Enantiomerically Pure Octahydronaphthalenone and Octahydroindenone: Elaboration of the Substrate Overcame the Specificity of Yeast-Mediated Reduction

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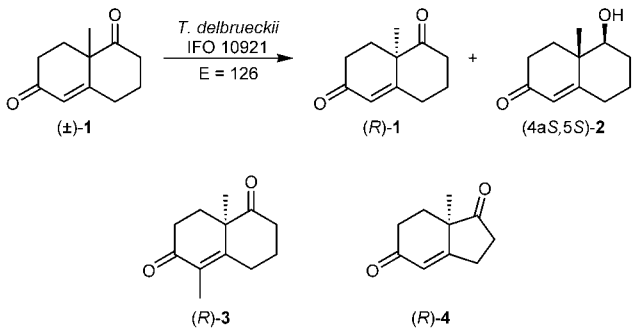
Abstract: Substrate specificity on the reduction of bicyclic diketones with a yeast strain, *Torulaspora delbrueckii* IFO10921, was investigated. Although this yeast efficiently reduces the isolated carbonyl group involved in the (*S*)-enantiomer of Wieland–Miescher ketone with high enantioselectivity (E = 126), the introduction of a substituent on the octahydronaphthalene skeleton as well as the structural change into an octahydroindene skeleton retarded the enzymatic reduction and the enantioselectivity fell to E = 5–16. Further structural variation into a bicyclo[3.3.0] skeleton led to an exclusive 1,4-conjugate reduction of the α,β-unsaturated carbonyl group, and the above results suggested the participation of plural oxidoreductive enzymes in the whole cell. In turn, among the 2,2-disubstituted cycloalkanediones there were found good substrates to give the corresponding hydroxy ketone equivalents by yeast-mediated reduction. The products were isolated as cyclic hemiacetals, such as (1*S*,6*S*)-3-ethyl-3-hydroxy-6-methyl-2-oxabicyclo[4.4.0]decan-7-one and (1*S*,6*S*)-3-hydroxy-3,6-dimethyl-2-oxabicyclo[4.3.0]nonan-7-one. In addition, the reduction worked well with use of an air-dried, long-term preservable cell preparation. The subsequent chemical transformation warranted the stereochemistry and the stereochemical purity of the products, which are related to octahydronaphthalenone and octahydroindenone systems that, in turn, are of considerable value as starting materials for terpenoid synthesis.

Keywords: chiral building block; desymmetrization; kinetic resolution; prochiral substrate; reduction; terpenoid; whole cell biocatalyst; yeast

Introduction

Torulaspora delbrueckii IFO10921 was found to be a yeast strain that enables the reduction of racemic Wieland–Miescher ketone (**1**) with high enantioselectivity as shown in Scheme 1.[1] The enantiomerically enriched products of this kinetic resolution have been utilized as the starting material for naturally occurring terpenoids.[2,3] Recent efforts towards microorganism- and enzyme-mediated preparation of mono- and bicyclic chiral building blocks of natural product synthesis[4] prompted us to investigate the scope and limitation of this yeast-mediated reduction. Based on an extensive study of substrate specificity, here we report a successful approach towards the preparation of (*R*)-**3** and (*R*)-**4**, as well as their synthetic equivalents.



Scheme 1.

Results and Discussion

Attempted Reduction of Bicyclic Substrates

In their pioneering work, Prelog and co-workers disclosed the microbial production of the Wieland–Miescher ketone (**1**) and its analogues.[5] In the subsequent years, Inayama, Shimizu and co-workers extensively studied the substrate specificity on the related compounds.[6] In turn, recently, Kodama applied baker's yeast reduction to the Wieland–Miescher ketone (**1**) and its hydroindenone analogue, Hajos–Parrish ketone (**4**).[7] Our first candidates were the analogous substrates

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Table 1. Yeast-mediated reduction of carbonyl compounds towards the kinetic resolution of racemic substrates.

Entry	Substrate	Product				Recovery		E value
			Yield [%]	% de	% ee	Yield [%]	% ee	
1	**3**	(4a*S*,5*S*)-**9a**	6	73.9	87.7	87	3.4	16
2	**4**	(1*S*,7a*S*)-**10a**	20	61.8	63.8	78	15.5	5
3	**5**	no reaction						
4	**6**	(3a*S*,4*S*)-**11a**	46	70.2	68.0	54	56.9	9

[(±)-**3** to (±)-**8**]; however, the results were rather disappointing and somewhat confusing as shown in Table 1.

Compared with a high level of enantiomer recognition,[8] E = 126 for (±)-**1**, the enantiomeric ratio dropped to 16 (for **3**, entry 1), 5 (for **4**, entry 2), and 9 (for **6**, entry 4). The introduction of a dimethyl substituent even into a rather remote position (C-3) in **1** severely retarded the reduction, which resulted in the lack of reaction of **5**. The above results suggested that plural enzymes work[9] in the whole cell of *T. delbrueckii*, and the activity of oxidoreductive enzyme which was responsible for the highly enantioselective and efficient reduction in (*S*)-**1** is easily suppressed, as being susceptible to the introduction of substituents as **3** and **5**, or the change in the skeleton of the vinylogous diketone system.

Further dramatic alteration of the leading role was observed in the case of the bicyclo[3.3.0] system in which both carbocycles were replaced with a cyclopentane ring. The reduction of isolated carbonyl groups so far consistently observed in the substrates (**1**, **3**, **4**, **6**) was suppressed, and the 1,4-conjugate reduction[10] of the α,β-unsaturated carbonyl group proceeded as shown in Scheme 2. This enzyme showed not only enantiofacial selection, but also enantiomeric kinetic resolution, and both the product (1*S*,5*R*)-**12** and the recovered substrate

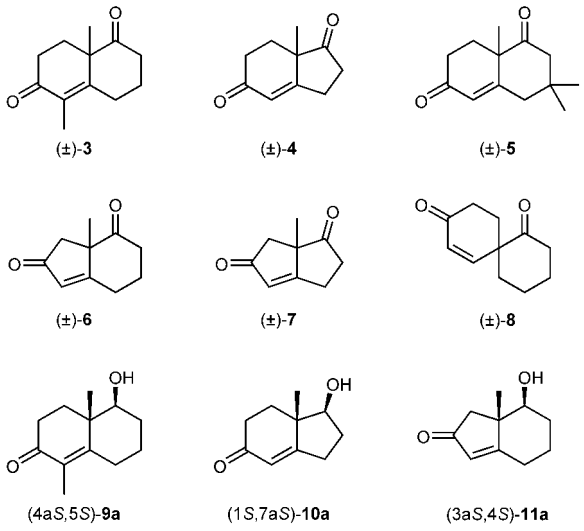
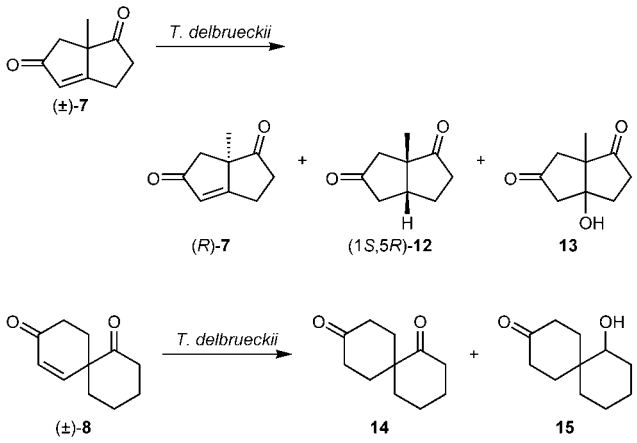


Figure 1.



