohkura, T.; Akita, H.; Oishi, T.; Iitaka, Y.; Inayama, S. *Chem. Pharm. Bull.* **1991**, *39*, 2973−2979.

(8) Prelog, V.; Acklin, W. *Helv. Chim. Acta* **1956**, *39*, 748−757.

(9) Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119−130. MacLeod, R.; Prosser, H.; Fikentscher, L.; Lanyi, J.; Mosher, H. S. *Biochemistry* **1964**, *3*, 838−846.

(10) Ramachandran, S.; Newman, M. S. *Organic Syntheses;* Wiley & Sons: New York, 1973, V, 486-489.

(11) This yeast strain was isolated from soil and identified by Deutsche Sammlung von Mikroorganismen und Zellikulturen GbmH (DSMZ). It was deposited at the Institute for Fermentation, Osaka (IFO), and now available from this Institute, 2-17-85 Juso-honmachi, Yodogawa-ku, Osaka 532-8686, Japan. For details, see the Experimental Section.

1% of the substrate concentration (Table 1, entries 2−5). In the best case (Table 1, entry 5), the enantiomeric ratio ($E = [V_{max}(fast)/K_m(fast)]/[V_{max}(slow)/K_m(slow)]$), developed by Sih and co-workers[12] as the index for the efficiency of a kinetic resolution, was 126. Under the reaction conditions, with an insufficient amount of the cells (Table 1, entry 4) and/or a low glucose concentration (Table 1, entry 3), the selectivity became considerably low (16−20).

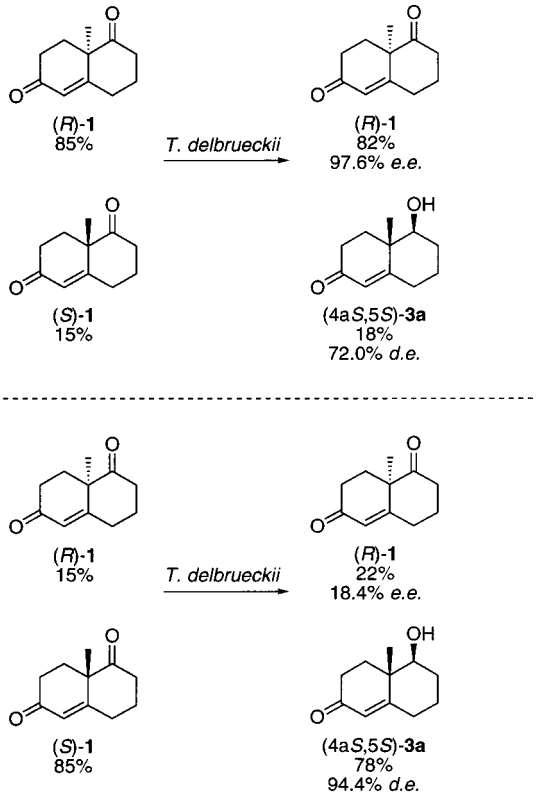
***C. melibiosica* IAM 4488-Mediated Preferential Reduction of the (*R*)-Enantiomer.** In contrast to *T. delbrueckii*, a yeast strain, *C. melibiosica* IAM 4488[13] preferentially reduced the (*R*)-**1** with an *E* of 15 (Scheme 3). It was noteworthy that the activity and selectivity were only expressed during the logarithmic growing phase of the yeast. For this reason, the incubation conditions were elaborated, and the reduction was initiated by the addition of the substrate several hours later than the inoculation of the strain to the incubation medium. The other way was for the incubation to be carried out for a prolonged period. After the substrate was mixed with the collected cells in a glucose medium, the mixture was shaken for 1 week, with the periodic addition of glucose every day. In this case, the reduction started suddenly, 4−5 days after the initiation of the attempted reaction.

Neither procedure described above, however, could be performed in a highly reproducible manner. In the former case, the smooth initiation of the reduction seemed to strongly depend on the cell concentration. If the addition of substrate was too early, no further growth of the yeast was observed, probably due to the toxicity of the substrate. On the other hand, the reduction was very slow when the addition of the substrate was delayed. This situation compelled us to give up any further attempts to carry out the reaction on a large scale.

Preparative-Scale Synthesis of Enantiomerically Pure Wieland−Miescher Ketone with *T. delbrueckii*. As mentioned earlier, the reproducibility of the rate of the reduction and the growth of *T. delbrueckii* in a large-scale incubation were very high. This yeast-mediated reduction was then applied to enantiomerically pre-enhanced forms of **1**, which were prepared by an established proline-mediated asymmetric Robinson annulation. The reduction of (*R*)-**1** (70% ee) with *T. delbrueckii* gave (*R*)-**1** (97.6% ee) in 82% yield, along with (4a*S*,5*S*)-**3a** (72.0% de) in 18% yield, as shown in Scheme 4. Enantiomerically pure (*R*)-**1** was obtained by the single recrystallization of recovered **1**. On the other hand, the reduction of (*S*)-**1** (70% ee) gave (4a*S*,5*S*)-**3a** (94.4% de) in 78% yield, along with (*R*)-**1** (18.4% ee) in 22% yield as shown in Scheme 4. Pure (4aS,5S)-**3a** was obtained by the single recrystallization of this material. Both procedures worked very preparatively in several 10 g scales of the substrate, and the products were obtained in nearly quantitative yields. Enantiomerically pure (*S*)-**1** was obtained in a high yield by Jones' oxidation of (4a*S*,5*S*)-**3a** followed by single recrystallization of the product.

