Enzymatic Preparation of Enantiomerically Pure Alkan-2- and -3-ols by Lipase-Catalysed Hydrolysis with *Pseudomonas cepacia* in the Presence of Organic Media

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Pseudomonas cepacia lipase-catalysed hydrolysis of the acetates of racemic alkan-2- and -3-ols was carried out in the presence of organic media. Good to high enantioselectivity was observed in an acetone–water solvent system, the system leading to (*R*)-alcohols of 80–96% ee. Enantioselectivity can be affected by the presence of the double bond in the unsaturated acetate prepared from an alkenol. The increased enantioselectivity observed for an acetone–water reaction system was utilized in the synthesis of optically active natural products possessing alkan-2-ol skeletons, such as (2*R*,6*R*,10*R*)-6,10,14-trimethylpentadecan-2-ol, the natural form of the sex pheromone of *Corcyra cephalonica*, and (*R*)-sulcatol, an aggregation pheromone of ambrosia beetles.

Lipase, one of the classes of biocatalysts, have been widely used over the past five years in organic synthesis, because of their ability to catalyse many potentially important reactions including hydrolysis, esterifications, and transesterifications and to transform not only natural substrates, but also to accept and convert structurally related organic molecules.[1] Lipase-catalysed enantioselective transformations, in a water or organic solvent system, would now be regarded as a routine procedure in synthetic chemistry.[2]

Although there are a number of papers on the lipase-catalysed resolution of secondary alcohols, relatively little has been reported about the enantioselective hydrolysis of esters of aliphatic secondary alcohols,[3,4] namely alkan-2- and -3-ols, which do not have any functional groups other than the hydroxyl function.[5] Recently we showed that the 2,2,2-trichloroethyl carbonate derivatives of long-chain alkan-2-ols, such as pentadecan-2-ol, were hydrolysed in high enantioselectivity by lipase from a *Pseudomonas* sp. and that the hydrolysis could be successfully applied to the synthesis of (2*R*,6*S*,10*S*)-6,10,14-trimethylpentadecan-2-ol, one of the stereoisomers of the sex pheromone of *Corcyra cephalonica*.[5] In the case of short-chain alkan-2-ol derivatives, however, it was sometimes difficult to separate the short-chain alcohol product from each hydrolysed mixture containing 2,2,2-trichloroethanol. Another disadvantage is the fact that the hydrolysis of the trichloroethyl carbonates derived from alkan-3-ols proceeds with lower enantioselectivity than does that of the corresponding alkan-2-ol derivatives.

In an effort to investigate the effects of organic media on the enantioselectivity of lipase-catalysed hydrolysis and to prepare chiral alkan-2- and -3-ols with high enantiomeric purity, we have carried out the enzymatic hydrolysis of the acetates of several alkan-2- and -3-ols by using lipase PS (lipase from *Pseudomonas cepacia*, Amano PS)* in organic–water mixed solvent systems.

Results and Discussion

We first attempted the resolution of (±)-tridecan-2-ol **1** in a water system by lipase PS. However, the enzymatic hydrolysis of its corresponding acetate (±)-**1a** gave the alcoholic product and the remaining ester with low enantiomeric purity. As exemplified in Table 1, the hydrolysis of acetate (±)-**1a** was therefore carried out by the addition of acetone, methanol, diethyl ether, or hexane as the organic medium. Among these solvent systems, acetone–phosphate buffer was the most effective for improving the enantioselectivity, the alcohol (*R*)-**1** of 96% ee being obtained. Similarly, enzymatic hydrolysis of (±)-pentadecan-2-yl acetate in an acetone–water system produced (*R*)-pentadecan-2-ol with an enantiomeric purity of 95% ee in 34% conversion; the analogous hydrolysis in phosphate buffer yielded the same alcohol with 74% ee. The acetate **2a**, which was prepared from the relatively short-chain alcohol, octan-2-ol **2**, was submitted to the action of lipase PS in both water and organic–water solvent systems. A water system gave the alcohol (*R*)-**2** with a low enantiomeric purity of 40%, whereas the enantioselectivity was enhanced in all of the organic–water systems examined, the ee of (*R*)-**2** reaching a maximum of 81% in an acetone–water system. These data for alkan-2-ols indicate that the long-chain alkanol acetates can be hydrolysed more enantioselectively than can the short-chain homologues.

