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Enzyme-Catalysed Kinetic Resolution of 4-*endo*-Hydroxy-2-Oxabicyclo[3.3.0]oct-7-en-3-one and Employment of the Pure Enantiomers for the Synthesis of Anti-viral and Hypocholestemic Agents

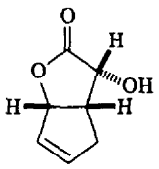
Rosemary A. MacKeith,[a] Ray McCague,[b] Horacio F. Olivo,[a] Stanley M. Roberts,[a] Stephen J.C. Taylor[b] and Hui Xiong[a]

[a]*Department of Chemistry, Exeter University, Exeter, Devon EX4 4QD, U.K.*
[b]*Research Division, Chiroscience Ltd, Science Park, Cambridge CB4 4WE, U.K.*

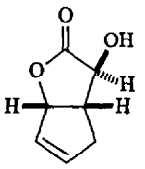
Abstract—The *endo*-hydroxylactone (±)-(1) was resolved by enantioselective acetylation using *Candida cylindracea* lipase or preferentially *Pseudomonas fluorescens* lipase (pfl). Alternatively the corresponding butyrate (±)-(3) was hydrolysed with pfl to give the ester (+)-(1*S*,4*R*,5*S*)-(3) and the alcohol (–)-(1*R*,4*S*,5*R*)-(1). The latter compound was converted into carbovir (–)-(1*R*,4*S*)-(12) while the ester (+)-(3) was transformed into the δ-lactone (+)-(3*R*,5*S*)-(18). The *exo*-hydroxylactone (±)-(2) was resolved less efficiently by a trans-esterification process employing pfl and vinyl acetate.

Introduction

The cycloaddition of glyoxylic acid and cyclopentadiene gives access to the racemic[1] hydroxylactones (1) and (2).[2a] The *endo*-hydroxylactone (1) predominates over the *exo*-hydroxylactone (2) [ratio (1):(2), 4:1] and the two compounds can be separated by chromatography over silica. Alternatively the major component can be obtained pure by fractional crystallization.[2b,2c]



(1)



(2)

The hydroxylactone (1) was resolved using *Pseudomonas fluorescens* lipase (pfl) working in the hydrolysis or esterification mode (Scheme I). Thus the lactone (±)-(1) was converted into the racemic butyrate ester (±)-(3) and hydrolysed with pfl in aqueous buffer allowing for periodic addition of sodium hydroxide solution to maintain the solution close to pH 7. The hydrolysis is rapid (10–20 h depending on the scale) and highly enantioselective (both products showing > 92 % *e.e.*). Moreover the products (–)-(1*R*,4*S*,5*R*)-(1)† and (+)-(1*S*,4*R*,5*S*)-(3) are easy to separate. Thus extraction of the aqueous solution with hexane afforded the butyrate (+)-(3); further extraction with ethyl acetate furnished the hydroxylactone (–)-(1). The absolute configuration of the latter compound was established by selected chemical transformations (*vide infra*).

The biocatalytic hydrolysis of the butyrate ester (±)-(3) was scaled up to utilize kilogram amounts of substrate; Amano Lipase PS was used as the catalyst and the isolation procedure was modified so that chromatography was avoided. By centrifugation of the biotransformation mixture, virtually all of the unreacted ester, which remained as a separate oil phase, was removed, thereby allowing facile extraction and crystallization of the formed hydroxylactone. Crystallization also enhanced the *e.e.* from 97 to > 99 %, the *e.e.* being determined by HPLC of the *p*-toluate ester on a (L)-leucine Pirkle HPLC column.

The enantiomeric ratio (E value) for the butyrate ester hydrolysis was typically E › 200. This compares with a slightly lower value (E = 85–100) for the acetate ester (4). Both acetate and butyrate esters were found to undergo non-enzymic ring-opening of the lactone under the aqueous conditions of the hydrolytic biotransformation and this was found to be pH dependent, occurring more slowly at lower pH. When the pH was controlled automatically at pH 7 during the biotransformation, this hydrolysis contributed to significant product yield loss on scale-up. This loss was much greater for the more water soluble acetate and therefore the butyrate ester was the substrate of choice.

As an alternative process the hydroxylactone (±)-(1) can be acetylated with equally exquisite selectivity using pfl in vinyl acetate (containing a small amount of triethylamine) to provide, after 20 h, optically active hydroxylactone (+)-(1*S*,4*R*,5*S*)-(1) (47 %; 100 % *e.e.*) and the acetate (–)-(1*R*,4*S*,5*R*)-(4) (50 %, 92.4 % *e.e.*). *Candida cylindracea* lipase (ccl) also catalysed the same trans-esterification over

†When first mentioned in this text a compound will be given a full descriptor, e.g. (–)-(1*R*,4*S*,5*R*)-(1); thereafter in the discussion and experimental sections reference will sometimes be made to this compound using just the sign of the optical rotation [e.g. (–)-(1)].

a much longer reaction time with opposite but lower selectivity. Thus after 250 h in vinyl acetate containing ccl the racemic lactone (±)-(**1**) gave the ester (+)-(**4**) (8 % > 90 % *e.e.*) and the recovered alcohol (–)-(**1**) (92 %, 6 % *e.e.*).

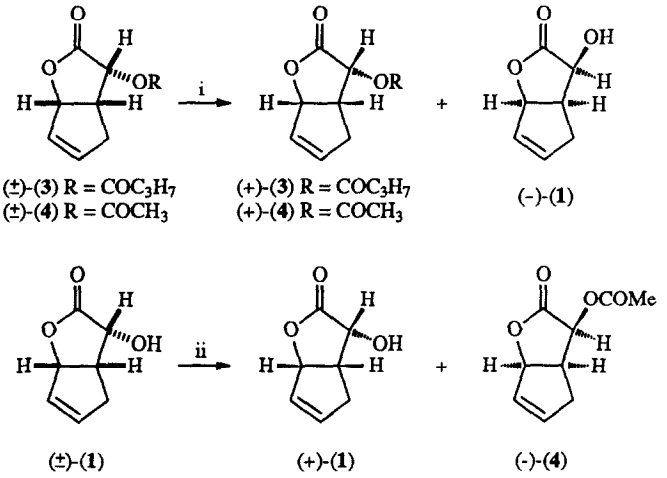
Although biocatalytic esterification appears at first sight to be more attractive than the hydrolytic mode for resolution of the hydroxylactone (±)-(**1**), in practice the enzymic hydrolysis was preferred. Comparison of the two methods showed that the hydrolysis strategy was much the faster, required less enzyme and gave purer product after work-up.

