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The Synthesis of (*R*)-(+)-Lipoic Acid using a Monooxygenase-Catalysed Biotransformation as the Key Step

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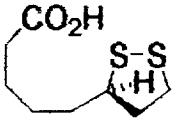
Abstract—2-(2-Acetoxyethyl)cyclohexanone (**4**) was converted into the lactone (−)-(**5**) regio- and enantioselectively using 2-oxo-Δ^3-4,5,5-trimethylcyclopentenyl acetyl-CoA monooxygenase, an NADPH-dependent Baeyer–Villiger monooxygenase from camphor grown *Pseudomonas putida* NCIMB 10007. The lactone (−)-(**5**) was converted into (*R*)-(+)-lipoic acid in six steps. In contrast cyclopentanone monooxygenase, an NADPH-dependent Baeyer–Villiger monooxygenase from cyclopentanol-grown *Pseudomonas* sp. NCIMB 9872 selectively oxidized the (*S*)-enantiomer of the ketone (**4**) giving better access to optically enriched, naturally occurring lipoic acid.

Introduction and Background Information

In 1951, Reed and co-workers reported the isolation of a crystalline growth-promoting enzyme cofactor from processed insoluble liver residue.[1] This compound was named α-lipoic acid due to its high lipid solubility and acidic nature ($pK_a = 4.7$). The chemical structure of α-lipoic acid was determined in the early 1950s[2] and its absolute configuration was confirmed to be (*R*) in 1983, when Golding synthesized the complementary enantiomer from (*S*)-malic acid.[3]



Since its discovery, lipoic acid has been found to be widely distributed in animal and plant tissue.[4] It is an important prosthetic group in various biological systems[5] and displays an extremely high level of biological activity. It plays an important role as a catalyst in the oxidative decarboxylation of pyruvate to acetate,[6] acts as a protein-bound transacylating cofactor of several multienzymic α-keto acid dehydrogenase complexes[7] and also participates in oxidative phosphorylation.[8] Since lipoic acid exhibits a high level of biological activity, its use in the treatment of various diseases has been investigated. The racemate was used to treat various liver diseases including liver poisoning.[9] The effects of lipoic acid on rats suffering from hepatitis,[10] pancreatitis[11] and induced carcinomas[12] have been investigated with positive results. It was found to be very effective in the treatment of several liver toxicoses caused by active peptide from the poisonous fungus *Amanita phalloides*.[13] Lipoic acid was shown to have protective and curative effects in heavy-metal poisoned animals.[14] It was also found to be a potent growth promoting factor which stimulated reparative regeneration of soft tissues.[11]

Lipoic acid also protects against ionizing radiation-induced damage to DNA and its components.[15] It has been demonstrated to regulate metabolic disturbances caused by *Escherichia coli* endotoxin in rabbits[16] and to prevent hair loss during chemotherapy in rats.[17] A potentially important property of lipoic acid is indicated by its potent ability to reduce the blood-sugar level of diabetic rabbits.[18] There are indications that the two enantiomeric forms of lipoic acid do not exhibit the same biological activity; generally the naturally occurring (*R*)-enantiomer is much more active than the (*S*)-enantiomer.[19] It is therefore desirable for any synthesis of lipoic acid to provide efficient access to the pure (*R*)-enantiomer.

Previously recorded Syntheses of Lipoic Acid

A considerable number of syntheses of racemic lipoic acid have been described since its discovery[20] and the material is commercially available. Initially, naturally occurring (+)-lipoic acid was obtained through classical resolution of a racemic intermediate in the synthetic route.[21] In 1983, the first asymmetric synthesis of lipoic acid was demonstrated by Golding from (*S*)-malic acid. However, the product was found to be the antipode of the naturally occurring (+)-lipoic acid. Nevertheless, this synthesis confirmed the absolute configuration of the naturally occurring (+)-lipoic acid as (*R*).[3] Subsequently, various other asymmetric syntheses have been reported. In 1985 Johnson reported a total synthesis of (*R*)-(+)-lipoic acid starting from (2*S*,4*S*)-2,4-pentanediol.[22] In 1986 Sutherland achieved a total synthesis of (*R*)-(+)-lipoic acid

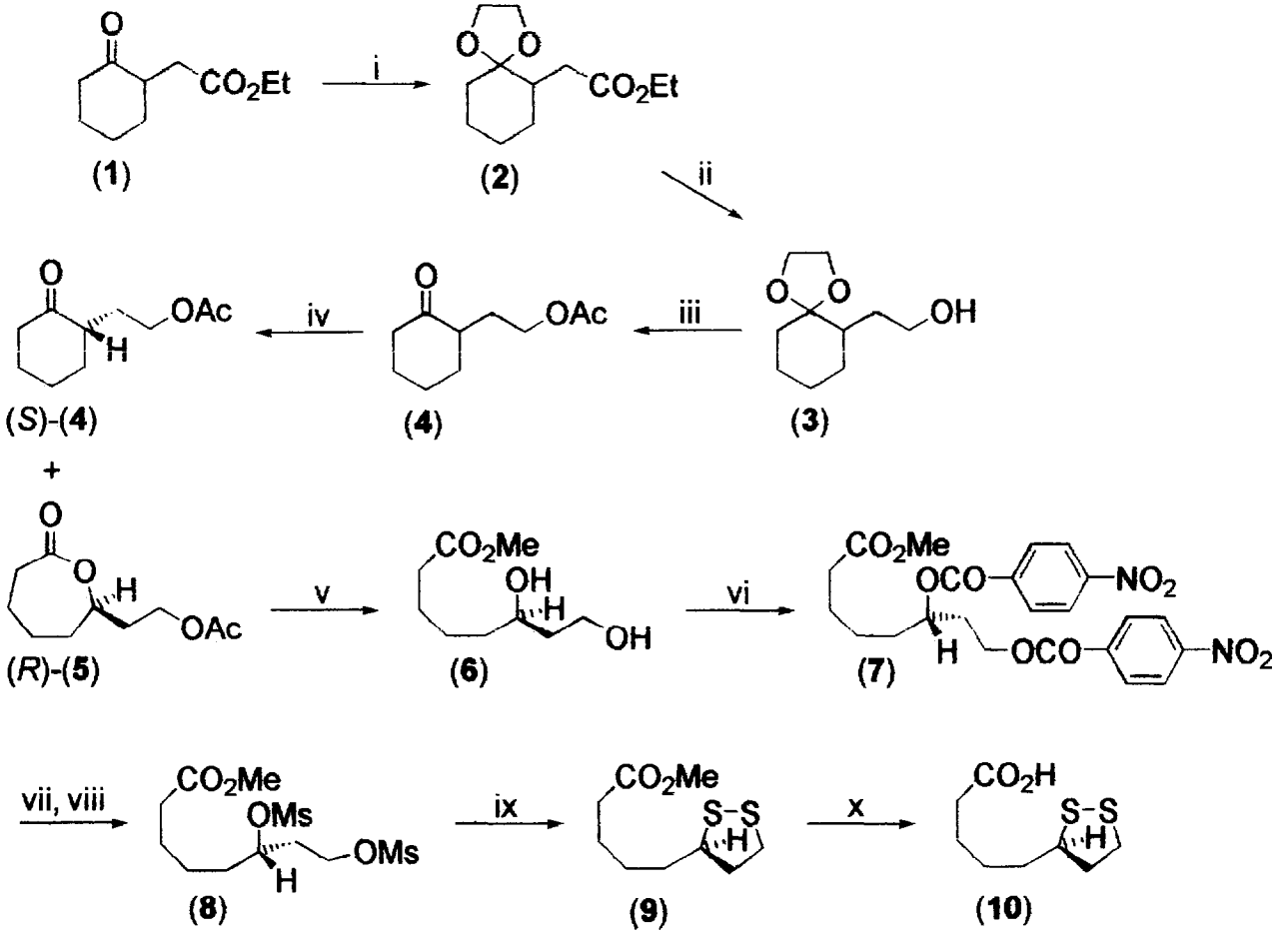
through a Sharpless asymmetric epoxidation.[23] In 1987 Rama Rao demonstrated the use of a derivative of D-mannitol,[24] and D-glucose[25] as starting materials for the enantiospecific syntheses of (*R*)-(+)-lipoic acid. D-Menthone was described as a recyclable chiral auxiliary for a stereospecific synthesis of (*R*)-(+)-lipoic acid.[26] In 1988 Golding finally achieved access to both enantiomers of lipoic acid from (*S*)-malic acid.[27] Subsequently, both Gopalan[28] and Bhalerao[29] have reported approaches towards a synthesis of (*R*)-(+)-lipoic acid via bakers' yeast asymmetric reductions. Finally, Tolstikov has also synthesized (*R*)-(+)-lipoic acid, from a 'chiral pool' starting material, di-*O*-benzyl-D-arabinal.[30]

