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Enzymatic Resolution, Desymmetrization, and Dynamic Kinetic Asymmetric Transformation of 1,3-Cycloalkanediols

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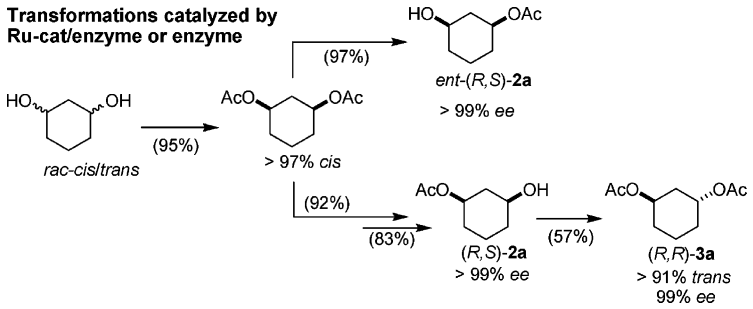

An efficient desymmetrization of *cis*-1,3-cyclohexanediol to (1*S*,3*R*)-3-(acetoxy)-1-cyclohexanol ((*R*,*S*)-**2a**) was performed via *Candida antarctica* lipase B (CALB)-catalyzed transesterification, in high yield (up to 93%) and excellent enantioselectivity (ee's up to >99.5%). (*R*,*R*)-Diacetate ((*R*,*R*)-**3a**) was obtained in a DYKAT process at room temperature from (1*S*,3*R*)-3-acetoxy-1-cyclohexanol ((*R*,*S*)-**2a**), in a high *trans*/*cis* ratio (91:9) and in excellent enantioselectivity of >99%. Metal- and enzyme-catalyzed dynamic transformation of *cis/trans*-1,3-cyclohexanediol using PS-C gave a high diastereoselectivity for *cis*-diacetate (*cis/trans* = 97:3). The (1*R*,3*S*)-3-acetoxy-1-cyclohexanol (*ent*-(*R*,*S*)-**2a**) was obtained from *cis*-diacetate by CALB-catalyzed hydrolysis in an excellent yield (97%) and selectivity (>99% ee). By deuterium labeling it was shown that intramolecular acyl migration does not occur in the transformation of *cis*-monoacetate to the *cis*-diacetate.

Introduction

Enantiomerically enriched 1,3-cycloalkanediols are precursors for various building blocks in asymmetric synthesis,[1] and recently, the interest of 1,3-cyclohexanediol derivatives as substructures in the pharmaceutical area has increased.[2] Methods for diastereo- and enantioselective synthesis of 1,3-cyclohexanediols have been developed; however, they suffer from moderate yields and low selectivity.[3] A few examples of desymmetrization of *cis*-1,3-cyclohexanediol using enzymatic resolution have previously been reported.[4,5]

The racemic *cis*/*trans* mixture of 1,3-cyclohexanediol is an inexpensive and readily available chemical. An efficient enzy-

(1) (a) Diaz, M.; Gotor-Fernandez, V.; Ferrero, M.; Fernandez, S.; Gotor, V. *J. Org. Chem.* **2001**, *66*, 4227−4232. (b) Diaz, M.; Ferrero, M.; Fernandez, S.; Gotor, V. *J. Org. Chem.* **2000**, *65*, 5647−5652.

(2) There are many patents in the pharmaceutical area using 1,3-cyclohexanediol as the substructure; for some examples, see: (a) Stapper, C.; Glombik, H.; Falk, E.; Goerlitzer, J.; Gretzke, D.; Keil, S.; Schaefer, H.-L.; Wendler, W. DE 10308352, 2004. (b) Holla, W.; Keil, S. WO 2004076390, 2004. (c) Chomczynski, P. US 2003232893, 2003.

(3) (a) Horowitz, A.; Rajbenbach, L. A. *Chem. Commun. (London)* **1967**, *23*, 1234. (b) Johnson, M. R.; Rickborn, B. *Chem. Commun. (London)* **1968**, *18*, 1073. (c) Tamao, K.; Nakajima, T.; Sumiya, R.; Arai, H.; Higuchi, N.; Ito, Y. *J. Am. Chem. Soc.* **1986**, *108*, 6090. (d) Evans, D. A.; Fu, G. C.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1988**, *110*, 6917. (e) Taqui Khan, M. M.; Shukla, R. S. *J. Mol. Catal.* **1989**, *54*, 45. (f) Brinkman, J. A.; Nguyen, T. T.; Sowa, J. R., Jr. *Org. Lett.* **2000**, *2*, 981. (g) Kim, T. J.; Kwak, B. S. KR 2003092889, 2003. (h) Ichikawa, S.; Urata, H.; Suzuki, S. EP 1333019, 2003.

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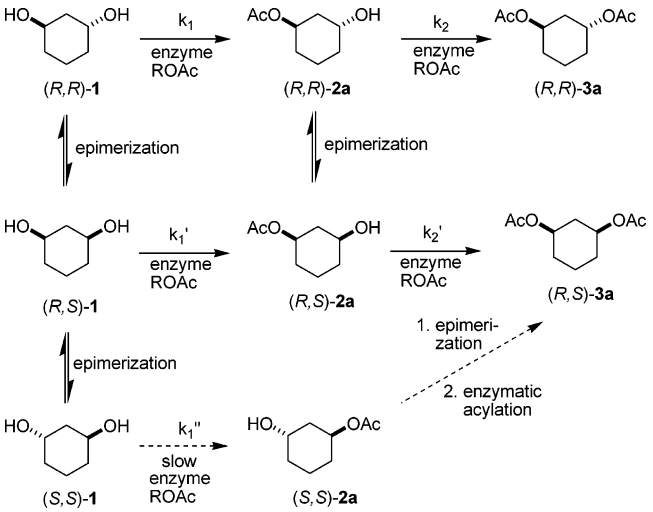
matic resolution of this mixture and analogous racemic cyclic 1,3-diols, possibly as a dynamic process involving epimerization, would provide a useful method for the synthesis of enantiomerically enriched 1,3-cycloalkanediols. We have recently developed methods for dynamic kinetic resolution (DKR) and dynamic kinetic asymmetric transformations (DYKAT) of alcohols and diols, respectively, by combining a ruthenium catalyst (for racemization/epimerization) and an enzyme catalyst (for resolution).[6] Previous studies on DYKAT of acyclic 1,3-diols have shown that the reaction suffers from moderate diastereoselectivity due to formation of *meso*-diacetate,[7] and it was shown that this was caused by an intramolecular acyl migration in the *syn*-1,3-diol monoacetates.[7c,8] This may also be a problem in the cyclic 1,3-diols.

