Synthesis and enzyme-catalysed reductions of 2-oxo acids with oxygen containing side-chains

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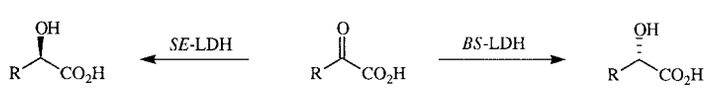
A series of novel 2-oxo esters with protected alcohols at the 3-, 4-, 5-, 6- and 7-positions has been prepared either *via* coupling of an aldehyde with an organometallic reagent (Zn, In or Cr) or *via* a one-carbon homologation of the precursor acid. A one-pot dual enzyme system was used to convert the simpler 2-oxo acids (with a single MOM ether at either C-3 or C-4) into enantiopure protected 2,3- and 2,4-dihydroxy acids in good yields, but in the cases of the more complex trisubstituted substrates, significant decomposition occurred. Biotransformations have proved valuable for the enantioselective synthesis of protected 2,6,7-trihydroxyhept-3-enoic acids.

Introduction

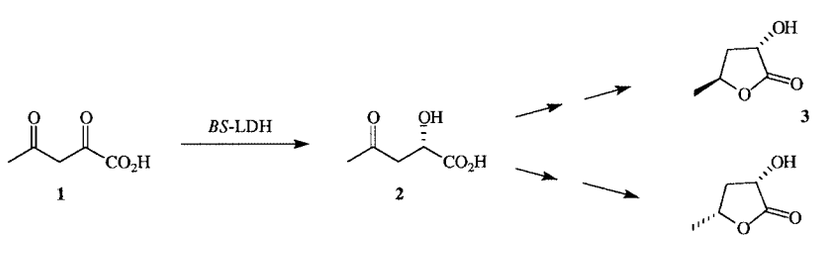
2-Hydroxy acids are valuable building blocks for use in the synthesis of complex molecules and may be prepared in good yields and excellent enantioselectivities by the lactate dehydrogenase (LDH) catalysed reduction of 2-oxo acids. These reactions are simple to perform on a multigram scale.[1] L- and D-LDHs have been obtained from various mammalian and bacterial sources and exhibit reasonably broad substrate specificities. For example, L-LDH from *Bacillus stearothermophilus* (*BS*-LDH) and D-LDH from *Staphylococcus epidermidis* (*SE*-LDH) have been used in the enantioselective synthesis of a range of 2-hydroxy acids with saturated and unsaturated hydrocarbon side-chains as well as those containing halides and aromatic rings (Scheme 1).[2]

2-Hydroxy acids with further oxygen containing substituents in the side-chain would be useful building blocks for a wide range of natural products and oxygen containing heterocycles but interestingly there have been only two reports of the LDH-catalysed reduction of such a 2-oxo acid.[3] Casy[3b] has shown that 2,4-dioxo acids, *e.g.* **1**, may be reduced to the corresponding (*S*)-2-hydroxy acids, *e.g.* **2**, with *BS*-LDH (Scheme 2). A further selective reduction of the 4-ketone either with DIBAL-H or tetramethylammonium triacetoxyborohydride gave, after lactonisation, the (3*S*,5*S*)- and (3*S*,5*R*)-3-hydroxy-5-substituted-γ-lactones (*e.g.* **3** and **4**, respectively) in good yields. We now report our investigations on the synthesis of a series of novel 2-oxo acids with side-chains containing protected alcohols at the C-3 to C-7 positions. Studies on the reduction of each 2-oxo acid with two oxidoreductases, *BS*-LDH and a D-hydroxyisocaproate dehydrogenase are also described.

Although we have found that good yields of (*R*)-2-hydroxy acids may be obtained from 2-oxo acids using *SE*-LDH, the reactions can be too slow to be of practical use in the synthesis of multigram quantities of (*R*)-2-hydroxy acids.[4] D-2-Hydroxy-4-methylvalerate dehydrogenase (also known as D-hydroxyisocaproate dehydrogenase[5]) from *Lactobacillus delbrueckii* subsp. *bulgaricus* (*LB*-hicDH) has more favourable kinetic parameters than D-LDHs for certain substrates.[6] Holbrook and co-workers have used genetic engineering to prepare a rational series of mutants of this enzyme and so analysed the important residues involved in its active site.[7] We have shown that the H205Q mutant (exchange of the His-205 for Gln) exhibits broader substrate specificity than LDHs. For example, L-leucine, valine and phenylalanine derived 2-oxo acids **5**, **6** and **7** are not reduced with either *BS*- or *SE*-LDH but give excellent yields of the corresponding (*R*)-2-hydroxy acids with the H205Q mutant of *LB*-hicDH (Scheme 3).[8] This broader substrate specificity combined with a significantly enhanced rate of reduction with *LB*-hicDH compared with *SE*-LDH (for example, reduction of 1 mmol of (*S*)-4-(Cbz-amino)-2-oxopentanoic acid **8** took just 4 hours with the H205Q mutant compared with 4 days with *SE*-LDH) has exciting potential in organic synthesis. Thus the use of *LB*-hicDH as well as *BS*-LDH was investigated in the studies reported herein.



Scheme 1 For example: R = Me, Et, Pr, cyclopropyl, benzyl, *p*NO_2-PhCH_2, ClCH_2, H_2C=CHCH_2CH_2, CH_3CH=CH.



Scheme 2

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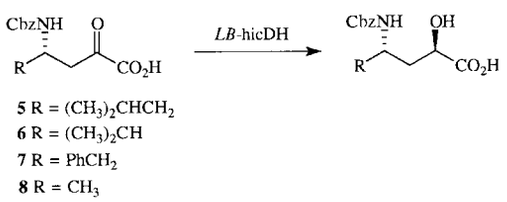
Results and discussion

Our first target compounds were 2-oxo esters **17** and **18** with a methoxymethyl ether at either C-3 or C-4 (Scheme 4). The methoxymethyl protecting group for the alcohols was selected as it is stable to a range of conditions, in particular to the basic conditions for the hydrolysis of the 2-oxo ester to the corresponding 2-oxo acid required as an enzyme substrate, but the group may be removed under mild conditions.

There has been considerable interest in the synthesis of 4-hydroxy-2-keto acids which occur in a variety of important natural products including *N*-acetylneuramic acid[9] and 3-deoxy-D-*manno*-oct-2-ulosonic acid (KDO).[10] Several methods have been developed for the construction of 4-hydroxy-2-oxo esters and derivatives thereof[11] and we favoured a general approach that could be used for the synthesis of both 3- and 4-hydroxy-2-oxo esters as shown in Scheme 4. Treatment of methyl (*R*)-lactate **9** with chloromethyl methyl ether and diisopropylethylamine followed by hydrolysis of the ester **11** with lithium hydroxide gave acid **13**. Many methods are known for the conversion of carboxylic acids to 2-oxo esters.[12] However, we required a strategy which would maintain the stereochemical integrity of the molecule and we favoured a method involving ozonolysis of β-ketocyanophosphoranes originally described by Wasserman and Ho.[13] Treatment of the carboxylic acid **13** with (cyanomethylene)triphenylphosphorane in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) and 4-dimethylaminopyridine (DMAP) followed by ozonolysis of **15** in methanol and dichloromethane gave the required 2-oxo ester (*R*)-**17**. Methyl (*R*)-4-methoxymethoxy-2-oxopentanoate (*R*)-**18** was prepared from ethyl (*R*)-3-hydroxybutanoate **10** using an analogous approach.



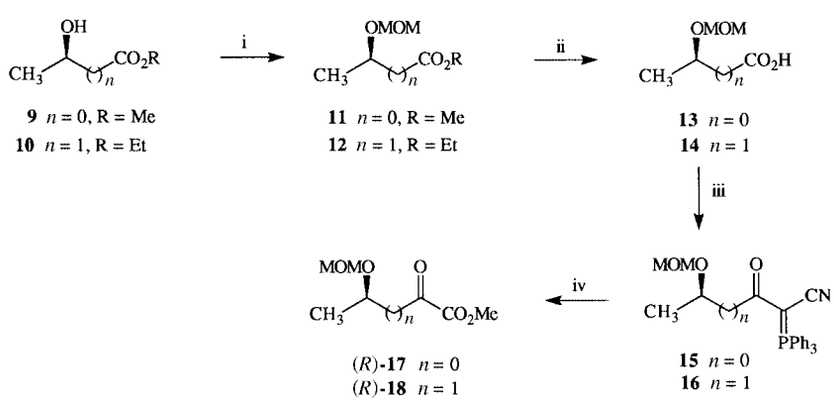
5 R = $(CH_3)_2CHCH_2$
6 R = $(CH_3)_2CH$
7 R = $PhCH_2$
8 R = CH_3

Scheme 3

It was then necessary to hydrolyse the 2-oxo esters (*R*)-**17** and (*R*)-**18** to the corresponding 2-oxo acids prior to the key oxidoreductase catalysed reaction. This may be achieved by saponification with sodium hydroxide,[14] but a much more convenient method proved to be a dual enzyme procedure to hydrolyse the ester and reduce the ketone in a one-pot process. Both *BS*-LDH and *LB*-hicDH require the co-factor NADH which may be recycled using a formate–formate dehydrogenase (FDH) protocol developed by Shaked and Whitesides[15] which allows the reactions to be performed economically on a multigram scale. In a typical procedure the 2-oxo ester was incubated with a lipase from *Candida rugosa* prior to the addition of the dehydrogenase (either *BS*-LDH or *LB*-hicDH), FDH and the requisite co-factors (Scheme 5). After work-up the products were methylated with diazomethane prior to purification by flash chromatography.