As shown in Table 2, the analogous lipase-catalysed resolution of the two alkan-3-ols (±)-tridecan-3-ol **3** and (±)-octan-3-ol **4** was performed in organic–water solvent systems. Each hydrolysis of the acetates **3a** and **4a** proceeded with higher enantioselectivity in acetone–phosphate buffer than in other solvent systems, resulting in the alcohol (*R*)-**3** of 80% ee and the alcohol (*R*)-**4** of 81% ee. The observed enantioselectivity for these two acetates was less than that of the corresponding alkan-2-yl acetates. In addition, similar resolution of an alken-3-ol, (±)-hept-6-en-3-ol **5**, was examined. The enzymatic hydrolysis of the unsaturated acetate **5a** with lipase PS showed higher enantioselectivity than did those of the saturated acetates **3a** and **4a**, the alcohol (*R*)-**5** of 89% ee being obtained in an acetone–water system (Table 3). A similar increase in enantioselectivity was observed for (*E*)-7-methylnon-6-en-3-yl acetate **6a**, which is the aggregation pheromone of the square-necked grain beetle. Lipase PS-catalysed hydrolysis of the unsaturated pheromone in an acetone–water system yielded the corresponding (*R*)-alcohol **6** with an enantiomeric purity of 98% ee.[6] This remarkable enantioselectivity observed for these

* *Pseudomonas cepacia* lipase was previously incorrectly classified as *Pseudomonas fluorescens* lipase (lipase P Amano).[3,5]

Table 1 Enzymatic hydrolysis of alkan-2-yl acetates in organic–water solvent systems[a]

(±)-**1a** $n = 10$; (±)-**2a** $n = 5$ —lipase PS→ (*R*)-**1** $n = 10$; (*R*)-**2** $n = 5$ + (*S*)-**1a** $n = 10$; (*S*)-**2a** $n = 5$

Acetate	Organic solvent	Time (t/h)	Conversion (%)	Yield (%)/ee (%) (*R*)-**1**	Yield (%)/ee (%) (*S*)-**1a**	*E*
(±)-**1a**	None[b]	24	37	30/76	50/49	12
	Acetone	19	32	26/96	52/41	65
	Methanol	47	39	31/83	53/36	15
	Ethylene glycol	13	38	30/78	50/31	11
	Diethyl ether	52	32	25/90	53/28	25
	Hexane	84	30	20/69	55/13	6

Acetate	Organic solvent	Time (t/h)	Conversion (%)	Yield (%)/ee (%) (*R*)-**2**	Yield (%)/ee (%) (*S*)-**2a**	*E*
	None[b]	14	46	40/40	43/31	3
	Acetone	20	33	30/81	40/32	13
	Methanol	22	43	30/69	45/45	9
	Ethylene glycol	10	38	31/54	45/35	5
	Diethyl ether	53	44	35/55	50/46	5
	Hexane	47	33	30/63	43/31	7

[a] Reactions were carried out in a mixture of organic solvent (6 cm^3) and phosphate buffer (9 cm^3). [b] Reaction was run in phosphate buffer (15 cm^3).

