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BIOCHEMICAL PREPARATIONS OF BOTH THE ENANTIOMERS OF METHYL 3-HYDROXYPENTANOATE AND THEIR CONVERSION TO THE ENANTIOMERS OF 4-HEXANOLIDE, THE PHEROMONE OF *TROGODERMA GLABRUM*†

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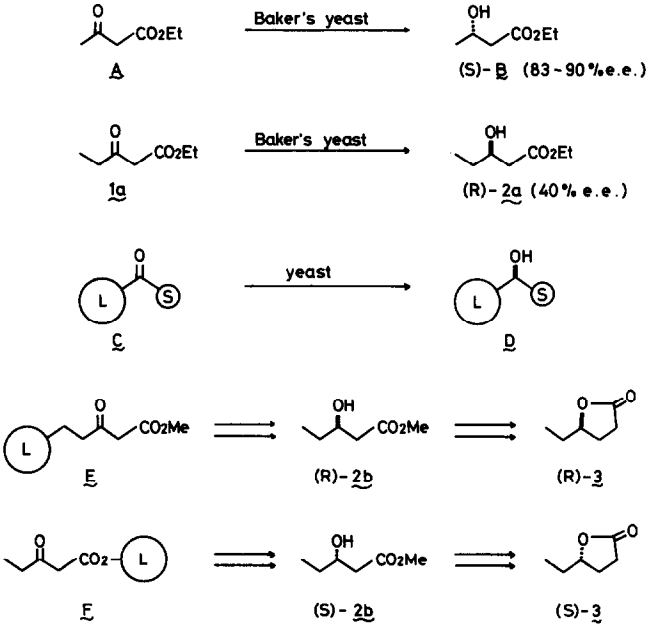
Abstract—Both the enantiomers of methyl 3-hydroxypentanoate were prepared by microbial asymmetric reduction of 3-oxopentanoic esters. Conversion of methyl 3-hydroxypentanoate to 4-hexanolide, the pheromone of *Trogoderma glabrum*, was achieved.

Utilisation of biochemical systems is one of the effective methods in asymmetric synthesis.[1] Usually only one enantiomer is produced by a particular organism. For example, baker's yeast reduces ethyl acetoacetate **A** to the (*S*)-enantiomer of ethyl 3-hydroxybutanoate **B**.[2] This is indeed the basis for using a biochemical system in chiral synthesis. When both the enantiomers are necessary, however, one must rely on the Walden inversion to obtain the unavailable antipode. It is therefore desirable to develop some methods capable of producing both the enantiomers by simple biochemical processes. Another drawback in bioorganic synthesis is that the optical yield in a biochemical reaction is not always satisfactory. An example is the reduction of ethyl 3-oxopentanoate **1a** with baker's yeast giving ethyl (*R*)-3-hydroxypentanoate **2a** of only 40% e.e.[3] This poor result can be explained by the so-called Prelog rule as shown in the process **C** → **D**.[4] The CO group of **C**, flanked by a large bulky group L and a small group S, is reduced asymmetrically with yeast to yield **D**. If the bulkiness of L is not so different from that of S, the product **D** will be of low optical purity. It then should be possible to obtain both the enantiomers of an alcohol by changing the size of the two substituents attached to the CO group, desirably in high optical yield, if one can adjust the difference between the bulkiness of the two substituents in an optimal condition.



Scheme 1.

The enantiomers of 3-hydroxypentanoic esters **2** are useful starting materials for natural products synthesis.[3,5,6] An insect pheromone such as 4-hexanolide **3**[7] may be readily synthesised from **2b**. To synthesise both the enantiomers of **3**, it became

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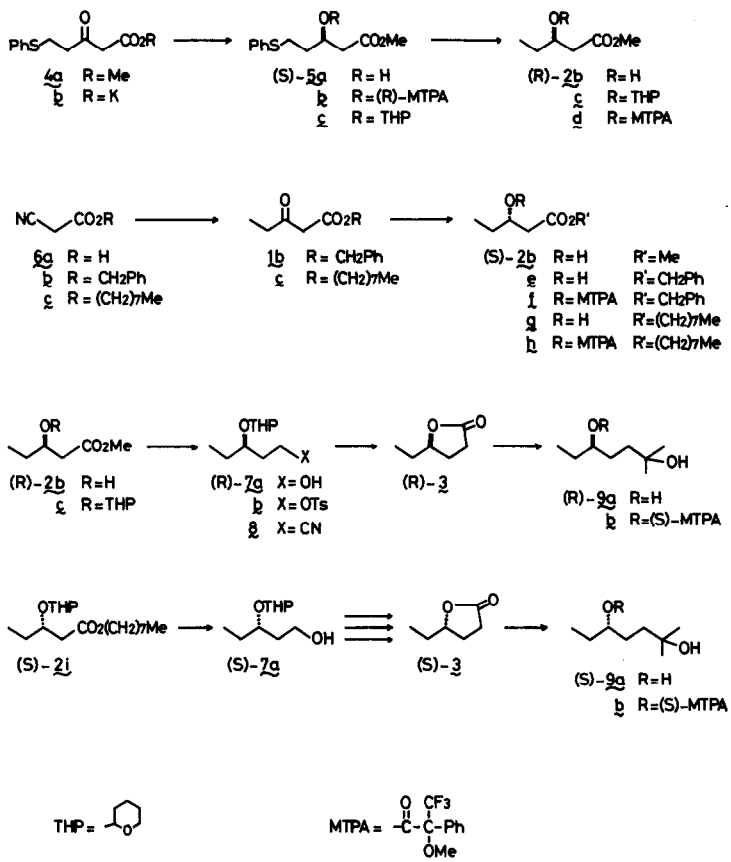

Scheme 2.

necessary to prepare the enantiomers of **2**. We reasoned that the biochemical reduction of **E** with a bulky group L at the end of the alkyl chain would give rise to (*R*)-**2b** after reductive removal of L. On the other hand, reduction of **F** with a bulky group L at the end of the ester group would afford (*S*)-**2b** after replacing L with Me. This paper describes the methods by which both (*R*)- and (*S*)-**2b** were prepared and further converted into the enantiomers of 4-hexanolide **3**.

