DOI: 10.1002/chem.200600257

Highly Efficient Synthesis of Enantiopure Diacetylated C_2-Symmetric Diols by Ruthenium- and Enzyme-Catalyzed Dynamic Kinetic Asymmetric Transformation (DYKAT)

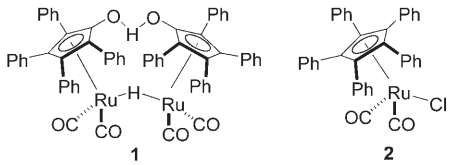
Belén Martín-Matute,[a, b] Michaela Edin,[a] and Jan-E. Bäckvall*[a]

Abstract: Highly efficient synthesis of enantiopure diacetates of 2,4-pentanediol and 2,5-hexanediol starting from commercially available mixtures of the diols (*dl*/*meso* ≈1:1) has been realized by combining a fast ruthenium-catalyzed epimerization with an enzymatic transesterification. The in situ coupling of these two processes produces the diacetates in high yield in >99% enantiomeric excess.

Keywords: diols · dynamic kinetic asymmetric transformations · enzymes · kinetic resolution · racemization

Introduction

Great achievements in catalytic asymmetric synthetic transformations using transition metals, enzymes, and organocatalysts have been reported in recent years.[1] However, kinetic resolution (KR) of racemic mixtures is still the most common way to prepare enantiomerically pure compounds on an industrial scale.[2] Enzymatic KR suffers, as does all resolution, from being limited to a maximum theoretical yield of 50%. In situ racemization of the slow-reacting enantiomer leads to deracemization by dynamic kinetic resolution (DKR), and makes a 100% yield of enantiopure product possible. The number of examples of chemoenzymatic DKR that combine an enzymatic KR with an in situ racemization method has increased during the past few years.[3–11] In particular, we have performed DKR of a variety of *sec*-alcohols by combining KR with an in situ racemization catalyzed by Shvo's[12] ruthenium complex (**1**).[3] More recently, we have employed a more effective racemization catalyst (**2**)[13] that, in combination with an enzyme, allows us to perform DKR of *sec*-alcohols under very mild reaction conditions.[5,11b] The ruthenium-catalyzed racemization of the *sec*-alcohols involves hydrogen transfer via a ketone and the mechanism of hydrogen transfer with catalysts **1** and **2** has recently been investigated.[5b,14]



An enzyme-catalyzed kinetic asymmetric transformation (KAT) of a commercial mixture of diols (*dl*/*meso* ≈1:1) would give, in the best case, a maximum theoretical yield of 25% of one enantiomer.[15] Therefore, the development of new synthetic methods to obtain enantiopure diols in 100% yield is highly desirable. The combination of KATs with an in situ racemization/epimerization leads to a dynamic kinetic asymmetric transformation (DYKAT) and makes a theoretical yield of 100% possible. As part of our ongoing program we have recently reported the DYKAT of diols by employing *Candida antarctica* lipase B (CALB) for the resolution and a ruthenium complex (**1** or **2**) for the epimerization.[16,17]

Despite the high enantiomeric excess obtained in the DYKAT of 1,3- and 1,4-symmetrical diols, this process showed low diastereoselectivity for 2,4-pentanediol (**3**) and 2,5-hexanediol (**4**) in their transformation to **5** and **6**, respectively (Scheme 1). In our previous studies, we concluded that the reason for the poor diastereoselectivity ((*R*,*R*):*meso* = 38:62) for the former diol is a rapid 1,3-acyl-

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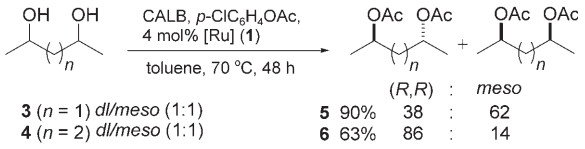
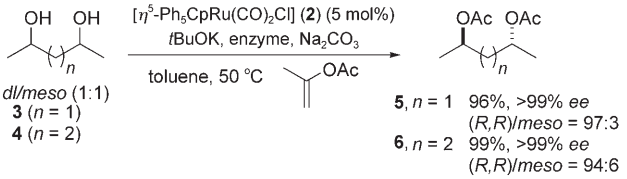


Scheme 1. Shvo- and CALB-catalyzed DYKAT of diols.

migration in the *syn*-1,3-diol monoacetate intermediate,[18] and for the latter diol (**4**) the low selectivity ((*R*,*R*):*meso* = 86:14) is due to a low enantioselectivity of the enzyme for the monoacylated intermediates, resulting in the formation of anti-Kazlauskas[19] products (i.e., acylation at an (*S*)-alcohol site). It is highly desirable to find a solution to the problem with the poor diastereoselectivity in Scheme 1 due to the importance of optically pure C_2-symmetric diols, as sources of chiral auxiliaries and ligands. Furthermore, 1,4-diols have been used in the preparation of enantiopure *trans*-2,5-disubstituted pyrrolidines.[20]