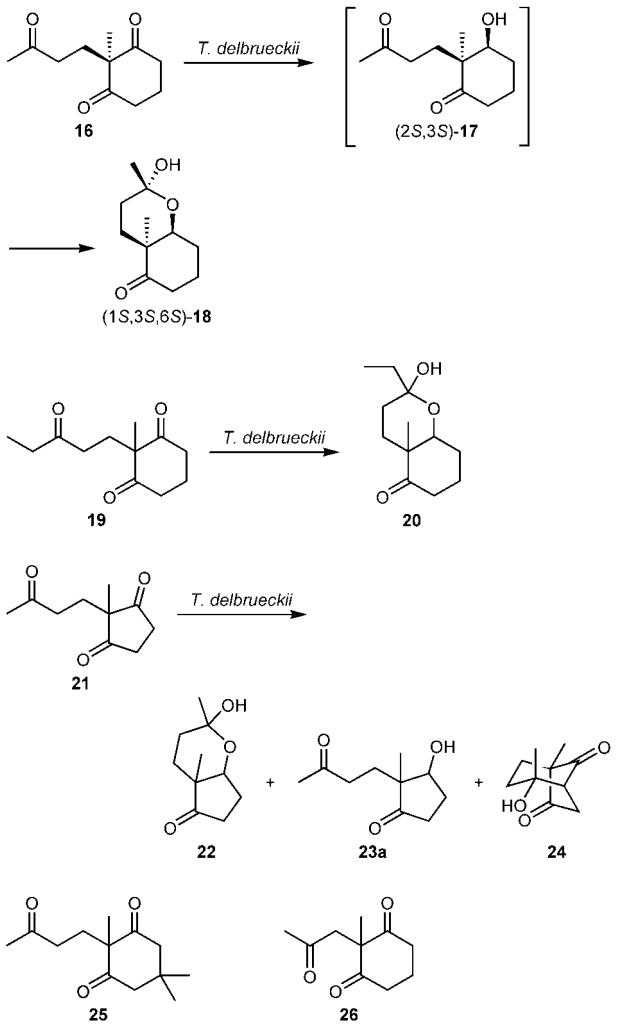
Scheme 2.

(*R*)-**7** were obtained in enaniomerically pure state (Table 2, entry 3). A side reaction is the spontaneous non-enzymatic 1,4-hydration of the unsaturated ketone to give **13**, which was observed even in a buffer solution without any microorganism (entry 4). This reaction lowered the yield of the recovered substrate (*R*)-**7** after prolonged incubation; however, the hydration product **13** could be isolated and recycled to the starting material after the dehydration under appropriate conditions. In contrast to the bicyclic diketone, the yeast-mediated reduction of a spiro diketone **8** proceeded with no enantioselectivity, together with the further reduced product, a hydroxy ketone **15**.

Table 2. Yeast-mediated 1,4-conjugate reduction of (±)-**7**.

Entry	Time [h]	Product		Recovery		**13**
		Yield [%]	% ee	Yield [%]	% ee	Yield [%]
1	3	21	> 99.9	57	36.8	22
2	6	34	> 99.9	32	89.8	34
3	18	38	> 99.9	17	> 99.9	45
4[a]	18	–	–	26	–	74

[a] Blank experiment: substrate was incubated in a buffer solution without yeast cells.



Scheme 3.

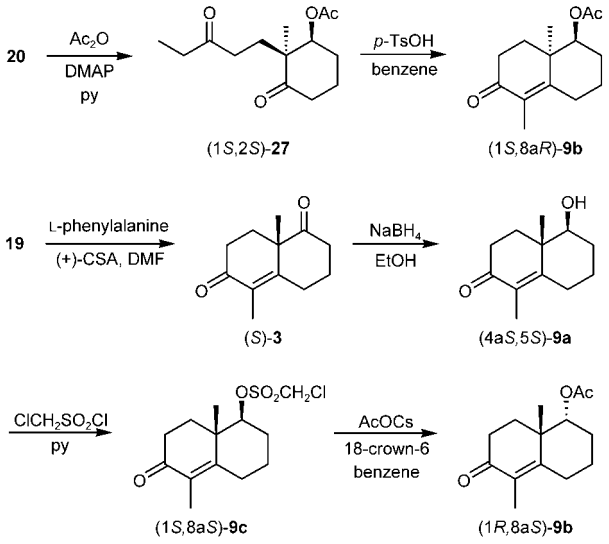
Reduction of Cyclic Triketones

Our previous study also revealed that *T. delbueckii* catalyzes the enantiotopic group-selective reduction of triketone **16**[1] to give a cyclic hemiacetal (1*S*,3*S*,6*S*)-**18**, the equivalent of (2*S*,3*S*)-**17** in high enantioselectivity. As expected, the reduction of the analogous triketone **19** worked well to give hemiacetal **20** in 56% yield. Cyclopentanetrione **21** was also accepted by this microorganism and the desired product **22** was obtained in 63% yield. In contrast to our previous examples, two byproducts were isolated in this case. The first was the open-chain form of hydroxy ketone **23a** (7%), whose relationship between the hydroxy group and the methylene side chain was *trans*. The determination of stereochemistry is shown in the following section. Judged from the ratio of the formation of **22** and **23a**, the enantiotopic group selectivity was *ca.* 9.3:1. Our *T. delbrueckii* preferred the pro-(*R*)-carbonyl group. The formation of minor isomer **23a** is compatible with the findings with a microorganism with an opposite pro-(*S*)-preference, brewer's yeast.[11] Another side product **24** (14%) is probably due to the non-enzymatic intramolecular aldol reaction; however, this aspect was not pursued in the present work. The introduction of a dimethyl group (**25**) and one methylene group subtraction between the quaternary center and the side-chain carbonyl group (**26**) only resulted in the very low activity of enzymes.