Preparation of methyl (R)-3-*hydroxypentanoate* **2b**

A known keto ester **4a**[8] was chosen as our substrate for biochemical reduction. This was readily prepared by alkylating the dianion of methyl acetoacetate with phenylthiomethyl iodide.[8] The PhS group seemed to be bulky enough and its reductive removal with Raney Ni is well known. We first tried the reduction of the corresponding K salt **4b** with baker's yeast, because Hirama *et al.* reported that the K salt of β-keto acids could be reduced efficiently by treatment with baker's yeast.[9] Saponification of **4a** in MeOH with KOH aq gave an aq soln of **4b**. This was added to the fermenting baker's yeast. After 35 hr the product was isolated and esterified with CH_2N_2 to give (+)-**5a**. Its optical purity was estimated to be 100% by the HPLC analysis of the corresponding (*R*)-α-methoxy-α-trifluoromethylphenylacetate (MTPA ester) **5b**.[10] With this gratifying result several attempts were made to obtain optically pure (+)-**5a** in high yield. Unfortunately, however, decomposition of the substrate during the alkaline hydrolysis of **4a** yielded Ph_2S_2 as a byproduct and the highest yield of (+)-**5a** realised by us was only 7.7%.

We therefore abandoned this approach and attempted the direct reduction of **4a** with various yeasts. After isolating the product **5a**, its optical purity was determined by the HPLC analysis of the corresponding MTPA ester **5b**. As shown in Table 1, different species of yeast brought about differences in the yield, the optical purity and the sign of the optical rotation of the product **5a**. The $[\alpha]_D$ value was not such a reliable criterion of the optical purity because of the possible contamination of a small amount of highly optically active byproducts. The highest optical yield was realised with a thermophilic yeast *Pichia terricola* KI 0117 giving (+)-**5a** of 94–100% e.e.

So as to establish its absolute configuration, a sizable amount of (+)-**5a** was demanded. Therefore the reduction of **4a** with *Pichia terricola* KI 0117 was carried out on a preparative scale employing 5 g of **4a**. The desired (+)-**5a** (100% e.e.) was obtained in 26% yield based on the amount of the consumed **4a**. 4-Phenylthio-2-butanone was also obtained as a byproduct and 36% of **4a** was recovered unchanged. Then the OH group of (+)-**5** was protected as a THP ether **5c**. Treatment of **5c** with Raney Ni W-7 resulted in desulfurisation to give **2c**. Removal of the THP protecting group of **5c** yielded (−)-**2b**, $[\alpha]_D^{20}$ −32.9° ($CHCl_3$), identical with the known (*R*)-**2b** [lit.[5] $[\alpha]_D^{22}$ −36.9° ($CHCl_3$)]. The absolute configuration of (+)-**5a** was therefore assigned to *S*. The present sample of (*R*)-**2b** was of 90.4% e.e. as determined by the HPLC analysis of the corresponding MTPA ester **2d**. The absolute configuration of the β-hydroxy ester **5a**

Table 1. Reduction of methyl 5-phenylthio-3-oxopentanoate **4a** to methyl 5-phenylthio-3-hydroxypentanoate **5a** with several types of yeast

Yeast	Reaction time (hr)	The product **5a**			
		Yield (%)	$[\alpha]_D$ ($CHCl_3$) (°)	Optical purity (% e.e.)	Absolute configuration
Saccharomyces cerevisiae NCYC 240	35	35.7	+13.8	65.1	*S*
S. uvarum IFO 1225	36.5	57.7	−13.8	68.8	*R*
S. carlsbergensis IFO 0565	34	28.3	+18.2	85.2	*S*
S. bailii KI 0116	44.5	34.7	+5.7	26.5	*S*
Pichia terricola KI 0117	32.5	53.2	+18.3	94.0	*S*
Baker's yeast	3.25	88.0	−1.25	41.5	*R*

produced by six different yeasts was assigned as shown in Table 1 by scrutinising the HPLC analytical data of **5b** derived from **5a**. Four out of six kinds of the examined yeasts produced (*S*)-**5a**, while baker's yeast and *Saccharomyces uvarum* IFO 1225 afforded (*R*)-**5a**. The enantioselectivity of the enzyme(s) responsible for the reduction of **4a** to **5a** was different between these two groups of yeasts.

The above result demonstrated that the yeast reduction of **4a** to **5a** gives optically pure (*S*)-**5a** which can be converted to (*R*)-**2b**. However, this approach seems to be impractical in view of the unsatisfactory chemical yield in the yeast reduction step. A far more efficient preparative method of (*R*)-**2b** is to utilise the β-oxidation of pentanoic acid with *Candida rugosa* IFO 0750.[11]

Preparation of methyl (S)-3-*hydroxypentanoate* **2b**

In order to prepare highly optically pure (*S*)-**2b**, it was necessary to select a suitable bulky group as L in the formula **F**. Very recently Zhou *et al.* reported that the stereochemistry of yeast reduction of γ-chloroacetoacetic esters can be altered from *S* to *R* by modifying the size of the ester grouping.[12] They obtained a highly optically pure (*R*)-hydroxy ester by reducing octyl γ-chloroacetoacetate.[12]

We therefore prepared benzyl and octyl esters (**1b** and **1c**) of 3-oxopentanoic acid and examined their yeast reduction. Esterification of cyanoacetic acid **6a** with either benzyl alcohol or *n*-octyl alcohol yielded **6b** or **6c**. These were separately treated with EtMgI to give the required substrates **1b** and **1c**. Reduction of the benzyl keto ester **1b** with baker's yeast gave (+)-**2e** in 85% yield, whose optical purity was estimated to be 45% by the HPLC analysis of the corresponding MTPA ester **2f**. Similarly reduction of the n-octyl keto ester **1c** with baker's yeast gave (+)-**2g** in 64% yield. The optical purity of (+)-**2g** was 97.4%. The absolute configuration of (+)-**2g** was determined to be *S* by its conversion to (*S*)-**2b** (93% e.e.) by successive alkaline hydrolysis, acidification and esterification with CH_2N_2. The absolute configuration of (+)-**2e** was also shown to be *S* by the same procedure.