An Attempt for the Asymmetric Synthesis of Enantiomerically Pure Wieland−Miescher Ketone with *T. delbrueckii*. The above-mentioned yeast strain,

Scheme 4. Preparative-Scale Reduction of Enantiomerically Pre-enhanced Forms of 1 with *T. delbrueckii* IFO 10921



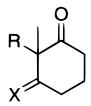
T. delbrueckii, effectively reduced a somewhat sterically hindered carbonyl group that is located adjacent to a quaternary carbon atom. This result prompted us to extend this reduction to other related substrates. The importance of asymmetrization of prochiral diketones, which would produce a new quaternary chiral center, has been pointed out by a number of organic chemists, and pioneering studies have been reported on the subject of yeast-mediated asymmetric reductions of cyclic substrates.[14,15] Along these lines, the substrate candidates were selected, as shown in Figure 1. In contrast to the result on the Wieland−Miescher ketone (**1**) as described above, the action of *T. delbrueckii* showed discrepancy on other substrates shown in Table 2. As judged from entries 1−6 (Table 2), it seems that this yeast strain shows a higher affinity to the substrate bearing 1,3-dicarbonyl functionalities and its vinylogous structure.

(12) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299.

(13) This yeast strain is available from the IAM culture collection, Institute of Molecular Cell Biosciences at the University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan.

(14) Zhou, W.-S.; Huang, D.-Z.; Wang, Z.-Q. *Kexue Tongbao* **1980**, *25*, 741−742. Zhou, W.-S.; Huang, D.-Z.; Dong, Q.-C.; Zhuang, Z.-P.; Wang, Z.-Q. *Chem. Nat. Prod. Proc. Sino-Am. Symp.* **1980**, 299−301. Zhou, W.-S.; Huang, D.-Z.; Dong, Q.-C.; Wang, Z.-Q. *Huaxue Xuebao* **1982**, *40*, 648−656. Zhou, W.-S.; Zhuang, Z.-P.; Wang, Z.-Q.*Huaxue Xuebao* **1982**, *40*, 666−669. Brooks, D. W.; Grothaus, P. G.; Irwin, W. L. *J. Org. Chem.* **1982**, *47*, 2820−2821. Lu, Y.; Barth, G.; Kieslich, K.; Strong, P. D.; Duax, W. L.; Djerassi, C. *J. Org. Chem.* **1983**, *48*, 4549−4554. Brooks, D. W.; Mazdiyasni, H.; Chakrabarti, S. *Tetrahedron Lett.* **1984**, *25*, 1241−1244. Yanai, M.; Sugai, T.; Mori, K. *Agric. Biol. Chem.* **1985**, *49*, 2373−2377. Dai, W.-M.; Zhou, W.-S. *Tetrahedron* **1985**, *41*, 4475−4482. Mori, K.; Mori, H. *Tetrahedron* **1985**, *41*, 5487−5493. Brooks, D. W.; Mazdiyasni, H.; Sallay, P. *J. Org. Chem.* **1985**, *50*, 3411−3414. Brooks, D. W.; Woods, K. W. *J. Org. Chem.* **1987**, *52*, 2036−2039. Brooks, D. W.; Madiyasni, H.; Grothaus, P. G. *J. Org. Chem.* **1987**, *52*, 3223−3232. Mori, K.; Fujiwhara, M. *Tetrahedron* **1988**, *44*, 343−354. Mori, K.; Mori, H. *Organic Syntheses;* Wiley & Sons: New York, 1993, VIII, 312−315. Sakakibara, M.; Ogawa-Uchida, A. *Biosci. Biotechnol. Biochem.* **1995**, *59*, 1300−1303.

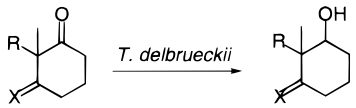
(15) Zhou, W.-S.; Wang, Z.-Q.; Zhang, H.; Fei, R.-Y.; Zhuang, Z.-P. *Huaxue Xuebao* **1985**, *43*, 168−173.



1: X = R = [CHC(=O)CH_2CH_2]
2: X = O, R = $CH_2CH_2COCH_3$
4: X = H_2, R = $CH_2CH_2CO_2CH_3$
5: X = O, R = CH_2CO_2H
6: X = O, R = $CH_2CO_2CH_3$
7: X = O, R = $CH_2CO_2CH_2C_6H_5$

Figure 1. Candidate of substituted cyclohexenones for asymmetric reduction with *T. delbrueckii* IFO 10921.