Somewhat surprisingly the *exo*-lactone (±)-(**2**) was not readily acetylated using pfl in vinyl acetate. Only after one week is a substantial amount (63.8 %, 49.7 % *e.e.*) of acetate (–)-(1*R*,4*R*,5*R*)-(**5**) formed together with the corresponding optically active *exo*-hydroxylactone (+)-(1*S*,4*S*,5*S*)-(**2**) (33.1 %, 93.2 % *e.e.*). The absolute configurations of the products obtained were established by correlation with the benzoate (+)-(1*S*,4*S*,5*S*)-(**6**) obtained from the *endo*-hydroxylactone (Scheme II).

In our programmes of research aimed at the synthesis of natural products we have found that pfl tends to preferentially catalyse reactions on a wide variety of secondary alcohols (or the derived esters) as shown in Figure 1.[10] The *endo*-hydroxylactone (±)-(**1**) conforms to this pattern. However, as a salutory lesson in the potential over-use of such crude models, the *exo*-hydroxylactone (the slow-reacting system) does not behave in the normal fashion.[11]

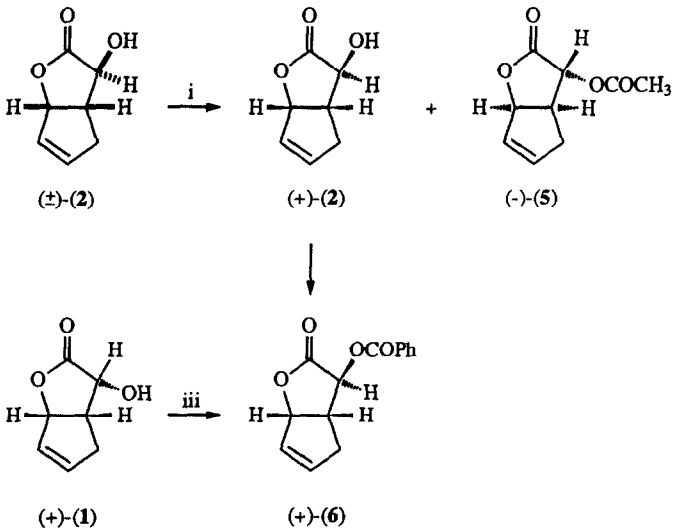
Up to this point we have demonstrated that the hydroxylactone (**1**) is a compound that is very simple to prepare and easy to resolve; in the following paragraphs we begin to show that it is potentially useful for the synthesis of a wide variety of different products.[12]

For example, reduction of the lactone (–)-(**1**) furnishes the triol (–)-(**7**) in excellent yield provided a carefully controlled work-up procedure is employed (Scheme III). Cleavage of the vicinal diol unit with sodium periodate, followed by sodium borohydride reduction gave a diol



Reagents and conditions: i. Pfl, H_2O, phosphate buffer solution pH 7, NaOH; ii. Pfl, vinyl acetate, Et3N.

Scheme I.



Reagents and Conditions: i. Pfl, vinyl acetate; ii. PhCOCl, Et_3N, CH_2Cl_2; iii. $PhCO_2H$, DEAD, Ph_3P, THF.

Scheme II.

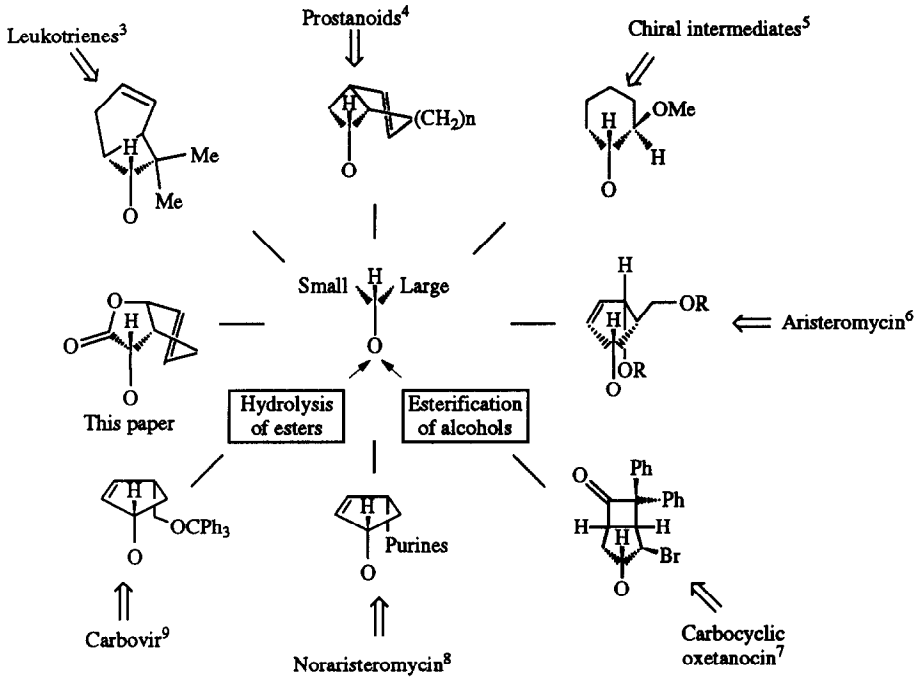
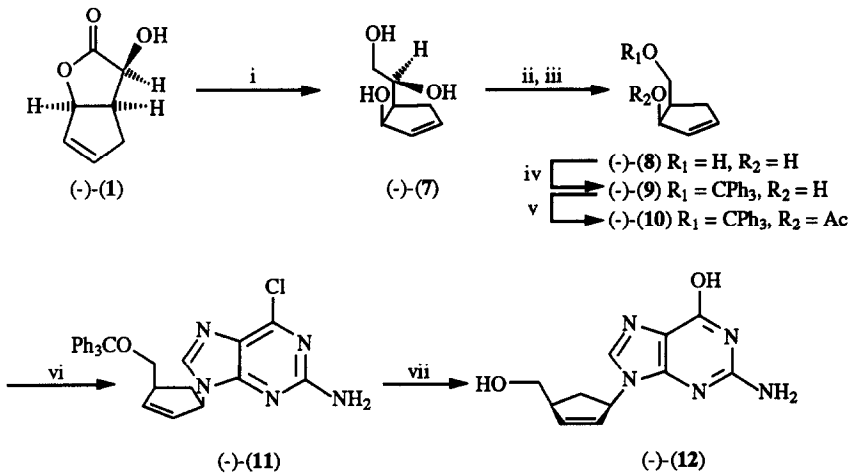


Figure 1. Preferential substitution pattern for hydrolysis/esterification reactions involving some cyclohexanol, cyclopentanol and cyclobutanol derivatives catalyzed by pfl.