Yeast reduction of octyl 3-oxopentanoate **1b** is indeed a very simple preparative method for (*S*)-3-hydroxypentanoic esters of high optical purity. Very recently Dr. J. Hasegawa of Kanegafuchi Chemical Industry Co., Ltd. kindly informed us that he could also prepare methyl (*S*)-3-hydroxypentanoate **2b** (76% e.e.) by microbial β-oxidation of pentanoic acid.[13]

Synthesis of the enantiomers of 4-*hexanolide* **3**

4-Hexanolide **3** was isolated and identified as a component of the pheromone secreted by female dermestid beetle *Trogoderma glabrum*.[7] *Trogoderma granarium* was reported to respond to (*R*)-(+)-**3**, but to neither (*S*)-(−)-**3** nor (±)-**3**.[14] There exist several syntheses of the enantiomers of **3**. The first synthesis as reported by Ravid *et al.* started from glutamic acid and led to the highly optically pure enantiomers of **3**, $[\alpha]_D^{20}$ ±53.2° (MeOH), although in a small amount [37.4 mg of (*R*)-(+)-**3**].[14] Later Vigneron and Bloy prepared (*R*)-(+)-**3**, $[\alpha]_D^{25}$ +51.5° (MeOH), by asymmetric reduction of an acetylenic ketone.[15] Another asymmetric reduction by Midland and Tramontano yielded (*R*)-**3** of 87% e.e.[16] Two bioorganic syntheses of **3** were reported employing yeast reduction: Bernardi *et al.* prepared (*S*)-**3** of ~80% e.e.[17] and Naoshima *et al.* obtained (*R*)-**3** of 83% e.e.[18] Thus only Ravid *et al.* were successful in preparing both the enantiomers of **3** in a highly optically pure state.[14] Our success in securing both the enantiomers of **2** by bioorganic synthesis made us attempt a simple synthesis of the enantiomers of **3** of ≥95% e.e. in quantities sufficient for thorough biological work.

Our synthesis is straightforward as shown in Scheme 2 involving the one-carbon elongation of alkyl 3-hydroxypentanoate **2**. Protection of the OH group of (*R*)-**2b** (100% e.e.) as a THP ether in the conventional manner gave (*R*)-**2c**. This was reduced with LAH to (*R*)-**7a**, whose tosylate **7b** was treated with NaCN in DMF to give (*R*)-**8**. Alkaline hydrolysis of (*R*)-**8** was followed by acidification to give (*R*)-4-hexanolide **3** (897 mg), $[\alpha]_D^{21}$ +53.1° (MeOH), in 51% overall yield from (*R*)-**2b**. Similarly, (*S*)-**3** was prepared from (*S*)-**2g** of 97% e.e. Reduction of (*S*)-**2i** with LAH gave (*S*)-**7a** and n-octyl alcohol. After chromatographic purification to remove *n*-octyl alcohol, (*S*)-**7a** was converted to (*S*)-4-hexanolide **3** (1.335 g), $[\alpha]_D^{21}$ −50.6° (MeOH).

In order to determine the optical purity of the enantiomers of **3**, they were treated with MeMgI to give the enantiomers of a diol **9a** (*cf* ref. 19). The NMR measurements at 100 MHz of the enantiomers of **9a** in the presence of *tris*[3-(trifluoromethylhydroxymethylene)-*d*-camphorato]europium (III) [Eu(tfc)$_3$] revealed them to be highly optically pure (>95% e.e.). More accurate determination of the optical purity was made possible by the HPLC analysis of the corresponding MTPA esters **9b**. By this method (*R*)-**3** was shown to be of 100% e.e., while the optical purity of (*S*)-**3** was 95%.

In conclusion both the enantiomers of 3-hydroxypentanoic esters were prepared by yeast reduction, which served as the starting materials in the synthesis of 4-hexanolide, the pheromone component of the dermestid beetle.

EXPERIMENTAL

All b.ps were uncorrected. IR spectra were measured as film on a Jasco A-102 spectrometer unless otherwise stated. NMR spectra were recorded at 60 MHz with TMS as an internal standard on a Hitachi R-24A spectrometer. Optical rotations were measured on a Jasco DIP-140 polarimeter. GLC analyses were performed on a Jeol-20K or Hitachi 163 gas chromatograph.

Methyl 5-phenylthio-3-oxopentanoate **4a**

This was prepared from α-iodothioanisole and methyl acetoacetate in 74.1% yield,[8] ν_{max} 1745 (s), 1720 (s), 1655 (w), 1630(w), 1585(m), 740(s), 690(s) cm^{-1}; $\delta(CCl_4)$ 2.50–3.20(4H, m), 3.28 (2H, s), 3.60 (3H, s), 7.15 (5H, br s).

Methyl (±)-5-phenylthio-3-hydroxypentanoate (±)-5a

A soln of **4a** (1g, 4.2 mmol) in MeOH (10 ml) was stirred and cooled with an icebath. To this was added $NaBH_4$ (1 g, 2.65 mmol) portionwise at 2°. The mixture was stirred for 1.5 hr at 2–4°. The excess $NaBH_4$ was destroyed by the addition of AcOH. The mixture was poured into brine and extracted with EtOAc. The extract was washed with sat $NaHCO_3$ soln and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed over SiO_2 (20 g) to give 0.44 g (46.6%) of (±)-**5a**, n_D^{23} 1.5405; ν_{max} 3400 (br m), 1740 (s), 1585 (m), 1440(s), 1285(m), 1200(m), 1175(m), 740(m), 690(m) cm^{-1}; δ ($CDCl_3$) 1.6–2.0 (2H, m), 2.45 (2H, d, J = 6 Hz), 3.03 (2H, t, J = 7 Hz), 3.32(1H, s), 3.65(3H, s), 3.90–4.40(1H, m), 7.26(5H, s). (Found: C, 58.80; H, 6.86. Calc for $C_{12}H_{16}O_3S$: C, 59.97; H, 6.71%.)