Table 2. Reduction of Carbonyl Compounds with *T. delbrueckii*



entry	substrate[a]	wet cells (mg/mL)	time (h)	convn (%)	yield (%)	syn/anti of the product[b]
1	(±)-**1**	60	2	26	26	1.4:98.6
2	(±)-**4**	300	20	44	24	1:1.9
3	**5**	60	24	0		
4	**6**	60	24	23	20	1:2.5
5	**7**	60	72	37	21	1:5.9
6	**2**	60	24	100	60	>99.9:0.1

[a] Substrate concentration = 1.0%. [b] Syn means that the substituent R and the substituent hydroxyl group are located in a syn relationship.

Table 3. Reduction of Prochiral Diketone 2 with *T. delbrueckii*

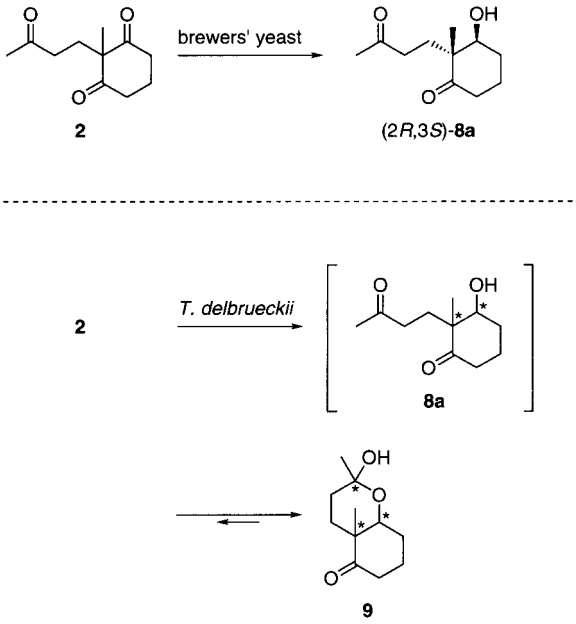
entry	time (h)	yield (%)	method of purification[a]
1	16	29	A
2	16	52	B
3	12	46	B
4	20	57	B
5	24	60	B
6	36	51	B

[a] A: Crude product was extensively chromatographed over silica gel for purification; B. Crude product was roughly chromatographed and then recrystallized for purification.

The reduction of monoketone (Table 2, entry 2) was very slow, and the product was obtained only in very low yield as a diastereomeric mixture. The introduction of a carboxymethyl group also had a deleterious effect on the reducing enzyme system of this yeast (Table 2, entry 3). The reduction worked by the protection of the carboxyl group as its esters in entries 4 and 5 (Table 2), while no high diastereomeric selectivity was observed in either case.

Finally, we attempted the *T. delbrueckii*-catalyzed reduction of 2-methyl-2-(3-oxobutyl)-1,3-cyclohexanedione (**2**, entry 6). The reaction was carefully monitored by thin-layer chromatographic analysis, because all of the three carbonyl groups were susceptible to reduction and the product would be a complex mixture. The exploration of both the incubation conditions and the workup procedure is summarized in Table 3. One major product was observed along with the progress of incubation (Table 3, entries 1−5), which, after prolonged incubation, began to decrease (Table 3, entry 6), allowing other more polar materials to become dominant. The workup and purification of the major product from the incubation mixture, at the appropriate stage, was examined. To our surprise, the major product was not identical to that which was expected, the (2*R*,3*S*) form of 3-hydroxy-2-methyl-2-(3-oxobutyl)cyclohexanone (**8a**) that had been reported as the product by the reduction of **2** with brewers' yeast.[15] By the elucidation of its ^{1}H NMR spectrum, the structure of the product was suggested to be **9** (Scheme 5).

Scheme 5. Reduction of 2 with *T. delbrueckii* IFO 10921



Of the three carbonyl groups of **2**, one on the carbocyclic skeleton was preferentially reduced, and the initially formed hydroxy diketone spontaneously cyclized via intramolecular hemiacetal formation to give **9**. Due to the acid-labile nature of **9**, the recovery of the product from the incubation broth was best (60%) in the following workup procedure: the crude product was roughly purified by chromatography through a short column of silica gel and subsequently recrystallized as shown in entry 5 (Table 3). The crystalline nature made the isolation of **9** possible in a highly pure state, and the relative configuration of **9** was unambiguously determined, by X-ray crystallographic analysis, to be (1*R**,6*R**,7*R**), as shown in Figure 2.

The next task was to determine the absolute configuration of **9**. This was accomplished by a chemical correlation of **9** to (1*S*,8a*R*)-**3b** as shown in Scheme 6. First, hemiacetal **9** was treated with acetic anhydride and pyridine to give acetate **8b**. In this case, the acetylation occurred almost exclusively on the secondary hydroxy group with a concomitant hemiacetal ring-opening reaction. The acid-catalyzed Robinson annulation reaction of **8b** was performed according to the reported procedure,[15] which resulted in bicyclic acetate **3b**. At this stage, the sign of rotation of **3b** ($[\alpha]^{20}_D$ −93.0 (chloroform)) was compared to that of an authentic sample ($[\alpha]^{20}_D$ −92.0 (chloroform)) derived from (4a*R*,5*S*)-**3b**, which was obtained by a *C. melibiosica*-catalyzed reduction of **1**. As the ee of (1*S*,8a*R*)-**3b** was confirmed to be >99%, by a chiral stationary phase HPLC analysis, it was revealed that the stereoselectivity of the reduction of prochiral triketone **2** was high.

