Enzymatic Baeyer–Villiger oxidations of some bicyclo[2.2.1]heptan-2-ones using monooxygenases from *Pseudomonas putida* NCIMB 10007: enantioselective preparation of a precursor of azadirachtin

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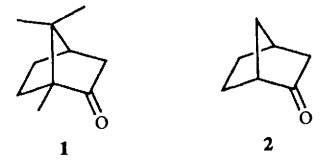
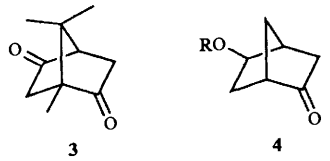
Two monooxygenases MO1 (NADH dependent) and MO2 (NADPH dependent) isolated from *Ps. putida* NCIMB 10007 [grown on (+)-camphor] have been utilized as biocatalysts in Baeyer–Villiger oxidations. The former enzyme oxidized the racemic ketones **9**, **10** and **14** into the optically active lactones **15**–**17**. The ketone **9** is not oxidized by MO2 but the ketones **10** and **14** gave the optically active lactones **16** and **17**. Whole-cell preparations of *Ps. putida* degraded the ketone **9** but transformed the racemic ketones **10** and **14** into the optically active lactones **16** and **17**. All the lactones possess the same absolute configuration: 1*S*, 5*S*, 6*R*. (+)-MO1 [the isozyme which metabolizes (+)-camphor], oxidized the ketone **10** but not the ketone **9**. Conversely, (−)-MO1 [the isozyme which metabolizes (−)-camphor], catalysed the oxidation of the ketone **9** but not the ketone **10**. Co-factor recycling was effected using dehydrogenase enzymes in preparative-scale experiments. The optically active lactone **17** is an intermediate in the synthesis of compound **5**, an important precursor of azadirachtin **6**.

Catalytic asymmetric oxidations are very useful in the synthesis of chiral building blocks.[1] Various reagents and metal catalysts have been developed for Baeyer–Villiger (B.–V.) oxidations,[2] but very few of them lead to optically active compounds.[3] In fact, most of the enantioselective B.–V. oxidations have been achieved with the help of enzymes.[4] In the last few years, we have been interested in enzymatic Baeyer–Villiger transformations using *Pseudomonas putida* NCIMB 10007 as the source of these enzymes.[5–7] Based upon the activity of *Ps. putida* on compounds such as camphor[8] **1**, norcamphor[7] **2** and diketocamphane[8] **3**, we have recently developed an interest in utilizing the bicyclic ketones **4** as potential substrates.



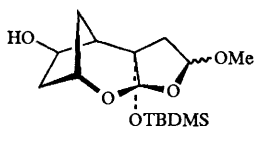
1 **2**



3 **4**

Ketones of type **4** can be transformed into the acetal **5**,[9] an advanced precursor of the potent antifeedant and growth regulator agent azadirachtin[10] **6**.

Herein we report a thorough study of the synthetic potential of the monooxygenases isolated from *Ps. putida* NCIMB 10007 in providing optically active lactones from bicyclic ketones of type **4**.



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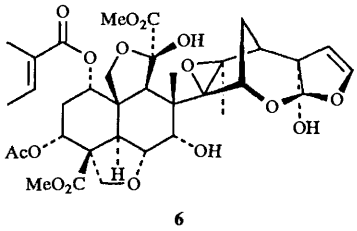

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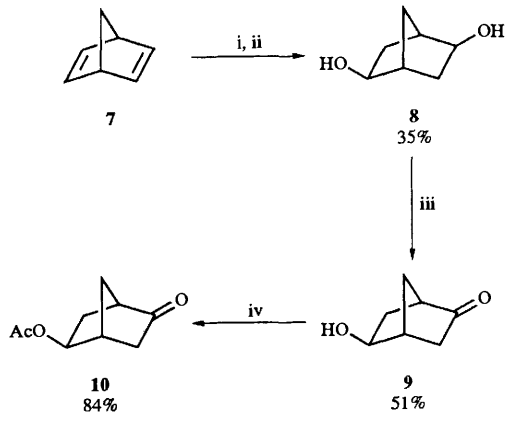
Results and discussion

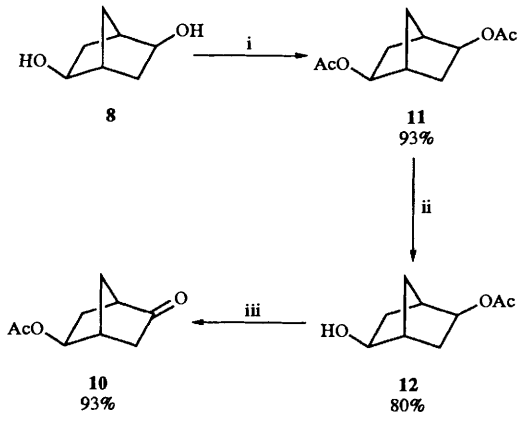
Compounds **9** and **10** were prepared using the hydroboration of norbornadiene **7** as the key step[11] (Scheme 1). The *exo,exo*-diol **8** is obtained in a pure state (≥99% by GC) after several recrystallisations. Careful oxidation of the diol **8** using Jones' reagent gave the hydroxy ketone **9** in 51% yield. Acetylation of **9** under the usual conditions afforded the ester **10**.



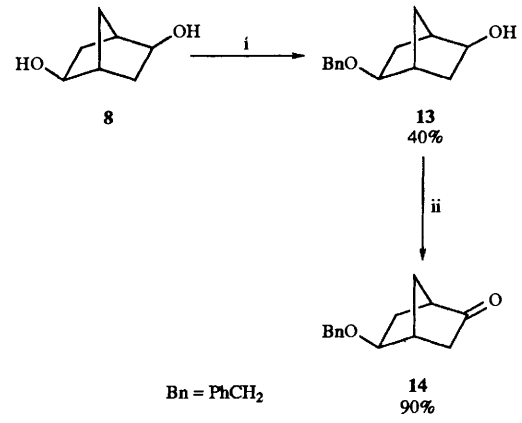
Scheme 1 *Reagents and conditions:* i, BH_3–THF; ii, H_2O_2, NaOH; iii, H_2CrO_4, acetone, 0 °C; iv, Ac_2O, DMAP, Py

An alternative preparation of the ester **10** was developed (Scheme 2). Acetylation of the diol **8** furnished compound **11** which was selectively hydrolysed by porcine pancreatic lipase (PPL) to afford the racemic monoacetate **12**. Oxidation of the alcohol **12** gave the ketone **10**.