Optically active forms of methyl 5-phenylthio-3-hydroxypentanoate **5a**

(a) *Yeast reduction via the K salt* **4b**. To a stirred soln of **4a** (1 g, 4.2 mmol) in MeOH (6.8 ml) was added KOH soln (0.623 N, 6.75 ml) and the mixture was stirred overnight at room temp. During that period a white solid of Ph_2S_2 precipitated. The soln was concentrated *in vacuo* to give a soln of **4b**. This was added to a shaken suspension of baker's yeast (Oriental Yeast Co. dry yeast, 14 g) in sucrose soln (24 g in 200 ml of tap water) at 30°. Each 24 g of sucrose was added to the flask after 6 and 18 hr and the reduction was continued for 35 hr at 30°. Ether was added to the medium. The mixture was filtered through Celite. The filtrate was saturated with NaCl, adjusted to pH 2 with conc HCl and extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo* to give the crude hydroxy acid. This was treated with CH_2N_2 in ether to give crude (+)-**5a** (0.54 g), which was purified by SiO_2 chromatography to give (+)-**5a** (77.7 mg, 7.7%), $[\alpha]_D^{22}$ + 16.3° (c = 1.085, $CHCl_3$). Its IR and NMR spectra were identical with those of the racemate. HPLC analysis of **5b** (column, Partisil 5, 25 cm × 4.6 mm; solvent, n-hexane–THF–MeOH (6000 : 100 : 1); press, 30 kg/cm^2; flow rate, 1 ml/min; detector SPD-1, 217 nm): Rt 91 min (100%). The diastereomer [(+)-**5a** + (*S*)-MTPA] was eluted at Rt 100 min. The HPLC analysis of the MTPA ester of the hydroxy esters related to **5a** was carried out in the same manner as described here to determine the optical purity of the hydroxy esters.

(b) *Direct yeast reduction of* **4a**. Baker's yeast (Oriental dry yeast, 15 g) was added to a soln of sucrose (30 g) in tap water (200 ml) and the suspension was shaken at 30° for 10 min. A suspension of **4a** (1g, 4.2 mmol) in 0.2% Triton X-100 soln (20 ml) was added to the yeast suspension and the mixture was shaken at 30°. After 2 hr sucrose (12 g) was added to the mixture. The reaction was stopped after 3.25 hr in total by the addition of ether. The mixture was filtered through Celite. The filtrate was saturated with NaCl and extracted with ether. The extract was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 1.22 g of crude **4a**. This was chromatographed over SiO_2 (20 g) to give 890 mg (88.3%) of (−)-**4a**, $[\alpha]_D^{24}$ − 1.25° (c = 1.283, $CHCl_3$); optical purity = 41.5%.

(c) *Reduction of* **4a** *with various brewer's yeasts and thermophilic yeasts.* Each of the yeasts shown in Table 1 was precultivated in a medium (200 ml) containing malt extract (4 g), peptone (0.2 g) and glucose (4 g) for 1.5 days at 30° (40° in the case of thermophilic yeasts) with shaking. This was added to 1.8 l. of the same medium and the cultivation was continued for 1.5 days at 30° on a gyrorotary shaker. Then the yeast cells were collected by centrifugation (3000 rpm, 5 min) and washed out by a glucose soln (10 g in 100 ml of pure water) warmed at 30° (or 40°). To the resulting yeast suspension was added a suspension of **4a** (500 mg, 2.1 mmol) in 0.2% Triton X-100 (10 ml) and the mixture was shaken at 30° (or 40°). The rate of reduction was monitored by the TLC analysis of the culture medium. An appropriate amount of glucose was added occasionally. When the rate of reduction became very slow, the reaction was stopped by the addition of ether. The mixture was filtered through Celite. The filtrate was saturated with NaCl and extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo* to give crude **5a**, which was purified by SiO_2 chromatography. The results are summarised in Table 1.

(d) *Reduction of* **4a** *in a preparative scale employing* Pichia terricola KI 0117. The yeast was precultivated in eight Sakaguchi flasks containing 100 ml each of the culture medium containing malt extract (2 g), peptone (0.1 g) and glucose (2 g) for 1.5 days at 40°. A culture medium containing malt extract (10 g), glucose (10 g), NH_4NO_3 (5 g) and peptone (1 g) in water (1 l.) was prepared and 1.8 l. of it was placed in a 5 l.-flask. The precultivated suspension of the yeast (200 ml) was added to the 5 l.-flask. Four 5 l.-flasks were used for the large-scale preparation of the yeast-cells. The flasks were shaken at 37° on a gyrorotary shaker. After 1 day, malt extract (20 g) was added to each of the flasks. The flasks were shaken at 37° for another day. Then the yeast-cells were collected by centrifugation. The cells were added to a new medium and shaken for another day. This procedure was repeated five times to give *ca* 240 g of the wet cells of *Pichia terricola* KI 0117. This was suspended in a sucrose soln (200 g in 1.5 l. of pure water). A suspension of **4a** (5 g) in 0.2% Triton X-100 soln (80 ml) was added to the yeast and the mixture was stirred at 40°. Glucose was added occasionally and after 48 hr, the reaction was stopped by the addition of ether. The mixture was filtered and the filtrate was extracted with ether. The ether extract was washed with brine and dried ($MgSO_4$). The filter-cake (yeast cells) was added to EtOAc and stirred for 6–7 hr. It was then filtered through Celite and the EtOAc soln was separated. The EtOAc soln was washed with brine and dried ($MgSO_4$). The combined ether–EtOAc soln was concentrated *in vacuo* to give 4.7 g of a crude oil. This was chromatographed over SiO_2 (80 g) to give the recovered **4a** (1.8 g), 4-phenylthio-2-butanone (0.58 g) and (+)-**5a** (839 mg, 26.0% yield based on the consumed **4a**), n_D^{19} 1.5384; $[\alpha]_D^{19}$ + 16.8° (c = 1.045, $CHCl_3$) (in other runs: 18.8°, 18.2°). Its IR and NMR spectra were identical with those of (±)-**5a**. (Found: C, 59.85; H, 6.79. Calc for $C_{12}H_{16}O_3S$: C, 59.97; H, 6.71%.)