Results and Discussion

The racemic ketone (**1**) was protected as the acetal (**2**) (Scheme 1), before reduction using lithium aluminium hydride converted the ester group into the alcohol moiety of compound (**3**). Acetylation and deprotection furnished the ketoester (**4**) in 61% yield from **1**.

meta-Chloroperbenzoic acid transformed the ketone (**4**) into racemic lactone (**5**) (98% yield), while optically active lactone (**5**) was obtained by enantioselective oxidation of ketone (**4**) using monooxygenase enzymes (Table 1).

A mixture of the two NADH-dependent diketocamphane monooxygenase isozymes[31] from *Pseudomonas putida* 10007[32] (MO 1) proved to be a poor catalyst for the oxidation, giving a conversion of ketone (**4**) to lactone (**5**) of just 8% after 7 h (Table 1, Entry 1). In contrast, the partially purified NADPH-dependent 2-oxo-Δ^3-4,5,5-trimethylcyclopentenyl acetyl-CoA monooxygenase from *Pseudomonas putida* NCIMB 10007[33] (MO 2) rapidly gave (*R*)-(−)-lactone (**5**) (40% conversion, 83% enantiomeric excess after 3 h) (Table 1, Entry 2). A second experiment, run for a shorter time period (2 h), gave a reduced amount of lactone (35%) of roughly the same optical purity (81% e.e.). The NADH-dependent and the NADPH-dependent cyclohexanone monooxygenases from cyclohexanol-grown *Xanthobacter autotrophicus* NCIMB 10811[34] catalyzed the oxidation of racemic ketone (**4**) into optically pure (*R*)-(−)-lactone (**5**) (Table 1, Entries 4 and 5). However, the substrate proved to be toxic to the *X. autotrophicus* monooxygenase enzymes at concentrations above 1.1 mM, higher ketone loading resulting in lower levels of lactone formation.



Scheme 1. Reagents and conditions: i, ethylene glycol, *p*-TsOH, toluene, Dean–Stark, reflux; ii, $LiAlH_4$, diethyl ether, 0–25 °C; iii, $(CH_3CO)_2O$, pyridine, DMPA then HCl, MeOH; iv, 2-oxo-Δ^3-4,5,5-trimethylcyclopentenylacetyl-CoA monooxygenase, NADPH, G-6-P, G-6-PDH; v, NaOMe, MeOH; vi, *p*-$NO_2C_6H_4CO_2H$, PPh_3, DEAD, THF; vii, K_2CO_3, MeOH; viii, MsCl, Et_3N, CH_2Cl_2, 0 °C; ix, $Na_2S \cdot 9H_2O$, sulfur, DMF, in the dark, 80 °C; x, 0.1 M KOH, MeOH.

Table 1. Biotransformation of (±)-2-(2′-acetoxyethyl)cyclohexanone (**4**) using various biocatalysts isolated from a variety of microorganisms

	1[a] MO 1	2[b] MO 2	3[a] Purified MO 2[c]	4[a] CHMO (NADH)[d]	5[a] CHMO (NADPH)[e]	6[b] CPMO CPMO
[S] (mM)	3	10.9	2.7	1.1	1.1	5.4
[S] (mg ml^{-1})	0.6	2	0.5	0.2	0.2	1
Time (h)	7	3¼	12	3	3	½
Ketone (**4**)	(*S*)-(+)	(*S*)-(+)	(*S*)-(+)	(*S*)-(+)	(*S*)-(+)	(*R*)-(−)
% Unreacted	81	19	56	83	66	38
% Yield	—	13	—	—	—	37
e.e.$_s$	8	75	60	27	61	68
Lactone (**5**)	(*R*)-(−)	(*R*)-(−)	(*R*)-(−)	(*R*)-(−)	(*R*)-(−)	(*S*)-(+)
% Conversion	8	36	43	17	34	62
% Yield	—	34	—	—	—	59
e.e.$_p$	90	83	78	>98	>98	42
E_p	~20	~17	~14	>100	>100	~5

[a]Analytical scale biotransformation.
[b]Preparative scale biotransformation.
[c]Purified **MO 2**: NADPH-dependent 2-oxo-Δ^3-4,5,5-trimethylcyclopentenylacetyl-CoA monooxygenase (**MO 2**) was further purified using a Q-Sepharose column.[33]
[d]CHMO (NADH): NADH-dependent cyclohexanone monooxygenase was isolated from *Xanthobacter autotrophicus* NCIMB 10811[34] and partially purified using a Q-Sepharose column.
[e]CHMO (NADPH): NADPH-dependent cyclohexanone monooxygenase was isolated from *Xanthobacter autotrophicus* NCIMB 10811[34] and partially purified using a Q-Sepharose column.
[f]CPMO: NADPH-dependent cyclopentanone monooxygenase was isolated from *Pseudomonas* sp. NCIMB 9872 and purified to homogeneity.[36]

Hence a preparative scale biotransformation was carried using partially purified NADPH-dependent monooxygenase from *P. putida* 10007, recycling NADP using glucose-6-phosphate and glucose-6-phosphate dehydrogenase. The lactone (*R*)-(−)-(**5**) $[\alpha]_D^{25}$ −52 (*c* 5; $CHCl_3$) was isolated in 34% yield and the enantiomeric excess (83%) was estimated by gas chromatography using Lipodex E[38] as the chiral stationary phase. Other compounds isolated were the recovered (*S*)-ketone (**4**) (13%, 75% e.e.), a single (unassigned) diastereoisomer of 2-(2-acetoxyethyl)cyclohexan-1-ol (11%), and a mixture of diastereoisomers (3:1 ratio) of 2-(2-hydroxyethyl)cyclohexan-1-ols (19%). The stereochemistries of the alcohols were not elucidated.

