# Microbial Oxidation of 7*endo*-Methylbicyclo[3.2.0]hept-2-en-6-one, 7,7-Dimethylbicyclo[3.2.0]hept-2-en-6-one and 2*exo*-Bromo-3*endo*-hydroxy-7,7-dimethylbicyclo[3.2.0]heptan-6-one using *Acinetobacter* NCIMB 9871

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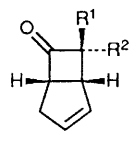
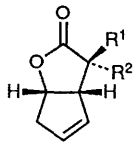
A bio-Baeyer–Villiger reaction using *Acinetobacter* NCIMB 9871 and the bicycloheptanone **2** provided the corresponding substituted oxabicyclooctanones **6** and **7**. Similarly the ketones **3** and **9** furnished the lactones **8** and **10** respectively: the lactones **6**, **7** and **10** were obtained in states of high optical purity.



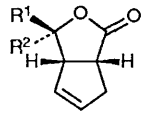
There has been considerable recent interest in the oxidation of cyclic ketones into lactones using monooxygenase enzymes contained in whole-cell systems.[1] While monocyclic systems have been shown to undergo this microbiological ring-expansion reaction successfully[2] much of the work has been focussed on the oxidation of bicyclic ketones. Thus various bicyclo[2.2.1]heptanones have been oxidized to the corresponding oxabicyclo[3.2.1]octanones.[3] The microbial oxidation of bicyclo[3.2.0]hept-2-en-6-one **1** using *Acinetobacter* NCIMB 9871 has been studied by Furstoss[4] and Knowles.[5] It was shown that both enantiomers of the starting material were oxidized, one enantiomer provided the 2-oxabicyclo[3.3.0]oct-6-en-3-one **4** while the other enantiomer furnished the 3-oxabicyclo[3.3.0]oct-6-en-2-one **5**. In this paper we report the results obtained on the oxidation of some derivatives of bicyclo-[3.2.0]hept-2-en-6-one with *Acinetobacter* NCIMB 9871.[6]



**1**; $R^1 = R^2 = H$
**2**; $R^1 = H$; $R^2 = Me$
**3**; $R^1 = R^2 = Me$



**4**; $R^1 = R^2 = H$
**6**; $R^1 = H$; $R^2 = Me$

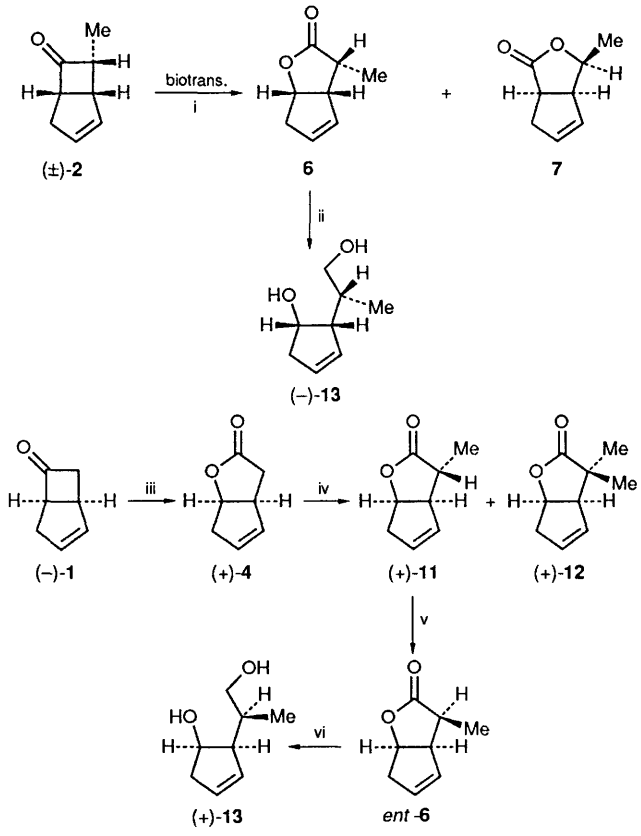


**5**; $R^1 = R^2 = H$
**7**; $R^1 = H$; $R^2 = Me$
**8**; $R^1 = R^2 = Me$

## Results

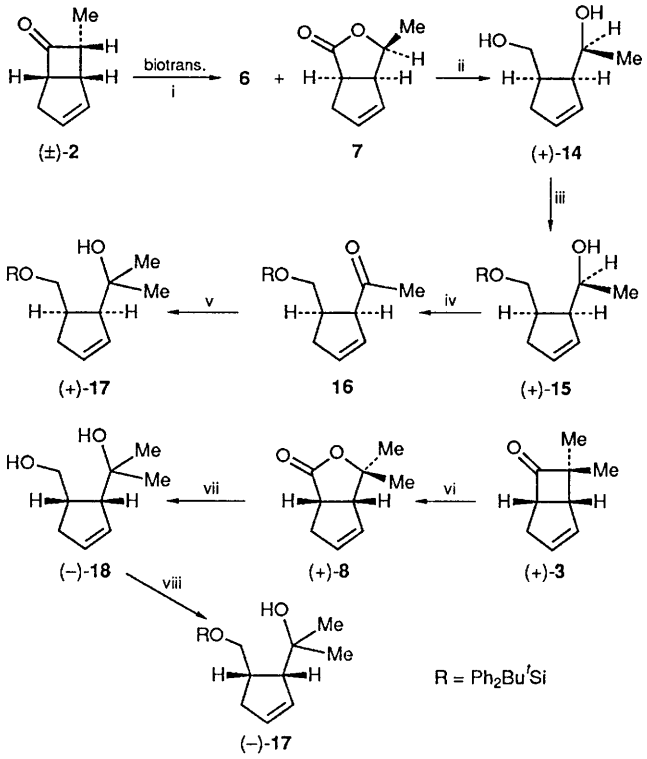
7*endo*-Methylbicyclo[3.2.0]hept-2-en-6-one **2**[7] was oxidized by *Acinetobacter* NCIMB 9871 to give almost equal amounts of the lactones **6** and **7** in 55% yield. The lactones were optically pure judging from NMR experiments employing tris[3-(hepta-fluoropropylhydroxymethylene)-(+)-camphorato]europium-(III). The absolute configuration of the lactone **6** was established by correlation with (−)-bicyclo[3.2.0]hept-2-en-6-one (−)-**1**[8] as outlined in Scheme 1 while the identity of the lactone **7** was confirmed by correlation with (+)-7,7-dimethylbicyclo[3.2.0]-hept-2-en-6-one (+)-**3**[9] (Scheme 2). Thus the oxidation of the ketone **2** with the *Acinetobacter* sp. closely matched the result obtained by other workers with the parent ketone **1**. It is noteworthy that peracetic acid oxidation of the ketone **2** gave (±)-**6** and (±)-**7** in the ratio of 1:10 (75% yield).[10]

