Lipase AKG mediated resolutions of α,α-disubstituted 1,2-diols in organic solvents; remarkably high regio- and enantio-selectivity

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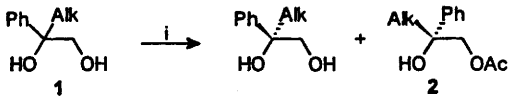
Diols 1, which contain adjacent tertiary and primary hydroxy groups, can be selectively mono-acylated at the primary hydroxy group by many lipases in organic solvents. Since the reaction does not take place at the chiral tertiary centre itself, observed enantioselectivities are usually low. Only the combination of one lipase, lipase AKG (Amano, *Pseudomonas sp.*), with selected substrates gives high enantioselectivities (*E* 20 to >200). Also, the solvent and acyl donor employed influences the outcome. On the basis of the results of lipase AKG towards substrates 1 an active site model for this specific lipase has been developed, which can account for the results obtained. Full experimental details on the synthesis of diols 1 and enzymatic preparation of acetates 2 are given. Also, the absolute stereochemistry of the enzymatically prepared diols 1 has been established by independent synthesis from (*R*)-mandelic acid.

Optically pure 1,2-diols are important building blocks in asymmetric synthesis. They are easily converted to epoxides,[1] aziridines[2] and amino alcohols,[3] all compounds which have shown their value in asymmetric synthesis in recent years. Applications of this type of vicinal diol as ligands in metal-catalysed enantioselective reactions are known as well,[4] and such diols have been used for the determination of the enantiomeric excess of chiral ketones by acetal formation.[5] Straightforward methodologies have been developed to prepare some vicinal diols in enantiopure form, an example being (*R*)-1-phenylethane-1,2-diol.[6] However, 1,2-diols having a tertiary (rather than the usual secondary) chiral centre adjacent to a primary hydroxy group cannot be prepared following these strategies. Optically pure tertiary diols of the general structure **1** (Scheme 1) have only been reported infrequently.



1 **2**

Scheme 1 *Reagents and conditions:* i, lipase, diisopropyl ether, vinyl acetate

One report comes from a German patent in which it is stated that the compounds **1** serve as building blocks for the synthesis of triazole derivatives, which show strong fungicide and growth regulating activities.[7] These activities depend on the absolute configuration of the chiral centre. Other routes to optically pure diols of type **1** have been reported, but all rely on the use of expensive (optically pure) reagents and/or laborious multi-step procedures.[8]

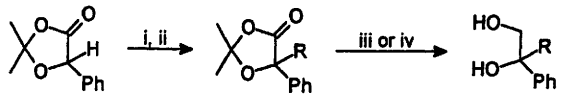
A catalytic route to prepare vicinal diols in (usually very high) enantiomerically enriched form is the *cis*-dihydroxylation of alkenes as described by Sharpless.[9] Although this methodology has also been applied to several *gem*-disubstituted alkenes, only one diol of type **1** (derived from α-methylstyrene) has been prepared in good enantiomeric excess.[10] We became interested in developing an expeditious route to such diols and at the same time wanted to test the scope of some recently developed enzyme methodologies. Since we already had experience with the use of esterases and lipases for the kinetic resolution of α-alkylated mandelic and lactic acids,[11] α-mercapto acids,[12] hydroxyfuranones[13] and hydroxypyrrolinones[14] we wondered whether we could use a similar approach for the resolution of racemic α-alkylated glycols **1**. In a preliminary communication we reported that for diols **1a–e**, lipase-catalysed acylation always proceeds at the primary hydroxy group to give **2**. Acylation of the tertiary hydroxy group was never observed.[15] Good *E* values could be obtained by proper choice of enzyme and conditions. This work is described here in detail.

In the work that has been reported in preliminary form it was shown that fifteen commercially available lipases were capable of catalysing the model reaction as shown in Scheme 1, although in a number of cases very long reaction times (up to one month) were needed to achieve reasonable conversions. As the reaction site is relatively far removed from the chiral centre, usually low chiral discriminations ($E < 5$) were observed using these lipases.† Only one, lipase AKG, showed an acceptable combination of reactivity coupled with enantioselectivity (*E* 20–200) for substrates **1** bearing certain types of alkyl substituents at the tertiary centre. The striking common feature among these substrates was the presence of unsaturation in the α-alkyl chain. As there seems to be something unique about the combination of lipase AKG and these substrates **1** we hoped we could gain more insight in this process of recognition by preparing a range of diols **1** and mapping the selectivity of lipase AKG towards these substrates.

Results and discussion

Synthesis of diols 1

We particularly desired phenyl substituted derivatives **1**, which could be readily prepared, usually in good yield, by the strategy shown in Scheme 2. By transacetalization of mandelic acid with dimethoxypropane, dioxolanone **3** can be prepared in nearly



3 **4** **1**

Scheme 2 *Reagents and conditions:* i, LDA, THF, −78 °C; ii, RX −78 to 20 °C; iii, $LiAlH_4$, THF, heat; iv, $NaBH_4$, MeOH, Bu^tOH, heat

† Lipases A (*Aspergillus niger*), AKG (*Pseudomonas sp.*), AY (*Candida rugosa*), CE (*Humicola lanuginesa*), D (*Rhizopus delemar*), G (*Penicillium camembertii*), GC (*Geotrichum candidum*), L (*Candida lipolytica*), M (*Mucor javanicus*), N (*Rhizopus niveus*), PS (*Pseudomonas cepacia*) and R (*Penicillium roqueforti*) from Amano as well as *Hog pancreatine* and *Candida cylindracae* (*rugosa*) from Sigma and *Candida antarctica* from Novo were tested.

Table 1 Preparation of diols **1** from dioxolanone **3**

Entry	Compound	R	Overall yield (%)
1	**1a**	Et	83
2	**1b**	Pr	52
3	**1c**	$CH_2CH{=}CH_2$	73
4	**1d**	(*E*)- and (*Z*)-$CH_2CH{=}CHCH_3$	60
5	**1e**	CH_2Ph	92
6	**1f**	CH_2-*p*-MePh	81
7	**1g**	$CH_2CH{=}CHPh$	67
8	**1h**	1-Naphthyl-CH_2	21
9	**1i**	$CH_2CH_2CH_2Ph$	64

quantitative yield.[11b] Previously we had shown that such dioxolanones can be easily α-alkylated by deprotonation with lithium diisopropylamide (LDA) followed by electrophilic trapping of the enolate formed using various alkyl halides.[11b] The products, the α-alkylated dioxolanones **4**, can subsequently be reduced to yield the desired diols **1** (Table 1) in overall yields of pure material ranging from 52–92% (with the exception of **1h**) based on **3**. This strategy was also shown to work for other α-hydroxy acids (*e.g.* lactic acid and phenyllactic acid) and some α-mercapto acids (thiolactic acid and thiomandelic acid); these results are not described in this paper. The alkylation is, however, limited to primary alkyl halides and allylic or benzylic halides. Secondary as well as branched primary alkyl halides fail to react probably due to steric repulsion, and not surprisingly, tertiary halides also do not serve as alkylating agents. Alkyl halides used without success in the reaction of Scheme 2 were isopropyl iodide, *tert*-butyl iodide, isoamyl bromide, phenylethyl bromide, cyclohexyl bromide and 2-bromomethyldioxolane. Reaction with 1-chloromethylnaphthalene to give **4h** proceeded only partially even at room temperature overnight. No further attempt was made to improve the yield.

Usually, $LiAlH_4$ can be employed for the reduction of **4** to **1**. However, for **4g**, partial reduction of the conjugated double bond took place as well, giving rise to a mixture of **1g** and **i**. On catalytic hydrogenation (Pd/C) this mixture was completely converted to **1i**. Selective formation of **1g** can be achieved by reduction of **4g** with $NaBH_4$ in a mixture of MeOH and Bu^tOH;[16] the conjugated alkene fragment remains intact under these conditions.

Lipase-catalysed resolution of diols 1

Lipase AKG-catalysed resolutions were carried out with substrates **1** (0.3 mmol) and the lipase (50% m/m) in a 3:1 mixture of diisopropyl ether (dipe) and vinyl acetate (va). A magnetic stirrer was used. Conversions[17] and enantiomeric excesses of both starting material **1** and product **2** were determined by analysis of aliquots at regular intervals by chiral HPLC (Daicel OJ column). Usually, by modification of eluent and flow rate all 4 peaks arising from the enantiomers of **1** and **2** could be detected in a single run. An example is depicted in Fig. 1.