Methyl (S)-5-phenylthio-3-tetrahydropyranyloxypentanoate **5c**

p-TsOH (10 mg) was added to a stirred soln of (+)-**5a** (800 mg, 3.33 mmol; prepared with *P. terricola*) and dihydropyran (500 mg, 5.95 mmol) in dry CH_2Cl_2 (15 ml). The mixture was stirred for 1.2 hr at room temp, poured into sat $NaHCO_3$ soln (15 ml) and extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo* to give an oil. This was chromatographed over SiO_2 (5 g) to give (*S*)-**5c** (1.09 g, quantitative yield), ν_{max} 1740 (s), 1585 (m), 1025 (s) cm^{-1}. This was employed in the next step without further purification.

Methyl (R)-3-*tetrahydropyranyloxypentanoate* **2c**

Raney Ni W-7 (9.0 g) was added to a soln of (*S*)-**5c** (1.09 g, 3.36 mmol) in MeOH (150 ml) and the mixture was stirred for 1.5 hr at room temp. It was then filtered through Celite and the solid was washed with THF. The combined filtrate and washings were concentrated *in vacuo* and the residue was partitioned between ether and sat $NaHCO_3$ soln. The ether soln was separated and the aq layer was extracted with ether. The ether extract was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo* to give (*R*)-**2c** (677.5 mg, 91.6%), ν_{max} 1740 (s), 1190 (m), 1170 (m), 1130 (m, sh), 1115 (m), 1070 (m), 1020 (s) cm^{-1}. The IR spectrum was identical with that of an authentic sample.[5,6]

Methyl (R)-3-*hydroxypentanoate* **2b**

A drop of N HCl soln was added to a soln of (*R*)-**2c** (677 mg, 3.13 mmol) in MeOH (15 ml) and the mixture was stirred for 2 hr at room temp. It was then neutralised with sat $NaHCO_3$ soln (1 ml) and concentrated *in vacuo*. The residue was diluted with water and extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by SiO_2 chromatography followed by distillation to give (*R*)-**2b** (183.6 mg, 44.4%), $n_D^{20.3}$ 1.4241; $[\alpha]_D^{20.3} - 32.9°$ (c = 1.00, $CHCl_3$) [lit.[5] $[\alpha]_D^{22} - 36.9°$ (c = 1.31, $CHCl_3$) (100% e.e.)]. The IR and NMR spectra coincided with the authentic spectra,[5] optical purity: 90.4%.

Benzyl cyanoacetate **6b**

A mixture of **6a** (98.5% purity; 16.8 g, 195 mmol), $PhCH_2OH$ (20 g, 185 mmol), C_6H_6 (100 ml) and conc H_2SO_4 (0.3 ml) was heated under reflux with azeotropic removal of water for 3 hr. After cooling, it was diluted with ether. The organic soln was washed with water, sat $NaHCO_3$ soln and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was distilled to give 28.5 g (91%) of **6b**, b.p. 150°/1.7 mm, $n_D^{20.3}$ 1.5239; [lit.[20] b.p. 100–105°/0.2 mm, n_D^{25} 1.5173]; ν_{max} 2260 (w), 1750 (s), 1335 (s), 1265 (s), 1210 (s), 1180 (s) cm^{-1}; δ (CCl_4) 3.28 (2H, s), 5.12 (2H, s), 7.22 (5H, s).

Octyl cyanoacetate **6c**

Esterification of **6a** (98.5% purity; 18 g, 208 mmol) with 1-octanol (24 g, 184.6 mmol) was carried out in the same manner as described above to give 31.6 g (86.8%) of **6c**, b.p. 95°/0.11 mm, $n_D^{20.4}$ 1.4345; ν_{max} 2260 (m), 1750 (s), 1335 (s), 1265 (s), 1185 (s) cm^{-1}; δ ($CDCl_3$) 0.88 (3H, deformed t), ~1.0–~2.0 (12H, br), 3.43 (2H, s), 4.19 (2H, t, J = 6 Hz). (Found: C, 67.17; H, 9.77; N, 6.92. Calc for $C_{11}H_{19}O_2N$: C, 66.97; H, 9.71; N, 7.10%.)

Benzyl 3-*oxopentanoate* **1b**

A soln of EtMgI was prepared from EtI (28 g, 179.5 mmol) and Mg (4 g, 164.6 mmol) in dry ether (40 ml). To this was added during 50 min a soln of **6b** (11.4 g, 65.1 mmol) in dry ether (40 ml) with vigorous stirring at 20°. After the addition, the mixture was stirred for 15 min and left to stand overnight at room temp. The mixture was carefully poured into an ice-cooled sat NH_4Cl soln (70 ml). The flask was rinsed with sat NH_4Cl soln and dil HCl. The resulting two-phase mixture was adjusted to pH 1–2 with conc HCl and stirred vigorously for 3 hr at room temp. It was then extracted with ether. The ether soln was washed with 5% $Na_2S_2O_3$ soln, sat $NaHCO_3$ soln and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue (12.5 g) was chromatographed over SiO_2 (180 g) and distilled to give 8.07 g (60.2%) of **1b**, b.p. 93–103°/0.16 mm, n_D^{20} 1.5034; ν_{max} 1745 (s), 1720 (s), 1650 (m), 1630 (m), 1305 (s), 1240 (s), 1155 (s) cm^{-1}; δ ($CDCl_3$) 1.03 (3H, t, J = 7 Hz), 2.52 (2H, q, J = 7 Hz), 3.44 (2H, s), 5.14 (2H, s), 7.34 (5H, s). (Found: C, 69.89; H, 6.84. Calc for $C_{12}H_{14}O_3$: C, 69.88; H, 6.84%.)