Theoretically, in an efficient enzymatic kinetic asymmetric transformation (KAT) of racemic *cis*/*trans*-1,3-cycloalkanediols, by an (*R*)-selective lipase, the products should be (*S*,*S*)-diol, (*R*,*R*)-diacetate, and (*R*,*S*)-monoacetate. However, if intramolecular acyl migration occurs in the (*R*,*S*)-monoacetate (*cis*-diol monoacetate), all monoacetate would be converted to (*R*,*S*)-diacetate (*meso*), since the released *R*-alcohol would be rapidly enzymatically acylated. Also, a nonselective acylation (according to Kazlauskas' rule[9]) of the (*S*)-alcohol in the (*R*,*S*)-monoacetate would lead to (*R*,*S*)-diacetate (*meso*). In a dynamic kinetic asymmetric transformation (DYKAT) of 1,3-cyclohexanediols, the products formed could therefore be a mixture of (*R*,*R*)-diacetate and (*R*,*S*)-diacetate.

By favoring or disfavoring the formation of the (*R*,*S*)-diacetate in the dynamic process with combined ruthenium and enzyme catalysis it should be possible to make either the (*R*,*S*)-diacetate or the (*R*,*R*)-diacetate, respectively, in high selectivity. Thus, if $k_2' \ll k_2$ in the DYKAT of 1,3-cyclohexanediol (Scheme 1) and if epimerization is fast no diacetate (*R*,*S*)-**3a** would be produced. This could occur if enzymatic (*S*)-acylation is slow and if a possible acyl migration in (*R*,*S*)-**2a** is shut down. In this case, the reaction would be shifted toward the (*R*,*R*)-diacetate ((*R*,*R*)-**3a**); epimerization of the monoacetate (*R*,*S*)-**2a** followed by enzymatic acylation of the monoacetate (*R*,*R*)-**2a** would give diacetate (*R*,*R*)-**3a** (Scheme 1).

On the other hand, if $k_2' \gg k_2$ the reaction would be shifted toward the *meso*-diacetate ((*R*,*S*)-**3a**) provided that epimerization is fast (Scheme 1). Under these conditions, a *cis*/*trans* diol mixture **1** could in principle be transformed into pure (*R*,*S*)-diacetate ((*R*,*S*)-**3a**). Thus, it should be possible to prepare either chiral (*R*,*R*)-diacetate ((*R*,*R*)-**3a**) or achiral *meso*-diacetate ((*R*,*S*)-**3a**), depending on the reaction conditions. Under dynamic

SCHEME 1. Reaction Pathway for Formation of *trans*/*cis*-Diacetates



conditions, i.e., in the presence of an epimerization catalyst, there is an additional pathway for the formation of the (*R*,*S*)-diacetate: low selectivity (low *E* value) in the first enzymatic acylation of the *trans*-diol (i.e., k_1 only slightly larger than k_1'') would give a mixture of (*R*,*R*)-monoacetate ((*R*,*R*)-**2a**) and (*S*,*S*)-monoacetate ((*S*,*S*)-**2a**). Subsequent epimerization of the latter monoacetate followed by enzymatic acylation would lead to the formation of *cis*-(*R*,*S*)-diacetate ((*R*,*S*)-**3a**).

Results and Discussion

Preparation of Starting Materials. To study the acyl migration, and the *E* values for the different steps, pure *cis*- and *trans*-diol were required. A commercially available mixture of *trans*/*cis*-1,3-cyclohexanediol was converted to the dibenzoates[10] followed by separation with flash chromatography. The pure *cis*- and *trans*-dibenzoates were collected and hydrolyzed, which afforded the *cis*- and *trans*-diols in high yields (Scheme 2).

Kinetic Resolution and Desymmetrization. Because of the possibility of forming the *cis*-(*R*,*S*)-diacetate ((*R*,*S*)-**3a**) from the (*S*,*S*)-diol in the case of low selectivity, it was desirable to determine the *E* value for the first acylation step of the *trans*-diol. Screening of several enzymes in different solvents[11] in the kinetic resolution (KR) of *trans*-**1** showed a very poor selectivity ($E_1 \leq 2$) in all cases. The best result was obtained with *Candida antarctica* lipase B (CALB) in toluene, which gave $E_1 = 2$ (Scheme 3).

However, the second step, i.e., enzymatic resolution of racemic *trans*-monoacetate, showed a much higher enantioselectivity ($E_2 = 48$) with CALB in toluene. Based on these results, we conclude that large amounts of *cis*-diacetate ((*R*,*S*-**3a**) are expected to be formed from DYKAT of *trans*/*cis*-diol due to low selectivity in the first step of the enzymatic resolution of *trans*-diol (*trans*-**1**).

(4) (a) Mattson, A.; Orrenius, C.; Oehrner, N.; Unelius, C. R.; Hult, K.; Norin, T. *Acta Chem. Scand.* **1996**, *50*, 918. (b) Garel, L.; Gelo-Pujic, M.; Schlama, T. WO 2005040394, 2005.

(5) For an example of nonenzymatic desymmetrization of *meso*-1,3-cyclohexanediol, see: Kawabata, T.; Stragies, R.; Fukaya, T.; Nagaoka, Y.; Schedel H.; Fuji, K. *Tetrahedron Lett.* **2003**, *44*, 1545.

(6) (a) Pàmies, O.; Bäckvall, J.-E. *Trends Biotechnol.* **2004**, *22*, 130−135. (b) Pàmies, O.; Bäckvall, J.-E. *Chem. Rev.* **2003**, *103*, 3247−3261. (c) Pàmies, O.; Bäckvall, J.-E. *Curr. Opin. Biotechnol.* **2003**, *14*, 407−413.

(7) (a) Mattson, A.; Öhrner, N.; Hult, K.; Norin, T. *Tetrahedron: Asymmetry* **1993**, *4*, 925. (b) Persson, B. A.; Huerta, F. F.; Bäckvall, J.-E. *J. Org. Chem.* **1999**, *64*, 5237. (c) Edin, M.; Bäckvall, J.-E. *J. Org. Chem.* **2003**, *68*, 2216.

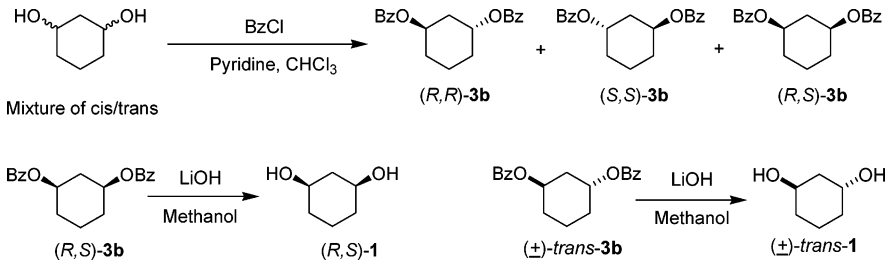
(8) A related acyl transfer has been observed in lipase-catalyzed hydrolysis of diacetates of *meso*-1,3-diols (2-substituted 1,3-propane diols): Liu, K. K.-C.; Nozaki, K.; Wong, C.-H. *Biocatalysis* **1990**, *3*, 169−177.

(9) Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656−2665.