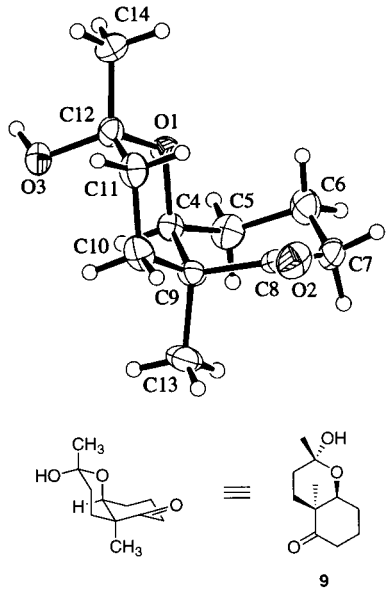
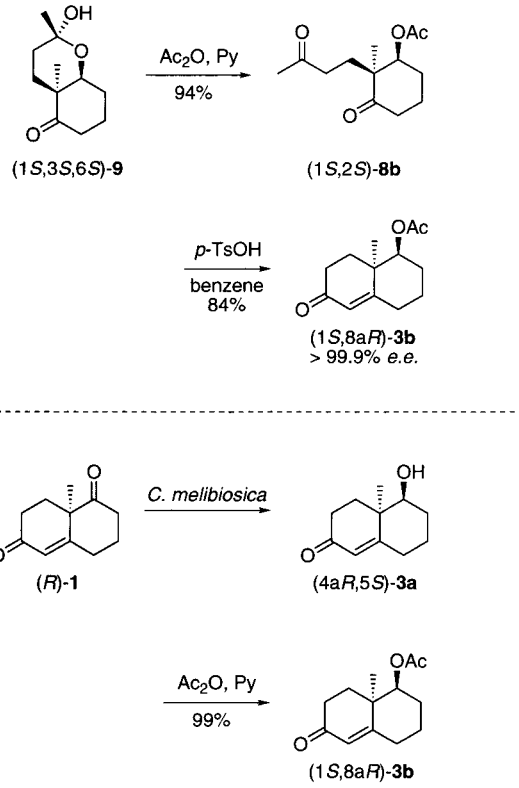


Figure 2. Structure of **9** determined by X-ray analysis.

Scheme 6. Determination of the Absolute Configuration of 9



Conclusion

The two *T. delbrueckii*-catalyzed approaches, kinetic resolution and asymmetrization, worked very well in a preparative scale. These two methodologies would be new entries to the preparation of useful starting materials in an enantiomerically pure form toward natural product synthesis.

Experimental Section

Screening of Microorganisms. The screening of microorganisms was performed as follows: The microorganisms from stock cultures and soil samples were incubated in a glucose medium (containing glucose (50 mg), malt extract (50 mg), peptone (70 mg), yeast extract (50 mg), KH_2PO_4 (30 mg), and K_2HPO_4 (20 mg), at pH 6.5, total volume of 10 mL) for 2 days at 30 °C. Then to the mixture were added (±)−**1** (20 mg) and glucose (100 mg), and the mixture was shaken on a reciprocal shaker (250 cpm) for several days at 30 °C. The progress of the reduction was confirmed by TLC analysis (silica gel, developed with hexanes/ethyl acetate 1:2). Each reaction mixture was worked up separately, in the same way as described below. The reaction mixture was filtered through a Celite pad and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The recovered substrate was purified by preparative TLC (hexanes/ethyl acetate 1:2, R_f = 0.3−0.4). The ee of **1** was estimated from HPLC analysis. HPLC (column, Chiralcel OJ, 0.46 cm × 25 cm, hexane/2-propanol 9:1; flow rate of 0.5 mL/min): t_R = 49.6 min for (*R*)-**1**, 54.7 min for (*S*)-**1**.[16]

Identification of Microorganism. *T. delbrueckii* IFO10921 was identified[11] to the species level as *T. delbrueckii* Lindner based on morphological and physiological characteristics. Morphology: colony on potato dextrose agar butyrous, smooth, creme colored. Cells: globose, mostly 5 μm ϕ; budding multipolar. Pseudomycelium and true mycelium absent; no sexual reproduction detected. Good growth at 37 °C. Utilization of C- and N-sources: anaerobic, glucose +; aerobic, glucose +; α-methyl glycoside −; galactose −; salicin −; sorbose +; cellobiose −; rhamnose −; maltose −; dulcit −; lactose −; inositol −; melibiose −; mannitol +; sucrose +; sorbitol +; trehalose +; glycerol −; inulin +; erythritol −; melezitose −; D-arabinose −; raffinose +; L-arabinose −; starch −; ribose −; xylitol +; D-xylose −; gluconate −; L-xylose −; 2-keto-gluconate +; adonitol −; 5-keto-gluconate −; nitrate −. From these results, the strain was identified as *T. delbrueckii* Lindner.