Table 2 Enzymatic hydrolysis of alkan-3-yl acetates in organic–water solvent systems[a]

(±)-**3a** $n = 9$; (±)-**4a** $n = 4$ —lipase PS→ (*R*)-**3** $n = 9$; (*R*)-**4** $n = 4$ + (*S*)-**3a** $n = 9$; (*S*)-**4a** $n = 4$

Acetate	Organic solvent	Time (t/h)	Conversion (%)	Yield (%)/ee (%) (*R*)-**3**	Yield (%)/ee (%) (*S*)-**3a**	*E*
(±)-**3a**	None[b]	71	45	32/56	54/45	5
	Acetone	49	46	35/80	42/32	11
	Methanol	72	41	30/54	53/35	5
	Ethylene glycol	22	49	46/52	40/43	5
	Ether	75	35	29/58	50/33	5
	Hexane	95	33	30/55	58/32	5

Acetate	Organic solvent	Time (t/h)	Conversion (%)	Yield (%)/ee (%) (*R*)-**4**	Yield (%)/ee (%) (*S*)-**4a**	*E*
(±)-**4a**	None[b]	58	37	30/63	62/46	7
	Acetone	54	32	29/81	64/45	15
	Methanol	32	43	40/72	57/57	11
	Ethylene glycol	29	40	36/60	56/49	6
	Diethyl ether	98	40	39/67	50/58	9
	Hexane	144	40	39/69	53/58	10

[a] Reactions were carried out in a mixture of organic solvent (6 cm^3) and phosphate buffer (9 cm^3). [b] Reaction was run in phosphate buffer (15 cm^3).

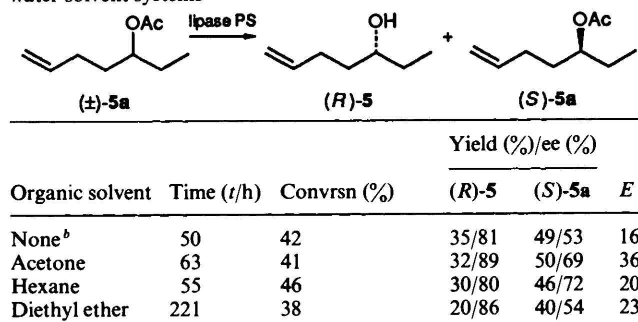
two unsaturated acetates, compared with the saturated analogues, can be attributable to the presence of the double bond.*

Similarly enhanced enantioselectivity of enzyme-catalysed hydrolysis in the addition of hydrophilic organic solvents such as methanol, dimethylformamide (DMF), or acetone, has also been observed by others;[8,†] several substrates, including 3-substituted dimethylglutarates, 3-acyloxypropionates, or 1-phenylalkan-1-yl acetates, were each hydrolysed. Although the reasons for the present observation in the presence of hydrophilic acetone are not clear, it seems to be a reasonable

* Very recently, an enhanced enantioselectivity in PPL-catalysed transesterification has been observed for unsaturated substrates such as α-phenylethyl alcohol, propargylic alcohols, and (*E*)-allylic alcohols.[7]

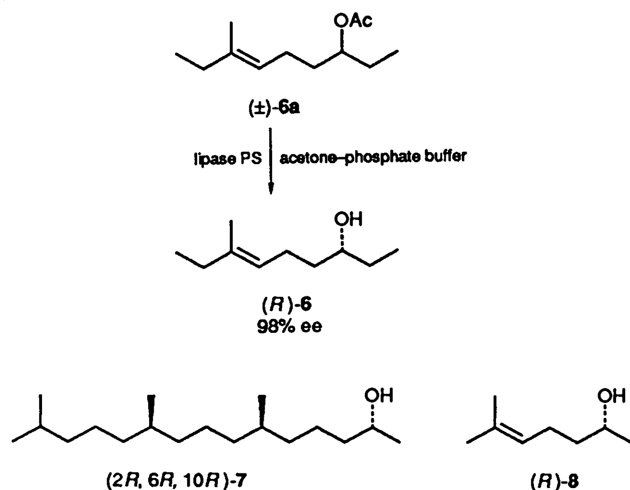
† It has also been found that enzyme enantioselectivity for the esterification and the transesterification can be controlled, enhanced, or reversed by appropriate selection of the reaction medium.[9] In addition to being of mechanistic and theoretical interest, these observations make it possible for organic chemists to control the enantioselectivity of enzymes by changing the reaction solvent; that is, by a method common in synthetic chemistry rather than in biological science such as protein engineering.