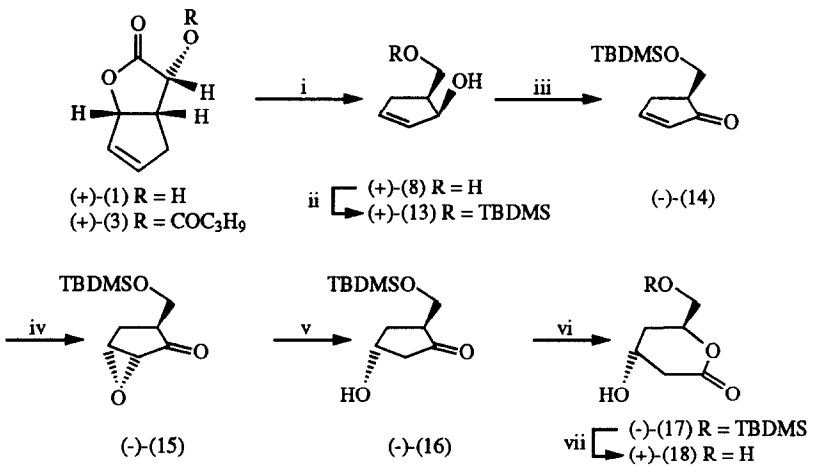


Reagents and Conditions: i. $LiAlH_4$, THF, heat, 2 h; ii. $NaIO_4$, Et_2O–H_2O, 0 °C, 1 h; iii. $NaBH_4$, EtOH, 0 °C, 1 h; iv. Ph_3CCl, DMAP, Et_3N, CH_2Cl_2; v. Ac_2O, pyridine; vi. 2-Amino-6-chloropurine, NaH, THF, $Pd(PPh_3)_4$; vii. ref 14.

Scheme III.

which was selectively tritylated at the primary hydroxyl group and then acetylated to furnish the ester (–)-(1*R*,2*R*)-(**10**). Employment of Trost-style chemistry using the substrate (–)-(**10**), deprotonated 2-amino-6-chloropurine and a Pd(0) catalyst afforded the cyclopentene derivative (–)-(1*R*,4*S*)-(**11**) and this compound was modified to give the anti-HIV agent carbovir (–)-(1*R*,4*S*)-(**12**).[2b]

Contrarywise the hydroxylactone (+)-(**1**) or the butyrate (+)-(**3**) can be elaborated, to provide synthons for hypocholestemic agents.[13] Thus lithium aluminium hydride reduction of both of these compounds gave the triol (+)-(**7**) (Scheme IV). Periodate cleavage, sodium borohydride reduction and selective silylation at the primary hydroxyl group followed by mild oxidation produced the ketone (–)-(5*S*)-(**14**). Attempted Baeyer–Villiger oxidations of the enone (**14**) proved fruitless, so, instead, the compound was epoxidized under basic conditions. This led to addition of peroxide from the more exposed face so as to form the oxirane (–)-(2*R*,3*R*,5*S*)-(**15**). This epoxide was reduced with aluminium amalgam to afford the hydroxylactone (–)-(2*S*,4*R*)-(**16**), which underwent smooth Baeyer–Villiger oxidation using *meta*-chloroperoxybenzoic acid to give the δ-lactone (–)-(3*R*,5*S*)-(**17**). This lactone was deprotected using aqueous acetic acid in tetrahydrofuran (THF) to provide the known diol (+)-(3*R*,5*S*)-(**18**),[15] a highly sought after synthon for the preparation of potent hypocholestemic agents.



Reagents and Conditions: i. see Scheme III; ii. TBDMSCl, Et_3N, CH_2Cl_2; iii. PCC, CH_2Cl_2; iv. H_2O_2, NaOH; v. Al(Hg), THF–H_2O; vi. MCPBA, $NaHCO_3$, CH_2Cl_2; vii. AcOH, H_2O.

Scheme IV.

In summary the lactones (+)-(1) and (–)-(1), as well as the corresponding esters, are now readily available on the kilogram scale and provide potential new starting points for the synthesis of a wide variety of fine chemicals.

Experimental Section

IR Spectra were recorded on a Perkin-Elmer 881 or a Nicolet Magna-IR 550 spectrometer. Optical rotations were measured on an Optical Instrument A-1000 polarimeter. ^1H and ^{13}C NMR spectra were recorded on either a Bruker AM-250 or a Bruker AC-300 spectrometer. Chemical shifts are given in ppm and coupling constants in Hertz. Mass spectra were recorded at the S.E.R.C. Mass Spectrometry Centre, Swansea on a VG ZAB-F spectrometer (high resolution), or a VG 12-253 spectrometer (low resolution). Elemental analyses were performed by Butterworth Laboratories, Ltd.

*Enzyme resolution of (±)-4-*endo*-butanoyloxy-2-oxabicyclo[3.3.0]oct-7-en-3-one 3*