This second route gave a better overall yield of the keto ester **10** (69% against 43% starting from the diol **8**). Compound **14** (Scheme 3) was prepared by monobenzylation of the diol **8** and oxidation of the remaining hydroxy group using pyridinium chlorochromate (PCC).



Scheme 2 *Reagents and conditions:* i, Ac_2O, DMAP, Py; ii, PPL, buffer; iii, PCC, CH_2Cl_2



Scheme 3 *Reagents and conditions:* i, NaH, Bu_4NI, BnBr, DMF; ii, PCC, CH_2Cl_2

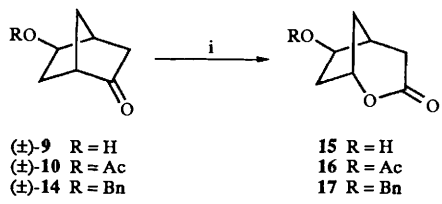
Table 1 Optimisation of the conditions for the Baeyer–Villiger oxidation involving substrate **10** and MO1

Subs. conc./ mmol dm^{-3}	pH	T/°C	Co-solvent	Lactone (%)[a]
20	7.1	22	—	18
20	7.1	30	—	25
20	7.1	40	—	9
10	7.1	30	—	31
10	8.1	30	—	39
10	8.1	30	ethanol[b]	40

[a] Determined by GC using a non-polar column. [b] 0.5% v/v.

It has been well established that *Ps. putida* when grown on (+)-camphor gives rise to two sets of monooxygenases,[8] one (labelled MO1) whose natural function is to metabolize a ketocamphane and the second (labelled MO2) which is necessary further down the metabolic pathway[12] to oxidize an α,β-unsaturated cyclopentanone. Optimisation studies were undertaken with a crude mixture of protein containing MO1 (NADH dependent) acting upon substrate **10** (Table 1). The best conversion into lactone **16** was obtained at 30 °C using a substrate concentration of 10 mmol dm^{-3} in Tris buffer pH 8.1 in the presence of ethanol (0.5%). Under these optimum conditions, using a mixture of MO1 and MO2 (NADPH dependent) on an analytical scale (Scheme 4), substrates **9, 10** and **14** yielded results as presented in Table 2. Note that the racemic lactones were prepared as standards using *m*-chloroperbenzoic acid (MCPBA).[9]

Good conversions of ketones into lactones (≥31%) were



(±)-**9** R = H
(±)-**10** R = Ac
(±)-**14** R = Bn

15 R = H
16 R = Ac
17 R = Bn

Scheme 4 *Reagents and conditions:* i, Crude mixture of MO1 and MO2, NADH or NADPH, Tris buffer. *NB* MO1 cannot use NADPH as the co-factor; similarly MO2 cannot use NADH as the co-factor.

Table 2 Analytical scale oxidations of ketones **9**, **10** and **14** to form the lactones **15**, **16** and **17** using a crude mixture of MO1 and MO2 over 24 h (see Scheme 4)

Enzyme	Substrate	Product	Conversion (%)[a]	ee lactone (%)[b]
MO1	(±)-**9**	**15**	31	≥95
MO2	(±)-**9**	**15**	4	ND[c]
MO1	(±)-**10**	**16**	40	≥95
MO2	(±)-**10**	**16**	39	≥95
MO1	(±)-**14**	**17**	31	ND[c]
MO2	(±)-**14**	**17**	35	ND[c]

[a] Determined by GC with a non-polar column. [b] Determined by GC with a chiral column. [c] ND: not determined.

Table 3 Preparative-scale oxidations of the ketones **9**, **10** and **14** to form the lactones **15**, **16** and **17** using *Ps. putida* or a crude mixture of MO1 and MO2 over 24 h

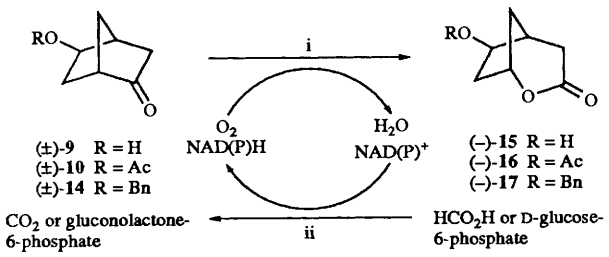
Org./Enz.	Substrate	Product	Yield (conv.[a]) (%)	ee lact. (%)[b] (abs. conf.)
10007	(±)-**9**	DC[c]	ND[c]	ND[c]
MO1	(±)-**9**	(−)-**15**	11 (14)	≥95 (1*S*,5*S*,6*R*)
10007	(±)-**10**	(−)-**16**	27 (28)	≥95 (1*S*,5*S*,6*R*)
MO1	(±)-**10**	(−)-**16**	35 (38)	≥95 (1*S*,5*S*,6*R*)
10007	(±)-**14**	(−)-**17**	11 (12)	≥95 (1*S*,5*S*,6*R*)
MO1	(±)-**14**	(−)-**17**	39 (42)	≥95 (1*S*,5*S*,6*R*)
MO2	(±)-**14**	(−)-**17**	50 (58)	87 (1*S*,5*S*,6*R*)

[a] Determined by GC with a non-polar column. [b] Determined by GC with a chiral column. [c] DC: decomposition products; ND: not determined.

obtained for all these runs except for the MO2-catalysed oxidation of the ketone **9** where only 4% of the lactone **15** was produced. This low conversion demonstrates the importance of having an acetyl or a benzyl group on the hydroxy moiety for oxidation by the enzyme MO2. Enantiomeric excesses (ees) were all excellent (over 95%; determined by GC using a Lipodex D stationary phase). In the case of the conversion of the ketone **14** into the lactone **17**, the reaction was scaled up in order to determine the ee of the product.

