Enzyme-Catalysed Hydrolyses of Some Meso-Diesters

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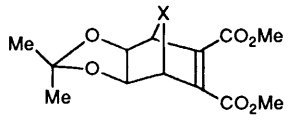
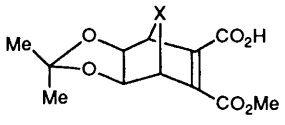
The diester **7** is hydrolysed, using porcine pancreatic lipase as catalyst, to give the monoester **8**. The latter compound has been converted into the known azido alcohol **9** and into the purine derivative **10**. Pig liver esterase (ple) catalysed hydrolysis of the diester **11** gave the acid **13** not the enantiomer **12** as previously reported. The structure of **13** was determined by its conversion into the compound *ent*-**10**. The diesters **17** and **18** are converted into the mono-esters **20** and **21** respectively using ple: hydrolysis of the diester **16** yields a mono-acid of high optical purity. The structure of this mono-acid is believed to be that shown in formula **19**.

The use of hydrolytic enzymes in synthetic organic chemistry is now becoming widespread [1] with pig liver esterase and porcine pancreatic lipase emerging as two of the most popular catalysts for the hydrolysis of carboxylic acid esters.

The ability of esterases and lipases to convert *meso*-diesters into optically active mono-esters has been observed on a number of occasions.[2] For example Ohno has effectively 'asymmetrized' [3] bicyclic compounds of type **1** to produce the corresponding mono-esters **2**.[4] *meso*-Diesters of the type **3** are

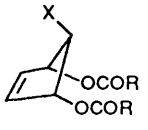


1 X = O or CH_2

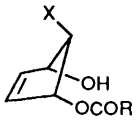


2 (e.e. 77–88%)

preferentially hydrolysed to the 1*S*,4*R*-mono-ester **4** using ple (86% e.e. for R = Me)[5] and to the 1*R*,4*S*-enantiomer (50–>99% e.e.) using *Candida cylindracea* lipase, porcine pancreatic lipase,[5] or electric eel acetylcholinesterase.[6] Furthermore Johnson and Penning have shown that the latter enzyme selectively hydrolysed the diester **5** to the alcohol **6**.[7]



3
X = H; R = Me, Et, Pr



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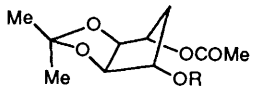
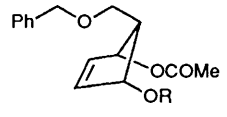


5 R = COMe
6 R = H

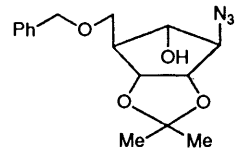
In this paper we show that the hydrolyses of other *meso*-diesters, related to compound **1** or compound **3**, with pig liver esterase (ple) or porcine pancreatic lipase (ppl) take place often with a high degree of selectivity, that the enzyme-catalysed reactions can be carried out on a large scale if necessary, and that some of the products can be used in multi-stage syntheses.

Results and Discussion

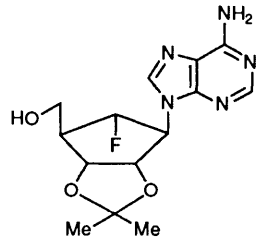
The diacetate **7** is prepared from cyclopentadiene in three steps.[8] Hydrolysis using ppl at pH 7 gave the mono-ester **8** (96%) in an essentially optically pure state (e.e. >95%) as judged by NMR spectroscopy employing a chiral shift reagent. The reaction is simple to perform on a ten gram scale. The absolute configuration of the mono-ester **8** was established by its conversion into the azido alcohol **9**,[9] a compound that has been prepared previously in optically active form by Martin *et al.* (using a classical resolution procedure) and transformed into the natural product (−)-aristeromycin.[10] The azido alcohol **9** has also been converted into the nucleoside analogue **10** (Scheme 1).



