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On the Mechanism of the Unexpected Facile Formation of *meso*-Diacetate Products in Enzymatic Acetylation of Alkanediols

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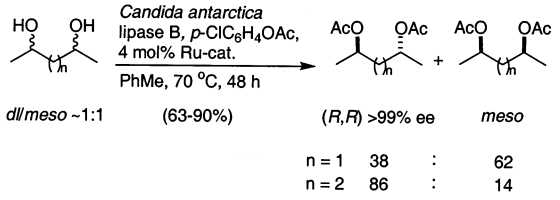
The mechanism of the unexpected facile formation of *meso*-diacetate previously observed in the enzymatic resolution of *dl*/*meso* mixtures of 2,4-pentanediol and 2,5-hexanediol with *Candida antarctica* lipase B has been elucidated. It was found that the formation of *meso*-diacetate proceeds via different mechanisms for the two diols. Enzyme-catalyzed acylation of AcO-d_3 labeled (*R*)-monoacetates of *meso*-2,4-pentanediol and *meso*-2,5-hexanediol and analysis of the *meso*-diacetates obtained show that the former reaction proceeds via intramolecular acyl migration while the latter occurs via direct *S*-acylation of the alcohol. For the (*R*)-monoacetate of (*R*,*S*)-2,4-pentanediol the intramolecular acyl migration was fast and therefore direct *S*-acylation by the external acyl donor is suppressed. For the hexanediol monoacetate the rate ratio (pseudo *E* value) between (5*R*,2*R*)- and (5*R*,2*S*)-5-acetoxy-2-hexanol was experimentally determined to be $k_{R,R}/k_{R,S} = 25$, which is about 10−20 times lower than the *E* value for 2-pentanol and 2-octanol. In a preliminary experiment it was demonstrated that facile acyl migration in the 1,3-diol derivative can be utilized to prepare *syn*-1,3-diacetoxynonane (>90% syn) in high enantioselectivity (>99% ee) via a chemoenzymatic dynamic kinetic asymmetric transformation of a *meso*/*dl* mixture of 1,3-nonanediol.

Introduction

As part of our ongoing program on the combined enzyme- and transition metal-catalyzed dynamic kinetic resolution (DKR) of various substrate classes,[1] a procedure for diols was recently reported.[2] Despite the excellent enantiomeric excess obtained, this process showed moderate to low diastereoselectivity for certain diols, which gave also the *meso*-compound containing an *S*-acylated hydroxyl group (anti-Kazlauskas product) (Scheme 1).[2] This is unexpected since *Candida antarctica* lipase B (CALB) usually exhibits a high preference for (*R*)-alcohols,[1,3] assuming the order of preference of the α-hydroxyl substituents agrees with large-small; CALB-catalyzed acylations normally lead to product formation in strict agreement with Kazlauskas' rule.[4]

Also, in a report on the kinetic resolution (KR) of diols using the same enzyme, considerable amounts of the *meso*-diacetate were produced employing 2,4-pentanediol as the substrate.[5] Two possible mechanisms have been

SCHEME 1. DKR of Diols



proposed to account for the unexpected facile acylation of the (*S*)-alcohol function: (i) an intramolecular acyl transfer from the (*R*)-acetate to the (*S*)-alcohol in the monoacylated (*R*,*S*)-diol with subsequent enzyme-catalyzed acylation of the (*R*)-hydroxyl group released (cf. path A, Scheme 2) and (ii) direct acylation of the (*S*)-alcohol due to a lower enantioselectivity for the monoacylated diol (cf. path B, Scheme 2).

Because of the unexpectedly large proportion of *meso*-diacetate produced in the dynamic kinetic resolution of acyclic diols, we decided to study the mechanism of the reaction. In the present work we have employed deuterium labeling and shown that the pentanediol derivative follows the first pathway (via intramolecular acyl transfer), whereas the hexanediol proceeds via the second pathway (direct acylation).

Results and Discussion

By deuterium labeling of the acetate group in (*R*)-monoacetate of the *meso*-diol it would be possible to

(1) (a) Huerta, F. F.; Minidis, A. B. E.; Bäckvall, J.-E. *Chem. Soc. Rev.* **2001**, *30*, 321. (b) Persson, B. A.; Larsson, A. L. E.; Le Ray, M.; Bäckvall, J.-E. *J. Am. Chem. Soc.* **1999**, *121*, 1645. (c) Larsson, A. L. E.; Persson, B. A.; Bäckvall, J.-E. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1211.

(2) Persson, B. A.; Huerta, F. F.; Bäckvall, J.-E. *J. Org. Chem.* **1999**, *64*, 5237.

(3) Anderson, E. M.; Larsson, K. M.; Kirk, O. *Biocatal. Biotransform.* **1998**, *16*, 181.

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

(5) Mattson, A.; Öhrner, N.; Hult, K.; Norin, T. *Tetrahedron: Asymmetry* **1993**, *4*, 925.