Results of the lipase AKG-catalysed resolution of diols **1** are presented in Table 2. Once again it is stressed that 14 other lipases showed hardly any enantioselectivity towards these substrates.[15] From these results it is clear that the presence of unsaturation in the α-alkyl chain is essential for chiral discrimination. For compounds having a totally aliphatic side-chain (entries 1, 2 and 11), barely any chiral discrimination is observed ($E < 3$). For compounds having an allylic (entries 3, 4, 5 and 9) or benzylic (entries 6, 7 and 8) fragment, moderate ($E = 15$) to excellent ($E > 200$) enantiomeric ratios are observed, a borderline case being the naphthyl derivative **1h** (entry 10).

Also we examined whether substituents on the aromatic ring

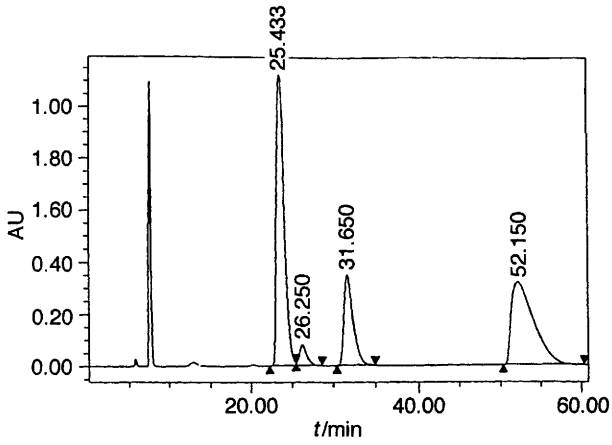
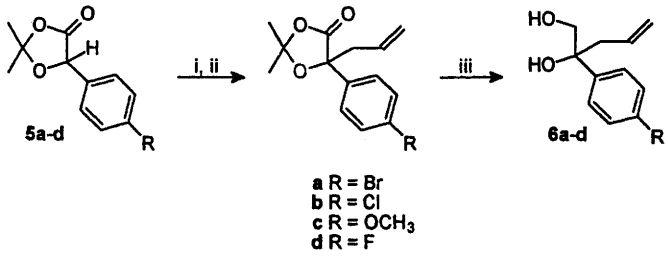


Fig. 1 HPLC diagram of **1c** (1st and 3rd eluting peaks) and **2c** (2nd and 4th eluting peaks)

would have influence on the chiral discrimination. Dioxolanones **5a–d** derived from substituted mandelic acids[18] were converted to α-allyl diols **6a–d** in 78–84% yield (Scheme 3). The



Scheme 3 *Reagents and conditions:* i, LDA, THF, −78 °C; ii, allyl bromide, −78 to 20 °C; iii, $LiAlH_4$, THF, heat

replacement of the proton at the *para* position by a larger substituent had a dramatic influence. Whereas **1c** showed an *E* of 15 (entry 1, Table 3), derivatives **6a–c** were not enantioselectively recognized by lipase AKG (entries 2, 3 and 4, Table 3). Only the fluorine derivative **6d** shows moderate enantioselectivity ($E = 8$, entry 5), although the rate of acylation is slow. As fluorine is comparable in size to a proton we suspect that the presence of larger substituents on the aromatic ring makes the fit of these substrates in the active site of lipase AKG less good (probably disrupting the catalytic machinery in the sense of the observations of Kazlauskas *et al.* for *Candida rugosa* lipase)[19] and therefore decreases chiral discrimination.

From the data described in Tables 2 and 3 is is clear that lipase AKG, in pronounced contrast to many other lipases, is capable of giving good discrimination for specific substrates. Although the exact reasons for the specificity of this lipase are not known, the unsaturation in the side chain is without doubt important. Also, substitution in the aromatic ring is not well tolerated by lipase AKG. Therefore, we concluded that this lipase must have, compared to the other lipases, something specific in its active site giving stabilizing interactions with the unsaturated part of diol **1**. However, since a 3D-structure of the active site of this specific lipase is not available, we only have recourse to an empirical model to account for these results.

An empirical active site model for lipase AKG

One way to envision interactions between the active site of an enzyme and a substrate is on the basis of 'pockets', which give an indication of the size and the shape of the molecules tolerated in the active site. The dimensions of these pockets are determined by the results of series of molecules tested. In this way a model has been developed by Jones for Pig Liver Esterase (PLE)-catalysed kinetic resolutions in aqueous media.[20] This

Table 2 Lipase AKG-catalysed resolution of diols **1** in dipe

Entry	Substrate	Reaction time/h	Conversion (%)	Ee **1** (%)	Ee **2** (%)	*E*
1	**1a**	17	13	< 2	7	1
2	**1b**	24	8	< 2	< 2	< 1
3	**1c**	44	27	31	82	15
4	**1c**	120	60	94	62	15
5	(*E*)-**1d**[a]	72	59	94	65	16
6	(*Z*)-**1d**[a]	72	59	57	40	4
7	**1e**	48	50	95	95	> 100
8[b]	**1e**	72	50	97	97	> 200
9[b]	**1f**	144	49	90	94	100
10	**1g**	114	58	> 99	73	60
11	**1h**	114	62	87	54	9
12	**1i**	138	41	19	27	2

[a] Mixture of (*E*)- and (*Z*)-**1d** used; conversion followed separately by chiral GC. [b] Benzene was employed as solvent.

Table 3 Lipase AKG-catalysed resolution of diols **6** in dipe

Entry	Substrate	Reaction time/h	Conversion (%)	Ee diol (%)	Ee acetate (%)	*E*
1	**1c**	44	27	31	82	15
2	**6a**	48	14	5	31	2
3	**6b**	72	8	5	56	4
4	**6c**	24	11	4	34	2
5	**6d**	96	22	21	74	8

model is known to have a high predictive value in enzymatic hydrolysis experiments using this esterase. In recent years, Jones has explored the boundaries of his model to make its dimensions more accurate. His latest update of the model is now considered by him to be the final one in which the exact dimensions of the pockets are known.[21] To make such an accurate model one has to have a large series of results available, stemming from different types of molecules. This is possible for PLE because it is one of the most frequently applied biocatalysts in organic chemistry. The successful application of lipase AKG for resolution of racemates has, however, seldom been reported in literature. There are, however, some reports on lipase AK (also from Amano); lipase AKG is the same enzyme but has a different physical form.[22] For lipase AK a crude model[23] for resolution of secondary alcohols has been proposed in which it is postulated that the active site is 'flat' because of its preference for the resolution of nearly planar (unsaturated) molecules. It is indeed striking that most molecules that are resolved with good chiral recognition ($E > 20$) by lipase AK are unsaturated somewhere in their backbone. Lipase AK also fulfils the general empirical rule for lipases that the enantiomer of a secondary alcohol that reacts fastest is the one with the small (or medium) substituent on the left and the large group on the right with the hydroxy group oriented towards the reader.[19,23]

Also, the molecules found to be resolved reasonably by lipase AKG have some kind of unsaturation in the α-alkyl side chain, giving it conformational rigidity and a certain degree of flatness. Unfortunately, the model of lipase AK as proposed by Burgess[23] is not applicable to diols **1** because it pertains to the resolution of secondary alcohols in which the hydroxy group is directly positioned at the chiral centre. In diols **1** the chiral centre is positioned two atoms away from the reaction site, making a translation of the diols **1** in the Burgess model necessary to position the reaction site properly. After translation the position of the different alkyl chains in the described pockets no longer gives any concrete information concerning the stereoselectivity of the process.

A model for lipase AKG which suits diols **1** was set up as follows. In analogy to the Burgess model,[23] one of the pockets of lipase AKG is assumed to be flat, in which only nearly planar side chains, in an extended conformation, can be positioned. This pocket is designated as the F (flat) pocket. The site where reaction takes place, at the primary hydroxy group, is depicted as the R (reaction) pocket. A third pocket in which the phenyl group is positioned is the S (small) pocket. The remaining tertiary hydroxy group may be stabilized by polar amino acid residues in the active site.