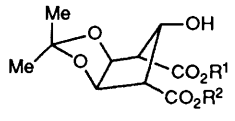
7 R = COMe
8 R = H



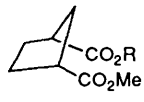
9



10

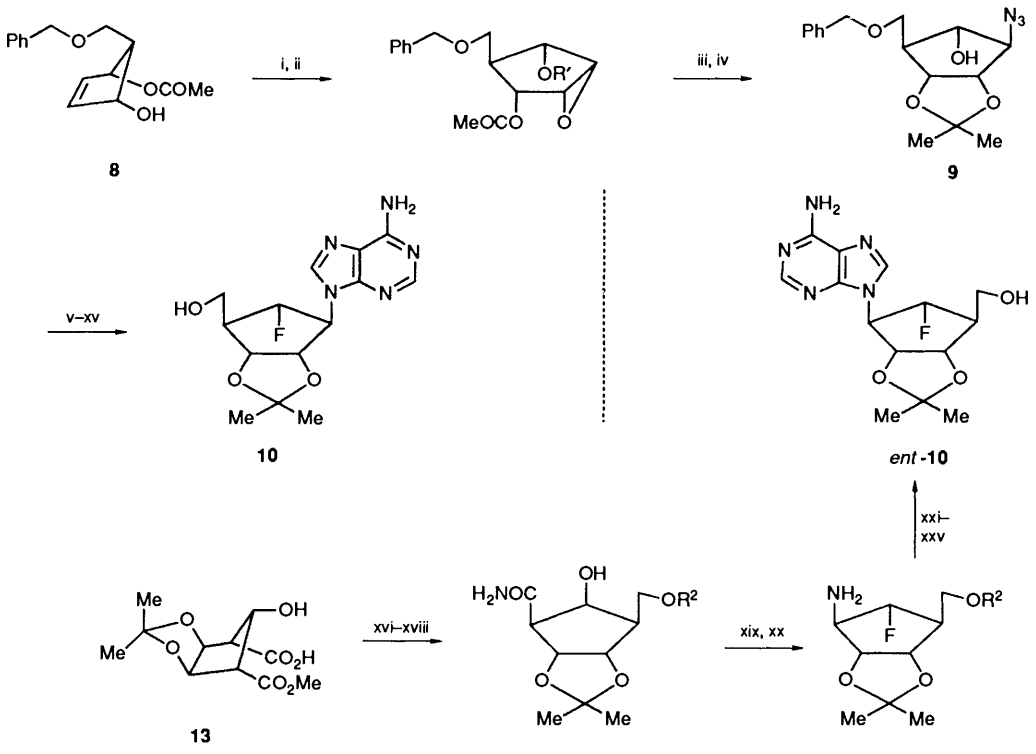


11 $R^1 = R^2$ = Me
12 R^1 = Me, R^2 = H
13 R^1 = H, R^2 = Me



14 R = Me
15 R = H

The diester **11** has been described previously and the compound has been hydrolysed using ple as the catalyst. The structure of the hydrolysis product ($[\alpha]_D$ = +5.5, *c* 0.51 dioxane) was purported to be the mono-ester **12**.[11] We have repeated this enzyme-catalysed reaction, on a multi-gram scale, and have obtained a product with a similar $[\alpha]_D$ value (+6.4, *c* 0.55 dioxane) in 96% yield. However this compound was shown to possess the structure **13** by conversion into *ent*-**10** (Scheme 1).[9] The CD spectra of **10** and *ent*-**10** are reproduced in



Scheme 1 *Reagents and conditions:* i, *meta*-Chloroperoxybenzoic acid, CH_2Cl_2, 0 °C–room temp., 24 h (80%); ii, $MeOCH_2CH_2OCH_2Cl$, Pr^i_2NEt, CH_2Cl_2, room temp., 48 h (81%); iii, 20% K_2CO_3–MeOH, room temp., 2 h (95%); iv (a), NaN_3, NH_4Cl, EtOH, H_2O (4:1), reflux, 3 d; iv (b), 2,2-dimethoxypropane, TsOH (cat.), 2 h (92%); v, Me_2BBr, CH_2Cl_2, Et_2O (10:1) −78 °C, 2 h (75%); vi, $(CF_3SO_2)O$, pyridine, CH_2Cl_2, 0 °C, 0.5 h; vii, $PhCO_2Li$, $HCONMe_2$, room temp., 0.5 h (90%); viii, 2% K_2CO_3–MeOH, room temp., 2 h (95%); ix, Et_2NSF_3, CH_2Cl_2, 0 °C–room temp., 2 h (81%); x, Lindlar cat., H_2, EtOH, room temp., 3 h (75%); xi, 4,6-Dichloro-5-nitropyrimidine, Et_3N, CH_2Cl_2, room temp., 3 h (83%); xii, Raney nickel, H_2, EtOH, room temp., 0.5 h (62%); xiii, Diethoxymethylacetate, reflux, 5 h (91%); xiv, liq. NH_3, 24 h (97%); xv, $Pd(OH)_2$, C_6H_{10}, EtOH (1:2) reflux, 12 h (83%); xvi, BH_3SMe_2, tetrahydrofuran, 0 °C–room temp., 18 h (100%); xvii, *tert*-Butylchlorodiphenylsilane, imidazole, $HCONMe_2$, room temp 3.5 h (85%); xviii, Me_2AlNH_2 prepared *in situ*, CH_2Cl_2, 0–35 °C, 26 h (73%); xix, Et_2NSF_3, CH_2Cl_2, −78 °C, 10 min (72%); xx, bis(Trifluoroacetoxy)iodobenzene, CH_3CN, pyridine, H_2O, dark, room temp., 6.5 h (72%); xxi, 4,6-Dichloro-5-nitropyrimidine, Et_3N, CH_2Cl_2, room temp., 18 h (53%), xxii, Raney nickel, H_2, dioxane, room temp., 12 h (66%); xxiii, Diethoxymethylacetate, reflux, 18 h (68%); xxiv, Bu_4NF, tetrahydrofuran, H_2O, room temp., 16 h; xxv, liq. NH_3, 20 bar, 70 °C, 22 h (23% for steps xxiv and xxv).