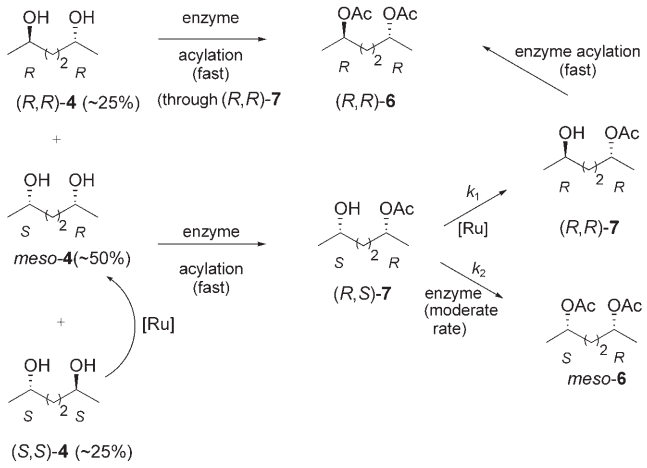
Here we report a detailed study of the enzyme-catalyzed acylation (kinetic asymmetric transformation) of 2,5-hexanediol (**4**) and its monoacetates, and also of those intermediates produced under dynamic conditions, that is, in the presence of the ruthenium catalyst. The use of a fast racemization/epimerization Ru complex (**2**), together with a better understanding of the enzyme-catalyzed acylations, has made it possible to develop a very efficient DYKAT providing a method for the synthesis of (*R*,*R*)-2,5-hexanediol diacetate (**6**). Also, by using Ru complex **2**, the DYKAT of 2,4-pentanediol (**3**) can be performed at a lower temperature (50 °C). These new reaction conditions outrun the acyl migration, avoiding the formation of unwanted *meso*-diacetate **5**. Both (*R*,*R*)-diacetates, **5** and **6**, can be prepared in excellent enantiomeric excess (*ee*), diastereomeric excess (*de*), and yield (Scheme 2).



Scheme 2. DYKAT of diols catalyzed by an enzyme and ruthenium complex **2**.

Results and Discussion

Kinetic studies of 2,5-hexanediol (4): We first turned our attention to the DYKAT of 2,5-hexanediol. A simplified overview of the mechanism is shown in Scheme 3. Diol (*R*,*R*)-**4** reacts fast with the acyl donor in the presence of the enzyme to give monoacetate (*R*,*R*)-**7**, which is immediately acylated again yielding diacetate (*R*,*R*)-**6**. The *meso*-diol will form monoacetate (*R*,*S*)-**7** very fast. This reaction can follow two pathways: 1) it can be epimerized with the Ru catalyst to yield monoacetate (*R*,*R*)-**7**, or 2) it can be enzymatically



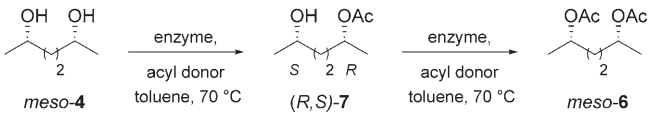
Scheme 3. DYKAT of 2,5-hexanediol (**4**).

acylated (at a moderate rate) at the (*S*)-alcohol site (anti-Kazlauskas acylation) to yield diacetate *meso*-**6**. The diol (*S*,*S*)-**4** is essentially nonreactive (vide infra) in the system and it would have to be epimerized to *meso*-**4** before any transformation can occur.

The ratio of the rates of formation of (*R*,*R*)-**7** and *meso*-**6** from (*R*,*S*)-**7** is shown in Equation (1).

$$\frac{\text{formation of } (R,R)\text{-}\mathbf{7}}{\text{formation of } \textit{meso}\text{-}\mathbf{6}} = \frac{k_1[\text{Ru}]}{k_2[\text{Acyl-enzyme}]} \quad (1)$$

The DYKAT process would become efficient if the above ratio could be significantly increased. We envisioned two approaches: 1) the use of a more selective enzyme, which gives less (*S*)-acylation of the monoacetate (*R*,*S*)-**7** (smaller k_2) than CALB, or 2) the use of a faster epimerization catalyst, so that competing (*S*)-acylation of (*R*,*S*)-**7** does not occur. A simple and fast experiment to test different enzymes is to study the enzyme-catalyzed acylation of diol *meso*-**4** (Scheme 4). We were looking for an enzyme that would efficiently catalyze the first acylation to form monoacetate (*R*,*S*)-**7**, but would not undergo acylation of the remaining (*S*)-alcohol affording *meso*-diacetate (*meso*-**6**) ($k_2 \ll k_1$).



Scheme 4. Enzyme-catalyzed KAT of *meso*-**4**.

Pure *meso* diol (*meso*-**4**) was prepared as previously described (see Supporting Information).[15d,18] From our experience,[3] we knew that only two *R*-selective lipases[21] have been successfully combined with an in situ racemization/epimerization for DKR of alcohols: *Candida antarctica* lipase B (CALB) and *Pseudomonas cepacia* lipase (PS-C "Amano II"). Figure 1 shows the kinetics of CALB- and PS-C-cat-

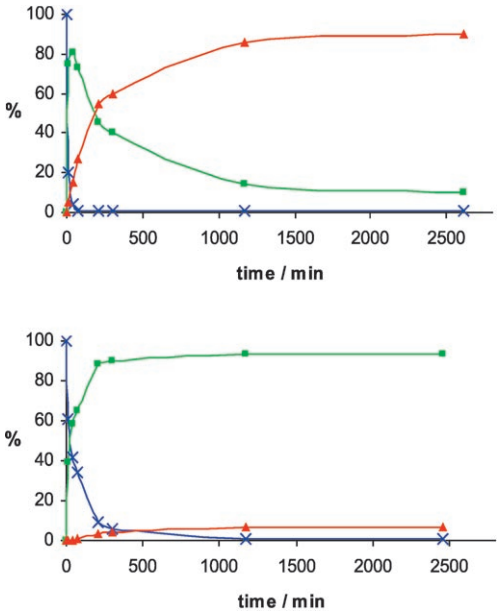
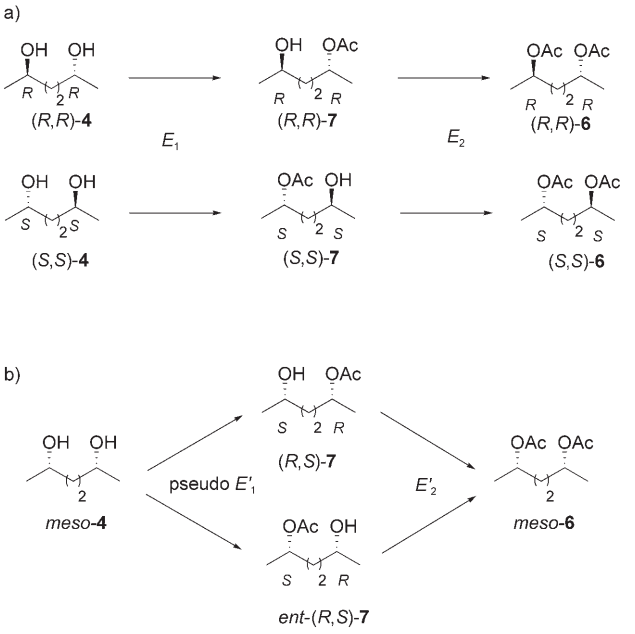


