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Kinetic resolution of *trans*-2-acetoxycycloalkan-1-ols by lipase-catalysed enantiomerically selective acylation

Viktória Bódai,[a,b] Olivér Orovecz,[a] György Szakács,[b] Lajos Novák[a] and László Poppe[a,*]

[a]*Institute for Organic Chemistry and Research Group for Alkaloid Chemistry of the Hungarian Academy of Sciences, Budapest University of Technology and Economics, H-1111 Budapest, Gellért tér 4., Hungary*
[b]*Department of Agricultural Chemical Technology, Budapest University of Technology and Economics, Gellért tér 4, H-1111 Budapest, Hungary*

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Abstract—Kinetic resolution of a series of racemic *trans*-cycloalkane-1,2-diol monoacetates *rac*-**2a–d** was performed by enantiomerically selective transesterification with vinyl acetate catalysed by commercial and our own-prepared fungal lipases to yield diacetates (*R,R*)-**3a–d** and monoacetates (*S,S*)-**2a–d** in high enantiomeric purity. The monoacetates (*R,R*)-**2a–d** were also prepared from the racemic diacetates *rac*-**3a–d** by lipase-catalysed hydrolysis.

1. Introduction

Enantiopure *trans*-cycloalkane-1,2-diols **1a–d** are useful as building blocks in the synthesis of optically active crown ethers[1] and as chiral auxiliaries for various asymmetric reactions.[2,3] Recently, (*S,S*)-cyclohexane-1,2-diol (*S,S*)-**1b** have been applied in mechanistic studies of rearrangement reactions.[4]

Optically active derivatives of *trans*-cycloalkane-1,2-diols **1a–d** were previously prepared by enzyme-catalysed acylation of the racemic diols *rac*-**1a–d** with lipase from *Pseudomonas* sp. (SAM II)[5] or with lipase from *Pseudomonas cepacia* (lipase PS, Amano)[6] (Fig. 1).

On the other hand, enzymatic hydrolyses of the corresponding racemic diacetates *rac*-**3a–d** with SAM II[5] or with porcine liver esterase (PLE)[7] have also been described (Fig. 2). Highly enantiomerically selective hydrolyses of *rac*-**3a**,[8] *rac*-**3a**[9,10] or *rac*-**3a–c**[11] were performed with lipase from *Pseudomonas fluorescens* (lipase P, Amano).

PLE-catalysed hydrolysis of racemic diacetates *rac*-**3a–d** was only moderately enantiomerically selective and yielded monoacetates **2a–d** in variable configuration and enantiomeric composition.[7] Although the parameters of the sequential kinetic resolution of racemic *trans*-diacetoxycyclohexane *rac*-**3a** by hydrolysis with PLE and lipase P were precisely optimized,[12] hydrolysis could not provide both diacetates and monoacetates in high yield and enantiomeric purity. The ambiguous configuration assignment of the five-membered monoacetate [(+)-**2a** was assigned as (*R,R*)-[9,10]] indicates the uncertainty of the configuration for monoacetates **2a–d** obtained by enzymatic hydrolysis from diacetates **3a–d**.

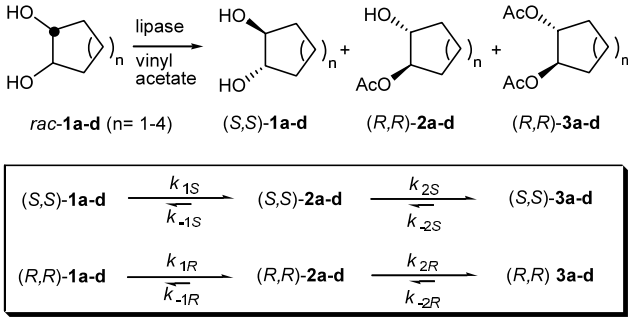

Figure 1. Enzymatic acylation of *trans*-cycloalkane-1,2-diols *rac*-**1a–d**.

Irrespective of whether starting from racemic diols *rac*-**1a–d** (Fig. 1) or diacetates *rac*-**3a–d** (Fig. 2), these

* Corresponding author. Tel.: +36-1 463 2229; fax: +36-1 463 3297; e-mail: poppe@mail.bme.hu

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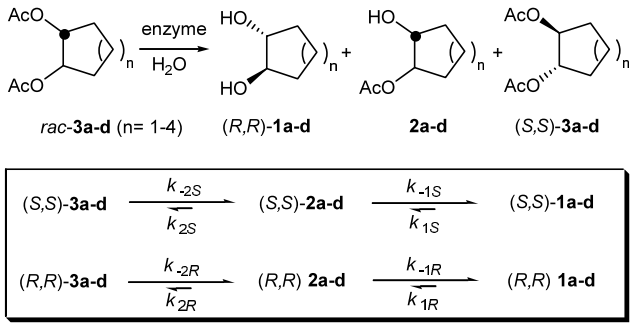

Figure 2. Enzyme-mediated hydrolysis of *trans*-cycloalkane-1,2-diol diacetates *rac*-**3a**–**d**.