In the preparative runs (Table 3) involving the enzymes MO1 and MO2, the co-factor was recycled using either formate and formate dehydrogenase or D-glucose-6-phosphate and glucose-6-phosphate dehydrogenase (Scheme 5). In addition to the experiments involving MO1 and MO2 a whole-cell biotransformation with *Ps. putida* NCIMB 10007 was performed as described in Table 3.

It is clear that the ketone **9** is not a good substrate. The whole cells caused extensive decomposition and MO1, on a preparative scale, gave a low conversion into the lactone **15** (14%). Instead a high percentage (53%) of reduction products was obtained indicating the presence of a dehydrogenase in the protein mixture. Whole cell and MO1 runs with substrate **10** gave better results. The whole-cell system gave 28% of lactone **16** and MO1 38% of lactone **16** (determined by GC using BP1 as



Scheme 5 *Reagents and conditions:* i, Crude mixture of MO1 and MO2, Tris or phosphate buffer, ethanol, 30 °C; ii, formate dehydrogenase from *Candida boidinii* or glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides*

Table 4 Oxidations of the ketones **9** and **10** into the lactones **15** and **16** using (+)-MO1 and (−)-MO1 over 24 h

Enzyme	Substrate	Product	Conversion (%)[a]	ee lactone (%)[b]
(+)-MO1	(±)-**9**	(−)-**15**	0	—
(−)-MO1	(±)-**9**	(−)-**15**	33	≥95
(+)-MO1	(±)-**10**	(−)-**16**	35	≥95
(−)-MO1	(±)-**10**	(−)-**16**	0	—

[a] Determined by GC using a non-polar column. [b] Determined by GC using a chiral column.

the stationary phase). Yields of isolated lactone were very similar (26 and 35%). The enantiomeric excesses for the lactones **16** were excellent (over 95% as determined by GC using Lipodex D as the stationary phase).

Substrate **14** was smoothly converted into the lactone **17** using the enzymes but not the whole cells. In the latter case the major compounds formed were reduction products (54%). The ees for the lactone **17** are good to excellent (≥87%). The smaller enantioselectivity of MO2 can be explained by the fact that the conversion was 58%. The ees were measured by GC after deprotection of the hydroxy group by catalytic hydrogenation using palladium-on-charcoal as the catalyst.

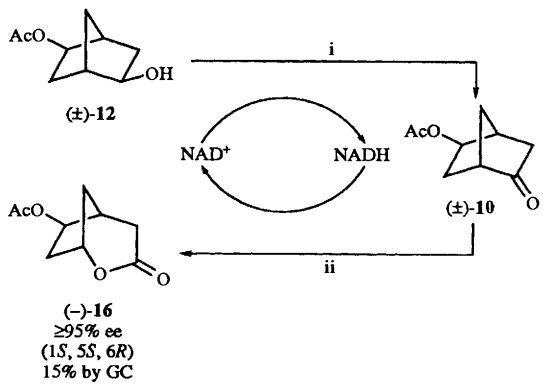
The absolute configuration of the lactone **15** was determined by comparison of the specific rotation with the literature value.[9] The lactones **16** and **17**, after hydrolysis or catalytic hydrogenation, were correlated with the lactone **15**. We found that all the lactones that were formed possess the same absolute configuration, *i.e.* 1*S*,5*S*,6*R*.

Further purification of protein MO1 led to the isolation of two isozymes as previously described.[13] One isozyme oxidizes (+)-camphor [(+)-MO1] while the other metabolizes (−)-camphor [(−)-MO1]. Very interestingly these two isozymes have opposite selectivity towards the ketones **9** and **10** (Table 4). The ketone **9** is a substrate for (−)-MO1 but not for (+)-MO1. Conversely, oxidation of the ketone **10** is catalysed by (+)-MO1 but not by (−)-MO1. Once again, the ees are excellent (over 95%) and, as expected, the absolute configuration is 1*S*,5*S*,6*R* in both cases.

Co-factor recycling can be effected by employing another coupled enzyme system, *i.e.* a NADH-dependent dehydrogenase (HLADH) and a monooxygenase (MO1) working in tandem with two closely related substrates (Scheme 6).

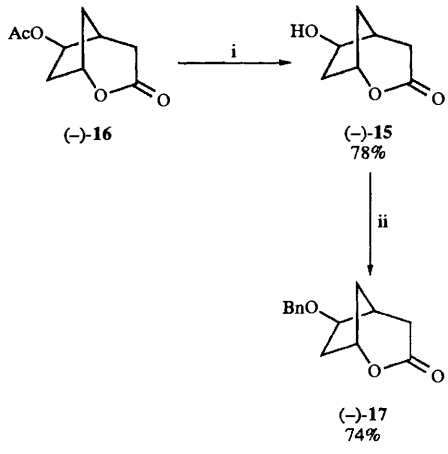
After 22 h 15% of the lactone **16** was present according to GC methods. The low percentage of lactone is probably due to the time-dependent loss in activity of MO1 during the slow oxidation of the alcohol **12**. In fact, it was shown in a separate experiment that after a few hours the ketone **10** is not further oxidized by MO1.

In summary the enzyme MO1 (comprising two isozymes) is the best monooxygenase for the biotransformation of bicyclic



Scheme 6 *Reagents and conditions:* i, HLADH, Tris buffer, ethanol, 30 °C; ii, crude mixture of MO1

compounds. This can be easily rationalized by examining the metabolic pathway of the (+)-camphor.[12] The protein MO1 is induced to metabolize a chiral bicycloalkanone while the protein MO2 is raised to oxidize a monocyclic ketone. Depending on the structural composition of the substrates only one of the two isozymes catalyses the oxidation into the lactone. Biotransformation of the ketone **14** leads to the optically active intermediate **17** previously used in the synthesis of compound **5**, an important precursor of azadirachtin[9] **6**. The lactone **17** can be prepared, with the same efficiency in two steps, from the lactone **16** (Scheme 7). Mild conditions were used in the latter



Scheme 7 *Reagents and conditions:* i, KCN, EtOH 95%; ii, Ag_2O, BnBr, DMF

conversion to avoid decomposition of the lactone moiety; potassium cyanide[14] was employed for the hydrolysis step and silver(I) oxide[15] for the benzylation.