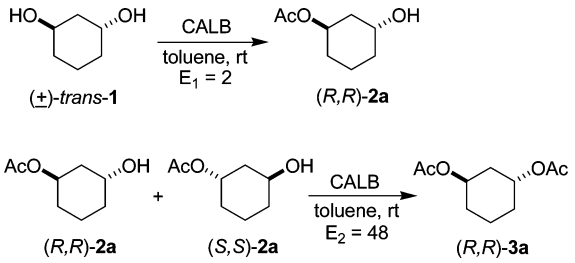
(10) Kikuchi, Y.; Kato, Y.; Tanaka, Y.; Toi, H.; Aoyama, Y. *J. Am. Chem. Soc.* **1991**, *113*, 1349.

(11) Enzymes investigated were as follows: *Aspergillus niger*, CALB = *Candida antarctica* lipase B, CRL = *Candida rugosa* lipase, PS-C, PS-D = *Pseudomonas cepacia* lipase, PF = *Pseudomonas fluorescens* lipase, PPL = porcine pancreas lipase). Solvents employed were as follows: TBME = *tert*-butyl methyl ether, toluene, and THF combined with CALB and also cyclohexane.

SCHEME 2. Separation of *trans*- and *cis*-1,3-Cyclohexanediol from *Trans/Cis* Mixture



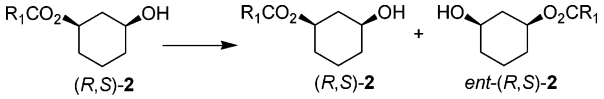
SCHEME 3. *E* Values for Enzymatic Acylation of *trans*-1,3-Cyclohexanediol



To obtain information concerning a possible acyl migration, the enzymatic desymmetrization of *meso*-diol (*R*,*S*)-**1** to *cis*-monoacetate (*R*,*S*)-**2a** was studied, using different enzymes and solvent. The results are given in Table 1.

CALB showed good activity, and (*R*)-monoacetate (*R*,*S*)-**2a** was obtained in high yields and excellent enantioselectivity (98 to >99,5% ee) in several solvents (Table 1, entries 1 and 7−10). In the case of *Candida rugosa* lipase (CRL), desymmetrization to enantiomerically enriched (*S*)-monoacetate **2a** occurred in good yield and high enantioselectivity (Table 1, entry 5). This (*S*)-selective acylation by a lipase is unusual and was also observed for porcine pancreas lipase (PPL) and *Aspergillus* sp. (Aspergillus), although in the latter cases the reaction was very slow (Table 1, entries 4 and 6). *Pseudomonas cepacia* lipase PS-C showed good activity, although considerable formation of the diacetate was observed and the selectivity was poor (entry 2). Also, *Pseudomonas fluorescens* lipase (PF) showed a poor selectivity, and the reaction was slow (entry 3.) On the basis of these results, we decided to study the formation of *cis*-diacetate ((*R*,*S*-**3a**) in the CALB-catalyzed acylation of *cis*-1,3-cyclohexandiol (*R*,*S*-**1**).

TABLE 2. Racemization of (*R*,*S*)-2*a*



2a = Me (>99.5% ee)
2b = Et (99% ee)

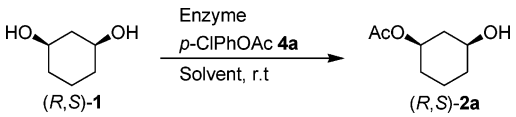
entry	conditions[b]	solvent	*T* (°C)	time (h)	% ee of **2**	$t_{1/2}$[c] (h)
1	(*R*,*S*)-**2a** + CALB	toluene	25	24	89	95
2	(*R*,*S*)-**2a** + CALB	toluene	70	5	21	3.5
3	(*R*,*S*)-**2a**	toluene	70	24	99.5	
4	(*R*,*S*)-**2a** + CSA	toluene	25	24	95	
5	(*R*,*S*)-**2a** + CALB	TBME	60	5	71	8.25
6	(*R*,*S*)-**2a** + CALB	TBME	25	24	83	38
7	(*R*,*S*)-**2a** + CALB	DIPE	70	5	6	2.25
8	(*R*,*S*)-**2a** + CALB	cyclohexane	70	3.5	3	1.3
9	(*R*,*S*)-**2b** + CALB	toluene	70	5	42	4.5

[a] Unless otherwise noted, all reactions were performed on a 0.2 mmol scale with 12 mg of CALB (*Candida antarctica* lipase) in 1 mL of solvent with >99.5% ee of the *cis*-diol monoacetate. [b] TBME = *tert*-butyl methyl ether, DIPE = diisopropyl ether. [c] $t_{1/2}$ (h) = time at 50% ee, determined by GC using a CP-Chirasil-Dex CB column using racemic compounds as references.

Racemization Studies of Monoacetate (*R*,*S*)-2a and (*R*,*S*)-2b. To probe the possible involvement of an intramolecular acyl transfer in the formation of the *meso*-diacetate we studied the racemization of (*R*,*S*)-**2a**. Intramolecular acyl migration in (*R*,*S*)-**2a** would lead to *ent*-(*R*,*S*)-**2a** and hence racemization.

The results presented in Table 2 show that the combination of high temperature and enzyme is important for the racemization to occur. In the cases where CALB was used at both 25 and 70 °C, there was a significant increase in the rate of racemization. At 25 °C after 24 h, the enantiomeric excess of

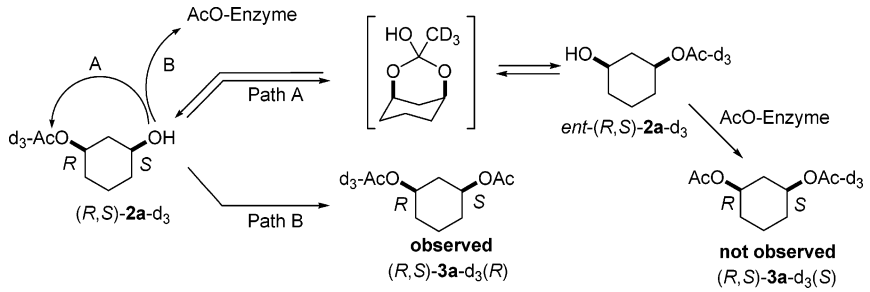
TABLE 1. Desymmetrization of (*R*,*S*)-1 Using Enzymatic Acylation*a*



entry	enzyme[b]	enzyme (mg)	solvent	time (h)	yield[c] (%)	diacetate (%)	% ee[d]
1	CALB	6	toluene	3.5	83		>99.5 (*R*)
2	PS-C	3	toluene	6	67	28	38 (*R*)
3	PF	3	toluene	6	15	1	53 (*R*)
4	PPL	75	toluene	6	<5		7 (*S*)
5	CRL	12	toluene	6	68	8	93 (*S*)
6	Aspergillus	75	toluene	6	<5		43 (*S*)
7	CALB	6	THF	3.5	85	3	>99.5 (*R*)
8	CALB	6	TBME[e]	3.5	93	7	>99.5 (*R*)
9	CALB	6	DIPE[f]	3.5	85	3	>99.5 (*R*)
10	CALB	6	cyclohexane	3.5	21	5	98 (*R*)

[a] Unless otherwise noted, all reactions were performed on a 0.1 mmol scale, with 0.3 mmol of **4a** (51 mg) in 0.5 mL of solvent at rt. [b] CALB = *Candida antarctica* lipase B, PS-C = *Pseudomonas cepacia* lipase, PF = *Pseudomonas fluorescens* lipase, PPL = porcine pancreas lipase, CRL = *Candida rugosa* lipase, Aspergillus = *Aspergillus* sp. [c] Determined by ^{1}H NMR. [d] Determined by GC using a CP-Chirasil-Dex CB column using racemic compounds as references. [e] TBME = *tert*-butyl methyl ether. [f] DIPE = diisopropyl ether.