Figure 1. CALB- (top) and PS-C-catalyzed (bottom) reaction of *meso*-**4**. ×: % *meso*-**4**, ■: % (*R*,*S*)-**7**, ▲: % *meso*-**6**.



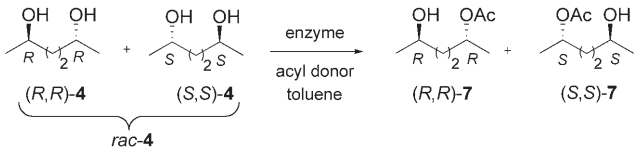
Scheme 5. Sequential esterifications of a) *rac*-**4** and b) *meso*-**4**.

alyzed reactions of diol *meso*-**4** as a function of time. Both KRs were performed at 70 °C by using three equivalents of *p*-chlorophenyl acetate as the acyl donor in toluene. It was observed that CALB showed very high activity for both acylations: after 1 h the diol *meso*-**4** had been completely transformed to a 4:1 mixture of monoacetate (*R*,*S*)-**7** and diacetate *meso*-**6**, and after 44 h the reaction mixture consisted of only 10% of monoacetate (*R*,*S*)-**7** and 90% of *meso*-**6**. In contrast, both acylations, in particular the second, were much slower for the reaction catalyzed by PS-C "Amano II"; after 45 min essentially no diacetate had been formed (<1%) and GC analysis showed a mixture of diol *meso*-**4** and monoacetate (*R*,*S*)-**7** in a ratio of 42:58. After 44 h there was still very little diacetate *meso*-**6** (7%) and the main product was monoacetate (*R*,*S*)-**7** (Figure 1, bottom).

Because of the better results obtained with PS-C lipase for the reaction of *meso*-**4** (Figure 1), it was of interest to compare selectivity and activity of both enzymes for all the enzyme-catalyzed transformations involved in the acylation of 2,5-hexanediol **4**. The esterification of diol **4** is not a simple single-step reaction, but involves two sequential esterifications in which each step can exhibit selectivity. Furthermore, the relative configuration of the two stereogenic centers may also affect the selectivity of each of the acetylations (Scheme 5). Therefore, the *meso*- and *rac*-diols were studied separately.

The selectivity of the enzymes was measured by quantifying the enantiomeric ratio (*E*) for the different steps of the enzymatic transformations.[22] The *E* value only applies to enantiomers, therefore, pure racemic diol (*rac*-**4**), and racemic mixtures of monoacetates had to be prepared. Diol *rac*-**4**, diol *meso*-**4**, a racemic mixture of monoacetates (*R*,*R*)-**7** and (*S*,*S*)-**7**, and a racemic mixture of monoacetates (*R*,*S*)-**7** and *ent*-(*R*,*S*)-**7** were prepared as described in the Supporting Information.

First we studied the consecutive kinetic resolutions of *rac*-**4** (Scheme 6). For molecules containing two alcohols that



Scheme 6. KR of (*R*,*R*)-**4** and (*S*,*S*)-**4**.

can be acylated by the enzyme, the reaction does not stop at the chiral monoacetate stage, but rather undergoes a second acylation to yield diacetates. This complicates the determination of the selectivity of the enzyme for the first acylation. In the case of *rac*-**4**, the second acylation is very fast, and when aliquots were taken at different reaction times, a mixture of diol, monoacetate, and diacetates was detected by chiral GC analysis. In the case of PS-C, the reaction was performed at 70 °C. After 15 min, the reaction was stopped. The diols were separated from the mixture of monoacetates and diacetates, which were collected together, by silica gel chromatography. The *ee* of the diols (18% *ee*) was measured after chemical acylation to the diacetates. The monoacetate/diacetate fraction was analyzed by GC, and showed a 1.9:1 mixture of monoacetate (*R*,*R*)-**7** in >99% *ee* and diacetate (*R*,*R*)-**6** in >99% *ee*. This shows that the E_1 (PS-C) is >200 for the monoacylation of *rac*-**4**. Similar studies were performed for the reaction catalyzed by CALB. In this case, due the higher activity of this enzyme, the reaction was performed at room temperature for only 2.5 min. Also in this case a mixture of enantiomerically pure monoacetate (*R*,*R*)-

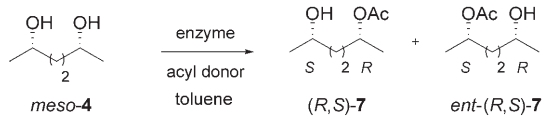
7 (>99% *ee*) and diacetate *R*,*R*)-**6** (>99% *ee*) was obtained, therefore E_1 (CALB) is also >200. The results are shown in Scheme 6 and Table 1. The conclusion is that both enzymes show very high enantioselectivity for the first acylation.

