Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

E. coli cells expressing the Baeyer-Villiger Monooxygenase 'MO14' (*ro*03437) from *Rhodococcus jostii* RHA1 catalyse the gram-scale resolution of a bicyclic ketone in a fermentor

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Supporting Information

Section S1: *Chiral Analysis of Biotransformation of Bicyclo[3.2.0]hept-2-en-6-one by E. coli cells expressing the MO14 gene. Determination of Absolute Configuration of Product Lactone and Residual Ketone Substrate.*

Cells of *E. coli* expressing the *ro*03437 gene encoding MO14 catalyse the regio- plus enantio-selective resolution of racemic **1** to yield (1*S*, 5*R*)-**1** and a single lactone enantiomer (1*S*, 5*R*)-**2** as illustrated in **Figures 1** and **3**.

The analytical resolution of the four possible lactone regioisomers/enantiomers from Baeyer-Villiger oxidation of **1** was achieved using a BGB-173 chiral GC column, with a temperature gradient of 90°C to 134 °C at 1°C min^{-1} (**Figure S1**).

Baeyer-Villiger oxidation of racemic **1** by acetic acid/hydrogen peroxide yields the racemic 3-oxa and 2-oxa lactone pairs in a ratio of approximately 1: 10 (**Figure S1**, top). The absolute configuration of the lactone produced by MO14 was determined by comparison with the results of biotransformation of **1** by cells of *E. coli* expressing the cyclohexanone monooxygenase (CHMO) from *Acinetobacter calcoaceticus* NCIMB 9871, also expressed in *E. coli* and described by us in Szolkowy *et al.*, (2009) *Chembiochem*, **10**, 1208. This biotransformation has also been extensively characterised independently by many other groups, including, initially, that of Furstoss [Alphand *et al.* (1989) *Tetrahedron Lett.*, **30**, 3663]. CHMO yields the 3-oxa lactone (1*R*, 5*S*)-**3** and the 2-oxa lactone (1*S*, 5*R*)-**2** in approximately equal amounts (**Figure S1**, middle). Thus the elution order and

retention times of the lactone products were: (1*R*, 5*S*)-(-)-**3**, 36.7 min.; (1*S*, 5*R*)-(+)-**3**), 37.0 min.; (1*S*, 5*R*)-(-)-**2**, 41.8 min.; (1*R*, 5*S*)-(+)-**2**, 42.4 min.