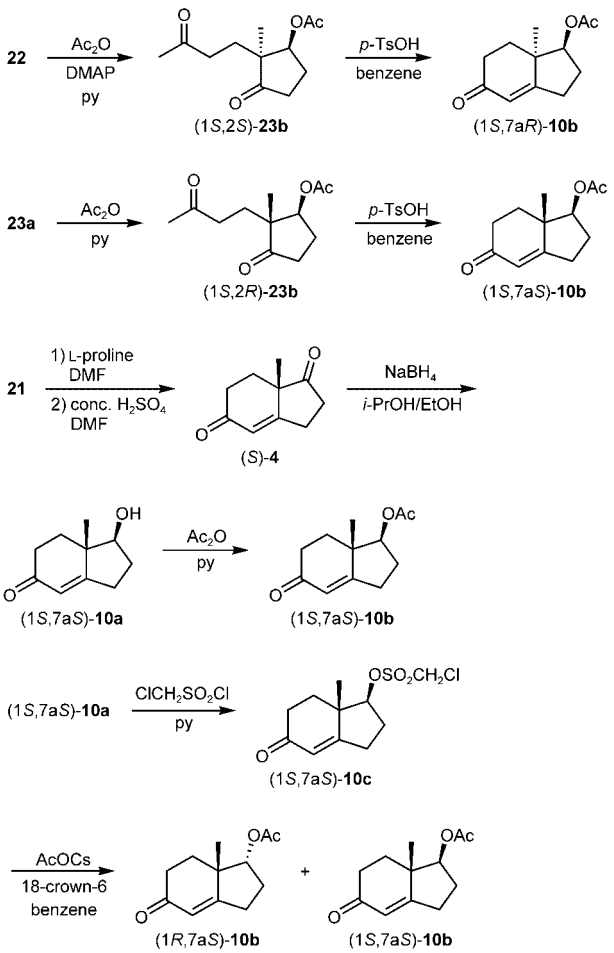
Transformation of Octahydronaphthalenone and Octahydroindenone

The next task was the derivation of cyclic hemiacetals of the bicyclic octahydronaphthalenone and octahydroindenone *via* hemiacetal ring-opening reaction and the subsequent intramolecular aldol reaction.[1] This transformation would unambiguously establish both the stereochemistry and the enantiomeric purities of hemiacetals **20** and **22**.

First, the hemiacetal **20** was acetylated with concomitant ring-opening to give the open-chain acetate **27** in 98% yield. Treatment of **27** with *p*-TsOH provided a bicyclic acetate **9b** in 83% yield as shown in Scheme 4. The suggested (1*S*,8a*R*) configuration[12] of **9b** was confirmed by the comparison with an authentic sample obtained as follows. The triketone **19** was submitted to asymmetric Robinson annulation in the presence of L-phenylalanine to give (*S*)-**3**.[13] The isolated ketone was reduced to give an alcohol (4a*S*,5*S*)-**9a** whose angular methyl group and hydroxy group are located in a *syn* relationship.[13] The stereochemistry of the secondary alcohol was inverted *via* a chloromethanesulfonate[14] (1*S*,8a*S*)-**9c** to give (1*R*,8a*S*)-**9b**. The ^{1}H NMR spectrum of this sample and the sign of rotation (see Experimental Section) were compared with those of the product from microbial origin, whose absolute configuration was confirmed to be (1*S*,8a*R*). In turn, the absolute config-



Scheme 4.



Scheme 5.

uration of cyclic hemiacetal **20** was (1*S*,6*S*). The ee of the product was 98.7%, determined at the stage of (1*S*,8a*R*)-**9b** by HPLC analysis with Chiralcel OJ, and this could be further enhanced to >99.9% by recrystallization of (1*S*,6*S*)-**20**.

In a similar manner, the hemiacetal **22** was converted to a bicyclic acetate (1*S*,7a*R*)-**10b** in a total 70% yield as shown in Scheme 5, whose absolute and relative configuration was determined by the comparison with an authentic sample.[14,15] Independently, hydroxy ketone **23a**, the by-product of yeast-mediated reduction, was converted to (1*S*,7a*S*)-**10b** and the absolute configuration of **23a** was unambiguously determined. In this case, the raw material of the yeast-mediated reaction showed the enantiomeric homogeneity.

Preparation of Long-Term Preservable Cells

If preservable whole cells of this yeast were available, the applicability of this yeast-mediated reduction in synthetic organic chemistry would greatly increase. Three contrasting methods of preparation were tried: 1) freeze-drying at low temperature under high vacuum; 2) oven-drying for a short period; 3) air-drying at ambient temperature. The activity of the preparations was compared on the Wieland–Miescher ketone **1** and the results are shown in Table 3. As can clearly be seen, air-dried cells (entry 4) were the most effective both in terms of activity and enantioselectivity, accompanied with the maintenance of a co-factor regeneration. The E value (123, entry 4) in the kinetic resolution of racemic Wieland–Miescher ketone **1** was nearly equal with that of the freshly harvested cells (126, entry 6). Moreover, the air-dried cells showed a long-term stability. The activity was almost same, even after storage in a refrigerator (4 °C) for sixty days (entry 5).

This air-dried cell preparation was also effective for the reduction of prochiral triketones **16**, **19** and **21** as shown in Table 4, in regard to both the activity of enzymes as well as the regio- and enantioselectivity. Also, the activity of enzymes responsible to 1,4-reduction of (±)-**7** was conserved.

Table 4. Reduction of prochiral triketones with air-dried cells.

Substrate	Product	Air-dried Cell		Fresh Cell	
		Yield [%]	% ee	Yield [%]	% ee
16	(1*S*,3*S*,6*S*)-**18**	62	99.4	60	>99.9
19	(1*S*,6*S*)-**20**	50	97.8	56	98.7
21	(1*S*,6*S*)-**22**	51	99.8	74	>99.9

Table 3. Reduction of (±)-**1** with dried cells towards the kinetic resolution of racemic substrate.

Entry	Method of preparation	Storage [days]	Cell wt. [g][a]	Reaction time [h]	Conversion [%]	E value
1	Freeze-dry	0	3.0	12	46.4	50
2	Freeze-dry	7	3.0	8	34.6	61
3	Oven-dry	0	1.2	30	11.3	29
4	Air-dry	0	1.2	4	41.3	123
5	Air-dry	60	1.2	4	37.8	110
6	Fresh Cells	–	0.6	2	26.3	126

[a] Cell weight per 100 mg of the substrate.

Conclusion

As mentioned above, the substrate specificity of *Torulaspora delbrueckii* and this yeast-mediated reduction offer a new way towards enantiomerically pure octahydronaphthalenone and octahydroindenone. Since a long-term preservable form of air-dried whole cell preparation was effective, this yeast-mediated reduction should be widely applied in the future.

