Hydrolytic enzymes in the synthesis of carba-sugars: application of the lipase from *Pseudomonas fragi*

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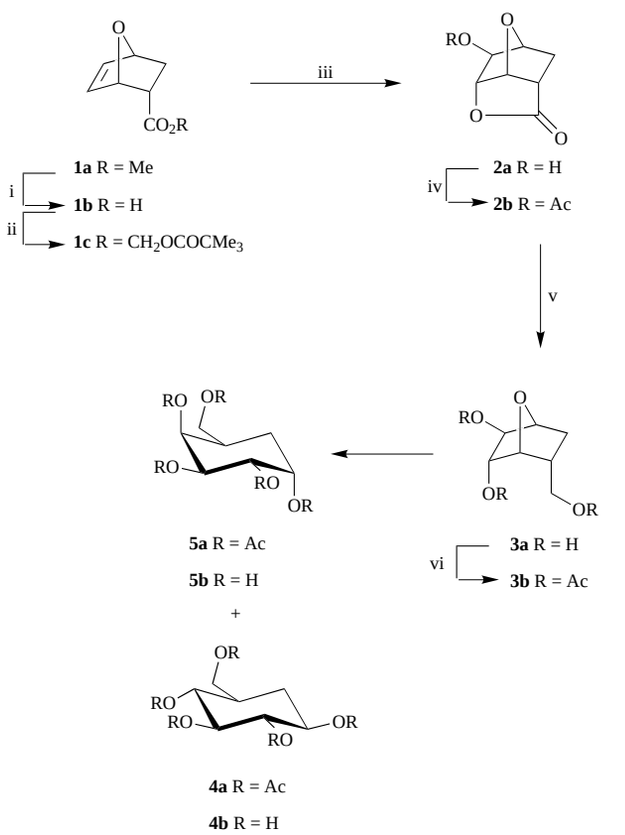
Pig liver esterase-catalysed hydrolysis of (±)-*endo*-2-methoxycarbonyl-7-oxabicyclo[2.2.1]hept-5-ene 1a provides (−)-*endo*-7-oxabicyclo[2.2.1]hept-5-ene-2-carboxylic acid 1b and (+)-*endo*-2-methoxycarbonyl-7-oxabicyclo[2.2.1]hept-5-ene 1a in 36 and 57% ee, respectively. Transesterification between (±)-2-*exo*-hydroxy-4,8-dioxatricyclo[4.2.1.0^{3,7}]nonan-5-one 2a and vinyl acetate catalysed by the lipase from *Pseudomonas fragi* gives the acetate (−)-2b and alcohol (+)-2a in 91 and 94% ee, respectively. Optically pure acetate (−)-2b is obtained after removal of contaminating racemate from the impure acetate by crystallisation of (−)-2b of 91% ee (EtOH).

Introduction

Pseudo-sugars (carba-sugars) are carbocyclic analogues of monosaccharides in which the ring oxygen has been replaced by a methylene group. The term 'pseudo-sugar' was proposed by McCasland *et al*. to describe the first synthetic analogue, DL-pseudo-α-talopyranose.[1] Recently, the use of the prefix 'carba' preceded by the appropriate locant instead of 'pseudo' was recommended by Suami and Ogawa for the purpose of indexing.[2] Carba-sugars are widely distributed in Nature, either in monosaccharide form or incorporated into oligosaccharides. Thus 5a-carba-α-D-galactopyranose was isolated from a fermentation broth of *Streptomyces* species MA-4145.[3] It was shown to inhibit the growth of *Klebsiella pneumoniae* MB-1264, although the potency of the antibiotic was rather low. Before the discovery of 5a-carba-α-D-galactopyranose, the pseudo-trisaccharidic validamycin antibiotics, widely used as farming antibiotics in Japan, had been isolated from a fermentation broth of *Streptomyces hygroscopicus* var. *limoneus*.[2,4] Validamycin A, a major component of the validamycin complex, exhibited strong inhibitory activity against sheath blight of rice and damping off of cucumber seedlings owing to infection by *Pellicularia sasakii* and *Rhizoctonia solani*. Carba-sugar-containing oligosaccharides with enzyme-inhibitory activity include acarbose,[5] adiposin[6] and the trestatins.[7] The carba analogues of α-DL-glucopyranose, and β-DL-glucopyranose have been used as glucose analogues in studies of the mechanism of glucose-stimulated insulin release by pancreatic islets.[8] Carba-sugars may find use as non-nutritive artificial sweeteners.[9] The carba-analogue of β-DL-glucose is as sweet as D-glucose and the sweetness of both the carba analogue of α-DL-galactose was 0.4–0.5 that of sucrose.