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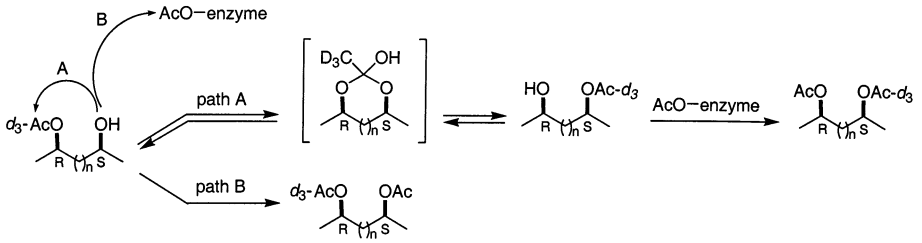
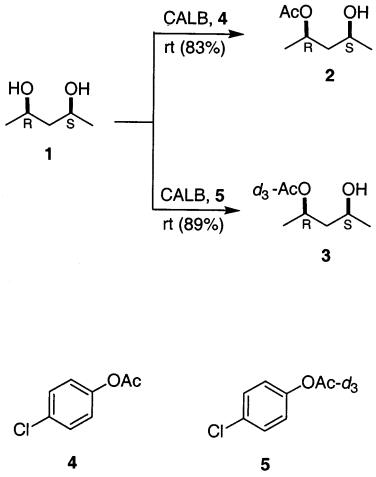
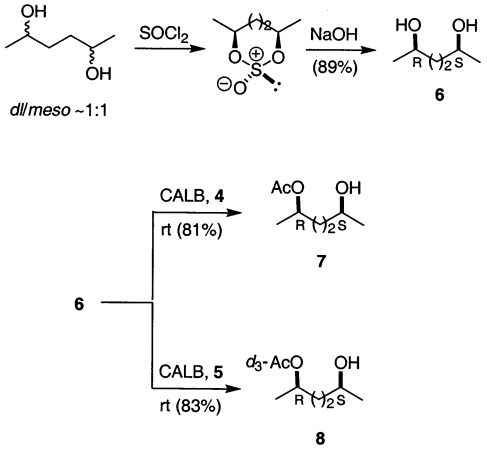
SCHEME 2. Deuterium Labeling to Distinguish between Acyl Transfer and Direct Enzymatic Acylation



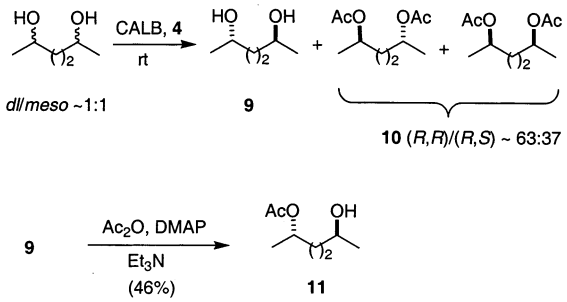
SCHEME 3. Stereoselective Preparation of (*R*)-Monoacetates of *meso*-2,4-Pentanediol



SCHEME 4. Stereoselective Preparation of (*R*)-Monoacetates of *meso*-2,5-Hexanediol



SCHEME 5



differentiate between the intramolecular acyl transfer pathway (path A, Scheme 2) and the direct acylation route (path B, Scheme 2). In the intramolecular pathway the deuterated acetoxy group would be transferred from the (*R*)-alcohol to the (S)-alcohol group via a cyclic intermediate. The (*R*)-alcohol function released would be rapidly acylated via the enzyme and give diacetate deuterated in the *S*-position. The pathway via direct acylation would give a diacetate in which deuterium is retained in the *R*-position.

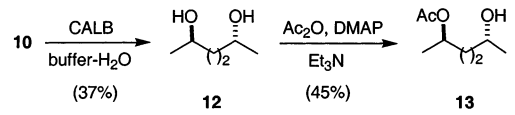
A. Preparation of Starting Materials and Reference Compounds. Pure *meso*-diol **1** was obtained by flash chromatography of the commercially available *dl*/*meso*-diol. Enzymatic acylation of **1** employing CALB and acyl donor **4** with careful monitoring of the reaction (TLC) gave monoacetate **2** in high selectivity. Analogously, stereoselective monoacylation of diol **1** by CALB and deuterium-labeled acyl donor **5** afforded deuterated monoacetate **3** (Scheme 3).

The nonlabeled and labeled (*R*)-monoacetates of *meso*-hexanediol were prepared in analogy with the pentanediols. Pure *meso*-diol **6** was prepared from a commercially available *dl*/*meso*-diol (*dl*/*meso* ~1:1) by converting the isomers into cyclic sulfites as described in the literature,[6] followed by separation and hydrolysis. CALB-catalyzed acylation of diol **6** with acyl donor **4** gave monoacetate **7**, while the same reaction employing labeled acyl donor **5** furnished monoacetate **8** (Scheme 4).