Table 1. KR of (*R*,*R*)-**4** and (*S*,*S*)-**4**.[a]

Enzyme	*t* [min]/ *T* [°C]	*ee* [%] of **4**[b]	*ee* [%] of **7**[c,d]	E_1
PS-C "Amano II"	22/70	18 (*S*,*S*)	>99 (*R*,*R*)	>200
CALB	2.5/RT	8 (*S*,*S*)	>99 (*R*,*R*)	>200

[a] For reaction conditions, see Experimental Section. [b] Determined by GC after chemical diacetylation. [c] Determined considering that also diacetate (*R*,*R*)-**6** was produced in 99% *ee*. [d] *ee* determined by GC.

As in the case of *rac*-**4**, the enzymatic reaction of *meso*-**4** may not stop at the chiral monoacetate stage (**7**), but can undergo a second acylation yielding diacetate *meso*-**6** (Scheme 5b). Scheme 7 shows the desymmetrization of *meso*-**4** to



Scheme 7. Desymmetrization of *meso*-**4**.

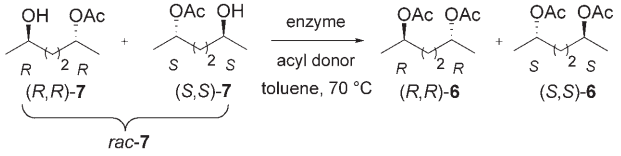
monoacetates **7**. Since this is not a kinetic resolution we cannot obtain an enantiomeric ratio (*E* value). However, we can define a pseudo-*E* value (E'_1).[23] When PS-C was employed as the enzyme, the desymmetrization was performed at 70°C. However, due to a fast second acylation when employing CALB (cf. Figure 1, top), the desymmetrization was performed at room temperature in this case. In both cases, the formation of diacetate was not detected after 15 min; GC analysis showed a mixture of only monoacetate and diol in each case. The pseudo E'_1 values are shown in Table 2, and in both cases they are >199. Thus, for both enzymes, the desymmetrization of *meso*-**4** is highly selective.

Table 2. Desymmetrization of *meso*-**4**.[a]

Enzyme	*t* [min]/ *T* [°C]	*ee* [%] of (*R*,*S*)-**7**[b]	Conversion [%][c]	Pseudo E'_1
PS-C "AmanoII"	15/70	>99	39	>199
CALB	15/23	>99	26	>199

[a] For reaction conditions, see Experimental Section. [b] Determined by GC. [c] Determined ^{1}H NMR spectroscopy.

Kinetic studies of 2,5-hexanediol monoacetates (7): The second enantiomeric value (E_2) for the transformation of (*rac*)-**4** to diacetate involves monoacetates as substrates and was determined in toluene at 70°C. A racemic mixture of monoacetates (*R*,*R*)-**7** and (*S*,*S*)-**7** was subjected to enzymatic acylation in the presence of *p*-chlorophenyl acetate (3 equiv) as the acyl donor (Scheme 8). Both of the enzymes, PS-C and CALB showed very high selectivity, in particular PS-C, which was found to have an E_2 value of 268. The corresponding E_2 value for CALB was 94. The results are summarized in Table 3.



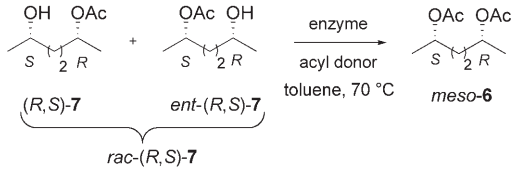
Scheme 8. KR of (*R*,*R*)-**7** and (*S*,*S*)-**7**.

The selectivity of the second acylation of the *meso* series (E'_2; Scheme 9) was determined by KR of the monoacetate *rac*-(*R*,*S*)-**7** (racemic mixture of (*R*,*S*)-**7**/*ent*-(*R*,*S*)-**7**). The KR was carried out at 70°C for both enzymes (Table 4). The E'_2 values obtained are rather low, 26 for PS-C and 7 for CALB. It is of interest to compare these results with those obtained for the kinetic resolution of (*R*,*R*)-**7**/(*S*,*S*)-**7** (Scheme 8), in which the enantiomeric ratio (E_2) is large;

Table 3. KR of *rac*-**7** ((*R*,*R*)-**7**/(*S*,*S*)-**7**).[a]

Enzyme	*t* [min]/ *T* [°C]	*ee* [%] of **7**[b]	*ee* [%] of **6**[b]	Conversion [%][c]	E_2
PS-C "Amano II"	30/70	31 (*S*,*S*)	>99 (*R*,*R*)	24	268
CALB	30/70	93 (*S*,*S*)	93 (*R*,*R*)	50	94

[a] For reaction conditions, see Experimental Section. [b] Determined by GC. [c] Determined applying the formula: conversion = $ee_s/(ee_s+ee_p)$.



Scheme 9. KR of monoacetates *rac*-(*R*,*S*)-**7**.

Table 4. KR of monoacetates *rac*-(*R*,*S*)-**7**.[a]

Enzyme	*t* [min]/ *T* [°C]	*ee* [%] of **7**[b]	Conversion [%][c]	E'_2
PS-C "Amano II"	30/70	27	23	26
CALB	30/70	63	51	7

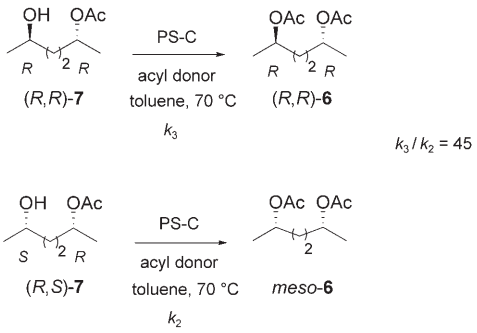
[a] For reaction conditions, see Experimental Section. [b] Determined by GC. [c] Determined by ^{1}H NMR spectroscopy.