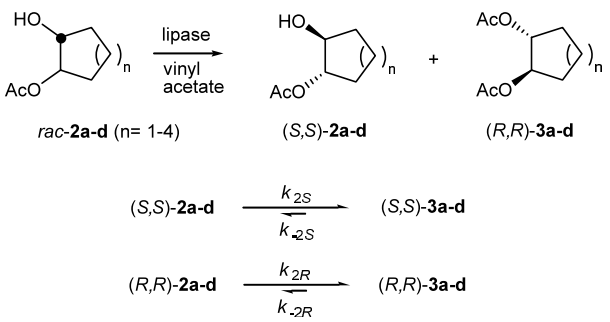

Figure 3. Enzymatic acylation of *trans*-2-acetoxycycloalkan-1-ols *rac*-**2a**–**d**.

enzymatic processes were sequential kinetic resolutions in which three fractions of diols **1a**–**d**, monoacetates **2a**–**d** and diacetates **3a**–**d** were produced in variable yields and enantiomeric compositions. Even if the processes are virtually irreversible—thus the back reactions would not significantly influence the overall results—their outcome is dependent on the relative measures of four independent rate constants (k_{1S}, k_{1R}, k_{2S}, and k_{2R} for acylation; or k_{-2S}, k_{-2R}, k_{-1S} and k_{-1R}, for hydrolysis). Analysis of the data available for acylation reactions[5,6] indicated that k_{2S} is by far the slowest step, thus (*R*,*R*)-**3a**–**d** was produced almost exclusively as a diacetate (Fig. 1). Systematic evaluation of the rate constants for the hydrolysis of *rac*-**3b**[12] indicated k_{-2S}—the formal back reaction rate of k_{2S}—as the slowest step in hydrolysis. As a result, accumulation of (*S*,*S*)-**3b** in the unreacted diacetate fraction was observed (Fig. 2).

Previously, we have systematically compared the six possible kinetic resolution strategies—acylations of the 1,2-diols, 1-acetates or 2-acetates and hydrolyses of the 1,2-diacetates, 1-acetates or 2-acetates—for acyclic 1,2-diol derivatives.[13] Among these reactions, the lipase-catalysed acylation of 2-acetoxy-1-ols proved to be the fastest and most selective.

Due to the kinetic analysis of the sequential kinetic resolutions of cyclic 1,2-diols *rac*-**1a**–**d** by acylation or by hydrolysis of their diacetates *rac*-**3a**–**d**—together with the results for lipase-catalysed kinetic resolutions of the acyclic 1,2-diols—implying acylation of the racemic monoacetates *rac*-**2a**–**d** as the most selective process, we thought it worthwhile investigating this unprecedented simple kinetic resolution (Fig. 3).

Herein we report the results on simple kinetic resolution of racemic *trans*-2-acetoxycycloalkan-1-ols *rac*-**2a**–**d** with a wide selection of lipases.

2. Results and discussion

Preparation of the racemic monoacetates *rac*-**2a**–**d** from the easily available diols *rac*-**1a**–**d** was performed using Ac_2O/Na_2CO_3 as reagents.[12] Another high yield method using $YbCl_3$ (10%)/Ac_2O (10 equiv.) for preparation of monoacetates from symmetric 1,2-diols has recently been published.[14]

With the racemic *trans*-monoacetates *rac*-**2a**–**d** in hand, the enantiomer selectivity of the enzyme-catalysed acylations of racemic *trans*-2-acetoxycycloalkan-1-ols *rac*-**2a**–**d** was screened with a wide selection of lipases (Tables 1–4).

The first and most extensive enzymatic acylation screen was carried out with 73 commercial and our own-isolated fungal enzymes for the racemic *trans*-2-acetoxycyclohexan-1-ol *rac*-**2b** (Table 2). As a result, numerous, highly enantiomerically selective enzymes for this acylation reaction were found. As expected from the previously discussed results, lipases from the *Pseudomonas* strains (lipase PS, *P. fluorescens* lipase, lipase AK) were quite selective, exhibiting E >500. Also, several fungal lipases (Lipozyme IM, Lipozyme IM 20, Lipozyme TL IM, and a number of our novel preparations from thermophilic fungi[15]) exhibited excellent selectivities, with E >500 as well.

Next, the 30 most effective enzymes in the screen on the six-membered monoacetate *rac*-**2b** were tested with the five- and seven-membered monoacetates *rac*-**2a** and *rac*-**2c** (Tables 1 and 3, respectively). As expected, the enzymes which performed best on the six-membered monoacetate *rac*-**2b** were also quite selective towards the five- and seven-membered analogues *rac*-**2a** and *rac*-**2c**, thus enabling the preparation of the highly enantiopure diacetates (*R*,*R*)-**3a**,c and monoacetates (*S*,*S*)-**2a**,**c**.

The lipases from the *Candida* strains (lipase from *C. rugosa*, *C. cylindracae*, lipase A from *C. antarctica* and lipase AY) turned out to be only moderately (*R*)-selective in acylations of the six- and seven-membered monoacetates *rac*-**2b**,**c** with formation of (*R*,*R*)-**3b**,**c** (Tables 2 and 3). Interestingly, *C. rugosa* lipase and lipase AY exhibited low but opposite enantiomer preference for the five-membered monoacetate *rac*-**2a** and catalysed the formation of (*S*,*S*)-**3a** (Table 1).

Finally, lipase-catalysed acylation of the eight-membered monoacetate *rac*-**2d** was studied with those lipases which were most selective in acylations of *rac*-**2a**–**c** (Table 4).