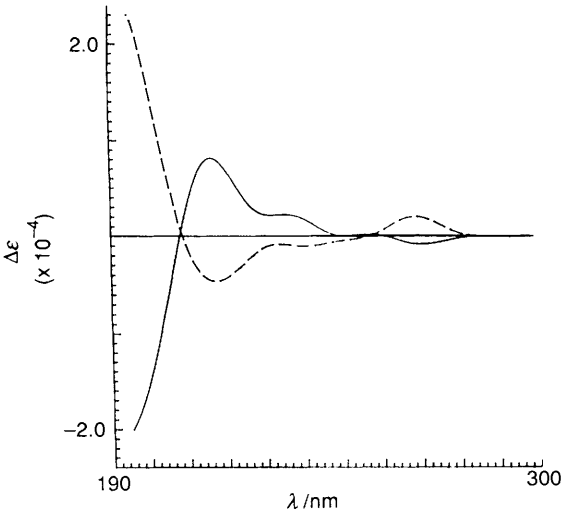


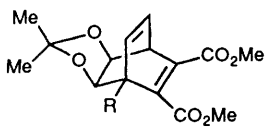
Fig. 1

Fig. 1. While the unsubstituted diester **14** was hydrolysed by ple at pH 7 to afford mainly the mono-ester **15** with low enantiomeric excess (34%),[12] the ester **13** was shown to be produced in an essentially optically pure state (e.e. >95%) by NMR spectroscopy using a chiral shift reagent.

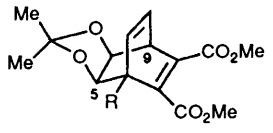
The conversion of the acetate **8** into the puridine derivative **10** and the transformation of the ester **13** into *ent*-**10** involve series of reactions (Scheme 1), full experimental details for which will be published in separate papers. Suffice it to say that the two synthetic routes are stereocontrolled and, if we assume Martin's results[10] are correct, then Zemlicka *et al.*[11] have mis-assigned the structure of the product of ple catalysed hydrolysis of the diester **11**.

The diester **16** was prepared in high yield by [4 + 2] cycloaddition of dimethyl acetylene dicarboxylate and 3,3-dimethyl-2,4-dioxabicyclo[4.3.0]nona-6,8-diene.[13] Similarly the diesters **17** and **18** were formed on refluxing dimethyl acetylene dicarboxylate with 3-fluoro- and 3-trifluoromethyl-3,3-dimethyl-2,4-dioxabicyclo[4.3.0]nona-6,8-diene respectively.[13] The compounds **17** and **18** possess the absolute configuration shown.[14] Hydrolysis of the diester **17** with ple in phosphate buffer (pH 8) gave the mono-ester **20** (84% yield) which was converted into the mixed diester **22**. The structure of the latter compound was elucidated by NMR spectroscopy and NOE studies. Thus NOEs were observed between 5-H and the protons of the methyl ester moiety (0.8%) and between 9-H and protons of the *tert*-butyl group (1.8%). Hydrolysis of the diester **18** with ple furnished the mono-ester **21**; the structure of the ester **21** was elucidated by the observation of an NOE between the protons of the ester group and 5-H (0.7%), but not between the ester group and 9-H.

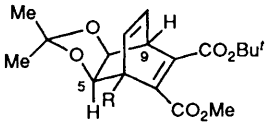
The diester **16** was hydrolysed by ple in phosphate buffer to give an optically active sample of the mono-ester **19**. A racemic sample of ester **19** was obtained by mono-methylation of the



16 R = H
17 R = F
18 R = CF_3



19 R = H
20 R = F
21 R = CF_3



22 R = F
23 R = H

adduct formed by reaction of acetylene dicarboxylic acid and 3,3-dimethyl-2,5-dioxabicyclo[4.3.0]nona-6,8-diene. The racemic and optically active samples of ester **16** were converted into the mono-methyl mono-*tert*-butyl ester **23**. The enantiomers of (±)-**23** were separated by analytical HPLC using a chiral column (Chiralcel OD) and isopropyl alcohol (1%) in hexane as eluent. Using the same analytical method the sample of the ester **23** prepared by the enzyme catalysed hydrolysis was shown to be substantially enriched in one enantiomer (>90% e.e.). It was not possible for us to identify the major enantiomer unequivocally, but it seems highly likely, based on the results described above and on Ohno's earlier work, that the absolute configuration of the major enantiomer is that described by formula **19**.