From experiments with diols **6** with lipase AKG we know that an increase of the bulk at the *para* position of the aromatic ring leads to loss of enantiomeric discrimination (although the reactivity remains about the same). Probably the aryl group is too large to fit well in the S pocket. The dimensions of the F pocket are not known.

In Fig. 2 the model for lipase AKG, based on the results of the diols described above, is depicted and both enantiomers of diol **1c** are placed in this model. It can be seen that the (*R*)-enantiomer gives an acceptable fit of the flat allyl group in the F pocket, and of the phenyl group in the S pocket. Positioning of the (*S*)-enantiomer in the same model, however, leads to a loose fit of the allyl group in the S pocket and improper positioning of the phenyl group in the F pocket. The fit for this enantiomer is not ideal and therefore the (*R*)-enantiomer will be acylated by preference, provided that the enantiomer that is bound most strongly is also acylated most rapidly. This latter point seems reasonable but has not been proven.

From the same model it can be concluded that aliphatic (staggered) side chains such as ethyl and propyl do not give a good fit in the F pocket for the (*R*)-enantiomers since they are not flat, whereas for the (*S*)-enantiomer the phenyl group does not give a good fit in this pocket. Therefore, **1a** and **b** are not acylated selectively. Introduction of a sterically demanding group at the *para* position of the aromatic ring (as for **6a–c**) destabilizes interaction of the phenyl group in the S pocket (the substrate is too large), and neither of the enantiomers will be acylated preferentially, resulting in low chiral recognition. The best evidence for this model, however, stems from the results obtained for α-benzyl diol **1e**. Lipase AKG shows an $E > 200$ (entry 7, Table 2) for this substrate. The (*R*)-enantiomer would give a perfect fit in our active site model for lipase AKG, whereas for the (*S*)-enantiomer both the phenyl group and the benzyl group fall outside the boundaries of the S and F pockets. Therefore, the chiral recognition for this specific diol is both

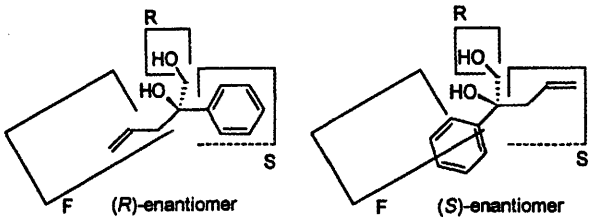


Fig. 2

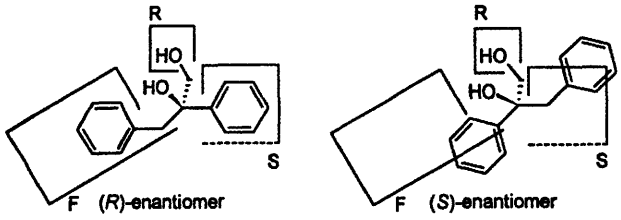


Fig. 3

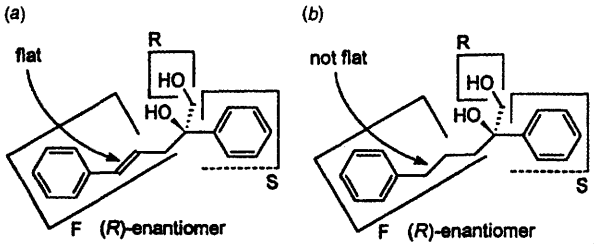


Fig. 4

expected and found to be very high (Fig. 3). Interestingly, other lipases tested only gave enantiomeric ratios of $E < 6$ for this specific substrate.

Following the same arguments as for **1e**, the *p*-tolyl substituted diol **1f** should be recognized as well with high enantiodiscrimination by lipase AKG. Indeed **1f** is resolved with an enantiomeric ratio of $E = 100$ in benzene. At 51% conversion the remaining alcohol had an ee of 96% (entry 8, Table 2).

The cinnamyl derivative **1g** is also resolved smoothly by lipase AKG ($E = 60$, entry 9, Table 2) as predicted by the model. The unsaturated and flat cinnamyl chain should easily fit in the F pocket, and the phenyl group in the S pocket [Fig. 4(*a*)]. Catalytic hydrogenation of the double bond of **1g** leads to **1i**. The alkyl chain of this compound is unsaturated, has a zig-zag conformation, and therefore does not meet the flatness requirements of the F pocket. Also, a good fit of this carbon chain in the S pocket is unlikely due to steric constraints. From the model chiral recognition is therefore expected to be low [Fig. 4(*b*)]. From the result in Table 2 (entry 11) it is clear that this is indeed the case. An enantiomeric ratio of only 2 is found for **1i**. Moreover, the overall reactivity decreases.

There might even be a physical basis for this model. Helpful discussions with Dr D. Lang and Professor B. W. Dijkstra[24] at our university have provided us with insight in the actual shape and size of the active site of *Pseudomonas* lipases. They have determined the structure of both the open and the closed form of a lipase from *Pseudomonas glumae*.[25] Although the chance that this specific lipase is identical to lipase AKG is small, there might be enough homology to justify comparison of our model with this 3D-structure. It is clear that in the active site of *P. glumae*, there is, relatively close to the catalytic machinery and the oxyanion hole, a rather polar position which could accommodate the tertiary hydroxy group. Moreover, there are two hydrophobic regions which can accommodate the alkyl substituents. One (smaller) hydrophobic region is positioned to the back and could bind the phenyl group; a larger hydrophobic region is positioned to the front. This region might accommodate larger alkyl fragments, in our case the benzyl group. In this specific lipase this hydrophobic region is wide enough to accommodate all kinds of hydrophobic groups. Lipase AKG might, however, have an additional insertion in the peptide sequence and therefore this specific region might be deformed in such a way that only flat molecules can be embedded. Also, there might be a hydrophobic opening (a flat tunnel) towards the core of the lipase in which the side chain might be embedded. Although speculative, this might be an explanation of the unique character of lipase AKG.

Solvent and acyl donor effects

The effects of the solvent and acylating agent on the reaction of Scheme 1 were also examined. As a test substrate diol **1c**, which was moderately ($E = 15$) resolved under the standard conditions, was examined. Ten solvents were tested, ranging from polar to apolar, for the resolution of **1c** using lipase AKG. On going from polar to more apolar solvents the reactivity *and* enantioselectivity of this particular reaction increase (Table 4). For example, in the polar solvent acetonitrile after 94 h only 16% conversion was achieved with an E of 5 (entry 1). After the same reaction time in benzene the conversion was already 42% and the corresponding E was 24 (entry 12). This good enantioselectivity in benzene remained constant during the reaction and at 52% conversion the remaining alcohol had an enantiomeric excess of 85%, which corresponds to an enantiomeric ratio of 22 (entry 13). From these experiments we conclude that benzene is the best solvent to carry out the resolution experiments, although our initial choice, the less carcinogenic dipe (entry 11), is a good alternative.

Although a nice trend can be observed going from polar to the more apolar solvents, a linear relationship between several solvent parameters, such as the relative permittivity (ε_r, Table 4), dipole moment and log P, and the enantiomeric ratio could not be found.

The acylating reagent is also known to have an effect on reactivity and enantioselectivity. Most often irreversible acylating reagents such as the enol esters, vinyl acetate or isopropenyl acetate are used for resolutions of alcohols, although reactive anhydrides, glycerol esters or activated esters can also be used. Therefore, five different acyl donors were tested for the resolution of diol **1c** employing lipase AKG in dipe. From Table 5 it can be seen that the initial choice, vinyl acetate, was indeed the best (entry 1). Although isopropenyl acetate gave a better chiral discrimination of $E = 23$ (entry 2) compared to vinyl acetate ($E = 14$), the reactivity was much lower. For this reason it is not attractive to use this specific acyl donor for resolution purposes. Ethyl acetate was unreactive (entry 3) and the activated ester trichloroethyl acetate as well as acetic anhydride were reactive but not enantioselective (entries 4 and 5). This lack of stereospecificity is the result of a competing non-catalysed reaction. In the absence of lipase AKG these latter two acylating reagents were shown to form spontaneously the acetate at room temperature. In contrast vinyl acetate gave, even after 10 days, not a trace of the product in the absence of a biocatalyst.