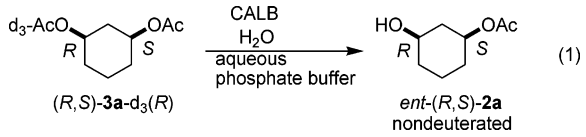
SCHEME 4. Deuterium Labeling To Distinguish between Acyl Migration and Enzymatic Acylation



(*R*,*S*)-**2a** was 89%; however, at 70 °C after 7 h the enantiomeric excess had dropped to 21% (Table 2, entry 1 vs 2). In the absence of enzyme no loss of enantioselectivity was observed after 24 h at elevated temperature (Table 2, entry 3). Addition of 20 mM camphorsulfonic acid (CSA), which is expected to facilitate acyl migration, resulted in only a slight decrease of the enantioselectivity from >99.5% to 95% ee after 24 h (Table 2, entry 4). The solvent effect in combination with CALB was then studied, and it was found that the fastest racemization takes place in cyclohexane, followed by DIPE and toluene (Table 2, entries 2, 7, and 8). TBME showed a comparable racemization rate as that in DIPE and toluene (entries 5 and 6). The racemization of the corresponding monopropionate **2b** was slower than racemization of **2a** (Table 2, entry 2 vs 9). This led us initially to believe that intramolecular acyl migration was involved in the formation of the *cis*-diacetate ((*R*,*S*-**3a**). However, the inability of (*R*,*S*)-**2a** to racemize in toluene in the absence of CALB was puzzling, and an intramolecular transesterification seemed more likely to explain the racemization of (*R*,*S*)-**2a** in the presence of CALB. To determine the exact mechanism for the formation of the diacetate further studies were required.

Origin of Formation of the *cis*-Diacetate (*R*,*S*)-3a. With deuterium labeling of the group in *cis*-diol monoacetate (*R*,*S*-**2a**-d_3) it is possible to determine whether the *cis*-diacetate (*R*,*S*)-**3a** is formed due to intramolecular acyl transfer or via direct enzymatic acylation as a result of low selectivity (Scheme 4). Involvment of an intramolecular acyl transfer would give *ent*-*R*,*S*-**2a**-d_3, which would be rapidly enzymatically acylated to the diacetate with deuterium in the (*S*)-position. On the other hand, direct acylation would produce the diacetate with the deuterium in the (*R*)-position.

The deuterated starting material in Scheme 4 was obtained from acylation of *cis*-1,3-cyclohexanediol with *p*-ClPhOAc-d_3 (**4a**-d_3), which afforded (*R*,*S*)-**2a**-d_3. The deuterium-labeled monoacetate (*R*,*S*)-**2a**-d_3 was acylated using CALB and *p*-chlorophenyl acetate (**4a**) to give the diacetate. Analysis of the diacetate showed that the deuterium has remained in the *R*-position ((*R*,*S*)-**2a**-d_3(*R*)), and hence, the diacetate has been obtained via direct enzymatic acylation of the (*S*)-alcohol as a result of low selectivity (mechanism B). The location of the deuterium in the diacetate was determined by (*R*)-selective hydrolysis by CALB in water.[12] The hydrolysis of the diacetate obtained using CALB in aqueous phosphate buffer afforded a nondeuterated monoacetate *ent*-(*R*,*S*)-**2a** (eq 1). The product was ~96% nondeuterated, and it was confirmed by chiral GC that the monoacetate (*ent*-(*R*,*S*)-**2a**) had the configuration opposite to that of the starting (*R*)-monoacetate ((*R*,*S*)-**2**), which shows that it is the (*S*)-monoacetate. This provides conclusive evidence that the diacetate obtained in Scheme 4 has the deuterium in the acetate group in the (*R*)-position and, hence, that intramolecular acetyl migration does not take place in (*R*,*S*)-**2a**.



The *cis*-1,3-diol derivatives can possess two conformations, a diaxial and a diequatorial conformation (Figure 1), of which the latter is the more stable one. For the parent diol the energy difference may not be so large due to stablilization of the diaxial form by hydrogen bonds.[13]

For an intramolecular acyl transfer, the alcohol and acetate group must be close to one another, and this requires that the alcohol and acetate are axial (Figure 1). In the intermediate for the intramolecular acetyl migration the oxygen substituents will be diaxial (Figure 2a), and furthermore, the dioxane ring will have two carbons of the cyclohexane ring as axial carbons (Figure 2b). For this reason, the energy of the acetyl migration intermediate is rather high for the cyclohexane system compared to the acyclic *syn*-1,3-diol system.[7c] This explains the unfavored intramolecular acyl migration in the 1,3-cyclohexanediol system.

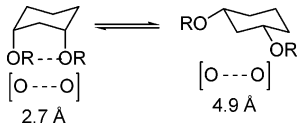


FIGURE 1. Conformations of 1,3-cyclohexanediol.

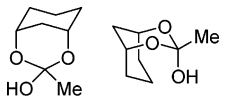


FIGURE 2. Conformation of acyl migration intermediate.

DYKAT of 1,3-Cyclohexanediol Using a Variety of Acyl Donors. Enzymatic acylation of the *cis*-diol (*R*,*S*)-**1** (vide supra) showed that the (*R*)-selectivity of the first acylation is high, and this led to efficient desymmetrization. If a first enzymatic acylation is allowed before the racemization catalyst is added, all *cis*-diol (*R*,*S*)-**1** would be converted to *cis*-monoacetate (*R*,*S*)-**2a** (Scheme 1). If $k_2 \gg k_2'$ in Scheme 1 and epimerization is fast, all monoacetate (*R*,*R*)-**2a** would be converted to (*R*,*R*)-**3a**. An in situ desymmetrization of the *cis*-diol before applying the DYKAT may therefore give mainly the (*R*,*R*)-diacetate ((*R*,S)-

(12) Lipases such as CALB are *R*-selective (according to Kazlauskas' rule) in the reaction of alcohol derivatives; e.g., see ref 7c.

(13) (a) Goodwin, J. C.; Hodge, J. E.; Nelson, E. C.; Warner, K. A. *J. Agric. Food Chem.* **1981**, *29*, 929. (b) Chen, X.; Walthall, D. A.; Brauman, J. I. *J. Am. Chem. Soc.* **2004**, *126*, 12614.