Pseudomonas fluorescens lipase (5.5 mg) was added to a solution of (±)-4-*endo*-butanoyloxy-2-oxabicyclo[3.3.0]oct-7-en-3-one **3** (248 mg, 1.18 mmol), contaminated with approximately 4 % (±)-*exo*- butyrate, in aqueous phosphate buffer (2.4 cm^3; 0.1 mol dm^{-3}; pH 7). The mixture was stirred at 35 °C for 7 h and aqueous sodium hydroxide solution (0.59 cm^3; 1 mol dm^{-3}) was added dropwise to the mixture at 30 min intervals to maintain the pH between 6.5 and 7. The mixture was stirred at room temperature for 16 h and the enzyme was then removed by filtration. The filtrate was diluted with water (2.5 cm^3) and extracted with ethyl acetate (10 x 5 cm^3) and the combined organic layers dried ($MgSO_4$) and concentrated under reduced pressure. The residue (164 mg) was purified by column chromatography (CH_2Cl_2–MeOH, 9:1) to give firstly (±)-*exo*- butyrate (9 mg, 3 %; R_f 0.41, 2:1, light petroleum–EtOAc) then (+)-(1*S*,4*R*,5*R*)-4-butanoyloxy-2-oxabicyclo[3.3.0]oct-7-en-3-one (+)-**3** (93 mg, 37 %) as a pale mobile oil; $[\alpha]_D^{28}$ + 8.5 ° (*c* 1.25 in CH_2Cl_2) (92 % *e.e.*); R_f 0.36 (light petroleum–ethyl acetate, 2:1); [Found: C, 62.7; H, 6.8 %; [M + H]$^+$ 211.0971. $C_{11}H_{14}O_4$ requires C, 62.8; H, 6.7 %; [M + H]$^+$ 211.0974]; υ_{max} ($CHCl_3$)/cm^{-1} 3027, 2973, 2938, 1786, 1747, 1360, 991 and 958; δ_H(300 MHz; $CDCl_3$) 6.22 (1 H, dt, *J* 5.7, 2.4, 7-H), 5.96 (1 H, dddd, *J* 5.7, 2.5, 2.2, 1.8, 8-H), 5.64 (1 H, d, *J* 9.4, 4-H), 5.38 (1 H, dt, *J* 6.6, 2.2, 1-H), 3.37 (1 H, dddd, *J* 9.4, 9.1, 6.6, 5.8, 5-H), 2.55 (1 H, ddddd, *J* 18.1, 5.8, 2.5, 2.4, 2.2, 6-H), 2.44 (2 H, t, *J* 7.4, 2'-H), 2.41 (1 H, m, 6-H), 1.71 (2H, m, 3'-H), 0.99 (3 H, t, *J* 7, CH_3); δ_C (63 MHz; $CDCl_3$) 172.4 and 172.0 (C, CO), 140.0 (CH), 128.0 (CH), 86.2 (CH), 69.4 (CH), 39.2 (CH), 35.7 (CH_2), 31.5 (CH_2,), 18.3 (CH_2) and 13.6 (CH_3); m/z (EI) 211 ([M + H]$^+$, 1 %), 83 (100), 78 (97), 67 (94). Later fractions contained (–)-(1*R*,4*S*,5*S*)-4-hydroxy-2-oxabicyclo[3.3.0]-oct-7-en-3-one (–)-**1** (39 mg, 24 %), mp 66–67.5 °C as a pale brown solid; $[\alpha]_D^{28}$ – 94 ° (*c* 1.0 in $CHCl_3$), (>98 % *e.e.*), R_f 0.08 (light petroleum–ethyl acetate, 2:1); IR, ^1H and ^{13}C NMR spectra were identical to those for the racemic compound.

*Large scale enzyme resolution of (±)-4-*endo*-butanoyloxy-2-oxabicyclo[3.3.0]oct-7-en-3-one 3*

The (±)-hydroxylactone ester **3** (2.391 kg, 4.5:1 ratio of *endo*:*exo* isomers) was stirred in phosphate buffer (20 L, 0.1 M KH_2PO_4) at 25 °C, and the pH adjusted to 6.6 using 10 M NaOH. Lipase (Amano PS, 120 g) was added as a slurry in 1 L phosphate buffer, then the mixture was stirred well to disperse the ester. The mixture was controlled at pH 6.6 by automatic addition of 10 M NaOH. After addition of 4.85 mol hydroxide, 10 % w/v NaCl was added, then excess butyrate ester and undissolved solids were removed by centrifugation. The supernatant was extracted 3 times with an equal volume of ethyl acetate, the organic extracts dried ($MgSO_4$), then concentrated under reduced pressure to 5 % of the original volume. Methyl-*tert*-butyl ether was added to induce crystallization and after chilling to 5 °C, the crystals were harvested by filtration,

then recrystallized from dichloromethane, yielding 396 g (–)-hydroxylactone (–)-(**1**) of >99 % *e.e.* The *e.e.* of the hydroxylactone was determined on the *para*-toluate ester separating enantiomers on a (L)-leucine Pirkle HPLC column (25 cm x 4.6 mm, eluting with 4 % ethanol in heptane, 2 cm^3/min 254 nm detection, retention times 14.9 and 15.9 min). Hydroxylactone esters were saponified with methanolic sodium hydroxide prior to derivatization.

*Enzyme resolution of (±)-4-*endo*-hydroxy-2-oxabicyclo-[3.3.0]oct-7-en-3-one* **1**

Pseudomonas fluorescens lipase (519.6 mg) was added to a solution of (±)-4-*endo*-hydroxy-2-oxabicyclo[3.3.0]oct-7-en-3-one (709.4 mg, 5.06 mmol) in vinyl acetate (46 cm^3) containing triethylamine (0.5 cm^3). The mixture was stirred vigorously for 20 h at room temperature and then the lipase was removed by filtration. The filtrate was concentrated under reduced pressure and the residue was separated by column chromatography (5:1, ether–light petroleum). The first compound eluted was (–)-4-*endo*-acetoxy-2-oxabicyclo[3.3.0]oct-7-en-3-one **4** ($[\alpha]_D^{28}$ –6.6 ° (*c* 1.0 in $CHCl_3$), 92.4 % *e.e.*, 461 mg, 2.53 mmol, 50.0 % yield) followed by (+)-4-*endo*-hydroxy-2-oxabicyclo-[3.3.0]oct-7-en-3-one was obtained ($[\alpha]_D^{28}$ + 101.4 ° (*c* 1.0 in $CHCl_3$), 100 % *e.e.*, 335.8 mg, 2.39 mmol, 47.3 % yield). 4-*endo*-Acetoxy-2-oxabicyclo[3.3.0]oct-7-en-3-one **4** had the following physical characteristics: R_f 0.26 (hexane-ethyl acetate, 4:1); υ_{max} (neat)/cm^{-1} 3028, 2942, 1781, 1372, 1230; δ_H (250 MHz; $CDCl_3$) 6.22 (1H, dt, *J* 3, 2), 5.95 (1H, m), 5.63 (1H, d, *J* 9.5), 5.38 (1H, dt, *J* 6.6, 2.0), 3.37 (1H, m), 2.58 (1H, m), 2.45 (1H, ddt, *J* 18, 9, 2.3), 2.2 (3H, s); δ_C (63 MHz; $CDCl_3$) 172.0 (CO), 169.6 (CO), 140.0 (CH), 128.0 (CH), 86.3 (CH), 69.6 (CH), 39.1 (CH), 31.5 (CH_2), 20.4 (CH_3); m/z (CI) 183 (100), 140 (10), 123 (45), 78 (40); [Found: 183.0657, $C_9H_{10}O_4$+H^+ requires 183.0657].