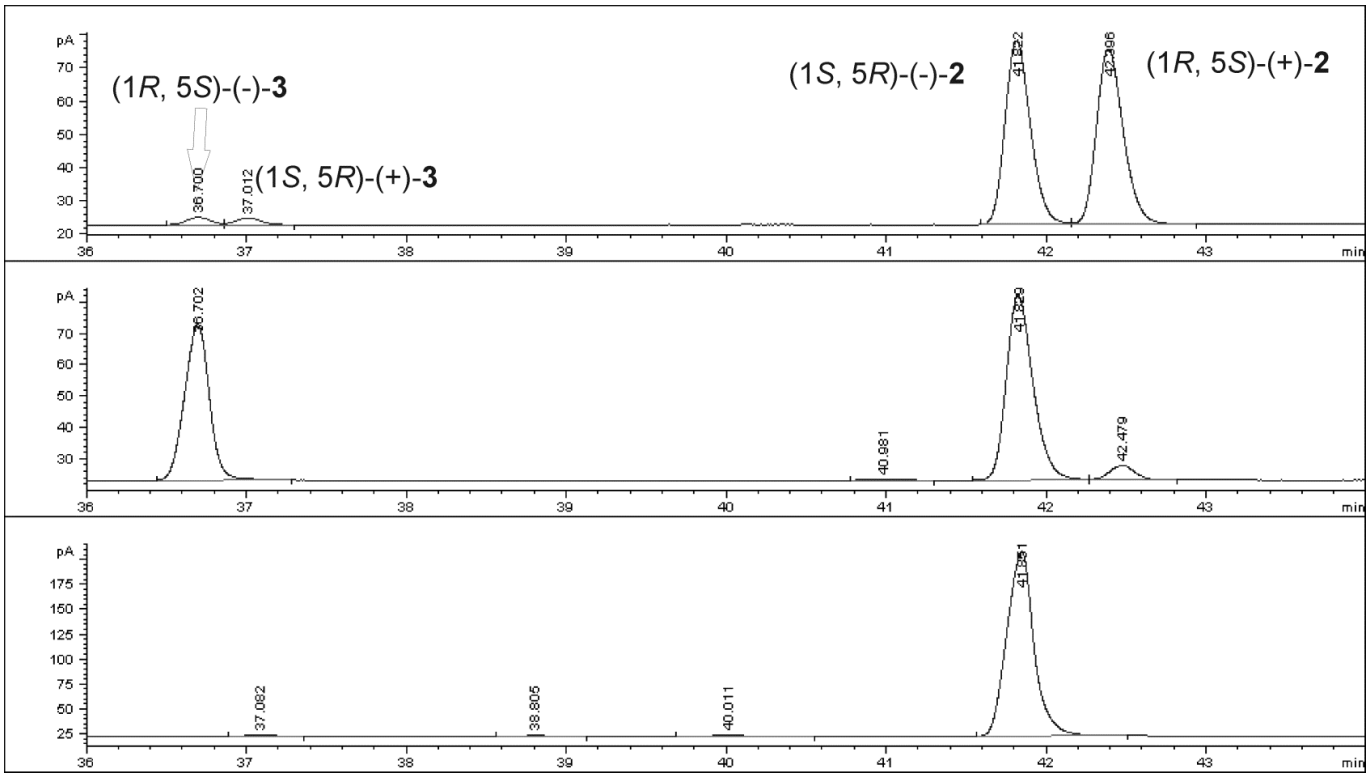

Figure S1. GC chromatograms illustrating the separation of regio- and enantiomeric lactone products of Baeyer-Villiger oxidation of racemic **1**. Top: Products from Baeyer-Villiger oxidation of racemic **1** using acetic acid/hydrogen peroxide; Middle: Products from biotransformation of **1** by cells expressing the cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* NCIMB 9871 [Szolkowy *et al.*, (2009) *Chembiochem*, **10**, 1208; Alphand *et al.* (1989) *Tetrahedron Lett.*, **30**, 3663]; Lactone product of biotransformation of racemic **1** by cells of *E. coli* expressing the *ro*03437 gene encoding MO14.

For the residual ketone, if the absolute configuration of the MO14 lactone product is (1*S*, 5*R*)-**2**, then the residual enantiomer must be (1*S*, 5*R*)-(-)-**1**, as the stereochemistry at carbon atoms C1 and C5 in each enantiomer of the starting material is fixed. The progress of the resolution of racemic **1** can also be followed by chiral GC, using the BGB-175 column and a gradient of 100°C to 127°C with

2°C min^{-1}. Retention times for enantiomers were: (1*R*, 5*S*)-(+)-**1,** 11.6 min.; (1*S*, 5*R*)-(-)-**1**, 12.4 min. The progress of the resolution is shown in **Figure S2**.