The formation of the cyclohexanols was circumvented using more extensively purified NADPH-dependent monooxygenase (the optical purity of the lactone was found to be similar, Table 1, Entry 3) but the amount of purified enzyme that was readily available precluded its use on a preparative scale.

The optically enriched lactone (*R*)-(−)-(**5**) was converted into the (6*R*)-dihydroxyoctanoate (+)-(**6**) $[\alpha]_D^{24}$ +3 (*c* 5; $CHCl_3$) [lit.[27] $[\alpha]_D^{18}$ +4.2 (*c* 5.2; $CHCl_3$)]. The diol (6*R*)-(+)-(**6**) was subjected to a Mitsunobu reaction to invert the stereochemistry at C-6; thus reaction of (6*R*)-(+)-(**6**) with *para*-nitrobenzoic acid, triphenylphosphine and diethyl azodicarboxylate gave the triester (6*S*)-(+)-(**7**). Hydrolysis and mesylation of (6*S*)-(+)-(**7**) furnished the (6*S*)-dimesylate (+)-(**8**) $[\alpha]_D^{25}$ +16 (*c* 5; $CHCl_3$) [lit.[39] $[\alpha]_D^{23}$ +18.5 (*c* 0.29; $CHCl_3$)]. Introduction of the disulfide bridge was effected using sodium sulfide nonahydrate and sulfur to give (*R*)-(+)-methyl lipoate (**9**) $[\alpha]_D^{26}$ +81 (*c* 2; C_6H_6) [lit.[27] $[\alpha]_D^{23}$ +97 (*c* 1.8; C_6H_6)] which was hydrolysed using aqueous potassium hydroxide to give enantiomerically enriched (*R*)-(+)-lipoic acid (**10**) mp 44–46 °C, $[\alpha]_D^{25}$ +87.32 (*c* 0.071; C_6H_6) [lit.[23] mp 44–46 °C, lit,[35] $[\alpha]_D^{23}$ +104 (*c* 0.88; C_6H_6)].

The route described in Scheme 1 represents an effective way of making lipoic acid. However it is obvious that the route to the dextrorotatory enantiomer could be shortened by two steps if the bio-oxidation of (±)-(**4**) produced the (*S*)-lactone (**5**) [rather than the (*R*)-lactone] thereby eliminating the need for the Mitsunobu inversion.

Preliminary results showed that this foreshortening of the pathway can be achieved. Thus the purified cyclopentanone monooxygenase from cyclopentanol-grown *Pseudomonas* sp. NCIMB 9872 transformed the (*S*)-ketone (**4**) preferentially; after 30 min 59% of (*S*)-lactone (**5**) (42% e.e.) was obtained together with recovered (*R*)-ketone (**4**) (37% yield, 68% e.e.) (Table 1, Entry 6). Methanolysis of this sample of the lactone gave the (6*S*)-diol (**6**) $[\alpha]_D^{25}$ −2 (*c* 0.5; $CHCl_3$) [lit.[27] $[\alpha]_D^{22.5}$ −3.9 (*c* 2.3; $CHCl_3$)]. Thus by using this NADPH-dependent Baeyer–Villiger monooxygenase from *Pseudomonas* sp. NCIMB 9872 to perform the required kinetic resolution, the conversion of racemic ketoester (**1**) into optically enriched (+)-lipoic acid (**10**) involves just nine steps. Optimization of the conditions for the use of *Pseudomonas* sp. NCIMB 9872 on ketone (**4**) and analogues has been reported in a separate paper[37] and the search for other monooxygenases which selectively oxidize the (*S*)-ketone (**4**) to the required lactone is presently underway.[38]

Experimental

Diethyl ether and tetrahydrofuran (THF) were dried and distilled from sodium metal and benzophenone. Ethyl acetate was dried and distilled over phosphorus pentoxide. Dichloromethane was dried and distilled over calcium hydride. Triethylamine was dried, distilled and stored over potassium hydroxide. Commercially available *meta*-chloroperbenzoic acid (MCPBA) (50–60% w/w) was dissolved in CH_2Cl_2 and dried over $MgSO_4$. The solvent was evapd under red. pres. to give MCPBA (80–90% w/w). Other reagents and solvents were used as commercially supplied. Thin layer chromatography (TLC) was performed on pre-coated glass plates (Merck silica gel 60 F 254). The plate was visualized using UV light (254 nm), *para*-anisaldehyde, potassium permanganate or cerium (IV) ammonium molybdate followed by heating. Flash CC was performed over silica gel (Merck silica gel 60, 40–63 μm). Gas chromatography (GC) was performed with a Shimadzu GC-14A gas chromatograph equipped with a capillary column, BP1 (25 m), using helium as the carrier gas. Chiral gas chromatography (GC) was performed with a Shimadzu GC-14A gas chromatograph equipped with a chiral capillary column, Lipodex® E (25 m), using helium as the carrier gas. ^1H, ^{13}C and ^{19}F NMR spectra were recorded on a Brüker AM 250 or AM 300 spectrometer using deuterium lock. Chemical shifts (δ) are quoted in ppm and coupling constants (J) in Hz. IR spectra were recorded on a Nicolet Magna-IR 550 Spectrometer on liquid films between sodium chloride plates. Mass spectra were recorded on a VG ZAB-F spectrometer at the S.E.R.C. Mass Spectrometry Centre, Swansea, or a Kratos Profile HV 3000 spectrometer at the University of Exeter. Optical rotations were measured on an Optical Activity AA-1000 polarimeter and specific rotations were quoted in units of 10^{-1} deg cm^2 g^{-1}. Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. Optical density was measured on a Spectronic 20 spectrophotometer. Centrifugation was performed with a M.S.E. Cool Spin 2 centrifugator. Sonication was performed using an M.S.E. Soniprep 150 sonicator.