The proposed preferential hydrolysis of diester **16** to mono-ester **19** is supported by the fact that appropriate orientation of the tricyclic molecule fits snugly into the active site of ple as modelled according to the instructions of Jones *et al.*[15] The alternative arrangement which would lead to the hydrolysis of the other ester group is shown by the model to give rise to more severe unfavourable interactions.

Experimental

General.—Unless noted otherwise, all starting materials were obtained from commercial suppliers and were used without further purification. Benzene, diethyl ether, and tetrahydrofuran (THF), were distilled from sodium-benzophenone ketyl immediately prior to use. Dry dichloromethane was obtained by distillation from phosphorus pentoxide. Light petroleum refers to the fraction boiling in the range 40–60 °C. This and ethyl acetate were distilled prior to use. Flash chromatography was carried out using silica gel 60 H (Merck 7385). TLC was performed on Merck 60F-254 (0.25 mm thickness, Art. 5715), glass-backed silica gel plates. M.p.s were carried out on an 'Electrothermal' device and are uncorrected. IR spectra were recorded on a Perkin-Elmer 881 grating infrared spectrophotometer. Optical rotations were performed on a Thorn NPL Automatic Polarimeter Type 243 [α] values are given in 10^{-1} deg cm^2 g^{-1}. CD spectra were measured on 0.2 mm cylindrical silica cells on a JASCO J600 Spectropolarimeter. Data are reported for acetonitrile solutions with absorbance one at the wavelength maximum (λ 270 nm) in a 1 cm cell. Noise reduction was achieved with the Fourier Transform routine associated with the JASCO software. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM250 spectrophotometer, *J* values are given in Hz. Low-resolution mass spectra were run using a VG 12–253 Low Resolution instrument. High-resolution mass spectra were run at the SERC Mass Spectrometry Centre, Swansea, using a VG ZAB-E High Resolution instrument.

(+)-(1α,2β,3α)-1-*Acetoxy*-2-[(*phenylmethoxy*)*methyl*]*cyclopent*-4-*en*-3-*ol*.—The *meso*-diacetate **7** (11.49 g, 37.8 mmol) was suspended in pH 7, 0.1 mol dm^{-3} phosphate buffer (250 cm^3) and porcine pancreatic lipase (2 g) was added. The resultant mixture was stirred for 7 d at room temp. The suspension was extracted with diethyl ether (4 × 150 cm^3), the ethereal layers combined, dried over anhydrous magnesium sulphate, filtered and the solvent evaporated. The resulting crude residue was purified by flash column chromatography over silica gel, using 2:1 hexane–ethyl acetate as eluent to give the *acetate* **8** (9.50 g, 96%) as a colourless oil; $([\alpha]_D^{23}$ +63.2, *c* 1.0, $CHCl_3$); δ_H(250 MHz; $CDCl_3$), 2.01 (3 H, s, $COCH_3$), 2.09 (1 H, brd, *J* 6.0, OH), 2.29 (1 H, dddd, *J* 7.5, 5.0, 4.5 and 4.5, 2-H), 3.63 (1 H, dd, *J* 9.0, 7.5, $BuOCH_2$), 3.77 (1 H, dd, *J* 9.0, 5.0, $BuOCH_2$), 4.57 (2 H, s, $PhCH_2$), 4.60 (1 H, m, 3-H), 5.40 (1 H, dm, *J* 4.5, 1-H), 5.88 (1 H, ddd, *J* 6.2, 2.0 and 2.0, 4-H), 6.04 (1 H, ddd, *J* 6.0, 2.0 and 2.0, 5-H) and 7.30 (5 H, m, Ph); δ_C(62.9 MHz; $CDCl_3$) *inter alia*: 21.1 (CH_3), 55.3 (CH), 69.85, 73.4 (2 × CH_2), 77.4, 78.95 (2 × CH), 131.75, 137.5 (2 × CH), 138.15 (C) and 170.9 (CO); ν_{max}(film)/cm^{-1} 3440 (OH), 3066 (Ar, CH), 2929 (CH) and 1733 (CO) [Found (M^+ + NH_4) 280.154 88. $C_{15}H_{18}O_4$ requires (M + NH_4) 280.154 88].