Table 1. Lipase-catalysed acylation of racemic *trans*-2-acetoxycyclopentan-1-ol *rac*-**2a**[a]

Enzyme	Time (h)	c (%)	**2a**		**3a**	
			Config.	Ee (%)[b]	Config.	Ee (%)[c]
Lipase TUB **3b**	24	50	(*SS*)	>99	(*R*,*R*)	>99
Lipase PS	168	50	(*SS*)	>99	(*R*,*R*)	>99
Lipozyme IM	168	43	(*SS*)	76	(*R*,*R*)	>99
Pseudomonas fluorescens lipase	168	49	(*SS*)	>99	(*R*,*R*)	>99
Lipase A	72	47	(*SS*)	93	(*R*,*R*)	>99
PPL	168	26	(*SS*)	36	(*R*,*R*)	>99
Lipozyme IM 20	168	40	(*SS*)	67	(*R*,*R*)	99
Lipase TUB 11b	168	31	(*SS*)	44	(*R*,*R*)	99
Lipase AK	24	51	(*SS*)	98	(*R*,*R*)	94
Novozyme 435	72	54	(*SS*)	>99	(*R*,*R*)	85
Lipase TUB 25b	168	47	(*SS*)	80	(*R*,*R*)	90
Lipozyme TL IM	168	49	(*SS*)	89	(*R*,*R*)	93
Lipase TUB 7b	168	26	(*SS*)	37	(*R*,*R*)	90
Lipase M	168	10	(*SS*)	9	(*R*,*R*)	84
Candida antarctica lipase A	24	92	(*SS*)	57	(*R*,*R*)	5
Candida rugosa lipase	168	26	(*RR*)	9	(*S*,*S*)	25
Lipase AY	168	28	(*RR*)	18	(*S*,*S*)	46

[a] Results with enzymes of c >10% within 168 h. For reaction details, see Section 3.
[b] The ee_{2a} (estd. error: ±0.6%) and c (estd. error: ±1.3%) values were determined by GC on an HP Chiral column.
[c] The ee_{3a} value was calculated from c and ee_{2a}.

Table 2. Lipase-catalysed acylation of racemic *trans*-2-acetoxycyclohexan-1-ol *rac*-**2b**[a]

Enzyme	Time (h)	c (%)	(*S*,*S*)-**2b** Ee (%)[b]	(*R*,*R*)-**3b** Ee (%)[b]	E[c]
Lipase TUB **3b**	48	50	>99.5	>99.8	>250
Lipase PS	48	49	98.3	>99.8	>250
Lipozyme IM	48	42	68.0	>99.8	>250
Lipase TUB 30b	48	44	77.2	>99.8	>250
Lipase AK	48	49	99.3	99.6	>250
Lipozyme IM 20	48	37	56.3	>99.8	>250
Pseudomonas fluorescens lip.	48	27	33.5	>99.8	>250
Lipase TUB 6b	48	7	4.9	>99.8	>100
Lipozyme TL IM	24	34	43.3	>99.8	>100
PPL	48	10	4.3	>99.8	>100
Lipase M	48	8	2.9	>99.8	>100
Novozyme 435	48	48	89.4	99.7	>100
Lipase TUB 31b	72	46	83.2	99.7	>100
Lipase R	48	7	1.5	96.1	54
Lipase TUB 25b	48	24	25.6	94.8	50
Lipase TUB 7b	48	17	18.5	92.5	31
Candida antarctica lipase A	48	38	33.6	64.6	6.8
Candida cylindracae lipase	48	10	2.1	64.8	5.0
Lipase TUB 10a	48	10	2.0	60.6	4.3
Lipase AY	48	7	1.3	54.0	3.5
Candida rugosa lipase	48	8	1.1	46.7	2.9

[a] Results with enzymes of c >5% within 48 h. For reaction details, see Section 3.
[b] The *ee* values of (*S*,*S*)-**2b** (estd. error: ±0.3%) and (*R*,*R*)-**3b** (estd. error: ±0.2%) and c (estd. error: ±1.2%) were determined by GC on an HP Chiral column.
[c] Degree of enantiomer selectivity (E) was calculated from c and ee_{3b},[16] and confirmed by independent calculation from ee_{2b} and ee_{3b}.[17] (Due to the sensitivity of experimental errors, E values calculated in the 500–5000 range are reported as >100, and E values calculated above 5000 are given as >250.)

In accordance with the previously reported results,[5,6] enzymatic acylation of *trans*-2-acetoxycyclooctan-1-ol *rac*-**2d** proved to be significantly less selective and yielded the optically active monoacetate (*S*,*S*)-**2d** and diacetate (*R*,*R*)-**3d** in only moderate enantiomeric purity.

To demonstrate the synthetic applicability of these processes and to obtain an unambiguous configuration/optical rotation correlation—especially for the five-membered monoacetate **2a**—the lipase AK-catalysed acylations of the racemic *trans*-2-acetoxy-cycloalkan-1-ols *rac*-**2a**–**d**

Table 3. Lipase-catalysed acylation of racemic *trans*-2-acetoxycycloheptan-1-ol *rac*-**2c**[a]

Enzyme	Time (h)	*c* (%)	(*S*,*S*)-**2c** Ee (%)[b]	(*R*,*R*)-**3c** Ee (%)[b]	*E*[c]
Novozyme 435	48	50	>98	>99	>250
Lipase AK	48	49	>98	>99	>250
Lipase TUB **3b**	168	50	>98	>99	>250
Lipase PS	168	48	>98	>99	>250
Pseudomonas fluorescens lipase	168	44	63	>99	>250
Lipozyme TL IM	168	43	57	>99	>100
Lipozyme IM	168	39	46	>99	>100
Lipozyme IM 20	168	34	31	>99	>100
Lipase TUB 11b	168	19	7	>99	>100
Lipase M	168	13	3	>99	>100
Candida cylindracae lipase	168	13	4	70	6.4
Candida rugosa lipase	168	11	2	53	3.5
Candida antarctica lipase A	48	42	9	38	2.8
Lipase AY	168	10	1	33	2.0

[a] Results with enzymes of *c* >10% within 168 h. For reaction details, see Section 3.