Extensive studies have been carried out on the synthesis of carba-sugars,[1,10–26] including methods based on biotransformations.[27,28]

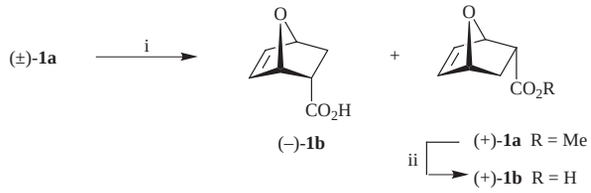
endo-7-Oxabicyclo[2.2.1]hept-5-ene-2-carboxylic acid **1b** is a versatile starting material in carba-hexopyranose synthesis. Ogawa's group has demonstrated the versatility of this compound as a building block for carba-sugar synthesis.[12–14] For projected syntheses of carba-oligosaccharides, we needed to prepare optically pure carba-glucopyranose **4b** and carba-galactopyranose **5b** (Scheme 1). A common strategy for the synthesis of enantiomerically pure compounds **4b** and **5b** is *via* the resolution of their precursor (±)-**1b**.[18] Resolution of racemate (±)-**1b** has been achieved by diastereoisomer crystallisation using (*R*)-(+)- or (*S*)-(−)-α-methylbenzylamine.[18] This method is laborious and time consuming since several crystallisations are required in order to obtain enantiomerically pure products. In addition, only one enantiomer was obtained in each resolution. Accordingly, we decided to explore the applicability of hydrolytic enzymes in the synthesis of enantiomerically pure carba-sugars. Enzymic hydrolysis of ester **1a** catalysed by pig liver esterase (PLE) and enzymic resolution of lactone **2a** *via* transesterification using the lipase from *Pseudomonas fragi* as the catalyst were studied.



Scheme 1 *Reagents and conditions*: i, NaOH; ii, chloromethyl pivalate, K_2CO_3, crown ether; iii, formic acid, H_2O_2; iv, pyridine, acetic anhydride; v, $LiAlH_4$; vi, pyridine, acetic anhydride, DMAP

Results and discussion

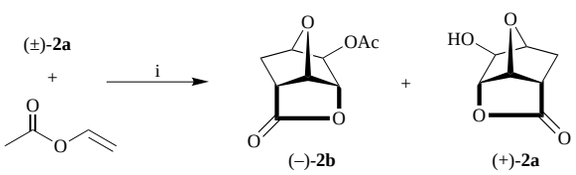
Acid **1b** was readily available by hydrolysis of the methyl ester **1a**,[12] which was itself obtained by catalysed Diels–Alder cycloaddition between furan and methyl acrylate.[29] Treatment of acid **1b** with hydrogen peroxide in formic acid gave the lactone **2a**,[13,18] which was reduced to triol **3a**[13] by $LiAlH_4$ (Scheme 1). PLE catalysed hydrolysis of ester **1a** at pH 7.0 (Scheme 2) gave acid (−)-**1b** in 40% yield [36% enantiomeric



Scheme 2 *Reagents and conditions*: i, PLE, phosphate buffer pH 7.0, 54% conv.; ii, NaOH

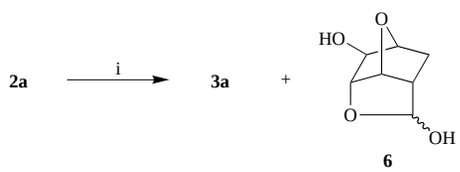
excess (ee)]. The unchanged ester (+)-**1a** was hydrolysed to acid (+)-**1b** in 48% yield (57% ee). Owing to our limited success with PLE, several lipases were screened for their ability to hydrolyse ester **1a**. These were lipase PS and the lipases from *Rhizopus delemar*, *Pseudomonas fragi*, *Aspergillus niger*, *Candida rugosa* and porcine pancreas. However, with none of these lipases were satisfactory results obtained. It was suspected that the steric congestion at the chiral centre inhibited enzymic hydrolysis. Accordingly, an alternative strategy was attempted. In order to relieve the steric hindrance, the pivaloyloxymethyl derivative **1c** was prepared. This was subjected to PLE-catalysed hydrolysis at pH 7.0. However, the hydrolysis was found to proceed with complete lack of stereodiscrimination.