(±)-6-(*Ethoxycarbonylmethyl*)-1,4-*dioxaspiro*[4.5]*decane* (2).[39] Under an argon atmosphere, ethylene glycol (7.6 ml, 135.87 mmol) and *para*-toluenesulfonic acid (516 mg, 2.72 mmol) were added to a soln of (±)-2-(ethoxycarbonylmethyl)cyclohexanone (**1**) (5.00 g, 27.17 mmol) in toluene (136 ml). The mixture was heated to reflux in a Dean–Stark apparatus for 4 h and then allowed to cool to room temperature and quenched with Na_2CO_3 (5 g) and a satd Na_2CO_3 soln (150 ml). The organic phase was sepd from the aq phase which was extracted with EtOAc (3 × 100 ml). The combined organic phases were washed with a satd Na_2CO_3 soln (100 ml) and brine (100 ml). The organic phase was dried over $MgSO_4$ and the solvent evapd under red. pres. to give the crude product which was purified using flash column chromatography with hexane:ethyl acetate (9:1) to give (±)-6-(*ethoxycarbonylmethyl*)-1,4-*dioxaspiro*[4.5]*decane* (**2**) (4.59 g, 74% yield) as a colourless oil. R_f 0.18 (hexane:ethyl acetate, 9:1). (Found: $[M]^+$ 228.1353. $C_{12}H_{20}O_4$ requires M 228.1362); η_{max} (neat)/cm^{-1} 2981, 2937, 2861, 1734 (C=O), 1293 (C-O); δ_H (250 MHz, $CDCl_3$) 4.22 (2 H, q, $J_{4',5'}$ 7.0 Hz, H-4′), 3.98–3.86 (4 H, m, H-2 and H-3), 2.55 (1 H, dd, J_{gem} 15.0, $J_{1',6}$ 5.0 Hz, H-1′), 2.22 (1 H, m, H-6), 2.05 (1 H, dd, J_{gem} 15.0, $J_{1',6}$ 7.5 Hz, H-1′), 1.82–1.72 (2 H, m, CH_2), 1.70–1.58 (2 H, m, CH_2), 1.54–1.29 (4 H, m, 2 × CH_2) and 1.25 (3 H, t, $J_{5',4'}$ 7.0, H-5′); δ_C (62.9 MHz, $CDCl_3$) 173.3 (C-2′), 109.9 (C-5), 64.7 (CH_2O), 64.5 (CH_2O), 60.0 (C-4′), 41.7 (C-6), 34.5 (CH_2), 34.4 (CH_2), 30.0 (CH_2), 24.5 (CH_2), 23.7 (CH_2) and 14.2 (C-5′).

(±)-6-(2′-*Hydroxyethyl*)-1,4-*dioxaspiro*[4.5]*decane* (3).[39] Under an argon atmosphere, a solution of (±)-6-(ethoxycarbonylmethyl)-1,4-dioxaspiro[4.5]decane (**2**) (1.00 g, 4.39 mmol) in Et_2O (22 ml) was cooled to 0 °C. $LiAlH_4$ (333 mg, 8.77 mmol) was added and the mixture was stirred at room temperature for 3 h. The mixture was cooled to 0 °C, quenched with EtOAc (10 ml) and 2 M NaOH soln (50 ml). The mixture was extracted with $CHCl_3$ (3 × 50 ml) and the combined organic extracts dried over $MgSO_4$. The solvent was evapd under red. pres. to give the crude product which was purified using flash CC with hexane:EtOAc (3:7) to give (±)-6-(2′-*hydroxyethyl*)-1,4-*dioxaspiro*[4.5]-*decane* (**3**) (767 mg, 94% yield) as a colourless oil. R_f 0.30 (hexane:EtOAc, 3:7). (Found: $[M]^+$ 186.1258. $C_{10}H_{18}O_3$ requires M 186.1256); η_{max} (neat)/cm^{-1} 3427 (OH), 2936, 2879, 1446, 1160 (C-O), 1090 (C-O), 1055 (C-O); δ_H (300 MHz, $CDCl_3$) 4.00–3.90 (4 H, m, H-2 and H-3), 3.74–3.52 (2 H, m, H-2′), 2.30 (1 H, br s, OH), 1.92–1.56 (5 H, m, H-6 and 2 × CH_2) and 1.54–1.20 (6 H, m, 3 × CH_2); δ_C (75.5 MHz, $CDCl_3$) 110.6 (C-5), 64.6 (CH_2O), 64.3 (CH_2O), 61.6 (C-2′), 41.9 (C-6), 34.3 (CH_2), 32.3 (CH_2), 30.5 (CH_2), 24.6 (CH_2) and 23.7 (CH_2).

(±)-2-(2′-*Acetoxyethyl*)*cyclohexanone* (4).[39] Under an argon atmosphere, DMAP (259 mg, 2.12 mmol) and Ac_2O (3 ml, 31.75 mmol) were added to a soln of (±)-6-(2′-hydroxyethyl)-1,4-dioxaspiro[4.5]decane (**3**) (3.94 g, 21.17 mmol) in pyridine (106 ml). The mixture was stirred at room temperature for 1 h. The pyridine was evapd under red. pres. and the residue was diluted with MeOH (100 ml). The mixture was cooled to 0 °C and 2 M HCl (250 ml) added. The mixture was stirred at room temperature for 1 h and then extracted with $CHCl_3$ (3 × 200 ml). The combined organic extracts were washed with 2 M HCl (200 ml) and brine (300 ml). The organic phase was dried over $MgSO_4$ and the solvent was evapd under red. pres. to give the crude product which was purified using flash CC with hexane:EtOAc (3:1) to give (±)-2-(2′-*acetoxyethyl*)-*cyclohexanone* (**4**) (3.45 g, 88% yield) as a colourless oil. GC column temp. 150 °C, injector temp. 280 °C, detector temp. 280 °C, RT 4.9 min (99.9%). Chiral GC column temp. 105 °C, injector temp. 200 °C, detector temp. 250 °C, RT 32.7 min (2*R*)-(−)-(**4**) (49.9%) and 33.5 min (2*S*)-(+)-(**4**) (50.1%). R_f 0.34 (hexane:

EtOAc, 7:3). (Found: $[M]^+$ 184.1098. $C_{10}H_{16}O_3$ requires M 184.1099); η_{max} (neat)/cm^{-1} 2937, 2862, 1740 (C=O), 1711 (OC=O), 1243 (C-O); δ_H (300 MHz, $CDCl_3$) 4.16–4.00 (2 H, m, H-2′), 2.46–2.21 (3 H, m, H-2 and H-6), 2.20–2.03 (3 H, m, H-1′ and CH_2), 2.01 (3 H, s, CH_3, Ac), 1.92–1.77 (1 H, m, H-1′), 1.76–1.55 (2 H, m, CH_2) and 1.54–1.30 (2 H, m, CH_2); δ_C (75.5 MHz, $CDCl_3$) 212.0 (C-1), 171.0 (CO, Ac), 62.6 (C-2′), 47.4 (C-2), 42.1 (CH_2), 34.2 (CH_2), 38.6 (CH_2), 28.0 (CH_2), 25.1 (CH_2) and 20.9 (CH_3, Ac).