[b] The *ee* values of (*S*,*S*)-**2c** (estd. error: ±0.6%) and (*R*,*R*)-**3c** (estd. error: ±0.5%) and *c* (estd. error: ±1.4%) were determined by GC on HP Chiral column.

[c] Degree of enantiomer selectivity (*E*) was calculated from *c* and ee_{3c},[16] and confirmed by independent calculation from ee_{2c} and ee_{3c}.[17] (Due to the sensitivity of experimental errors, *E* values calculated in the 500–5000 range are reported as >100, and *E* values calculated above 5000 are given as >250.)

Table 4. Lipase-catalysed acylation of racemic *trans*-2-acetoxycyclooctan-1-ol *rac*-**2d**[a]

Enzyme	Time (h)	*c* (%)	(*S*,*S*)-**2d** Ee (%)[b]	(*R*,*R*)-**3d** Ee (%)[c]
Novozyme 435	24	97	99	4
Lipase PS	168	80	95	26
Lipase TUB **3b**	24	72	94	42
Lipase AK	24	71	91	41
Lipozyme TL IM	24	78	83	27
Lipozyme IM	168	60	43	32

[a] A selection of the best performing enzymes on *rac*-**2a**–**c** was only tested. For reaction details, see Section 3.

[b] The ee_{2d} (estd. error: ±1.2%) and *c* (estd. error: ±1.6%) values were determined by GC on Beta-DEX 225 column.

[c] The ee_{3d} value was calculated from *c* and ee_{2d}.

were performed on a preparative scale (300–500 mg) as well.

The preparative scale experiments proved that the simple kinetic resolution method from the racemic of the five-, six- and seven-membered monoacetates *rac*-**2a**–**c** can be applied for the preparation of highly enantiopure monoacetates (*S*,*S*)-**2a**–**c** and diacetates (*R*,*R*)-**3a**–**c** (Table 5, >97% ee for the product and remaining substrate fractions at $c \sim 0.5$). Unfortunately, acylation of the eight-membered monoacetate *rac*-**2d** gave the (*S*,*S*)-monoacetate (*S*,*S*)-**2d** only in lower enantiomeric excess (94% ee).

The configuration of the products was determined by an established method;[6] by conversion of the products of the acylation reaction **2a**–**d** and **3a**–**d** into their parent diols **1a**–**d** of known absolute configuration by hydrolysis. Thus, monoacetates **2a**–**d** were hydrolysed to (*S*,*S*)-diols (*S*,*S*)-**1a**–**d** and the hydrolysis of diacetates **3a**–**d** yielded (*R*,*R*)-diols (*R*,*R*)-**1a**–**d**. It is noteworthy, that a discrepancy was found between ours and the previously reported[9,10] specific rotations for **2a**. The configuration of (+)-**2a** ([α] in $CHCl_3$) was assigned as (*R*,*R*)-,[9,10] whereas in our hand (*S*,*S*)-**2a** gave a positive rotation in acetonitrile. This might be related to the selection of the solvent (we have measured inaccurate and unreproducible results for monoacetates **2a** in chloroform due to solubility issues which were eliminated by using acetonitrile).

To prepare the opposite enantiomers of the monoacetates, lipase AK-catalysed hydrolysis from the racemic diacetates *rac*-**3a**–**d** was applied.

Results of a small scale preliminary hydrolysis screen of *rac*-**3b** in purely aqueous systems (unbuffered water, 0.2 M phosphate buffers pH 7, pH 7.5 and pH 8) or in the same systems but containing 50% (v/v) acetone showed, that hydrolysis in unbuffered water was the fastest and most selective. Therefore, the preparative scale hydrolyses were also performed in unbuffered water (Table 6). The results indicate that the enantiomer selectivity of the hydrolysis is lower than that which can be achieved in the corresponding acylation reaction: Thus the monoacetates (*R*,*R*)-**2a**–**d** prepared by hydrolysis of the diacetates *rac*-**3a**–**d** were obtained in lower enantiomeric excesses as their enantiomers (*S*,*S*)-**2a**–**d** prepared by acylation. In addition, traces of the diols **1a**–**d** were also observed in the hydrolytic reactions.

The usefulness of our methods in the preparation of both enantiomeric forms of the monoacetates of *trans*-cycloalkane-1,2-diols (*S*,*S*)- and (*R*,*R*)-**2a**–**d** in high chemical and enantiomeric purity was further confirmed by the finding that in our hands the enantiomers of *trans*-2-acetoxycyclohexan-1-ol (*S*,*S*)- and (*R*,*R*)-**2b** were solids (mp 59.5–61°C and mp 57–59°C, respectively; lit.,[9] oil; lit.,[18] mp 28°C).

Table 5. Preparative scale acylation of racemic *trans*-2-acetoxycyclo-alkan-1-ols *rac*-**2a–d** by lipase AK[a]

Substrate	Time (h)	(*S*,*S*)-**2**		(*R*,*R*)-**3**	
		Yield (%)	Ee (%)	Yield (%)	Ee (%)
rac-**2a**	12	33	>99	43	>98
rac-**2b**	10	36	97	37	>99
rac-**2c**	18	36	>99	40	>98
rac-**2d**	24	43	94	44	N.d.[b]

[a] For reaction details see Section 3.
[b] Not determined.

Table 6. Preparative scale hydrolysis of racemic *trans*-1,2-diacetoxy-cycloalkanes *rac*-**3a–d** by lipase AK[a]

Substrate	Time (h)	(*R*,*R*)-**2**		
		Yield (%)	Ee (%)[b]	$[\alpha]_D^{22}$
rac-**3a**	30	28	96	−28.2[c]
rac-**3b**	24	21	99	−43.8[d]
rac-**3c**	24	37	>98	−21.9[c]
rac-**3d**	30	25	9	−0.9[c]

[a] For reaction details see Section 3.
[b] Determined by GC on HP Chiral or Beta-DEX 225.
[c] *c* 1.0 in acetonitrile.
[d] *c* 1.0 in chloroform.