In contrast to the above results the microbiological oxidation of the dimethyl ketone **3** gave the lactone **8** (63%) of low optical purity (29% e.e.). (Peracetic acid oxidation of **3** gave a 56% yield of the same lactone.)

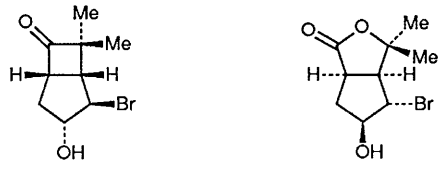


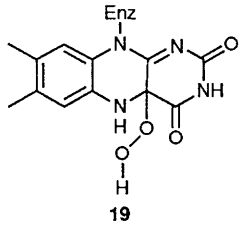

**Scheme 1** *Reagents and conditions:* i, *Acinetobacter* NCIMB 9871, 30 °C, 55% combined yield of **6** and **7**; ii, $LiAlH_4$, $Et_2O$, room temperature, 64%; iii, $MeCO_2H$, $H_2O_2$, 0–5 °C, 75%; iv, $(Me_3Si)_3NLi$, THF, −65 °C then MeI, −65 °C to −20 °C, 68% of (+)-**11** and 9% of (+)-**12**; v, $(Me_3Si)_3NLi$, THF, −65 °C then MeOH, −65 °C to −20 °C, 15%; vi, $LiAlH_4$, $Et_2O$, room temperature, 78%.

Derivatization of the unsaturated ketone **3** gave the bromohydrin (±)-**9**,[11] a substrate which was oxidized enantioselectively by the *Acinetobacter* sp. Thus incubation of the ketone **9** with the microorganism for 3 h gave the lactone (−)-**10** (25%; e.e. >98%) and recovered (+)-(1*R*,5*S*)-ketone **9** (51%; e.e. = 46%). A longer reaction time (7 h) provided the lactone (−)-**10** (31%; e.e. >98%) and (1*R*,5*S*)-ketone **9** (30%; e.e. = 85%). Peracetic acid oxidation of **9** gave the lactone **10** in 61% yield. The absolute configurations of the bromohydrins **9** and **10** were established by appropriate derivatization of the optically active ketone (+)-**3**.[9]




**Scheme 2** *Reagents and conditions:* i, *Acinetobacter* NCIMB 9871, 30 °C, 55% overall yield of **6** and **7**; ii, $LiAlH_4$, $Et_2O$, room temperature, 66%; iii, $Ph_2Bu^tSiCl$, imidazole, $CH_2Cl_2$, room temperature, 69%; iv, $(COCl)_2$, DMSO, $Et_3N$, $CH_2Cl_2$, −78 °C, 68%; v, MeLi, $Et_2O$, 0 °C, 56%; vi, $MeCO_2H$, $H_2O_2$, 0 °C, 56%; vii, $LiAlH_4$, $Et_2O$, room temperature, 89%; viii, $Ph_2Bu^tSiCl$, Imidazole, DMAP, $CH_2Cl_2$, room temperature, 72%.




## Discussion

The three substrates that were used in the microbiological oxidation process follow different reaction pathways. The ketone **3** is oxidized with high regioselectivity and low enantioselectivity, the ketone **9** is oxidized with high regio- and enantio-selectivity while the enantiomers of the ketone **2** are oxidized to isomeric lactones of high optical purity.

One explanation of these facts is that there are two monooxygenase enzymes in *Acinetobacter* NCIMB 9871. One enzyme produces the 2-oxabicyclooct-6-en-3-one while the second enzyme catalyses the formation of the 3-oxabicyclooct-6-en-2-one. The first enzyme preferentially biotransforms the (5*S*)-ketone while the second accepts the (5*R*)-ketone as the preferred substrate. Neither enzyme is entirely selective and if the two modes of ring expansion are not energetically equivalent then both enantiomers of the ketone are processed by one of the proteins to provide a single lactone of low optical purity.

However there is little hard evidence for the presence of two monooxygenase isozymes in the *Acinetobacter* sp. In addition, the partially purified protein used by Knowles *et al.*, *does* process both enantiomers of the ketone **1**, and an alternative scenario involving one enzyme with a complex active site must be considered.

The monooxygenase from *Acinetobacter* NCIMB 9871 utilizes flavin 4a-hydroperoxide **19** as the oxidant. It seems reasonable to assume that the hydroperoxide or the corresponding anion must approach the bicyclo[3.2.0]heptanone system from the more open *exo* face. Furthermore, steric requirements seem to dominate over electronic factors to determine the outcome of the bio-Bayer–Villiger reactions.