(±)-7-(2′-*Acetoxyethyl*)-2-*oxepanone* (5). Under an argon atmosphere, Na_2CO_3 (1.78 g, 21.20 mmol) and 80–90% w/w MCPBA (4.95 g, 28.26 mmol) were added to a solution of (±)-2-(2′-acetoxyethyl)cyclohexanone (**4**) (2.60 g, 14.13 mmol) in CH_2Cl_2 (70 ml) at room temperature. The mixture was stirred at room temperature for 3 h, diluted with CH_2Cl_2 (80 ml) and washed sequentially with a satd Na_2SO_3 soln (3 × 100 ml), distilled water (100 ml) and brine (100 ml). The organic phase was dried over $MgSO_4$. After filtration, the solvent was evapd under red. pres. to give the crude product which was purified by flash CC using hexane:EtOAc (2:3) as eluent to give (±)-7-(2′-*acetoxyethyl*)-2-*oxepanone* (**5**) (2.76 g, 98% yield) as a colourless oil. GC column temp. 150 °C, injector temp. 280 °C, detector temp. 280 °C, RT 10.0 min (99.9%). Chiral GC column temp. 160 °C, injector temp. 200 °C, detector temp. 250 °C, RT 14.8 min (7*R*)-(−)-(**5**) (48.1%) and 15.3 min (7*S*)-(+)-(**5**) (48.4%). R_f 0.40 (hexane:EtOAc, 7:3). (Found: $[M]^+$ 200.1051. $C_{10}H_{16}O_4$ requires M 200.1048); (Found: $[M+H]^+$ 201.1121. $C_{10}H_{17}O_4$ requires M+H 201.1127); η_{max} (neat)/cm^{-1} 2936, 2864, 1747 (C=O), 1255 (C-O); δ_H (300 MHz, $CDCl_3$) 4.36 (1 H, ddd, $J_{7,1'}=8.6$, $J_{7,1'}=8.6$, $J_{7,6}=4.0$, H-7), 4.29–4.10 (2 H, m, H-2′), 2.72–2.52 (2 H, m, H-3), 2.04 (3 H, s, CH_3, Ac) and 2.03–1.53 (8 H, m, H-1′, H-4, H-5 and H-6); δ_C (75.5 MHz, $CDCl_3$) 175.1 (C-2), 170.9 (CO, Ac), 77.0 (C-7), 60.7 (C-2′), 35.5 (CH_2), 34.9 (CH_2), 34.7 (CH_2), 28.2 (CH_2), 22.9 (CH_2) and 20.9 (CH_3, Ac).

Biotransformation of (±)-2-(2′-acetoxyethyl)cyclohexanone (4) using MO 2 isolated from (±)-camphor-grown *Pseudomonas putida* NCIMB 10007

Following the procedure for the preparative scale biotransformation using the partially purified MO 2 preparation described in the preceding paper,[38,40] (±)-2-(2′-acetoxyethyl) cyclohexanone (**4**) (660 mg, 3.59 mmol), ([S] 11 mM or 2 mg ml^{-1}) was transformed into (7*R*)-(−)-7-(2′-*acetoxyethyl*)-2-*oxepanone* (**5**) in 3 h. 2-(2′-*Acetoxyethyl*)*cyclohexan*-1-*ol* (**11**) and 2-(2′-*hydroxyethyl*)*cyclohexan*-1-*ol* (**12**) were also formed as by-products. After work-up and purification using flash column chromatography with hexane:EtOAc (gradient 25–60–100% of EtOAc), the products were isolated in the following order:

(2*S*)-(+)-2-(2′-*Acetoxyethyl*)*cyclohexanone* (4). Isolated as a colourless oil [19% (GC), 88 mg, 13% yield]. $[\alpha]_D^{25}$ +2 (*c* 2; $CHCl_3$). 75% e.e. (determined by chiral GC). GC column temp. 150 °C, injector temp. 280 °C, detector temp. 280 °C, RT 4.9 min (98.4%). R_f 0.34 (hexane:EtOAc, 7:3). The ^{1}HNMR spectrum obtained was identical to the one obtained for the racemic sample prepared above.

2-(2′-*Acetoxyethyl*)*cyclohexan*-1-*ol* (11). Isolated as a single diastereoisomer and as a colourless oil [23% (GC), 76 mg, 11% yield]. GC (same conditions) RT 5.2 min (97.2%). R_f 0.55 (hexane:EtOAc, 3:7). (Found: $[M]^+$ 186.1258. $C_{10}H_{18}O_3$ requires M 186.1256); η_{max} (neat)/cm^{-1} 3467 (OH), 2932, 2858, 1739 (C=O), 1245 (C-O); δ_H (300 MHz, $CDCl_3$) 4.10 (2 H, ddd, $J_{2',1'}=6.6$, $J_{2',1'}=6.6$, $J_{2',2}=1.2$ Hz, H-2′), 3.84 (1 H, m, H-1), 2.01 (3 H, s, CH_3, Ac), 1.80–1.67 (3 H, m, H-2 and CH_2) and 1.67–1.14 (8 H, m, 4 × CH_2); δ_C (75.5 MHz, $CDCl_3$) 171.2 (CO, Ac), 68.9 (C-1), 62.9 (C-2′), 38.2 (C-2), 33.0 (CH_2), 30.7 (CH_2), 26.6 (CH_2), 24.9 (CH_2), 21.0 (CH_3, Ac) and 20.4 (CH_2).

(7*R*)-(−)-7-(2′-*Acetoxyethyl*)-2-*oxepanone* (5). Isolated as a colourless oil [36% (GC), 241 mg, 34% yield]. $[\alpha]_D^{25}$ −52 (*c* 5; $CHCl_3$). 83% e.e. (determined by chiral GC). $E_p \sim 17$. GC (same conditions) RT 10.0 min (95.3%). R_f 0.40 (hexane:EtOAc, 7:3). The ^{1}H NMR spectrum obtained was identical to a racemic sample prepared chemically.

An inseparable mixture of *syn* and *anti* 2-(2′-*hydroxyethyl*)*cyclohexan*-1-*ol* (**12**) in the ratio of 3:1 or 1:3 (determined by ^{13}C NMR) was isolated as a colourless oil [20% (GC), 96 mg, 19% yield]. GC (same conditions) RT 3.5 min (100%). R_f 0.15 (hexane:EtOAc, 3:7). (Found: $[M]^+$ 144.1156. $C_8H_{16}O_2$ requires M 144.1150); η_{max} (neat)/cm^{-1} 3346 (OH), 2931, 2859, 1448, 1063; δ_H (300 MHz, $CDCl_3$) 3.84 (1 H, m, H-1), 3.78–3.52 (0.3 H, m, H-1 and 2 H, m, H-2′), 3.50 (2.0 H, br s, CH_2OH and $CHOH$ and 0.6 H, br s, CH_2OH and $CHOH$), 3.18 (0.6 H, ddd, $J_{2',1'}=9.8$, $J_{2',1'}=9.8$, $J_{2',2}=4.6$ Hz, H-2′) and 2.00–0.90 (11 H, m, H-1′, H-2, H-3, H-4, H-5 and H-6 and 3.3 H, m, H-1′, H-2, H-3, H-4, H-5 and H-6); δ_C (75.5 MHz, $CDCl_3$) 74.9 and 69.5 (C-1), 61.4 and 60.5 (C-2′), 44.3 and 39.1 (C-2), 38.0 (CH_2), 35.5 (CH_2), 34.6 (CH_2), 32.6 (CH_2), 32.5 (CH_2), 27.3 (CH_2), 25.6 (CH_2), 24.9 (CH_2), 24.6 (CH_2) and 21.1 (CH_2).