Reduction of (±)−1 with *T. delbrueckii* IFO10921. A small portion of the yeast cells of *T. delbrueckii* IFO10921 grown on the agar-plate culture was aseptically inoculated into a glucose medium (containing glucose (5.0 g), peptone (2.0 g), yeast extract (0.5 g), KH_2PO_4 (0.3 g), and K_2HPO_4 (0.2 g), at pH 6.5, total volume of 100 mL) and then incubated for 2 days at 30 °C. The wet cells were harvested by centrifugation (3 000 rpm) and washed with phosphate buffer (0.1 M, pH 6.5). The combined wet cells (6.0 g) were resuspended in a reaction medium (containing glucose (2.0 g), phosphate buffer (0.1 M, pH 6.5), total volume of 100 mL) in a 500 mL shaking culture (Sakaguchi) flask, together with (±)−**1** (1.02 g, 5.72 mmol), and shaken on a reciprocal shaker (186 cpm) for 2 h at 30 °C. The reaction mixture was filtered through a Celite pad and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was charged on a silica gel column (50 g). Elution with hexanes/ethyl acetate (3:1 to 2:1) afforded (*R*)-**1** (759 mg, 74%): IR $\tilde{\nu}_{max}$ 1710, 1665, 1615 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.44 (3H, s), 1.61−1.79 (1H, m), 2.06−2.20 (3H, m), 2.35−2.54 (4H, m), 2.65−2.78 (2H, m), 5.85 (1H, d, J = 1.8 Hz). Its IR and NMR spectra were identical to those reported previously.[4] On the basis of HPLC analysis, the ee of (*R*)-**1** was estimated to be 34.8%.

Further elution of the column (hexanes/ethyl acetate 1:1) afforded (4a*S*,5*S*)-**3a** (275 mg, 26%): IR $\tilde{\nu}_{max}$ 3420, 1655, 1615, 1055 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.19 (3H, s), 1.31−1.49 (1H, m), 1.62−1.93 (5H, m), 2.13−2.50 (5H, m), 3.41 (1H,

(16) The authors observed an inversion of the retention time of (*R*)-**1** and (*S*)-**1** on Chiralcel OJ. First, a commercial new column shows good separation by eluting with hexane/2-propanol (9:1). The repeatedly used column tended to showed low separation and sometimes, surprisingly, an inverted t_R (min) of 40.8 for (*S*)-**1** and 43.5 for (*R*)-**1** by eluting with hexane/2-propanol (15:1). However, washing the exhausted column with ethanol again showed a sharp separation of two enantiomers, t_R (min) = 49.6 for (*S*)-**1** and 54.7 for (*R*)-**1**. From this example, we concluded that the condition of the chiral stationary phase column should always be maintained and those who use the elution pattern on chiral stationary phase for the determination of the absolute configuration should be very careful. We strongly recommend the combination of the measurement of the sign of the optical rotation.

dd, J = 4.3, 11.4 Hz (4a*S*,5*S*)), 3.65 (1H, dd, J = 2.5, 2.6 Hz (4a*R*,5*S*)), 5.78 (1H, d, J = 1.2 Hz). Its IR and NMR spectra were identical with those reported previously.[17] Judging from the area of signals at δ 3.43 for (4a*S*,5*S*)-**3a** and δ 3.65 for (4a*R*,5*S*)-**3a**, the de of (4a*S*,5*S*)-**3a** was estimated to be 96.2%.

A small portion of (4a*S*,5*S*)-**3a** was oxidized with Jones' reagent and was converted to (*S*)-**1**. On the basis of HPLC analysis, the ee of (*S*)-**1** was estimated to be 97.8%.

(*R*)-3,4,8,8a-Tetrahydro-8a-methyl-1,6(2*H*,7*H*)-naphthalenedione (*R*)-1. The large-scale incubation of *T. delbrueckii* IFO10921 was carried out as follows: A seed culture (100 mL) was prepared in the same glucose medium as described above for 30 h at 30 °C. The resulting mixture was aseptically poured into another glucose medium (containing glucose (100 g), peptone (40 g), yeast extract (10 g), KH_2PO_4 (6.0 g), K_2HPO_4 (4.0 g), at pH 6.5, total volume of 2000 mL) in a 5000 mL Erlenmeyer cultivating flask with two internal projections and further incubated for 40 h at 30 °C. At this stage, its OD (660 nm) reached 61−67. The wet cells were harvested by centrifugation (5 000 rpm) and washed with phosphate buffer (0.1 M, pH 6.5). The combined wet cells (60 g) were resuspended in a reaction medium (containing glucose (20 g), phosphate buffer (0.1 M, pH 6.5), total volume of 1000 mL) in a 5000 mL Erlenmeyer cultivating flask with two internal projections, together with (*R*)-**1** (70% ee, 10.0 g, 56.1 mmol), and shaken on a gyrorotary shaker (186 rpm) for 6 h at 30 °C. The reaction mixture was filtered through a Celite pad and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was charged on a silica gel column (450 g). Elution with hexanes/ethyl acetate (3:1 to 1:1) afforded (*R*)-**1** (8.22 g, 82%) as a solid. On the basis of HPLC analysis, the ee of (*R*)-**1** was estimated 97.6%.