On-going work in these laboratories involves complete purification, crystallisation, and analysis by X-ray diffraction of the monooxygenases isolated from *Pseudomonas putida* NCIMB 10007 and an investigation of new applications of these enzymes in organic synthesis on a substantial scale.

Experimental

Diethyl ether and tetrahydrofuran were dried and distilled from sodium–benzophenone. Ethyl acetate was distilled from phosphorus pentoxide. Light petroleum (bp 40–60 °C) and dichloromethane were distilled from calcium hydride. Chloroform was washed with water, dried over potassium carbonate and distilled from calcium chloride. Other reagents and solvents were used as commercially supplied. Thin layer chromatography (TLC) was performed on pre-coated glass plates (Merck silica gel

60 F254). The plate was visualized using a mixture of ceric sulfate and ammonium molybdate or a potassium permanganate dip followed by heating. Flash column chromatography was performed over silica gel (Merck silica gel 60, 40–63 mm). Gas chromatography was performed with a Shimadzu GC-14A gas chromatograph equipped with a capillary column: BP1 (25 m), Lipodex D (25 m). ^1H, ^{13}C and ^{19}F NMR spectra were recorded on a Bruker AM 300 spectrometer using the deuterium lock, for $CDCl_3$ solutions. 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 a liquid film between sodium chloride plates. Mass spectra were recorded on a Kratos Profile HV 3000 spectrometer. Optical rotations were measured on an Optical Activity AA-1000 polarimeter and are recorded in units of 10^{-1} deg cm^2 g^{-1}. Solutions of compounds in organic solvents were dried using anhydrous magnesium sulfate.

***exo,exo*-Bicyclo[2.2.1]heptane-2,5-diol (±)-8**[11]

Bicyclo[2.2.1]hepta-2,5-diene **7** (9.20 g, 100 mmol) was dissolved in THF (100 cm^3) under argon. The solution was cooled at 0 °C and borane–THF complex (100 cm^3, 100 mmol) was slowly added to it. After the addition the solution was warmed to room temperature and held at that temperature for 2 h. Aqueous sodium hydroxide (2 mol dm^{-3}; 40 cm^3) and hydrogen peroxide (30%; 40 cm^3) were then slowly added to it at room temperature. The resulting mixture was heated at 65 °C for 4 h after which the solvents were removed by evaporation under reduced pressure and the residue was dissolved in ethanol. The insoluble material was filtered off and the solution evaporated under reduced pressure. The residue was crystallized twice from ethanol–diethyl ether and in order to raise the yield, the mother solutions were purified by flash chromatography and crystallized from ethanol–diethyl ether to give the title diol **8** (35%) as a white solid, mp 183.0–183.5 °C (lit.,[11] 183.5–184.5 °C) (Found: M$^+$, 128.083 42. $C_7H_{12}O_2$ requires M^+, 128.083 73); $\delta_H(CD_3OD)$ 4.80 (2 H, s, OH), 3.58 (2 H, dd, J 2, 6.5, 2-H, 5-H), 2.11 (2 H, m, 1-H, 4-H), 1.55 (2 H, s, 2 × 7-H), 1.46 (2 H, dd, J 6.5, 14, 3-H, 6-H) and 1.22 (2 H, m, 3-H, 6-H); $\delta_C(CD_3OD)$ 74.24 (CH), 44.12 (CH), 37.28 (CH_2) and 30.94 (CH_2).

***exo*-5-Hydroxybicyclo[2.2.1]heptan-2-one (±)-9**

A solution of the diol **8** (497 mg, 3.88 mmol) in acetone (50 cm^3) was stirred at 0 °C whilst chromic acid (CrO_3 2 mol dm^{-3} in 30% H_2SO_4; 1.8 cm^3) was added very slowly to it over a period of 1 h. The mixture was then stirred at 0 °C for a further 1 h after which the solution was decanted and the residue washed with acetone. The decanted solution and acetone washings were combined, dried (K_2CO_3), filtered and evaporated under reduced pressure at room temperature to give a crude oil. This was purified by flash chromatography using diethyl ether to afford the title ketone **9** (51%) as an oil (Found: M$^+$, 126.067 72. $C_7H_{10}O_2$ requires M^+, 126.068 08); ν_{max}(neat)/cm^{-1} 3427, 2972, 1746, 1407, 1343, 1305, 1183, 1142, 1093, 1061, 1002, 958, 907 and 763; $\delta_H(CDCl_3)$ 4.08 (1 H, d, J 6.5, 5-H), 2.56 (2 H, m, 1-H, 4-H), 2.00 (3 H, m, 3-H, 6-H, 7-H), 1.68 (3 H, m, 3-H, 6-H, 7-H) and 1.86 (1 H, br s, OH); $\delta_C(CDCl_3)$ 216.50 (CO), 72.62 (CH), 48.95 (CH), 43.38 (CH), 39.99 (CH_2), 35.68 (CH_2) and 33.61 (CH_2).

***exo*-5-Acetoxybicyclo[2.2.1]heptan-2-one (±)-10**

A mixture of compound **9** (32 mg, 0.254 mmol), *N*,*N*-dimethylaminopyridine (catalytic amount) and acetic anhydride (43 mg, 0.421 mmol) in dry pyridine (1 cm^3) was stirred at room temp. under argon for 5 h. After dilution with chloroform the mixture was washed with hydrochloric acid (2 mol dm^{-3}) and brine, dried ($MgSO_4$), filtered and concentrated by evaporation under reduced pressure. The resulting crude oil was purified by flash chromatography with diethyl ether–light petroleum (2:3) as eluent to afford the title bicyclic ketone **10** (84%) as an oil (Found: M$^+$, 168.079 36. $C_9H_{12}O_3$ requires M^+, 168.078 64); ν_{max}(neat)/cm^{-1} 2982, 1752, 1441, 1410, 1376, 1361, 1298, 1248, 1182, 1141, 1050, 1020, 978 and 962; $\delta_H(CDCl_3)$ 4.84 (1 H, dd, J 1, 7, 5-H), 2.71 (1 H, m, 1-H), 2.57 (1 H, m, 4-H), 2.25 (1 H, m, 3-H), 2.03 (3 H, s, CH_3), 1.92 (1 H, m, 6-H), 1.81 (1 H, dd, J 4.5, 18.5, 3-H) and 1.72 (3 H, m, 6-H, 2 × 7-H); $\delta_C(CDCl_3)$ 215.15 (CO), 170.43 (CO), 74.80 (CH), 48.71 (CH), 40.67 (CH), 39.96 (CH_2), 34.27 (CH_2), 33.34 (CH_2) and 21.11 (CH_3).