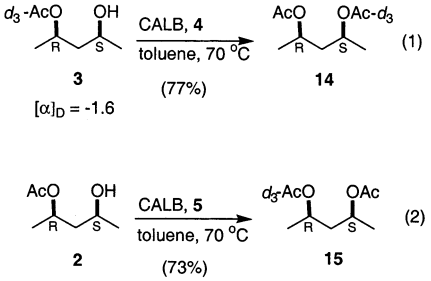
The stereoisomeric monoacetates **11** and **13** were also prepared from the commercially available *dl*/*meso*-2,5-hexanediol mixture via diol **9** and diacetates **10**, respectively. The high *R*-selectivity of CALB in esterification as well as in hydrolysis of esters was taken advantage of. Reaction of the commercial diol with enzyme and acyl donor **4** overnight afforded diacetates **10** and unchanged (*S*,*S*)-diol **9**, easily separable by flash chromatography. Diacetates **10** were isolated in a ratio of (*R*,*R*)/(*R*,*S*) ~ 63:37. The significant production of (*R*,*S*)-diacetate can most likely be explained by the direct anomalous *S*-acylation found for monoacetates of 2,5-hexanediol (vide infra). This reaction gave also a monoacetate fraction containing mainly the (*R*)-monoacetate of the (*R*,*S*)-diol ((*R*,*S*)/(*S*,*S*) = 95:5). Since this fraction was not used for further studies, it was discarded and its structure omitted in the scheme (Scheme 5). Optically pure monoacetate **7** was instead prepared as depicted in Scheme 4 (vide supra). Chemical acetylation of **9** afforded monoacetate **11** (Scheme 5). Enzymatic hydrolysis of diacetate fraction **10** furnished (*R*,*R*)-diol **12**, which was chemically transformed into monoacetate **13** (Scheme 6). Although chemoenzymatic routes to the pure enantiomers of hexanediol have been reported, our approach to combine enzymatic esterification and hydrolysis is to the best of our knowledge a novel approach toward these structures.

(6) Caron, G.; Kazlauskas, R. J. *Tetrahedron: Asymmetry* **1994**, *5*, 657.

SCHEME 6



SCHEME 7. Results from Enzymatic Acylation of Monoacetates

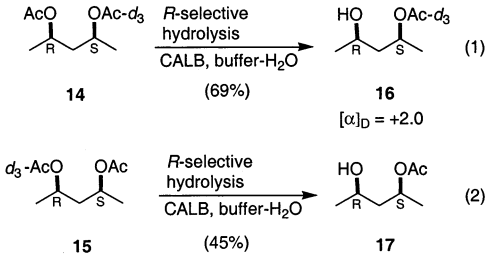


B. Studies of Enzymatic Acylation of the (*R*)-Monoacetate of (*R,S*)-2,4-Pentanediol. To distinguish between the two mechanisms outlined in Scheme 2 we studied the enzymatic acylation of specifically deuterated monoacetate **3** and nondeuterated monoacetate **2** with acyl donor **4** and **5**, respectively. Enzyme-catalyzed acetylation of (*R*)-monoacetate **3**, specifically trideuterated in the acetyl group, with CALB and nondeuterated acyl donor **4** gave diacetate **14**, which now has the deuterium at the (*S*)-acetate and no deuterium in the original (*R*)-acetate (Scheme 7, eq 1). Analogously, enzymatic acetylation of nondeuterated (*R*)-monoacetate **2** with deuterated acyl donor **5** afforded diacetate **15**, where the deuterated acetoxy group of the acyl donor ends up in the *R*-position (Scheme 7, eq 2).

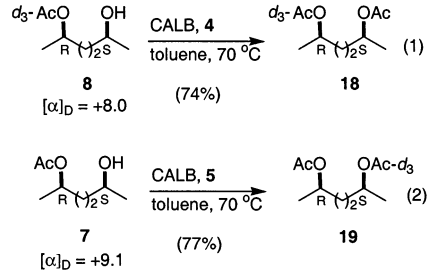
These results unambiguously show that an intramolecular acetyl migration accounts for the formation of the (*S*)-acetate. Apparently, the acyl migration in the syn isomer (*R,S*) is relatively fast in this case, which is consistent with the observation[2] that there was more *meso*-diacetate than (*R,R*)-product in the combined ruthenium- and enzyme-catalyzed DKR of the *dl*/*meso*-diol (*dl*/*meso* ~1:1). In the latter study the relative amount of *meso* configuration in the product was higher than in that of the starting material, suggesting that the acyl migration is faster than the ruthenium-catalyzed epimerization.

The analysis of diacetates **14** and **15** and determination of the location of deuterium is difficult to make by conventional methods. These pseudo *meso* compounds are formally enantiomers of one another and differ only in the deuterium labeling. To analyze these compounds we took advantage of the fact that lipases can hydrolyze *meso*-diacetates with high *R*-selectivity (Kazlauskas' rule considered). Enzymatic hydrolysis of diacetate **14** in a buffered aqueous solution afforded **16** (Scheme 8). It was found that the optical rotation of **16** had the opposite sign to that of the starting monoacetate **3**. The analogous enzymatic hydrolysis of **15** furnished **17**. Analysis of monoacetates **16** and **17** by ^{1}H NMR and MS showed that the former is deuterated in the acetoxy group whereas the latter is nondeuterated. A small amount of nondeuterated acetate (ca. 13%) in **16** and deuterated acetate (ca 8%) in **17** was observed. This shows that the reaction proceeds to ~90% via intramolecular acyl migration (path A, Scheme 2) and to ~10% via the direct acyl transfer pathway (path B, Scheme 2).