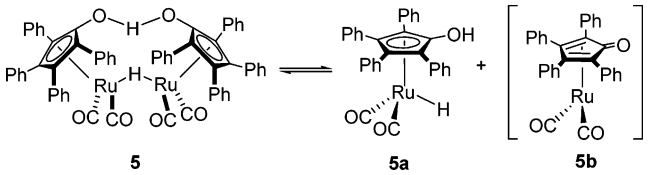
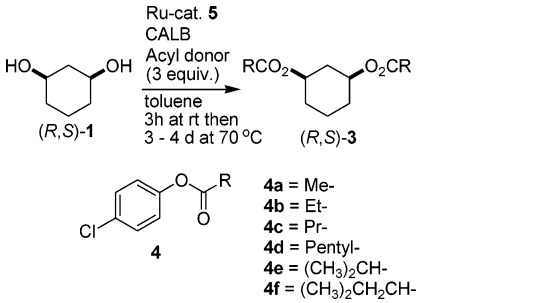


FIGURE 3. Dissociation of the precatalyst **5** to form the active species **5a** and **5b**.

TABLE 3. DYKAT of (1*S*,3*R*)-Cyclohexandiol (1)[a]



entry	acyl donor	time (h)	yield of diacetates **3**[b] (%)	*cis*/*trans*[c]	% ee[d]
1	**4a** (R = Me−)	72	>95	65:35	97
2	**4b** (R = Et−)	72	>95	49:51	98
3	**4c** (R = Pr−)	96	>95	81:19	
4	**4d** (R = pentyl−)	96	>95	87:13	
5	**4e** (R = $(CH_3)_2CH$−)	72	>95	80:20	
6	**4f** (R = $(CH_3)_2CH_2CH$−)	96	>95	92:8	

[a] Unless otherwise noted, all reactions were performed on a 0.2 mmol scale using 6.5 mg of CALB, 5 mol % of catalyst **5**, 0.6 mmol of acyl donor (**4a**−**f**), and 2.5 mg of Na_2CO_3 in 1 mL of toluene. The reaction mixture was stirred at rt for 3 h and 69 or 93 h at 70 °C. Hydrogen source 2,4-dimethyl-3-pentanol (**6**) (0.5 equiv/hydroxyl group), was added after 24 h. [b] Determined by ^{1}H NMR. [c] Diastereomeric ratio, determined by ^{1}H NMR. [d] Enantiomeric excess of *trans*-diester, determined by GC using a CP-Chirasil-Dex CB column using racemic compounds as references.

3a) provided that epimerization is fast. The desymmetrization-DYKAT sequence was accomplished by allowing *cis*-diol **1**, CALB, ruthenium catalyst **5,** and *p*-chlorophenylacetate (**4a**) to react at room temperature in toluene for 3 h and then increasing the temperature to 70 °C. At room temperature, catalyst **5** is inactive, and therefore there will be no epimerization during the first 3 h. The dimeric ruthenium precatalyst **5** is activated by heat and then it is dissociated into the two active species **5a** and **5b** (Figure 3).

The results from this study (Table 3) show that using larger R groups on the acyl donor favors the formation of the *cis*-(*R*,*S*)-diesters (except in one case) and not as we expected, leading to an increased selective formation of the *trans*-diacetate. This may be a result of slow epimerization by the catalyst, in combination with a decreased ratio of k_2/k_2' (cf. Scheme 1). The *cis*-diacetate is the predominant product, with acyl donor **4f**, giving a *cis*/*trans* ratio of 92:8.

Due to the high temperature (70 °C) required for racemization catalyst **5**, a moderate to low selectivity was obtained in the DYKAT process (Table 3). Because of the low selectivity obtained, we decided to study the enzymatic acylation of (*R*,*S*)-**2a** in the presence of ruthenium catalyst **7** (Figure 4). The latter catalyst was recently found to efficiently racemize alcohols at room temperature.[14]

The results from the DYKAT employing CALB and **7** show that it is possible to obtain the (*R*,*R*)-diacetate ((*R*,*R*)-**3a**) from

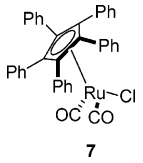
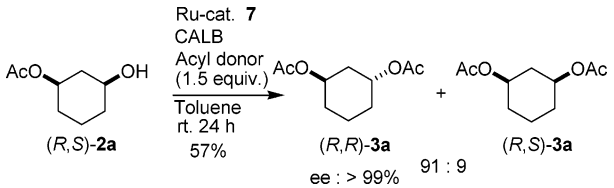
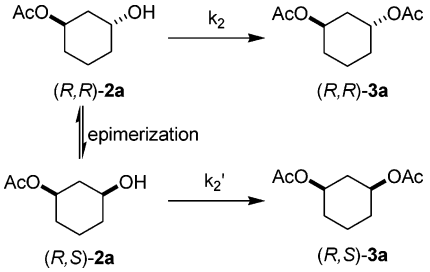


FIGURE 4. Ruthenium catalyst **7** for racemization at 25 °C.

SCHEME 5. DYKAT of (1*S*,3*R*)-3-(Acetoxy)-1-cyclohexanol ((*R*,*S*)-2a)



SCHEME 6. DYKAT of (*R*,*S*)-2a ($k_2 > k_2'$)



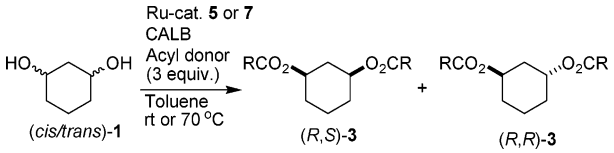
(*R*,*S*)-**2a** in a high *trans*/*cis* ratio and with excellent enantioselectivity (Scheme 5). In this case, k_2 is larger than k_2', which makes it possible to prepare enantiomerically pure (*R*,*R*)-diacetate ((*R*,*R*)-**3a**).

However, if k_2' can be made larger than k_2, the formation of *cis*-diacetate (*R*,*S*)-**3a**)) would be favored over (*R*,*R*)-diacetate ((*R*,*R*)-**3a**); i.e., the rate of the enzymatic (*S*)-acylation in the *cis*-monoacetate (*R*,*S*)-**2a**)) should be faster then the (*R*)-acylation of the *trans*-monoacetate (*R*,*R*)-**2a**)). For the purpose of preparing the *cis*-diacetate (*R*,*S*-**3a**), we started from a *cis*/*trans*-cyclohexanediol mixture. The low selectivity of the first step is of less importance since (*S*,*S*)-monoacetate ((*S*,*S*)-**2a**) can be epimerized and enantiomerically acylated forming the *cis*-diacetate (*R*,*S*)-**3a**)). In initial experiments of DYKAT of *cis*/*trans*-cyclohexanediol, we combined the KAT of the diols, using an enzyme and the *p*-chlorophenylacetate (**4a**)**,** with a ruthenium-catalyzed epimerization process via hydrogen transfer employing the dimeric Ru precatalyst **5** in toluene at 70 °C. It appears that elevated temperature favors the formation of *cis*-diacetate (*R*,*S*)-**3a**)) (cf. Table 3). The DYKAT of *cis*/*trans*-cyclohexanediol was additionally studied at room temperature using Ru catalyst **7** and isopropenyl esters (Scheme 6). These results are summarized in Table 4.