Table 3 Enzymatic hydrolysis of unsaturated acetate **5a** in organic-water solvent systems[a]



Organic solvent	Time (t/h)	Convrsn (%)	Yield (%)/ee (%) (R)-5	Yield (%)/ee (%) (S)-5a	E
None[b]	50	42	35/81	49/53	16
Acetone	63	41	32/89	50/69	36
Hexane	55	46	30/80	46/72	20
Diethyl ether	221	38	20/86	40/54	23

[a] Reactions were carried out in a mixture of organic solvent (6 cm^3) and phosphate buffer (9 cm^3). [b] Reaction was run in phosphate buffer (15 cm^3).



explanation to assume that this phenomenon arises from a conformational change of the lipase enzyme induced by a particular interaction between the lipase and the organic medium.

The present enzymatic strategy in an acetone–water system was explored in the synthesis of two kinds of optically active natural products, (2*R*,6*R*,10*R*)-6,10,14-trimethylpentadecan-2-ol **7** and (*R*)-6-methylhept-5-en-2-ol **8**. The former saturated alcohol, which belongs to a class of long-chain alkan-2-ols, is believed to be the natural form of the sex pheromone of *Corcyra cephalonica*,[10] and the latter unsaturated alcohol, called sulcatol, which is classified as a short-chain alkan-2-ol, is an aggregation pheromone of ambrosia beetles.[11]

For the synthesis of the alcohol (2*R*,6*R*,10*R*)-**7** a diastereoisomeric alcohol, *viz* (2*RS*,6*R*,10*R*)-**7**, which was prepared starting from (*R*)-β-citronellol (*R*)-**9** and methyl (*S*)-3-hydroxy-2-methylpropionate (*S*)-**12**, was converted into the corresponding acetate **7a** (Scheme 1).[5] Acetate **7a** was hydrolysed with lipase PS in acetone–phosphate buffer to give the alcohol (2*R*,6*R*,10*R*)-**7** of 94% ee, which, after conversion into its acetate **7a**, was resubmitted to lipase hydrolysis (Scheme 2). The resulting alcohol (2*R*,6*R*,10*R*)-**7** showed an enantiomeric purity of almost 100%. (±)-Sulcatol (**8**) was resolved by the analogous two-step enzymatic pathway. Unsaturated acetate **8a** was therefore initially hydrolysed with lipase PS, and the (*R*)-alcohol **8** of 93% ee was obtained. This alcohol was immediately transformed into its acetate, and the latter was submitted to the second hydrolysis with lipase PS to give (*R*)-sulcatol **8** of almost 100% ee (Scheme 3).*

* A biochemical synthesis of (*R*)-sulcatol **8** has been effected by a two-step procedure involving initial microbial reduction with *Pichia miso* IAM 4682 followed by PPL-catalysed acylation.[12]

Experimental

General.—IR spectra were determined on a Fourier transform Perkin-Elmer 1720 IR spectrometer. ^1H NMR spectra were obtained on a Fourier-transform Hitachi R-1500 (60 MHz) spectrometer for $CDCl_3$ solutions with Me_4Si as internal standard. Gas chromatography was carried out on a Hitachi G-3000 gas chromatograph equipped with a PEG-20M 25 m × 0.25 mm WCOT fused silica capillary column. Optical rotations were measured on a Horiba SEPA-200 high-sensitivity polarimeter; $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. Column chromatography was performed with 70–230 mesh silica gel (Merck Kieselgel 60 Art. No. 7734) and 230–400 mesh silica gel (Merck Kieselgel 60 Art. No. 9385). Analytical samples were prepared by a combination of column chromatography and micro-vacuum distillation with a Kugelrohr distillation apparatus.