Our final effort was to find an efficient extraction system for the separation of the resulting monoacetate **2a–d** and diacetate **3a–d** fractions, which might be useful for the elaboration of chromatography-free purification processes or even for the development of continuously operating tube reactor-extractor systems. To solve this task, a systematic search was made to find the most selective hexane—a water/methanol two-phase extraction system using a hexane solution of equimolar mixture of the six-membered mono- and diacetates **2b** and **3b** (Table 7).

Table 7. Extractive separation of 2-acetoxycyclohexan-1-ol **2b** and 1,2-diacetoxycyclohexane **3b**[a]

MeOH (%) In aqueous phase[a]	**3b** (%) In hexane phase[b]	**2b** (%) In hexane phase[b]	K_{3b}/K_{2b}[c]
0	8.1	88.0	83
10	8.5	92.8	138
20	7.6	92.5	149
30	6.3	88.6	115
40	6.1	80.6	64
50	3.5	68.4	59
60	2.9	50.9	34
70	1.8	35.7	30
80	1.8	24.3	17
90	2.6	21.0	10

[a] Hexane solution (1 ml) of **2b** (0.1 mmol) and **3b** (0.1 mmol) was extracted with a water–MeOH mixture (1 ml).
[b] Amounts of **2b** and **3b** (%) are relative to those which were present in the original hexane solution, and were determined by GC on HP Chiral column using undecene as internal standard.
[c] Ratio of distribution coefficients *K* for **3b** and **2b**.

The results indicated, that an extraction with 20% MeOH–water system is optimal for the separation of the mixture of **2b** and **3b** with a selectivity factor (K_{3b}/K_{2b}) of 149. This selectivity enables an efficient, extractive separation of the products without chromatography.

3. Experimental

3.1. Materials and methods

3.1.1. Analytical methods. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C; $CDCl_3$; TMS; ppm on δ scale). IR spectra were taken on a Specord 2000 spectrometer in film and the wavenumbers reported in cm^{-1}. GC analyses were carried out on Agilent 4890D or HP 5890 instruments equipped with FID detector using H_2 as the carrier gas (injector: 250°C, detector: 250°C, head pressure: 12 psi). For the separations HP Chiral (30 m×0.32 mm×0.25 μm film of 20% permethylated β-cyclodextrin, HP Part No.: 190916-B213) with a 1:50 split ratio; or Beta-DEX 225 (30 m×0.25 mm×0.25 μm film, Supelco Column No.: 16161-0413) with a 1:80 split ratio were used. Optical rotations were determined on a Perkin Elmer 241 polarimeter. TLC was carried out on Kieselgel 60 F_{254} (Merck) sheets. Spots were visualized by treatment with 5% ethanolic phosphomolybdic acid solution and heating of the dried plates. Solvents used were freshly distilled.

3.1.2. Reagents and solvents. *trans*-Cyclopentane-1,2-diol *rac*-**1a**, *trans*-cyclohexane-1,2-diol *rac*-**1b** and vinyl acetate were products of Aldrich. *trans*-Cycloheptane-1,2-dol *rac*-**1c**[19] and *trans*-cyclooctane-1,2-diol *rac*-**1d**[20] were prepared by literature procedures.

3.1.3. Biocatalysts. Lipase A, lipase AK, lipase AY, lipase M, lipase PS and lipase R were obtained from Amano Europe. Lipozyme IM, Lipozyme IM 20, Lipozyme TL IM, Novozyme 435 and *Candida antarctica* lipase A were products of Novozymes, Denmark. Lipases from *Candida rugosa* and *Pseudomonas fluorescens* were purchased from Fluka. PPL and lipase from *Candida cylindracae* were obtained from Sigma. A selection of extracellular hydrolases from various thermophilic fungi (lipases TUB 1-52a,b) were isolated as acetone dried supernatants of shake flask fermentations.[15]

3.2. Racemic *trans*-2-acetoxycycloalkan-1-ols, *rac*-2a–d

To a suspension of racemic diol *rac*-**1a**–**d** (17.2 mmol) and Na_2CO_3 (5.48 g, 52 mmol) in EtOAc (60 ml), Ac_2O (0.052 mol) was added at room temperature over a period of 15 min. After stirring for 14 h, the mixture was poured into 40 ml of ice-water, and the aqueous phase extracted with EtOAc (2×40 ml). The combined organic extracts were dried over $MgSO_4$ and concentrated. Separation of the residue by chromatography (silica gel, 33–100% gradient of EtOAc in hexane) gave the monoacetate *rac*-**2a**–**d** as a colourless oil.

3.2.1. *trans*-2-Acetoxycyclopentan-1-ol, *rac*-2a. Yield: 1.36 g, 55%; IR: 3448, 2968, 1736, 1376, 1244, 1140, 1092; ^{1}H NMR: 1.45–1.7 (4H, m), 1.8–1.9 (1H, m), 1.99 (3H, s, CO-CH_3), 2.0–2.1 (1H, m), 3.29 (1H, m, OH), 4.00 (1H, br s, C(1)H), 4.40 (1H, m, C(2)H); ^{13}C NMR: 21.09, 21.36, 29.77, 32.22, 77.30, 83.04, 171.45; Anal. calcd for $C_7H_{12}O_3$: C, 58.32, H, 8.39. Found: C, 58.49, H, 8.32%.