Experimental Section

All mps are uncorrected. IR spectra were measured as films for oils or KBr disks for solids on a Jasco FT/IR-410 spectrometer. ^{1}H NMR spectra were measured in $CDCl_3$ at 270 MHz on a Jeol JNM EX-270 or at 400 MHz on a Jeol JNM GX-400 spectrometer. HPLC data were recorded on SSC-3461 and SSC-5410 (Senshu Scientific Co., Ltd) liquid chromatographs. GLC data were recorded on a GC-353 (GL Science Co., Ltd) gas chromatograph. Optical rotation values were recorded on a Jasco DIP 360 polarimeter. Merck silica gel 60 F_{254} thin-layer plates (1.05744) and silica gel 60 (spherical; 100–210 μm, 37558–79) from Kanto Chemical Co., were used for preparative thin-layer chromatography and column chromatography, respectively. Peptone and yeast extract were purchased from Kyokuto Pharmaceutical Co., for the cultivation of the microorganism.

Preincubation of *T. delbrueckii* IFO10921

A small portion of yeast cells of *T. delbrueckii* IFO10921 grown on the agar-plate culture was aseptically inoculated to a glucose medium [containing glucose (5.0 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 100 mL] in a 500-mL Erlenmeyer cultivating flask with two internal projections and then shaken on a gyrorotary shaker (180 rpm) 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 weight of combined wet cells was *ca*. 3 g from 100 mL of the broth.

Reduction of (±)-1,4a-Dimethyl-4,4a,7,8-tetrahydronaphthalene-2,5(3*H*,6*H*)-dione (3)

The combined wet cells of *T. delbrueckii* IFO10921 (0.6 g) incubated as described above were resuspended in a reaction medium [containing glucose (0.2 g), phosphate buffer (0.1 M, pH 6.5), total volume of 10 mL] in a test tube (21 mm × 20 cm), together with (±)-**3**[16] (100 mg, 0.520 mmol) and shaken on a reciprocal shaker (250 cpm) for 36 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 saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate, and concentrated under vacuum. The residue was charged on a silica gel column (13 g). Elution with hexane/ethyl acetate (5/1 to 2/1) afforded (*R*)-**3**; yield: 87.1 mg (87%); $[\alpha]_D^{23}$: −2.8 (*c* 0.89, methanol) {lit.[13] $[\alpha]_D$: −140 (*c* 0.200, methanol)}; IR: ν_{max} = 1712, 1665, 1611 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.43 (s, 3H), 1.82 (s, 3H), 2.06–2.19 (m, 3H), 2.38–2.52 (m, 3H), 2.62–2.74 (m, 2H), 2.83–2.93 (m, 2H). Its IR and NMR spectra were identical with those reported previously.[13] Based on the HPLC analysis [column, Daicel Chiralcel OJ, 0.46 cm × 25 cm; hexane/2-propanol (9/1); flow rate 0.5 mL/min]: *t*R = 26.7 min for (*R*)-**3**, 28.7 min for (*S*)-**3**, the ee of (*R*)-**3** was determined to be 3.4%.

Further elution of the column [hexane/ethyl acetate (1/1)] afforded (4a*S*,5*S*)-**9a**; yield: 6.0 mg (6%); IR: ν_{max} = 3427, 1653, 1606 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.18 (s, 3H), 1.30–1.49 (m, 1H), 1.64–1.94 (m, 8H), 2.02–2.15 (m, 2H), 2.32–2.61 (m, 2H), 2.65–2.76 (m, 1H), 3.42 [dd, *J* = 4.4, 11.7 Hz, (4a*S*,5*S*), 1H], 3.60 [m, (4a*R*,5*S*), 1H]. Its IR and NMR spectra were identical with those reported previously.[13] Judging from the area of signals at δ = 3.42 for (4a*S*,5*S*)-**9a** and δ = 3.60 for (4a*R*,5*S*)-**9a**, the de of (4a*S*,5*S*)-**9a** was determined to be 73.9%. A very small portion of (4a*S*,5*S*)-**9a** was oxidized with the Jones reagent and the resultant (*S*)-**3** was directly analyzed by HPLC to have 87.7% ee.

Reduction of (±)-7a-Methyl-2,3,7,7a-tetrahydro-1*H*-indene-1,5(6*H*)-dione (4)

The enone (±)-**4**[16] (49.4 mg, 0.301 mmol) was incubated with cells (0.3 g) for 3 h at 30 °C. After the work-up, the crude residue was purified by preparative thin-layer chromatography [developed with hexane/ethyl acetate (1/1)].

(*R*)-**4**; yield: 38.4 mg (78%); $[\alpha]_D^{21}$: −45.4 (*c* 0.77, toluene) {lit.[7] $[\alpha]_D^{25}$: −355 (*c* 1.0 toluene)}; IR: ν_{max} = 1744, 1699, 1665 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.32 (s, 3H), 1.85 (ddd, *J* = 6.2, 13.4, 13.4 Hz, 1H), 2.12 (ddd, *J* = 2.5, 5.0, 13.4 Hz, 1H), 2.36–2.61 (m, 3H), 2.70–3.04 (m, 3H), 5.98 (d, *J* = 1.6 Hz, 1H). Its IR and NMR spectra were identical with those reported previously.[15] Based on the GLC analysis [column, Supelco BETA DEX™ 225, 30 m × 0.25 mm × 0.25 μm; flow rate 50 mL/min; pressure 180 kPa; oven temperature 160 °C]: *t*R = 58.8 min for (*S*)-**4**, 60.8 min for (*R*)-**4**, the ee of (*R*)-**4** was determined to be 15.5%.

(1*S*,7a*S*)-**10a**; yield: 9.8 mg (20%); IR: ν_{max} = 3410, 1669, 1651 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.14 (s, 3H), 1.70–1.87 (m, 3H), 2.08–2.19 (m, 2H), 2.21–2.61 (m, 3H), 2.67–2.76 (m, 1H), 3.85 [m, (1*S*,7a*S*), 1H], 3.99 [m, (1*S*,7a*R*), 1H], 5.79 [s, (1*S*,7a*S*), 1H], 5.91 [s, (1*S*,7a*R*), 1H]. Its IR and NMR spectra were identical with those reported previously.[7] Judging from the area of signals at δ = 3.85 for (1*S*,7a*S*)-**10a** and δ = 3.99 for (1*S*,7a*R*)-**10a**, the de of (1*S*,7a*S*)-**10a** was determined to be 61.8%. The alcohol (1*S*,7a*S*)-**10a** was converted to (*S*)-**4**, $[\alpha]_D^{23}$: +229.2 (*c* 0.075, toluene). The ee was confirmed to be 63.8% by GLC analysis as described above.