*A priori*, both enantiomers of the bicyclic systems can ring expand to give 2-oxa- and 3-oxa-bicyclo[3.3.0]octanones (Fig. 1). In fact enantiomer **A** gives the 2-oxabicyclo[3.3.0]octan-3-one system for $R^1 = R^2 = H$ and for $R^1 = H$, $R^2 = Me$. However this lactone system is not formed for the dimethylated compound, $R^1 = R^2 = Me$; instead the 3-oxabicyclo[3.3.0]-octan-2-one is produced. We suggest that the *exo*-methyl group interferes with the antiperiplanar alignment of the carbon–carbon and oxygen–oxygen bonds that is necessary for the production of the former lactone.

On formation of the 2*exo*-bromo-3*endo*-hydroxy derivative **9**, enantiomer **A** is no longer able to form a lactone. We propose that the 2*exo*-bromine atom is the culprit, forming an unfavourable interaction with the enzyme surface.

For enantiomer **B** ring expansion to the 2-oxabicyclo[3.3.0]-alkan-3-one is not observed in any case, so obviously the necessary intermediate requires a highly undesirable orientation of the bicyclic compound in the active site. In contrast, the intermediate leading to the 3-oxabicyclo[3.3.0]alkan-2-one is particularly favoured since all four compounds **1**–**3**, **9** can be accommodated.

In future work it would be interesting to synthesize and biotransform 7*exo*-methylbicyclo[3.2.0]hept-2-en-6-one and 3*endo*-hydroxy-7,7-dimethylbicyclo[3.2.0]heptan-6-one in order to try to refine our rather crude qualitative picture of the monooxygenase(s) from *Acinetobacter* NCIMB 9871.

## Experimental

Diethyl ether and tetrahydrofuran (THF) were dried and distilled over sodium metal and benzophenone. Ethyl acetate was distilled over phosphorus pentoxide. Light petroleum (60–80 °C) and dichloromethane were distilled over calcium hydride. Chloroform was washed with water, dried over $K_2CO_3$ and distilled over calcium chloride. Triethylamine was dried and distilled over potassium hydroxide. Other reagents and solvents were used as commercially supplied.

Thin layer chromatography was performed on pre-coated glass plates (Merck silica gel 60F 254). The plates were visualized using *p*-anisaldehyde and UV light (254 nm). Flash column chromatography was performed over silica (Merck silica 60, 40–53 nm).

$^1H$ and $^{13}C$ NMR spectra were run on a Bruker AM 250 FT machine in deuteriochloroform solution unless stated otherwise. Chemical shifts ($\delta$) are quoted in ppm with tetramethylsilane (0.00 ppm) as reference and $J$ values are in Hz. The chiral shift reagent used for the determination of enantiomeric excess (e.e.) was tris[3-(heptafluoropropyl-hydroxymethylene)-(+)-camphorato]europium(III). 60 MHz $^1H$ NMR spectra were recorded on a Hitachi Perkin-Elmer high resolution R-24A NMR spectrometer, 100 MHz, $^1H$ and $^{13}C$ spectra were recorded on a Jeol FX 100 spectrometer, IR spectra were recorded on a Perkin-Elmer 881 infrared

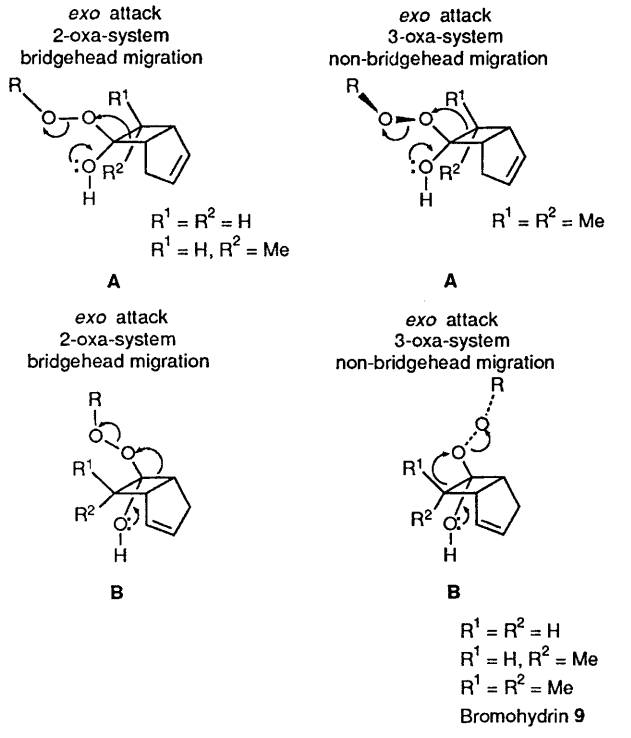



**Fig. 1**

spectrometer and optical rotations were measured on a Thorn automation NPL automatic polarimeter type 243. Rotations are recorded in $10^{-1}$ deg cm$^2$ g$^{-1}$.

*General Procedure for Baeyer–Villiger Biotransformation of Bicycloalkanones* **2**, **3** *and* **9** *with* Acinetobacter NCIMB 9871.—*Acinetobacter* NCIMB 9871 was grown up according to the procedure described by Trudgill.[12] Six litres of the medium was inoculated, grown to an optical density of 0.9 (Spectronic 20 Spectrophotometer) and the cells harvested by centrifugation (3800 r.p.m.) at 30 °C for 10 min. The cells were resuspended in phosphate buffer (350 ml, pH 7.2) and the suspension was used immediately for biotransformation or frozen in small aliquots (15 ml) for later use.