Octyl 3-*oxopentanoate* **1c**

A soln of EtMgI was prepared from EtI (69 g, 422.3 mmol) and Mg (10 g, 411.5 mmol) in dry ether (80 ml). To this was added a soln of **6c** (32.4 g, 164.4 mmol) in dry ether (80 ml). Subsequent manipulation and workup as described above gave 29.9 g (79.8%) of **1c**, b.p. 96°/0.18 mm, $n_D^{19.9}$ 1.4373; ν_{max} 1745 (s), 1720 (s), 1650 (m), 1630 (m), 1310 (m), 1250 (s), 1230 (s), 1160 (s) cm^{-1}; δ ($CDCl_3$) 0.65–1.80 (18H, br m), 2.54 (2H, q, J = 7 Hz), 3.38 (2H, s), 4.08 (2H, t, J = 6 Hz). (Found: C, 68.49; H, 10.56. Calc for $C_{13}H_{24}O_3$: C, 68.38; H, 10.59%.)

Benzyl (+)-3-*hydroxypentanoate* **2e**

Dry baker's yeast (Oriental yeast, 7 g) was added to a soln of sucrose (15 g) in tap water (100 ml) at 30°. The mixture was shaken at 30° for 10 min. A suspension of **1b** (500 mg, 2.43 mmol) in 0.2% Triton X-100 (10 ml) was added to the yeast suspension and the mixture was shaken at 30°. After 1 hr an additional amount of sucrose (10 g) was added. The shaking culture was continued for 4.5 hr after the addition of **1b**. Then the fermentation was stopped by the addition of ether. The mixture was filtered. The filtrate was saturated with NaCl and extracted with ether. The ether soln was washed with brine and dried ($MgSO_4$). The filter cake (yeast cells) was placed in a flask and mixed with EtOAc. The mixture was stirred for 6–7 hr and filtered. The EtOAc layer was separated, washed with brine and dried ($MgSO_4$). The combined ether–EtOAc soln was concentrated to give 790 mg of a crude oil. This was purified by SiO_2 chromatography to give 428 mg (85%) of **2e**, $[\alpha]_D^{20.7}$ + 11.3° (c = 1.167, $CHCl_3$); ν_{max} 3450 (br s), 1735 (s), 1165 (s) cm^{-1}; δ ($CDCl_3$) 0.93 (3H, t, J = 7 Hz), 1.44 (2H, q, J = 7 Hz), 2.44 (1H, d, J = 2 Hz), 2.53 (1H, s), 2.95 (1H, br s, —OH), 3.93 (1H, m), 5.15 (2H, s), 7.37 (5H, s). Optical purity (as determined by the HPLC analysis of **2f**): 45%. (Found: C, 69.27; H, 7.74. Calc for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74%.)

Methyl (S)-3-*hydroxypentanoate* **2b** *from* **2e**

To a soln of (+)-**2e** (400 mg, 1.92 mmol) in 95% EtOH (25 ml) was added KOH soln (0.18 g in 20 ml water). The mixture was stirred for 2 hr at room temp, concentrated *in vacuo* to remove EtOH and extracted with ether to remove $PhCH_2OH$. The aq layer was acidified with dil HCl to pH 2 and extracted with EtOAc. The EtOAc soln was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was treated with ethereal CH_2N_2. The excess CH_2N_2 was destroyed with AcOH. The ether soln was washed with sat $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was distilled to give 152 mg (60%) of **2b**, b.p. 90–100° (bath temp)/35 mm, n_D^{20} 1.4230; $[\alpha]_D^{20}$ + 16.5° (c = 0.907, $CHCl_3$). From this $[\alpha]_D$ value the optical purity of this sample of (*S*)-**2b** was estimated to be 44.7% which was in good agreement with the value (45%) obtained by the HPLC analysis of **2f**. The IR and NMR spectra of (*S*)-**2b** were identical with those of (*R*)-**2b**.[5]

Octyl (+)-3-*hydroxypentanoate* **2g**

Dry baker's yeast (7 g) was added to a soln of sucrose (15 g) in tap water (100 ml) at 30°. The mixture was shaken for 10 min at 30°. Then a suspension of **1c** (500 mg, 2.19 mmol) in 0.2% Triton X-100 (10 ml) was added to the yeast suspension. The mixture was shaken at 30°. After 1 hr sucrose (10 g) was added. The fermentation was continued for 4.5 hr. Fifteen batches of the mixture (7.5 g of **1c** was reduced) was combined and worked up as usual to give 11.0 g of a crude oil. This was purified by chromatography over SiO_2 (110 g) followed by distillation to give 4.82 g (63.7%) of (+)-**2g**, b.p. 100–101.5°/0.24 mm, $n_D^{20.4}$ 1.4371; $[\alpha]_D^{20.4}$ + 22.1° (c = 1.079, $CHCl_3$); ν_{max} 3470 (br m), 1740 (s), 1175 (s) cm^{-1}; δ ($CDCl_3$) 0.65–1.90 (20H, m), 2.36 (1H, d, J = 2 Hz), 2.46 (1H, s), 2.90 (1H, br, —OH), 3.77–4.20 (3H, m); optical purity (as determined by the HPLC analysis of **2h**): 97.4%. (Found: C, 67.76; H, 11.47. Calc for $C_{13}H_{26}O_3$: C, 67.78; H, 11.38%.)