Transesterification between alcohols and esters catalysed by lipases is commonly used to resolve secondary and tertiary alcohols. Accordingly, it was anticipated that lactone **2a**, through its secondary alcohol function, might be resolved by lipase-catalysed transesterification. Through assays using the lipases used in the attempted kinetic resolution of ester **1a**, it was found that lactone **2a** was a good substrate for the lipase from *Ps. fragi*. Transesterification was carried out in Bu^{t}OMe by using 11% acetone as co-solvent, vinyl acetate as the acyl donor and the lipase from *Ps. fragi* as the biocatalyst (Scheme 3). After 16 h, acetate (−)-**2b** was obtained in 47% yield (94% of theoretical) and 91% ee, and the unchanged substrate (+)-**2a** was obtained in 47% yield (94% of theoretical) and 94% ee. Traces of enantiomer (+)-**2b** in (−)-**2b** in these products were selectively removed by recrystallisation (EtOH). Thus, optically pure ester (−)-**2b** was obtained by removal of contaminating racemate by crystallisation from the acetate (−)-**2b** of 91% ee.



Scheme 3 *Reagents and conditions*: i, lipase from *Ps. fragi*, acetone, Bu^{t}OMe

Reduction of lactone **2a** was carried out using $LiAlH_4$ (Scheme 4).[13] When reduction was carried out with one mol equiv. of $LiAlH_4$ at room temp. for 2 h, triol **3a** (58%) and lactol **6** (28%) were isolated as crystalline solids. (Triol **3a** has previously been reported as a syrup.[13]) Obviously, the reduction was not complete. When the same reduction was carried out at room temp. for 20 h with 2 mol equiv. of $LiAlH_4$, the yield of triol increased to 74% and that of lactol **6** dropped to 14%.



Scheme 4 *Reagents and conditions*: i, $LiAlH_4$, THF

In summary, PLE-catalysed hydrolysis of ester **1a** provided ester (+)-**1a** and acid (−)-**1b** in low yield and low ee. Lipase-catalysed transesterification between hydroxy lactone **2a** and vinyl acetate afforded acetate (−)-**2b** and alcohol (+)-**2a** in good yield and good ee. Optically pure acetate (−)-**2b** could be obtained by crystallisation of the small amount of contaminating racemate from the acetate (−)-**2b** of 91% ee. Reduction of lactone **2a** using the literature method provided triol **3a** and lactol **6** which have not previously been isolated and adequately characterised.

The lipase from *Ps. fragi* has been little used in biotransformations. It has been shown to be very effective in catalysing esterifications between a wide range of carboxylic acids and alcohols.[30,31] Its primary structure has been elucidated.[32] The sequence contains 135 amino acids and is considerably shorter than that of other *Ps.* lipases such as that from *Ps. glumae* (319 amino acid residues[33]) and *Ps. cepacia* (320 amino acid residues[34]). However, it retains the active-site sequence Gly-His-Ser-X-Gly typical of other mammalian microbial lipases.[32] It clearly warrants further study as a useful biotransformation enzyme.

Experimental

^{1}H NMR spectra were recorded on Bruker APC-400 or AC-250 MHz spectrometers. ^{13}C NMR spectra were determined at 62.9 MHz on the Bruker AC-250 spectrometer. *J*-Values are given in Hz. Optical rotations were determined using an AA-1000 polarimeter (Optical Activity Ltd) with a 2 dm cell. Optical rotations are given in units of 10^{-1} deg cm^2 g^{-1}. Mass spectra were recorded with a Kratos MS80 spectrometer. Mps were determined with a Gallenkamp melting point apparatus and are uncorrected. The lipases from *C. rugosa*, *Rh. delemar*, *Ps. fragi* and *A. niger* were obtained from Biocatalysts Ltd., Pontypridd, UK; the lipase PS from the Amano Pharmaceutical Company, Osaka, Japan; and porcine pancreatic lipase from the Sigma Chemical Company.