Reduction of (±)-4,5-Dihydro-7a-methyl-2*H*-indene-2,7(1*H*,6*H*)-dione (6)

The enone (±)-**6** (50.0 mg, 0.305 mmol) was incubated with cells (0.3 g) for 18 h 30 °C. After the work-up, the crude residue was purified by preparative thin-layer chromatography [developed with hexane/ethyl acetate (1/1)].

(*R*)-**6**; yield: 27.2 mg (54%); $[\alpha]_D^{22}$; +25.5 (*c* 0.95, chloroform) {lit.[17] $[\alpha]_D^{20}$: +47.3 (*c* 0.42, chloroform)}; IR: ν_{max} = 1712, 1683, 1623 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.53 (s, 3H),

1.66 – 1.79 (m, 1H), 2.14 (d, $J = 19.0$ Hz, 1H), 2.23 – 2.31 (m, 1H), 2.42 – 2.48 (m, 1H), 2.69 – 2.83 (m, 2H), 2.84 – 2.90 (m, 1H), 3.20 (d, $J = 19.0$ Hz, 1H), 5.81 (s, 1H). Its IR and NMR spectra were identical with those reported previously.[17] Based on the HPLC analysis [column, Daicel Chiralcel OJ, 0.46 cm × 25 cm; hexane/2-propanol (48/1); flow rate 0.5 mL/min]: tR = 102.9 min for (*S*)-**6**, 112.4 min for (*R*)-**6**, the ee of (*R*)-**6** was determined to be 56.9%.

(3a*S*,4*S*)-**11a**; yield: 23.1 mg (46%); IR: $\nu_{max} = 3413$, 1683, 1618 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.23$ (s, 3H), 1.33 – 1.45 (m, 1H), 1.62 – 2.86 (m, 8H), 3.46 [dd, $J = 4.4$, 11.2 Hz, (3a*S*,4*S*), 1H], 3.90 [br s, (3a*R*,4*S*), 1H], 5.83 [d, $J = 2.0$ Hz, (3a*S*,4*S*), 1H], 5.90 [d, $J = 1.5$ Hz, (3a*R*,4*S*), 1H]. Judging from the area of signals at $\delta = 3.46$ for (3a*S*,4*S*)-**11a** and $\delta = 3.90$ for (3a*R*,4*S*)-**11a**, the de of (3a*S*,4*S*)-**11a** was determined to be 70.2%. The alcohol (3a*S*,4*S*)-**11a** was converted to (*S*)-**6**, $[\alpha]_D^{22}$: – 37.7 (*c* 0.185, chloroform). The ee was confirmed to be 68.0% by HPLC analysis as described above.

Reduction of (±)-1-Methylbicyclo[3.3.0]oct-5-ene-2,7-dione (7)

Treatment of (±)-**7** (69.7 mg, 0.464 mmol) with cells (0.4 g) for 18 h at 30 °C, and subsequent purification afforded the products shown in Scheme 2.

(1*S*,5*R*)-**12**; yield: 27.0 mg (38%). A bulb-to-bulb distillation gave an analytical sample of (1*S*,5*R*)-**12**; bp 110 – 120 °C/2.0 mmHg. This solidified upon standing to give needles, mp 59.0 – 60.0 °C; $[\alpha]_D^{20}$: + 144.8 (*c* 0.67, chloroform); IR: $\nu_{max} = 1745$, 1733 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.22$ (s, 3H), 1.66 (ddd, $J = 8.8$, 14.4, 21.6 Hz, 1H), 2.03 – 2.31 (m, 3H), 2.38 – 2.53 (m, 3H), 2.56 – 2.64 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 21.4, 25.0, 36.1, 43.3, 44.2, 46.0, 52.6, 215.7, 219.9; anal. calcd. for $C_9H_{12}O_2$: C 71.03, H 7.95; found: C 71.01, H 7.94.

For the determination of the ee of this product by means of chromatographic method, authentic (±)-(1*S**,5*R**)-**12** was prepared in the following manner. A mixture of (±)-**7**, ethanol and a catalytic amount of palladium on charcoal was vigorously stirred for 1 h at room temperature under H_2. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated under vacuum to afford (±)-(1*S**,5*R**)-**12**. The GLC analysis [column, TCI Chiraldex B-PM, 30 m × 0.25 mm × 0.125 μm; flow rate 50 mL/min; pressure 180 kPa; oven temperature 120 °C]: tR = 86.7 min over 89.7 min. In turn, an authentic specimen (1*R*,5*S*)-**12**, prepared in the same manner from (*R*)-**7** coincided with the peak of 86.7 min. The absolute configuration of **12** of microbial origin was unambiguously confirmed to be (1*S*,5*R*) and its ee was determined to be >99.9%.

(*R*)-**7**; yield: 11.5 mg (16%); $[\alpha]_D^{21}$: – 78.5 (*c* 0.18, chloroform) {lit. [18] $[\alpha]_D^{20}$: – 104 (*c* 0.80, chloroform) for (*R*)-**7** (79% ee)}; IR: $\nu_{max} = 1752$, 1713, 1634 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.35$ (s, 3H), 2.32 (d, $J = 18.1$ Hz, 1H), 2.45 (ddd, $J = 8.8$, 8.8, 19.2 Hz, 1H), 2.60 (d, $J = 18.1$ Hz, 1H), 2.92 – 3.17 (m, 3H), 5.97 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 23.3$, 24.5, 38.3, 44.7, 56.8, 126.1, 184.6, 207.4, 212.3. Its IR and NMR spectra were identical with those reported previously.[18] Based on the HPLC analysis [column, Daicel Chiralcel OJ, 0.46 cm × 25 cm; hexane/2-propanol (9/1); flow rate 0.5 mL/min]: tR = 46.5 min for (*S*)-**7**, 49.4 min for (*R*)-**7**, the ee of (*R*)-**7** was determined to be >99.9%.

13; yield: 45.4 mg (46%); IR: $\nu_{max} = 3451$, 1742, 1733 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.20$ (s, 3H), 2.17 – 2.29 (m, 4H), 2.40 – 2.49 (m, 2H), 2.55 – 2.74 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 15.6$, 32.0, 35.3, 46.6, 50.6, 56.4, 82.8, 212.9, 218.1.

Reduction of (±)-Spiro[5.5]undec-7-ene-1,9-dione (8)

Treatment of (±)-**8**[19] (308 mg, 1.73 mmol) with cells (1.8 g) for 15 h at 30 °C, and subsequent purification afforded the products shown in Scheme 2.