Methyl (+)β-*Carboxy*-7β-*hydroxy*-3,3-*dimethyl*-2,4-*dioxabicyclo*[3.3.0]*octane*-8β-*carboxylate* **13**.—Pig liver esterase (5030 units) in ammonium sulphate (Sigma) was added to a solution of *meso*-diester **11** (3.02 g, 11.1 mmol) in phosphate buffer (60 ml, pH 6.85). The suspension was stirred at room temp. with the pH being adjusted to 6.85 at regular intervals using 1 mol dm^{-3} aqueous sodium hydroxide solution. After 96 h, no starting material remained and the system was acidified to pH 3.0 using 1 mol dm^{-3} aqueous orthophosphoric acid. Extraction was carried out immediately using diethyl ether (5 × 50 cm^3) and dichloromethane (5 × 50 cm^3). The combined organic phases were dried ($MgSO_4$) and the solvent removed under reduced pressure to give mono-ester **13** as a white crystalline solid (2.79 g, 96%), m.p. 159–162 °C (lit.,[10] 162–164 °C); $[\alpha]_D$ +6.38 (*c* 0.548 in dioxane); δ_H 1.35 (3 H, s, CH_3), 1.53 (3 H, s, CH_3), 3.06–2.96 (2 H, m, 6-H and 8-H), 3.76 (3 H, s, OCH_3), 4.85 (1 H, t, *J* 4, 7-H), 5.16–5.08 (2 H, m, 1-H and 5-H) and 5.51 (1 H, s, OH); δ_C 27.1, 24.6 (2 × CH_3), 52.3 (OCH_3), 55.9, 55.8 (C-6, C-8), 76.1 (C-7), 80.7, 80.6 (C-1 and C-5), 114.1 (C-3) and 171.5 (CO); ν_{max}($CHCl_3$)/cm^{-1} 3488 (OH) and 1717 (CO_2H, CO_2Me) [Found: (M^+ + H), 261.0974. Calc. for $C_{11}H_{16}O_7$: (M + H) 261.0974].

Addition of Acetylene Dicarboxylic acid to 3,3-*Dimethyl*-2,5-*dioxabicyclo*-[4.3.0]*nona*-6,8-*diene*.—The diene (0.152 g, 1 mmol) and acetylene dicarboxylic acid (0.114 g, 1 mmol) were dissolved in THF. The mixture was refluxed for 2 d under nitrogen. The solvent was removed by distillation at reduced pressure to give the crude product as a yellow solid, which was subjected to column chromatography using as eluent ethyl acetate–methanol–glacial acetic acid (250:25:1). This afforded the *adduct* as a white solid (0.127 g, 48%, m.p. 207 °C); δ_H(CD_3OD) 1.24 (3 H, s, CH_3), 1.28 (3 H, s, CH_3), 4.30 (2 H, s, OCH × 2), 4.61 (2 H, br s, CH × 2) and 6.30 (2 H, m, HC=C*H* × 2); δ_C(CD_3OD) 25.85 (s, CH_3), 26.1 (CH_3), 46.9 (OCH × 2), 79.5 (CH × 2), 114.2 (OCO), 132.8 (HC=*C*H × 2), 145.95 (C=*C*CO_2 × 2) and 170.0 (C=O × 2), *m/z* (CI) 284 [(M + NH_4)$^+$, 62%], 267 [(M + H)$^+$, 22%], 223 (6), 184 (100), 167 (57), 147 (2), 136 (1), 101 (65), 93 (2), 85 (14) and 58 (2) [Found: (M^+ + H) 267.0869; $C_{13}H_{14}O_6$ requires (M + H) 267.0868].

Preparation of (±)-*Ester* **19**.—The diacid prepared above (0.133 g, 0.5 mmol) was dissolved in THF (10 cm^3). To this was added, at ambient temperature, an ethereal solution of diazo-

methane (1.25 cm^3, approx. 0.5 mmol). The reaction mixture was allowed to stand for several hours. The solvent was removed by distillation at reduced pressure to give the crude product as a yellow oil, which was subjected to column chromatography using as eluent ethyl acetate in light petroleum (1:4) and then ethyl acetate in light petroleum (3:1) containing 0.85% glacial acetic acid. This afforded the *ester* **19** as a pale yellow solid (0.063 g, 45%); δ_H 1.24 (3 H, s, CH_3), 1.32 (3 H, s, CH_3), 3.88 (3 H, s, OCH_3), 4.30–4.40 (3 H, br m, CH and OCH × 2), 4.54 (1 H, m, CH), 6.30–6.43 (2 H, br m, HC=C*H* × 2) and 10.54 (1 H, br s, CO_2H); δ_C 25.5 (CH_3), 25.6 (CH_3), 44.9 (CH), 45.0 (CH), 53.2 (OCH_3), 77.9 (OCH), 78.0 (OCH), 113.8 (OCO), 130.6 (HC=*C*H), 131.7 (HC=*C*H), 142.2 (C=*C*), 143.8 (C=*C*), 166.3 (C=O) and 167.2 (C=O); *m/z* (CI) 298 [$(M + NH_4)^+$, 41%], 281 [$(M + H)^+$, 77%], 265 (1), 255 (1), 223 (2), 198 (37), 181 (100), 163 (4), 100 (21) and 85 (12) [Found: (M^+ + H) 281.1025; $C_{14}H_{16}O_6$ requires (M^+ + H) 281.1025].