Methyl (S)-3-*hydroxypentanoate* **2b** *from* **2g**

To a soln of (+)-**2g** (500 mg, 2.17 mmol) in 95% EtOH (25 ml) was added KOH soln (0.17 g in 15 ml water). The mixture was stirred for 1.5 hr at room temp. Subsequent workup and methylation of the product as described for the preparation of (*S*)-**2b** from **2e** gave 129 mg (45%) of (*S*)-**2b**, $n_D^{20.7}$ 1.4230; $[\alpha]_D^{20.7}$ + 34.4° (c = 0.990, $CHCl_3$); optical purity (as determined by the HPLC analysis of **2d**): 93.3%.

Octyl (S)-3-*tetrahydropyranyloxypentanoate* **2i**

p-TsOH (100 mg) was added to a soln of (*S*)-**2g** (8.13 g, 35.4 mmol) and dihydropyran (5.05 g, 60.1 mmol) in dry CH_2Cl_2 (100 ml). The mixture was stirred for 1 hr at room temp. It was then washed with sat $NaHCO_3$ soln and brine, dried (K_2CO_3 and $MgSO_4$) and concentrated *in vacuo* to give 10.8 g (97.2%) of (*S*)-**2i**. A portion of it was distilled to give an analytical sample. b.p. 132–133°/0.22 mm, $n_D^{20.4}$ 1.4493; $[\alpha]_D^{20.4}$ + 5.12° (*c* = 1.035, $CHCl_3$); ν_{max} 1740 (s), 1170 (s), 1135 (s), 1115 (s), 1075 (s), 1030 (s), 1020 (s), 1000 (s) cm^{-1}; δ ($CDCl_3$) 0.70–1.10 (6H, m), 1.10–2.00 (20H, m), 1.41–1.63 (2H, m), 3.30–4.20 (5H, br, m), 4.60–4.76 (1H, br). (Found: C, 69.07; H, 10.73. Calc for $C_{18}H_{34}O_4$: C, 68.75; H, 10.90%.)

1,3-*Pentanediol-3-THP ether* **7a**

(a) (S)-*Isomer*. A soln of (*S*)-**2i** (10.5 g, 33.5 mmol) in dry ether (25 ml) was gradually added during 72 min to a stirred and ice-cooled suspension of LAH (1 g) in dry ether (80 ml) at 2–3°. The stirring was continued for 4 hr at room temp. The excess of LAH was destroyed by cautious addition of water (1 ml) to the stirred and ice-cooled mixture. 10% NaOH soln (1 ml) and water (3 ml) were successively added to the mixture. After stirring for 1 hr at room temp, the mixture was filtered through Celite. The filter cake was washed thoroughly with ether and THF. The combined filtrate and washings were dried (K_2CO_3 and $MgSO_4$) and concentrated *in vacuo* to give 11.38 g of a mixture of **7a** and 1-octanol. This was chromatographed over SiO_2 (200 g). Elution with n-hexane–EtOAc (10:1–7:1) removed 1-octanol. Further elution with n-hexane–EtOAc (1:1–1:2) yielded (*S*)-**7a** (5.57 g, 88.4%). A portion of it was distilled to give an analytical sample, b.p. 86–88°/2.1 mm, $n_D^{19.3}$ 1.4564; $[\alpha]_D^{19.3}$ + 21.3° (*c* = 0.953, $CHCl_3$). The IR and NMR spectra coincided with those of an authentic (*S*)-**7a**.[6]

(b) (*R*)-*Isomer*. In the same manner (*R*)-**2c** (100% e.e.; 3.34 g, 15.4 mmol) was reduced with LAH in ether (40 ml) to give 2.51 g (86.4%) of (*R*)-**7a**, b.p. 90–90.9°/3.0 mm, $n_D^{19.4}$ 1.4565; $[\alpha]_D^{19.4}$ − 16.8° (*c* = 0.895, $CHCl_3$). The IR and NMR spectra were identical with the authentic spectra.[6]

1,3-*Pentanediol-3-THP ether-1-tosylate* **7b**

(a) (S)-*Isomer*. *p*-TsCl (9.63 g, 50.4 mmol) was added portionwise during 11 min to a stirred and ice-cooled soln of (*S*)-**7a** (5.46 g, 29.0 mmol) in dry C_5H_5N (25 ml). The stirring was continued for 4 hr under ice-cooling. The mixture was poured into ice-water and extracted with ether. The ether soln was washed with water, sat $CuSO_4$ soln, water, sat $NaHCO_3$ soln and brine, dried (K_2CO_3 and $MgSO_4$) and concentrated *in vacuo* to give 9.45 g (quantitative) of (*S*)-**7b**, ν_{max} 1600 (s), 1190 (s), 1180 (s) cm^{-1}. This was employed in the next step without further purification.

(b) (R)-*Isomer*. In the same manner as above (*R*)-**7a** (2.51 g, 13.3 mmol) and *p*-TsCl (4.32 g, 22.7 mmol) yielded 4.45 g (97.5%) of (*R*)-**7b**, which showed an identical IR spectrum with that of (*S*)-**7b**.

4-*Tetrahydropyranyloxyhexanenitrile* **8**

(a) (S)-*Isomer*. NaCN (2 g, 40.8 mmol) was added to a soln of (*S*)-**7b** (9.45 g, 29.0 mmol) in dry DMF (35 ml). The mixture was stirred for 46 hr at room temp. It was then poured into water and extracted with ether. The ether soln was washed with water, sat $NaHCO_3$ soln and brine, dried (K_2CO_3 and $MgSO_4$) and concentrated *in vacuo*. The residue was distilled to give 5.97 g (quantitative) of (*S*)-**8**, b.p. 112–117°/6 mm, $n_D^{20.8}$ 1.4508; $[\alpha]_D^{20.4}$ + 45.1° (*c* = 0.962, $CHCl_3$); ν_{max} 2240 (m), 1130 (s), 1120 (s), 1075 (s), 1030 (s), 995 (s) cm^{-1}; δ ($CDCl_3$) 0.88 and 0.92 (total 3H, each t, J = 7 Hz), 1.25–2.05 (10H, m), 2.27–2.64 (2H, m), 3.20–4.10 (3H, m), 4.44–4.73 (1H, br). (Found: C, 66.58; H, 9.57; N, 6.98. Calc for $C_{11}H_{19}O_2N$: C, 66.97; H, 9.71; N, 7.10%.)