Typically the bicyclic ketone, optionally predissolved in dimethylformamide, was added to the rapidly shaken cell suspension under aerobic conditions at 30 °C. The bioconversion was monitored by GC analysis of aliquots (1 ml) of the reaction mixture after centrifugation. On completion of the reaction the cells were removed by centrifugation (3800 r.p.m.) for 30 min and the supernatant was thoroughly extracted with ethyl acetate (4 × 200 ml). The combined extracts were dried ($MgSO_4$), filtered and the solvent removed to give a crude residue which was purified by flash column chromatography.

*Biotransformation of* 7endo-*methylbicyclo*[3.2.0]*hept*-2-*en*-6-*one* **2**. The (±)-ketone **2** (145 mg, 1.19 mmol) was oxidized, over 12 h, to give, after work up and flash column chromatography (ethyl acetate–light petroleum, 1:1), a mixture of the partially separable lactones (1S,4R,5S)-4-*methyl*-2-*oxabicyclo*[3.3.0]*oct*-6-*en*-3-*one* **6** and (1R,4S,5S)-4-*methyl*-3-*oxabicyclo*[3.3.0]*oct*-6-*en*-2-*one* **7** [(1*S*,4*R*,5*S*)-**6**:(1*R*,4*S*,5*S*)-**7**; 42:47] (91 mg, 55%) as a colourless oil. Both lactones were shown to be optically pure, >98% e.e. Lactone **6**: $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3049, 2933, 1770 (C=O), 1611 (C=C); $\delta_H$ 5.9–5.7 (2 H, m, 6-H and 7-H), 5.04–4.98 (1 H, m, 1-H), 3.58–3.48 (1 H, m, 5-H), 2.90–2.84 (1 H, m, 4-H), 2.82–2.78 (2 H, m, 2 × 8-H) and 1.2 (3 H, d, *J* 7.0, $CH_3$); $\delta_C$ 180.33 (C=O), 130.72 and 127.42 (C-6 and C-7), 80.62 (C-1), 50.65 (C-4), 37.41 (C-5), 39.73 (C-8) and 12.02 ($CH_3$); *m*/*z* (CI) 156 ([M + $NH_4$]$^+$, 100%), 172 (88) and 139 ([M + H]$^+$, 5) [Found: M$^+$ + $NH_4$; 156.1025. $C_8H_{14}NO_2$ requires *M* + $NH_4$, 156.1025]. Lactone **7**: $\nu_{max}$ as for lactone **6**; $\delta_H$ 5.9–5.6 (2 H, m, 6-H and 7-H), 4.74 (1 H, quintet, *J* 11.0, 7.0, 4-H), 3.58–3.48 (1 H, m, 5-H), 3.22 (1 H, dt, *J* 7.0, 1.5, 1-H), 2.90–2.60 (2 H, m, 2 × 8-H) and 1.4 (3 H, d, *J* 7.0, $CH_3$); $\delta_C$ 180.33 (C=O), 133.58 and 127.00 (C-6 and C-7), 78.06 (C-4), 51.06 and 43.91 (C-1 and C-5), 36.62 (C-8) and 17.09 ($CH_3$).

*Biotransformation of* (±)-7,7-*dimethylbicyclo*[3.2.0]*hept*-2-*en*-6-*one* **3**. The (±)-dimethyl ketone **3** (200 mg, 1.47 mmol) was oxidized over 4 h to give, after work-up and flash column chromatography (light petroleum–ethyl acetate, 4:1) 4,4-*dimethyl*-3-*oxabicyclo*[3.3.0]*oct*-6-*en*-2-*one* **8** (141 mg, 63%), 29% e.e. for the (−)-(1*R*,5*S*) enantiomer, $[\alpha]_D^{24}$ −12° (*c* 0.59, $CHCl_3$). Authentic optically pure material has $[\alpha]_D^{24}$ +41 (*c* 0.59, $CHCl_3$), for the (1*S*,5*R*)-enantiomer.

*Biotransformation of* 2exo-*Bromo*-3endo-*hydroxy*-7,7-*dimethylbicyclo*[3.2.0]*heptan*-6-*one* **9**. The (±)-bromohydrin **9** (204 mg, 0.87 mmol) was oxidized and two equal volume aliquots were taken at 3 h (50% substrate utilized by GC analysis) and at 7 h. After work-up and purification of the crude residues by flash column chromatography (light petroleum–ethyl acetate, 7:3) recovered starting ketone **9** and the product (−)-lactone **10** were obtained. Incubation over 3 h gave (+)-(1*R*,5*S*)-ketone **9** (53 mg, 51%), 46% e.e.; $[\alpha]_D^{27}$ +41° (*c* 0.48, $CHCl_3$) and (−)-(1*R*,5*S*)-lactone **10** (23 mg, 25%), >98% e.e.; $[\alpha]_D^{27}$ = −46 (*c* 0.24, $CH_2Cl_2$). Incubation over 7 h gave (+)-(1*R*,5*S*)-ketone **9** (31 mg, 30%), 85% e.e.: $[\alpha]_D^{25}$ = +81 (*c* 0.48, $CHCl_3$) and (−)-(1*R*,5*S*)-lactone (1*R*,5*S*)-6-*bromo*-7-*hydroxy*-4,4-*dimethyl*-3-*oxabicyclo*[3.3.0]*octan*-2-*one* **10** (32.5 mg, 43%), >98% e.e.; $[\alpha]_D^{25}$ −45 (*c* 0.24, $CH_2Cl_2$). Physical data for compound **10** were as follows: $\nu_{max}(CHCl_3)/cm^{-1}$ 3603 (OH) 2987 and 1760 (C=O); $\delta_H$ 4.36–4.26 (1 H, m, 7-H), 4.04 (1 H, t, *J* 6.6, 6.1, 6-H), 3.28 (1 H, ddd, *J* 10.2, 9.5, 4.0, 1-H), 2.95 (1 H, dd, *J* 9.5, 6.6, 5-H), 2.55 (1 H, ddd, *J* 14.0, 10.2, 6.3, 8*endo*-H), 2.4 (1 H, d, OH), 2.07 (1 H, m, 8*exo*-H), 1.56 (3 H, s, $CH_{3exo}$) and 1.46 (3 H, s, $CH_{3endo}$); $\delta_C$ 178.33 (C=O), 84.18 (C-4), 79.61 (C-7), 57.19 (C-5), 53.95 (C-6), 42.81 (C-1), 34.17 (C-6), 30.42 ($CH_{3exo}$) and 23.84 ($CH_{3endo}$); *m*/*z* (CI) 266 ([M + $NH_4$]$^+$, 100%), 251 (23), 186 (53) and 170 ([M + H]$^+$ − Br, 15) [Found: M$^+$ + $NH_4$, 266.0392. $C_9H_{17}BrNO_3$ requires *M* + $NH_4$, 266.0392].