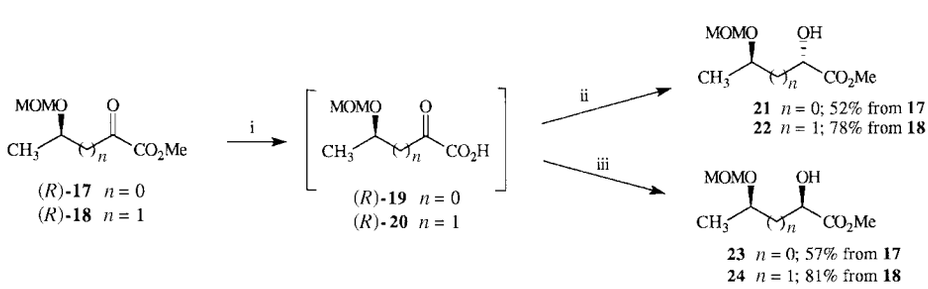
Hydrolysis of (*R*)-**17** followed by reduction of the resultant 2-oxo acid (*R*)-**19** catalysed by either *BS*-LDH or *LB*-hicDH gave, after methylation, the (2*S*,3*R*)- and (2*R*,3*R*)-hydroxy esters **21** and **23** respectively in good yields over the 3 steps and each as a single diastereomer (Scheme 5). No racemisation was apparent at C-3. Similarly, hydrolysis of methyl (*R*)-4-methoxymethoxy-2-oxopentanoate (*R*)-**18** and reduction of (*R*)-**20** catalysed by either *BS*-LDH or *LB*-hicDH gave, after methylation, good yields of (2*S*,4*R*)- and (2*R*,4*R*)-hydroxy esters **22** and **24** respectively. Hence it is apparent that 2-oxo acids with a single MOM substituent at either C-3 or C-4 are efficiently reduced by the oxidoreductases to the corresponding 2-hydroxy acid with complete stereocontrol.

We have previously found that incubation of racemic 3-methoxymethoxy-2-oxobutanoic acid with leucine dehydrogenase led not only to reductive amination of the ketone but also to a kinetic resolution.[16] To investigate whether a similar difference in reactivity of (*R*)- and (*S*)-**19** with the oxidoreductases *BS*-LDH and *LB*-hicDH was apparent, methyl (*S*)-3-methoxymethoxy-2-oxobutanoate (*S*)-**17** was prepared using the same approach as for the (*R*)-enantiomer. Lipase catalysed hydrolysis of the ester and reduction of the resultant 2-oxo acid catalysed by either *BS*-LDH or *LB*-hicDH gave, after methylation, the (2*S*,3*S*)- and (2*R*,3*S*)-diastereomers **25** and **26** respectively in good yields (Scheme 6). Hence, unlike leucine



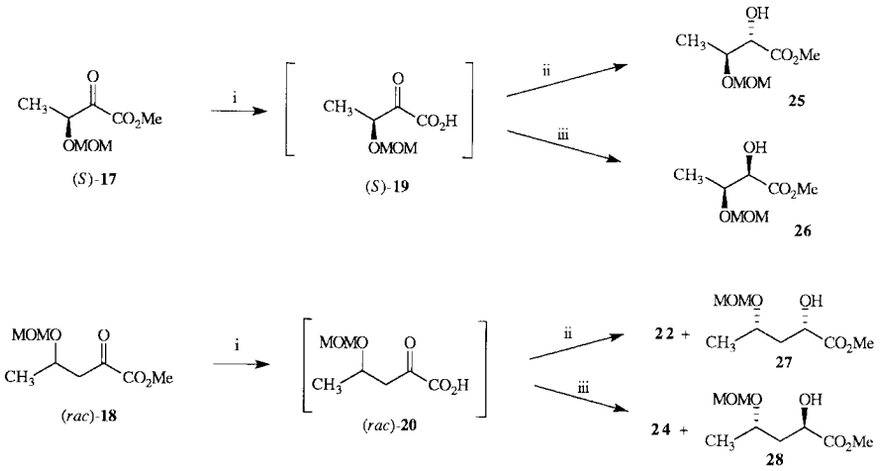
9 *n* = 0, R = Me
10 *n* = 1, R = Et
11 *n* = 0, R = Me
12 *n* = 1, R = Et
13 *n* = 0
14 *n* = 1
15 *n* = 0
16 *n* = 1
(*R*)-**17** *n* = 0
(*R*)-**18** *n* = 1

Scheme 4 *Reagents*: i, CH_3OCH_2Cl, either $^iPr_2EtN$ or NaH; ii, LiOH; iii, Ph_3PCHCN, EDCI, DMAP; iv, O_3, MeOH, CH_2Cl_2.

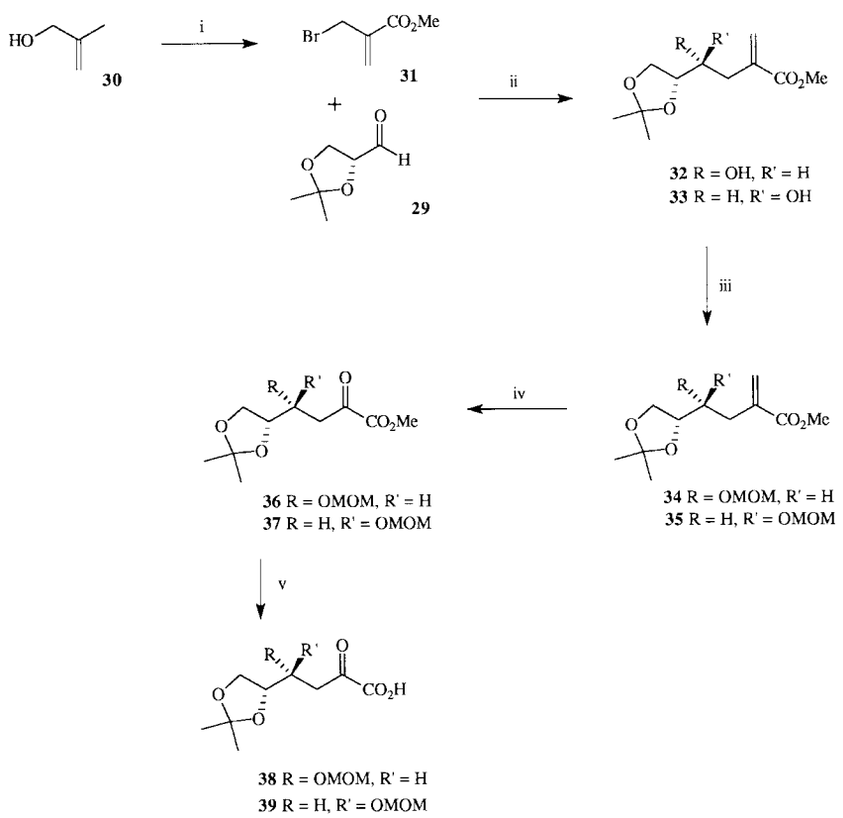


(*R*)-**17** *n* = 0
(*R*)-**18** *n* = 1
(*R*)-**19** *n* = 0
(*R*)-**20** *n* = 1
21 *n* = 0; 52% from **17**
22 *n* = 1; 78% from **18**
23 *n* = 0; 57% from **17**
24 *n* = 1; 81% from **18**

Scheme 5 *Reagents*: i, *Candida rugosa* lipase; ii, a) *BS*-LDH, NADH, FDH; b) CH_2N_2; iii, a) *LB*-hicDH, NADH, FDH; b) CH_2N_2.



Scheme 6 *Reagents*: i, *Candida rugosa* lipase; ii, a) *BS*-LDH, NADH, FDH; b) CH_2N_2; iii, a) *LB*-hicDH, NADH, FDH; b) CH_2N_2.

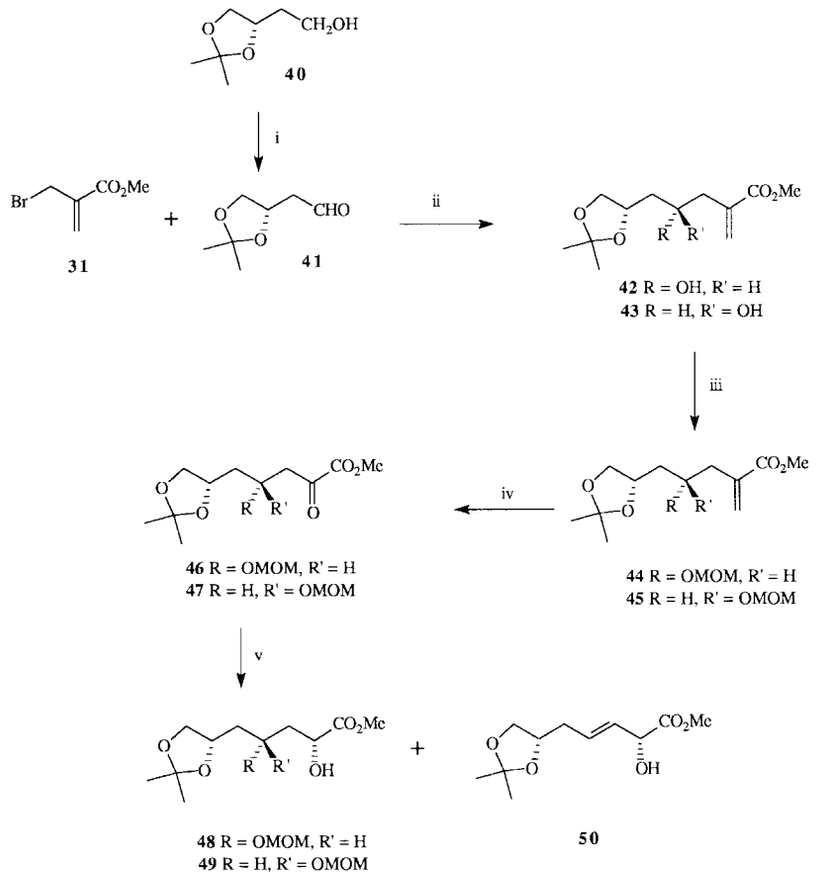


Scheme 7 *Reagents*: i, PBr_3, ether; ii, Zn, NH_4Cl, THF, H_2O; iii, MOMCl, EtN^iPr_2, CH_2Cl_2; iv, O_3, CH_2Cl_2; v, aq. LiOH.

dehydrogenase, *BS*-LDH and *LB*-hicDH showed no obvious difference in rate of reduction of the two enantiomers of **19**.

Racemic methyl 4-methoxymethoxy-2-oxopentanoate (*rac*)-**18** was also prepared and subjected to the dual enzyme catalysed hydrolysis and reduction procedure. The *BS*-LDH and *LB*-hicDH catalysed reductions of (*rac*)-**20** both gave a 1 : 1 mixture of the expected diastereomers as shown in Scheme 6 indicating that there was no significant difference in the rate of reduction of the two enantiomers of 2-oxo acid **20**. The mixture of (2*S*,4*R*)- and (2*S*,4*S*)-hydroxy esters **22** and **27** was treated with hydrochloric acid in THF giving the corresponding 2-hydroxy-4-methyl-γ-lactones **4** and **3**, which were readily separated by flash chromatography thus giving an alternative approach to these enantiopure γ-lactones to that outlined in Scheme 2.