In the first reaction, using CALB as the enzyme, the *trans*/*cis* ratio of the substrate diol, which is 37:63, was increased to 43:56 for the diacetate. The *trans*-diacetate (*R*,*R*)-**3a** was obtained in 94% ee (Table 4, entry 1). In the above desymmetrization of *cis*-diol **1** using PS-C as the enzyme, we had observed that the first acylation is followed by a fast second acylation to give the *cis*-diacetate (*R*,*S*)-**3a** (Table 1, entry 2).

(14) (a) Csjernyik, G.; Bogár, K.; Bäckvall, J.-E. *Tetrahedron Lett.* **2004**, *45*, 6799. (b) Martín-Matute, B.; Edin, M.; Bogár, K.; Bäckvall, J.-E. *Angew. Chem., Int. Ed.* **2004**, *43*, 6535. (c) Martín-Matute, B.; Edin, M.; Bogár, K.; Kaynak, F. B.; Bäckvall, J.-E. *J. Am. Chem. Soc.* **2005**, *127*, 8817.

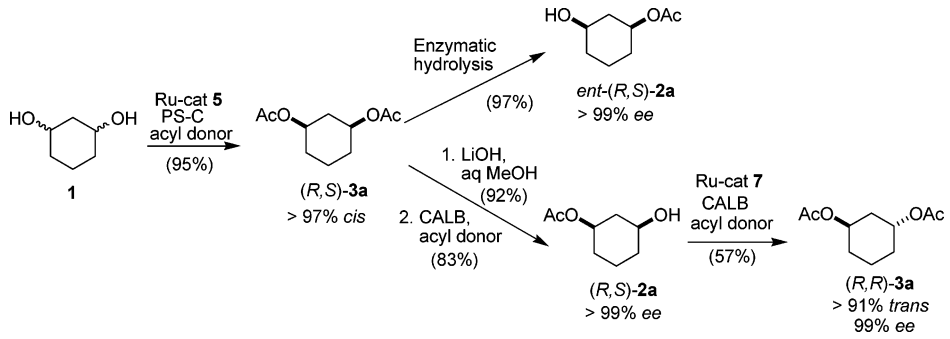
TABLE 4. DYKAT of *Cis*/*Trans* Mixture 1[a]



entry	method	enzyme	Ru−cat.	*T* (°C)	R group	time (h)	yield of diacetate **3a**[b] (%)	dr[c] *cis*/*trans*	% ee[d]
1	A	CALB	**5**	70	Me	72	>95	56:43	94
2	A	PS-C	**5**	70	Me	72	>95	97:3[e]	92
3	B	CALB	**7**	25	Me	24	41	33:66	96
4	B	CALB	**7**	25	Pr	24	26	22:78	>98

[a] Unless otherwise noted, all reactions were performed on a 0.2 mmol scale using 6.5 mg of CALB, 5 mol % of catalyst, and 0.6 mmol of acyl donor in 1 mL of toluene. Method A: *p*-ClPhOAc **4a** as acyl donor, **5** as catalyst, and 2.5 mg of Na_2CO_3 was stirred at 70 °C for 72 h. Hydrogen source, 2,4-dimethyl-3-pentanol (**6**) (0.5 equiv/hydroxyl group), was added after 24 h. Method B: Isopropenyl acetate or butyrate as acyl donor, **7** as catalyst, 6 mol % of KO^{t}Bu (0.5 M in THF), and 10 mg of Na_2CO_3 was stirred at rt. [b] Determined by ^{1}H NMR. [c] Diastereomeric ratio, determined by ^{1}H NMR. [d] Enantiomeric excess of *trans*-diester, determined by GC using a CP-Chirasil-Dex CB column using racemic compounds as references. [e] The dr was determind by GC; the *trans*-diacetate was not detectable using ^{1}H NMR.

SCHEME 7. Stereoselective Synthesis of (*R*,*S*)-3a, *ent*-(*R*,*S*)-2a, (*R*,*S*)-2a. and (*R*,*R*)-3a from a Racemic *Cis*/*Trans* Mixture of 1,3-Cyclohexanediol (1) via Enzyme and Metal-Catalyzed Transformations



This indicates that k_2' is quite large for this enzyme and presumably larger than k_2. Applying PS-C in the dynamic process with ruthenium and enzyme catalysis resulted in diastereoselective formation of *cis*-diacetate of 97:3 (Table 4, entry 2). In this way, one can obtain the *cis*-diacetate (*R*,*S*)-**3a** in high yield and high diastereoselectivity from a mixture of *trans*/*cis*-diol **1**. On the other hand, the use of catalyst **7** at room temperature favored the formation of *trans*- over *cis*-diacetate. Thus, enzymatic acetylation under dynamic conditions afforded a cis:trans ratio of 33:66, wheras a butyrate in the acyl donor gave a *cis*/*trans* ratio of 22:78. In both cases, the enantioselectivity of the *trans*-diacetate (*R*,*R*)-**3a**) was high (96−98% ee). The increased predominance for *trans* product can be a result of higher selectivity of the enzyme at lower temperature.

Conclusions

A high diastereoselectivity for *cis*-diacetate (*cis*/*trans* = 97:3) was obtained in the metal- and enzyme-catalyzed transformation of *trans*/*cis*-diol, under dynamic conditions. We have managed to obtain the (*R*,*R*)-diacetate ((*R*,*R*)-**3a**) from (1*S*,3*R*)-3-acetoxy-1-cyclohexanol (**2a**) in a high *trans*/*cis* ratio (91:9) and in excellent enantioselectivity of >99%. Desymmetrization of *cis*-diol to *cis*-monoacetate was successfully accomplished in up to >99.5% ee and yields up to 93% for (1*S*,3*R*)-3-(acetoxy)-1-cyclohexanol (**2a**) using CALB. The formation of the *meso*-diacetate was initially believed to be a result of intramolecular acylmigration. However, by use of deuterium labeling it was shown that intramolecular acyl migration does not occur in the transformation of *cis*-monoacetate (*R*,*S*)-**2a** to *cis*-diacetate (*R*,*S*)-**3a**. By combination of these methods, a commercially available racemic *cis*/*trans* mixture of 1,3-cyclohexanediol (**1**) can be transformed into (*R*,*S*)-**3a**, *ent*-(*R*,*S*)-**2a**, (*R*,*S*)-**2a**, and (*R*,*R*)-**3a** (Scheme 7).