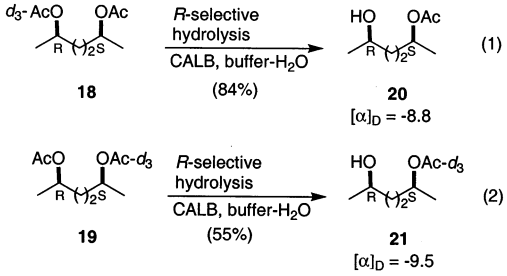
SCHEME 8. Analysis of Deuterated Diacetates



SCHEME 9. Results from Enzymatic Acylation of Monoacetates



SCHEME 10. Analysis of Deuterated Diacetates



The presence of three deuteriums in the acetoxy group of compound **16** (Scheme 8, eq 1) establishes that diacetate **14** has the d_3-acetate in the *S*-position. Furthermore, the lack of deuterium in product **17** shows that the isotope incorporation is at the (*R*)-acetate in diacetate **15** (Scheme 8, eq 2).

These results prove that the enzyme-catalyzed formation of the *meso*-diacetate from *meso*-2,4-pentanediol proceeds via mechanism A, where the second acylation is preceded by an intramolecular acyl transfer (Scheme 2). This acyl transfer releases the fast-reacting (*R*)-alcohol, which is subsequently rapidly acylated by the enzyme. A related acyl transfer has been observed in lipase-catalyzed hydrolysis of diacetates of *meso*-1,3-diols (2-substituted 1,3-propane diols).[7] Analysis of monoacetates **16** and **17** by chiral GC revealed a slightly lower enantiomeric excess (ee 90−92%) compared to that of the starting materials **3** and **2**, respectively (94% ee). This is probably due to a minor contribution from nonselective hydrolysis by the enzyme, or to intramolecular acyl migration (path A) in **16** and **17** during the reaction.

C. Studies of Enzymatic Acylation of the (*R*)-Monoacetate of (*R,S*)-2,5-Hexanediol. To make the analogous study of a 1,4-related diol, deuterium-labeled

(7) Liu, K. K.-C.; Nozaki, K.; Wong, C.-H. *Biocatalysis* **1990**, *3*, 169.

monoacetate **8** of *meso*-hexanediol was acetylated in the presence of the enzyme. Interestingly, this substrate furnished diacetate **18**, which now has the labeling retained in the *R*-position (Scheme 9, eq 1). Likewise, acetylation of nondeuterium-labeled monoacetate **7** under the same reaction conditions, using the deuterated acyl donor, gave diacetate **19** where the deuterated acetoxy group from the acyldonor is found in the *S*-position of the diacetate (Scheme 9, eq 2).

Diacetates **18** and **19** were analyzed again by *R*-selective hydrolysis with use of CALB in aqueous phosphate buffer (Scheme 10). The formation of (*S*)-monoacetates **20** and **21** was confirmed by the optical rotation, which now had the opposite sign to that of the starting (*R*)-monoacetates **8** and **7**. This unambiguously confirms the *R*-selectivity of the enzyme hydrolysis. Analysis of monoacetates **20** and **21** by ^{1}H NMR and MS showed that the former is nondeuterated and that the latter is trideuterated in the acetoxy group. The lack of deuterium in product **20** (Scheme 10, eq 1) establishes that the label was in the *R*-position of diacetate **18**. Analogously, the presence of deuterium in monoacetate **21** (Scheme 10, eq 2) shows that the deuterated acetate was in the *S*-position of diacetate **19**.

These results prove that mechanism B operates for the hexanediol and that the *meso*-diacetate is obtained through a *direct enzymatic* acylation of the (*S*)-alcohol. The possibility of a direct chemical acetylation was ruled out since no reaction could be observed in the absence of enzyme, neither on the isomeric mixture of 2,5-hexanediols nor on the pure (*R*)-monoacetate. In addition, no acyl migration could be detected when monoacetate **8** was heated in toluene at 70 °C for 24 h in a control experiment.

D. Kinetic Studies of Enzymatic Acylation of 2,5-Hexanediol Monoacetates. Because of the unexpected facile direct enzymatic acylation of the (*S*)-alcohol function of the (*R*)-monoacetate of *meso*-2,5-hexanediol it was of interest to study the kinetics of the process. The relatively easy enzymatic acylation of the (*S*)-alcohol of this monoacetate suggests that there is an additional interaction between the enzyme and the acetoxy group of the 5-acetoxy-2-hexanol. This interaction could facilitate acylation of the latter diol monoacetate and lead to a much lower rate ratio (pseudo *E* value) between the (*S*)-2-ol and the (*R*)-2-ol of the monoacetate, compared to that of the parent 2-hexanol. It was therefore of interest to determine the relative rate of the (2*R*)- and (2*S*)-alcohols when there is an acetate in the 5-position. Kinetic studies of the diastereomeric (2*R*,5*R*)-5-acetoxy-2-hexanol and (2*S*,5*R*)-5-acetoxy-2-hexanol were performed and the rates were compared for the approximate first-order acetylation reactions. This gave a pseudo *E* value of 25 for the enzyme (Scheme 11 and Figure 1). This value is at least 1 order of magnitude smaller than that for 2-hexanol.[8] It is interesting to note that a related moderate enantioselectivity with CALB was observed for *tert*-butyl 4-hydroxypentanoate ($E = 10$)[9] and ethyl