14; yield: 248 mg (80%); IR: $\nu_{max} = 1704$, 1698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.60 – 1.68$ (m, 2H), 1.74 – 1.82 (m, 4H), 1.87 – 1.93 (m, 2H), 2.28 – 2.38 (m, 4H), 2.44 – 2.52 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 21.1$, 28.0, 33.2, 37.7, 38.6, 39.2, 48.0, 211.2, 215.0; anal. calcd. for $C_{11}H_{16}O_2$: C 73.30, H 8.95; found: C 73.16, H 8.75.

15; yield: 30.7 mg (10%); IR: $\nu_{max} = 3448$, 1707 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.17 – 1.63$ (m, 8H), 1.69 – 2.05 (m, 5H), 2.33 (br s, 4H), 3.53 (d, $J = 7.8$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 21.1$, 23.2, 27.4, 30.4, 31.2, 34.2, 36.8, 75.4, 212.7.

Reduction of 2-Methyl-2-(3-oxopentyl)-1,3-cyclohexanedione (19)

Treatment of **19** (1.03 g, 4.88 mmol) with cells (6.0 g) for 24 h at 30 °C, and subsequent purification afforded (1*S*,6*S*)-**20**; yield: 578 mg (56%). This was employed for the next step without further purification. Recrystallization from hexane/ethyl acetate gave an analytical sample of (1*S*,6*S*)-**20** as needles, mp 107.0 – 108.0 °C; $[\alpha]_D^{20}$: – 102.0 (*c* 1.03, chloroform); IR: $\nu_{max} =$ 3490, 1698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.87$ (t, $J =$ 7.3 Hz, 3H), 0.93 – 1.00 (m, 1H), 1.13 (s, 3H), 1.41 – 1.67 (m, 5H), 1.84 (s, 2H), 1.99 – 2.28 (m, 4H), 2.40 – 2.59 (m, 1H), 4.20 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 7.62$, 21.3, 24.4, 26.5, 27.4, 29.1, 36.0, 37.9, 47.4, 75.2, 97.3, 214.4; anal. calcd for $C_{12}H_{20}O_3$: C 67.89, H 9.50; found: C 67.80, H 9.13.

Reduction of 2-Methyl-2-(3-oxobutyl)-1,3-cyclopentanedione (21)

Treatment of **21** (1.51 g, 8.29 mmol) with cells (9.0 g) for 24 h at 30 °C, and subsequent purification afforded the products shown in Scheme 3.

(1*S*,6*S*)-**22**; yield: 961 mg (63%). This was employed for the next step without further purification. Recrystallization from hexane/ethyl acetate gave an analytical sample of (1*S*,6*S*)-**22** as needles, mp 61.0 – 63.0 °C; $[\alpha]_D^{19}$: + 44.4 (*c* 1.02, chloroform); IR: $\nu_{max} = 3490$, 1698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 0.95 (s, 3H), 1.24 – 1.33 (m, 1H), 1.35 (s, 3H), 1.60 (ddd, $J = 2.4$, 2.4, 13.6 Hz, 1H), 1.75 (ddd, $J = 4.9$, 13.6, 13.6 Hz, 1H), 1.90 – 2.00 (m, 3H), 2.11 – 2.20 (m, 1H), 2.34 – 2.38 (m, 2H), 4.26 (d, $J = 3.9$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 21.5$, 24.5, 25.5, 30.3, 31.8, 33.8, 48.2, 76.8, 95.3, 220.8; anal. calcd for $C_{10}H_{16}O_3$: C 65.19, H 8.75; found: C 65.46, H 8.79.

24; yield: 205 mg (14%); IR: $\nu_{max} = 3247$, 3168, 1767, 1726 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.07$ (s, 3H), 1.40 (s, 3H), 1.53 – 1.88 (m, 5H), 2.53 (dd, $J = 7.3$, 19.5 Hz, 1H), 2.79

(d, $J = 7.8$ Hz, 1H), 3.04 (d, $J = 19.5$ Hz, 1H). Its IR and NMR spectra were identical with those reported previously.[20]

(2*R*,3*S*)-**23a**; yield: 105 mg (7%); $[\alpha]_D^{19}$: −49.4 (*c* 1.15, chloroform) {lit.[11] $[\alpha]_D^{8}$: −45.4 (*c* 2.03, chloroform)}; IR: $\nu_{max} = 3448$, 2968, 1732, 1714 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 0.98$ (s, 3H), 1.67 (ddd, $J = 7.3$, 7.3, 14.6 Hz, 1H), 1.78 (ddd, $J = 7.3$, 7.3, 14.6 Hz, 1H), 1.88 (dddd, $J = 7.3$, 7.3, 9.8, 12.3 Hz, 1H), 2.15 (s, 3H), 2.17–2.31 (m, 2H), 2.42–2.56 (m, 3H), 4.00 (dd, $J = 6.3$, 6.3 Hz, 1H). Its IR and NMR spectra were identical with those reported previously.[11]

(1*S*,2*S*)-2-Methyl-3-oxo-2-(3-oxopentyl)cyclohexyl Acetate (27)

A mixture of (1*S*,6*S*)-**20** (263 mg, 1.24 mmol), anhydrous pyridine (4 mL), acetic anhydride (4 mL), and a catalytic amount of 4-dimethylaminopyridine was stirred for 16 h at room temperature. The reaction mixture was poured into water and extracted with ethyl acetate. The combined organic layer was washed with 6 M hydrochloric acid, water, saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate, and concentrated under vacuum. The residue was charged on a silica gel column (10 g). Elution with hexane/ethyl acetate (4/1 to 2/1) afforded (1*S*,2*S*)-**27**; yield: 308 mg (98%). This was employed for the next step without further purification. A bulb-to-bulb distillation gave an analytical sample of (1*S*,2*S*)-**27** as an oil, bp 170–180 °C/2.0 mmHg; $[\alpha]_D^{20}$: +40.8 (*c* 1.12, chloroform); IR: $\nu_{max} =$ 1738, 1712, 1235 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.02$ (t, $J = 7.3$ Hz, 3H), 1.07 (s, 3H), 1.63–1.71 (m, 1H), 1.80 (ddd, $J =$ 5.4, 5.4, 15.2 Hz, 1H), 1.90–2.13 (m, 7H), 2.15–2.24 (m, 1H), 2.27–2.42 (m, 5H), 4.85 (dd, $J = 3.4$, 7.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 7.9$, 19.1, 20.5, 21.1, 25.7, 25.9, 36.1, 36.3, 37.5, 52.1, 78.2, 170.0, 210.6, 212.0; anal. calcd for $C_{14}H_{22}O_4$: C 66.12, H 8.72; found: C 66.05, H 8.77.