268 for PS-C and 94 for CALB. These results show that the relative stereochemistry of the neighboring acetate group is of importance for the enantioselectivity in the enzymatic acylation of the diol monoacetates to diacetates; in contrast

with the very slow acylation of monoacetate (*S*,*S*)-**7** in Scheme 8, acylation of the (*S*)-alcohol in monoacetate (*R*,*S*)-**7** seems to be facile.

It is also of interest to determine the relative rate of acylation of (5*R*)- and (5*S*)-alcohols when there is a (2*R*)-acetate present, that is, to determine the relative rate of enzymatic acylation of (*R*,*R*)-**7** and (*R*,*S*)-**7**. These two monoacetates will be produced in higher concentrations than the other monoacetates in the reaction mixture of the DYKAT process, because of the excellent enantioselectivity (E_1 and pseudo E'_1) in the acylation of the diols. Monoacetates (*R*,*R*)-**7** and (*R*,*S*)-**7** were prepared according to a previously described procedure[18] with a slight modification (see Supporting Information).

The enzymatic acylation of monoacetate (*R*,*R*)-**7** was compared with that of monoacetate (*R*,*S*)-**7** for the approximate first-order acetylation reaction (Scheme 10). The kinetic studies when employing PS-C as the enzyme gave a ratio k_3/k_2 of 45 (Figure 2), which can be compared to the *E* values obtained for the monoacetates (E_2 and E'_2). We have previously carried out the analogous kinetic experiment for the CALB-catalyzed reaction and a ratio k_3/k_2 of 25 was observed.[18] Once again, the PS-C lipase gave a more selective reaction than CALB.



Scheme 10. PS-C-catalyzed acylation of (*R*,*R*)-**7** and of (*R*,*S*)-**7**.

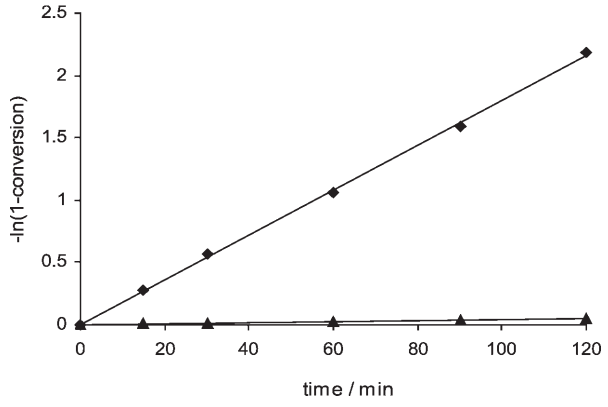
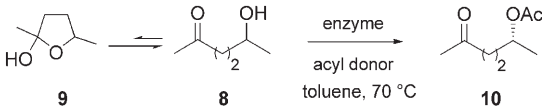


Figure 2. PS-C rate of acetylation of (*R*,*R*)-**7** (◆) and of (*R*,*S*)-**7** (▲). Reactions performed at 70 °C in toluene using *p*-chlorophenylacetate as acyl donor (3 equiv) and 30 mg of enzyme per mmol of **7**.

Kinetic studies of keto intermediates formed in the DYKAT of 2,5-hexanediol (4): The above studies show that in general *Pseudomonas cepacia* lipase shows higher selectivity than *Candida antarctica* lipase B. However, the enzyme- and ruthenium-catalyzed DYKAT gives only slightly higher *de* for the reaction catalyzed by *Pseudomonas cepacia* lipase compared to the reaction catalyzed by *Candida antarctica* lipase B (vide infra). We therefore next considered the intermediates that are formed in the reaction mixture under DYKAT conditions. It is important to note that during the racemization of *sec*-alcohols catalyzed by ruthenium complexes, a hydrogen-transfer process takes place, and ketones are formed as reaction intermediates. The concentration of ketones in the reaction mixtures increases as the reaction temperature increases.[24] In the case of 1,4-diols, the oxidation of one of the hydroxyl groups gives rise to γ-hydroxyketones **8**. The enantioselectivity of the enzymatic acylation of hydroxyketone **8** may influence the overall enantioselectivity and diastereoselectivity of the DYKAT process, since the ketoacetate formed finally will be converted to diacetate (after ketone reduction and subsequent acylation). Thus, we decided to study the kinetic resolution of compounds **8**. In this case, the conversion of the enzyme-catalyzed KR was measured by NMR spectroscopy. Since it was difficult to measure the conversion due to the formation of cyclic hemiacetals **9** (Scheme 11), the kinetic resolutions were run three times for each enzyme, which gave more reliable *E* values (Table 5). It is interesting to note that both enzymes gave very low *E* values for the kinetic resolution of γ-hydroxyketone **8** and PS-C showed no selectivity at all ($E=1.3$). A similar negative effect of a keto function in the δ-position of a 2-alkanol was recently observed,[17b,25] and an *E* value of 2.2 for the CALB-catalyzed KR of Ph(CO)-$(CH_2)_2$CH(OH)Me was obtained. The pseudo *E* value for the corresponding 1,4-diol, PhCH(OH)$(CH_2)_2$CH(OH)Me, was 285.[17b] This drastic drop of the enantioselectivity on changing from 1,4-diol to 4-hydroxyketone is intriguing. Computational and modeling studies carried out in our group have shown that hydrogen bonding to the ketone is



Scheme 11. KR of 5-hydroxy-2-hexanone (**8**).