Further purification by recrystallization from hexanes/ethyl acetate afforded (*R*)-**1** as needles: mp 48.6−49.4 °C (lit.[4] mp 49−50 °C); $[\alpha]^{27}_D = -95.2$ (*c* 1.03, toluene) (lit.[4] $[\alpha]^{25}_D = +97.3$ (*c* 1.0 toluene) for (*S*)-**1**). Its IR and NMR spectra were identical with those of (*R*)-**1** as descrived above. On the basis of HPLC analysis, the ee of (*R*)-**1** was estimated to be 99.5%. Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 73.80; H, 8.06.

Further elution of the column (hexanes/ethyl acetate 1:1) afforded (4a*S*,5*S*)-**3a** (1.84 g, 18%). Its IR and NMR spectra were identical with those of (4a*S*,5*S*)-**3a** as descrived above. On the basis of the NMR spectrum, the de of (4a*S*,5*S*)-**3a** was estimated to be 72.0%.

(4a*S*,5*S*)-4,4a,5,6,7,8-Hexahydro-5-hydroxy-4a-methyl-2(3*H*)-naphthalenone (4a*S*,5*S*)-3a. The large-scale incubation of *T. delbrueckii* IFO10921 and the reaction were carried out in a manner similar to the procedure as described above. The combined wet cells (60 g) were resuspended in a reaction medium (containing glucose (20 g), phosphate buffer (0.1 M, pH 6.5), total volume of 1000 mL) in a 5000 mL Erlenmeyer cultivating flask with two internal projections, together with (*S*)-**1** (70% ee, 10.0 g, 56.1 mmol), and shaken on a gyrorotary shaker (186 rpm) for 14 h at 30 °C. The extraction and workup were performed as described above. The residue was charged on a silica gel column (450 g). Elution with hexanes/ethyl acetate (3:1 to 1:1) afforded (*R*)-**1** (2.21 g, 22%) as a solid. Its IR and NMR spectra were identical with those of (*R*)-**1** as described above.On the basis of HPLC analysis, the ee of (*R*)-**1** was estimated to be 18.4%.

Further elution of the column (hexanes/ethyl acetate 1:1) afforded (4a*S*,5*S*)-**3a** (7.91 g, 78%) as a solid. On the basis of the NMR spectrum, the de of (4a*S*,5*S*)-**3a** was estimated to be 94.4%.

Further purification by recrystallization from hexanes/ethyl acetate afforded (4a*S*,5*S*)-**3a** as needles: mp 44.0−46.2 °C (lit.[17] mp 45−48 °C); $[\alpha]^{24}_D = +191.6$ (*c* 1.05, benzene) (lit.[8] $[\alpha]^{25}_D = +203$ (*c* 1.55 benzene)). Its IR and NMR spectra were identical with those of (4a*S*,5*S*)-**3a** as described above. On the basis of the NMR spectrum, the de of (4a*S*,5*S*)-**3a** was estimated to be >99.9%.

A small portion of (4a*S*,5*S*)-**3a** was oxidized with Jones' reagent and was converted to (*S*)-**1** as a solid. On the basis of HPLC analysis, the ee of (*S*)-**1** was estimated to be 99.0%. Further purification by recrystallization from hexanes/ethyl acetate afforded (*S*)-**1** as needles: mp 45.0−47.0 °C; $[\alpha]^{25}_D = +95.5$ (*c* 0.98, toluene). Its IR and NMR spectra were idential with those of (*R*)-**1** as described above. On the basis of HPLC analysis, the ee of (*S*)-**1** was estimated to be >99.9%.

(4a*R*,5*S*)-Hexahydro-5-hydroxy-4a-methyl-4,4a,5,6,7,8-hexahydro-2(3*H*)-naphthalenone (4a*R*,5*S*)-3a. A small portion of the yeast cells of *C. melibiosica* IAM4488 grown on the agar-plate culture was aseptically inoculated into a glucose medium (containing glucose (1.0 g), peptone (2.0 g), yeast extract (0.5 g), KH_2PO_4 (0.3 g), K_2HPO_4 (0.2 g), at pH 6.5, total volume of 100 mL), and the culture was shaken on a reciprocal shaker (186 cpm) at 30 °C. After 12 h, (±)−**1** (198 mg, 1.11 mmol) was added to the mixture, and it was shaken for 68 h at 30 °C. The extraction and workup were performed as described above. The residue was charged on a silica gel column (20 g). Elution with hexane/ethyl acetate (3:1 to 1:1) afforded (*S*)-**1** (129 mg, 65%) as a solid. On the basis of HPLC analysis, the ee of (*S*)-**1** was estimated to be 39.2%. Its IR and NMR spectra were identical with those of (*R*)-1, as described above.