(−)-(1′S,2′S,2R)-2-(2′-*Hydroxycyclopent*-4′-*enyl*)*propan*-1-*ol* **13** *and* (+)-(1S,1′S,2′R)-1-[2′-*Hydroxymethylcyclopent*-4′-*enyl*]*ethanol* **14**.—Lithium aluminium hydride (220 mg, 1 equiv.) was stirred in dry diethyl ether (8 ml) under nitrogen for 5 min. A solution of the biotransformation product lactones **6** and **7** (800 mg, 5.79 mmol) in dry diethyl ether (11 ml) was added dropwise to the mixture. The reaction was stirred for 1 h under a nitrogen atmosphere and then quenched with water (10 ml), which was added dropwise with cooling (ice bath). Diethyl ether (50 ml) was added and the solution was filtered, the residue being washed with more ether. The filtrate was dried ($MgSO_4$) and the solvent evaporated. Flash column chromatography (chloroform–methanol, 19:1) of the crude residue gave the *title compounds:* (−)-**13** (250 mg, 30%) as a white crystalline solid m.p. 50–52 °C and (+)-**14** (287 mg, 35%) as a colourless oil, with some mixed fractions. For the alcohol (−)-**13** $[\alpha]_D^{25}$ −54 (*c* 0.4, MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3615 (OH), 3434 and 2932 (OH); $\delta_H$ 5.80–5.62 (2 H, m, 4′-H and 5′-H), 4.48–4.36 (1 H, m, 2′-H), 4.00 (2 H, br s, 2 × OH), 3.54–3.40 (2 H, m, 2 × 1-H), 2.70–2.52 (2 H, m, 2-H and 1′-H), 2.36–2.00 (2 H, m, 2 × 3′-H) and 0.98 (3 H, d, *J* 7.0, $CH_3$); $\delta_C$ 129.80 and 129.60 (*C*H=*C*H), 73.00 (C-2′), 65.79 (C-1), 53.78 (C-1′), 42.21 (C-3′), 33.96 (C-2) and 16.85 ($CH_3$); *m*/*z* (CI), 143 ([M + H]$^+$, 20%), 285 (22), 160 (100) and 125 (10) [Found: M$^+$ + $NH_4$, 160.1338. $C_8H_{18}NO_2$ requires *M* + $NH_4$ 160.3338]. For compound (+)-**14**: $[\alpha]_D^{25}$ + 131 (*c* 0.32, MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3615 (OH), 3437

(OH), 3045, 2927 and 1613; $\delta_H$ 6.00–5.80 (2 H, m, 4-H and 5-H), 4.11 (1 H, qd, *J* 6.5, 2.0, 1-H), 3.94 (1 H, dd, *J* 10.5, 4.0, C*H*$_2$OH), 3.83 (1 H, dd, *J* 10.5, 6.0, C*H*$_2$OH), 2.82–2.74 (1 H, m, 1′-H), 2.62–2.45 (1 H, m, 2′-H), 2.42–2.31 (2 H, m, 2 × 3′-H) and 1.23 (3 H, d, *J* 6.5, CH$_3$); $\delta_C$ 134.50 and 128.40 (*C*H=*C*H), 67.26 (C-1), 62.29 (*C*H$_2$OH), 53.42 (C-1′), 42.54 (C-2′), 34.73 (C-3′) and 22.18 (CH$_3$).

*Peracetic acid oxidation of* (±)-7endo-*methylbicyclo*[3.2.0]-*hept-2-en-6-one* **2**.—The ketone **2** (200 mg, 1.64 mmol) was oxidized with peracetic acid as described for ketone (+)-**3**. Flash column chromatography (light petroleum–ethyl acetate, 4:1) of the crude product gave an inseparable mixture of the lactones **6** and **7** in a 1:10 ratio respectively (169 mg, 75%) as a colourless oil.

*Peracetic acid oxidation of* (±)-2exo-*bromo*-3endo-*hydroxy*-7,7-*dimethylbicyclo*[3.2.0]*heptan-6-one* **9**. The bromohydrin **9** (200 mg, 0.86 mmol) was oxidized as described previously to give, after purification by flash column chromatography (light petroleum–ethyl acetate, 3:2), the lactone **10** (131 mg, 61%) as a white crystalline solid, m.p. 135 °C.