3.2.2. *trans*-2-Acetoxycyclohexan-1-ol, *rac*-2b. Yield: 1.66 g, 61%; IR: 3432, 2936, 2896, 1732, 1384, 1232, 1080, 1040; ^{1}H NMR: 1.2–1.36 (4H, m), 1.62–1.71 (2H, m), 1.95–2.05 (2H, m), 2.06 (3H, s, CO-CH_3), 2.53 (1H, m, OH), 3.52 (1H, mc, C(1)H), 4.56 (1H, mc, C(2)H); ^{13}C NMR: 21.40, 23.84, 23.94, 30.03, 33.11, 72.57, 78.09, 171.04; Anal. calcd for $C_8H_{14}O_3$: C, 60.74; H, 8.92. Found: C, 60.56; H, 8.88%.

3.2.3. *trans*-2-Acetoxycycloheptan-1-ol, *rac*-2c. Yield: 1.36 g, 46%; IR: 3448, 2936, 2888, 1736, 1452, 1376, 1248, 1028; ^{1}H NMR: 1.35–1.50 (4H, m), 1.52–1.69 (4H, m), 1.71–1.82 (2H, m), 2.03 (3H, s, CO-CH_3), 2.88 (1H, s, OH), 3.67 (1H, m, C(1)H), 4.64 (1H, m, C(2)H); ^{13}C NMR: 21.38, 22.73, 22.86, 28.00, 30.19, 32.69, 75.47, 81.56, 171.12; Anal. calcd for $C_9H_{16}O_3$: C, 62.77; H, 9.36. Found: C, 62.91; H, 9.29%.

3.2.4. *trans*-2-Acetoxycyclooctan-1-ol, *rac*-2d. Yield: 0.99 g, 31%; IR: 3416, 2936, 2888, 1732, 1368, 1248, 1052, 1040; ^{1}H NMR: 1.35–1.50 (2H, m), 1.61–1.83 (10H, m), 1.98 (3H, s), 2.13 (1H, s, OH), 3.78 (1H, m, C(1)H), 4.84 (1H, m C(2)H); ^{13}C NMR: 21.58, 22.03, 22.94, 27.39, 30.09, 30.48, 33.52, 71.31, 74.39, 170.21; Anal. calcd for $C_{10}H_{18}O_3$: C, 64.49; H, 9.74. Found: C, 64.56; H, 9.82%.

3.3. Racemic *trans*-1,2-diacetoxycycloalkanes, *rac*-3a–d

To a solution of *rac*-**1a**–**d** (8.7 mmol) in dry Et_3N (2.53 g, 25 mmol), Ac_2O (1.77 g, 17.4 mmol) was added over a period of 15 min and the mixture stirred at room temperature for 24 h. The resulting mixture was then poured into cold water (20 ml), and the aqueous phase extracted with hexane (3×20 ml). The combined organic extracts were washed with 5% HCl (10 ml), saturated $NaHCO_3$ (10 ml) and brine (10 ml). The solution was dried over $MgSO_4$ and concentrated. Separation of the residue by chromatography (silica gel, 33–100% gradient of EtOAc in hexane) gave the diacetate *rac*-**3a**–**d** as a colourless oil.

3.3.1. *trans*-1,2-Diacetoxycyclopentane, *rac*-3a. Yield: 1.16 g, 72%; IR: 2976, 1748, 1372, 1252, 1088, 1040; ^{1}H NMR: 1.34–1.41 (2H, m), 1.48–1.54 (2H, m), 1.79 (6H, s, 2 CO-CH_3), 1.8–1.89 (2H, m), 4.82 (2H, br s, C(1)H and C(2)H); ^{13}C NMR: 20.49, 21.08, 29.90, 78.28, 169.10; Anal. calcd for $C_9H_{14}O_4$: C, 58.05; H, 7.58. Found: C, 57.96; H, 7.62%.

3.3.2. *trans*-1,2-Diacetoxycyclohexane, *rac*-3b. Yield: 1.39 g, 80%; IR: 2944, 2896, 1740, 1368, 1232, 1044; ^{1}H NMR: 1.26–1.42 (4H, m), 1.65–1.73 (2H, m), 2.01 (6H,s, 2 CO-CH_3), 2.03 (2H, m), 4.78 (2H, mc, C(1)H and C(2)H); ^{13}C NMR: 21.21, 23.49, 30.17, 73.63, 170.09; Anal. calcd for $C_{10}H_{16}O_4$: C, 59.98; H, 8.05. Found: C, 60.01; H, 8.01%.

3.3.3. *trans*-1,2-Diacetoxycycloheptane, *rac*-3c. Yield: 1.47 g, 79%; IR: 2936, 2838, 1740, 1372, 1240, 1032, 992; ^{1}H NMR: 1.44–1.65 (4H, m), 1.58–1.66 (4H, m), 1.72–1.79 (2H, m), 1.97 (6H, s, 2 CO-CH_3), 4.92 (2H, mc, C(1)H and C(2)H); ^{13}C NMR: 21.20, 22.82, 28.31, 30.37, 76.76, 169.84; Anal. calcd for $C_{11}H_{18}O_4$: C, 61.66; H, 8.47. Found: C, 61.59; H, 8.43%.

3.3.4. *trans*-1,2-Diacetoxycyclooctane, *rac*-3d. Yield: 1.39 g, 70%; IR: 2936, 2838, 1736, 1368, 1248, 1032, 1020, 980; ^{1}H NMR: 1.43–1.51 (2H, m), 1.62–1.86 (10H, m), 1.97 (6H, s, 2 CO-CH_3), 4.84 (2H, m, C(1)H and C(2)H); ^{13}C NMR: 21.46, 22.53, 27.23, 30.13, 73.90, 168.88; Anal. calcd for $C_{12}H_{20}O_4$: C, 63.14; H, 8.83. Found: 63.21; H, 8.78%.