General procedure for assignment of configurations and determination of enantiomeric excess

Assignment of the absolute configurations of acid (−)-**1b** and alcohol (+)-**2a** was based on the optical rotations as compared with the values of the known compounds.[18] Ees of acids (−)-**1b** and (+)-**1b** were determined by ^{1}H NMR spectroscopy in $CDCl_3$ in the presence of one mol equiv. of (*S*)-(−)-α-methylbenzylamine. The ee of alcohol (−)-**2b** was determined directly by ^{1}H NMR spectroscopy in $CDCl_3$ in the presence of 3 mol equiv. of (*R*)-(−)-1-(9-anthryl)-2,2,2-trifluoroethanol. The ee of alcohol (+)-**2a** was determined by conversion of the alcohol into the acetate (+)-**2b**, then by ^{1}H NMR spectroscopy in $CDCl_3$ in the presence of 3 mol equiv. of (*R*)-(−)-1-(9-anthryl)-2,2,2-trifluoroethanol.

(±)-*endo*-7-Oxabicyclo[2.2.1]hept-5-ene-2-carboxylic acid 1b[12]

To a mixture of esters *endo*- and *exo*-**1a**[29] in the ratio 2.7 : 1 (50 g, 0.32 mol) was added 10% aq. NaOH (170 cm^3). The resulting mixture was stirred at room temp. for 20 h before being acidified (conc. HCl) and extracted with dichloromethane (5 × 100 cm^3). The organic phase was dried ($MgSO_4$), and evaporated under reduced pressure to give a mixture of acids *endo*- and *exo*-**1b** as a solid (41.4 g, 91%). Fractional crystallisation (EtOAc–light petroleum 100–120 °C) afforded acid *endo*-**1b** as a crystalline solid (24.7 g, 59%), mp 96–98 °C (lit.,[12] 98–99.5 °C) (Found: M^+, 140.0473. Calc. for $C_7H_8O_3$: *M*, 140.0473); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 3055 (=C–H) and 1709 (C=O); δ_H(250 MHz; $CDCl_3$) 1.56 [1 H, dd, *J* 3.90 and 11.33, H-3 (*endo*)], 2.13 [1 H, ddd, *J* 4.83, 9.19 and 11.33, H-3 (*exo*)], 3.17 (1 H, ddd, *J* 3.90, 4.73 and 9.19, H-2), 5.05 (1 H, dd, *J* 1.75 and 4.83, H-4), 5.19 (1 H, ddd, *J* 0.58, 1.63 and 4.73, H-1), 6.30 (1 H, dd, *J* 1.63 and 5.81, H-6) and 6.46 (1 H, dd, *J* 1.75 and 5.81, H-5); δ_C($CDCl_3$) 28.27 (C-3), 42.69 (C-2), 78.53 (C-1), 79.00 (C-4), 132.55 (C-6), 137.12 (C-5)

and 178.04 (C=O); *m*/*z* (relative abundance) (EI) 140 (M^+, 8%), 123 (48), 111 (55), 97 (25), 95 (60), 85 (62) and 83 (100).

(±)-2-*exo*-Hydroxy-4,8-dioxatricyclo[4.2.1.0^{3,7}]nonan-5-one 2a[13]

To a solution of acid **1b** (10.0 g, 71 mmol) in formic acid (20 cm^3) was added H_2O_2 (35%; 25 cm^3). The resulting mixture was heated at 45 °C until effervescence occurred. It was then kept at room temp. for 30 min. The mixture was evaporated under reduced pressure to give a solid. Recrystallisation (EtOH) afforded title lactone **2a** as a crystalline solid (5.1 g, 46%). The mother liquor was evaporated to dryness and the residue was recrystallised (EtOH) to give a second crop of lactone **2a** (1.4 g, 12.6%), giving a combined yield of 6.5 g (58.6%), mp 112–113 °C (lit.,[13] 112–113 °C) (Found: M^+, 156.0423. Calc. for $C_7H_8O_4$: *M*, 156.0423); ν_{max}(THF)/cm^{-1} 3430 (O–H) and 1796 (C=O); δ_H(250 MHz; D_2O) 1.85 [1 H, dd, *J* 1.94 and 13.66, H-9 (*endo*)], 2.06 [1 H, ddd, *J* 5.23, 11.22 and 13.66, H-9 (*exo*)], 2.64 (1 H, ddd, *J* 1.94, 4.94 and 11.22, H-6), 3.83 (1 H, s, H-2), 4.45 (1 H, d, *J* 4.94, H-3), 4.49 (1 H, d, *J* 5.23, H-1) and 5.32 (1 H, t, *J* 4.94, H-7); δ_C(D_2O) 33.34 (C-9), 39.54 (C-6), 76.67 (CH–O), 81.67 (CH–O), 83.56 (CH–O), 86.21 (CH–O) and 181.44 (C=O); *m*/*z* (rel. ab.) (EI) 156 (M^+, 12%), 138 (32), 127 (26), 110 (22), 97 (100), 84 (88), 73 (50), 69 (82), 60 (42) and 55 (74).