***exo,exo*-2,5-Diacetoxybicyclo[2.2.1]heptane (±)-11**

A mixture of the diol **8** (100 mg, 0.781 mmol), *N*,*N*-4-dimethylaminopyridine (catalytic amount) and acetic anhydride (270 mg, 2.64 mmol) in dry pyridine (2 cm^3) was stirred at room temperature under argon for 4 h. After dilution with chloroform the mixture was washed with hydrochloric acid (2 mol dm^{-3}) and brine, dried ($MgSO_4$), filtered and concentrated by evaporation under reduced pressure. The resulting crude oil was purified by flash chromatography with diethyl ether–light petroleum (3:7) as eluent to afford the title bicycloheptane **11** (93%) as an oil; ν_{max}(neat)/cm^{-1} 2977, 1743, 1441, 1377, 1362, 1307, 1290, 1238, 1189, 1069, 1014, 985, 963, 934, 756, 631 and 605; $\delta_H(CDCl_3)$ 4.51 (2 H, dd, J 2, 7, 2-H, 5-H), 2.34 (2 H, m, 1-H, 4-H), 1.98 (6 H, s, 2 × CH_3), 1.67 (2 H, dd, J 7, 14, 3-H, 6-H), 1.54 (2 H, s, 7-H) and 1.42 (2 H, m, 3-H, 6-H); $\delta_C(CDCl_3)$ 170.59 (CO), 75.90 (CH), 40.45 (CH), 34.21 (CH_2), 31.98 (CH_2) and 21.20 (CH_3).

***exo,exo*-5-Acetoxybicyclo[2.2.1]heptan-2-ol (±)-12**

A mixture of the bicycloheptane **11** (87 mg, 0.41 mmol) and porcine pancreatic lipase (PPL; 802 mg, 8758 units) in Tris buffer pH 7.1 (50 mmol dm^{-3}; 21 cm^3) was stirred at room temp. After 15 h the solution was adjusted to pH 7.1 and after 42 h it was diluted with ethanol and the resulting suspension cooled to 0 °C. The solids present were filtered and the filtrate was evaporated under reduced pressure. The resulting residue was dissolved in chloroform–ethanol (9:1) to give a solution which was filtered and evaporated under reduced pressure to afford a crude oil. This was purified by flash chromatography with diethyl ether–light petroleum (3:2) as eluent to afford the title bicyclic heptanol (80%) as an oil (Found: M$^+$, 170.094 56. $C_9H_{14}O_3$ requires M^+, 170.094 294); ν_{max}(neat)/cm^{-1} 3357, 2972, 2900, 1745, 1437, 1374, 1359, 1288, 1242, 1079, 1062, 1025, 1005 and 988; $\delta_H(CDCl_3)$ 4.47 (1 H, d, J 7, 5-H), 3.71 (1 H, d, J 6.5, 2-H), 2.32 (1 H, d, J 4.5, 4-H), 2.18 (1 H, d, J 4.5, 1-H), 1.99 (3 H, s, CH_3), 1.83 (1 H, s, OH), 1.55 (4 H, m, 2 × 7-H, 3-H, 6-H) and 1.32 (2 H, m, 3-H, 6-H); $\delta_C(CDCl_3)$ 170.85 (CO), 76.25 (CH), 73.35 (CH), 43.27 (CH), 40.51 (CH), 36.81 (CH_2), 34.37 (CH_2), 31.19 (CH_2) and 21.27 (CH_3).

***exo*-5-Acetoxybicyclo[2.2.1]heptan-2-one (±)-10**

A solution of compound **12** (208 mg, 1.224 mmol) in dry dichloromethane (3.5 cm^3) was stirred at 0 °C under argon whilst pyridinium chlorochromate (PCC) (410 mg, 1.902 mmol) was added to it. The mixture was then allowed to warm to room temp. over 4 h after which it was deposited onto silica. The title compound was eluted with diethyl ether–light petroleum (2:3) in 93% yield, after purification. The physical data were the same as above.

***exo,exo*-5-Benzyloxybicyclo[2.2.1]heptan-2-ol (±)-13**

A solution of compound **8** (600 mg, 4.68 mmol) in dry dimethylformamide (13 cm^3) was added to a suspension of sodium hydride (95%; 130 mg, 5.15 mmol) in dry DMF (10 cm^3) at 0 °C under argon. The mixture was stirred for 1 h after which tetrabutylammonium iodide (catalytic amount) and benzyl

bromide (1.22 g, 7.13 mmol) were added to it. After the reaction had been stirred at room temp. for a further 2 d under argon, methanol and distilled water were added to it and then extracted with chloroform. The extract was dried ($MgSO_4$), filtered and evaporated under reduced pressure to give a crude oil. This was purified by flash chromatography with diethyl ether–light petroleum (1:1) as eluent to afford the title bicyclic heptanol **13** (40%) as an oil (Found: M^+, 218.130 65. $C_{14}H_{18}O_2$ requires M^+, 218.130 68); ν_{max}(neat)/cm^{-1} 3389, 3031, 2960, 2935, 2880, 1496, 1453, 1440, 1352, 1207, 1181, 1095, 1066, 1012, 982, 927, 734 and 697; δ_H($CDCl_3$) 7.33 (5 H, m, Ph), 4.49 (1 H, d, *J* 12, C*H*Ph), 4.43 (1 H, d, *J* 12, C*H*Ph), 3.68 (1 H, m, 5-H), 3.37 (1 H, t, *J* 4.5, 2-H), 2.42 (1 H, d, *J* 5, 4-H), 2.16 (1 H, m, 1-H), 1.69 (1 H, br s, OH), 1.60 (2 H, m, 3-H, 7-H), 1.47 (1 H, ddd, *J* 1.5, 7, 14, 6-H), 1.44 (2 H, m, 3-H, 7-H) and 1.28 (1 H, dddd, *J* 1.5, 2, 5, 14, 6-H); δ_C($CDCl_3$) 138.82 (=C), 128.34 (=CH), 127.54 (=CH), 127.41 (=CH), 80.77 (CH), 73.95 (CH), 70.41 (CH_2), 43.22 (CH), 39.49 (CH), 37.10 (CH_2), 34.35 (CH_2) and 30.84 (CH_2).