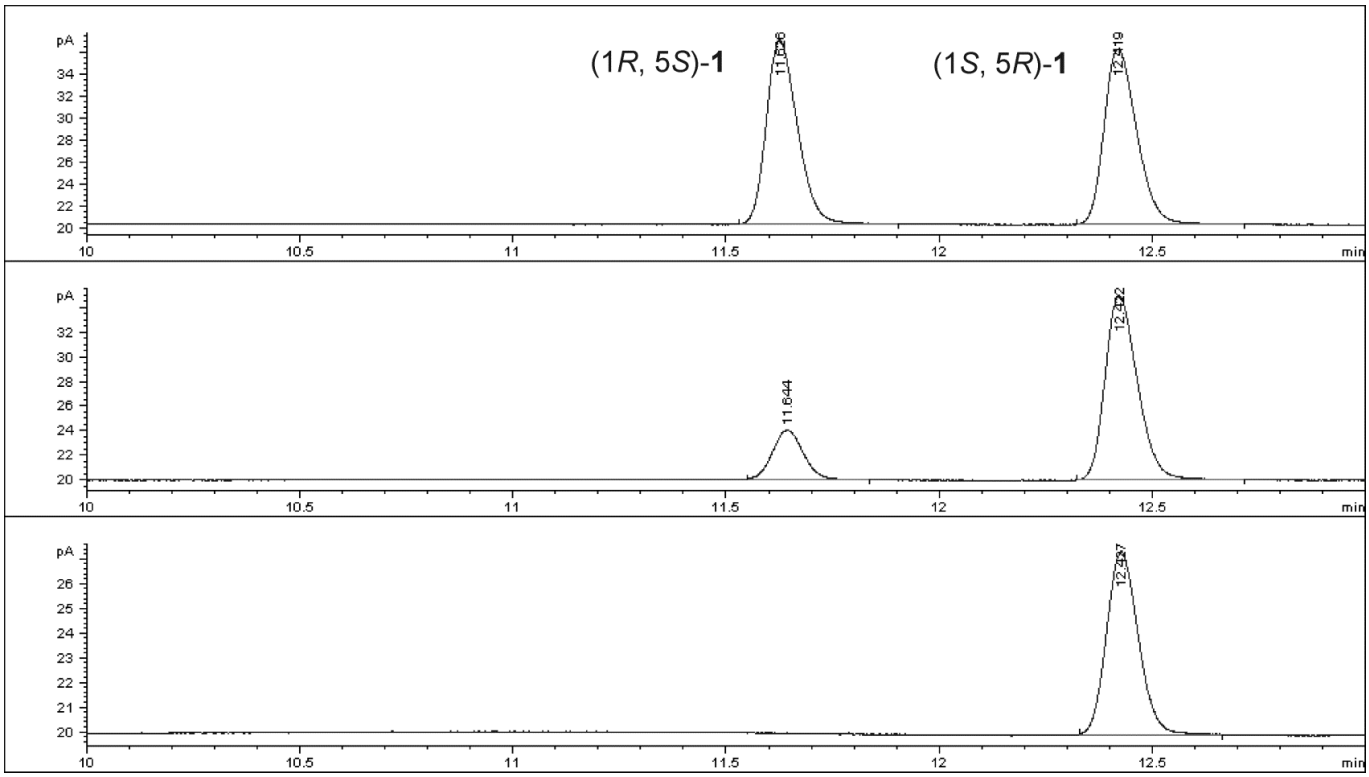

Figure S2. GC chromatograms illustrating the resolution of (1*S*, 5*R*)-**1**, from racemic **1** by cells of *E. coli* expressing the *ro*03437 gene encoding MO14. Top: t = 0 h; Middle: t = 12 h; Bottom: t = 16 h.

Section S2: *NMR Spectra for (1S,5R)-2-Oxabicyclo[3.3.0]oct-6-en-3-one*

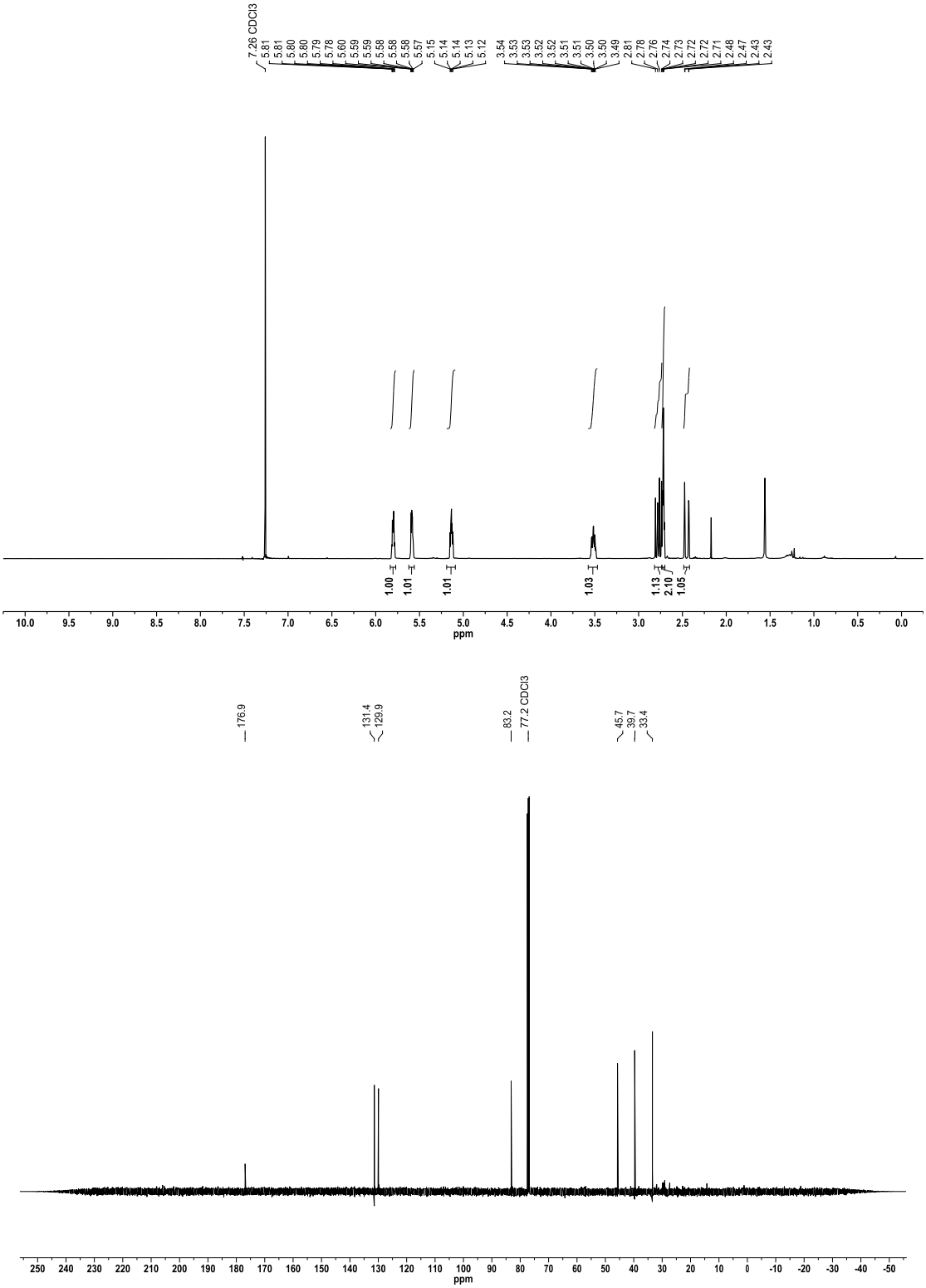

Figure S3. Top: ^{1}H NMR spectrum; Bottom: ^{13}C spectrum.