Establishment of the absolute configurations of diols 1

Although the model developed for lipase AKG is crude, it has a predictive value for this series of structurally similar diols. Since all these diols have to fit in the same fashion in this model, the stereochemical fate of the reaction has to be constant throughout the series. This means that all the remaining diols after resolution by lipase AKG should have the same absolute configuration. Our model, therefore, requires knowledge of the

Table 4 Lipase AKG-catalysed resolution of **1c** in different solvents

Entry	Solvent	ε_r	Reaction time/h	Conversion (%)	Ee **1c** (%)	Ee **2c** (%)	*E*
1	Acetonitrile	35.9	94	16	12	65	5
2	THF	7.58	94	18	16	71	7
3	EtOAc	6.02	94	19	17	71	7
4	Diethyl ether	4.20	69	31	35	79	11
5	Chloroform	4.81	164	30	33	77	11
6	CH_2Cl_2	8.93	164	24	26	84	13
7	Pentane	1.84	69	35	44	82	15
8	Hexane	1.88	69	29	34	83	15
9	mtbe[a]	4.50	138	35	44	80	15
10	Toluene	2.38	138	36	47	85	18
11	dipe	—	188	53	84	74	18
12	Benzene	2.27	94	42	62	87	24
13	Benzene	2.27	164	52	85	77	22

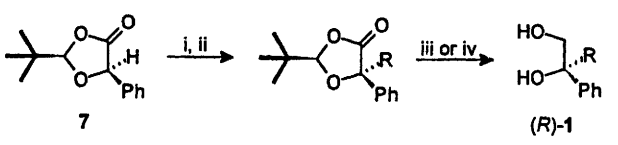
[a] Methyl *tert*-butyl ether.

Table 5 Lipase AKG-catalysed resolution of **1c** in dipe using several acyl donors

Entry	Acyl donor	Time/d	Conversion (%)	*E*
1	Vinyl acetate	5	30	14
2	Isopropenyl acetate	13	9	23
3	Ethyl acetate	20	< 2	—
4	Trichloroethyl acetate	8	18	2
5	Acetic anhydride	6	> 98	—

absolute configuration of the diols as a check of its validity. Unfortunately, only the optical rotation of **1c** (and corresponding absolute configuration) was known.[8c] Therefore we decided to prepare the diols in optically pure form by independent asymmetric synthesis, which would also allow determination of the absolute configuration.

The most trustworthy route to optically pure diols **1** is probably the Seebach strategy[26] as employed in the German patent.[7] This method consists of condensation of optically pure mandelic acid with pivalaldehyde with removal of water by azeotropic distillation. A *cis*/*trans* mixture of dioxolanones in about 90% de is obtained. The major *cis*-dioxolanone **7** can be obtained diastereoisomerically pure by a single crystallization. The enolate is generated with LDA, and the large *tert*-butyl group at the chiral acetal centre directs the incoming electrophile to the *trans* position. In this way nearly diastereoisomerically pure α-alkylated dioxolanones of unambiguous absolute configuration are obtained. Reduction subsequently yields the nearly enantiomerically pure diols (*R*)-**1**; the absolute configurations are unambiguous (Scheme 4).

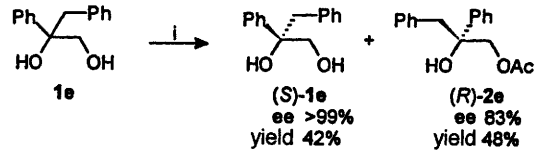


Scheme 4 *Reagents and conditions:* i, LDA, THF, −78 °C; ii, RX, −78 to 20 °C; iii, $LiAlH_4$, THF, heat; iv, $NaBH_4$, MeOH, Bu^{t}OH, heat

In this way (*R*)-diols **1c**,**e**,**f** and **g** were produced from **7** (overall 75–78% yield). Their enantiomeric purity was determined by chiral HPLC; the ees varied between 90–95%. It is clear that better enantiomeric purities can be obtained by our enzymatic approach. Subsequently, the retention times of the synthesized diols were compared with samples prepared from lipase AKG-catalysed resolution. In all cases the remaining diol of the resolution had the opposite enantiomeric composition compared to the diols prepared by chemical synthesis. Therefore, we can conclude that the enzymatically prepared diols always had the (*S*)-configuration (as also had been determined for **1c** by optical rotation) and that the acetate formed therefore must have the (*R*)-configuration. As expected, the stereochemical outcome of the lipase AKG-catalysed reactions is constant throughout the series of diols as is predicted by the model.

Preparative scale resolution of diols 1 and immobilization of lipase AKG

The reactions discussed so far were carried out on an analytical scale (0.3 mmol). We have looked at the problems involved in scale up. Scaling up to a preparative resolution is difficult due to a significant increase in reaction times. The main cause for this increase in (the already long) reaction times probably lies in mechanical denaturation of the biocatalyst. Procedures have been described to avoid this. For example, one can covalently bind lipases to polymeric carrier materials,[27] incorporate them into dialysis bags or membranes[28] and even into gels.[29] The simplest procedure is, however, to absorb them onto inert carrier materials such as Celite[30] or Hyflo SuperCell (HSC).[31] Absorption makes the lipase less sensitive to denaturation and therefore increases observed reaction speeds. Without immobilization, even after a reaction time of 1 month, a conversion of 50% was never reached for diol **1e**. However, by using lipase AKG on Celite (pH 7.00), diol **1e** (3 mmol) was converted in 47% yield to the acetate in 3 days. Whereas the *E* factor for this reaction on an analytical scale is > 200, the immobilized lipase AKG showed an enantiomeric ratio of 'only' 80. The obtained acetate **2e** had an enantiomeric excess of 94%. To obtain enantiomerically pure **2e** the conversion was allowed to reach 54% (4 days) and the remaining diol was indeed shown to have an enantiomeric purity of > 99% (Scheme 5).



Scheme 5 *Reagents and conditions:* i, lipase AKG (immobilized), benzene, vinyl acetate

Immobilization of lipase AKG on Celite at pH 8.00 gave about the same result. The enantiomeric ratio for this reaction was 64, but the reactivity decreased somewhat. A conversion of 46% was achieved only after 10 days. Also, for resolutions of hydroxyfuranones we had gained good experience with immobilizing lipases on inert carriers.[13] The best results for these reactions were obtained using HSC as carrier material. However, immobilized lipase AKG on HSC at pH 7.00 was less effective, as the *E* for the reaction described above dropped to 32.

Conclusions

We have shown that a large variety of tertiary glycols can be easily prepared from α-hydroxy acids such as mandelic acid, making use of simple chemical reagents and transformations. These diols can be regioselectively acylated by a number of lipases at the primary hydroxy group. Enantioselectivity for these types of acylations are in general, however, low. This is not surprising as reaction takes place relatively far away from the chiral centre. Only lipase AKG is capable of giving good chiral discrimination for a specific range of unsaturated substrates. Based on this series of substrates an active site model for lipase AKG has been devised, which seems to have a predictive value for this type of diols. Although a crystal structure is not available for the lipase, by comparison of our model with the structure of another *Pseudomonas* lipase we have shown that there might be an actual physical basis for the model. By independent synthesis the enantiomeric preference of lipase AKG throughout the series of diols is shown to be constant, as expected from the primitive model. Scaling the reactions up from 0.3 mmol to 3 mmol has proven to be successful as well. Reactivity can be enhanced significantly by immobilization of lipase AKG on Celite but it causes some loss in chiral discrimination.

A general point is the rather profound difference in reactivity and efficiency of enantioselection between various lipases even though most lipases seem to have common features for recognition of enantiomers.[19,23] Although their mode of action is closely related, most of them being serine proteases, there are clearly differences in the detailed shape of the surroundings of the active sites. Therefore, in surveys of new substrates a bank of *lipases should* always be investigated if acylation is being carried out. More subtle fine tuning can subsequently be accomplished by optimization of the organic solvent and acyl donor.