Table 5. KR of 5-hydroxy-2-hexanone (**8**).[a]

Enzyme	*t* [min]/*T* [°C]	*ee* [%] of **10**[b]	Conversion [%][c]	E_3[d]
PS-C "Amano II"	15/70	11	10	1.3
CALB	15/70	70	47	9

[a] See Experimental Section. [b] Determined by GC. [c] Determined by ^{1}H NMR spectroscopy. [d] Average of three runs.

responsible for the increased relative rate of acylation of the (*S*)-enantiomer of **8**.[26]

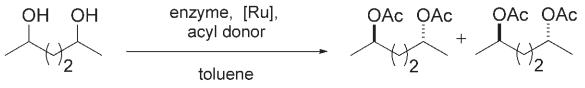
DYKAT of 2,5-hexanediol (4): The DYKAT of 2,5-hexanediol (**4**) was tested with both enzymes. During our initial studies on the DYKAT of diol **4**, we used Shvo's ruthenium complex (**1**) as the racemization catalyst and the reactions were run under the conditions required for dynamic kinetic resolution (DKR) by using ruthenium complex **1**, that is, 70 °C and with *p*-chlorophenylacetate as the acyl donor.[27] The results with complex **1** as the catalyst are shown in Table 6, entries 1 and 2. Thus, when CALB was employed as the enzyme, diacetate **6** was obtained in moderate yield as a mixture of (*R*,*R*)-**6** (99% *ee*) and *meso*-**6** (77:23).[28] PS-C gave slightly better results (Table 6, entry 2), yielding diacetate **6** in higher yield (72%) and a better (*R*,*R*)-**6**/*meso*-**6** ratio (89:11). In both cases, very long reaction times were required. More recently we have found that ruthenium complex **2** efficiently catalyzes racemization of *sec*-alcohols at room temperature, and, importantly, it is compatible with the use of isopropenyl acetate when performing DKR.[5] Catalyst **2** needs to be activated by a catalytic amount of *t*BuOK. We decided to combine this fast racemization catalyst (**2**) with PS-C "Amano II", since this enzyme gives better results in the kinetic studies than CALB (vide supra). The reactions using catalyst **2** were performed at 50 °C. When using 30 mg of PS-C per mmol of diol **4** (entry 3), a good (*R*,*R*)-/*meso*-**6** ratio was obtained (90:10) with 91% yield in 40 h. To decrease the reaction time, the DYKAT was performed with double amount of enzyme (60 mg per mmol of diol **4**; entry 4). Diacetate **6** was obtained in excellent yield (96%) and good (*R*,*R*)/*meso*-**6** ratio (92:8) in 20 h. When the amount of enzyme was further increased the reaction time was reduced to 6 h (entry 5) and **6** was obtained quantitatively with the same (*R*,*R*)/*meso*-**6** ratio as in entry 4. The results were not improved when the amount of enzyme employed was further increased (not shown in the table), or when 6 mol% of *t*BuOK was employed (entry 6). In the last case, although **6** was obtained in 97% yield, more of the undesired *meso*-**6** was produced, probably due to chemical acylation with the excess of base. When the catalyst loading was reduced to 2 mol%, the product was obtained quantitatively, but, once again, more of undesired *meso*-**6** was produced, possibly due to a slower racemization rate (entry 7). The DYKAT was also tested with ruthenium complex **2** and CALB as the enzyme, yielding diacetate **6** in excellent yields and much shorter reaction times (entries 8 and 9) than when the DYKAT was performed with catalyst **1** (cf. entry 1). However, the amount of CALB has to be reduced to 3 mg per mmol of diol **4** in order to obtain a high (*R*,*R*)/*meso*-**6** ratio (94:6) (entry 9). This ratio was not further improved when less than 3 mg of CALB were used.

Mechanistic consideration of DYKAT of 2,5-hexanediol (4): The (*R*,*R*)/*meso* ratio does not dramatically change when the amount of PS-C "Amano II" is increased. However, when the amount of CALB is increased, a considerable decrease of the (*R*,*R*)/*meso* ratio is observed. These results are in accordance with the faster undesired anti-Kazlauskas acylation of monoacetate (*R*,*S*)-**7** to *meso*-**6** for the CALB-catalyzed reaction compared to the PS-C-catalyzed reaction (see Figure 1 and Scheme 3).

Ruthenium catalyst **2** is a faster racemization/epimerization catalyst than Shvo's complex (**1**).[13] When the DYKAT is catalyzed by complex **2** and PS-C, the fast epimerization outruns the unwanted anti-Kazlauskas acylation of (*R*,*S*)-**7** to *meso*-**6** (i.e., $k_1 > k_2$, Scheme 3). With CALB as the enzyme and **2** as the ruthenium catalyst, this situation is achieved when the amount of enzyme is reduced with a factor 20 (Table 6, entry 9).