(b) (R)-*Isomer*. In the same manner as above (*R*)-**7b** (4.45 g, 13.0 mmol) and NaCN (900 mg, 18.4 mmol) gave 2.0 g (78%) of (*R*)-**8**, b.p. 115–116°/4.7 mm, $n_D^{20.6}$ 1.4495; $[\alpha]_D^{20.6}$ − 37.5° (*c* = 1.126, $CHCl_3$). The IR and NMR spectra were identical with those of (*S*)-**8**. (Found: C, 66.95; H, 9.43; N, 6.90. Calc for $C_{11}H_{19}O_2N$: C, 66.97; H, 9.71; N, 7.10%.)

4-*Hexanolide* **3**

(a) (S)-*Isomer*. NaOH (4.62 g, 115.5 mmol) in water (14 ml) was added to a soln of (*S*)-**8** (5.74 g, 29.0 mmol) in 2-methoxyethanol (40 ml). The mixture was stirred and heated under reflux for 21 hr. After acidification with 3N HCl to pH 2, the mixture was extracted with ether. The aq layer was saturated with NaCl and further extracted with ether. The ether soln was dried ($MgSO_4$) and concentrated to give 4 g of a crude oil. This was dissolved in MeOH (15 ml) and made alkaline with KOH soln (965 mg in 20 ml water). The mixture was stirred for 4.5 hr at room temp, evaporated *in vacuo* to remove MeOH and extracted with ether to remove neutral impurities. The aq layer was acidified with 3 N HCl to pH 2 and stirred for 1 hr at room temp. It was then extracted with ether. The ether soln was dried ($MgSO_4$) and concentrated *in vacuo* to give 1.7 g of crude (*S*)-**3**. This was distilled to give 1.3345 g (40.3%) of (*S*)-**3**, b.p. 102–106°/23 mm, $n_D^{20.7}$ 1.4325; $[\alpha]_D^{20.7}$ − 50.6° (*c* = 1.485, MeOH); ν_{max} 2980 (m), 2950 (m), 2890 (m), 1775 (s), 1460 (m), 1420 (w), 1385 (s), 1355 (m), 1285 (w), 1220 (m), 1185 (s), 1175 (s), 1130 (m), 1120 (m), 1100 (m), 1070 (w), 1055 (w), 1025 (m), 1015 (m), 970 (m), 955 (m), 900 (m), 850 (w), 805 (w), 775 (w) cm^{-1}; δ (CCl_4) 1.00 (3H, t, J = 7 Hz), 1.45–2.67 (6H, m), 4.10–4.54 (1H, m); GLC (column, 10% PEG-20M, 2 m × 4 mm; carrier gas, N_2; 1 kg/cm^2; column temp 100° + 10°/min): Rt 7 min 57 sec (100%). (Found: C, 62.74; H, 8.81. Calc for $C_6H_{10}O_2$: C, 63.13; H, 8.83%.)

(b) (R)-*Isomer*. In the same manner as above (*R*)-**8** (2.0 g, 10.1 mmol) yielded 897.3 mg (77.5%) of (*R*)-**3**, b.p. 113°/32 mm, n_D^{21} 1.4326; $[\alpha]_D^{21}$ + 53.1° (*c* = 1.005, MeOH). The IR and NMR spectra were identical with those of (*S*)-**3**. GLC (column, 5% PEG-20M, 2m × 4 mm; carrier gas, N_2; 1 kg/cm^2; column temp 100° + 10°/min): Rt 4 min 18 sec (99.3%). (Found: C, 63.00; H, 9.06. Calc for $C_6H_{10}O_2$: C, 63.13; H, 8.83%.)

Determination of the optical purity of **3**

(*R*)- or (*S*)-**3** (100 mg, 0.876 mmol) was treated with 5 eq of MeMgI to give 100 mg (78.1%) of (*R*)-**9a** or 120 mg (93.7%) of (*S*)-**9a**, respectively.

^{1}H-NMR *measurements at* 100 MHz (Jeol FX-100). (i) (*R*)-**9a** (4.3 mg) + Eu(tfc)$_3$ (21 mg, 0.8 eq) in CCl_4 (0.3 ml) and $CDCl_3$ (0.12 ml): δ 3.82 (3H, s), 4.55 (3H, s); (ii) (*S*)-**9a** (5.1 mg) + Eu(tfc)$_3$ (24.9 mg, 0.8 eq) in CCl_4 (0.3 ml) and $CDCl_3$ (0.12 ml): δ 4.25 (3H, s), 5.02 (3H, s); (iii) (±)-**9a** under the same condition; δ 4.27, 4.33, 5.10, 5.22. The optical purity of (*R*)-**9a** and that of (*S*)-**9a** were therefore > 95%.

HPLC analysis of **9b**. (*R*)- or (*S*)-**9a** was converted to the corresponding (*S*)-MTPA ester (*R*)- or (*S*)-**9b** in the usual manner[10] and they were analysed by HPLC (column, Partisil 5,25 cm × 4.6 mm; solvent, n-hexane–THF (10:1); flow rate 0.78 ml/min; press, 37 kg/cm^2; detection at 254 nm). The two diastereomers showed Rt 46 min 9 sec and 48 min 4 sec, respectively. (*R*)-**9b** was shown to be 100% pure. (*S*)-**9b** showed two peaks (97.45:2.55). The optical purity of (*R*)-**3** was therefore 100% and that of (*S*)-**3** was 95%. This was in almost complete agreement with the values estimated from the comparison of the $[\alpha]_D$ values of our samples with that (± 53.2°) reported by Ravid *et al.*[14]

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