(+)-(1R,5S)-2-*Oxabicyclo*[3.3.0]*oct-6-en-3-one* **4**. The (−)-(1*S*,5*R*)-ketone **1** (2 g, 18.5 mmol) was oxidized with peracetic acid at 0 °C for 6 h. Flash column chromatography (dichloromethane) of the crude residue gave an inseparable mixture of lactones (+)-**4** and **5** (1.72 g, 75%) in the ratio 95:5 as a white crystalline solid m.p. 42–44 °C; $[\alpha]_D^{22}$ +84 (*c* 0.84, $CHCl_3$); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3051, 2932 and 1763 (C=O); $\delta_H$ 5.80–5.52 (2 H, m, 6-H and 7-H), 5.16–5.07 (1 H, m, 1-H), 3.54–3.44 (1 H, m, 5-H), 2.82–2.65 (3 H, m, 2 × 4-H and 8*exo*-H) and 2.35 (1 H, dd, *J* 3.0, 18.0, 8*endo*-H).

(+)-(1R,4R,5R)-4-*Methyl*-2-*oxabicyclo*[3.3.0]*oct-6-en-3-one* **11**. A solution of the (+)-lactone **4** (1.00 g, 9.26 mmol) in dry THF (20.6 ml) was cooled to −65 °C and bis(trimethylsilyl)-lithium amide (1.0M solution in THF; 10.18 ml) was added dropwise with stirring, the temperature being maintained < −60 °C. The solution was stirred for 0.5 h before methyl iodide (1.24 g, 19 equiv.) was added dropwise. The mixture was warmed to −20 °C for 1.5 h. The reaction mixture was washed with a saturated aqueous ammonium chloride (2 × 10 ml) and the aqueous phase was back extracted with diethyl ether (3 × 20 ml). The combined organic phases were dried ($MgSO_4$) and evaporated. Flash column chromatography (light petroleum–ethyl acetate, 4:1) of the crude residue gave (+)-exo-*methyllactone* **11** (762 mg, 68%) and (+)-(1R,5R)-4,4-*dimethyl*-2-*oxabicyclo*[3.3.0]*oct-6-en-3-one* **12** (111 mg, 9%) both as colourless oils. For lactone **11**: $[\alpha]_D^{21}$ +73 (*c* 0.83, $CHCl_3$); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3052, 2918, 2880, 2844 and 1780 (C=O); $\delta_H$ 5.79–5.72 (1 H, m, 7-H), 5.63–5.57 (1 H, m, 6-H), 5.17–5.09 (1 H, m, 1-H), 3.13 (1 H, dddd, *J* 8.0, 4.0, 3.5 and 1.9, 5-H), 2.71–2.64 (2 H, m, 2 × 8-H), 2.53 (1 H, dq, *J* 7.6 and 1.9, 4-H) and 1.35 (3 H, d, *J* 7.6, $CH_3$); $\delta_C$ 179.93 (C=O), 131.02 and 129.54 (C-6 and C-7), 81.33 (C-1), 53.91 (C-5), 40.00 (C-4), 39.37 (C-8) and 17.45 ($CH_3$); *m/z* (CI) 277 ([2M + H]$^+$, 100%), 139 ([M + H]$^+$ 55) [Found: M$^+$ + H, 139.0759. $C_8H_{11}O_2$ requires *M* + H, 139.0759]. For lactone **12**: $[\alpha]_D^{23}$ +52° (*c* 0.72, $CHCl_3$); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3052, 2965, 2913, 2875 and 1758 (C=O); $\delta_H$ 5.79–5.57 (2 H, m, 6-H and 7-H), 5.02 (1 H, dt, *J* 5.5 and 2.5, 1-H), 3.16–3.09 (1 H, m, 5-H), 2.68–2.60 (2 H, m, 2 × 8-H), 1.29 (3 H, s, $CH_{3exo}$) and 1.2 (3 H, s, $CH_{3endo}$); $\delta_C$ 181.42 (C=O), 130.16 and 128.47 (C-6 and C-7), 79.39 (C-1), 5.08 (C-5), 42.27 (C, quaternary), 39.42 (C-8), 26.71 ($CH_{3exo}$) and 21.11 ($CH_{3endo}$); *m/z* (CI) 153 ([M + H]$^+$, 62%) and 170 ([M + NH$_4$]$^+$, 100) [Found: M$^+$ + H, 153.0909. $C_9H_{13}O_2$ requires *M* + H, 153.0915].

(1R,4S,5R)-4-*Methyl*-2-*oxabicyclo*[3.3.0]*oct-6-en-3-one* **6**. The (+)-*exo*-methyl ketone **11** (600 mg, 4.35 mmol) was stirred in dry tetrahydrofuran (9.8 ml) at −60 °C and bis(trimethylsilyl)lithium amide (1.0M solution in tetrahydrofuran; 4.78 ml) was added slowly. After 0.5 h methanol (3.3 ml, 19 equiv.) was added and the reaction mixture was warmed to −20 °C over 1 h. The reaction mixture was then quenched with saturated aqueous ammonium chloride (6 ml) and diluted with water (6 ml). The layers were separated and the aqueous layer was back extracted with diethyl ether (3 × 60 ml). The combined organic extracts were dried ($MgSO_4$) and evaporated. Flash column chromatography (light petroleum–ethyl acetate, 4:1) of the crude residue gave recovered starting material **11** (400 mg) and the *title compound* **6** (86 mg, 15%). (Data for this compound were identical with those obtained for the biotransformation product **6**).

(+)-(1′R,2′R,2S)-2-(2′-*Hydroxycyclopent*-4′-*enyl*)*propan*-1-*ol* **13**. (1*R*,4*S*,5*R*)-Lactone **6** (86 mg, 0.62 mmol) was reduced with lithium aluminium hydride (24 mg, 0.62 mmol) as described above. Flash column chromatography (ethyl acetate–light petroleum, 1:1) of the crude product gave the *title compound* (+)-**13** (68 mg, 78%) as a white crystalline solid m.p. 52–54 °C; $[\alpha]_D^{23}$ +58° (*c* 0.4, MeOH). [Compound spectral data were identical with those obtained for (−)-**13**, see above].