Experimental

All solvents were reagent grade and were dried and distilled prior to use, following standard procedures. All reagents were purchased from either Acros Chimica (previously Janssen Chimica), Aldrich, Merck or Fluka and used without purification unless stated otherwise. Lipase AKG was obtained from Amano and used as such. Melting points (uncorrected) were determined on a Mettler FP21 melting point apparatus equipped with a Mettler FP2 microscope. Optical rotations were determined at room temperature using a Perkin-Elmer 241 polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR spectra were recorded at room temperature on either a Varian Gemini-200 (200 MHz) or Varian VXR-300 (300 MHz) spectrometer. Chemical shifts are denoted in δ units (ppm), relative to tetramethylsilane (TMS) as internal standard or relative to residual solvent peaks; J values are given in Hz. ^{13}C NMR spectra (APT) were recorded on either a Varian Gemini-200 (50.32 MHz) or Varian VXR-300 (75.48 MHz) spectrometer. Chemical shifts are denoted in δ units (ppm) relative to $\delta(CDCl_3)$ 76.91. ^{19}F NMR spectra were recorded on a Varian Gemini-200 (188.143 MHz) spectrometer and are denoted in δ units (ppm), relative to $\delta(CFCl_3)$ 0. Chiral HPLC analysis was carried out using an analytical Daicel OJ column (25 × 0.46 cm, cellulose tris-toluate coated on silica) on a Waters 600 HPLC system equipped with a Waters 600E system controller and a Waters 991 photodiode array detector; MILLENNIUM™ 2010 chromatography manager was used as system software. Chiral GLC analysis was carried out using a Hewlett-Packard 5890A gas chromatograph equipped with a 50 m WCOT fused silica capillary GLC column coated with cyclodextrin-B-2,3,6-M-19 (Chrompack No. 7501) and a Hewlett-Packard 3396 series II integrator. Mass spectra were recorded on an AEI-MS-902 mass spectrometer by EI by Mr A. Kiewiet in our department. Elemental analyses were performed in the microanalytical group of this department by Mr H. Draaijer, Mr J. Ebels and Mr J. Hommes.

General procedure for the α-alkylation of dioxolanone 3

In an atmosphere of nitrogen, using predried glassware, diisopropylamine (3.50 cm^3, 25 mmol) was dissolved in 50 cm^3 of dry THF. After cooling to −80 °C, BuLi (14 cm^3, 1.6 M in hexane, 22 mmol) was added. The mixture was stirred for 15 min and then recooled to −80 °C. Dioxolanone **3**[11b] (3.84 g, 20 mmol) was dissolved in 20 cm^3 of dry THF and added dropwise to the lithium diisopropylamide (LDA) solution. After stirring for another 15 min the yellow enolate solution was cooled to −80 °C and a solution of the alkyl halide (22 mmol) in 10 cm^3 of THF was added dropwise. Under stirring, the reaction mixture was slowly allowed to reach room temperature (~3 h) and quenched with saturated aqueous NH_4Cl. The reaction mixture was extracted three times with diethyl ether (100 cm^3) and the combined organic layers were washed with brine. After drying (Na_2SO_4) and evaporation there remained a nearly quantitative yield of the α-alkylated dioxolanone **4**, which can be purified as described previously.[11b] Generally, however, the purity was checked by ^{1}H NMR and crude **4** was directly reduced to the corresponding diol **1**.

General procedure for the reduction of α-alkylated dioxolanones 4 to diols 1

In an atmosphere of nitrogen, using predried glassware, $LiAlH_4$ (2.28 g, 60 mmol) was suspended in 100 cm^3 of dry THF. Slowly a solution of **4** (20 mmol) in 25 cm^3 of dry THF was added under stirring. The solution was refluxed for 2 h and after cooling to room temperature excess $LiAlH_4$ was carefully destroyed using saturated aqueous NH_4Cl. The reaction mixture was filtered over Celite which was washed with 200 cm^3 of diethyl ether. The combined organic layers were washed with brine and dried (Na_2SO_4). After evaporation crude **1** was obtained; this was purified as indicated.

Preparation of 2-phenylbutane-1,2-diol 1a

Starting from **3** and ethyl iodide, following the procedures described above, there was obtained **1a** (2.77 g, 16.7 mmol, 83%) after bulb to bulb distillation (95 °C at 0.07 mmHg) as a colourless oil which solidified upon standing; mp 52.6–54.5 °C (lit.,[32] 56 °C) (Found: C, 72.54; H, 8.47. $C_{10}H_{14}O_2$ requires C, 72.25; H, 8.49%); $\delta_H(CDCl_3)$ 0.77 (3 H, t, J 6), 1.84 (2 H, m), 1.95 (1 H, br s), 2.73 (1 H, s), 3.65 (1 H, dd, J 6, J_{AB}11), 3.85 (1 H, d, J_{AB} 11), 7.27–7.44 (5 H, m); $\delta_C(CDCl_3)$ 7.38 (q), 31.09 (t), 70.35 (t), 77.48 (s), 125.59 (d), 126.97 (d), 128.33 (d), 143.70 (s) (HRMS: calc. 166.099. Found: 166.099); ee determination by chiral HPLC (hexane–propan-2-ol, 9:1, flow rate 1.0 ml min^{-1}), R_{t1} 11.8 min, R_{t2} 19.8 min.

Preparation of 2-phenylpentane-1,2-diol 1b

Starting from **3** and propyl iodide, following the procedures described above, there was obtained **1b** (1.94 g, 10.8 mmol, 52%) after bulb to bulb distillation (115 °C at 0.2 mmHg) as a colourless oil; $\delta_H(CDCl_3)$ 0.85 (3 H, t, J 7.2), 0.95–1.45 (2 H, m), 1.76 (2 H, m), 2.08 (1 H, br s), 2.83 (1 H, br s), 3.66 (1 H, d, J_{AB} 11), 3.82 (1 H, d, J_{AB} 11), 7.20–7.45 (5 H, m); $\delta_C(CDCl_3)$ 14.40 (q), 16.38 (t), 40.81 (t), 70.51 (t), 77.22 (s), 125.47 (d), 126.92 (d), 128.32 (d), 143.80 (s) (HRMS: calc. 180.115. Found: 180.115); ee determination by chiral HPLC (hexane–propan-2-ol, 9:1, flow rate 1.0 ml min^{-1}), R_{t1} 9.4 min, R_{t2} 11.2 min. Due to the hygroscopic character of this compound a correct elemental analysis could not be obtained.[33]

Preparation of 2-phenylpent-4-ene-1,2-diol 1c

Starting from **3** (50 mmol) and allyl bromide (55 mmol), following the procedures described above, there was obtained

1c (6.45 g, 36.2 mmol, 73%) as a colourless oil after bulb to bulb distillation (115 °C at 0.01 mmHg); $\delta_H(CDCl_3)$ 1.92 (1 H, dd, *J* 8.0 and 5.1; this is the signal for $HOCH_2$, which couples to the two diastereotopic methylene protons), 2.58–2.80 (3 H, m), 3.65–3.84 (2 H, m), 5.09–5.21 (2 H, m), 5.51–5.69 (1 H, m), 7.25–7.47 (5 H, m); $\delta_C(CDCl_3)$ 42.90 (t), 70.03 (t), 76.34 (s), 119.63 (t), 125.41 (d), 127.19 (d), 128.39 (d), 132.83 (d), 143.76 (s) [HRMS: calc. ($-H_2O$), 160.089. Found: 160.089]; ee determination by chiral HPLC (hexane–propan-2-ol, 9:1, flow rate 1.0 ml min^{-1}), R_{t1} 11.9 min, R_{t2} 16.0 min. Due to the hygroscopic character of this compound a correct elemental analysis could not be obtained.[33]

Preparation of 2-phenylhex-4-ene-1,2-diol 1d

Starting from **3** and *E*/*Z*-crotyl bromide, following the procedures described above, there was obtained an approximately 85:15 mixture of *E*/*Z* **1d** (2.40 g, 12.5 mmol, 60%) as a colourless oil after bulb to bulb distillation (120 °C at 0.08 mmHg); $\delta_H(CDCl_3)$ 1.61 (*E*) and 1.65 (*Z*) (3 H, s), 2.38–2.87 (4 H, m), 3.59–3.77 (3 H, m), 5.12–5.29 (1 H, m), 5.49–5.66 (1 H, m), 7.22–7.46 (5 H, m); $\delta_C(CDCl_3)$ for the major *E* isomer 17.98 (q), 41.60 (t), 69.88 (t), 76.32 (s), 124.97 (d), 125.51 (d), 127.01 (d), 128.27 (d), 130.57 (d), 143.91 (s) [HRMS: calc. ($-H_2O$), 174.104. Found: 174.104]; ee determination by chiral GLC, oven temp. 170 °C, R_{t1} 26.01 min, R_{t2} 26.20 min. Due to the hygroscopic character of this compound a correct elemental analysis could not be obtained.[33]