The *endo*-hydroxylactone (±)-**1** (30 mg, 2.14 x 10^{-4} mol) was acetylated with vinyl acetate (3 cm^3) in the presence of *Candida cylindracea* lipase (10 mg) at room temperature for 11 days to give *endo*-acetoxy lactone (+)-**4** (8 %) (> 90 % *e.e.*) and *endo*-hydroxylactone (–)-**1** (92 %) (6 % *e.e.*).

*Enzyme resolution of (±)-4-*exo*-hydroxy-2-oxabicyclo-[3.3.0]oct-7-en-3-one* **2**

Pseudomonas fluorescens lipase (1.10 g) was added to a solution of *exo*-(±)-4-hydroxy-2-oxabicyclo[3.3.0]oct-7-en-3-one **2** (1.57 g, 11.2 mmol) in vinyl acetate (66 cm^3). The mixture was stirred for 8 days at 30°C and then the enzyme was removed by filtration. The filtrate was concentrated under reduced pressure and the oily residue was purified by column chromatography (2:1 to 3:1 ether–light petroleum). The first fraction contained (–)-4-*exo*-acetoxy-2-oxabicyclo[3.3.0]oct-7-en-3-one (–)-**5** ($[\alpha]_D^{28}$ –44.6 °(*c* 1.0 in $CHCl_3$), 49.7 % *e.e.*, 1.30 g, 7.17 mmol, 63.8 % yield) which had the following properties: R_f 0.3 (hexane–ethyl acetate, 2:1); $[\alpha]_D^{28}$ –60° (*c* 1.0 in $CHCl_3$); υ_{max} (neat)/cm^{-1} 3029, 2939, 1780, 1750, 1373, 1230; δ_H (250 MHz; $CDCl_3$) 6.12 (1H, m), 5.90 (1H, m), 5.58 (1H, brd, *J* 7.8), 5.09 (1H, d, *J* 6.6), 3.05 (1H, ddd, *J* 14, 7, 3), 2.75 (1H, m), 2.15 (3H, s); δ_C (63 MHz; $CDCl_3$) 172.7 (CO), 170.0 (CO), 136.7 (CH), 129.3 (CH), 87.3 (CH), 74.9 (CH), 42.7 (CH), 36.7 (CH_2), 20.6 (CH_3); [Found: C, 59.24; H, 5.61. $C_9H_{10}O_4$ requires C, 59.34; H, 5.53]. Later fractions contained (+)-4-*exo*-hydroxy-2-oxabicyclo-[3.3.0]oct-7-en-3-one (+)-**2** ($[\alpha]_D^{28}$ + 70.9 ° (*c* 1.0 in $CHCl_3$), 93.2 % *e.e.*, 542 mg, 3.72 mmol, 33.1 % yield) R_f 0.31 (ethyl acetate–hexane, 3:2); υ_{max} (neat)/cm^{-1} 3427, 1764, 1184, 1116, 986; δ_H (250 MHz; $CDCl_3$) 6.1 (1H, m), 5.9 (1H, m), 5.55 (1H, m), 4.15 (1H, d, *J* 7), 3.5 (1H, bs), 3.05 (1H, m), 2.78 (1H, m), 2.55 (1H, m); δ_C (63 MHz; $CDCl_3$) 178.2 (CO), 136.9 (CH), 129.2 (CH), 87.5 (CH), 74.3 (CH), 44.3 (CH), 36.6 (CH_2); m/z (CI) 158 ($[M+NH_4]^+$, 100), 141 (10), 123 (15), 96 (25); [Found: C, 59.24; H, 5.74; $[M+NH_4]^+$ 158.0817. $C_7H_8O_3$ requires C, 60.00; H, 5.75; $[M+NH_4]^+$ 158.0817].

*(+)-(1S,4S,5R)-4-*exo*-Benzoyloxy-2-oxabicyclo[3.3.0]oct-7-en-3-one* **6**

Benzoyl chloride (2 cm^3) was added to a solution of 1*S*,4*S*,5*R*-(+)-4-*exo*-hydroxy-2-oxabicyclo[3.3.0]oct-7-en-3-one ($[\alpha]_D^{28}$ +80.1 °, 99.3 % *e.e.*, 111.7 mg, 0.797 mmol) in dry pyridine (2 cm^3). The solution was stirred for 1.5 h at room temperature under argon. The reaction mixture was purified by column chromatography (dichloromethane–hexane, 2 to 10:1) to give the *exo*-benzoyl ester as a colourless oil (169.7 mg, 0.699 mmol, 87.3 % yield): $[\alpha]_D^{28}$ + 78.4 ° (*c* 1.0 in $CHCl_3$); υ_{max} (neat)/cm^{-1} 1784, 1727, 1603, 1454, 1267; δ_H (250 MHz; $CDCl_3$) 8.08 (2H, m), 7.60 (1H, m), 7.45 (2H, m), 6.14 (1H, m), 5.94 (1H, m), 5.65 (1H, m), 5.30 (1H, d, *J* 6.5), 3.20 (1H, m), 2.80 (2H, m); δ_C (63 MHz; $CDCl_3$) 172.6 (CO), 165.6 (CO), 137.0 (CH), 133.7 (CH), 130.0 (CH), 129.3 (CH), 128.7 (C), 128.5 (CH), 87.4 (CH), 75.5 (CH), 42.9 (CH), 37.0 (CH_2); m/z (CI) 245 (1), 172 (35), 122 (55), 105 (100), 94 (35), 77 (78); [Found: 245.0814. $C_{14}H_{13}O_4$+H^+ requires 245.0828].

*Mitsunobu reaction of (1S,4R,5S)-4-*endo*-hydroxy-2-oxabicyclo[3.3.0]oct-7-en-3-one (+)-1*

Diethyl azodicarboxylate (602.1 mg, 3.45 mmol, 1.7 eq) in dry THF (10 cm^3) was added dropwise to a mixture of *endo*-(+)-(1*S*,4*R*,5*R*)-4-hydroxy-2-oxabicyclo[3.3.0]oct-7-en-3-one (+)-**1** ($[\alpha]_D^{28}$ + 100.5 ° (*c* 1.0 in $CHCl_3$), 100 % *e.e.*, 283.5 mg, 2.02 mmol, 1 eq.), triphenylphosphine (909.7 mg, 3.44 mmol, 1 eq.) and benzoic acid (743.9 mg, 6.09 mmol, 3 eq.) in dry THF (20 cm^3). The mixture was stirred overnight at room temperature. After solvents were evaporated under reduced pressure the residue was purified by column chromatography (ethyl acetate–light petroleum, 1:4) to give *exo*-(+)-(1*S*,4*S*,5*R*)-benzoate ester (+)-**6** ($[\alpha]_D^{28}$ + 73.7 ° (*c* 1.0 in $CHCl_3$), 421.4 mg, 1.27 mmol, 85.2 % yield), R_f 0.26 (benzene–ether, 35:1).