(1*S*,8a*R*)-1,2,3,4,6,7,8,8a-Octahydro-5,8a-dimethyl-6-oxonaphthyl Acetate (9b)

A mixture of (1*S*,2*S*)-**27** (191 mg, 0.750 mmol), benzene (15 mL), and a catalytic amount of *p*-toluenesulfonic acid was stirred under reflux for 10 h with 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 washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under vacuum. The residue was charged on a silica gel column (12 g). Elution with hexane/ethyl acetate (4/1 to 2/1) afforded (1*S*,8a*R*)-**9b**; yield: 147 mg (83%). This was employed for the next step without further purification. Recrystallization from hexane/ethyl acetate gave an analytical sample of (1*S*,8a*R*)-**9b** as needles, mp 78.0–78.5 °C; $[\alpha]_D^{21}$: −90.2 (*c* 1.00, chloroform) {lit.[12] $[\alpha]_D^{30}$: −79 (*c* 1.4, chloroform) for (1*S*,8a*R*)-**9b**}; IR: $\nu_{max} = 1726$, 1664, 1606, 1243 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.28$ (s, 3H), 1.46 (ddd, $J =$ 3.3, 5.2, 13.3 Hz, 1H), 1.63–1.77 (m, 3H), 1.79 (s, 3H), 1.84–2.01 (m, 1H), 2.04 (m, 3H), 2.11–2.20 (m, 2H), 2.41–2.55 (m, 2H), 2.73–2.79 (m, 1H), 4.80 (dd, $J = 2.9$, 2.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 11.3$, 20.1, 21.3, 22.2, 25.6, 26.8, 30.8, 33.7, 39.7, 77.7, 130.7, 158.8, 170.3, 198.3; anal. calcd for $C_{14}H_{20}O_3$: C 71.16, H 8.53; found: C 70.99, H 8.55.

For the confirmation of the absolute configuration of **9b** of microbial origin, an authentic (1*R*,8a*S*)-**9b** was prepared as described in Scheme 4;[13,14] $[\alpha]_D^{19}$: +77.0 (c 0.915, chloroform) for (1*R*,8a*S*)-**9b**. HPLC analysis [column, Daicel Chiralcel OJ, 0.46 cm × 25 cm; hexane/2-propanol (9/1); flow rate 0.5 mL/min]: *t*R = 31.4 min over 34.5 min. In turn, an authentic specimen (1*R*,8a*S*)-**9b** showed a major peak at 31.4 min and that was revealed to be 84.3% ee. The absolute configuration of **9b** of microbial origin was unambiguously confirmed to be (1*S*,8a*R*) and its ee was determined to be >99.9%.

(1*S*,2*S*)-2-Methyl-3-oxo-2-(3-oxobutyl)cyclopentyl Acetate (23b)

A mixture of (1*S*,6*S*)-**22** (305 mg, 1.66 mmol), anhydrous pyridine (4 mL), acetic anhydride (3 mL), and a catalytic amount of 4-dimethylaminopyridine was stirred for 24 h at room temperature. The extraction and work-up were performed as described above. The residue was charged on a silica gel column (20 g). Elution with hexane/ethyl acetate (4/1 to 1/2) afforded (1*S*,2*S*)-**23b**; yield: 371 mg (99%). This was employed for the next step without further purification. Recrystallization from hexane/ethyl acetate gave an analytical sample of (1*S*,2*S*)-**23b** as needles, mp 76.5–77.5 °C; $[\alpha]_D^{28}$: +51.9 (*c* 1.08, chloroform); IR: $\nu_{max} = 1731$, 1706, 1245 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.03$ (s, 3H), 1.70 (ddd, $J =$ 5.0, 5.0, 19.5 Hz, 1H), 1.88 (ddd, $J = 5.0$, 5.0, 15.2 Hz, 1H), 1.97–2.05 (m, 1H), 2.07 (s, 3H), 2.13 (s, 3H), 2.24–2.49 (m, 4H), 2.57 (ddd, $J = 5.9$, 10.3, 17.6 Hz, 1H), 5.11 (dd, $J = 5.1$, 5.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 19.5$, 21.1, 24.3, 25.3, 30.1, 34.4, 37.9, 50.9, 79.1, 170.2, 207.8, 218.3; anal. calcd for $C_{12}H_{18}O_4$: C 63.70, H 8.02; found: C 63.83, H 8.06.

(1*S*,7a*R*)-2,3,5,6,7,7a-Hexahydro-7a-methyl-5-oxo-1*H*-indenyl Acetate (10b)

A mixture of (1*S*,2*S*)-**23b** (191 mg, 0.844 mmol), benzene (15 mL), and a catalytic amount of *p*-toluenesulfonic acid was stirred under reflux for 77 h with azeotropic removal of water. The extraction and work-up were performed as described above. The residue was charged on a silica gel column (20 g). Elution with hexane/ethyl acetate (4/1 to 1/1) afforded (1*S*,7a*R*)-**10b**; yield: 124 mg (71%). A bulb-to-bulb distillation gave an analytical sample of (1*S*,7a*R*)-**10b** as an oil, bp 150 °C/2.0 mmHg; $[\alpha]_D^{27}$: −5.5 (*c* 1.11, chloroform); IR: $\nu_{max} = 1741$, 1671, 1241 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta =$ 1.20 (s, 3H), 1.71 (ddd, $J = 2.0$, 5.4, 13.2 Hz, 1H), 1.89 (ddd, $J =$ 4.0, 8.4, 15.0 Hz, 1H), 2.03 (s, 3H), 2.04–2.11 (m, 1H), 2.25–2.42 (m, 2H), 2.52 (ddd, $J = 5.4$, 14.4, 17.8 Hz, 1H), 2.64 (ddd, $J = 1.7$, 8.5, 19.5 Hz, 1H), 2.70–2.76 (m, 1H), 5.04 (d, $J =$ 4.9 Hz, 1H), 5.86 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 21.1, 21.7, 28.3, 28.5, 28.8, 33.0, 46.7, 81.3, 122.8, 170.2, 174.6, 198.5; anal. calcd for $C_{12}H_{16}O_4$: C 69.21, H 7.74; found: C 68.95, H 7.74.

For the determination of the absolute configuration of **10b** of microbial origin, an authentic (1*R*,7a*S*)-**10b** was prepared as described in Scheme 5;[14,15] $[\alpha]_D^{20}$: +9.5 (*c* 0.18, chloroform) for (1*R*,7a*S*)-**10b**. HPLC analysis [column, Daicel Chiralcel OJ,

0.46 cm × 25 cm; hexane/2-propanol (15/1); flow rate 0.5 mL/min]: tR = 62.5 min over 72.3 min. In turn, an authentic specimen (1*R*,7a*S*)-**10b** showed a major peak at 62.5 min and that was revealed to be 98.1% ee. The absolute configuration of **10b** of microbial origin was unambiguously confirmed to be (1*S*,7a*R*) and its ee was determined to be >99.9%.