exo-5-Benzyloxybicyclo[2.2.1]heptan-2-one (±)-14

A solution of compound **13** (272 mg, 1.25 mmol) in dry dichloromethane (5 cm^3) was stirred at 0 °C under argon whilst PCC (434 mg, 2.01 mmol) was added to it. The mixture was then allowed to warm to room temp. over 3 h after which it was deposited onto silica. The product was eluted with diethyl ether–light petroleum (2:3) to afford the title bicyclic heptanone **14** (90%) as an oil (Found: M^+, 216.115 63. $C_{14}H_{18}O_2$ requires M^+, 216.115 03); ν_{max}(neat)/cm^{-1} 2975, 1748, 1454, 1354, 1183, 1143, 1097, 1073, 1028, 991, 737 and 698; δ_H($CDCl_3$) 7.36 (5 H, m, Ph), 4.55 (1 H, d, *J* 12.5, C*H*Ph), 4.50 (1 H, d, *J* 12.5, C*H*Ph), 3.73 (1 H, br d, *J* 6.5, 5-H), 2.78 (1 H, br d, *J* 5, 1-H), 2.57 (1 H, d, *J* 5, 4-H), 2.04 (1 H, ddd, *J* 1, 5, 18, 3-H), 2.03 (1 H, m, 7-H), 1.92 (1 H, ddd, *J* 2, 6.5, 14, 6-H), 1.78 (1 H, m, 6-H), 1.69 (1 H, dd, *J* 4, 18, 3-H) and 1.68 (1 H, m, 7-H); δ_C($CDCl_3$) 216.50 (CO), 138.28 (=C), 128.44 (=CH), 127.66 (=CH), 127.55 (=CH), 79.68 (CH), 70.88 (CH_2), 48.72 (CH), 40.14 (CH_2), 39.77 (CH), 34.07 (CH_2) and 33.34 (CH_2).

exo-6-Hydroxy-2-oxabicyclo[3.2.1]octan-3-one (±)-15[9]

To a stirred mixture of compound **9** (90 mg, 0.71 mmol) and *m*-chloroperoxybenzoic acid (193 mg, 1.12 mmol) in dichloromethane (2 cm^3) at room temperature was added a catalytic amount of toluene-*p*-sulfonic acid. The solution was kept in the dark for 3 h after which it was diluted with diethyl ether washed with saturated aqueous solutions of sodium sulfite and sodium hydrogen carbonate, dried ($MgSO_4$), filtered and evaporated under reduced pressure. The crude residue was purified by flash chromatography with methanol–diethyl ether (1:14) as eluent to afford the title bicyclic octanone **15** (76%) as an oil; ν_{max}(neat)/cm^{-1} 3433, 2085, 1711, 1640, 1385, 1249, 1204, 1075, 1040 and 974; δ_H($CDCl_3$) 4.88 (1 H, m, 1-H), 4.31 (1 H, d, *J* 6.5, 6-H), 2.76 (1 H, dd, *J* 6, 19, 4-H), 2.57 (1 H, ddd, *J* 2.5, 6.5, 16, 7-H), 2.49 (1 H, ddd, *J* 1.5, 2, 19, 4-H), 2.40 (1 H, m, 5-H), 2.13 (1 H, dq, *J* 2.5, 13.5, 8-H), 1.93 (1 H, dd, *J* 1, 13, 8-H), 1.88 (1 H, dddd, *J* 1.5, 2, 5, 16, 7-H) and 1.25 (1 H, s, OH); δ_C($CDCl_3$) 169.52 (CO), 80.26 (CH), 75.49 (CH), 44.30 (CH_2), 41.09 (CH), 36.79 (CH_2) and 32.46 (CH_2).

exo-6-Acetoxy-2-oxabicyclo[3.2.1]octan-3-one (±)-16

A mixture of compound **15** (29 mg, 0.20 mmol), *N*,*N*-4-dimethylaminopyridine (catalytic amount) and acetic anhydride (38 mg, 0.372 mmol) in dry pyridine (0.5 cm^3) was stirred at room temperature under argon for 80 min after which it was diluted with chloroform and washed with hydrochloric acid (2 mol dm^{-3}) and brine, dried ($MgSO_4$), filtered and evaporated under reduced pressure to give a crude oil. This was purified by flash chromatography with diethyl ether as eluent to afford the title bicyclic ketone **16** (85%) as an oil (Found: M^+, 184.072 94. $C_9H_{12}O_4$ requires M^+, 184.073 56); ν_{max}(neat)/cm^{-1} 2974, 1736, 1431, 1378, 1322, 1248, 1196, 1172, 1140, 1071, 1025, 978 and 923; δ_H($CDCl_3$) 5.06 (1 H, dd, *J* 2.5, 7, 6-H), 4.88 (1 H, m, 1-H), 2.79 (1 H, dd, *J* 6, 19, 4-H), 2.66 (1 H, ddd, *J* 1, 7, 16, 7-H), 2.60 (1 H, m, 4-H), 2.54 (1 H, m, 5-H), 2.02 (3 H, s, CH_3), 2.00 (2 H, m, 8-H) and 1.97 (1 H, m, 7-H); δ_C($CDCl_3$) 170.26 (CO), 168.66 (CO), 79.53 (CH), 77.51 (CH), 41.69 (CH_2), 38.34 (CH), 36.72 (CH_2), 33.11 (CH_2) and 21.01 (CH_3).

exo-6-Benzyloxy-2-oxabicyclo[3.2.1]octan-3-one (±)-17[9]