The reaction temperature also has an important effect on the outcome of the DYKAT of diol **4**. When the reactions were analyzed by GC at about 50% conversion, different reaction intermediates were detected depending on the reac-

Table 6. DYKAT of diol **4** (*dl*/*meso* ≈1:1).[a]

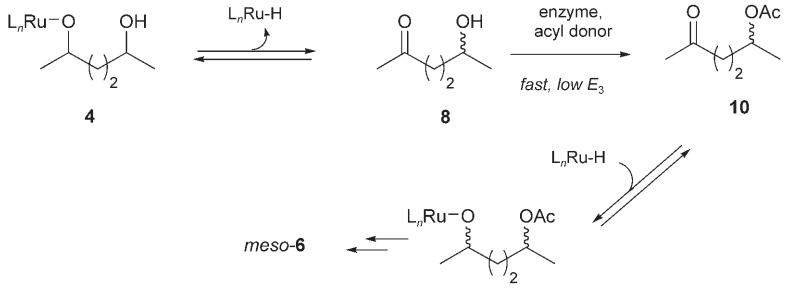


4 *dl*/*meso* (1:1) (*R*,*R*)-**6** *meso*-**6**

Entry	Enzyme [mg per mmol of diol **4**]	Catalyst [mol%]	*t*BuOK [mol%]	*T* [°C]	*t* [h]	Yield of **6** [%][c]	*ee* [%][c]	(*R*,*R*)/*meso*[c]
1[b]	CALB (60)	**1** (5)	–	70	48	61	99	77:23
2[b]	PS-C (60)	**1** (5)	–	70	40	72	99	89:11
3	PS-C (30)	**2** (5)	5	50	40	91	99	90:10
4	PS-C (60)	**2** (5)	5	50	20	96	99	92:8
5	PS-C (80)	**2** (5)	5	50	6	>99 (95)[d]	>99	92:8
6	PS-C (80)	**2** (5)	6	50	6	97[e]	>99	83:17
7	PS-C (80)	**2** (2)	2	50	20	99[e]	>99	84:16
8	CALB (60)	**2** (5)	5	50	6	97	>99	80:20
9	CALB (3)	**2** (5)	5	50	21	>99	>99	94:6

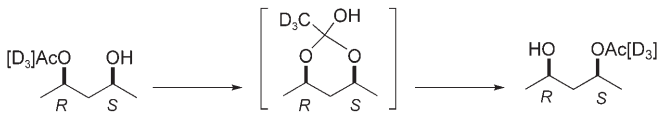
[a] Unless otherwise noted, Ru-catalyst **2** (2–5 mol%), CALB or PS-C, Na_2CO_3 (1 mmol) and KO*t*Bu (2–6 mol%) were stirred in toluene (2 mL) at RT for 6 min before adding the diol **4** (1 mmol). After 4 min isopropenyl acetate (3 mmol) was added and the mixture was stirred at 50 °C under an argon atmosphere. [b] A solution of diol **4** (0.2 mmol) and *p*-chlorophenyl acetate (0.6 mmol) in toluene (0.5 mL) was added to a mixture of Ru-catalyst **1** (5 mol%) and the enzyme, CALB or PS-C. The mixture was stirred at 70 °C under an argon atmosphere. [c] Determined by chiral GC. [d] In parenthesis isolated yield. [e] Yield determined by ^{1}H NMR spectroscopy.

tion temperature: for those DYKAT transformations performed at 70°C, the only intermediates observed were acetoxy ketones **10**, and for those performed at 50°C, the main intermediates were acetoxy alcohols (*R*,*R*)-**7** and (*R*,*S*)-**7**. In the latter case traces of acetoxy ketones **10** were also detected. During the ruthenium-catalyzed racemization, a hydrogen-transfer process takes place, and ketones are formed as reaction intermediates. The concentration of ketones in the reaction mixtures increases as the reaction temperature increases.[24] Although the formation of hydroxyketone intermediates **8** decreases with decreasing the temperature, it cannot be completely suppressed. The enzyme-catalyzed acylation of intermediates **8** takes place fast and with very low selectivity ($E_3 = 1.3$ and 9 for PS-C and CALB, respectively; see Table 5). As a consequence, a small amount of *meso*-**6** is always produced during the DYKAT (Scheme 12).



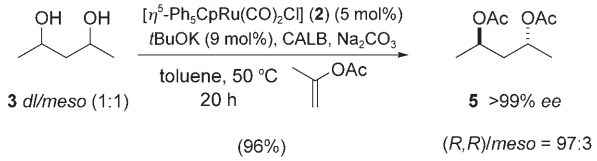
Scheme 12. Formation of *meso*-**6** from 5-hydroxy-2-hexanone **8**.

DYKAT of 2,4-pentanediol (3): In the DYKAT of 2,4-pentanediol (**3**) previously reported by our group, diacetate **5** was obtained in high yield (90%), but with the *meso* diacetate as the major product ((*R*,*R*)/*meso* = 38:62) (cf. Scheme 1).[16] In that DYKAT, the Shvo ruthenium catalyst was used for epimerization and therefore the reaction was performed at 70°C. From deuterium labeling studies, it was subsequently demonstrated that formation of *meso*-**5** is mainly due to a facile 1,3-acyl migration in the *syn*-1,3-diol monoacetate intermediates (Scheme 13).[18]



Scheme 13. Intramolecular acyl migration in a *syn*-1,3-diol monoacetate.

With the new and more efficient ruthenium complex **2**, DYKAT of *meso*/*dl*-2,4-pentanediol (**3**) was dramatically improved. At 50°C the epimerization outruns the acyl migration and a commercial stereoisomeric mixture of the diol (*meso*/*dl* = 1:1) was transformed to (*R*,*R*)-diacetate **5** in high yield (96%) with an excellent *ee* (>99%) and an excellent (*R*,*R*)/*meso* ratio (97:3) (Scheme 14).



Scheme 14. DYKAT of 2,4-pentanediol (**3**).