Our next goal was to prepare protected 4,5,6-trihydroxy-2-oxohexanoic acids required not only to examine the substrate specificities of the oxidoreductases but also to give access to 2-hydroxy acids which would be valuable building blocks for use in synthesis, for example, as a precursor to the marine natural product leptosphaerin.[17] The approach selected for the preparation of the required 2-oxo esters involved a Reformatsky type coupling[18] between (*R*)-2,3-*O*-isopropylideneglyceraldehyde **29** and the allylic bromide **31** (Scheme 7). Allylic alcohol **30** was simply prepared in 70% yield *via* the Baylis–Hillman reaction of methyl acrylate with paraformaldehyde in the presence of DABCO following a literature procedure.[19] Treatment of alcohol **30** with phosphorus tribromide gave the required bromide **31** in 84% yield. The zinc mediated coupling[20] of aldehyde **29** (prepared in two steps following a literature procedure[21]) with allylic bromide **31** gave a 2.5 : 1 mixture of alcohols **32** and **33** in 78% yield. Recently a similar mixture of these alcohols has been obtained by reaction of an allylboronate with aldehyde **29**.[22] The effects of other metals on the outcome of the coupling of allylic bromide **31** and aldehyde **29** were examined. We found that chromium chloride in THF[23]



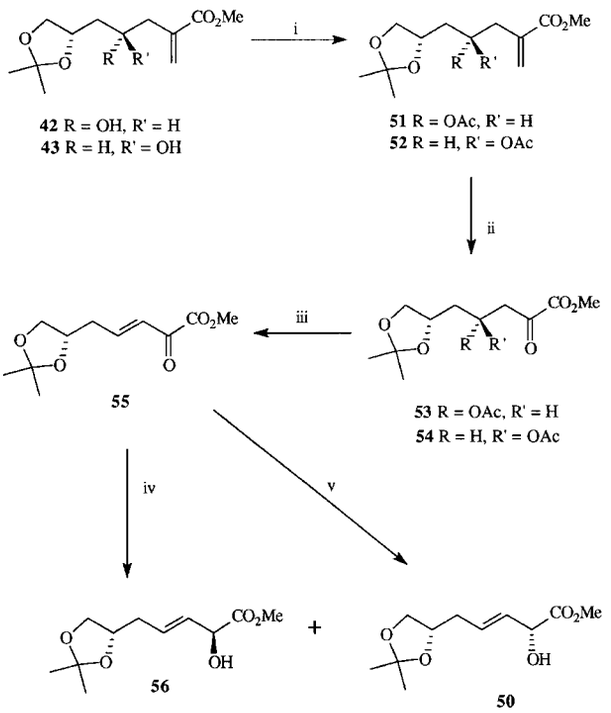
Scheme 8 *Reagents*: i, TPAP; ii, Zn, NH_4Cl, THF, H_2O; iii, MOMCl, EtN^iPr_2, CH_2Cl_2; iv, O_3, CH_2Cl_2; v, *Candida rugosa* lipase, *LB*-hicDH, FDH, NADH then CH_2N_2.

gave a 51% yield of **32** and **33**, whilst the use of indium in water[24] led to a 60% yield of the two alcohols. In each case a similar mixture of diastereomers was obtained.

Although we found that **32** and **33** could be separated by column chromatography, partial decomposition occurred on the silica. Hence, since we have already shown that both enantiomers of the MOM protected 4-hydroxy-2-oxo acid **20** are good substrates for both *BS*-LDH and *LB*-hicDH, it was decided at this stage to complete the synthesis of the required 2-oxo esters **36** and **37** with the mixture of diastereomers and to test them both as substrates for the oxidoreductases. The mixture of alcohols **32** and **33** were readily protected as the MOM ethers and then ozonolysis of **34** and **35** in dichloromethane gave a mixture of required 2-oxo esters **36** and **37** which were inseparable by flash chromatography. The mixture of 2-oxo esters was then subjected to the one-pot, dual enzyme-catalysed hydrolysis–reduction procedure, but with both *BS*-LDH and *LB*-hicDH no products were isolated from the reaction mixture and it was apparent that decomposition had occurred. It was not clear whether decomposition was occurring during the hydrolysis and/or the reduction. Thus the mixture of **36** and **37** was hydrolysed with aqueous lithium hydroxide to the corresponding 2-oxo acids **38** and **39** prior to incubation with the oxidoreductases. However, decomposition occurred again with both *BS*-LDH and *LB*-hicDH and no products were isolated from the reaction mixture.

The next two targets were 2-oxo esters **46** and **47** which contained 3-oxygenated substituents in the side-chain at the 4-, 6- and 7-positions, *i.e.* these compounds contain an extra methylene between the MOM ether and the protected diol compared with **36** and **37**. These were also prepared *via* a Reformatsky type coupling. Aldehyde **41** was prepared from the known alcohol **40**[25] (synthesised in 82% overall yield by diborane reduction of (*S*)-malic acid, followed by protection of the vicinal diol with acetone under acidic conditions). Oxidation of **40** under Swern conditions[26] gave a 70% yield of the known[27] aldehyde **41** whereas a quantitative yield was obtained with tetrapropylammonium perruthenate (TPAP)[28] (Scheme 8). Treatment of aldehyde **41** with allylic bromide **31** in the presence of zinc gave a 1 : 1 mixture of alcohols **42** and **43**. These were protected as their MOM ethers **44** and **45**, and oxidative cleavage of the double bond gave a mixture of the required 2-oxo esters. The mixture of **46** and **47** was then subjected to the one-pot dual enzyme catalysed hydrolysis–reduction procedure using *LB*-hicDH giving, after methylation and purification by column chromatography, a 1 : 1 mixture of the expected hydroxy esters **48** and **49** in just 14% yield and 4% of the β,γ-unsaturated-α-hydroxy ester **50**. Therefore, in contrast to the dual enzyme catalysed hydrolysis–reduction of the protected 4,5,6-trihydroxy-2-oxo esters **36** and **37**, which gave no isolable products, the protected 4,6,7-trihydroxy-2-oxo esters **46** and **47** gave some of the expected 2-hydroxy esters **48** and **49** as well as the unsaturated alcohol **50**, albeit in low yield.

Iodolactonisation of simple β,γ-unsaturated-α-hydroxy esters such as methyl (2*R*,3*E*)-2-hydroxypent-3-enoate have been shown to proceed with excellent stereocontrol,[29] thus unsaturated 2-hydroxy esters such as **50** have potential for the enantioselective synthesis of polyhydroxylated compounds. With this in mind the final target substrate for the biotransformations was the unsaturated 2-oxo ester **55** which was prepared from the mixture of alcohols **42** and **43** (Scheme 9). Acetylation of the alcohols, followed by ozonolysis of the resultant acetates **51** and **52** and elimination gave **55** in 90% overall yield. The one-pot lipase catalysed hydrolysis of the ester and reduction of the ketone using *BS*-LDH gave, after methylation and purification by flash chromatography, (2*S*)-hydroxy ester **56** in 45% yield over the 3 steps. When the reduction was carried out with *LB*-hicDH, (2*R*)-hydroxy ester **50** was isolated in 55% yield.



Scheme 9 *Reagents*: i, Ac_2O, py; ii, O_3, CH_2Cl_2; iii, SiO_2; iv, *Candida rugosa* lipase, *BS*-LDH, FDH, NADH then CH_2N_2; v, *Candida rugosa* lipase, *LB*-hicDH, FDH, NADH then CH_2N_2.

In conclusion, a series of novel 2-oxo esters with protected alcohols at the 3-, 4-, 5-, 6- and 7- positions have been prepared either *via* a one-carbon homologation of the precursor acid or *via* coupling of an aldehyde with an organometallic (Zn, In or Cr) reagent. Although the simple keto acids with a single substituent at C-3 and C-4 (*e.g.* **19** and **20**) were good substrates for the oxidoreductases, giving high yields of enantiopure protected 2,3- and 2,4-dihydroxy acids, in the cases of the more complex trisubstituted keto acids significant decomposition occurred under the reaction conditions. The use of the biotransformations has proved valuable for the enantioselective synthesis of the protected 2,6,7-trihydroxyhept-3-enoates **50** and **56**.

Experimental

General details

General experimental details have been reported.[30] All NMR spectra were run in deuteriochloroform with tetramethylsilane as the internal reference unless otherwise stated. Chemical shifts are reported in ppm on the δ scale. Coupling constants are quoted in Hz. Optical rotations were measured at 25 °C except where indicated otherwise. The enzymes were purchased and stored as follows. Lipase from *Candida rugosa* (CRL), Sigma, stored at 4 °C as a 1000 eU ml^{-1} solution in Tris buffer (5 mM); formate dehydrogenase (FDH) from *Candida boidinii*, Boerhinger, stored at 4 °C; lactate dehydrogenase from *Bacillus stearothermophilis*, Sigma, stored at −20 °C; β-nicotinamide adenine dinucleotide hydride (NADH), Genzyme, stored at −20 °C.

Ethyl (*R*)-3-methoxymethoxybutanoate 12

Ethyl (*R*)-3-hydroxybutanoate **10** (3.0 g, 23 mmol) was added to a solution of *N*-ethyldiisopropylamine (5.2 cm^3, 30 mmol) in dichloromethane (100 cm^3) at 0 °C under a nitrogen atmosphere. After 0.5 h, chloromethyl methyl ether (2.62 cm^3, 34.5 mmol) was added. The reaction mixture was warmed to room temperature and left stirring overnight. Water (80 cm^3) was then added and the reaction mixture acidified to pH 2 by the addition of 2 M hydrochloric acid (15 cm^3). The two layers were separated and the aqueous layer was washed with dichloromethane (40 cm^3). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 15% ethyl acetate in light petroleum gave *ethyl (R)-3-methoxymethoxybutanoate* **12** as a colourless oil (3.43 g, 86%). $[\alpha]_D$ −11.7 (*c* 0.7, $CHCl_3$); ν_{max}(film)/cm^{-1} 2980, 2935 and 1736 (CO); δ_H(400 MHz) 1.25 (3H, d, *J* 6, 4-H_3), 1.27 (3H, t, *J* 7, $CO_2CH_2CH_3$), 2.41 (1H, dd, *J* 15 and 5, 2-H*H*), 2.59 (1H, dd, *J* 15 and 8, 2-*H*H), 3.36 (3H, s, OCH_3), 4.14 (3H, m, 3-H and CO_2CH_2), 4.65 (1H, d, *J* 7, OC*H*HO) and 4.68 (1H, d, *J* 7, OCH*H*O); *m*/*z* (CI) 145.0867 ($[MH]^+$ − MeOH, $C_8H_{16}O_4$ requires 145.0865, 80%), 130 (10), 115 (49), 102 (14), 86 (61) and 84 (100).