Preparation of 2,3-diphenylpropane-1,2-diol 1e

Starting from **3** (18 mmol) and benzyl bromide, following the procedures described above, there was obtained **1e** (3.77 g, 16.5 mmol, 92%) as a white solid. Recrystallization from EtOAc–hexane afforded tiny white needles of pure **1e**; mp 75.2–75.7 °C (lit.,[34] 73 °C); $\delta_H(CDCl_3)$ 2.08 (1 H, br s), 2.61 (1 H, br s), 3.17 (2 H, dd, J_{AB} 14), 3.77 (1 H, d, J_{AB} 11), 3.87 (1 H, d, J_{AB} 11), 6.92–6.97 (2 H, m), 7.18–7.36 (8 H, m); $\delta_C(CDCl_3)$ 44.87 (t), 69.28 (t), 77.04 (s), 125.06 (d), 126.70 (d), 127.17 (d), 128.08 (d), 128.25 (d), 130.52 (d), 135.76 (s), 143.20 (s); ee determination as the monoacetate **2e** by chiral HPLC (hexane–propan-2-ol 9:1, flow rate 1.0 ml min^{-1}), R_{t1} 21.5 min, R_{t2} 27.9 min.

Preparation of 2-phenyl-3-*p*-tolylpropane-1,2-diol 1f

Starting from **3** and α-bromoxylene, following the procedure described above, **4f** was obtained as a yellow oil which was bulb to bulb distilled (150 °C at 0.01 mmHg) to yield **4f** (5.65 g, 19.1 mmol, 95%) contaminated with a small trace of α-bromoxylene. Subsequently **4f** (17 mmol) was reduced following the standard procedure to yield a white solid which was recrystallized from EtOAc–hexane to give pure **1f** (3.31 g, 13.7 mmol, 81%) as white needles; mp 91.5–91.7 °C (Found: C, 79.18; H, 7.49. $C_{16}H_{18}O_2$ requires C, 79.31; H, 7.49%); $\delta_H(CDCl_3)$ 1.84 (1 H, br s), 2.28 (3 H, s), 2.46 (1 H, s), 3.11 (1 H, d, J_{AB} 15), 3.20 (1 H, d, J_{AB} 15), 6.84 (2 H, d, *J* 8), 7.01 (2 H, d, *J* 8), 7.05–7.39 (5 H, m); $\delta_C(CDCl_3)$ 21.01 (q), 44.15 (t), 68.99 (t), 76.91 (s), 125.45 (d), 126.93 (d), 127.98 (d), 128.51 (d), 130.08 (d), 132.24 (s), 135.88 (s), 143.25 (s) [HRMS: calc. ($-H_2O$), 224.120. Found: 224.120]; ee determination by chiral HPLC (hexane–ethanol, 95:5, flow rate 1.0 ml min^{-1}), R_{t1} 21.2 min, R_{t2} 25.3 min.

Preparation of 2,5-diphenylpent-4-ene-1,2-diol 1g

Dioxolanone **4g**[11b] (920 mg, 2.98 mmol) was dissolved in 12 cm^3 of Bu^{t}OH, and $NaBH_4$ (285 mg, 7.5 mmol) was added. The mixture was brought to reflux and 2.4 cm^3 of MeOH was added in small portions over a period of 1 h. After refluxing for another 1 h, water was added and the solution partially evaporated at reduced pressure. The remaining water layer was extracted twice with ethyl acetate. The combined organic layers were washed with brine and, after drying (Na_2SO_4), evaporated to dryness. As determined from the 200 MHz ^1H NMR spectrum this residue consisted of 85% product and 15% of starting material. Pure **1g** (430 mg, 1.69 mmol, 67%) was isolated by column chromatography (silica, diethyl ether–hexane, 1:2) as a colourless oil which solidified upon standing; mp 70.0–71.5 °C (Found: C, 80.04; H, 7.17. $C_{17}H_{18}O_2$ requires C, 80.28; H, 7.13%); $\delta_H(CDCl_3)$ 2.12 (1 H, br s), 2.84 (3 H, m), 3.81 (2 H, br s), 6.01 (1 H, m), 6.50 (1 H, d, *J* 16), 7.20–7.50 (10 H, m); $\delta_C(CDCl_3)$ 42.23 (t), 69.93 (t), 76.66 (s), 124.16 (d), 125.44 (d), 126.17 (d), 127.25 (d), 127.41 (d), 128.47 (d), 134.45 (d), 136.90 (s), 143.24 (s) [HRMS: calc. ($-H_2O$), 206.120. Found: 206.120]; ee determination by chiral HPLC (hexane–propan-2-ol 9:1, flow rate 1.0 ml min^{-1}), R_{t1} 27.6 min, R_{t2} 41.0 min.

Preparation of 3-(naphthalen-1-yl)-2-phenylpropane-1,2-diol 1h

Following the procedure described above the lithium enolate from **3** (20 mmol) was generated. To this solution was added freshly distilled 1-chloromethylnaphthalene (3.88 g, 22 mmol) in dry THF. After stirring at room temperature overnight, saturated aqueous NH_4Cl was added and the mixture was extracted three times with diethyl ether. The organic layers were washed with brine and, after drying (Na_2SO_4), evaporated. There remained 6.98 g of material composed of **3**, **4h** and alkyl halide. Excess alkyl halide and **3** were distilled off in a Kugelrohr apparatus (80 °C at 0.01 mmHg) and the remaining yellow oil was filtered over silica using hexane–EtOAc (9:1). The crude **4h** obtained this way was reduced using $LiAlH_4$ (700 mg) following the standard procedure. After work-up a yellow oil was obtained which was purified by column chromatography (silica, diethyl ether) to give **1h** (1.18 g, 4.24 mmol, 21%) as a colourless solid. For analytical purposes a small portion was recrystallized from EtOAc–hexane to give tiny white needles; mp 73.5–74.5 °C (Found: C, 81.10; H, 6.55. $C_{19}H_{18}O_2$ requires C, 81.99; H, 6.52%); $\delta_H(CDCl_3)$ 1.85 (1 H, br s), 2.52 (1 H, s), 3.50 (1 H, d, J_{AB} 14), 3.81 (1 H, d, J_{AB} 14), 3.88 (2 H, m), 7.09 (1 H, d, *J* 6), 7.20–7.47 (8 H, m), 7.67–7.86 (2 H, m), 8.00–8.09 (1 H, m); $\delta_C(CDCl_3)$ 41.08 (t), 69.09 (t), 77.42 (s), 124.03 (d), 124.82 (d), 125.34 (d), 125.61 (d), 126.92 (d), 127.29 (d), 127.98 (d), 128.40 (d), 128.92 (d), 131.93 (s), 132.72 (s), 133.66 (s), 143.35 (s) (HRMS: calc. 278.131. Found: 278.131); ee determination by chiral HPLC (hexane–propan-2-ol, 9:1, flow rate 1.0 ml min^{-1}), R_{t1} 31.9 min, R_{t2} 41.1 min.

Preparation of 2,5-diphenylpentane-1,2-diol 1i

Starting from **4g** (10 mmol) following the procedure described above using $LiAlH_4$ there was obtained a mixture of **1g** and **1i**. This mixture was dissolved in 50 cm^3 of MeOH and hydrogenated in a Parr apparatus overnight in the presence of a catalytic amount Pd/C (10%). After filtration **1i** was obtained, which was purified by column chromatography (silica, diethyl ether) to give pure material (1.82 g, 7.11 mmol, 71%) as a colourless oil which solidified upon standing; mp 63.1–64 °C (Found: C, 79.42; H, 7.90. $C_{17}H_{20}O_2$ requires C, 79.65; H, 7.86%); $\delta_H(CDCl_3)$ 1.34–1.88 (4 H, m), 2.44 (2 H, br s), 2.49–2.61 (2 H, m), 3.63 (1 H, d, J_{AB} 11), 3.78 (1 H, d, J_{AB} 11), 7.09–7.38 (10 H, m); $\delta_C(CDCl_3)$ 24.72 (t), 36.01 (t), 37.96 (t), 70.58 (t), 77.17 (s), 125.50 (d), 125.73 (d), 127.00 (d), 128.24 (d), 128.38 (d), 141.84 (s), 143.15 (s) [HRMS: calc. ($-H_2O$), 238.136. Found: 238.136]; ee determination by chiral HPLC (hexane–propan-2-ol, 9:1, flow rate 1.0 ml min^{-1}, R_{t1} 28.4 min, R_{t2} 34.4 min.