(+)-(1S,1′S,2′R)-1-(2′-tert-*butyldiphenylsilyloxymethylcyclopent*-4′-*enyl*)*ethanol* **15**. *tert*-Butyldiphenylsilyl chloride (565 mg, 535 μl, 1 equiv.) and imidazole (279 mg, 2 equiv.) were added to a stirred solution of the (+)-diol **14** (287 mg, 2.05 mmol) in dry dichloromethane (8.5 ml). The reaction mixture was stirred for 0.5 h, filtered and evaporated. Flash column chromatography (light petroleum–ether acetate, 1:1) of the crude residue gave the *title compound* (+)-**15** (540 mg, 69%) as a viscous colourless oil; $[\alpha]_D^{20}$ +46 (*c* 0.4, $CH_2Cl_2$); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3567 (OH), 3466 (OH), 2933 and 2862; $\delta_H$ 7.9–7.4 (10 H, m, Ar), 6.10–5.80 (2 H, m, 4-H and 5-H), 4.26–4.12 (1 H, m, 1-H), 4.0–3.92 (2 H, m, C*H*$_2$OH), 2.84–2.76 (1 H, m, 1′-H), 2.62–2.50 (2 H, m, 2′-H and OH), 2.36–2.24 (2 H, m, 2 × 3′-H), 1.24 (3 H, d, *J* 7.0, $CH_3$) and 1.1 (9 H, br s, Bu$^t$); $\delta_C$ 134.82 and 134.47 (Ph, quat-C), 136.70, 136.63, 130.74, 130.03, 128.74 (Ar, C-4′ and C-5′), 67.85 (C-1′), 65.15 (*C*H$_2$OSi), 54.50 (C-1′), 44.18 (C-2′), 36.13 (C-3′), 27.93 ($CH_3$), 23.13 [C(*C*H$_3$)$_3$] and 20.28 [*C*(CH$_3$)$_3$] [Found: M$^+$ + H, 381.2270. $C_{24}H_{33}O_2Si$ requires *M* + H, 381.2250].

(1′S,2′R)-2′-tert-*Butyldiphenylsilyloxymethylcyclopent*-4′-*enyl methyl ketone* **16**. Dry dimethyl sulphoxide (90 μl, 1.16 mmol, 2.2 eq) was added to a stirred solution of oxalyl chloride (53 μl, 0.58 mmol, 1.1 equiv.) in dry dichloromethane (1.6 ml) the temperature being kept between −50 °C and −60 °C. The reaction was stirred for 2 min and the silyl ether (+)-**15** (200 mg, 0.53 mmol) in dichloromethane (0.53 ml) was added. Stirring was continued for 15 min whereupon triethylamine (370 μl, 2.6 mmol) was added and the reaction was stirred for 5 min. After warming to room temperature, water (2.3 ml) was added and the layers were separated; the aqueous phase was extracted with dichloromethane (3 × 3 ml). The combined organic extracts were washed with 1% aqueous hydrochloric acid (2 × 1 ml) and 5% w/v aqueous sodium carbonate (2 × 1 ml), dried ($MgSO_4$) and the solvent evaporated. Flash column chromatography (ethyl acetate–light petroleum, 1:16) of the crude residue gave the *title compound* **16** (136 mg, 68%) as a colourless oil; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3046, 2933 and 1704 (C=O); $\delta_H$(100 MHz) 7.80–7.30 (10 H, m, Ar), 6.06–5.50 (2 H, m, 4′-H and 5′-H), 3.80–3.40 (4 H, C*H*$_2$OSi, 2′-H and 1′-H), 3.00–2.00 (2 H, m, 2 × 3′-H), 2.10 (3 H, s, C*H*$_3$) and 1.00 [9 H, s, C(C*H*$_3$)$_3$]; $\delta_C$ 135.00, 134.80, 133.80, 133.00, 129.50, 122.50 (Ar and *C*H=*C*H), 67.00 (*C*H$_2$OSi), 64.00 (*C*H$_3$CO), 62.50 (C-1′), 45.00 (C-2′), 35.00 (C-3′), 27.00 [C(*C*H$_3$)$_3$] and 19.50 [*C*(CH$_3$)$_3$]; *m/z* (CI) 379 ([M + H]$^+$, 100%), 301 ([M − Ph]$^+$, 10) [Found: M$^+$, 379.2093. $C_{24}H_{31}O_2Si$ requires *M*, 379.2093].

(+)-(1′S,2′R)-2-(2′-tert-*Butyldiphenylsilyloxymethylcyclopent*-4′-*enyl*)*propan*-2-*ol* **17**. Methyllithium (1.4M solution in