Experimental Section

General Procedures for Synthesis of Starting Material. *cis*/*trans*-Dibenzoate (3b). The mixture of *dl*/*meso*-diols (5.0 g) was esterified with benzoyl chloride (15.1 g) and pyridine (10.3 g) in chloroform (24 mL) at room temperature for 24 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in CH_2Cl_2 (100 mL) and washed with 1 M HCl (50 mL). The organic phase was further washed with saturated aq Na_2CO_3 (2 × 25 mL), H_2O (25 mL), and brine (25 mL) and dried (Na_2SO_4), and the solvent was removed in vacuo. Flash chromatography (chloroform) afforded *trans*-**3b** (1.9 g, 14%) and *cis*-**3b**-dibenzoate (3.9 g, 28%) as pure isomers.[15] Also, a fraction of a mixture of *cis*- and *trans*-dibenzoate (5.8, 42%) was collected. *cis*-Dibenzoate: ^{1}H NMR ($CDCl_3$) δ 8.04−8.00 (m, 4H), 7.57−7.50 (m, 2H), 7.42−7.36 (m, 4H), 5.16−5.08 (m, 2H), 2.48−2.43 (m, 1H), 2.14−1.84 (m, 3H), 1.63−1.46 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 165.8, 132.8, 130.5, 129.6, 128.3, 70.8, 36.7, 30.8, 19.6. *trans*-Dibenzoate: ^{1}H NMR ($CDCl_3$) δ 8.08−8.04 (m, 4H), 7.59−7.52 (m, 2H), 7.47−7.40 (m, 4H), 5.49−5.43 (m, 2H), 2.14 (t, J = 5.5, 2H), 2.02−1.72 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 165.8, 132.9, 130.5, 129.6, 128.4, 70.6, 35.9, 30.4, 19.4.

***cis*-1,3-Cyclohexanediol (*cis*-1).** To a solution of the *cis*-**3b** (3.9 g, 12.0 mmol) in methanol (110 mL) at 0 °C was added LiOH (875 mg, 36.5 mmol). The mixture was stirred at room temperature overnight. The pH in the solution was adjusted to 4 using 2 M

(15) Fleming, I.; Lawrence, N. J. *J. Chem. Soc., Perkin Trans. 1* **1992**, *24*, 3309.

HCl (aq), and the methanol was concentrated in vacuo. The resulting water phases were saturated with NaCl (s) and extracted with EtOAc (4 × 250 mL). The combined organic phases were dried over $NaSO_4$ and concentrated in vacuo, and the residue was purified by flash chromatography (CH_2Cl_2 to EtOAc/acetone 3:1), affording the *cis*-diol (1.29 g, 91%) as white crystals.[10]

General Procedure for Desymmetrization of *cis*-1 Using Enzymatic Acylation (Table 1). In a typical experiment, *p*-ClC_6H_4-OAc **4a** (51 mg, 0.30 mmol) in toluene (1 mL) was degassed with argon for 1 min and added to a Schlenk tube containing *cis*-**1** (23.2 mg, 0.2 mmol) and CALB (6 mg). The mixture was stirred at room temperature and monitored by TLC. The mixture was filtered through a silica pad to remove the enzyme, and the silica pad was washed with Et_2O (3 × 3 mL). The filtrate was collected, the solvent was concentrated in vacuo, and the residue was analyzed by GC and ^{1}H NMR.

(1*S*,3*R*)-3-Acetoxy-1-cyclohexanol ((*R*,*S*)-2a) was prepared according to the procedure for the desymmetrization of *cis*-**1**, starting from *p*-ClC_6H_4OAc **4a** (510 mg, 3.0 mmol), *cis*-**1a** (232 mg, 2.0 mmol), and CALB (120 mg) in toluene (6 mL). After filtration, the silica pad was washed with Et_2O (3 × 30 mL), the combined solvent was concentrated in vacuo, and the residue was purified by flash chromatography (pentane/EtOAc 1:4 to EtOAc). **(*R*,*S*)-2a** (263 mg, 83%) was obtained as a colorless oil (>99% ee): ^{1}H NMR ($CDCl_3$) δ 4.79−4.68 (m, 1H), 3.75−3.66 (m, 1H), 2.27−2.16 (m, 1H), 2.04 (s, 3H), 1.92−1.72 (m, 3H), 1.44−1.18 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 170.6, 71.2, 68.7, 40.8, 34.5, 30.9, 21.6, 20.1.

General Procedure for Racemization of Monoesters (*R*,*S*)-2a and (*R*,*S*)-2b. In a typical experiment, the monoester (0.2 mmol) and CALB (12 mg) in toluene (1 mL) was stirred in a Schlenk tube under argon atmosphere at 70 °C. The racemization was monitored by GC. After standard workup, the mixture was filtered through a silica pad to remove the enzyme, the silica pad was washed with acetone (2 mL), and the filtrate was analyzed by GC.

(1*S*,3*R*)-3-(2,2,2-Trideuterioacetoxy)-1-cyclohexanol ((*R*,*S*)-2a-d_3). *p*-ClC_6H_4OAc-d_3 **(4a-d_3)** (582 mg, 3.3 mmol) in toluene (6.8 mL) was degassed with argon for 1 min and added to a Schlenk tube containing *cis*-**1** (260 mg, 2.2 mmol) and CALB (135 mg). The mixture was stirred at room temperature, monitored by NMR, and stirred for 9 h. The mixture was filtered through a silica pad to remove the enzyme, the enzyme was washed with Et_2O (3 × 30 mL), the solvent was concentrated in vacuo, and the residue was purified by flash chromatography (pentane/EtOAc 1:4 to EtOAc) affording **(*R*,*S*)-2a-d_3** (153 mg, 43%) as a colorless oil (>99% ee): ^{1}H NMR ($CDCl_3$) δ 4.81−4.69 (m, 1H), 3.76−3.64 (m, 1H), 2.27−2.18 (m, 1H), 2.04−2.00 (m, 0.3H), 1.94−1.78 (m, 3H), 1.46−1.20 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 170.4, 70.9, 68.4, 40.6, 34.3, 30.6, 21.1(m) 19.9.

General Procedure for 4-Chlorophenyl Acyl Donors (4). In a typical experiment, the appropriate acid chloride (5.5 mmol) was added dropwise to a solution of 4-chlorophenol (0.643 g, 5.0 mmol), Et_3N (1.52 g, 15 mmol), and DMAP (12 mg, 0.1 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature overnight. The solution was washed with 1 M HCl (3 × 8 mL), and the combined aqueous phases were reextracted with ether (3 × 8 mL). The combined organic phases were washed with saturated Na_2CO_3 (aq, 5 mL) and brine (4 mL) and dried over $MgSO_4$. The solvent was concentrated in vacuo, and the crude mixture was purified on silica (pentane/EtOAc gradient).

***p*-Chlorophenyl propionate (4b):** isolated as colorless oil; yield 0.698 g (76%); ^{1}H NMR ($CDCl_3$) δ 7.34 (dm, J = 9.1, 2H), 7.03 (dm, J = 9.1, 2H), 2.59 (q, J = 7.4, 2H), 1.26 (t, J = 7.4, 3H); ^{13}C NMR ($CDCl_3$) δ 172.7, 149.2, 131.0, 129.4, 122.9, 27.7, 9.0.