(+)-(1S,2S)-2-Hydroxymethylcyclopent-4-en-1-ol (+)-8

A solution of (+)-(1*S*,4*R*,5*S*)-4-hydroxy-2-oxabicyclo-[3.3.0]oct-7-en-3-one (+)-**1** (5.00 g, 35.7 mmol) in dry

tetrahydrofuran (40 cm^3) was added dropwise to a solution of lithium aluminium hydride in dry tetrahydrofuran (0.5 mol dm^{-3}; 72 cm^3) under nitrogen. The mixture was stirred at reflux for 2 h and then at room temperature for 16 h. Diethyl ether saturated with water (100 cm^3) was added dropwise, with vigorous stirring, followed by water (10 cm^3), at such a rate that gentle reflux was maintained, to give a grey precipitate. The mixture was stirred at reflux for 1 h and then allowed to stand for 15 min. The supernatant liquid was decanted and filtered through Celite filter agent. Tetrahydrofuran (300 cm^3) and water (10 cm^3) were added to the precipitate which was stirred at reflux for 1 h and then the hot mixture was filtered through Celite. The combined filtrates were concentrated under reduced pressure to give crude 5*S*-(1',2'-dihydroxyethyl)-cyclopent-2-en-1*R*-ol (+)-**7** (5.65 g) as a brown viscous oil: υ_{max} (neat)/cm^{-1} 3405, 3061, 2930, 2847, 1005; δ_H (250 MHz; $CDCl_3$) 6.05 (1H, m), 5.85 (1H, m), 4.85 (1H, m), 3.9 (1H, m), 3.6 (6H, m), 2.2 (2H, m); δ_C (63 MHz; $CDCl_3$) 135.1 (CH), 132.2 (CH), 76.0 (CH), 72.4 (CH), 65.5 (CH_2), 44.2 (CH), 33.2 (CH_2); m/z (CI) 144 (100), 109 (100); (Found: $[M+NH_4]^+$ 162.113. $C_7H_{12}O_3$ requires $[M+NH_4]^+$ 162.113).

Sodium metaperiodate (7.64 g, 35.7 mmol) was added to a vigorously stirred solution of the crude triol **7** (5.65 g) in a mixture of diethyl ether (300 cm^3) and water (300 cm^3) at 0 °C. After stirring for 1.5 h, ethylene glycol (0.65 cm^3, 12 mmol) was added and the mixture stirred at 0 °C for 1 h. The brown solution was then concentrated under reduced pressure to remove the diethyl ether and then ethanol (75 cm^3) was added. The mixture was cooled to 0 °C and sodium borohydride (1.35 g, 35.7 mmol) was added with vigorous stirring, accompanied by slight effervescence and formation of a small amount of precipitate. The mixture was stirred for 1 h at 0 °C and then concentrated under reduced pressure to a volume of 300 cm^3. The brown aqueous solution was saturated by addition of sodium chloride and then extracted with ethyl acetate (6 x 400 cm^3). The combined organic extracts were washed with brine (200 cm^3), dried (Na_2SO_4) and concentrated under reduced pressure. The residue (3.32 g), a pale yellow mobile oil, was purified by column chromatography (petroleum–ethyl acetate, 1:1, then to 100 % ethyl acetate) to give the title compound (+)-**8** (3.00 g, 73 %) as a very pale yellow mobile oil: R_f 0.35 (chloroform–methanol, 9:1); $[\alpha]_D^{28}$ + 121 ° (*c* 1.0 in $CHCl_3$); (Found: C, 62.55; H, 8.4; $[M+NH_4]^+$ 132.103. $C_6H_{10}O_2$ requires C, 63.1; H, 8.8; $[M+NH_4]^+$ 132.1025); υ_{max} (neat)/cm^{-1} 3296, 2937, 1416, 1351, 1215, 1032, 946; δ_H (250 MHz; $CDCl_3$) 6.01 (1H, m), 5.83 (1H, m), 4.92 (1H, m), 3.82 (2H, m), 3.64 (2H, m), 2.40–1.90 (3H, m); δ_C (63 MHz; $CDCl_3$) 134.5 (CH), 132.3 (CH), 76.9 (CH), 62.1 (CH_2), 42.5 (CH), 33.4 (CH_2); m/z (CI) 114 (100), 96 (25), 79 (35). The enantiomer (–)-(**8**) was obtained from (–)-(**1**) by an identical procedure.

*(+)-(1S,2S)-2-(Triphenylmethyloxymethyl)cyclopent-4-en-1-ol (+)-**9***

A solution of diol (+)-**8** (157 mg, 1.206 mmol), trityl chloride (370 mg, 1.32 mmol), DMAP (7 mg, 0.06 mmol) and triethylamine (0.185 cm^3, 1.32 mmol) in dichloromethane (10 cm^3) was stirred for 4.5 h. The solution was washed with NH_4Cl solution and brine, and dried over $MgSO_4$, filtered and solvent evaporated. The residue was purified by column chromatography on silica gel (hexane–ethyl acetate, 4:1) to give 368.3 mg (1.07 mmol, 89 %) of pure alcohol (+)-**9**: mp 100–103 °C; $[\alpha]_D^{28}$ +48 ° (*c* 1.0 in $CHCl_3$); υ_{max} (neat)/cm^{-1} 3063, 2930, 1488, 1447, 1216, 1061; δ_H (250 MHz; $CDCl_3$) 7.5 (6H, m), 7.4–7.2 (9H, m), 6.0 (1H, m), 5.88 (1H, m), 4.95 (1H, m), 3.43 (1H, dd, *J* 9, 5.5), 3.27 (1H, t, *J* 9), 2.6 (1H, m), 2.35 (1H, m), 2.1 (2H, m); δ_C (63 MHz; $CDCl_3$) 144.1 (3C), 134.8 (CH), 132.8 (CH), 128.6 (6CH), 128.0 (6CH), 127.1 (3CH), 86.9 (C), 77.0 (CH), 63.4 (CH_2), 42.0 (CH), 34.3 (CH_2); [Found: C, 84.50; H, 6.68. $C_{25}H_{24}O_2$ requires C, 84.24; H, 6.78]. Enantiomer (–)-(**8**) was converted into (–)-(**9**) using an identical procedure.