(±)-2-*exo*-Acetoxy-4,8-dioxatricyclo[4.2.1.0^{3,7}]nonan-5-one 2b

Acetic anhydride (4 cm^3) was added to a solution of lactone **2a** (0.3 g, 1.92 mmol) in pyridine (4 cm^3). The resulting solution was kept at room temp. for one day, then was concentrated under reduced pressure and the residue was diluted with EtOAc (100 cm^3). The resulting solution was washed successively with saturated aq. $NaHCO_3$ (3 × 10 cm^3), water (10 cm^3) and brine (10 cm^3), dried ($MgSO_4$), and evaporated under reduced pressure to give a brown liquid. This liquid was subjected to flash chromatography (EtOAc) to give *racemic acetate* **2b** as a crystalline solid, R_f(EtOAc) 0.66 (0.34 g, 89%), mp 166–167 °C (Found: $[M + H]^+$, 199.0606. $C_9H_{11}O_5$ requires *m*/*z*, 199.0606); ν_{max}(CH_2Cl_2)/cm^{-1} 1795 (C=O) and 1744 (C=O); δ_H(250 MHz; $CDCl_3$) 2.07 [1 H, dd, *J* 2.03 and 13.63, H-9 (*endo*)], 2.11 (3 H, s, OAc), 2.25 [1 H, ddd, *J* 5.38, 11.14 and 13.63, H-9 (*exo*)], 2.75 (1 H, dddd, *J* 0.87, 2.03, 4.65 and 11.14, H-6), 4.65 (1 H, ddd, *J* 0.87, 1.45 and 4.94, H-3), 4.70 (1 H, br d, *J* 5.38, H-1), 4.71 (1 H, br s, H-2) and 5.35 (1 H, dd, *J* 4.65 and 4.94, H-7); δ_C($CDCl_3$) 20.70 (Me), 33.43 (C-9), 38.61 (C-6), 78.33 (CH–O), 80.31 (CH–O), 80.63 (CH–O), 82.81 (CH–O), 169.81 (C=O) and 176.10 (C=O); *m*/*z* (rel. ab.) (CI) 216 ($[M + NH_4]^+$, 74%), 199 ($[M + H]^+$, 58) and 43 (100).

(±)-*endo*-5-*tert*-Butanoylmethoxycarbonyl-7-oxabicyclo[2.2.1]-hept-2-ene 1c

To a solution of bicyclic acid **1b** (3.0 g, 21 mmol) in dimethylformamide (DMF) (30 cm^3) were added potassium carbonate (3.2 g, 23 mmol), chloromethyl pivalate (4.0 g, 0.07 mol) and dibenzo-18-crown-6 (0.33 g, 4.4 mol %). The mixture was stirred at room temp. for one day, diluted with dichloromethane (150 cm^3), washed with water (10 × 30 cm^3), dried ($MgSO_4$), and evaporated under reduced pressure. The residue was subjected to flash chromatography (CH_2Cl_2–EtOAc, 10:1) to yield title ester **1c** as a crystalline solid. Recrystallisation (*n*-hexane) gave compound **1c** as needles (3.85 g, 72%), mp 58.5–59 °C (Found: C, 61.70; H, 7.22. $C_{13}H_{18}O_5$ requires C, 61.39; H, 7.14%); ν_{max}(CH_2Cl_2)/cm^{-1} 1746 (C=O); δ_H(250 MHz; $CDCl_3$) 1.21 (9 H, s, 3 × Me), 1.60 [1 H, dd, *J* 3.78 and 11.48, H-6 (*endo*)], 2.11 [1 H, dd, *J* 4.65, 9.09 and 11.48, H-6 (*exo*)], 3.14 (1 H, ddd, *J* 3.78, 4.87 and 9.09, H-5), 5.10 (1 H, dd, *J* 1.74 and 4.65, H-1), 5.17 (1 H, dd, *J* 1.74 and 4.87, H-4), 5.70 (2 H, AB quartet, *J* 5.52, OCH_2O), 6.21 (1 H, dd, *J* 1.74 and 5.81, H-3) and 6.44 (1 H, dd, *J* 1.74 and 5.81, H-2); δ_C($CDCl_3$) 26.76 (3 × Me), 28.31 (C-6), 38.66 (CMe_3), 42.65 (C-5), 78.54 (CH–O), 78.97 (CH–O), 79.32 (CH–O), 132.29 (C=C), 137.18 (C=C), 170.70 (C=O) and 176.94 (C=O); *m*/*z* (rel. ab.) (CI) 272 ($[M + NH_4]^+$, 65%), 204 (100), 187 (42), 174 (72), 157 (36), 123 (62), 102 (65), 85 (40), 68 (20) and 55 (42).