Esterification of (±)-*Ester* **19** *with* 2-*Methylpropan*-2-*ol*.—Racemic ester **19** (0.064 g, 0.23 mmol), 2-methylpropan-2-ol (0.073 g, 0.99 mmol) and a catalytic quantity of DMAP were dissolved in dichloromethane (1 cm^3). The solution was stirred and cooled to 0 °C in an ice bath. Dicyclohexylcarbodiimide (DCC) (0.075 g, 0.36 mmol), dissolved in dichloromethane (1 cm^3) was added dropwise over a period of 5 min under nitrogen. The mixture was slowly allowed to attain ambient temperature and stirred for 3 d. The reaction mixture was worked up by the method described below. This furnished the crude product which was subjected to column chromatography using as eluent ethyl acetate in light petroleum (1:4). This afforded the pure *product* **23** as a white crystalline solid (0.019 g, 24%, m.p. 85 °C); δ_H 1.25 (3 H, s, CH_3), 1.32 (3 H, s, CH_3), 1.48 [9 H, s, $C(CH_3)_3$], 3.77 (3 H, s, OCH_3), 4.18 (2 H, m, CH × 2), 4.37 (2 H, m, OCH × 2) and 6.38 (2 H, m, HC=C*H* × 2); δ_C 25.55 (CH_3), 25.75 (CH_3), 27.9 [$C(CH_3)_3$], 44.3 (CH), 52.0 (OCH_3), 78.2 (OCH), 82.2 [$C(CH_3)_3$], 113.5 (OCO), 131.2 (HC=*C*H), 131.4 (HC=*C*H), 139.4 (C=*C*), 142.3 (C=*C*), 164.5 (C=O) and 166.0 (C=O); *m/z* (CI), 337 [$(M + H)^+$, 50%], 298 (100), 281 (80), 198 (5), 181 (12), 163 (2), 100 (45), 85 (13) [Found: (M^+ + H) 337.1651. $C_{18}H_{24}O_6$ requires (M + H) 337.1651].

Enantioselective Hydrolysis of Diester **16** *by Pig Liver Esterase*.—To the diester **16** (0.10 g, 0.34 mmol) in aqueous buffer (10 cm^3, 100 mmol dm^{-3} potassium phosphate, pH 8) and acetone (5 cm^3) was added the enzyme (150 units, 115 mm^3 pig liver esterase). The reaction mixture was stirred overnight at ambient temperature. The reaction mixture was acidified (pH 3) by the addition of 2 mol dm^{-3} hydrochloric acid and extracted with ethyl acetate (6 × 10 cm^3). The combined organic extracts were dried over anhydrous magnesium sulphate, filtered, and the solvent removed by distillation at reduced pressure to give the *product* **19** as a white crystalline solid (0.092 g, 97%, m.p. 154 °C); ($[\alpha]_D^{25}$ −34; *c* 5.7, CH_2Cl_2); δ_H 1.25 (3 H, s, CH_3), 1.33 (3 H, s, CH_3), 3.88 (3 H, s, OCH_3), 4.34 (2 H, m, OCH × 2), 4.39 (1 H, m, CH), 4.61 (1 H, m, CH), 6.30–6.43 (2 H, br m, HC=C*H*) and 10.90 (1 H, br s, CO_2H); δ_C 25.5 (CH_3), 25.7 (CH_3), 45.0 (CH), 45.4 (CH), 53.5 (OCH_3), 77.9 (OCH), 78.0 (OCH), 113.8 (OCO), 130.6 (HC=*C*H), 131.7 (HC=*C*H), 141.8 (C=*C*), 145.0 (C=*C*), 165.4 (C=O) and 167.4 (C=O); *m/z* (CI), 298 [$(M + NH_4)^+$, 59%], 281 [$(M + H)^+$, 87%], 198 (56), 181 (100), 101 (22), 100 (13) and 85 (6) [Found: (M^+ + H) 281.1025. $C_{14}H_{16}O_6$ requires (M + H) 281.1025].