Methyl (*R*)-2-methoxymethoxypropionate 11

Methyl (*R*)-lactate **9** (5.0 g, 48 mmol) was slowly added to a solution of sodium hydride (46 mmol) in THF (70 cm^3) at 0 °C under a nitrogen atmosphere. Chloromethyl methyl ether (4.52 cm^3, 60 mmol) was added dropwise and, after 0.75 h, the reaction was warmed to room temperature. After 7 h, the reaction mixture was concentrated, acidified using 2 M HCl (20 cm^3) and extracted with ethyl acetate. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* and then purified by column chromatography giving *methyl (R)-2-methoxymethoxypropionate* **11** as a colourless oil (6.76 g, 95%). $[\alpha]_D^{24}$ +85.5 (*c* 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 1753 (CO); δ_H(270 MHz) 1.43 (3H, d, *J* 7, 3-H_3), 3.39 (3H, s, OCH_3), 3.75 (3H, s, CO_2CH_3), 4.24 (1H, q, *J* 7, 2-H), 4.68 (1H, d, *J* 7, OC*H*HO) and 4.71 (1H, d, *J* 7, OCH*H*O); *m*/*z* (CI) 117.0549 ($[MH]^+$ − MeOH, $C_6H_{12}O_4$ requires 117.0551, 4%), 117 (100), 89 (39) and 84 (32).

The above reaction was repeated using ethyl (*S*)-lactate (5.0 g, 42 mmol), giving ethyl (*S*)-2-methoxymethoxypropionate as a colourless oil (6.52 g, 95%). $[\alpha]_D^{23}$ −92.3 (*c* 1.0, $CHCl_3$) (lit.,[27] $[\alpha]_D^{22}$ −88.1 (*c* 2.9, $CHCl_3$)); δ_H(400 MHz) 1.29 (3H, t, *J* 7, OCH_2CH_3), 1.43 (3H, d, *J* 7, 3-H_3), 3.39 (3H, s, OCH_3), 4.21 (2H, q, *J* 7, CO_2CH_2), 4.24 (1H, q, *J* 7, 2-H), 4.69 (1H, d, *J* 7, OC*H*HO) and 4.72 (1H, d, *J* 7, OCH*H*O); *m*/*z* (CI) 131 ($[MH]^+$ − MeOH, 86%), 117 (20), 103 (42), and 84 (100).

(*R*)-3-Methoxymethoxybutanoic acid 14

Ethyl (*R*)-3-methoxymethoxybutanoate **12** (3.43 g, 19 mmol) was dissolved in methanol (20 cm^3) and cooled to 0 °C. Lithium hydroxide monohydrate (1.06 g, 24.7 mmol) in water (10 cm^3) was added and the reaction mixture warmed to room temperature. After 7 h, the reaction mixture was concentrated, acidified to pH 2 by the addition of 2 M hydrochloric acid (15 cm^3) and extracted with ethyl acetate (2 × 30 cm^3). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give *(R)-3-methoxymethoxybutanoic acid* **14** as a colourless oil (2.39 g, 83%). $[\alpha]_D$ −18.9 (*c* 0.5, $CHCl_3$); ν_{max}(film)/cm^{-1} 3451 (OH) and 1719 (CO); δ_H(270 MHz) 1.27 (3H, d, *J* 6, 4-H_3), 2.48 (1H, dd, *J* 15 and 5, 2-*H*H), 2.63 (1H, dd, *J* 15 and 8, 2-H*H*), 3.36 (3H, s, OCH_3), 4.16 (1H, m, 3-H), 4.68 (1H, d, *J* 7, OC*H*HO) and 4.70 (1H, d, *J* 7, OCH*H*O); *m*/*z* (CI) 117.0551 ($[MH]^+$ − MeOH, $C_6H_{12}O_4$ requires 117.0552, 36%), 107 (21), 91 (9), 87 (59) and 83 (100).

(*R*)-2-Methoxymethoxypropanoic acid 13

The above reaction was repeated using methyl (*R*)-2-methoxymethoxypropionate **11** (6.28 g, 42 mmol). Purification by flash column chromatography, eluting with 49% ethyl acetate in light petroleum gave *(R)-2-methoxymethoxypropanoic acid* **13** as a colourless oil (3.69 g, 65%). $[\alpha]_D^{25}$ +81.5 (*c* 1.6, $CHCl_3$); ν_{max}(film)/cm^{-1} 3485 (OH) and 1736 (CO); δ_H(270 MHz) 1.49 (3H, d, *J* 7, 3-H_3), 3.41 (3H, s, OCH_3), 4.28 (1H, q, *J* 7, 2-H), 4.72 (1H, d, *J* 7, OC*H*HO) and 4.75 (1H, d, *J* 7, OCH*H*O); *m*/*z* 135.0653 ($[MH]^+$, $C_5H_{10}O_4$ requires 135.0657, 12%), 117 (7), 103 (76), 89 (16), 84 (32) and 75 (100).

The above reaction was repeated using ethyl (*S*)-2-methoxymethoxypropionate (6.8 g, 42 mmol) giving *(S)-2-methoxymethoxypropanoic acid* as a colourless oil (3.82 g, 68%). $[\alpha]_D^{24}$ −83.0 (*c* 1.4, $CHCl_3$); spectroscopic data as for the (*R*)-enantiomer.

Methyl (*R*)-4-methoxymethoxy-2-oxopentanoate (*R*)-18

(Cyanomethylene)triphenylphosphonium chloride (8.05 g, 24 mmol) was dissolved in water (50 cm^3) and dichloromethane (50 cm^3). Sodium hydroxide (2.43 g, 61 mmol) in water (10 cm^3) was slowly added. After 0.3 h, the two layers were separated. The dichloromethane layer was dried ($MgSO_4$) and added to a solution of (*R*)-3-methoxymethoxybutanoic acid **14** (2.0 g, 13.5 mmol), DMAP (0.2 g) and EDCI (4.12 g, 22 mmol) in dichloromethane (100 cm^3) at 0 °C under a nitrogen atmosphere. After 0.5 h, the reaction mixture was warmed to room temperature and left stirring for 15 h. Water (100 cm^3) was added and the two layers separated. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 61% ethyl acetate in light petroleum gave a mixture of (*R*)-5-methoxymethoxy-3-oxo-2-(triphenylphosphoranylidene)hexanenitrile **16** and triphenylphosphine oxide.

The mixture of **16** and triphenylphosphine oxide from the previous reaction was dissolved in dichloromethane (100 cm^3) and methanol (100 cm^3) and cooled to −78 °C. Ozone was then bubbled through the solution until the reaction mixture turned blue. Nitrogen was then passed through the solution to remove excess ozone. The reaction mixture was warmed to room temperature and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 41% ethyl acetate in light petroleum gave *methyl (R)-4-methoxymethoxy-2-oxopentanoate* (*R*)-**18** as a colourless oil (1.20 g, 47% over two steps). $[\alpha]_D$ −17.3 (*c* 0.5, $CHCl_3$); ν_{max}(film)/cm^{-1} 1736 (br, 2 × CO); δ_H(270 MHz) 1.27 (3H, d, *J* 6, 5-H_3), 2.81 (1H, dd, *J* 17 and 5, 3-*H*H), 3.20 (1H, dd, *J* 17 and 8, 3-H*H*), 3.32 (3H, s, OCH_3), 3.89 (3H, s, CO_2CH_3), 4.26 (1H, dqd, *J* 8, 6 and 5, 4-H), 4.61 (1H, d, *J* 7, OC*H*HO) and 4.65 (1H, d, *J* 7, OCH*H*O); δ_C(75 MHz) 20.8 (C-5), 46.6 (C-3), 52.9 and 55.5 (each OCH_3), 69.6 (C-4), 95.5 (OCH_2O), 161.4 (C-1) and 192.0 (C-2); *m/z* (CI) 159.0662 ($[MH]^+$ − MeOH, $C_8H_{14}O_5$ requires 159.0657, 36%), 129 (100), 115 (21), 101 (14), 89 (11) and 84 (14).

Methyl (*R*)-3-methoxymethoxy-2-oxobutanoate (*R*)-17

The above reaction was repeated using (*R*)-2-methoxymethoxypropanoic acid **13** (1.82 g, 14 mmol). Purification by flash column chromatography, eluting with 40% ethyl acetate in light petroleum gave *methyl (R)-3-methoxymethoxy-2-oxobutanoate* (*R*)-**17** as a colourless oil (0.83 g, 35% over two steps). $[\alpha]_D^{24}$ +9.8 (*c* 1.2, $CHCl_3$); ν_{max}(film)/cm^{-1} 1746 (br, 2 × CO); δ_H(270 MHz) 1.44 (3H, d, *J* 7, 4-H_3), 3.34 (3H, s, OCH_3), 3.89 (3H, s, CO_2CH_3), 4.66 (1H, d, *J* 7, OC*H*HO), 4.71 (1H, d, *J* 7, OCH*H*O) and 4.73 (1H, q, *J* 7, 3-H); δ_C(100.5 MHz) 16.8 (C-4), 52.9 and 55.6 (each OCH_3), 74.9 (C-3), 96.4 (OCH_2O), 161.9 (C-1) and 193.7 (C-2); *m/z* (CI) 145.0504 ($[MH]^+$ − MeOH, $C_7H_{12}O_5$ requires 145.0501, 100%), 129 (13), 117 (45) and 84 (96).

The above reaction was repeated using (*S*)-2-methoxymethoxypropanoic acid (3.8 g, 28 mmol). Purification by flash chromatography, eluting with 40% ethyl acetate in light petroleum gave *methyl* (*S*)*-3-methoxymethoxy-2-oxobutanoate* as a colourless oil (1.80 g, 36% over two steps). $[\alpha]_D^{24}$ −10.5 (*c* 0.5, $CHCl_3$); spectroscopic data as for the (*R*)-enantiomer.