(±)-2-*exo*,3-*endo*-Dihydroxy-5-*endo*-hydroxymethyl-7-oxabicyclo[2.2.1]heptane 3a and (±)-2-*exo*,5-dihydroxy-4,8-dioxatricyclo[4.2.1.0^{3,7}]nonane 6

A solution of lactone **2a** (2.0 g, 13 mmol) in dry tetrahydrofuran (THF) (60 cm^3) was added dropwise to a stirred suspension of $LiAlH_4$ (0.49 g, 13 mmol) in dry THF (20 cm^3) at 0 °C. When the addition was complete, stirring was continued at room temp. for 2 h. The excess of reagent was destroyed by successive addition of water (0.5 cm^3), 15% aq. NaOH (0.5 cm^3) and water (1.5 cm^3). The mixture was filtered and the precipitate was washed with acetone–water (1:1, v/v; 100 cm^3). The filtrate was passed through a column of ion-exchange resin (Dowex 50-X8, H^+; 25 g) and evaporated under reduced pressure at 50 °C to give a syrup. Column chromatography on silica gel with EtOAc–MeOH (9:1, v/v) as the eluent yielded diol **6**, R_f 0.40 (0.58 g, 28%) and triol **3a**, R_f 0.21 (1.22 g, 58%) as crystalline solids. Recrystallisation from acetone gave diol **6** (0.31 g, 15%) and triol **3a** (0.81 g, 39%) as crystals; *triol* **3a** mp 90–91 °C (from acetone) (Found: $[M + H]^+$, 161.0814. $C_7H_{13}O_4$ requires *m*/*z* 161.0814); ν_{max}($CHCl_3$)/cm^{-1} 3214 br (OH); δ_H(250 MHz; D_2O) 1.28 [1 H, dd, *J* 6.11 and 12.58, H-6 (*endo*)], 2.03 [1 H, ddd, *J* 12.58, 12.58 and 6.68, H-6 (*exo*)], 2.25–2.40 (1 H, m, H-5), 3.65 (1 H, d, *J* 2.61, H-2), 3.75 (1 H, dd, *J* 6.97 and 11.39, C*H*$_2$OH), 3.82 (1 H, dd, *J* 6.11 and 11.39, C*H*$_2$OH), 3.97–4.00 (1 H, m, H-3), 4.33 (1 H, d, *J* 6.10, H-1) and 4.38 (1 H, t, *J* 4.94, H-4); δ_C(D_2O) 30.62 (C-6), 44.00 (C-5), 61.63 (CH_2OH), 79.87 (CH–O), 81.03 (CH–O), 83.44 (CH–O) and 86.02 (CH–O); *m*/*z* (CI) (rel. ab.) 178 ($[M + NH_4]^+$, 100%), 161 ($[M + H]^+$, 18), 143 (10) and 125 (10); *diol* **6**, mp 109–110 °C (Found: $[M + NH_4]^+$, 176.0923. $C_7H_{14}NO_4$ requires *m*/*z*, 176.0928); ν_{max}($CHCl_3$)/cm^{-1} 3362br (OH); δ_H(250 MHz; D_2O) 1.41 [1 H, dd, *J* 2.48 and 13.59, H-9 (*endo*)], 1.91 [1 H, ddd, *J* 5.82, 10.77 and 13.59, H-9 (*exo*)], 2.38 (1 H, ddd, *J* 2.48, 4.80 and 10.77, H-6), 3.6 (1 H, s, H-2), 4.10 (1 H, dd, *J* 1.46 and 4.80, H-3), 4.37 (1 H, br d, *J* 5.82, H-1) and 5.21–5.25 (2 H, m, H-5, H-7); δ_C(D_2O) 30.58 (C-9), 44.61 (C-6), 79.83, 81.29, 82.01, 84.03 and 102.12; *m*/*z* (CI) (rel. ab.) 176 ($[M + NH_4]^+$, 20%), 158 (M^+, 100) and 141 (60).