Esterification of (−)-*Ester* **19** *with* 2-*Methylpropan*-2-*ol*.—(−)-Ester **4** (0.092 g, 0.33 mmol), 2-methylpropan-2-ol (0.073 g, 0.99 mmol) and a catalytic quantity of 4-*N*,*N*-dimethylaminopyridine (DMAP) were dissolved in dichloromethane (1 cm^3). The solution was stirred and cooled to 0 °C in an ice bath. Dicyclohexylcarbodiimide (0.075 g, 0.36 mmol) dissolved in dichloromethane (1 cm^3) was added dropwise over a period of 5 min, under nitrogen. The mixture was stirred for a further 5 min at 0 °C and then stirred overnight at ambient temperature. The precipitate was removed by filtration and the filtrate washed successively with hydrochloric acid (0.5 mol dm^{-3}; 2 × 10 cm^3) and saturated aqueous sodium hydrogencarbonate solution (2 × 10 cm^3). Additional precipitate was removed by filtration and the two phases were separated. The organic phase was dried over anhydrous magnesium sulphate, filtered and the solvent removed by distillation at reduced pressure to give the crude product as a white crystalline solid (0.063 g) which was subjected to column chromatography using as eluent ethyl acetate in light petroleum (1:4). This afforded the *diester* **23** (0.036 g, 33%) as a semi-solid; δ_H 1.25 (3 H, s, CH_3), 1.32 (3 H, s, CH_3), 1.48 [9 H, s, $C(CH_3)_3$], 3.77 (3 H, s, OCH_3), 4.18 (2 H, m, CH × 2), 4.36 (2 H, m, OCH × 2) and 6.37 (2 H, m, HC=C*H* × 2); δ_C 25.55 (CH_3), 25.75 (CH_3), 27.9 [$C(CH_3)_3$], 44.3 (CH), 52.0 (OCH_3), 78.2 (OCH), 82.3 [$C(CH_3)_3$], 113.5 (OCO), 131.2 (HC=*C*H), 131.4 (HC=*C*H), 139.4 (C=*C*), 142.38 (C=*C*), 164.5 (C=O) and 166.0 (s, C=O); *m/z* (CI) 354 [$(M + NH_4]^+$, 3%), 337 [$(M + H)^+$, 99%], 321 (2), 298 (100), 281 (95), 237 (2), 198 (7), 181 (19), 163 (4), 100 (93) and 85 (34) [Found: (M^+ + H) 337.1651. $C_{18}H_{24}O_6$ requires (M + H) 337.1751].

Hydrolysis of the Diester **17** *by Pig Liver Esterase*.—To the diester **17** (0.40 g, 1.28 mmol) in aqueous buffer (10 cm^3, 100 mol dm^{-3} potassium phosphate, pH 8) was added the enzyme (*ca.* 500 units). The reaction mixture was stirred for 2 d at room temp. The reaction mixture was acidified (pH 2) by the addition of 2 mol dm^{-3} hydrochloric acid and extracted with ethyl acetate (6 × 10 cm^3). The combined organic extracts were dried over anhydrous magnesium sulphate, filtered, and the solvent removed by distillation at reduced pressure to give the *product* **20** as a white crystalline solid (0.32 g, 84%), m.p. 132 °C; $\delta_H(CDCl_3)$ 1.29 (3 H, s, CH_3), 1.36 (3 H, s, CH_3), 3.84 (3 H, s, OCH_3), 3.32–4.48 (2 H, br, m, CH and OCH), 4.58 (1 H, m, FCCHO), 6.36 (1 H, m, FCCH=C*H*) and 6.45 (1 H, m, FCC*H*=CH); $\delta_H(C_6D_6)$, 1.02 (3 H, s, CH_3), 1.26 (3 H, s, CH_3), 3.51 (3 H, s, CH_3), 3.91 (1 H, m, *J* 7.2 and 3.5), 4.25 (1 H, m, *J* 7.6 and 3.5, OCH), 4.52 (1 H, td, *J* 7.2, 7.2 and 1.3, FCCHO), 5.93 (1 H, dt, *J* 7.6, 5.6 and 5.6, FCCH=C*H*) and 6.29 (1 H, tm, *J* 8.9, 5.6, 1.6 and 1.4, FCC*H*=CH); $\delta_C(CDCl_3)$ 25.6 (CH_3), 41.75 (CH), 52.9 (OCH_3), 77.95 (d, *J* 5.49, OCH), 79.7 (d, *J* 18.66, FC*C*HO), 100.2 (d, *J* 202.5, CF), 115.4 (OCO), 130.25 (d, *J* 9.79, FCCH=*C*H), 130.95 (d, *J* 27.17, FC*C*H=CH), 149.4 (C=*C*–CO_2), 149.8 (C=*C*CO_2), 164.1 (*C*O_2CH_3) and 166.7 (CO_2H); *m/z* (CI) 316 [$(M + NH_4)^+$, 100%], 299 [$(M + H)^+$, 13%], 216 (15), 199 (4), 100 (16) and 85 (14) [Found: (M^+ + NH_4), 316.1196; $C_{14}H_{15}FO_6$ requires (M + NH_4) 316.1196].