(6*R*)-(+)-*Methyl* 6,8-*dihydroxyoctanoate* (6).[27] Under an argon atmosphere, NaOMe (12 mg, 0,24 mmol) was added to a solution of (7*R*)-(−)-7-(2′-acetoxyethyl)-2-oxepanone (**5**) (241 mg, 1.21 mmol) in MeOH (12 ml) at room temperature. The mixture was stirred at room temperature for 3 h, quenched with a satd NH_4Cl soln (40 ml) and extracted with $CHCl_3$ (3 × 40 ml). The combined organic extracts were washed with brine (40 ml) and dried over $MgSO_4$. The solvent was evapd under red. pres. to give the crude product which was purified by flash CC using EtOAc as eluent to give (6*R*)-(+)-*methyl* 6,8-*dihydroxyoctanoate* (**6**) (184 mg, 80% yield) as a colourless oil. $[\alpha]_D^{24}$ +3 (*c* 5; $CHCl_3$) [lit.[27] $[\alpha]_D^{18}$ +4.2 (*c* 5.2; $CHCl_3$)]. R_f 0.30 (EtOAc,

100%). (Found: $[M+H]^+$ 191.1282. $C_9H_{19}O_4$ requires M+H 191.1283); η_{max} (neat)/cm^{-1} 3375 (OH), 2942, 2866, 1738 (C=O), 1201 (C-O); δ_H (300 MHz, $CDCl_3$) 3.90–3.72 (3 H, m, H-6 and H-8), 3.64 (3 H, s, CH_3O), 2.69 (1 H, d, $J_{OH,6}=3.9$ Hz, CHO*H*), 2.55 (1 H, t, $J_{OH,8}=5.0$ Hz, CH_2OH), 2.31 (2 H, t, $J_{2,3}=7.5$ Hz, H-2), 1.74–1.54 (4 H, m, H-5 and H-7) and 1.54–1.25 (4 H, m, H-3 and H-4); δ_C (75.5 MHz, $CDCl_3$) 174.3 (C-1), 71.5 (C-6), 61.5 (C-8), 51.5 (CH_3O), 38.3 (CH_2), 37.2 (CH_2), 33.9 (CH_2), 25.0 (CH_2) and 24.7 (CH_2).

***(6S)-(+)-Methyl 6,8-bis(4-nitrobenzoxy)octanoate* (7).** Under an argon atmosphere, triphenylphosphine (1.016 g, 3.87 mmol) and 4-nitrobenzoic acid (647 mg, 3.87 mmol) were added to a soln of (6*R*)-(+)-methyl 6,8-dihydroxyoctanoate (**6**) (184 mg, 0.97 mmol) in THF (9.7 ml). DEAD (0.6 ml, 3.87 mmol) was added dropwise over 15 min to the stirred mixture at room temperature. The mixture was stirred at room temperature for 30 min. The solvent was evapd under red. pres. and the residue purified using flash CC with hexane:EtOAc (3:1) to give (6*S*)-(+)-*methyl* 6,8-*bis*(4-*nitrobenzoxy*)*octanoate* (**7**) (461 mg, 97% yield) as a clear yellow oil. $[\alpha]_D^{25}$ +45 (*c* 2; $CHCl_3$). R_f 0.24 (hexane:EtOAc, 7:3). (Found: $[M]^+$ 488.1419. $C_{23}H_{24}N_2O_{10}$ requires M 488.1431); η_{max} (neat)/cm^{-1} 3113, 3025, 2952, 2867, 1728 (C=O), 1608, 1529 (NO_2), 1351 (NO_2), 1293 (C-O), 1268 (C-O), 1108 (C-O); δ_H (300 MHz, $CDCl_3$) 8.30–8.10 (8 H, m, 8×CH, Ar), 5.38 (1 H, tt, $J_{6,5}=6.2$, $J_{6,7}=6.2$ Hz, H-6), 4.55–4.37 (2 H, m, H-8), 3.62 (3 H, s, CH_3O), 2.30 (2 H, t, $J_{2,3}=7.3$ Hz, H-2), 2.22 (2 H, dt, $J_{7,6}=6.2$, $J_{7,8}=6.2$ Hz, H-7), 1.92–1.75 (2 H, m, CH_2), 1.75–1.60 (2 H, m, CH_2) and 1.54–1.38 (2 H, m, CH_2); δ_C (75.5 MHz, $CDCl_3$) 173.7 (C-1), 164.5 (CO, bz), 164.2 (CO, bz), 150.60 (CNO_2, Ar), 150.57 (CNO_2, Ar), 135.5 (CCO_2, Ar), 135.3 (CCO_2, Ar), 130.7 (4×CH, Ar), 123.52 (2×CH, Ar), 123.49 (2×CH, Ar), 72.8 (C-6), 62.1 (C-8), 51.5 (CH_3O), 34.0 (CH_2), 33.7 (CH_2), 33.1 (CH_2), 24.7 (CH_2) and 24.6 (CH_2).

***(6S)-(−)-Methyl 6,8-dihydroxyoctanoate* (6).**[27] Under an argon atmosphere, anhydrous K_2CO_3 (130 mg, 0.94 mmol) was added to a soln of (6*S*)-(+)-methyl 6,8-bis(4-nitrobenzoxy)octanoate (**7**) (461 mg, 0.94 mmol) in MeOH (9.4 ml). The mixture was stirred at room temperature for 1 h and quenched with a satd NH_4Cl soln (50 ml). The mixture was extracted with $CHCl_3$ (3×50 ml) and the combined organic extracts were washed with brine (50 ml). The organic phase was dried over $MgSO_4$ and the solvent evapd under red. pres. to give the crude product which was purified using flash CC with EtOAc to give (6*S*)-(−)-*methyl* 6,8-*dihydroxyoctanoate* (**6**) (147 mg, 82% yield) as a colourless oil. $[\alpha]_D^{25}$ −3 (*c* 5; $CHCl_3$) [lit.,[27] $[\alpha]_D^{22.5}$ −3.9 (*c* 2.3; $CHCl_3$)]. R_f 0.30 (EtOAc, 100%). (Found: $[M+H]^+$ 191.1282. $C_9H_{19}O_4$ requires M+H 191.1283); η_{max} (neat)/cm^{-1} 3375 (OH), 2942, 2866, 1738 (C=O), 1201 (C-O); δ_H (300 MHz, $CDCl_3$) 3.90–3.72 (3 H, m, H-6 and H-8), 3.64 (3 H, s, CH_3O), 2.69 (1 H, d, $J_{OH,6}=3.9$ Hz, CHO*H*), 2.55 (1 H, t, $J_{OH,8}=5.0$ Hz, CH_2OH), 2.31 (2 H, t, $J_{2,3}=7.5$ Hz, H-2), 1.74–1.54 (4 H, m, H-5 and H-7) and 1.54–1.25 (4 H, m, H-3 and H-4); δ_C (75.5 MHz, $CDCl_3$) 174.3 (C-1), 71.5 (C-6), 61.5 (C-8), 51.5 (CH_3O), 38.3 (CH_2), 37.2 (CH_2), 33.9 (CH_2), 25.0 (CH_2) and 24.7 (CH_2).