(±)-2-*exo*,3-*endo*-Diacetoxy-5-*endo*-acetoxymethyl-7-oxabicyclo[2.2.1]heptane 3b

Acetic anhydride (20 cm^3) and 4-(dimethylamino)pyridine (DMAP) (10 mg) were added to a solution of triol **3a** (1.0 g, 6.25 mmol) in pyridine (15 cm^3). The resulting mixture was kept at room temp. for 16 h and was concentrated under reduced pressure (~15 cm^3). The residue was diluted with EtOAc (100 cm^3), and washed successively with HCl (1 M; 2 × 15 cm^3) and saturated aq. $NaHCO_3$ (6 × 15 cm^3). The organic phase was dried ($MgSO_4$), and concentrated under reduced pressure to give a yellow liquid. Purification of the liquid by flash chromatography (EtOAc) gave triacetate **3b** as a syrup (1.62 g, 91%) (Found: $[M + NH_4]^+$, 304.1396. $C_{13}H_{22}NO_7$ requires *m*/*z*, 304.1396); ν_{max}(film)/cm^{-1} 1747 (C=O); δ_H(250 MHz; $CDCl_3$) 1.33 [1 H, dd, *J* 6.10 and 12.79, H-6 (*exo*)], 2.03 (3 H, m, OAc), 2.05 (3 H, m, OAc), 2.10 (3 H, s, OAc), 2.13–2.26 [1 H, m, H-6 (*endo*)], 2.52–2.63 (1 H, m, H-5), 4.19 (1 H, dd, *J* 9.01 and 11.04, C*H*$_2$OAc), 4.35 (1 H, dd, *J* 7.85 and 11.04, C*H*$_2$OAc), 4.48 (1 H, br d, *J* 6.10, H-4), 4.65–4.70 (2 H, m, H-1, -2) and 5.04–5.08 (1 H, m, H-3); δ_C($CDCl_3$) 20.45 (OAc), 20.61 (OAc), 20.78 (OAc), 30.37 (C-6), 39.93 (C-5), 63.86 and 76.51 (O–C), 80.36 (O–C), 80.76 (O–C), 82.03 (O–C), 169.78 (C=O), 170.52 (C=O) and 170.60 (C=O); *m*/*z* (rel. ab.) (CI) 304 ($[M + NH_4]^+$, 64%), 287 ($[M + H]^+$, 12), 202 (10) and 52 (100).

PLE-catalysed hydrolysis of (±)-*endo*-5-methoxycarbonyl-7-oxabicyclo[2.2.1]hept-2-ene 1a

PLE (0.1 cm^3, 160 units) was added to a mixture of racemic

ester **1a** (1 g, 6.5 mmol) in phosphate buffer (0.1 M, pH 7.0; 50 cm^3). The mixture was stirred vigorously at room temp. and pH 7.0 was maintained through the reaction by continuous addition of aq. NaOH (0.1 M) using an autotitrator. The course of the reaction was monitored by the volume of aq. sodium hydroxide added and the reaction was stopped at 54% conversion (40 h, 34.8 cm^3 of aq. sodium hydroxide was added). The unchanged ester and acid were then separated by acid–base extraction. Saturated aq. $NaHCO_3$ (40 cm^3) was added to the mixture, which was then extracted with CH_2Cl_2 (3 × 40 cm^3). The aqueous phase was acidified (conc. HCl), then extracted with CH_2Cl_2 (4 × 40 cm^3). The combined organic extracts were dried ($MgSO_4$), and evaporated under reduced pressure to give acid (−)-**1b** as a solid (0.2 g, 40%, 36% ee); $[\alpha]_D^{29.2}$ −36.5 (*c* 0.1, $CHCl_3$). The dichloromethane fraction containing unchanged ester (+)-**1a** was concentrated under reduced pressure to give an oily liquid, to which 10% aq. sodium hydroxide (20 cm^3) was added. The resulting mixture was stirred at room temp. for 3 h, acidified (conc. HCl) and extracted with CH_2Cl_2 (3 × 40 cm^3). The combined organic extracts were dried ($MgSO_4$), and evaporated under reduced pressure to give acid (+)-**1b** as a solid (0.24 g, 48%, 57% ee); $[\alpha]_D^{30}$ +38.8 (*c* 0.12, $CHCl_3$). ^{1}H NMR data of enantiomeric acids (+)-**1b** and (−)-**1b** were identical with those of the racemic acid **1b**.