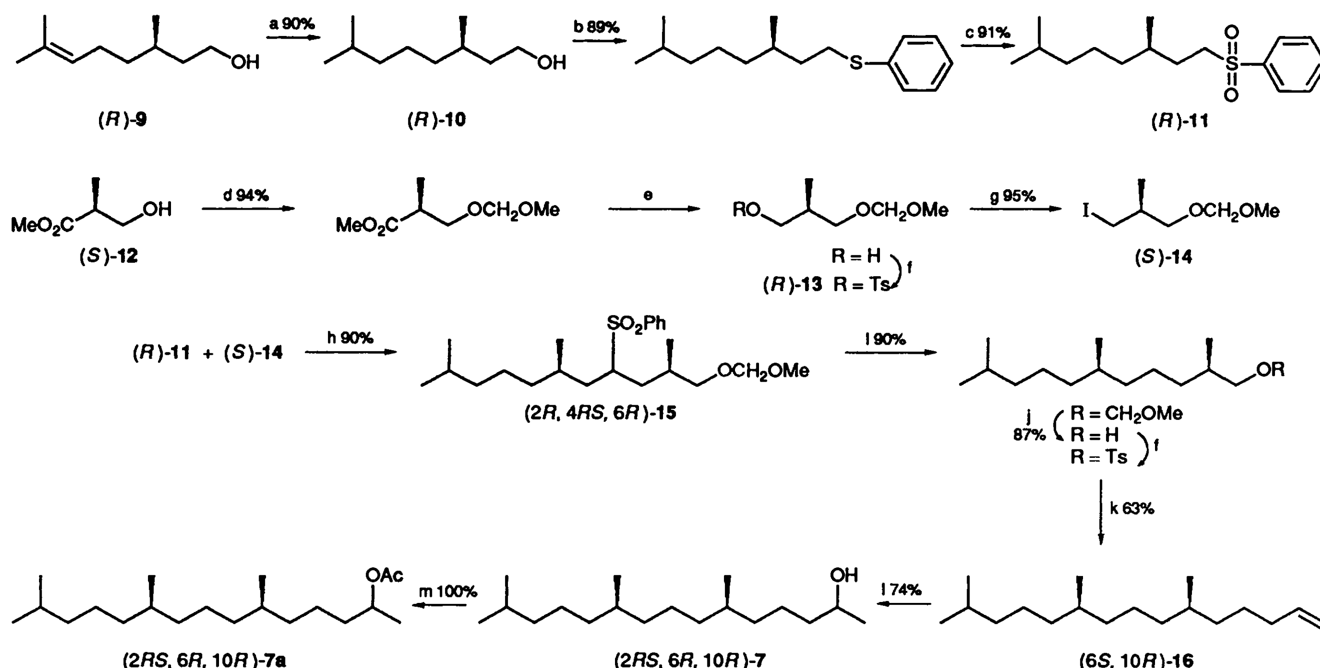
Determination of Enantiomeric Purity.—The enantiomeric purity of alcohol products, (*R*)-**1**, (*R*)-**2**, (2*R*,6*R*,10*R*)-**7**, and (*R*)-**8**, was determined by HPLC analysis of their α-methoxy-α-(trifluoromethyl)phenylacetic acid [(*R*)-MTPA] esters, using a Hitachi L-6250 liquid chromatograph equipped with a UV detector [column Partisil 5 (4.6 × 250 mm); eluent hexane–1,2-dichloroethane–methanol (900:10:0.1); flow rate 1.0 cm^3 min^{-1}; detection 220 nm]. The enantiomeric purity of alcohols (*R*)-**3**, (*R*)-**4**, and (*R*)-**5** was estimated after HPLC analysis of their 3,5-dinitrophenylurethane derivatives prepared by treatment with 3,5-dinitrophenyl isocyanate, using a Waters 510 liquid chromatograph equipped with a UV detector (254 nm). A Sumipax OA 2100 4.0 × 250 mm column was used with hexane–1,2-dichloroethane–ethanol (80:10:1); flow rate 1.0 cm^3 min^{-1}. The enantiomeric excesses of the acetates (*S*)-**1a** to (*S*)-**5a**, (2*S*,6*R*,10*R*)-**7a** and (*S*)-**8a** are based on those of their corresponding alcohols.