*(+)-(1S,5S)-1-Acetoxy-2-(triphenylmethyloxymethyl)-cyclopent-4-ene (+)-**10***

Alcohol (+)-**9** (106 mg, 0.3 mmol) was stirred in a mixture of pyridine (2 cm^3) and acetic anhydride (2 cm^3) overnight. Saturated aqueous ammonium chloride solution (10 cm^3) was added, and the solution was extracted with ether (10 cm^3). The organic layer was washed with sodium bicarbonate and brine. The organic layer was dried over $MgSO_4$, filtered and solvent evaporated to yield 120 mg (0.3 mmol, 100 % yield) of the acetyl ester (+)-**10**: R_f 0.35 (hexane–ethyl acetate, 9:1); $[\alpha]_D^{28}$ –96 ° (*c* 1.0 in $CHCl_3$); υ_{max} (neat)/cm^{-1} 3062, 3024, 1721, 1596, 1243, 1215; δ_H (250 MHz; $CDCl_3$) 7.5–7.4 (6H, m), 7.35–7.25 (9H, m), 6.1 (1H, m), 5.84 (1H, m), 5.78 (1H, m), 3.2 (2H, m), 2.7 (1H, m), 2.45 (1H, ddt, *J* 16, 8, 2), 2.2 (1H, dd, *J* 17, 7), 1.8 (3H, s); δ_C (63 MHz; $CDCl_3$) 170.5 (CO), 144.3 (CH), 137.2 (CH), 129.6 (3C), 128.8 (6CH), 127.7 (6CH), 126.9 (3CH), 86.5 (C), 78.4 (CH), 62.5 (CH_2), 41.5 (CH), 34.9 (CH_2), 21.0 (CH_3); m/z (EI) 321 (M^+–C_6H_5, 3), 243 (Ph_3C^+, 100), 165 (80); [Found: C, 81.81; H, 6.52. $C_{27}H_{26}O_3$ requires C, 81.38; H, 6.57]. Enantiomer (–)-(**10**) was synthesized from (–)-(**9**) by an identical procedure. Compound (–)-(**10**) was converted into carbovir (–)-(**12**) as prescribed.[14]

*(+)-(1S,2S)-2-(t-Butyldimethylsilyloxymethyl)cyclopent-4-en-1-ol (+)-**13***

Diol (+)-**8** (261.4 mg, 2.295 mmol) was dissolved in dry CH_2Cl_2 (10 cm^3). 4-Dimethyl-aminopyridine (11.2 mg), triethylamine (0.350 cm^3, 1.1 eq.) and *tert*-butyldimethylsilyl chloride (381 mg, 1.1 eq.) were added. The solution was stirred overnight. Thin layer chromatography showed two less polar products and traces of starting material. Water was added to the solution, and the aqueous layer was washed with aqueous ammonium chloride solution, then brine. The organic layer was dried with $MgSO_4$, filtered and evaporated. The mixture was separated by column chromatography (hexane–ethyl acetate, 9:1) to give 360 mg (1.577 mmol, 70 % yield) of the monoprotected diol (+)-**13**: R_f 0.43 (hexane–ethyl

acetate, 4:1); $[\alpha]_D^{28}$ + 57 ° (*c* 1.0 in $CHCl_3$); υ_{max} (neat)/cm^{-1} 3427, 3061, 2931, 1464, 1255, 1082; δ_H (250 MHz; $CDCl_3$) 5.93 (1H, m), 5.83 (1H, m), 4.85 (1H, m), 3.8 (2H, m), 2.9 (1H, brs), 2.55–2.10 (3H, m), 0.90 (9H, m), 0.10 (6H, m); δ_C (63 MHz; $CDCl_3$) 133.7 (CH), 133.1 (CH), 77.4 (CH), 63.2 (CH_2), 42.6 (CH), 34.0 (CH_2), 25.7 ($3CH_3$), 18.0 (C), –5.5 (CH_3), –5.6 (CH_3); m/z (CI) 228 (20), 211 (100), 132 (30); [Found: C, 63.08; H, 10.46; $[M+NH_4^+-H_2O]$ 228.1784. $C_{12}H_{24}O_2Si$ requires C, 63.10; H, 10.59, $[M+NH_4^+-H_2O]$ 228.1785].

*(–)-(S)-2-(t-Butyldimethylsilyloxymethyl)cyclopent-4-en-1-one (–)-**14***

Monoprotected cyclopentendiol (+)-**13** (140.4 mg, 0.6158 mmol) was diluted in CH_2Cl_2 (10 cm^3). Pyridinium chlorochromate (265 mg, 1.23 mmol) was added, and reaction stirred under argon at room temperature overnight. Ether (20 cm^3) was added, the solution decanted and filtered through florisil. The solvent was evaporated and the cyclopentenone purified by column chromatography (chloroform–ether, 99:1) to give the ketone **14** 118 mg (0.52 mmol, 85 % yield) : R_f 0.47 (chloroform); $[\alpha]_D^{28}$ –93 ° (*c* 1.0 in $CHCl_3$); υ_{max} (neat)/cm^{-1} 3020, 2934, 2861, 1713, 1592, 1474, 1257, 1118; δ_H (250 MHz; $CDCl_3$) 7.73 (1H, dt, *J* 5.8, 2.6), 6.17 (1H, dt, *J* 5.8, 2.1), 3.88 (1H, dd, *J* 19.2, 5.1), 3.84 (1H, dd, *J* 19.2, 3.8), 2.77 (2H, m), 2.45 (1H, m), 0.92 (9H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C (63 MHz; $CDCl_3$) 210.3 (CO), 164.4 (CH), 134.2 (CH), 62.3 (CH_2), 47.3 (CH), 32.7 (CH_2), 25.7 ($3CH_3$), 16.1 (C), –5.59 (CH_3), –5.61 (CH_3); m/z (CI) 227 (100), 211 (10), 169 (15); [Found: C, 63.34; H, 9.98; $[M+H]^+$ 227.1467. $C_{12}H_{22}O_2Si$ requires C, 63.66; H, 9.80; $[M+H]^+$ 227.1468].