A mixture of compound **14** (21 mg, 0.097 mmol) and *m*-chloroperoxybenzoic acid (26 mg, 0.15 mmol) in dichloromethane (0.5 cm^3) was stirred at room temp. A catalytic amount of toluene-*p*-sulfonic acid was then added and the solution kept in the dark for 2 h. It was then diluted with diethyl ether washed with saturated aqueous solutions of sodium sulfite and sodium hydrogen carbonate, dried ($MgSO_4$), filtered and evaporated under reduced pressure. The crude residue was purified by flash chromatography with diethyl ether–light petroleum (7:3) as eluent to afford the title bicyclic ketone (±)-**17** (98%) as an oil; ν_{max}(neat)/cm^{-1} 2943, 1734, 1496, 1453, 1375, 1312, 1244, 1194, 1139, 1079, 973, 906, 737 and 698; δ_H($CDCl_3$) 7.32 (5 H, m, Ph), 4.89 (1 H, m, 1-H), 4.48 (2 H, s, CH_2Ph), 3.96 (1 H, ddd, *J* 1, 2.5, 7, 6-H), 2.79 (1 H, dd, *J* 6, 19, 4-H), 2.62 (1 H, m, 5-H), 2.55 (1 H, ddd, *J* 2.5, 7, 16, 7-H), 2.46 (1 H, ddd, *J* 1.5, 2, 19, 4-H), 2.09 (1 H, m, 8-H), 2.02 (1 H, m, 7-H) and 1.94 (1 H, m, 8-H); δ_C($CDCl_3$) 169.36 (CO), 137.84 (=C), 128.50 (=CH), 127.82 (=CH), 127.58 (=CH), 82.57 (CH), 80.01 (CH), 71.15 (CH_2), 42.03 (CH_2), 37.34 (CH), 37.06 (CH_2) and 32.90 (CH_2).

Maintenance and growth of microorganisms

Pseudomonas putida (strain no. NCIMB 10007) was obtained from NCIMB (Aberdeen, UK) and maintained on nutrient agar slopes at 28 °C. The organism was routinely grown on a basal salts medium: NH_4Cl (2 g), K_2HPO_4 (8.2 g), KH_2PO_4 (3.1 g), yeast extract (0.1 g), tryptone (0.1 g), $MgSO_4 \cdot 7H_2O$ (0.4 g), $MnSO_4 \cdot H_2O$ (0.05 g), $CaCl_2 \cdot H_2O$ (0.01 g) and $NaMoO_4$ (0.01 g) was added prior to autoclaving the media at 15 psi for 15 min and then $FeSO_4 \cdot 7H_2O$ (0.05 g dm^{-3}) and (+)-camphor (2.5 g dm^{-3}) were added. A 100 cm^3 culture of bacteria was inoculated from a slope and after 24 h growth at 28 °C on an orbital shaker (150 rpm) was transferred to 1 dm^3 of media. After a further 24 h of growth this culture was used to inoculate 12 dm^3 of sterile media in a 20 dm^3 glass fermenter which was sparged with sterile air at 4 dm^3 min^{-1}. Growth of the organism was monitored by transferring 1 cm^3 aliquots of growing cell suspension to a plastic cuvette and reading the absorbance at 500 nm on a Spectronic 20 spectrophotometer. Cells were harvested after approximately 18 h of growth.

Partial purification of NADH and NADPH dependent monooxygenases from *Ps. putida* 10007

Cells were harvested by centrifugation at 4000 rpm for 30 min and the cell paste from 12 dm^3 of growth medium was resuspended in phosphate buffer (pH 7.1, 21 mmol dm^{-3}; 300 cm^3). The cells were disrupted by sonication at an amplitude of 22 μm for four 30 s periods with 1 min intervals in an ice-bath. The cell debris was removed by centrifugation (12 000 rpm, 20 min) and the supernatant taken to 30% ammonium sulfate saturation and left for 1 h at 0 °C. The precipitate was centrifuged (12 000 rpm, 20 min) and the pellet discarded. The supernatant was taken to 75% ammonium sulfate saturation and left for 1 h at 0 °C. The precipitate was centrifuged as above and the supernatant discarded. The pellet was dissolved in a minimum of phosphate buffer and dialysed against sequential 5 dm^3 portions of the same until all the native co-factor had been removed. The protein solution was then lyophilised and stored at 4 °C until required. This ammonium

sulfate cut contained both the NADH and the NADPH dependent monooxygenases used in the following biotransformations.

Separation of (+)-diketocamphane monooxygenase[(+)-MO1] and (−)-diketocamphane monooxygenase[(−)-MO1]

The crude enzyme preparation (see above), containing around 300 mg of protein, was loaded onto a Fast-Flow Q-Sepharose column which was eluted with a gradient of 0 to 0.5 mol dm^{-3} KCl in 21 mmol dm^{-3} phosphate buffer pH 7.1. Column fractions were assayed for (+) and (−)-camphor oxidation activity using the method of Jones *et al.*[13] Fractions containing protein capable of oxidizing (+)-camphor were eluted in the 0.20–0.25 mol dm^{-3} KCl region. Fractions containing protein capable of oxidizing (−)-camphor were eluted in the 0.35–0.40 mol dm^{-3} region. Fractions containing separated isoenzymes were pooled, the protein precipitated, dialysed and redissolved in a minimum of buffer to yield a (+)-camphor specific preparation [(+)-MO1] and a (−)-camphor specific preparation [(−)-MO1]. These enzymes were lyophilised and stored at 4 °C until required. The enantioselectivity of both isoenzymes towards (+) and (−)-camphor was confirmed by assay with each enantiomer.

General procedures for the whole-cell biotransformation using *Ps. putida* NCIMB 10007

Harvested cells were resuspended in a phosphate buffer solution (21 mmol dm^{-3}, pH 7.1; 1 dm^3) and the suspension was used immediately for biotransformations. The substrate (2 mmol dm^{-3}) which was predissolved in ethanol (1%) was added to the cell suspension. The whole mixture was put into a gyratory shaker at 30 °C (200 rpm). The biotransformation was monitored by periodic sampling of aliquots (0.5 cm^3) which were extracted with ethyl acetate (0.5 cm^3) and analysed by GC (BP1). After completion of the biotransformation the cells were removed by centrifugation (12 000 rpm, 20 min) and the supernatant was evaporated under reduced pressure at room temperature after addition of ethanol (99%). Chloroform–ethanol (9:1) was added to the residue. The solution was filtered and solvents were removed under reduced pressure to give a crude residue. The products were separated and purified by flash column chromatography. The pure lactone was fully characterized and injected on Lipodex D in order to determine the enantiomeric excess.