3.4. Analytical scale enzymatic acylation of the racemic *trans*-2-acetoxycycloalkan-1-ols, *rac*-2a–d

Enzyme (20 mg, for *rac*-**2a**: see Table 2; for *rac*-**2b**: see Table 1; for *rac*-**2c**: see Table 3; for *rac*-**2d**: see Table 4) was added to a solution of racemic *trans*-2-acetoxycycloalkan-1-ol *rac*-**2a**–**d** (20 mg) in hexane (1.5 ml) and vinyl acetate (0.5 ml) and the resulting suspension shaken in a sealed glass vial at 1000 rpm, at room temperature for the time indicated in Tables 1–4. The reaction was then analysed by GC. The conversion and enantiomeric composition of the resulting monoacetates (*S*,*S*)-**2a**–**d** and diacetates (*R*,*R*)-**3a**–**d** are listed in Tables 1–4.

3.4.1. GC analysis for acylation reactions from *trans*-2-acetoxy-cyclopentan-1-ol *rac*-2a. R_t (HP Chiral; 100–116°C, 2°C/min)/min: 5.76 (*S*,*S*)-**2a**, 6.27 (*R*,*R*)-**2a**, 6.98 (*S*,*S*)- and (*R*,*R*)-**3a**.

3.4.2. GC analysis for acylation reactions from *trans*-2-acetoxy-cyclohexan-1-ol *rac*-2b. R_t (HP Chiral; 100–115°C, 1°C/min)/min: 10.57 (*S*,*S*)-**2b**, 10.98 (*R*,*R*)-**2b**, 12.21 (*S*,*S*)-**3b**, 12.59 (*R*,*R*)-**3b**.

3.4.3. GC analysis for acylation reactions from *trans*-2-acetoxy-cycloheptan-1-ol *rac*-2c. R_t (HP Chiral; 100–136°C, 2°C/min)/min: 13.22 (*S*,*S*)-**2c**, 13.38 (*R*,*R*)-**2c**, 15.11 (*S*,*S*)-**3c**, 15.29 (*R*,*R*)-**3c**.

3.4.4. GC analysis for acylation reactions from *trans*-2-acetoxycyclooctan-1-ol *rac*-2d. R_t (Beta-DEX 225; 100–170°C, 2°C/min)/min: 28.62 (*S*,*S*)-**2d**, 28.79 (*R*,*R*)-**2d**, 33.21 (*S*,*S*)- and (*R*,*R*)-**3d**.

3.5. Preparative scale enzymatic acylation of racemic *trans*-2-acetoxycycloalkan-1-ols, *rac*-2a–d

Lipase AK (150 mg for *rac*-**2a**, *rac*-**2b** and *rac*-**2c**; 100 mg for *rac*-**2d**) was added to a solution of racemic *trans*-2-acetoxycycloalkan-1-ol (*rac*-**2a**: 300 mg, 2.08 mmol; *rac*-**2b**: 500 mg, 3.16 mmol; *rac*-**2c**: 300 mg, 1.74 mmol; *rac*-**2d**: 400 mg, 2.15 mmol) in vinyl acetate (3 ml for *rac*-**2a**, *rac*-**2c** and *rac*-**2d**; 5 ml for *rac*-**2b**) and the resulting suspension shaken at 1000 rpm at room temperature (12 h for *rac*-**2a**, 10 h for *rac*-**2b**; 18 h for *rac*-**2c**; 52 h for *rac*-**2d**). The enzyme was filtered off and the filtrate concentrated in vacuum. The residue was separated by vacuum-chromatography (silica gel, hexane–acetone 10:1 v/v) to yield the monoacetate (*S*,*S*)-**2a–d** and diacetate (*R*,*R*)-**3a–d**.

3.5.1. (*S*,*S*)-2-Acetoxycyclopentan-1-ol (*S*,*S*)-2a. Yield: 99 mg, 33% as colourless oil; $[\alpha]_D^{22}=+29.2$ (*c* 1.0 in acetonitrile) (lit.,[10] $[\alpha]_D^{20}=+22.3$ (*c* 1.12 in $CHCl_3$) and lit.,[9] $[\alpha]_D^{25}=+29.3$ (*c* 1.1 in $CHCl_3$) were reported for (*RR*)-**2a**); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**2a**.

3.5.2. (*R*,*R*)-1,2-Diacetoxycyclopentane (*R*,*R*)-3a. Yield: 168 mg, 43% as colourless oil; $[\alpha]_D^{22}=-29.7$ (*c* 1.0 in acetonitrile); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**3a**.

3.5.3. (*S*,*S*)-2-Acetoxycyclohexan-1-ol (*S*,*S*)-2b. Yield: 180 mg, 36% as white crystals; mp 59.5–61°C; $[\alpha]_D^{22}=+44.3$ (*c* 1.0 in $CHCl_3$); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**2b**.

3.5.4. (*R*,*R*)-1,2-Diacetoxycyclohexane (*R*,*R*)-3b. Yield: 231 mg, 37% as colourless oil; $[\alpha]_D^{22}=-13.7$ (*c* 1.0 in $CHCl_3$) (lit.,[21] $[\alpha]_D^{22}$ −10.7 (*c* 0.58 in $CHCl_3$), lit.,[22] $[\alpha]_D^{27}$ −12.5 (*c* 1.60 in $CHCl_3$)); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**3b**.