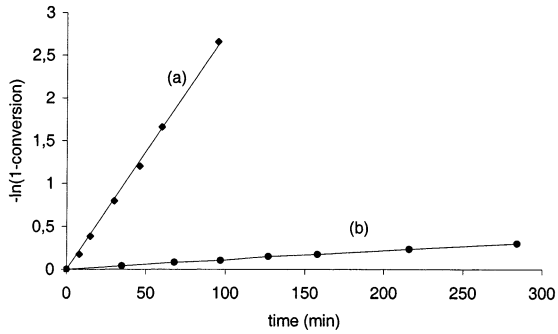
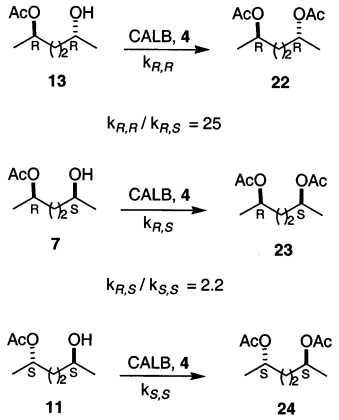


FIGURE 1. Rate of acetylation of (a) alcohol 13 and (b) alcohol 7.

SCHEME 11. Relative Rates of Stereoisomeric Monoacetates



3-hydroxybutanoate ($E = 31$),[10] where presumably the ester group leads to an additional interaction with the enzyme.

The effect of a neighboring ester group was further studied by examining whether the configuration of the neighboring acetate would influence the rate of the acylation of the alcohol. Remote recognition of a stereogenic carbon atom away from the reaction site has been reported for hydrolytic enzymes in enantioselective hydrolysis[11] and transesterification.[12] The relative rates of (2*S*,5*R*)-alcohol **7** (vide supra) and (2*S*,5*S*)-alcohol **11** were compared and in this case the kinetic studies resulted in a pseudo *E* value of 2.2 (Scheme 11 and Figure S1 available as Supporting Information). Comparison of the pseudo $E_{R,R/R,S} = 25$ with the pseudo $E_{R,S/S,S} = 2.2$, indicates a small but significant influence of the configuration at the acetoxy bearing carbon atom.[13]

E. Synthetic Application of the Acyl Migration Mechanism. The fact that the acetyl migration is fast in *syn*-1,3-diol monoacetates, as shown for **2** and **3**, could

(8) Rotticci, D.; Hæffner, F.; Orrenius, C.; Norin, T.; Hult, K. *J. Mol. Catal. B: Enzymol.* **1998**, *5*, 267.

(9) Runmo, A.-B. L.; Pàmies, O.; Faber, K.; Bäckvall, J.-E. *Tetrahedron Lett.* **2002**, *43*, 2983.

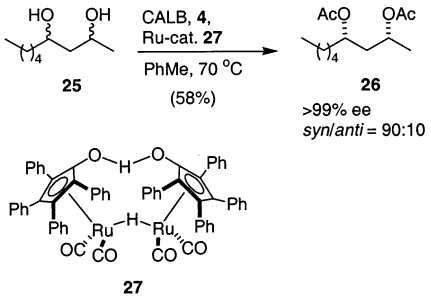
(10) Magnusson, A.; Hult, K.; Holmquist, M. *J. Am. Chem. Soc.* **2001**, *123*, 4354.

(11) (a) Mizuguchi, E.; Achiwa, K. *Tetrahedron: Asymmetry* **1993**, *4*, 2303 and references therein. (b) Hughes, D. L.; Bergan, J. J.; Amato, J. S.; Bhupathy, M.; Leazer, J. L.; McNamara, J. M.; Sidler, D. R.; Reider, P. J.; Grabowski, E. J. J. *J. Org. Chem.* **1990**, *55*, 6252.

(12) Ors, M.; Morcuende, A.; Jiménez-Vacas, M. I.; Valverde, S.; Herradón, B. *Synlett* **1996**, 449.

(13) To investigate the origin of the effect, the CALB-catalyzed acylation rate of (*S*,*S*)-diol monoacetate **11** was compared with the rate of monoacylation of (*S*,*S*)-diol **9** itself. It was found that they were acylated with the same rate (pseudo $E = 1$). The plot obtained is available as Supporting Information. This finding suggests that the electronegativity of the neighboring group is of importance.