General Procedure for Lipase-catalysed Hydrolysis of Acetates in an Acetone–Water System.—A mixture of each acetate (2.5–2.6 mmol), lipase PS (0.2 g), acetone (6 cm^3) and 0.1 mol dm^{-3} phosphate buffer (9 cm^3) was stirred at room temperature for the required period (see Tables 1–3). After filtration through Celite, each filtrate was extracted with diethyl ether and the extract was worked up. Purification of each crude product by column chromatography with hexane–ethyl acetate (30:1) gave (*R*)-alcohols and (*S*)-acetates, which were fully characterized on the basis of their spectral data. According to this procedure the following (*R*)-alcohols were prepared: (R)-**1**, 96% ee; $[\alpha]_D^{20}$ −8.75 (*c* 1.69, pentane); (*R*)-**2**, 80% ee; $[\alpha]_D^{20}$ −9.72 (*c* 0.37, pentane); (*R*)-**3**, 80% ee; $[\alpha]_D^{20}$ −6.05 (*c* 3.17, pentane); (*R*)-**4**, 81% ee; $[\alpha]_D^{20}$ −6.76 (*c* 3.25, pentane); (*R*)-**5**, 89% ee; $[\alpha]_D^{20}$ −10.38 (*c* 1.81, pentane).

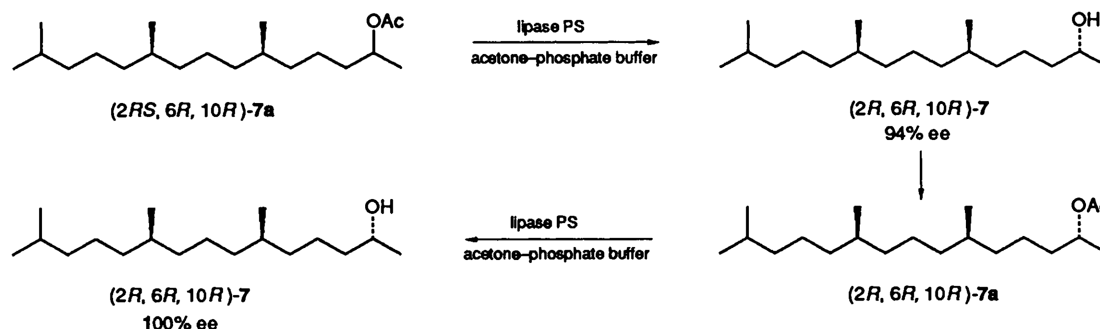
(R)-3,7-*Dimethyloctan*-1-*ol* (R)-**10**.—Citronellol (*R*)-**9** (5.0 g, 32 mmol, ~98.5% ee) was reduced by a combination of $CoCl_2$ (20.8 g, 160 mmol) and $LiAlH_4$ (7.30 g, 192 mmol) under argon according to the procedure described previously.[10] Distillation of the crude product gave title product (*R*)-**10** as a liquid (4.54 g, 90%), b.p. 67–69 °C (1.0 mmHg); $[\alpha]_D^{20}$ +6.02 (*c* 4.22, MeOH) {lit.,[5] $[\alpha]_D^{20}$ −6.10 (*c* 4.0, MeOH) for the (*S*)-enantiomer; lit.,[10] $[\alpha]_D^{20}$ +5.3 (neat, *d* 0.827)}.