Further elution of the column (hexanes/ethyl acetate 1:1) afforded (4a*R*,5*S*)-**3a** (62 mg 31%) as a solid. Judging from the NMR spectrum, as described above, the de of (4a*R*,5*S*)-**3a** was estimated to be 87.4%. Further purification by two recrystallizations from hexane/ethyl acetate afforded (4a*R*,5*S*)-**3a** as needles: mp 92.4−92.8 °C (lit.[8] mp 94−95 °C); $[\alpha]^{20}_D = -108.5$ (*c* 1.04, benzene) (lit.[8] $[\alpha]^{25}_D = -111$ (*c* 1.3, benzene)); IR $\tilde{\nu}_{max}$ 3435, 1645, 1605, 1065 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.23 (3H, s), 1.46−1.53 (1H, m), 1.66−2.11 (5H, m), 2.23−2.65 (5H, m), 3.41 (1H, dd, J = 4.3, 11.4 Hz (4a*S*,5*S*)), 3.65 (1H, dd, J = 2.5, 2.6 Hz (4a*R*,5*S*)), 5.85 (1H, d, J = 1.7 Hz). Its IR and NMR spectra were identical with those reported previously.[18] On the basis of the NMR spectrum, the de of (4a*R*,5*S*)-**3a** was estimated to be >99.9%. Anal. Calcd for $C_{11}H_{16}O_2$: C, 73.30; H, 8.95. Found: C, 73.29; H, 8.91.

(1*S*,3*S*,6*S*)-3-Hydroxy-2-oxa-3,6-dimethyl-bicyclo[4.4.0]-7-decanone (1*S*,3*S*,6*S*)-9. The incubation of *T. delbrueckii* IFO10921 was carried out in a manner similar to the procedure as described above. The combined wet cells (6.0 g) were resuspended in a reaction medium (containing glucose (2.0 g), phosphate buffer (0.1 M, pH 6.5), total volume of 100 mL) in a 500 mL shaking culture (Sakaguchi) flask, together with **2** (1.01 g, 5.14 mmol), and shaken on a reciprocal shaker (186 cpm) for 24 h at 30 °C. The reaction mixture was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was charged on a silica gel column (60 g). Elution with hexanes/ethyl acetate (4:1 to 1:1) afforded crude (1*S*,3*S*,6*S*)-**9** as a solid. Further purification by recrystallization from hexanes/ethyl acetate afforded (1*S*,3*S*,6*S*)-**9** (544 mg) as needles. The remaining mixture was charged on a silica gel column (20 g). Elution with hexanes/ethyl acetate (4:1 to 1:1) and subsequent recrystallization afforded further (1*S*,3*S*,6*S*)-**9** (68.9 mg). The combined yield of (1*S*,3*S*,6*S*)-**9** (613 mg) was 60%. This was employed for the next step without further purification.

Recrystallization from hexanes/ethyl acetate gave an analytical sample of (1*S*,3*S*,6*S*)-**9** as needles: mp 75.4−76.2 °C; $[\alpha]^{24}_D = -117.9$ (*c* 0.96, chloroform); IR $\tilde{\nu}_{max}$ 3460, 1690, 1080 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.13 (3H, s), 1.35 (3H, s), 1.41−1.48 (1H, m), 1.57−1.63 (2H, m), 1.71−1.74 (1H, m), 1.80−1.86 (1H, m), 1.88 (1H, s), 2.04−2.18 (3H, m), 2.24−2.29 (1H, m), 2.49−2.58 (1H, m), 4.21 (1H, dd, J = 2.2, 2.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.2, 24.4, 26.4, 27.5, 30.4, 32.0, 37.9, 47.2, 75.3, 95.8, 214.3. Anal. Calcd for $C_{11}H_{18}O_3$: C, 66.64; H, 9.15.

(17) Yeo, S.-K.; Hatae, N.; Seki, M.; Kanematsu, K. *Tetrahedron* **1995**, *51*, 3499−3506.

(18) Tables of atomic parameters, bond lengths, and bond angles for **9** have been deposited with the Cambridge Crystallographic Data Centre.

Found: C, 66.89; H, 9.09. Single-crystal X-ray diffraction measurement was performed with a Rigaku AFC-7R four-circle diffractometer with Mo Kα radiation (λ = 0.710 73 Å). The crystal data are as follows: $C_{11}H_{18}O_3$, M_r = 198.26, orthorhombic, $P2_12_12_1$, a = 11.420(5) Å, b = 15.451(5) Å, c = 6.126(3) Å, V = 1080.9(9) Å^3, Z = 4, μ = 0.087 mm^{-1}, D_x = 1.218 Mg m^{-3}, R = 0.054 for 1051 reflections.[18] The molecular structure is shown in Figure 2.