SCHEME 12. DYKAT of a 1,3-Diol



be used for synthetic transformations via a dynamic kinetic asymmetric transformation (DYKAT). For a 1,3-diol where only one of the alcohol functions (the least sterically hindered) can be enzymatically acylated under DYKAT conditions to give (*R*)-acetate, the other alcohol would be intramolecularly acylated via acyl migration. Because the acyl migration is favored in the *syn*-diol, the acetate formed via intramolecular acylation will be of *S*-configuration (see Scheme 12). The released (*R*)-alcohol will subsequently be rapidly acylated to give the *syn*-diacetate. Under dynamic conditions, where the alcohol functions will be epimerized by e.g. a metal catalyst, a racemic diastereomeric mixture of a 1,3-diol could be transformed into one enantiomer of the diacetate. In preliminary studies a racemic syn/anti mixture (syn/anti ~1:1) of 2,4-nonanediol (**25**) was treated with ruthenium catalyst **27**, CALB, and acyl donor **4** to give enantiomerically pure (>99% ee) *syn*-diacetate **26** (syn/anti 90:10) in 58% yield after 72 h. This shows that a dynamic process operates involving enzymatic resolution coupled with ruthenium-catalyzed epimerization and *syn*-acyl migration. In this process control of the absolute stereochemistry at both chiral centers is obtained. Optimization of this reaction and studies on its scope and limitations will be reported in due course.

Conclusions

We have been able to distinguish between the two previously proposed mechanisms for the formation of anti-Kazlauskas products observed in CALB-catalyzed acylation of 2,4-pentanediol and 2,5-hexanediol. Deuterium-labeling studies show that the former diol gives a monoacetate that is prone to undergo intramolecular acyl transfer (Mechanism A, Scheme 2), whereas the latter substrate is diacylated in a direct enzymatic fashion (Mechanism B, Scheme 2). For the hexandiol monoacetates **7** and **8** a neighboring effect of the acetate reduces the substrate specificity of the enzyme. The facile acyl migration in monoacylated 1,3-diols was applied to DYKAT of nonane-1,4-diol to give the enantiomerically pure *syn*-diacetate.

Experimental Section

General Methods. Analytical TLC was performed with use of aluminum plates precoated with silica gel 60 F_{254} and detected with UV light and phosphomolybdic acid (5% (w/v) in EtOH). Flash chromatography was carried out on 60 Å (35−70 μm) silica gel. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 or 300 MHz and at 100 or 75 MHz, respectively. Chemical shifts (δ) are reported in ppm, using the residual solvent peak in $CDCl_3$ (δ_H 7.26 and δ_C 77.0) as internal standard, and coupling constants (J) are given in Hz and without sign. Enantiomeric and diastereomeric excess were determined by analytical gas chromatography employing a CP-Chirasil-Dex CB column for all monoacetates as well as for diol **1**, and a JW Cyclodex-B column for diacetate **10**. Optical rotations, $[\alpha]_D$, were measured at the sodium D line and at ambient temperature. Mass spectra were recorded at an ionizing voltage of 30 eV (EI). All reactions were carried out under argon atmosphere in flame-dried glassware, except for those reactions utilizing a water buffer as solvent, which were run in air. Unless otherwise noted, reagents are commercially available and were used without further purification. Toluene was distilled from calcium hydride and stored over 4 Å molecular sieves. Methylene chloride (CH_2Cl_2) was distilled from calcium hydride prior to use. Aqueous 0.1 M phosphate buffer pH 7.5 was prepared in a standard fashion with KH_2PO_4 and 1 M NaOH. Acyl donor **4** was prepared according to a literature procedure,[1b] and acyl donor **5** was prepared by the same method employing acetyl chloride-d_3.[14] Solvents used for extractions and chromatography were of technical grade and were distilled before use. The CALB (*Candida Antarctica* lipase B) used was the immobilized commercially available Novozym 435.

***meso*-2,4-Pentanediol (1).**[15] Flash chromatography (Et_2O) of commercial *dl*/*meso*-2,4-pentanediol gave pure *meso*-2,4-pentanediol (**1**) (>99% de) as a pale yellow, highly viscous oil about to crystallize. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.05 (m, 2H), 3.03 (br s, 2H), 1.53 (m, 2H), 1.20 (d, J = 6.3, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 68.7, 46.1, 24.0.

***meso*-2,5-Hexanediol (6).** Commercial *dl*/*meso*-2,5-hexanediol (*dl*/*meso* ~1:1) was converted into cyclic sulfites according to a literature procedure.[6] After 1 h at room temperature the reaction mixture was concentrated on silica. Flash chromatography (pentane/Et_2O 10:1) gave a pure fraction of *meso*-sulfite, as confirmed by ^{1}H NMR.[6]

This sulfite (2.74 g, 16.7 mmol) was refluxed in 2 M NaOH (60 mL) for 1 h and the reaction mixture was allowed to cool to room temperature. The solution was saturated with solid NaCl and extracted with EtOAc (5 × 40 mL). The combined organic phases were concentrated in vacuo on silica and subjected to flash chromatography with a stepwise eluent gradient (pentane/EtOAc 1:1 → 0:1). Removal of the solvent in vacuo gave **1** (1.76 g, 89%) as white crystals. Its NMR spectra were in agreement with those previously reported.[15b] The ^{13}C NMR spectrum contained only signals corresponding to the *meso*-diol; no traces of the racemic diol were detected.[15b]