*(–)-(2R,3R,5S)-2,3-Epoxy-5-(t-butyldimethylsilyloxymethyl)cyclopentanone (–)-**15***

Cyclopentenone (–)-**14** (117.8 mg, 0.521 mmol) was diluted in methanol (5 cm^3) and the solution was cooled in an ice–salt bath. Hydrogen peroxide (0.2 cm^3, 30 %) was added, whereafter sodium hydroxide solution (0.5 cm^3) was added slowly. The solution was stirred for 1 hr. Sodium bisulfite solution was added and the aqueous layer was extracted with ethyl acetate. The organic layer was washed with brine and dried with $MgSO_4$, filtered and solvent evaporated. The residue was chromatographed (silica gel, hexane–ethyl acetate, 9:1) to give 55.6 mg (0.23 mmol, 44 % yield) of epoxide **15**: R_f 0.34 (hexane–ethyl acetate, 9:1); $[\alpha]_D^{28}$ –21 ° (*c* 1.0 in $CHCl_3$); υ_{max} (neat)/cm^{-1} 2935, 2861, 1750, 1474, 1364, 1121; δ_H (250 MHz; $CDCl_3$) 3.90 (2H, m), 3.69 (1H, dd, *J* 10.0, 3.3), 3.35 (1H, d, *J* 2.4), 2.42 (2H, m), 2.18 (1H, m), 0.85 (9H, s), 0.05 (6H, s); δ_C (63 MHz; $CDCl_3$) 209.3 (CO), 60.6 (CH_2), 56.8 (CH), 55.1 (CH), 43.1 (CH), 26.6 (CH_2), 25.7 ($3CH_3$), 16.1 (C), –5.59 (CH_3), –5.62 (CH_3); m/z (CI) 243 (100), 227 (35), 185 (40); [Found: C, 58.99; H, 9.24; $[M+H]^+$ 243.1416. $C_{12}H_{22}O_3Si$ requires C, 59.46; H, 9.15; $[M+H]^+$ 243.1416].

*(–)-(2S,4R)-2-(t-Butyldimethylsilyloxymethyl)-4-hydroxycyclopentanone (–)-**16***

Aluminium strips (62.2 mg, 2.37 mmol) were washed with sodium hydroxide solution, H_2O, $HgCl_2$, H_2O and THF according to Keck's procedure,[16] and added to a solution of epoxycyclopentanone **15** (82.4 mg, 0.34 mmol) in a mixture of THF–H_2O (11 cm^3, 10:1). The solution was stirred for 1.5 h and filtered through a pad of celite, and then the solvent was evaporated to yield β-hydroxycyclopentanone **16** (79.4 mg, 0.325 mmol, 96 % yield) : R_f 0.27 (hexane–ethyl acetate, 1:1); $[\alpha]_D^{28}$ –72 ° (*c* 1.0 in $CHCl_3$); υ_{max} (neat)/cm^{-1} 3440, 2935, 1747, 1473, 1257, 1104; δ_H (250 MHz; $CDCl_3$) 4.64 (1H, m), 3.98 (1H, dd, *J* 9.9, 3.8), 3.66 (1H, dd, *J* 9.9, 3.2), 2.54 (1H, m), 2.38–2.1 (4H, m), 1.8 (1H, bs), 0.9 (9H, s), 0.10 (6H, s); δ_C (63 MHz; $CDCl_3$) 217 (CO), 66.1 (CH), 61.6 (CH_2), 48.6 (CH_2), 47.9 (CH), 35.0 (CH_2), 25.8 ($3CH_3$), 18.2 (C), –5.8 ($2CH_3$); m/z (CI) 245 (100), 227 (80), 169 (20); [Found: C, 58.46; H, 9.88. $C_{12}H_{24}O_3Si$ requires C, 58.97; H, 9.90].

*(–)-(3R,5S)-5-(t-Butyldimethylsilyloxy)methyl-3-hydroxy-δ-valerolactone (–)-**17***

Dry dichloromethane (5 cm^3) was added to hydroxycyclopentanone **16** (75 mg, 0.307 mmol). Sodium bicarbonate (80 mg) and *meta*-chloroperoxybenzoic acid (50–60 %, 320 mg) were added. The suspension was stirred overnight then washed with sodium carbonate solution and brine. The organic layer was dried over magnesium sulfate, filtered and the solvent evaporated. The crude product was purified by column chromatography (silica gel, ethyl acetate–hexane, 3:2) to give 43.2 mg (0.166 mmol, 54 % yield) of the lactone **17**: R_f 0.25 (ethyl acetate–hexane, 3:2); $[\alpha]_D^{28}$ –2.5 ° (*c* 1.0 in $CHCl_3$); υ_{max} (neat)/cm^{-1} 3430, 2927, 2858, 1717, 1257, 1139, 1075; δ_H (250 MHz; $CDCl_3$) 4.73 (1H, m), 4.45 (1H, quint, *J* 4.0), 3.84 (1H, dd, *J* 11.1, 2.3), 3.73 (1H, dd, *J* 11.1, 3.6), 2.64 (1H, m), 2.4 (1H, bs), 1.98 (1H, dd, *J* 9.0, 4.0), 0.9 (9H, s), 0.1 (6H, s); δ_C (63 MHz; $CDCl_3$) 171.2 (CO), 76.6 (CH), 64.9 (CH_2), 62.3 (CH), 38.7 (CH_2), 31.6 (CH_2), 25.8 ($3CH_3$), 16.2 (C), –5.4 (CH_3), –5.5 (CH_3).

*(+)-(3R,5S)-3-Hydroxy-5-(hydroxymethyl)-δ-valerolactone (+)-**18***

The protected alcohol **17** (120 mg, 0.46 mmol) was stirred in a mixture of acetic acid, water and THF (4 cm^3, 2:1:1) for 1.5 h at 60 °C. The solvent was evaporated and the diol was purified by column chromatography ($CHCl_3$–MeOH, 85:15) to yield 35.7 mg (0.245 mmol, 53 % yield) of the diol (+)-**18**: $[\alpha]_D^{28}$ + 1.2 ° (*c* 1.0 in CH_3OH) {lit.[13] $[\alpha]_D^{29}$ + 1.8 ° (*c* 0.99 in CH_3OH}; υ_{max} (neat)/cm^{-1} 3391, 3016, 2934, 1716, 1389, 1257; δ_H (250 MHz; $CDCl_3$) 4.84 (1H, m), 4.48 (1H, m), 3.91 (1H, dd, *J* 12.2, 2.7), 3.68 (1H, dd, *J* 12.2, 4.7), 2.70 (2H, m), 1.95 (4H, m); δ_C (63 MHz; CD_3OD) 173.3 (CO), 78.5 (CH), 65.1 (CH_2), 63.3 (CH), 39.2 (CH_2), 32.3 (CH_2).

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