3.5.5. (*S*,*S*)-2-Acetoxycycloheptan-1-ol (*S*,*S*)-2c. Yield: 106 mg, 36% as colourless oil; $[\alpha]_D^{22}=+22.9$ (*c* 1.0 in acetonitrile); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**2c**.

3.5.6. (*R*,*R*)-1,2-Diacetoxycycloheptane (*R*,*R*)-3c. Yield: 156 mg, 40% as colourless oil; $[\alpha]_D^{22}=-13.1$ (*c* 1.0 in acetonitrile); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**3c**.

3.5.7. (*S*,*S*)-2-Acetoxycyclooctan-1-ol (*S*,*S*)-2d. Yield: 171 mg, 43% as colourless oil; $[\alpha]_D^{22}=+9.1$ (*c* 1.0 in acetonitrile); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**2d**.

3.5.8. (*R*,*R*)-1,2-Diacetoxycyclooctane (*R*,*R*)-3d. Yield: 215 mg, 44% as colourless oil; $[\alpha]_D^{22}=0$ (*c* 1.0 in acetonitrile); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**3d**.

3.6. Preparative scale enzymatic hydrolysis of racemic *trans*-1,2-diacetoxycycloalkanes, *rac*-3a–d

Lipase AK (150 mg for *rac*-**3a** and *rac*-**3b**; 75 mg for *rac*-**3c** and *rac*-**3d**) was added to racemic *trans*-2-diacetoxycycloalkane (*rac*-**3a**: 300 mg, 1.61 mmol; *rac*-**3b**: 500 mg, 2.50 mmol; *rac*-**3c**: 120 mg, 0.56 mmol; *rac*-**3d**: 150 mg, 0.66 mmol) in water (3 ml for *rac*-**3a** and *rac*-**3d**; 5 ml for *rac*-**3b**; 1.5 ml for *rac*-**3c**) and the resulting mixture shaken at 1000 rpm at room temperature (30 h for *rac*-**3a** and *rac*-**3d**; 24 h for *rac*-**3b** and *rac*-**3c**). The mixture was extracted with EtOAc (2×10 ml) and the combined extracts washed with brine (10 ml), dried over Na_2SO_4, and concentrated in vacuum. The residue was separated by vacuum-chromatography (silica gel, hexane–acetone 10:1 v/v) to yield the monoacetate (*RR*)-**2a–d** and diacetate (*SS*)-**3a–d**.

3.6.1. (*R*,*R*)-2-Acetoxycyclopentan-1-ol (*R*,*R*)-2a. Yield: 65 mg, 28% as colourless oil; $[\alpha]_D^{22}=-28.2$ (*c* 1.0 in acetonitrile); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**2a**.

3.6.2. (*S*,*S*)-1,2-Diacetoxycyclopentane (*S*,*S*)-3a. Yield: 154 mg, 51% as colourless oil; $[\alpha]_D^{22}=+23.2$ (*c* 1.0 in acetonitrile); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**3a**.

3.6.3. (*R*,*R*)-2-Acetoxycyclohexan-1-ol (*R*,*R*)-2b. Yield: 106 mg, 21% as white crystals; mp 57–59 (lit.,[9], oil; lit.,[18], mp 28°C); $[\alpha]_D^{22}=-43.8$ (*c* 1.0 in $CHCl_3$) (lit.[9] $[\alpha]_D^{22}=-37.5$ (*c* 1.1 in $CHCl_3$), lit.,[9] $[\alpha]_D^{28}=-30.2$ (*c* 1.60 in $CHCl_3$)); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**2b**.

3.6.4. (*S*,*S*)-1,2-Diacetoxycyclohexane (*S*,*S*)-3b. Yield: 94 mg, 24% as colourless oil; $[\alpha]_D^{22}=+9.5$ (*c* 1.0 in $CHCl_3$) (lit.,[21] $[\alpha]_D^{20}=+12.0$ (*c* 0.96 in $CHCl_3$), lit.,[12] $[\alpha]_D^{20}=+16.1$ (*c* 0.42 in MeOH)); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**3b**.

3.6.5. (*R*,*R*)-2-Acetoxycycloheptan-1-ol (*R*,*R*)-2c. Yield: 36 mg, 37% as colourless oil; $[\alpha]_D^{22}=-21.9$ (*c* 1.0 in acetonitrile); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**2c**.

3.6.6. (*S*,*S*)-1,2-Diacetoxycycloheptane (*S*,*S*)-3c. Yield: 52 mg, 43% as colourless oil; $[\alpha]_D^{22}=+3.0$ (*c* 1.0 in acetonitrile); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**3c**.

3.6.7. (*R*,*R*)-2-Acetoxycyclooctan-1-ol (*R*,*R*)-2d. Yield: 97 mg, 80% as colourless oil; $[\alpha]_D^{22}=-0.9$ (*c* 1.0 in acetonitrile); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**2d**.

3.6.8. (*S*,*S*)-1,2-Diacetoxycyclooctane (*S*,*S*)-3d. Yield: 11 mg, 7% as colourless oil; $[\alpha]_D^{22}=0$ (*c* 1.0 in acetonitrile); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**3d**.

4. Conclusion

In conclusion, for simple kinetic resolutions of the monoacetates of *trans*-cycloalkane-1,2-diols *rac*-**2a**–**d**, a number of highly enantiomeric selective enzymes were found. These enzymes enabled the preparation of highly enantiopure diacetates (*R*,*R*)-**3a**–**c** and monoacetates (*S*,*S*)-**2a**–**c** in good yields. Our screen also showed that most enzymes are significantly less selective towards the monoacetate of *trans*-cyclooctane-1,2-diol *rac*-**2d**.

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