Methyl (2*S*,4*R*)-2-hydroxy-4-methoxymethoxypentanoate 22

Methyl (*R*)-4-methoxymethoxy-2-oxopentanoate (*R*)-**18** (0.19 g, 1 mmol) was dissolved in 5 mM Tris buffer (40 cm^3). *Candida rugosa* lipase (10000 eU) was added and the pH adjusted to 7.5 by the addition of 1.0 M hydrochloric acid. The pH was maintained at a value between 7.0 and 7.5 by the addition of 0.1 M sodium hydroxide. The reaction was complete after 3 days. The solution was deoxygenated by bubbling a stream of nitrogen through for 1 h. Dithiotreitol (20 μl) was then added, followed by *BS*-LDH (10 mg), formate dehydrogenase (10 mg), sodium formate (1 g, 15 eq.) and NADH (10 mg). The reaction was left stirring under a nitrogen atmosphere with the pH kept constant at approximately 6.1 by the addition of 1.0 M hydrochloric acid. The reaction was complete after 2 days. The reaction mixture was acidified to pH 2 by the addition of 2 M hydrochloric acid and extracted with ethyl acetate (3 × 40 cm^3). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The resulting 2-hydroxy acid was dissolved in acetone (15 cm^3) and 0.5–0.6 M ethereal diazomethane (2.5 cm^3) was slowly added turning the solution a bright yellow colour. The solution was then concentrated *in vacuo*. Purification by flash column chromatography, eluting with 41% ethyl acetate in light petroleum gave *methyl (2S,4R)-2-hydroxy-4-methoxymethoxypentanoate* **22** as a viscous oil (0.15 g, 78%). $[\alpha]_D^{24}$ −20.6 (*c* 0.1, $CHCl_3$); ν_{max}(film)/cm^{-1} 3425 (OH) and 1732 (CO); δ_H(270 MHz) 1.23 (3H, d, *J* 6, 5-H_3), 1.71 (1H, ddd, *J* 14, 10 and 3, 3-*H*H), 1.99 (1H, ddd, *J* 14, 10 and 3, 3-H*H*), 3.40 (3H, s, OCH_3), 3.79 (3H, s, CO_2CH_3), 3.98 (1H, m, 4-H), 4.42 (1H, dd, *J* 10 and 3, 2-H), 4.66 (1H, d, *J* 7, OC*H*HO) and 4.73 (1H, d, *J* 7, OCH*H*O); *m/z* (CI) 161.0820 ($[MH]^+$ − MeOH, $C_8H_{16}O_5$ requires 161.0814, 5%), 141 (4), 131 (100), 113 (29), 101 (28) and 84 (91).

Methyl (2*R*,4*R*)-2-hydroxy-4-methoxymethoxypentanoate 24

The above reaction was repeated using *LB*-hicDH (1 cm^3) in place of *BS*-LDH. The reaction was complete after 1 day, giving after purification by flash column chromatography, eluting with 40% ethyl acetate in light petroleum, *methyl (2R,4R)-2-hydroxy-4-methoxymethoxypentanoate* **24** as a viscous oil (0.16 g, 81%). $[\alpha]_D$ −12.3 (*c* 0.1, $CHCl_3$); ν_{max}(film)/cm^{-1} 3435 (OH) and 1736 (CO); δ_H(270 MHz) 1.23 (3H, d, *J* 6, 5-H_3), 1.89–2.06 (2H, m, 3-H_2), 3.37 (3H, s, OCH_3), 3.78 (3H, s, CO_2CH_3), 3.97 (1H, m, 4-H), 4.29 (1H, t, *J* 5, 2-H), 4.59 (1H, d, *J* 7, OC*H*HO) and 4.62 (1H, d, *J* 7, OCH*H*O); *m/z* (CI) 161.0812 ($[MH]^+$ − MeOH, $C_8H_{16}O_5$ requires 161.0814, 5%), 131 (81), 113 (27), 101 (24), 86 (63) and 84 (100).

Methyl (2*S*,3*R*)-2-hydroxy-3-methoxymethoxybutanoate 21

The above reaction was repeated using 2-oxo ester (*R*)-**17** (95 mg, 0.54 mmol) and *BS*-LDH, giving, after purification by flash chromatography, *methyl (2S,3R)-2-hydroxy-3-methoxymethoxybutanoate* **21** (50 mg, 52%) as a colourless oil. $[\alpha]_D$ −20.5 (*c* 1.9, $CHCl_3$); ν_{max}(film)/cm^{-1} 3435 (br, OH), 2930 (CH) and 1735 (CO); δ_H(300 MHz) 1.24 (3H, d, *J* 6.6, 4-H_3), 3.07 (1H, br s, OH), 3.41 (3H, s, OMe), 3.81 (3H, s, CO_2CH_3), 4.00 (1H, qd, *J* 6.6 and 2.8, 3-H), 4.28 (1H, d, *J* 2.8, 2-H), 4.71 (2H, s, OCH_2O); δ_C(75 MHz) 15.8 (C-4), 52.4 and 55.8 (each OCH_3), 73.9 and 76.3 (C-2 and C-3), 96.1 (OCH_2O), 172.7 (C-1); *m/z* (CI) 147.0649 ($[MH]^+$ − MeOH. $C_6H_{11}O_4$ requires 147.0657, 46%), 117 (61), 87 (98), 85 (66) and 57 (100).

The above reaction was repeated using the enantiomeric 2-oxo ester (*S*)-**17** (105 mg, 0.60 mmol) and *LB*-hicDH, giving, after purification by flash chromatography, *methyl (2R,3S)-2-hydroxy-3-methoxymethoxybutanoate* **26** (60 mg, 57%) as a colourless oil. $[\alpha]_D$ +22.8 (*c* 0.8, $CHCl_3$); spectroscopic data as above.

Methyl (2*R*,3*R*)-2-hydroxy-3-methoxymethoxybutanoate 23

The above reaction was repeated with 2-oxo ester (*R*)-**17** (95 mg, 0.54 mmol) and *LB*-hicDH giving, after purification by column chromatography, *methyl (2R,3R)-2-hydroxy-3-methoxymethoxybutanoate* **23** (55 mg, 57%) as a colourless oil. $[\alpha]_D$ −36.8 (*c* 2.4, $CHCl_3$); ν_{max}(film)/cm^{-1} 3435 (br, OH), 2930

(CH), 1735 (CO); δ_H(300 MHz), 1.32 (3H, d, *J* 6.3, 4-H_3), 2.75 (1H, br s, OH), 3.32 (3H, s, OCH_3), 3.81 (3H, s, CO_2CH_3), 4.05–4.17 (2H, m, 2-H and 3-H), 4.57 (1H, d, *J* 7, OC*H*HO), 4.67 (1H, d, *J* 7, OCH*H*O); δ_C(75 MHz), 16.3 (C-4), 52.4 and 55.6 (each OCH_3), 73.8, 74.3 (C-2 and C-3), 94.9 (OCH_2O) and 173.3 (C-1); *m*/*z* (CI) 147.0655 ($[MH]^+ - MeOH$, $C_6H_{11}O_4$ requires 147.0657, 47%), 117 (53), 87 (82), 85 (55) and 57 (100).

The above reaction was repeated using the enantiomeric 2-oxo ester (*S*)-**17** (105 mg, 0.60 mmol) and *BS*-LDH giving after purification by flash chromatography *methyl (2S,3S)-2-hydroxy-3-methoxymethoxybutanoate* **25** (55 mg, 52%) as a colourless oil. $[a]_D$ +35.0 (*c* 1.0, $CHCl_3$); spectroscopic data as for **23**.

Lactonisation of a 1 : 1 mixture of hydroxy esters 22 and 27

2 M Hydrochloric acid (2.5 cm^3, 5.0 mmol) was added to a solution of a 1 : 1 mixture of the diastereomeric alcohols **22** and **27** (0.10 g, 0.52 mmol) in tetrahydrofuran (5 cm^3). The mixture was stirred for 16 h. The reaction mixture was concentrated and extracted with ethyl acetate (3 × 10 cm^3). The organic extracts were combined and evaporated. The crude reaction mixture was purified by flash column chromatography eluting with 40% ethyl acetate in light petroleum to give (3*S*,5*S*)-3-hydroxy-5-methyl-1-oxacyclopentan-2-one **3** as a clear oil (22 mg, 36%). $[a]_D$ −51.4 (*c* 0.1, MeOH) (lit.[31] $[a]_D$ −59.8 (*c* 1.0, MeOH)); δ_H(300 MHz) 1.42 (3H, d, *J* 6.6, CH_3), 1.89 (1H, br s, OH), 2.25 (1H, ddd, *J* 13.0, 8.0 and 4.0, 4-*H*H), 2.38 (1H, dt, *J* 13.0 and 8.0, 4-H*H*), 4.54 (1H, t, *J* 8.0, 3-H) and 4.81 (1H, dqd, *J* 8.0, 6.5 and 4.0, 5-H); *m*/*z* (CI) 117 ($[MH]^+$, 90%), 99 (100) and 71 (59). Further elution afforded (3*S*,5*R*)-3-hydroxy-5-methyl-1-oxacyclopentan-2-one **4** as a clear oil (22 mg, 36%). $[a]_D$ −2.7 (*c* 0.4, MeOH) (lit.[31] $[a]_D$ +3.6 (*c* 1.0, methanol) opposite enantiomer); δ_H(300 MHz) 1.48 (3H, d, *J* 6.1, CH_3), 1.87 (1H, dt, *J* 12.6 and 11.0, 4-*H*H), 2.29 (1H, br s, OH), 2.73 (1H, ddd, *J* 12.6, 8.4 and 5.1, 4-H*H*), 4.46–4.60 (2H, m, 3-H and 5-H); *m*/*z* (CI) 117 ($[MH]^+$, 94%), 99 (100) and 71 (61).

Methyl (5*R*)-4-hydroxy-5,6-(isopropylidenedioxy)-2-methylenehexanoates 32 and 33

Powdered zinc (0.92 g, 14.0 mmol) was added in one portion to a stirred solution of (*R*)-*O*-isopropylideneglyceraldehyde **29** (1.87 g, 14.34 mmol), methyl 2-(bromomethyl)acrylate **31** (1.98 g, 11.07 mmol), saturated aqueous ammonium chloride (12 cm^3) and THF (10 cm^3) at room temperature. After stirring for 18 h the reaction mixture was diluted with brine (10 cm^3), extracted into dichloromethane (3 × 25 cm^3), the combined dichloromethane extracts were washed with brine (10 cm^3), dried ($MgSO_4$) and the solvent evaporated to leave a yellow oil. The crude product was dissolved in a small volume of dichloromethane and passed through a plug of silica eluting with ethyl acetate to give a 2.5 : 1 mixture of diastereomers **32** and **33** as a pale yellow oil (2.0 g, 78%). Major diastereomer: δ_H (300 MHz; $CDCl_3$) 1.35 (3 H, s, acetonide), 1.43 (3 H, s, acetonide), 2.34 (1 H, ddd, *J* 14.3, 8.6 and 0.7, 3-*H*H), 2.71 (1 H, ddd, *J* 14.3, 3.3 and 1.1, 3-H*H*), 3.10 (1 H, br s, OH), 3.77 (3 H, s, CO_2CH_3), 3.80–4.20 (4 H, m, 4-H, 5-H, 6-H_2), 5.75 (1 H, d, *J* 1.1, 2-C*H*H) and 6.28 (1 H, d, *J* 1.4, 2-CH*H*); δ_C(75 MHz) 25.06, 26.40 (each CH_3), 35.94 (C-3), 51.95 (CO_2CH_3), 65.77 (C-6), 70.80, 78.05 (C-4 and C-5), 109.00 (acetonide *C*Me_2), 128.06 (2-CH_2), 136.58 (C-2) and 168.12 (C-1); *m*/*z* (EI) 215.0921 ($M^+ - CH_3$, $C_{10}H_{15}O_5$ requires 215.0919, 31%), 201 (5), 183 (4), 155 (10), 141 (31), 101 (100) and 59 (88).