(1*S*,2*R*)-2-Methyl-3-oxo-2-(3-oxobutyl)cyclopentyl Acetate (23b)

A mixture of (2*R*,3*S*)-**23a** (31.3 mg, 0.169 mmol), anhydrous pyridine (1 mL), and acetic anhydride (1.5 mL) was stirred for 12 h at room temperature. The extraction and work-up were performed as described above. The residue was purified by preparative thin-layer chromatography [developed with hexane/ethyl acetate (1/1)] to afford (1*S*,2*R*)-**23b**; yield: 29.0 mg (quant.). This was employed for the next step without further purification. $[\alpha]_D^{22}$: +11.1 (*c* 1.45, chloroform) {lit.[11] $[\alpha]_D^{27}$: +10.3 (*c* 3.58, chloroform)}; IR: ν_{max} = 1737, 1716, 1243 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.97 (s, 3H), 1.66 (ddd, *J* = 5.6, 9.0, 14.4 Hz, 1H), 1.77 (ddd, *J* = 5.9, 9.0, 14.6 Hz, 1H), 1.90–2.07 (m, 1H), 2.06 (s, 3H), 2.13 (s, 3H), 2.27–2.46 (m, 4H), 2.55 (ddd, *J* = 5.9, 9.1, 17.9 Hz, 1H), 5.18 (dd, *J* = 4.4, 4.4 Hz, 1H). Its IR and NMR spectra were identical with those reported previously.[11]

(1*S*,7a*S*)-2,3,5,6,7,7a-Hexahydro-7a-methyl-5-oxo-1*H*-indenyl Acetate (10b)

A mixture of (1*S*,2*R*)-**23b** (23.1 mg, 0.102 mmol), benzene (10 mL), and a catalytic amount of *p*-toluenesulfonic acid was stirred under reflux for 48 h with azeotropic removal of water. The extraction and work-up were performed as described above. The residue was purified by preparative thin-layer chromatography [developed with hexane/ethyl acetate (1/1)] to afford (1*S*,7a*S*)-**10b**; yield: 16.9 mg (79%); $[\alpha]_D^{20}$: +28.7 (*c* 0.86, chloroform) {lit.[11] $[\alpha]_D^{29}$: +28.6 (*c* 3.67, chloroform)}; IR: ν_{max} = 1739, 1670, 1240 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.18 (s, 3H), 1.82–1.92 (m, 2H), 2.23 (ddd, *J* = 2.4, 5.4, 13.1 Hz, 1H), 2.09 (s, 3H), 2.24–2.54 (m, 4H), 2.76 (dddd, *J* = 2.4, 2.4, 11.7, 19.5 Hz, 1H), 4.80 (dd, *J* = 7.8, 9.8 Hz, 1H), 5.80 (s, 1H). Its IR and NMR spectra were identical with those reported previously.[11] Based on the HPLC analysis [column, Daicel Chiralcel OJ, 0.46 cm × 25 cm; hexane/2-propanol (9/1); flow rate 0.4 mL/min]: tR = 33.5 min for (1*S*,7a*S*)-**10b**, 35.8 min for (1*R*,7a*R*)-**10b**, the ee of (1*S*,7a*S*)-**10b** was determined to be >99.9%.

Method of Preparation of Long-Term Preservable Cells

Air-drying: The incubation of *T. delbrueckii* IFO10921 was performed as described above. The freshly harvested wet cells (9 g) were resuspended in 20 mL of phosphate buffer (0.1 M, pH 6.5). The cell suspension was added to a solution of a commercially available skim milk (6 g) in 10 mL of phosphate buffer (0.1 M, pH 6.5) at 0 °C. The suspension was further stirred for 30 min at 0 °C, and then sprayed into a balloon, which was connected with an air-drier as shown in Figure 2. A domestic air-drier [A] for clothes (35 °C) was connected to a plastic bag [B], and the cell suspension [C] was sprayed inside and then dried. The cells were exposed to warm and dry air at 35 °C overnight, to give air-dried cells (*ca.* 6 g).

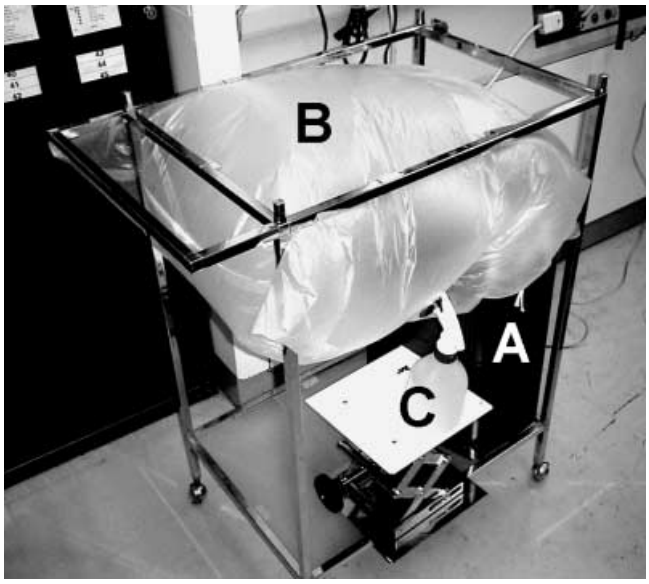


Figure 2. [A] Air-drier; [B] plastic bag; [C] cell suspension in a sprayer.

Freeze-drying: The wet cells of *T. delbrueckii* IFO10921 (15 g), skim milk (10 g) and glycerol (0.6 g) were resuspended in 50 mL of phosphate buffer (0.1 M, pH 6.5) at 0 °C. After stirring for 30 min at 0 °C, the suspension was frozen by immersion in liquid N_2, and then lyophilized at 0.03 torr overnight to give freeze-dried cells (*ca.* 13 g).

Oven-drying: The wet cells of *T. delbrueckii* IFO10921 (9 g) were resuspended in 20 mL of phosphate buffer (0.1 M, pH 6.5) at 0 °C. The thick suspension was spread on a silicone-coating cooking sheet in an as thin film as possible. This was dried on an electric hot plate for 15 min at 80 °C to give oven-dried cells (*ca.* 6 g).

Typical Procedure of Reduction with Air-Dried Cells

A mixture of **21** (100 mg, 0.548 mmol), glucose (0.2 g), air-dried cells (1.0 g), and phosphate buffer (0.1 M, pH 6.5), (10 mL) was shaken on a reciprocal shaker (250 cpm) in a test tube for 20 h at 30 °C, and subsequent purification afforded the product (1*S*,6*S*)-**22**; yield: 52.1 mg (51%). The ee of (1*S*,6*S*)-**22** was confirmed to be 99.8% according to the method as described before. Other results are summarized in Table 4.

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