General Procedure for Enzymatic Monoacylation of *meso*-Diols: (2*S*,4*R*)-4-(2,2,2-Trideuterioacetoxy)-2-pentanol (3). To a solution of diol **1** (0.398 g, 3.82 mmol) and acyl donor **5** (1.01 g, 5.82 mmol) in toluene (11.5 mL) was added CALB (0.229 g). The Schlenk flask was evacuated and filled with argon and this procedure was repeated three times before it was sealed and the mixture was stirred at room temperature. After 33 min the reaction mixture was filtered and the enzyme was washed with Et_2O. The filtrate was evaporated under vacuo and flash chromatography (stepwise gradient of pentane/Et_2O 1:1 → 1:2) of the residue afforded **3** (0.507 g, 89%) as a pale yellow oil about to crystallize. $[\alpha]^{24}_D$ −1.6 (*c* 3.0, $CHCl_3$); ee 94%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.04 (app sextet, J = 6.3, 1H), 3.89 (br app sextet, J = 6.2, 1H), 2.00 (m, 0.4H), 1.83 (app dt, J = 7.9, 14.0, 1H), 1.59 (app dt, J = 5.1, 14.0, 1H), 1.26 (d, J = 6.2, 3H), 1.21 (d, J = 6.2, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 69.4, 65.6, 45.2, 23.7, 20.9 (m), 20.3.

(2*S*,4*R*)-4-Acetoxy-2-pentanol (2). The same procedure was used to give a colorless oil of **2**. Yield 83%. ee 94%; ^{1}H

(14) Acetyl-d_3 chloride, 99+ atom % D, was purchased from Aldrich company. However, ^{1}H NMR showed the bottle to contain about 35% CD_2HCOCl, which is why a proton signal from the acetyl group in the deuterated monoacetates is reported.

(15) (a) Gordillo, B.; Hernández, J. *Org. Prep. Proced. Int.* **1997**, *29*, 195. (b) Ikeda, H.; Sato, E.; Sugai, T.; Ohta, H. *Tetrahedron* **1996**, *52*, 8113.

NMR (300 MHz, $CDCl_3$) δ 5.04 (app sextet, J = 6.4, 1H), 3.89 (app sextet, J = 6.3, 1H), 2.04 (s, 3H), 1.84 (app dt, J = 7.7, 14.4, 1H), 1.59 (app dt, J = 5.0, 14.4, 1H), 1.26 (d, J = 6.4, 3H), 1.21 (d, J = 6.3, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 69.5, 65.5, 45.1, 23.7, 21.3, 20.2.

(2*S*,5*R*)-5-Acetoxy-2-hexanol (7). Isolated as a pale yellow oil. Yield 81%. $[\alpha]^{29}_D$ +9.1 (*c* 1.00, $CHCl_3$) (lit.[16] $[\alpha]^{25}_D$ +9.6 (*c* 1, $CHCl_3$)); ee >99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.90 (app sextet, J = 6.2, 1H), 3.79 (app sextet, J = 6.2, 1H), 2.03 (s, 3H), 1.62 (m, 2H), 1.46 (m, 2H), 1.22 (d, J = 6.4, 3H), 1.19 (d, J = 6.2, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 71.0, 67.6, 34.8, 32.1, 23.5, 21.3, 19.9.

(2*S*,5*R*)-5-(2,2,2-Trideuterioacetoxy)-2-hexanol (8). Isolated as a pale yellow oil. Yield 83%. $[\alpha]^{23}_D$ +8.0 (*c* 1.27, $CHCl_3$); ee >99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.90 (app sextet, J = 6.2, 1H), 3.79 (app sextet, J = 6.2, 1H), 2.00 (m, 0.4H), 1.63 (m, 2H), 1.46 (m, 2H), 1.22 (dd, J = 6.2, 1.5, 3H), 1.19 (dd, J = 5.9, 1.5, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 71.0, 67.7, 34.9, 32.1, 23.5, 20.8 (m), 19.9.

(2*S*,5*S*)-2,5-Hexanediol (9) and Diacetates 10. To a stirred solution of *dl*/*meso*-hexanediol ~1:1 (0.303 g, 2.56 mmol) and acyl donor **4** (1.31 g, 7.69 mmol) in toluene (8 mL) was added at room temperature CALB (0.154 g). The Schlenk flask was evacuated and filled with argon and the procedure was repeated three times before the flask was sealed. The reaction mixture was stirred at room temperature overnight and was filtered. The enzyme was washed with Et_2O and the filtrate was concentrated in vacuo. Flash chromatography with pentane/EtOAc (stepwise gradient 1:1 → 0:1) provided unchanged alcohol **9** (0.064 g, 21%) as white crystals. $[\alpha]^{24}_D$ +34.4 (*c* 9.0, $CHCl_3$) (lit.[17] $[\alpha]^{25}_D$ +35.1 (*c* 9.5, $CHCl_3$)); the ^{1}H NMR and ^{13}C NMR spectra are consistent with those previously reported[15b,16] and the ^{13}C NMR shows no traces of diastereoisomeric *meso*-diol.[15b] From this column was also isolated a fraction of pure monoacetate (0.159 g, 39%) as a colorless oil. ee >99%; de 89%; (*R*,*S*)/(*S*,*S*) = 95:5.