***(6S)-(+)-Methyl 6,8-bis(methylsulfonyloxy)octanoate* (8).**[27,30] Under an argon atmosphere, triethylamine (324 μl, 2.32 mmol) and methanesulphonyl chloride (132 μl, 1.70 mmol) were added to a soln of (6*S*)-(−)-methyl 6,8-dihydroxyoctanoate (**6**) (147 mg, 0.77 mmol) in CH_2Cl_2 (3.9 ml) at 0 °C. The mixture was stirred at 0 °C for 30 min, then quenched with ice water (10 ml) and extracted with CH_2Cl_2 (10 ml). The extract was washed with 2 M HCl acid (10 ml), satd Na_2CO_3 soln (10 ml) and brine (10 ml). The organic phase was dried over $MgSO_4$ and the solvent evapd under red. pres. to give the crude product which was purified using flash CC with hexane:EtOAc (2:3) to give (6*S*)-(+)-*methyl* 6,8-*bis*(*methylsulfonyloxy*)*octanoate* (**8**) (247 mg, 92% yield) as a clear yellow oil. $[\alpha]_D^{25}$ +16 (*c* 5; $CHCl_3$) [lit.[30] $[\alpha]_D^{23}$ +18.5 (*c* 0.29; $CHCl_3$)]. R_f 0.30 (hexane:EtOAc, 3:2). (Found: $[M-CH_3O]^+$ 315.0581. $C_{10}H_{19}O_7S_2$ requires M-CH_3O 315.0572); η_{max} (neat)/cm^{-1} 3029, 2944, 2871, 1734 (C=O), 1354 (SO_2-O), 1174 (SO_2-O); δ_H (300 MHz, $CDCl_3$) 4.88 (1 H, m, H-6), 4.38–4.30 (2 H, m, H-8), 3.66 (3 H, s, CH_3O), 3.047 (3 H, s, CH_3SO_2), 3.043 (3 H, s, CH_3SO_2), 2.33 (2 H, t, $J_{2,3}=7.2$ Hz, H-2), 2.10 (2 H, m, H-7), 1.84–1.58 (4 H, m, H-3 and H-5) and 1.52–1.38 (2 H, m, H-4); δ_C (75.5 MHz, $CDCl_3$) 173.7 (C-1), 78.5 (C-6), 65.6 (C-8), 51.5 (CH_3O), 38.7 (CH_3SO_2), 37.4 (CH_3SO_2), 34.6 (CH_2), 34.0 (CH_2), 33.6 (CH_2), 24.4 (CH_2) and 24.2 (CH_2).

***(R)-(+)-Methyl lipoate* (9).**[27] Under an argon atmosphere, finely grounded sodium sulfide nonahydrate (189 mg, 0.79 mmol) and sulfur (25 mg, 0.79 mmol) were dissolved in DMF (14 ml). The mixture was heated to 80 °C in the dark for 1 h. A soln of (6*S*)-(+)-methyl 6,8-bis(methylsulfonyloxy)octanoate (**8**) (247 mg, 0.71 mmol) in DMF (2 ml) was added dropwise to the mixture at 80 °C over 25 min. The mixture was stirred at 80 °C for 2 h in the dark, cooled to room temperature and diluted with water (70 ml). The mixture was extracted with hexane (2×100 ml) and EtOAc (2×100 ml). The combined organic extracts were washed with brine (150 ml) and dried over $MgSO_4$. The solvent was evapd to give the crude product which was purified using flash CC with hexane:EtOAc (4:1) to give (*R*)-(+)-methyl lipoate (**9**) (130 mg, 83% yield) as a clear yellow oil. $[\alpha]_D^{26}$ +81 (*c* 2; benzene) [lit.[27] $[\alpha]_D^{24}$ +97 (*c* 1.8; benzene)]. R_f 0.30 (hexane:EtOAc, 4:1). (Found: $[M]^+$ 220.0594. $C_9H_{16}O_2S_2$ requires M 220.0592); η_{max} (neat)/cm^{-1} 2934, 2857, 1737 (C=O), 1436, 1255 (C-O), 1197, 1173; δ_H (300 MHz, $CDCl_3$) 3.66 (3 H, s, CH_3O), 3.55 (1 H, m, H-6), 3.22–3.04 (2 H, m, H-8), 2.45 (1 H, m, H-7), 2.31 (2 H, t, $J_{2,3}=7.3$ Hz, H-2), 1.90 (1 H, m, H-7), 1.76–1.57 (4 H, m, H-3 and H-5) and 1.57 - 1.36 (2 H, m, H-4); δ_C (75.5 MHz, $CDCl_3$) 173.9 (C-1), 56.3

(C-6), 51.5 (CH_3O), 40.2 (CH_2), 38.5 (CH_2), 34.6 (CH_2), 33.8 (CH_2), 28.7 (CH_2) and 24.7 (CH_2).

***(R)*-(+)-*Lipoic acid* (10).**[27] KOH (99 mg, 1.77 mmol) was dissolved in water (17.7 ml) and added to a soln of (*R*)-(+)-methyl lipoate (**9**) (130 mg, 0.59 mmol) in MeOH (17.7 ml). The mixture was stirred at room temperature for 3 h and then washed with $CHCl_3$ (20 ml) to remove the organic impurities. The aqueous phase was acidified with 2 M HCl (50 ml) and extracted with Et_2O (3 × 50 ml). The combined organic extracts were washed with brine (50 ml) and dried over $MgSO_4$. The solvent was evapd under red. pres. to give the crude product which was recrystallized from hexane to give (*R*)-(+)-lipoic acid (**10**) (85 mg, 70% yield) as yellow needles. GC column temp. 200 °C, injector temp. 280 °C, detector temp. 280 °C, RT 5.3 min (100%). $[\alpha]_D^{25}$ +87.32 (*c* 0.071; benzene)* [lit.[35] $[\alpha]_D^{23}$ +104 (*c* 0.88; benzene)]; mp 44–46 °C (lit.[23] mp 46–48 °C). Optical purity 83% (determined by comparing the observed $[\alpha]_D$ and the literature $[\alpha]_D$). (Found: $[M]^+$ 206.0430. $C_8H_{14}O_2S_2$ requires M 206.0435); η_{max} ($CHCl_3$)/cm^{-1} 3400–2500 (OH), 2929, 2857, 1706 (C=O), 1466, 1436, 1409, 1252, 1204; δ_H (300 MHz, $CDCl_3$) 3.57 (1 H, m, H-6), 3.23–3.07 (2 H, m, H-8), 2.46 (1 H, m, H-7), 2.38 (2 H, t, $J_{2,3}$ = 7.3 Hz, H-2), 1.91 (1 H, m, H-7), 1.80–1.60 (4 H, m, H-3 and H-5) and 1.60–1.40 (2 H, m, H-4); δ_C (75.5 MHz, $CDCl_3$) 179.5 (C-1), 56.3 (C-6), 40.2 (CH_2), 38.5 (CH_2), 34.6 (CH_2), 33.8 (CH_2), 28.6 (CH_2) and 24.4 (CH_2).