(R)-3,7-*Dimethyl*-1-*phenylsulfonyloctane* [(R)-**11**].—This compound was prepared from the alcohol (*R*)-**10** according to the reported procedure:[5] $[\alpha]_D^{20}$ −7.89 (*c* 3.55, MeOH) {lit.,[5] $[\alpha]_D^{20}$ +8.16 (*c* 2.65, MeOH) for the (*S*)-enantiomer}. The IR and ^1H NMR spectra were identical with those of its (*S*)-isomer.[5]

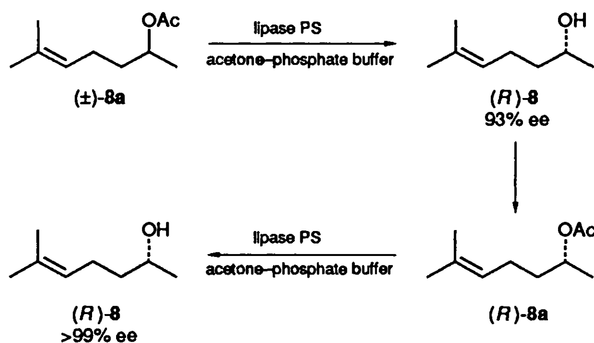
(S)-1-*Iodo*-3-*methoxymethoxy*-2-*methylpropane* [(S)-**14**].—Methyl (*S*)-3-hydroxy-2-methylpropionate [(*S*)-**12**, ~100%



Scheme 1 (*a*) $CoCl_2$, $LiAlH_4$, tetrahydrofuran (THF); (*b*) $(PhS)_2$, Bu_3P, CH_2Cl_2; (*c*) *m*-chloroperbenzoic acid, CH_2Cl_2; (*d*) $MeOCH_2Cl$, Pr^i_2NEt, CH_2Cl_2; (*e*) $LiAlH_4$, Et_2O; (*f*) TsCl, pyridine; (*g*) NaI, butan-2-one; (*h*) LDA, hexamethylphosphoric triamides; (*i*) Na–Hg, MeOH; (*j*) aq. HCl, MeOH; (*k*) $CH_2{=}CHCH_2CH_2$ MgBr, Et_2O) (*l*) $Hg(OAc)_2$, aq. THF; $NaBH_4$, aq. NaOH; (*m*) AcCl, pyridine, Et_2O.



Scheme 2



Scheme 3

ee] was converted by the reported procedure[5] into the tosate **13**, which, in turn, was treated with NaI in butan-2-one to give title compound (*S*)-**14**; $[\alpha]_D^{20}$ +8.63 (*c* 3.80, MeOH) {lit.,[5] $[\alpha]_D^{20}$ −9.14 (*c* 2.89, MeOH) for the corresponding (*R*)-bromide}. The IR and ^{1}H NMR spectra of compound (*S*)-**14** were indistinguishable from those of its corresponding (*R*)-bromide.

(2R,4RS,6R)-1-*Methoxymethoxy*-2,6,10-*trimethyl*-4-*phenylsulfonylundecane* [(2R,4RS,6R)-**15**].—This phenylsulfonyl derivative was prepared by the coupling of (*R*)-**11** and (*S*)-**14** in the presence of lithium diisopropylamide (LDA) according to the known procedure.[5] Compound **15** was identified by comparison of its IR and ^{1}H NMR spectra with those of the (2*S*,4*RS*,6*S*)-isomer.[5]

(2RS,6R,10R)-6,10,14-*Trimethylpentadecan*-2-*ol* [(2RS,6R,10R)-**7**].—According to the reported procedure[5] compound (2*R*,4*RS*,6*R*)-**15** was converted, *via* alkene (6*S*,10*R*)-**16**, into a diastereoisomeric alcohol (2*RS*,6*R*,10*R*)-**7**, which was characterized by comparison of its IR and ^{1}H NMR spectra with those of the (2*RS*,6*S*,10*S*)-isomer.[5]