Hydrolysis of Diester **18** *by Pig Liver Esterase*.—To the trifluoromethyl diester **18** (0.05 g, 0.14 mmol) in aqueous buffer solution (10 cm^3, 100 mmol dm^{-3} potassium phosphate, pH 8) was added the enzyme (*ca.* 500 units, purchased from Biocatalysts). The reaction mixture was stirred for 2 d at ambient temperature. The reaction mixture was worked up by the method described above. This furnished the *product* **21** as a viscous yellow oil (0.035 g, 73%); $\delta_H(CDCl_3)$ 1.29 (3 H, s, CH_3), 1.34 (3 H, s, CH_3), 3.80 (3 H, s, OCH_3), 4.41–4.52 (2 H, br m, CH and OCH), 4.63 (1 H, m, F_3CCCHO), 6.37 (1 H, d, *J* 7.3, HC=C*H*), 6.57 (1 H, m, HC=C*H*) and 8.35 (1 H, br s, CO_2H); $\delta_H(C_6D_6)$ 0.98 (3 H, s, CH_3), 1.22 (3 H, s, CH_3), 3.55 (3 H, s, OCH_3), 3.99 (1 H, m, *J* 6.8 and 3.2, OCH), 4.34 (1 H, m, *J* 7.2 and 3.2, CH), 4.58 (1 H, d, *J* 6.8, F_3CCCHO), 6.09–6.24 (2 H, br m, *J* 7.5, HC=C*H* × 2) and 7.50 (1 H, br s, CO_2H); $\delta_C(CDCl_3)$ 25.4 (CH_3), 25.7 (CH_3), 42.3 (CH), 53.0 (OCH_3), 59.0 (q, *J* 27.9,

F_3CC), 78.5 (OCH × 2), 115.3 (OCO), 125.3 (q, *J* 281.4 CF_3), 126.9 (q, *J* 3.0, $F_3CCCH{=}CH$), 133.7 ($F_3CCH{=}$), 146.0 (C=*C*) and 165.4 (*C*=O); $\delta_C(C_6D_6)$ 25.4 (CH_3), 25.8 (CH_3), 42.7 (CH), 52.6 (OCH_3), 59.5 (q, *J* 27.8, F_3CC), 78.7 (OCH), 78.9 (F_3CCCHO), 115.2 (OCO), 126.2 (q, *J* 281.3, CF_3), 126.9 (q, *J* 2.9, $F_3CCCH{=}CH$), 134.0 ($F_3CCCH{=}CH$), 136.8 ($F_3CCCCO_2CH_3$), 146.6 ($F_3CCC{=}CCO_2H$), 165.45 (C=O) and 167.3 (C=O); *m*/*z* (CI) 366 [$(M + NH_4)^+$, 100%], 349 [$(M + H)^+$, 7%), 266 (32), 136 (2), 101 (23), 100 (16), 85 (9), 58 (2) [Found: ($M^+ + NH_4$), 366.1164; $C_{15}H_{15}F_3O_6$ requires ($M + NH_4$) 366.1164].

Esterification of Ester **20** *with 2-Methylpropan-2-ol.*—The ester **20** (0.213 g, 0.715 mmol), 2-methylpropan-2-ol (0.12 g, 1.619 mmol) and DMAP (0.076 g, 0.622 mmol) were dissolved in dichloromethane (10 cm^3). The solution was stirred and cooled in an ice bath to 0 °C. Dicyclohexylcarbodiimide (0.172 g, 0.834 mmol) dissolved in dichloromethane (4 cm^3), was added dropwise over a period of 10 min, under an atmosphere of nitrogen. The reaction mixture was slowly allowed to attain ambient temperature and stirred for 7 d. The reaction mixture was worked up by the method described above. This furnished the crude product which was subjected to column chromatography using as eluent ethyl acetate in light petroleum (1:4). This afforded the pure *product* **22** as an oil (0.114 g, 41%); δ_H 1.25 (3 H, s, CH_3), 1.32 (3 H, s, CH_3), 1.42 [9 H, s, $C(CH_3)_3$], 3.80 (3 H, s, OCH_3), 4.30 (1 H, m, CH), 4.37 (1 H, m, OCH), 4.52 (1 H, td, *J* 7.3, 7.3 and 1.4, FCCHO), 6.26–6.36 (1 H, brm, FCCH=C*H*) and 6.36–6.45 (1 H, brm, FCC*H*=CH); δ_C 25.6 (CH_3), 25.65 (CH_3), 27.8 [$C(CH_3)_3$], 41.8 (d, *J* 1.9, CH), 52.4 (OCH_3), 78.0 (d, *J* 5.65, OCH), 79.9 (d, *J* 18.7, FCCHO), 82.6 [$C(CH_3)_3$], 100.1 (d, *J* 201.5, CF), 115.0 (OCO), 130.5 (d, *J* 9.8, FCCH=CH), 131.0 (d, *J* 27.2, FCCH=CH), 133.1 (d, *J* 4.7, $FCC{=}CCO_2CH_3$), 145.65 (d, *J* 25.25, $FCC{=}CCO_2CH_3$), 161.4 (d, *J* 1.65, C=O) and 164.301 (d, *J* 1.7, C=O); *m*/*z* (CI) 372 [$(M + NH_4)^+$, 37%], 355 [$(M + H)^+$, 28%], 316 (100), 299 (29), 216 (6), 100 (28), 85 (12) [Found: ($M^+ + H$) 355.1557. $C_{18}H_{23}FO_6$ requires ($M + H$) 355.1557].

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