diethyl ether; 0.198 mmol, 141 μl) was added slowly to a stirred solution of the ketone **16** (50 mg, 0.13 mmol) in dry diethyl ether (289 μl) at 0 °C. After 0.5 h the mixture was quenched with water (1 ml) and the layers were separated. The water phase was extracted with ether (3 × 1 ml) and the combined organic extracts were dried ($MgSO_4$) and evaporated. Flash column chromatography (light petroleum–ethyl acetate, 1:1) of the crude residue gave recovered starting material **16** (4.2 mg) and the *title compound* (+)-**17** (29 mg, 56%) as a colourless oil; $[\alpha]_D^{22}$ +24 (*c*, 0.2, $CHCl_3$); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3449 (OH), 3071, 3029, 2934 and 2861; $\delta_H$ 7.90–7.40 (10 H, m, Ar), 5.86–5.70 (2 H, m, 4′-H and 5′-H), 4.07 (1 H, dd, *J* 11.0 and 9.0, C*H*$_2$OH), 3.76 (1 H, dd, *J* 11.0 and 5.0, C*H*$_2$OH), 2.94–2.86 (1 H, m, 1′-H), 2.68–2.54 (1 H, m, 2′-H), 2.28–1.04 (3 H, m, OH and 2 × 3′-H), 1.27 (6 H, 2 s, 2 × $CH_3$) and 1.10 [9 H, s, C($CH_3$)$_3$]; $\delta_C$ 135.67, 134.82, 127.78 (Ph*C*–H), 132.78 (Ph–*C*), 129.85 and 129.63 (*C*H=*C*H), 72.86 (C-2), 64.98 (*C*H$_2$OH), 58.25 (C-1′), 44.34 (C-2′), 35.38 (C-3′), 30.68 and 27.03 (2 × *C*H$_3$), 26.83 [C(*C*H$_3$)$_3$] and 19.16 [*C*(CH$_3$)$_3$]; *m/z* (CI) 395 ([M + H]$^+$, 15%), 121 (PhSiO, 100); (EI) 199 ($Ph_2SiOH$, 80) and 121 (PhSiO, 100) [Found: M$^+$ + H, 395.2406. $C_{12}H_{35}O_2Si$ requires *M* + H, 395.2406].

(+)-(1S,5R)-4,4-*Dimethyl*-3-*oxabicyclo*[3.3.0]*oct*-6-*en*-2-*one* **8**. A solution of the ketone (+)-**3** (130 mg, 0.95 mmol) in glacial acetic acid (0.8 ml) at 0 °C was slowly added to an ice cold mixture of 30% aqueous hydrogen peroxide (0.5 ml) and glacial acetic acid (0.5 ml). The reaction mixture was stirred at 0–5 °C for 48 h before dilution with dichloromethane (5 ml). The organic layer was separated and washed with water (3 × 2 ml), dried ($MgSO_4$) and evaporated. Flash column chromatography (light petroleum–ethyl acetate, 7:3) of the crude residue gave the *title compound* (+)-**8** (81 mg, 56%) as a colourless oil; $[\alpha]_D^{27}$ +41 (*c* 0.59, $CHCl_3$); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3048, 2923, 2863 and 1754 (C=O); $\delta_H$ 5.86–5.60 (2 H, m, 6-H and 7-H), 3.40–3.20 (2 H, m, 1-H and 5-H), 2.80–2.60 (2 H, m, 2 × 8-H) and 1.4 (6 H, 2 s, 2 × $CH_3$); $\delta_C$ 179.66 (C=O), 132.75 and 128.56 (C-6 and C-7), 82.27 (C-4), 56.63 (C-1), 43.56 (C-5), 36.93 (C-8), 29.37 (*C*H$_{3exo}$) and 24.32 (*C*H$_{3endo}$); *m/z* (CI) 153 ([M + H]$^+$, 75%) and 170 ([M + $NH_4$]$^+$, 100) [Found: M$^+$ + H, 153.0916. $C_9H_{13}O_2$ requires *M* + H, 153.0916].

(−)-(1′R,2′S)-2-(2′-*hydroxymethylcyclopent*-4′-*enyl*)*propan*-2-*ol* **18**. The (+)-lactone **8** (59 mg, 0.39 mmol) was reduced with lithium aluminium hydride (15 mg, 1 equiv.) in dry diethyl ether (0.85 ml) over 3 h as described above. Flash column chromatography (light petroleum–ethyl acetate, 3:2) of the crude residue gave the *title compound* (−)-**18** (54 mg, 89%) as a colourless oil; $[\alpha]_D^{21}$ −74.5° (*c* 0.27, $CHCl_3$); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3611 (OH), 3442 (OH) and 2936; $\delta_H$ 5.94–5.66 (2 H, m, 4′-H and 5′-H), 3.94 (1 H, dd, *J* 11.5 and 10.0, C*H*$_2$OH), 3.74 (1 H, dd, *J* 11.5 and 4.0, C*H*$_2$OH), 2.90–2.78 (1 H, m, 1′-H), 2.72–2.54 (1 H, m, 2′-H), 2.34–2.00 (2 H, m, 2 × 3′-H) and 1.31–1.20 (6 H, 2 s, 2 × $CH_3$); $\delta_C$ 132.44 and 132.32 (CH=CH), 74.45 (C-2), 63.15 (*C*H$_2$OH), 56.91 (C-1′), 44.84 (C-2′), 35.24 (C-3′) and 31.28 and 26.50 (2 × $CH_3$).

(−)-(1′R,2′S)-2-(2′-tert-*Butyldiphenylsilyloxymethylcyclopent*-4′-*enyl*)*propan*-2-*ol* **17**. A solution of the (−)-diol **18** (43 mg, 0.27 mmol) in dry dichloromethane (0.4 ml) was added to a stirred solution of triethylamine (30 mg, 41 μl, 1.1 equiv.), *tert*-butyldiphenylsilyl chloride (81.6 mg, 77 μl, 1.1 equiv.) and 4-*N*,*N*-dimethylaminopyridine (catalytic) in dichloromethane (0.5 ml) at room temperature. The reaction mixture was stirred for 16 h, diluted with dichloromethane (5 ml) and washed with water (3 × 2 ml), dilute hydrochloric acid (2 ml) and saturated aqueous sodium hydrogencarbonate (2 ml). The aqueous phases were back extracted with dichloromethane (2 ml). The combined organic phases were dried ($MgSO_4$) and evaporated. Flash column chromatography (light petroleum–ethyl acetate, 9:1) of the crude residue gave the *title compound* (−)-**17** (77 mg, 72%) as a colourless oil; $[\alpha]_D^{22}$ −24 (*c* 0.2 $CHCl_3$).

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