Preparation of 5-(4-bromophenyl)-2,2-dimethyl-1,3-dioxolan-4-one 5a

A mixture of 4-bromomandelic acid[18] (20.0 g, 86.6 mmol) and acetone (25 g) in 100 cm^3 of benzene containing a catalytic amount of H_2SO_4 was azeotropically refluxed for 8 h. After cooling, the mixture was washed three times with saturated aqueous $NaHCO_3$ followed by brine. After drying (Na_2SO_4) the solution was evaporated to dryness to yield **5a** (13.24 g, 48.9 mmol, 57%); mp 62.0–63.0 °C (lit.,[35] 65–66 °C); $\delta_H(CDCl_3)$

1.66 (3 H, s), 1.71 (3 H, s), 5.34 (1 H, s), 7.33–7.37 (2 H, m), 7.50–7.56 (2 H, m); $\delta_C(CDCl_3)$ 26.07 (q), 27.19 (q), 75.09 (d), 111.13 (s), 122.82 (s), 127.93 (d), 131.82 (d), 133.52 (s), 172.06 (s).

Preparation of 5-(4-chlorophenyl)-2,2-dimethyl-1,3-dioxolan-4-one 5b

Following the same procedure as for **3** there was obtained, from 4-chloromandelic acid[36] (10.0 g, 53.6 mmol) and dimethoxypropane (6.76 g, 65 mmol), **5b** (10.07 g, 44.5 mmol, 83%) after recrystallization from diethyl ether–hexane; mp 72–73 °C (Found: C, 58.07; H, 4.92; Cl, 15.82. $C_{11}H_{11}ClO_3$ requires C, 58.29; H, 4.89; Cl, 15.64%); $\delta_H(CDCl_3)$ 1.68 (3 H, s), 1.72 (3 H, s), 5.37 (1 H, s), 7.35–7.47 (5 H, m); $\delta_C(CDCl_3)$ 26.08 (q), 27.19 (q), 75.14 (d), 111.16 (s), 127.60 (d), 128.90 (d), 132.87 (s).

Preparation of 5-(4-methoxyphenyl)-2,2-dimethyl-1,3-dioxolan-4-one 5c

Following the same procedure as for **3** there was obtained, from 4-methoxymandelic acid[36] (20.0 g, 0.11 mol) and dimethoxypropane (13.5 g, 0.13 mol), a quantitative yield of **5c** (24.4 g, 0.11 mol) as an oil which solidified upon standing; mp 35–36 °C; $\delta_H(CDCl_3)$ 1.65 (3 H, s), 1.71 (3 H, s), 3.79 (3 H, s), 5.34 (1 H, s), 6.91–6.95 (2 H, m), 7.34–7.38 (2 H, m); $\delta_C(CDCl_3)$ 25.95 (q), 27.19 (q), 55.26 (q), 75.75 (d), 110.62 (s), 114.17 (d), 126.67 (s), 128.13 (d), 160.06 (s), 171.45 (s).

Preparation of 5-(4-fluorophenyl)-2,2-dimethyl-1,3-dioxolan-4-one 5d

Following the same procedure as for **3** there was obtained, from 4-fluoromandelic acid[36] (20.0 g, 117 mmol) and dimethoxypropane (15.6 g, 150 mmol), **5d** (22.59 g, 108 mmol, 92%) after recrystallization from diethyl ether–hexane; mp 71.0–71.2 °C (Found: C, 62.61; H, 5.40; F, 9.04. $C_{11}H_{11}FO_3$ requires C, 62.85; H, 5.27; F, 9.04%); $\delta_H(CDCl_3)$ 1.68 (3 H, s), 1.72 (3 H, s), 5.37 (1 H, s), 7.06–7.16 (2 H, m), 7.42–7.49 (2 H, m); $\delta_C(CDCl_3)$ 26.05 (q), 27.20 (q), 75.22 (d), 110.97 (s), 115.50 (d), 115.94 (d), 128.14 (d), 128.31 (d), 161.13 (s), 164.83 (s); $\delta_F(CDCl_3)$ 165.22 (HRMS: calc. 210.069. Found: 210.069).

Preparation of 2-(4-bromophenyl)pent-4-ene-1,2-diol 6a

Following the same procedures as for diols **1** there was obtained, from **5a** (5.00 g, 18.4 mmol) and allyl bromide, **6a** (3.87 g, 15.0 mmol, 82%). An analytical sample was prepared by recrystallization from benzene–hexane; mp 62.0–62.5 °C (Found: C, 51.50; H, 5.08; Br, 31.13. $C_{11}H_{13}BrO_2$ requires C, 51.38; H, 5.10; Br, 31.08%); $\delta_H(CDCl_3)$ 2.37 (2 H, br s), 2.60 (1 H, s), 2.64 (1 H, s), 3.66 (1 H, d, J_{AB} 11.3), 3.74 (1 H, d, J_{AB} 11.3), 5.14–5.17 (2 H, m), 5.47–5.68 (1 H, m), 7.27–7.33 (2 H, m), 7.46–7.53 (2 H, m); $\delta_C(CDCl_3)$ 42.81 (t), 69.75 (t), 76.00 (s), 119.98 (s), 127.37 (d), 131.44 (d), 132.37 (d), 142.39 (s) [HRMS: calc. ($-H_2O$), 237.999. Found: 237.999]; ee determination by chiral HPLC (hexane–propanol, 9:1, flow rate 1.0 ml min^{-1}), R_{t1} 8.9 min, R_{t2} 9.6 min.

Preparation of 2-(4-chlorophenyl)pent-4-ene-1,2-diol 6b

Following the same procedure as for diols **1** there was obtained, from **5b** (4.53 g, 20 mmol) and allyl bromide, **6b** (3.30 g, 15.5 mmol, 78%), which was purified by bulb to bulb distillation (160 °C at 0.05 mmHg); $\delta_H(CDCl_3)$ 1.93 (1 H, br s), 2.62–2.66 (3 H, m), 3.72 (2 H, m), 5.10–5.18 (2 H, m), 5.48–5.66 (1 H, m), 7.27–7.40 (5 H, m); $\delta_C(CDCl_3)$ 42.85 (t), 69.85 (t), 76.35 (s), 119.99 (s), 126.97 (d), 128.49 (d), 132.40 (d), 141.88 (s) [HRMS: calc. ($-H_2O$), 194.050. Found: 194.050]; ee determination by chiral HPLC (hexane–propan-2-ol 9:1, flow rate 0.4 ml min^{-1}), R_{t1} 22.6 min, R_{t2} 25.6 min. Due to the hygroscopic character of this compound a correct elemental analysis could not be obtained.[33]

Preparation of 2-(4-methoxyphenyl)pent-4-ene-1,2-diol 6c

Following the same procedures as for diols **1** there was obtained, from **5c** (4.11 g, 18.5 mmol) and allyl bromide, after bulb to bulb distillation (200 °C at 2 mmHg) pure **6c** as a colourless oil (3.25 g, 15.6 mmol, 84%); $\delta_H(CDCl_3)$ 1.86 (1 H, dd, J 8.1 and 5.1), 2.56 (1 H, s), 3.62–3.78 (2 H, m), 3.82 (3 H, s), 5.09–5.19 (2 H, m), 5.52–5.65 (1 H, m), 6.87–6.95 (2 H, m), 7.27–7.38 (2 H, m); $\delta_C(CDCl_3)$ 43.03 (t), 55.27 (q), 70.22 (t), 113.68 (d), 119.51 (s), 126.67 (d), 133.03 (d) [HRMS: calc. ($-H_2O$) 190.099. Found: 190.099]; ee determination by chiral HPLC (hexane–propan-2-ol, 8:2, flow rate 1.0 ml min^{-1}), R_{t1} 12.4 min, R_{t2} 14.4 min. Due to the hygroscopic character of this compound no correct elemental analysis could be obtained.[33]