General procedures for analytical scale oxidations using MO1 or MO2

The ketone (10 mmol dm^{-3}) was stirred (gyratory shaker, 200 rpm) in Tris buffer (pH 7.1 or 8.1, 50 mmol dm^{-3}) at 30 °C containing 7–10 mg cm^{-3} of the crude monooxygenase, an equimolar amount of NADH or NADPH and ethanol (0.5%). The biotransformation was monitored by periodic sampling of aliquots (0.2 cm^3) which were extracted with ethyl acetate (0.2 cm^3) and analysed by GC (BP1). When the reaction had proceeded to either the required stage (around 45% bioconversion) or showed no signs of continuing below this level, ethanol was added and solvents were removed by evaporation under reduced pressure at room temperature. A mixture of chloroform and ethanol (9:1) was added to the residue and filtered off. This solution was injected on BP1 and Lipodex D in order to determine the final composition and the enantiomeric excess of the lactone.

General procedures for preparative-scale oxidations using MO1 or MO2

The ketone (4–10 mmol dm^{-3}) was stirred (gyratory shaker, 200 rpm) in Tris buffer (pH 7.1 or 8.1, 50 mmol dm^{-3}) at 30 °C containing 7–12 mg cm^{-3} of the crude monooxygenase, a catalytic amount of NADH or NADPH (10%) and ethanol (0.5%). The co-factor NADH was recycled by using formate dehydrogenase (0.2 units cm^{-3}) and sodium formate (20 mmol dm^{-3}). Glucose-6-phosphate dehydrogenase (1 unit cm^{-3}) and D-glucose-6-phosphate (10 mmol dm^{-3}) were used to recycle NADPH. The biotransformation was monitored by periodic sampling of aliquots (0.2 cm^3) which were extracted with ethyl acetate (0.2 cm^3) and analysed by GC (BP1). When the reaction had proceeded to either the required stage (around 45% bioconversion) or showed no signs of continuing below this level, ethanol (99%) was added to the mixture and the solvents were removed by evaporation under reduced pressure at room temperature. The resulting residue was dissolved in chloroform–ethanol (9:1) and the solution filtered and then injected on BP1 in order to determine the final composition. The solution was then evaporated under reduced pressure and the resulting crude residue purified by flash chromatography. The pure lactone was fully characterized and injected on Lipodex D in order to determine the ee.

Double biotransformation involving HLADH and MO1

A mixture of compound **12** (2.5 mg, 0.015 mmol), NAD^+ (2.1 mg, 0.0032 mmol), horse liver dehydrogenase (21 mg, 35 units) and crude monooxygenase (11 mg) from *Pseudomonas putida* NCIMB 10007 in Tris buffer pH 8.1 (1.5 cm^3) was stirred at 30 °C for 22 h after which it was diluted with ethanol (5 cm^3) and evaporated under reduced pressure at room temperature. The resulting residue was dissolved in chloroform–ethanol (9:1; 2 cm^3) and the solution filtered and injected on GC. GC results: (BP1) lactone **16**: 15%, alcohol **12**: 22%, ketone **10**: 37%, and 26% of other materials; (Lipodex D) ee of lactone **16** ≥95%.

***exo*-6-Hydroxy-2-oxabicyclo[3.2.1]octan-3-one (−)-15**

A mixture of compound (−)-**17** (15 mg, 0.0647 mmol) and palladium-on-charcoal (10%, wet; 14 mg) in ethanol (99%; 1 cm^3) under hydrogen was stirred for 7 h at room temperature. The solid present was filtered off and washed with ethanol and the combined ethanolic solutions were evaporated under reduced pressure to give a crude oil. This was purified by flash chromatography with diethyl ether–methanol (14:1) as eluent to afford the title bicyclic ketone **15** (98%) as an oil. The physical data were the same as above.

***exo*-6-Hydroxy-2-oxabicyclo[3.2.1]octan-3-one (−)-15**

A mixture of compound (−)-**16** (10 mg, 0.0543 mmol) and potassium cyanide (47 mg, 0.723 mmol) in ethanol (95%; 0.2 cm^3) was stirred for 24 h after which it was diluted with chloroform, washed with a saturated aqueous sodium hydrogen carbonate, diluted further with toluene and evaporated under reduced pressure to give a crude oil. This was purified by flash chromatography with diethyl ether–methanol (14:1) as eluent to afford the title bicyclic ketone (−)-**13** (78%) as an oil. The physical data were the same as above.

***exo*-6-Benzyloxy-2-oxabicyclo[3.2.1]octan-3-one (−)-17**

A mixture of compound (−)-**15** (5 mg, 0.0352 mmol), silver(I) oxide (20 mg, 0.0863 mmol) and benzyl bromide (22 mg, 0.129 mmol) in dimethylformamide (0.07 cm^3) was stirred in the dark under argon for 3 d after which it was diluted with diethyl ether, filtered and evaporated under reduced pressure to give a crude oil. This was purified by flash chromatography with diethyl ether–light petroleum (7:3) as eluent to afford the title bicyclic ketone (−)-**17** (74%) as an oil. The physical data were the same as above.

Measurements of the enantiomeric excesses of the lactones and physical data for the chiral lactones

The ees were measured using a Lipodex D stationary phase. The

lactone **17** was transformed into the lactone **15** before injection onto a Lipodex D gc column. Specific rotations and ees (GC) for the lactones were as follows: (−)-(1*S*,5*S*,6*R*)-**15**, $[\alpha]_D$ −13.4 (*c* 0.67, $CHCl_3$) [lit.,[9] −16.3 (*c* 0.24, $CHCl_3$)], ee ≥95%. (−)-(1*S*,5*S*,6*R*)-**16**, $[\alpha]_D$ −14.7 (*c* 1.8, $CHCl_3$), ee ≥95%. (−)-(1*S*,5*S*,6*R*)-**17**, $[\alpha]_D$ −17.0 (*c* 1.2, $CHCl_3$), ee ≥87%.

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