Conclusion

We have performed kinetic studies of all the enzyme-catalyzed steps involved in the KAT of 2,5-hexanediol (**4**), and also of those intermediates formed under dynamic conditions (i.e., in the presence of a transfer-hydrogenation ruthenium complex) using two different enzymes, *Candida antarctica* lipase B (CALB) and *Pseudomonas cepacia* lipase (PS-C "Amano II"). Both enzymes show very high selectivity for the acylation of diols to monoacetates and for the acylation of (*R*,*R*)/(*S*,*S*)-monoacetate. However, the enantioselectivity of CALB- or PS-C-catalyzed acylation of alcohols bearing a carbonyl group or an (*R*)-acetoxy group at the δ-position is rather low. The reason for this anomalous behavior is currently being investigated in our laboratories.[26] Important improvements of the DYKAT of symmetrical diols have been achieved by using a highly efficient catalyst (**2**) to achieve very fast epimerization. In the DYKAT of 2,5-hexanediol **4** catalyzed by **2**, the faster epimerization and the lower reaction temperature reduce the concentration of intermediates that could evolve by anomalous enzyme-catalyzed *S*-acylations, such as monoacetate (*R*,*S*)-**7** and hydroxyketone **8**. (*R*,*R*)-Diacetate **6** is now obtained in quantitative yield with excellent *ee* (>99%) and high (*R*,*R*)/*meso* ratio (94:6). DYKAT of 2,4-pentanediol (**3**) by using catalyst **2** was also performed at 50°C. At this temperature, the epimerization outruns the acyl migration and a commercial stereoisomeric mixture of the diol was transformed to 96% of (*R*,*R*)-diacetate **5** with an excellent *ee* (>99%) and an excellent (*R*,*R*)/*meso* ratio (97:3).

Experimental Section

General: All reactions were carried out under dry argon atmosphere in flame-dried glassware. Solvents were purified and dried with standard procedures. To control the water activity, the enzymes were stored in a sealed container with a saturated solution of LiCl for a minimum of 24 h. Flash chromatography was carried out on 60 Å (35–70 μm) silica gel. ^{1}H and ^{13}C NMR spectra were recorded at 400 or 300 MHz and at 100 or 75 MHz, respectively. Enantiomeric excess and diastereomeric excess were determined by analytical gas chromatography employing a CP-Chirasil-Dex CB chiral capillary column.

DYKAT of 2,4-pentanediol (3): Ruthenium complex **2** (32 mg, 0.05 mmol), CALB (6 mg), and Na_2CO_3 (106 mg, 1 mmol) were placed in a Schlenk flask. The flask was evacuated and filled with argon and then toluene (2 mL) was added. Addition of *t*BuOK (0.5 M in THF; 180 μL, 0.09 mmol) to the yellow suspension resulted in a color change to orange. The mixture was then stirred for 6 min, and then 2,4-pentanediol (110 μL, 1 mmol) was added. On the addition of the diol the mixture turned red. After 4 min, isopropenyl acetate (330 μL, 3 mmol) was added and the flask was placed in an oil bath at 50 °C. After 20 h the reaction mixture was filtered and analyzed: 96 % yield, (*R*,*R*)/*meso* = 97:3 (achiral GC, CP-Sil 8 CB column, constant column flow: 1.8 mL min^{-1}, hydrogen carrier gas. Temperature program: 50 °C for 2 min, then up to 180 °C with 5 °C min^{-1}; then up to 300 °C with 80 °C min^{-1} and keep for 5 min. Retention times: (*R*,*R*)-**5** = 12.32, *meso*-**5** = 13.36 min), > 99 % *ee* (chiral GC, CP-Chirasil-Dex column, constant flow: 1.8 mL min^{-1}, hydrogen carrier gas. Temperature program: 90 °C for 3 min, then up to 115 °C with 3 °C min^{-1}; then up to 200 °C with 80 °C min^{-1} and keep for 8 min. Retention times: (*R*,*R*)-**5** = 6.67, *meso*-**5** = 7.96 min. (*S*,*S*)-**5** (not detected in reaction mixtures) = 6.20).[29]

General procedure for the DYKAT of 2,5-hexanediol (4): A solution of *t*BuOK (0.5 M in THF; 100 μL, 0.05 mmol) was added to a 10 mL Schlenk flask. The THF was carefully removed under vacuum and the flask filled with argon. PS-C "Amano II" (80 mg), Na_2CO_3 (106 mg, 1 mmol), and ruthenium catalyst **2** (32 mg, 0.05 mmol) were quickly added. The Schlenk flask was evacuated and filled with argon. After addition of toluene (2 mL) the mixture turned dark orange. After 6 min **4** (123 μL, 1 mmol) was added, and after 4 min isopropenyl acetate (330 μL, 3 mmol) was added. The reaction mixture was stirred for 6 h at 50 °C, and then filtered, concentrated, and analyzed: > 99 % yield, (*R*,*R*)/*meso* = 92:8, > 99 % *ee* (chiral GC, CP-Chirasil-Dex column). Purification by column chromatography (SiO_2; pentane/diethyl ether 98:2) afforded (*R*,*R*)-2,5-diacetoxyhexane as a colorless oil (193 mg, 95 % yield, (*R*,*R*)/*meso* = 92:8, > 99 % *ee* (chiral GC, CP-Chirasil-Dex column, constant flow: 1.8 mL min^{-1}, hydrogen carrier gas. Temperature program: 70 °C for 3 min, then up to 140 °C with 2 °C min^{-1}; then up to 180 °C with 100 °C min^{-1} and keep for 10 min. Retention times: (*R*,*R*)-**6** = 19.51, *meso*-**6** = 17.70 min, (*S*,*S*)-**6** (not detected in reaction mixtures) = 14.6). The NMR spectra were in agreement with those previously reported in the literature.[27b]

Acknowledgements

Financial support from the Swedish Research Council and the Swedish Foundation for Strategic Research is gratefully acknowledged.

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Received: February 23, 2006
Published online: June 26, 2006