Methyl (5*R*)-5,6-(isopropylidenedioxy)-4-(methoxymethoxy)-2-oxohexanoates 36 and 37

A solution of a mixture of **32** and **33** (2.0 g, 8.71 mmol) in dichloromethane (3 cm^3) was added in one portion to a stirred solution of diisopropylethylamine (2.0 cm^3, 11.6 mmol) in dichloromethane (4 cm^3) at 0 °C under an atmosphere of nitrogen. The mixture was allowed to warm to room temperature, then chloromethyl methyl ether (0.9 cm^3, 11.6 mmol) was added dropwise over 20 min and stirring continued for a further 8 h. The resulting solution was diluted with dichloromethane (20 cm^3), washed with 1 M hydrochloric acid (10 cm^3) and the washings extracted with dichloromethane (2 × 20 cm^3). The combined organic phase was washed with brine (10 cm^3), dried (Na_2SO_4) and the solvent evaporated to leave a diastereomeric mixture of the methoxymethyl ethers **34** and **35** (2.05 g, 86%) as a yellow oil, which was used in the next reaction without further purification. A small portion was purified by flash chromatography for characterisation. Major, less polar product: $[a]_D^{25}$ 16.0 (*c* 1.32 in $CHCl_3$); ν_{max} (neat)/cm^{-1} 2947 (C-H), 1731 (C=O), 1646 (C=C), 1438 and 1380; δ_H(300 MHz) 1.36 and 1.43 (each 3 H, each s, 2 × CH_3), 2.42 (1 H, dd, *J* 14.0 and 8.5, 3-*H*H), 2.55 (1 H, ddd, *J* 14.0, 4.1 and 0.8, 3-H*H*), 3.33 (3 H, s, OCH_3), 3.79 (3 H, s, CO_2CH_3), 3.75–4.17 (4 H, m, 4-H, 5-H, 6-H_2), 4.61 (1 H, d, *J* 6.9, OC*H*HO), 4.74 (1 H, d, *J* 6.9, OCH*H*O), 5.72 (1 H, d, *J* 0.9, =C*H*H) and 6.25 (1 H, d, *J* 0.9, =CH*H*); δ_C(75 MHz) 25.13 and 26.28 (2 × CH_3), 34.18 (C-3), 51.85 and 55.66 (2 × OCH_3), 65.76 (C-6), 76.32, 77.78 (C-4 and C-5), 96.63 (OCH_2O), 109.29 (acetonide-*C*Me_2), 127.90 (=CH_2), 136.47 (C-2) and 167.23 (C-1). Minor, more polar product: $[a]_D^{25}$ 12.7 (*c* 0.97 in $CHCl_3$); δ_H(300 MHz) 1.35 and 1.42 (each 3 H, each s, 2 × CH_3), 2.41 (1 H, dd, *J* 14.2 and 7.9, 3-*H*H), 2.70 (1 H, ddd, *J* 14.2, 4.2 and 1.1 3-H*H*), 3.32 (3 H, s, OCH_3), 3.76 (3 H, s, CO_2CH_3), 3.81–4.60 (4 H, m, 4-H, 5-H, 6-H_2), 4.61 (1 H, d, *J* 6.9, OC*H*HO), 4.65 (1 H, d, *J* 6.9, OCH*H*O), 5.72 (1 H, d, *J* 0.9, =C*H*H) and 6.24 (1 H, d, *J* 0.9, =CH*H*); δ_C(75 MHz) 25.34, 26.49 (2 × CH_3), 34.43 (C-3), 51.99, 55.88 (2 × OCH_3), 65.94 (C-6), 76.36 and 77.53 (C-4 and C-5), 96.84 (OCH_2O), 109.47 (acetonide-*C*Me_2), 128.13 (=CH_2), 136.75 (C-2) and 167.47 (C-1); *m*/*z* of mixture (CI) 275 (MH^+, 16%), 261 (22), 243 ($M^+ - OMe$, 100), 217 (35), 185 (70) and 113 (78).

Ozone was bubbled through a solution of a mixture of **34** and **35** (5.32 g, 19.54 mmol) in dichloromethane (200 cm^3) at −78 °C for 1 h until the solution turned blue. Nitrogen was then bubbled through to remove excess ozone, then dimethyl sulfide (6 cm^3) was added and stirring continued for 10 min at −78 °C and 0.5 h at room temperature. The solvent was removed *in vacuo* to leave a yellow oil which was purified by column chromatography (light petroleum–ethyl acetate, 3 : 1) to give a mixture of diastereomers **36** and **37** as a colourless oil (3.36 g, 63%). Major product: δ_H(300 MHz) 1.32 and 1.38 (each 3H, each s, 2 × CH_3), 3.13 (2H, d, *J* 5.6, 3-H_2), 3.31 (3H, s, OCH_3), 3.87 (3H, s, CO_2CH_3), 3.85–4.15 (4H, m, 4-H, 5-H, 6-H_2), 4.65 (1H, d, *J* 7, OCH*H*O) and 4.68 (1H, d, *J* 7, OC*H*HO); δ_C(75 MHz) 25.34 and 26.51 (2 × CH_3), 42.26 (C-3), 53.25 and 56.04 (each -OCH_3), 67.21 (C-6), 76.44 and 77.42 (C-4 and C-5), 97.13 (OCH_2O), 110.03 (O*C*Me_2O), 161.35 (C-1) and 191.32 (C-2). Minor diastereomer: δ_H(300 MHz) 1.34, 1.42 (each 3H, each s, 2 × CH_3), 2.95 (1H, dd, *J* 17.0 and 3.4, 3-*H*H), 3.18 (1H, dd, *J* 17.0 and 7.7, 3-H*H*), 3.31 (3H, s, OCH_3), 3.88 (3H, s, CO_2CH_3), 3.86–4.33 (4H, m, 4-H, 5-H, 6-H_2), 4.63 (1H, d, *J* 7, OCH*H*O) and 4.71 (1H, d, *J* 7, OC*H*HO); δ_C(75 MHz) 25.45 and 26.71 (2 × CH_3), 40.84 (C-3), 53.61 and 56.36 (each -OCH_3), 65.76 (C-6), 74.79 and 76.77 (C-4 and C-5), 97.78 (OCH_2O), 110.34 (O*C*Me_2O), 161.64 (C-1) and 191.86 (C-2). For the mixture, *m*/*z* (CI) 277.1298 (MH^+, $C_{12}H_{21}O_7$ requires 277.1287, 5%), 245 (35), 215 (43) and 187 (100).

Coupling aldehyde 41 and allylic bromide 31

Powdered zinc (0.85 g, 13 mmol) was added to a stirred solution of aldehyde **41** (1.44 g, 10 mmol) and bromide **31** (2.31 g, 13 mmol) in saturated aqueous ammonium chloride (15 cm^3) and tetrahydrofuran (15 cm^3). The solution was stirred at room temperature for 8 h. The mixture was diluted with brine (30

cm^3), extracted with dichloromethane (3×50 cm^3), and the combined organic extracts were washed with brine (50 cm^3), dried over sodium sulfate, filtered and concentrated *in vacuo* to give unsaturated alcohols **42** and **43** as a 1:1 diastereomeric mixture by ^1H NMR spectroscopy. Purification by column chromatography eluting with a 30% solution of ethyl acetate in light petroleum effected partial separation of the diastereomers giving the less polar alcohol (0.33 g, 14%); a mixture of alcohols **42** and **43** (0.67 g, 28%) and the more polar product (0.31 g, 13%) were obtained all as pale yellow oils.

Less polar product: $[a]_D^{25}$ +8.8 (*c* 1.4, MeOH); ν_{max}(film)/cm^{-1} 3415 (br, OH), 2930 (CH), 1720 (CO) and 1630 (C=C); δ_H(270 MHz), 1.36 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.70 (2H, m, 5-H_2), 2.51 (2H, m, 3-H_2), 3.29 (1H, br s, OH), 3.57 (1H, dd, *J* 8.0 and 7.3, 7-*H*H), 3.77 (3H, s, CO_2CH_3), 3.98 (1H, m, 4-H), 4.09 (1H, dd, *J* 8.0 and 5.9, 7-H*H*), 4.28 (1H, m, 6-H), 5.70 (1H, dd, *J* 2.6 and 1.1, 2-C*H*H) and 6.26 (1H, d, *J* 1.5, 2-CH*H*); δ_C(75 MHz) 25.61 (CH_3), 26.89 (CH_3), 39.98 and 40.20 (C-3 and C-5), 52.01 (CO_2CH_3), 69.35 (C-4 or C-6), 69.65 (C-7), 76.69 (C-4 or C-6), 109.31 (OCO), 127.94 (2-CH_2), 136.99 (C-2) and 167.85 (C-1); *m*/*z* (EI) 229.1080 ($M^+ - CH_3$, $C_{11}H_{17}O_5$ requires 229.1076, 7%), 197 (100), 155 (12), 137 (58), 129 (12), 119 (11), 109 (12), 101 (24), 97 (72), 91 (21), 87 (22), 81 (26), 67 (52) and 69 (56).