(1*S*,2*S*)-2-Methyl-3-oxo-2-(3-oxobutyl)cyclohexyl Acetate (1*S*,2*S*)-8b. A mixture of (1*S*,3*S*,6*S*)-**9** (746 mg, 3.76 mmol), anhydrous pyridine (6 mL), and acetic anhydride (6 mL) was stirred for 6 days at room temperature and further stirred for 2 days at 35 °C. The reaction mixture was poured into water and acidified to pH 2 by adding 2 M hydrochloric acid. The reaction mixture was extracted with ethyl acetate. The combined organic layer was successively washed with 2 M hydrochloric acid, water, saturated aqueous sodium hydrogen carbonate solution, and brine; dried over anhydrous sodium sulfate; and concentrated in vacuo. The residue was charged on a silica gel column (20 g). Elution with hexanes/ethyl acetate (15:1 to 4:1) afforded (1*S*,2*S*)-**8b** (847 mg, 94%) as a solid. This was employed for the next step without further purification.

Recrystallization from hexanes/ethyl acetate gave an analytical sample of (1*S*,2*S*)-**8b** as needles: mp 48.4−48.8 °C; $[\alpha]^{20}_D$ = +42.9 (*c* 1.03, chloroform); IR $\tilde{\nu}_{max}$ 1735, 1715, 1235 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (3H, s), 1.64−2.09 (6H, m), 2.05 (3H, s), 2.12 (3H, s), 2.20−2.41 (4H, m), 4.87 (1H, dd, J = 3.3, 6.7 Hz). Its IR and NMR spectra were identical with those of (1*R*,2*R*)-**8b**, reported previously.[19] Anal. Calcd for $C_{13}H_{20}O_4$: C, 64.98; H, 8.39. Found: C, 65.06; H, 8.48.

(1*S*,8a*R*)-1,2,3,4,6,7,8,8a-octahydro-8a-methyl-6-oxonaphthyl Acetate (1*S*,8a*R*)-3b. A mixture of (1*S*,2*S*)-**8b** (60.8 mg, 0.253 mmol), benzene (6 mL), and a catalytic amount of *p*-toluenesulfonic acid was stirred under reflux for 2 days with an azeotropic removal of water. The reaction mixture was poured into saturated aqueous sodium hydrogen carbonate solution and extracted with ethyl acetate. The combined organic layer was successively washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was charged on a silica gel column (3 g). Elution with hexanes/ethyl acetate (10:1 to 1:1) afforded (1*S*,8a*R*)-**3b** (47.0 mg, 84%). The ee of (1*S*,8a*R*)-**3b** was estimated from HPLC analysis. HPLC (column, Chiralcel OJ; hexane/2-propanol 9:1; flow rate of 0.5 mL/min]: t_R = 41.1 min for (1*R*,8a*S*)-**3b,** 46.7 min for (1*S*,8a*R*)-**3b**. Accordingly, the ee of (1*S*,8a*R*)-**3b** was estimated to be >99.9%.

Further purification by a bulb-to-bulb distillation afforded (1*S*,8a*R*)-**3b** as an oil: bp 160 °C/1.8 mmHg (bath temperature); $[\alpha]^{20}_D$ = −93.0 (*c* 1.58, chloroform); IR $\tilde{\nu}_{max}$ 1730, 1670, 1620, 1240 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.31 (3H, s), 1.51 (1H, ddd, J = 3.3, 5.0, 13.2 Hz), 1.69−2.01 (4H, m), 2.06 (3H, s), 2.19 (1H, ddd, J = 5.3, 13.5, 13.5 Hz), 2.29−2.56 (4H, m), 4.85 (1H, dd, J = 2.6, 2.6 Hz), 5.84 (1H, d, J = 1.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 21.2, 21.8, 25.9, 30.9, 31.7, 33.9, 39.5, 76.9, 126.3, 166.3, 170.1, 198.6. Anal. Calcd for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 70.17; H, 8.00.

For the determination of the absolute configuration of the product, the corresponding (1*S*,8a*R*)-3b was prepared in the following manner: A mixture of (4a*R*,5*S*)-**3a** (199 mg, 1.10 mmol), anhydrous pyridine (2 mL), and acetic anhydride (1 mL) was stirred for 2 days at room temperature. The reaction mixture was subsequently poured into water and acidified to pH 2 by adding 2 M hydrochloric acid. The reaction mixture was extracted with ethyl acetate. The combined organic layer was washed with 2 M hydrochloric acid, water, saturated aqueous sodium hydrogen carbonate solution, and brine; dried over anhydrous sodium sulfate; and concentrated in vacuo. The residue was charged on a silica gel column (15 g). Elution with hexanes/ethyl acetate (10:1 to 4:1) afforded (1*S*,8a*R*)-**3b** (242 mg, 99%), $[\alpha]^{20}_D$ = −92.0 (*c* 0.64, chloroform).

For the determination of the ee of the product, the corresponding (±)-(1*S**,8a*S**)-**3b** was prepared in the reported procedure from (±)-**1**.[20,21]

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(19) Renouf, P.; Poirier, J.-M.; Duhamel, P. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1739−1745.

(20) Ward, D. E.; Rhee, C. K.; Zoghaib, W. M. *Tetrahedron Lett.* **1988**, *29*, 517−520. Ward, D. E.; Rhee, C. K. *Can. J. Chem.* **1989**, *67*, 1206−1211.

(21) Shimizu, T.; Hiranuma, S.; Nakata, T. *Tetrahedron Lett.* **1996**, *37*, 6145−6148.