The Acetate of (2RS,6R,10R)-6,10,14-*Trimethylpentadecan*-2-*ol* **7**, *compound* [(2RS,6R,10R)-**7a**].—This compound was prepared from the corresponding alcohol (2*RS*,6*R*,10*R*)-**7** (2.2 g, 8.14 mmol) and acetyl chloride (1.0 g, 12.3 mmol) in dry diethyl ether (50 cm^3) in the presence of pyridine (1.0 g). Purification of the product by column chromatography with hexane–ethyl acetate (20:1) gave (2*RS*,6*R*, 10*R*)-**7a** with almost quantitative yield: ν_{max}(neat)/cm^{-1} 2927, 1741, 1463, 1373, 1245, 1129, 1023, 949 and 737; δ_H 0.80–1.57 (36 H, br m), 2.02 (3 H, s) and 4.85 (1 H, m) (Found: C, 76.8; H, 12.9. $C_{20}H_{40}O_2$ requires C, 76.86; H, 12.90%).

Synthesis of Compound (2R,6R,10R)-**7** *by Enzymatic Hydrolysis of the Acetate* (2RS,6R,10R)-**7a**.—A mixture of the acetate

(2*RS*,6*R*,10*R*)-**7a** (1.0 g, 3.7 mmol), lipase PS (0.4 g), acetone (12 cm^3), and 0.1 mol dm^{-3} phosphate buffer (18 cm^3) was stirred for 37 h at room temperature. GC analysis showed that conversion was ~35%. After filtration through Celite, the filtrate was extracted with diethyl ether and the extract was worked up. Purification by column chromatography with hexane–ethyl acetate (30:1) gave the alcohol (2*R*,6*R*,10*R*)-**7** (0.23 g, 26.6%) with 94% ee, $[\alpha]_D^{20}$ −5.70 (*c* 4.82, pentane). This alcohol was reconverted into its corresponding acetate (2*R*,6*R*,10*R*)-**7a** (0.197 g, 0.63 mmol) and the latter was added to a mixture of lipase PS (0.1 g), acetone (2.4 cm^3), and phosphate buffer (3.6 cm^3). The mixture was stirred for 33 h at room temperature; GC showed a conversion of 73%. Column chromatography as already described gave the enantiomeric alcohol (2*R*,6*R*,10*R*)-**7** (0.12 g, 70.6%) with almost 100% ee, $[\alpha]_D^{20}$ −6.54 (*c* 4.93, pentane) {lit.,[10] $[\alpha]_D^{18}$ −6.4 (*c* 1.07, pentane)}. The IR and ^{1}H NMR spectra were identical with those reported previously.[10]

Synthesis of the Alcohol (R)-**8** *by Enzymatic Hydrolysis of Racemic Acetate* (±)-**8a**.—Acetate (±)-**8a** (2.0 g, 11.76 mmol), which was prepared from racemic alcohol (±)-**8** in the usual manner, was added to a mixture of lipase PS (0.8 g), acetone (24 cm^3), and 0.1 mol dm^{-3} phosphate buffer (36 cm^3), and the mixture was stirred for 41 h at room temperature. GC showed a conversion of 32%. Column chromatography with hexane–ethyl acetate (30:1) gave the alcohol (*R*)-**8** (0.43 g, 28.5%) with 93% ee, $[\alpha]_D^{20}$ −15.83 (*c* 2.88, EtOH). This alcohol was reconverted into its acetate (*R*)-**8a** (0.22 g, 1.29 mmol), and the latter was treated with lipase PS (0.1 g) in a mixture of acetone (2.4 cm^3) and phosphate buffer (3.6 cm^3). The mixture was stirred for 35 h at room temperature; the conversion reached 61%. Column chromatography as described gave the alcohol (*R*)-**8** (0.096 g, 58%) with >99% ee, $[\alpha]_D^{20}$ −19.55 (*c* 3.14, EtOH) {lit.,[12] $[\alpha]_D^{23}$ −16.7 (*c* 1.18, EtOH)}. The IR and ^{1}H NMR spectra were identical with those of authentic racemic alcohol (±)-**8**.

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