More polar product: $[a]_D^{25}$ −15.0 (*c* 1.2, MeOH); ν_{max}(film)/cm^{-1} 3415 (br, OH), 2930 (CH), 1720 (CO) and 1630 (C=C); δ_H(270 MHz), 1.36 (3H, s, CH_3), 1.41 (3H, s, CH_3), 1.65 (1H, ddd, *J* 14.0, 9.0 and 5.0, 5-*H*H), 1.80 (1H, ddd, *J* 14.0, 7.2 and 3.1, 5-H*H*), 2.43 (1H, ddd, *J* 13.9, 8.0 and 0.9, 3-*H*H), 2.60 (1H, ddd, *J* 13.9, 4.2 and 1.1 3-H*H*), 2.79 (1H, br s, OH), 3.57 (1H, t, *J* 8.0, 7-*H*H), 3.77 (3H, s, CO_2CH_3), 3.98 (1H, m, 4-H), 4.09 (1H, dd, *J* 8.0 and 6.0, 7-H*H*), 4.33 (1H, m, 6-H), 5.69 (1H, d, *J* 1.3, 2-C*H*H) and 6.26 (1H, d, *J* 1.3, 2-CH*H*); δ_C(75 MHz) 25.63 (CH_3), 26.97 (CH_3), 40.04, 40.50 (C-3 and C-5), 52.12 (CO_2CH_3), 67.77 (C-4 or C-6), 69.53 (C-7), 73.68 (C-4 or C-6), 108.69 (OCO), 128.11 (2-CH_2), 137.05 (C-2) and 168.08 (C-1); *m*/*z* (EI) 229.1071 ($M^+ - CH_3$, $C_{11}H_{17}O_5$ requires 229.1076, 11%), 197 (100), 155 (16), 137 (61), 109 (22), 101 (28), 97 (73), 91 (32), 87 (34), 81 (26), 67 (54) and 69 (60).

When the above reaction was repeated with no attempt to separate the diastereomers, **42** and **43** were obtained in 75% yield.

Protection of alcohols 42 and 43 to give 44 and 45

The following reactions were carried out on the individual diastereomers for characterisation purposes only, but because of problems of separating **42** and **43**, the majority of the material was converted to the methoxymethyl ethers **44** and **45** as a mixture of diastereomers.

A solution of the less polar alcohol from the previous experiment (0.32 g, 1.3 mmol) in dichloromethane (0.5 cm^3) was added to a stirred solution of diisopropylethylamine (0.34 cm^3, 1.9 mmol) in dichloromethane (0.7 cm^3) at 0 °C under nitrogen. The solution was stirred for 5 min then allowed to reach room temperature. Chloromethyl methyl ether (0.14 cm^3, 1.9 mmol) was added dropwise over 20 min and the solution was allowed to stir at room temperature overnight. The mixture was diluted with dichloromethane (10 cm^3), washed with 1 M hydrochloric acid (10 cm^3) and the washings were extracted with dichloromethane (3×10 cm^3). The combined organic phases were washed with brine, dried over sodium sulfate and the solvent was removed *in vacuo*. The crude reaction mixture was purified by column chromatography to afford the methoxymethyl ether as a colourless oil (0.267 g, 71%). $[a]_D^{25}$ +25.0 (*c* 2.73, $CHCl_3$); ν_{max}(film)/cm^{-1} 2950 (CH), 1720 (CO) and 1630 (C=C); δ_H(270 MHz; $CDCl_3$), 1.35 (3H, d, *J* 0.6, CH_3), 1.40 (3H, d, *J* 0.6, CH_3), 1.70 (1H, ddd, *J* 14.1, 6.6 and 5.1, 5-*H*H), 1.93 (1H, dt, *J* 14.1 and 6.3, 5-H*H*), 2.58 (2H, m, 3-H_2), 3.34 (3H, s, CH_2OCH_3), 3.50 (1H, t, *J* 7.8, 7-*H*H), 3.76 (3H, s, CO_2CH_3), 3.83 (1H, m, 4-H), 4.08 (1H, dd, *J* 7.8 and 5.9, 7-H*H*), 4.27 (1H, m, 6-H), 4.58 (1H, d, *J* 7.0, OC*H*HO), 4.63 (1H, d, *J* 7.0, OCH*H*O), 5.67 (1H, dd, *J* 1.7 and 0.2, 2-C*H*H) and 6.24 (1H, d, *J* 1.7, 2-CH*H*); δ_C(75.5 MHz) 25.80 (CH_3), 26.97 (CH_3), 37.62, 37.93 (C-3 and C-5), 51.96 and 55.74 (each OCH_3), 69.64 (C-7), 72.91, 73.57 (C-4 and C-6), 95.56 (OCH_2O), 108.63 (OCO), 128.02 (2-CH_2), 136.87 (C-2) and 167.44 (C-1); *m*/*z* (EI) 273.1335 ($M^+ - CH_3$, $C_{13}H_{21}O_6$ requires 273.1338, 9%), 243 (10), 207 (11), 197 (11), 189 (4), 167 (16), 149 (40), 137 (13), 113 (12), 101 (27), 91 (100), 84 (68), 69 (67) and 57 (45).

The above reaction was repeated on the more polar alcohol from the previous experiment to give a colourless oil (0.25 g, 72%). $[a]_D^{25}$ −14.0 (*c* 3.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2950 (CH), 1720 (CO) and 1630 (C=C); δ_H(270 MHz) 1.35 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.65 (1H, ddd, *J* 14.0, 9.0 and 5.0, 5-*H*H), 1.78 (1H, ddd, *J* 14.0, 7.9 and 3.7, 5-H*H*), 2.53 (1H, ddd, *J* 14.0, 6.5 and 1.0, 3-*H*H), 2.61 (1H, ddd, *J* 14.0, 6.0 and 1.0, 3-H*H*), 3.38 (3H, s, OCH_3), 3.52 (1H, t, *J* 7.7, 7-*H*H), 3.76 (3H, s, CO_2CH_3), 3.95 (1H, m, 4-H), 4.07 (1H, dd, *J* 7.7 and 5.9, 7-H*H*), 4.23 (1H, m, 6-H), 4.65 (1H, d, *J* 7.0, OC*H*HO), 4.69 (1H, d, *J* 7.0, OCH*H*O), 5.65 (1H, d, *J* 1.4, 2-C*H*H) and 6.24 (1H, d, *J* 1.4, 2-CH*H*); δ_C(75 MHz) 25.79 (CH_3), 27.01 (CH_3), 37.94, 38.86 (C-3 and C-5), 51.96 and 55.83 (each OCH_3), 69.90 (C-7), 73.10, 73.70 (C-4 and C-6), 95.92 (OCH_2O), 108.57 (OCO), 127.81 (2-CH_2), 136.87 (C-2) and 167.43 (C-1); *m*/*z* (EI) 273.1339 ($M^+ - CH_3$, $C_{13}H_{21}O_6$ requires 273.1338, 14%), 243 (15), 207 (14), 197 (15), 189 (18), 167 (17), 149 (58), 137 (16), 113 (19), 101 (35), 91 (42), 84 (100), 69 (89) and 57 (50).

The above reaction was repeated on the mixture of diastereomers **42** and **43** giving a mixture of **44** and **45** in 85% yield.

Ozonolysis of a mixture of unsaturated esters 44 and 45

Ozone was bubbled through a stirred solution of **44** and **45** (0.58 g, 2 mmol) in dichloromethane (30 cm^3) at −78 °C until the solution turned blue. Oxygen was then bubbled through the solution until the blue colouration disappeared. Dimethyl sulfide (3.5 cm^3) was added to the solution which was then allowed to warm to room temperature and stir for a further 5 min. The solvent was evaporated and the crude product purified by column chromatography to afford a mixture of 2-oxo esters **46** and **47** as a colourless oil (0.48 g, 82%). ν_{max}(film)/cm^{-1} 3455 (OH), 2935 (CH) and 1735 (CO); δ_H(300 MHz) 1.32, 1.34, 1.38, 1.40 (each 3H, each s, $4 \times CH_3$), 1.70–2.00 (4H, m, $2 \times$ 5-H_2), 3.00–3.30 (4H, m, $2 \times$ 3-H_2), 3.31 (3H, s, OCH_3), 3.33 (3H, s, OCH_3), 3.48–3.57 (2H, m, $2 \times$ 7-*H*H), 3.88 (6H, s, $2 \times CO_2CH_3$), 4.03–4.12 (2H, m, $2 \times$ 7-H*H*), 4.14–4.36 (4H, m, $2 \times$ 4-H and $2 \times$ 6-H) and 4.58–4.66 (4H, m, $2 \times OCH_2O$); δ_C(75 MHz) 25.7, 25.8, 26.9, 27.0 (each CH_3), 38.0, 39.7 ($2 \times$ C-5), 44.1, 45.2 ($2 \times$ C-3), 52.9, 53.0 ($2 \times CO_2CH_3$), 55.7, 55.7 ($2 \times OCH_3$), 69.5, 69.7 ($2 \times$ C-7), 71.4, 72.3, 72.3, 72.8 ($2 \times$ C-4 and $2 \times$ C-6), 96.2, 96.6 ($2 \times OCH_2O$), 108.9, 109.1 ($2 \times$ OCO), 161.2, 161.3 ($2 \times$ C-1), 191.5 and 191.9 ($2 \times$ C-2); *m*/*z* (CI) 259.1180 ($[MH]^+ - CH_3OH$, $C_{12}H_{19}O_6$ requires 259.1182, 3%), 231 (5), 229 (4), 213 (16), 201 (6), 171 (30), 153 (26), 129 (22), 111 (28), 101 (76), 93 (27), 85 (25), 71 (22) and 59 (100).