PLE-catalysed hydrolysis of (±)-*endo*-5-*tert*-butanoylmethoxycarbonyl-7-oxabicyclo[2.2.1]hept-2-ene 1c

A solution of racemic ester **1c** (0.4 g, 1.57 mmol) in *tert*-BuOH (12 cm^3) and PLE (0.2 cm^3) were added to phosphate buffer (0.1 M, pH 7.0; 25 cm^3). The mixture was stirred vigorously at room temp. and pH 7.0 was maintained through the reaction by continuous addition of NaOH (0.1 M) using an autotitrator. After 3.5 h the mixture was diluted with saturated aq. $NaHCO_3$ (15 cm^3) and extracted with CH_2Cl_2 (3 × 20 cm^3). The aqueous phase was acidified (conc. HCl) and extracted with CH_2Cl_2 (4 × 30 cm^3). The combined organic extracts were dried ($MgSO_4$), and evaporated under reduced pressure to give acid **1b** as a solid (0.04 g, 18%, 0% ee). The CH_2Cl_2 fraction was concentrated under reduced pressure to give an oily liquid, to which 10% aq. sodium hydroxide (10 cm^3) was added. The resulting mixture was stirred at room temp. for 20 h before being acidified (conc. HCl), and extracted with dichloromethane (3 × 40 cm^3). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give acid **1b** as a solid (0.14 g, 35%, 0% ee). The acid had a ^{1}H NMR spectrum identical with that of racemic acid **1b**.

Lipase-catalysed transesterification of (±)-2-*exo*-hydroxy-4,8-dioxatricyclo[4.2.1.0^{3,7}]nonan-5-one 2a

Lactone **2a** (2.35 g, 15 mmol) was dissolved in acetone (25 cm^3). To the solution were added Bu^{t}OMe (200 cm^3), vinyl acetate (1.7 g, 19.7 mmol) and lipase from *Ps. fragi* (55 mg). The mixture was stirred vigorously at room temp. for 3 days. It was filtered, and evaporated under reduced pressure. The residue was dissolved in the minimum amount of acetone and subjected to column chromatography (EtOAc–light petroleum 40–60 °C, 5:2, v/v) to give acetate (−)-**2b** as a powder, R_f 0.45 [1.4 g, 47% (97% theoretical), 91% ee] and alcohol (+)-**2a** as a powder, R_f 0.20 [1.1 g, 47% (94% theoretical), 94% ee]; acetate (−)-**2b** had $[\alpha]_D^{24}$ −86.4 (*c* 0.1, $CHCl_3$); alcohol (+)-**2a** had $[\alpha]_D^{23}$ +36.8 (*c* 0.1, EtOH). Both alcohol (+)-**2a** and acetate (−)-**2b** gave ^{1}H NMR spectra identical with those of racemic compounds **2a** and **2b**, respectively.

Purification of (−)-2-*exo*-acetoxy-4,8-dioxatricyclo[4.2.1.0^{3,7}]-nonan-5-one (−)-2b

Acetate (−)-**2b** (0.2 g, 91% ee) was dissolved in boiling ethanol (22 g). The resulting solution was kept at room temp. for two days. The solids (14 mg) were filtered off {$[\alpha]_D^{24}$ −2.7 (*c* 0.2, EtOH)}. The mother liquor was concentrated under reduced pressure. The residue was subjected to flash chromatography (EtOAc–light petroleum 40–60 °C, 10:4, v/v) to give optically pure acetate (−)-**2b** (0.17 g); $[\alpha]_D^{26}$ −89.4 (*c* 0.5, EtOH).

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