***p*-Chlorophenyl butyrate (4c):** isolated as pale yellow oil; yield 0.873 g (88%); ^{1}H NMR ($CDCl_3$) δ 7.33 (dm, J = 8.8, 2H), 7.03 (dm, J = 8.8, 2H), 2.53 (t, J = 7.4, 2H), 1.78 (app. hextet, J = 7.4, 2H), 1.04 (t, J = 7.4, 3H); ^{13}C NMR ($CDCl_3$) δ 171.9, 149.2, 131.0, 129.4, 122.9, 36.1, 18.4, 13.6.

***p*-Chlorophenyl hexanoate (4d):** isolated as pale yellow oil; yield 0.655 g (58%); ^{1}H NMR ($CDCl_3$) δ 7.33 (dm, J = 8.9, 2H), 7.02 (dm, J = 8.9, 2H), 2.54 (t, J = 7.4, 2H), 1.82−1.69 (m, 2H), 1.46−1.30 (m, 4H), 0.98−0.86 (m, 3H); ^{13}C NMR (CDCl3) δ 172.0, 149.2, 131.0, 129.4, 122.9, 34.3, 31.2, 24.5, 22.3, 13.9.

Gereral Procedure for Preparation of Diesters of 1,3-Cyclohexandiol. To a solution of the *cis*- or *trans*-diol **1** (69 mg, 0.6 mmol) and DMAP (1.4 mg, 0.01 mmol) in dichloromethane (1 mL) was added the desired acid chloride (3.0 mmol) dropwise at 0 °C. The reaction was stirred at room temperature overnight. The solution was evaporated and purified by flash chromatography (pentane/CH_2Cl_2). The yields of the diester were >90%.

***cis*-Diacetate *cis*-3a:**[16] ^{1}H NMR ($CDCl_3$) δ 4.80−4.68 (m, 2H), 2.29−2.19 (m, 1H), 2.02 (s, 6H), 2.00−1.79 (m, 3H), 1.52−1.18 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 170.2, 70.5, 37.1, 30.7, 21.2, 20.1.

General Procedure for Preparation of Racemic *cis*-3-Acetoxy-1-cyclohexanol (*cis*-2a). To a solution of the *cis*-diol **1** (58 mg, 0.5 mmol) in THF (5.5 mL) and pyridine (79 mg, 1.0 mmol) at 0 °C was added acetyl chloride (41 mg, 0.525 mmol) dropwise over 10 min. The reaction was stirred at 0 °C for 2 h and at room temperature for an additional 2 h. The solvent was concentrated in vacuo, and 1 M HCl (5.5 mL) and CH_2Cl_2 (10 mL) were added. The phases were separated, and the aqueous layer was collected and extracted with CH_2Cl_2 (5 × 20 mL). The combined organic phases were washed with saturated aq Na_2CO_3 (10 mL), water (10 mL) and brine (10 mL) and dried over $MgSO_4$. The solvent was concentrated in vacuo ,and the residue was purified by flash chromatography (CH_2Cl_2 to (CH_2Cl_2/acetone 2:1) affording (*rac*)-*cis*-**2a** (31 mg, 39%) as a pale yellow oil:[17] ^{1}H NMR ($CDCl_3$) δ 4.79−4.68 (m, 1H), 3.75−3.66 (m, 1H), 2.27−2.16 (m, 1H), 2.04 (s, 3H), 1.92−1.72 (m, 3H), 1.44−1.18 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 170.6, 71.2, 68.7, 40.8, 34.5, 30.9, 21.6, 20.1.

General Procedure for DYKAT of *cis*-1. In a typical experiment, *p*-chlorophenyl ester **4** (0.6 mmol) in toluene (1 mL) was degassed with argon for 1 min and added to a Schlenk tube containing *cis*-**1** (23.2 mg, 0.2 mmol), CALB (6.5 mg), and the ruthenium catalyst **5** (10.8 mg, 5 mol %). The mixture was stirred at room temperature for 3 h and then for 69 or 93 h at 70 °C. After 24 h, 2,4-dimethyl-3-pentanol was added as a hydrogen source (0.5 equiv/hydroxyl group). The mixture was filtered through a Celite pad to remove the enzyme. The solid was washed with acetone (3 × 2 mL), solvent was concentrated in vacuo, and the residue was analyzed by GC and ^{1}H NMR.

General Procedure for DYKAT of 1. In a typical experiment, *p*-ClC_6H_4OAc **4a** (102 mg, 0.6 mmol) in toluene (1 mL) was degassed with argon for 1 min and added to a Schlenk tube containing *cis*/*trans*-**1** (23.2 mg, 0.2 mmol), enzyme (6.5 mg), and the ruthenium catalyst **5** (10.8 mg, 5 mol %). The mixture was stirred at 70 °C for 24 h, after which time 2,4-dimethyl-3-pentanol was added as a hydrogen source (0.5 equiv/hydroxyl group). The mixture was stirred for additionally 48 h and worked up by filtering through a Celite pad to remove the enzyme. The solid was washed with acetone (3 × 2 mL), solvent was concentrated in vacuo, and the residue was analyzed by GC and ^{1}H NMR.

General Procedure for DYKAT of 1 or (*R*,*S*)-2a. A solution of KO-*t*-Bu (0.5 in THF; 48 μL, 12 mol %) was added to a 10 mL Schlenk tube. The THF was carefully removed under vacuum, and the flask was filled with argon. CALB (4 mg), Na_2CO_3 (8 mg, 0.08 mmol), and Ru catalyst **7** (12.8 mg, 5 mol % for each hydroxyl group) were added. The Schlenk flask was evacuated and filled with argon. Toluene (1 mL) was added, the mixture was stirred for 6 min, and *cis*/*trans*-cyclohexanediol (23 mg, 0.2 mmol) was added quickly. After an additional 4 min, isopropenyl acetate (65

(16) Hirata, T.; Izumi, S.; Aoki, M.; Gotoh, S.; Utsumi, R *Chirality* **1997**, *9*, 250.

(17) Fleming, I. M.; Henning, R.; Parker, D. C.; Plaut H. E.; Sanderson, P. E. J. *J. Chem. Soc., Perkin Trans. 1* **1995**, *4*, 317.

μL, 3 equiv) was added. After being stirred for 24 h at ambient temperature, the reaction mixture was analyzed by GC and ^{1}H NMR.

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Supporting Information Available: Experimental procedures and characterization data for compounds *trans*-**1**, (*R*,*S*)-**2b**, (*R*,*S*)-**3a**-d_3, (*R*,*S*)-**2a**, **4e**−**f**, *cis*-**3c**−**g**, *trans*-**3a**−**g**, *cis*-**2b**, and *trans*-**2b**. Copies of ^{1}H NMR and ^{13}C NMR spectra of compounds *cis*-**2a**,**b**, *trans*-**2a**, *cis*-**3a**−**g**, *trans*-**3a**−**g**, and **4b**−**f**. This material is available free of charge via the Internet at http://pubs.acs.org.

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