Hydrolysis of a mixture of 46 and 47 with *Candida rugosa* lipase followed by reduction catalysed by *LB*-hicDH

The biotransformations were carried out as described earlier using *LB*-hicDH. The reduction was extremely slow but was worked up after 2 days. The crude reaction product was methylated with ethereal diazomethane and purified by flash column chromatography eluting with 20% ethyl acetate in petroleum ether to give *methyl (2R,3E,6S)-6,7-O-isopropylidene-2,6,7-trihydroxyhept-3-enoate* **50** as a pale yellow oil (10 mg, 4%). $[a]_D^{25}$ −27.8 (*c* 2.4, $CHCl_3$); ν_{max}(film)/cm^{-1} 3450 (OH), 2985 (CH) and 1745 (CO); δ_H(300 MHz) 1.35, 1.42 (each 3 H, each s, $2 \times CH_3$), 2.33 (1H, dddt, *J* 14.2, 7.5, 6.8 and 1.2, 5-*H*H), 2.42 (1H, dddt, *J* 14.2, 6.8, 6.1 and 1.2, 5-H*H*), 3.57 (1H, dd, *J* 8.1

and 6.8, 7-*H*H), 3.81 (3H, s, CO_2CH_3), 4.02 (1H, dd, *J* 8.1 and 6.1, 7-H*H*), 4.16 (1H, tt, *J* 6.8 and 6.8, 6-H), 4.63 (1H, br dd, *J* 5.4 and 1.4, 2-H), 5.64 (1H, ddt, *J* 15.4, 5.8 and 1.2, 3-H), 5.88 (1H, dddd, *J* 15.4, 7.5, 6.8 and 1.4, 4-H); δ_C(75 MHz) 25.6, 26.9 (each CH_3), 36.4 (C-5), 52.9 (CO_2CH_3), 68.8 (C-7), 71.1 (C-2), 74.9 (C-6), 109.1 (OCO), 129.0, 129.1 (C-3 and C-4) and 172.0 (C-1); *m*/*z* (EI) 215.0917 ($M^+ - CH_3$, $C_{10}H_{15}O_5$ requires 215.0919, 31%), 155 (11), 129 (12), 123 (8), 113 (11), 101 (100), 95 (30), 86 (34) and 84 (47). Further elution with 50% ethyl acetate in light petroleum gave a mixture of hydroxy esters **48** and **49** (30 mg, 14%). ν_{max}(film)/cm^{-1} 3460 (OH), 2955 (CH) and 1740 (CO); δ_H(300 MHz), 1.34, 1.35, 1.40, 1.41 (each 3H, each s, 4 × CH_3), 1.74–1.88 (4H, m, 2 × 3-H_2 or 2 × 5-H_2), 1.92–2.16 (4H, m, 2 × 3-H_2 or 2 × 5-H_2), 3.37 (3H, s, OCH_3), 3.42 (3H, s, OCH_3), 3.51 (1H, t, *J* 7.5, 7-*H*H), 3.53 (1H, t, *J* 7.5, 7-*H*H), 3.78 (3H, s, CO_2CH_3), 3.79 (3H, s, CO_2CH_3), 3.98 (2H, m, 2 × 4-H or 2 × 6-H), 4.06 (1H, dd, *J* 7.3 and 5.9, 7-H*H*), 4.07 (1H, dd, *J* 7.3 and 5.9, 7-H*H*), 4.20 (2H, m, 2 × 4-H or 2 × 6-H), 4.33 (1H, dd, *J* 6.6 and 4.6, 2-H), 4.42 (1H, dd, *J* 10.3 and 2.9, 2-H), 4.57 (1H, d, *J* 6.8, OC*H*HO), 4.62 (1H, d, *J* 6.8, OC*H*HO), 4.71 (1H, d, *J* 6.8, OCH*H*O) and 4.75 (1H, d, *J* 6.8, OCH*H*O); δ_C(75 MHz), 25.8, 25.8, 27.0, 27.0 (each CH_3), 38.1, 38.3, 39.7, 40.2 (2 × C-3 and 2 × C-5), 52.5, 52.5 (2 × CO_2CH_3), 55.8, 56.0 (2 × OCH_3), 67.7, 68.0 (2 × C-2), 69.7, 69.7 (2 × C-7), 72.6, 72.7, 72.8, 73.3 (2 × C-4 and 2 × C-6), 96.3, 97.0 (2 × OCH_2O), 108.9, 108.9 (2 × OCO), 175.2 and 175.4 (2 × C-1); *m*/*z* (CI) 261.1345 ($[MH]^+ - CH_3OH$, $C_{12}H_{21}O_6$ requires 261.1338, 32%), 245 (50), 203 (100), 185 (30), 173 (82), 171 (48), 155 (70), 153 (64), 113 (72) and 101 (74).

Acetylation of alcohols 42 and 43

Acetic anhydride (0.5 ml, 5 mmol) was added to a stirred solution of diastereomeric alcohols **42** and **43** (0.244 g, 1 mmol) in anhydrous pyridine (0.9 ml, 11 mmol) at 0 °C. The solution was allowed to warm to room temperature and was stirred for a further 48 h. The solution was diluted with water (5 cm^3) and extracted with dichloromethane (3 × 10 cm^3), the organic extracts were dried over sodium sulfate and the solvent removed *in vacuo*. The crude product was purified by column chromatography to yield a mixture of acetates **51** and **52** as a colourless oil (0.180 g, 63%). ν_{max}(film)/cm^{-1} 2950 (CH), 1720 (CO) and 1630 (C=C); δ_H(300 MHz) 1.32, 1.35, 1.37 and 1.43 (each 3H, each s, 4 × -CH_3), 1.70–1.99 (4H, m, 2 × 5-H_2), 2.01 (6H, s, 2 × $OCOCH_3$), 2.38–2.80 (4H, m, 2 × 3-H_2), 3.50–3.75 (2H, m, 2 × 7-*H*H), 3.77 (6H, s, 2 × CO_2CH_3), 4.00–4.25 (4 H, m, 2 × 6-H and 2 × 7-H*H*), 5.12–5.22 (2 H, m, 2 × 4-H), 5.63 (2 H, m, 2 × 2-C*H*H) and 6.22 (2 H, m, 2 × 2-CH*H*); δ_C(75 MHz) 21.0, 21.1, 25.6, 25.7, 26.8 and 26.9 (each CH_3), 37.1, 37.3, 37.7 and 38.1 (2 × C-3 and 2 × C-5), 52.0 (2 × OCH_3), 69.3. 69.5, 69.9 and 70.2 (2 × C-4 and 2 × C-6), 72.9 and 73.1 (2 × C-7), 108.6 and 108.8 (2 × C-2), 127.6 and 127.7 (2 × 2-CH_2), 136.2 and 136.3 (2 × C-6′), 167.0, 167.1 (2 × C-1 or 2 × C-4), 170.3 and 170.4 (each CO); *m*/*z* (EI) 271.1177 ($M^+ - CH_3$, $C_{13}H_{19}O_6$ requires 271.1182, 18%), 265 (12), 263 (20), 246 (24), 244 (22), 197 (25), 191 (32), 169 (26), 153 (30), 137 (41), 129 (60), 84 (50), 79 (43), 69 (41) and 59 (100).

Methyl (3*E*,6*S*)-6,7-dihydroxy-6,7-*O*-isopropylidene-2-oxohept-3-enoate 55

Ozone was bubbled through a stirred solution of a mixture of acetates **51** and **52** (1.00 g, 3.5 mmol) in dichloromethane (70 cm^3) at −78 °C until the solution became blue. Oxygen was then bubbled through the solution until the blue colouration disappeared. Dimethyl sulfide (1.0 cm^3) was added to the solution which was then allowed to warm to room temperature and stir for a further 5 min. The solvent was evaporated and the crude product purified by dry flash column chromatography eluting with 70% ethyl acetate in light petroleum to give acetates **53** and **54** as a colourless oil (0.95 g, 94%) which were used immediately in the next step.

A solution of acetates **53** and **54** (0.95 g, 3.30 mmol) in chloroform (20 cm^3) was treated with silica (0.5 g). The reaction was allowed to stir at room temperature for 5 h after which the silica was removed by filtration and washed with copious amounts of a 70% solution of ethyl acetate in light petroleum. The filtrate was evaporated to give unsaturated 2-oxo ester **55** (0.72 g, 96%). $[\alpha]_D^{25}$ −1.0 (*c* 3.7, MeOH); ν_{max}(film)/cm^{-1} 2985 (CH) and 1740 (br, CO); δ_H(270 MHz) 1.36 and 1.43 (each 3H, each d, *J* 0.5, 2 × CH_3), 2.58 (2H, m, 5-H_2), 3.60 (1H, dd, *J* 8 and 6, 7-*H*H), 3.90 (3H, s, -CO_2CH_3), 4.08 (1H, dd, *J* 8 and 6, 7-H*H*), 4.27 (1H, quintet, *J* 6, 6-*H*), 6.76 (1H, dt, *J* 16 and 1, 3-H) and 7.18 (1H, dt, *J* 16 and 7, 4-H); δ_C(75 MHz) 25.27 and 26.58 (2 × CH_3), 37.07 (C-5), 52.16 (OCH_3), 68.70 (C-7), 73.95 (C-6), 109.44 ($C(CH_3)_2$), 127.58 (C-4), 148.41 (C-3), 162.60 (C-1) and 182.56 (C-2); *m*/*z* (EI) 213.0767 ($M^+ - CH_3$, $C_{10}H_{13}O_5$ requires 213.0763, 20%), 199 (63), 169 (10), 139 (30), 129 (20), 127 (16), 113 (18), 111 (18), 101 (100), 84 (60) and 59 (83).

Hydrolysis of 55 with *Candida rugosa* lipase followed by reduction catalysed by *BS*-LDH

The biotransformations were carried out as described earlier using *BS*-LDH. The crude reaction product was methylated with ethereal diazomethane and purified by flash column chromatography eluting with 20% ethyl acetate in light petroleum to give *methyl (2S,3E,6S)-6,7-O-isopropylidene-2,6,7-trihydroxyhept-3-enoate* **56** as a pale yellow oil (105 mg, 45%). $[\alpha]_D^{25}$ +51.5 (*c* 1.9, $CHCl_3$); ν_{max}(film)/cm^{-1} 3460 (OH), 2990 (CH) and 1745 (CO); δ_H(300 MHz) 1.35 and 1.42 (each 3H, each s, CH_3), 2.33 (1H, m, 5-*H*H), 2.42 (1H, m, 5-H*H*), 3.57 (1H, dd, *J* 8.0 and 7.0, 7-*H*H), 3.81 (3H, s, CO_2CH_3), 4.03 (1H, dd, *J* 8.0 and 6.0, 7-H*H*), 4.16 (1H, br quintet, *J* 6.5, 6-H), 4.65 (1H, br dd, *J* 5.5 and 1.5, 2-H), 5.64 (1H, ddt, *J* 15.5, 5.5 and 1.5, 3-H) and 5.90 (1H, dtd, *J* 15.5, 7.5 and 1.5, 4-H); δ_C(75 MHz) 25.6, 26.9 (each CH_3), 36.3 (C-5), 52.9 (CO_2CH_3), 68.8 (C-7), 71.1 (C-2), 75.0 (C-6), 109.1 ($C(CH_3)_2$), 129.0, 129.1 (C-3 and C-4) and 173.9 (C-1); *m*/*z* (EI) 215.0919 ($M^+ - CH_3$, $C_{10}H_{15}O_5$ requires 215.0919, 30%), 155 (5), 123 (86), 101 (100), 95 (25), 86 (44) and 84 (65).

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