Analytical scale biotransformation of (±)-2-(2-acetoxyethyl)cyclohexanone (4) using MO 1 isolated from (±)-camphor-grown *Pseudomonas putida* NCIMB 10007

Following the procedure for the analytical scale biotransformation using a mixture of the two partially purified diketocamphane monooxygenase isozymes (MO 1),[31] (±)-2-(2′-acetoxyethyl)cyclohexanone (**4**) (2 mg, 11 μmol) was oxidised to (7*R*)-(−)-7-(2′-acetoxyethyl)-2-oxepanone (**5**). After 7 h, the products were identified as listed in Table 1.

Analytical scale biotransformation of (±)-2-(2′-acetoxyethyl)cyclohexanone (4) using partially purified MO 2 isolated from (±)-camphor-grown *Pseudomonas putida* NCIMB 10007

NADPH (2 mg, 2.7 μmol) and (±)-2-(2′-acetoxyethyl)cyclohexanone (**4**) (0.5 mg, 2.7 μmol) were added to a solution of MO 2 (partially purified using a Q-Sepharose column)[33] in phosphate buffer (1 ml, 50 mM, pH 8.1). The mixture was agitated in an orbital incubator (200 rpm, 30 °C) and the transformation was monitored by GC. After 12 h, the products were identified as listed in Table 1.

*The specific rotation of the (*R*)-(+)-lipoic acid (**10**) was recorded at Birkbeck College, University of London (error ±0.01).

Analytical scale biotransformation of (±)-2-(2′-acetoxyethyl)cyclohexanone (4) using partially purified NADPH-dependent cyclohexanone monooxygenase (CHMO) isolated from cyclohexanol-grown *Xanthobacter autotrophicus* NCIMB 10811[34]

NADPH (1 mg, 1.1 μmol) and (±)-2-(2′-acetoxyethyl)-cyclohexanone (**4**) (0.2 mg, 1.1 μmol) were added to a soln of cyclohexanone monooxygenase (partially purified using a Q-Sepharose column) in phosphate buffer (1 ml, 50 mM, pH 8.1). The mixture was agitated in an orbital incubator (200 rpm, 30 °C) and the transformation was monitored by GC. After 3 h, the products were identified as listed in Table 1.

Preparative scale biotransformation of (±)-2-(2′-acetoxyethyl)cyclohexanone (4) using cyclopentanone monooxygenase (CPMO) isolated from cyclopentanol-grown *Pseudomonas* sp. NCIMB 9872

NADPH (91 mg, 0.11 mmol) and (±)-2-(2′-acetoxyethyl)cyclohexanone (**4**) (20 mg, 0.11 mmol) were added to a solution of cyclopentanone monooxygenase[36] (3.12 IU) in Trisma buffer (20 ml, 50 mM, pH 7.5). The mixture was agitated in an orbital incubator (200 rpm, 30 °C) for 45 min and then extracted with EtOAc (3 × 20 ml). The combined organic extracts were washed with brine (20 ml) and dried over $MgSO_4$. The solvent was evapd under red. pres. to give the crude product. Purification using flash CC with hexane:EtOAc (gradient 25–60% of EtOAc) afforded the following products.

***(2R)*-(-)-2-(2′-*Acetoxyethyl)cyclohexanone* (4).** The first product eluted and isolated as a colourless oil [38% (GC), 7 mg, 37% yield]. $[\alpha]_D^{25}$ −1 (*c* 0.5 in $CHCl_3$). 68% e.e. (determined by chiral GC). GC column temp. 150 °C, injector temp. 280 °C, detector temp. 280 °C, RT 4.9 min (100%). R_f 0.34 (hexane:EtOAc, 7:3). The ^{1}H NMR spectrum obtained was identical to the one obtained for a racemic sample.

***(7S)*-(+)-7-(2′-*Acetoxyethyl)-2-oxepanone* (5).** The second product eluted and isolated as a colourless oil [62% (GC), 13 mg, 59% yield]. $[\alpha]_D^{25}$ +30 (*c* 1; $CHCl_3$). 42% e.e. (determined by chiral GC). $E_p \sim 5$. GC (same conditions) RT 10.0 min (100%). R_f 0.40 (hexane:EtOAc, 7:3). The ^{1}H NMR spectrum obtained was identical to the one obtained for the equivalent racemic sample prepared chemically.

***(6S)*-(−)-*Methyl* 6,8-*dihydroxyoctanoate* (6).**[27] Under an argon atmosphere, NaOMe (3 mg, 0.06 mmol) was added to a soln of (7*S*)-(+)-7-(2′-acetoxyethyl)-2-oxepanone (**5**) (13 mg, 0.06 mmol) in MeOH (0.6 ml) at room temperature. The mixture was stirred at room temperature for 3 h, quenched with a satd NH_4Cl soln (5 ml) and extracted with $CHCl_3$ (3 × 5 ml). The combined extracts were washed with brine (5 ml) and dried over $MgSO_4$. The solvent was evapd under red. pres. to give the crude product which was purified by flash CC using EtOAc as eluent to give

(6*S*)-(−)-*methyl* 6,8-*dihydroxyoctanoate* (**6**) (8 mg, 63% yield) as a colourless oil. $[\alpha]_D^{25}$ −2 (*c* 0.5 in $CHCl_3$) [lit.[27] $[\alpha]_D^{22.5}$ −3.9 (*c* 2.3 in $CHCl_3$)]. R_f 0.30 (EtOAc, 100%). (Found: $[M+H]^+$ 191.1282. $C_9H_{19}O_4$ requires M+H 191.1283); η_{max} (neat)/cm^{-1} 3375 (OH), 2942, 2866, 1738 (C=O), 1201 (C-O); δ_H (300 MHz, $CDCl_3$) 3.90–3.72 (3 H, m, H-6 and H-8), 3.64 (3 H, s, CH_3O), 2.69 (1 H, d, $J_{OH,6}$=3.9 Hz, CHO*H*), 2.55 (1 H, t, $J_{OH,8}$=5.0 Hz, CH_2OH), 2.31 (2 H, t, $J_{2,3}$=7.5, H-2), 1.74–1.54 (4 H, m, H-5 and H-7) and 1.54–1.25 (4 H, m, H-3 and H-4); δ_C (75.5 MHz, $CDCl_3$) 174.3 (C-1), 71.5 (C-6), 61.5 (C-8), 51.5 (CH_3O), 38.3 (CH_2), 37.2 (CH_2), 33.9 (CH_2), 25.0 (CH_2) and 24.7 (CH_2).

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