Preparation of 2-(4-fluorophenyl)pent-4-ene-1,2-diol 6d

Following the same procedure as for diols **1** there was obtained from **5d** (4.20 g, 20 mmol) the α-allylated dioxolanone (4.04 g, 16.2 mmol, 81%) which was purified by bulb to bulb distillation (90 °C at 1 mmHg). This intermediate (10 mmol) was subsequently reduced to give, after bulb to bulb distillation (105 °C at 0.05 mmHg), **6d** (1.76 g, 8.98 mmol, 90%) as a colourless oil; $\delta_H(CDCl_3)$ 2.06 (1 H, br s), 2.55–2.72 (3 H, m), 3.67 (1 H, d, J_{AB} 11.1), 3.75 (1 H, d, J_{AB} 11.1), 5.09–5.18 (2 H, m), 5.49–5.70 (1 H, m), 7.01–7.11 (2 H, m), 7.27–7.45 (3 H, m); $\delta_C(CDCl_3)$ 42.96 (t), 69.93 (t), 75.90 (s), 114.94 (d), 115.35 (d), 119.83 (s), 127.12 (d), 127.28 (d), 132.55 (d) [HRMS: calc. ($-H_2O$) 178.079. Found: 178.079]; ee determination by chiral HPLC (hexane–ethanol 95:5, flow rate 0.7 ml min^{-1}), R_{t1} 18.8 min, R_{t2} 21.5 min. Due to the hygroscopic character of this compound a correct elemental analysis could not be obtained.[33]

General procedure for the enzymatic resolutions of diols 1 and 6

Diol (0.25 mmol) was dissolved in 1 cm^3 of organic solvent and 0.3 cm^3 of vinyl acetate. Lipase (20 mg) was added and the heterogeneous mixture was stirred at room temperature. The progress of the reaction was monitored by TLC (silica, diethyl ether–hexane, 1:2, staining alkaline $KMnO_4$ or phosphomolybdic acid in EtOH). At regular intervals aliquots of 0.1 cm^3 were taken which were filtered over 0.5 cm Celite in a Pasteur pipette. The Celite was washed with 1 cm^3 of CH_2Cl_2 and the filtrate was evaporated. The residue was dissolved in 1 cm^3 of Pr^iOH and analysed on a chiral Daicel OJ HPLC column as indicated. If the ees of both components could not be detected in a single run, the components were separated by preparative TLC.

General procedure for the diastereoselective synthesis of (*R*)-diols 1

In an atmosphere of nitrogen using predried glassware, diisopropylamine (1.26 cm^3, 9 mmol) was dissolved in 50 cm^3 of dry THF. After cooling to −80 °C BuLi (5.2 cm^3, 1.6 M in hexane, 8.2 mmol) was added. The mixture was stirred for 15 min and then recooled to −80 °C. *cis*-Dioxolanone **7**[26] (1.65 g, 7.5 mmol) was dissolved in 10 cm^3 of dry THF and added dropwise to the LDA solution. After stirring for another 30 min the yellow enolate solution was cooled to −80 °C, and a solution of the alkyl halide (8 mmol) in 10 cm^3 of THF was added dropwise. Under stirring the reaction mixture was slowly allowed to reach room temperature (~5 h) and quenched with saturated aqueous NH_4Cl. The reaction mixture was extracted three times with diethyl ether (100 cm^3) and the combined organic layers were washed with brine. After drying (Na_2SO_4) and evaporation, there remained a nearly quantitative yield of the α-alkylated dioxolanone. The purity was checked by ^1H NMR and it was subsequently reduced to the corresponding (*R*)-diol **1** by slowly adding it in solution in dry THF to $LiAlH_4$ (816 mg, 21 mmol) in 100 cm^3 of THF under inert conditions. After reflux for 2 h, excess hydride was destroyed by the careful addition of saturated aqueous NH_4Cl. The mixture was filtered over Celite, which was washed with 200 cm^3 of diethyl ether. The filtrate was washed with brine and dried (Na_2SO_4) to give

the crude, enantiomerically enriched diol **1** after evaporation. The diol was purified as described and its enantiomeric composition determined by chiral HPLC (OJ) as indicated for the racemate.

Purification of (*R*)-2-phenylpent-4-ene-1,2-diol 1c. This compound was purified by column chromatography (silica, diethyl ether–hexane, 1:2) to give pure (*R*)-**1c** (1.01 g, 5.67 mmol, 76%) as a colourless oil; NMR spectra in accordance with the racemate; ee 92%; $[\alpha]_D^{25}$ +47 (*c* 1.24 in $CHCl_3$) (lit.,[8c] $[\alpha]_D^{25}$ +49.5); op 95%.

Purification of (*R*)-2,3-diphenylpropane-1,2-diol 1e. This compound was purified by crystallization from diethyl ether–hexane to provide (*R*)-**1e** (1.34 g, 5.88 mmol, 78%) as a white solid; mp 62.2–63.2 °C (lit.,[7] 62 °C); NMR spectra in accordance with the racemate; ee 94% (determined as the mono acetate); $[\alpha]_D^{25}$ +76.5 (*c* 1.44 in $CHCl_3$).

Purification of (*R*)-2-phenyl-3-*p*-tolylpropane-1,2-diol 1f. This compound was purified by recrystallization from diethyl ether–hexane to give pure (*R*)-**1f** (1.40 g, 5.78 mmol, 77%) as a white solid; mp 83.8–84.2 °C; NMR spectra in accordance with the racemate; ee 93%; $[\alpha]_D^{25}$ +84.8 (*c* 1.12 in $CHCl_3$).

Purification of (*R*)-2,5-diphenylpent-4-ene-1,2-diol 1g. This compound was prepared *via* reduction of 3.5 mmol α-alkylated dioxolanone using $NaBH_4$ (as for the racemate) to give pure (*R*)-**1g** (670 mg, 2.64 mmol, 75%) after column chromatography (silica, diethyl ether–hexane, 1:1) as a colourless oil which solidified on prolonged standing; mp 49.6–50.2 °C; NMR spectra in accordance with the racemate; ee 90%; $[\alpha]_D^{25}$ +49.3 (*c* 0.70 in $CHCl_3$).

General procedure for the immobilization of lipase AKG

Lipase AKG (500 mg) was dissolved in 10 cm^3 of buffer. This was added to carrier material (2.0 g) (Celite or Hyflo SuperCell), which had been washed with water and buffer. After thorough mixing, the slurry was poured into a large petri-dish, and the water was evaporated overnight in the hood. The white powder thus obtained was stored in the refrigerator and used for resolution experiments.

Preparative scale resolution of diol 1e using immobilized lipase AKG

In a representative experiment, diol **1e** (684 mg, 3.0 mmol) was dissolved in 10 cm^3 of benzene and 3 cm^3 of vinyl acetate. Lipase AKG (immobilized on Celite at pH 7.00, 1.25 g) was added and the mixture was stirred at room temperature in a sealed bottle. The conversion was monitored by TLC. After 70 h the reaction mixture was filtered and the residue was washed with diethyl ether. The filtrate was evaporated and the residue was purified by column chromatography (silica, diethyl ether–hexane, 1:2) to give **1e** [339 mg, 1.49 mmol, 49%, ee 82% (as the monoacetate **2e** by chiral HPLC)] and **2e** [357 mg, 1.32 mmol, 44%, ee 94% (chiral HPLC)]; total yield 94%. From these data the conversion was determined to be 0.468 and the enantiomeric ratio 80.

The same experiment was repeated, but the reaction was now worked up after 93 h to give **1e** [228 mg, 1.26 mmol, 42%, ee >99% (as the monoacetate **2e** by chiral HPLC)] and **2e** [387 mg, 1.43 mmol, 48%, ee 83% (chiral HPLC)]; total yield 90%. From these data the conversion was determined to be 0.546 and the enantiomeric ratio 70.

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