The fastest running fractions from the above column contained diacetate, acyl donor **4**, and *p*-chlorophenol in a mixture. These fractions were concentrated in vacuo, then diluted with Et_2O (25 mL) and washed with 1 M NaOH (3 × 10 mL). The aqueous phases were extracted with Et_2O (2 × 20 mL) and the combined organic layers were washed with water and brine. Drying ($MgSO_4$), concentration, and flash chromatography (pentane/Et_2O stepwise gradient 7:1 → 3:1) gave diacetate **10** (0.186 g, 36%) as a pale yellow oil. (*R*,*R*)/(*R*,*S*) ~ 63:37.

(2*S*,5*S*)-5-Acetoxy-2-hexanol (11). To a stirred solution of diol **9** (52 mg, 0.44 mmol), DMAP (cat.), and Et_3N (0.123 mL, 0.88 mmol) in CH_2Cl_2 (5 mL) at room temperature was added Ac_2O (44 μL, 0.46 mmol) dropwise over 1 h. The reaction mixture was stirred at room temperature for 5 h, and then poured into 1 M HCl (5 mL). The layers were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with saturated aq Na_2CO_3, water, and brine. Drying ($MgSO_4$) followed by flash chromatography (pentane/Et_2O 1:2) furnished 24 mg (46%) of **11** as a colorless oil (>99% ee, 99% de). The spectral data were identical with those of its enantiomer **13**.

(2*R*,5*R*)-2,5-Hexanediol (12). Enzyme (0.197 g) was added to a stirred emulsion of diacetate fraction **10** (1.33 g, 6.56 mmol) in a 0.1 M phosphate buffer pH 7.5 (60 mL) and the reaction mixture was stirred at room temperature for 17 h. The reaction mixture was then filtered through a filter paper and the enzyme was washed with EtOAc. The aqueous phase was saturated with solid NaCl before the layers were separated and the aqueous phase extracted with EtOAc (4 × 40 mL). The combined organic phases were washed with brine, dried ($MgSO_4$), and concentrated. Flash chromatography (pentane/Et_2O 1:2 → EtOAc) gave **12** as white crystals (0.286 g, 37%). $[\alpha]^{23}_D$ −35.4 (*c* 9.0, $CHCl_3$) (lit.[16] $[\alpha]^{25}_D$ −35.7 (*c* 1, $CHCl_3$), lit.[18] $[\alpha]^{25}_D$ −39.6 (*c* 1, $CHCl_3$)); the ^{1}H NMR and ^{13}C NMR spectra were in accordance with those previously reported.[15b,16]

(2*R*,5*R*)-5-Acetoxy-2-hexanol (13). 13 was prepared from **12** as described for the preparation of compound **11** from **9**. Isolated as a pale yellow oil, partly crystallized. Yield 45% (>99% ee, 99% de). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.91 (app sextet, J = 6.2, 1H), 3.80 (br m, 1H), 2.02 (s, 3H), 1.76−1.38 (m, 4H), 1.22 (d, J = 6.4, 3H), 1.19 (d, J = 6.2, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 70.8, 67.6, 34.7, 32.0, 23.5, 21.3, 19.9.

General Procedure for Acylation of Diol Monoacetates: (2*S*,4*R*)-4-Acetoxy-2-(2,2,2-trideuterioacetoxy)pentane (14). To a stirred solution of diol monoacetate **3** (0.302 g, 2.02 mmol) and acyl donor **4** (0.521 g, 3.05 mmol) in toluene (6.1 mL) was added CALB (0.121 g). The flask was evacuated and filled with argon and this procedure was repeated three times before it was sealed and then heated in an oil bath at 70 °C. The reaction mixture was stirred for 18.5 h and then allowed to cool to room temperature. The mixture was filtered and the enzyme was washed with Et_2O (100 mL). The filtrate was washed with 1 M NaOH (3 × 40 mL). The washings were back-extracted with Et_2O (2 × 60 mL) and the combined organic phases were washed with water and brine, dried ($MgSO_4$), and concentrated. Flash chromatography (stepwise gradient of pentane/Et_2O 5:1 → 3:1) furnished **14** as a pale yellow oil (0.299 g, 77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.97 (app sextet, J = 6.2, 2H), 2.03 (s, 3H), 1.98 (m, 1H), 1.63 (m, 1H), 1.23 (d, J = 6.2, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 68.0, 67.9, 41.8, 21.3, 20.8 (m), 20.1.

(2*R*,4*S*)-4-Acetoxy-2-(2,2,2-trideuterioacetoxy)pentane (15). A pale yellow oil. Yield 73%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.95 (app sextet, J = 6.2, 2H), 2.01 (s, 3H), 1.97 (app dt, J = 7.2, 14.5, 1H), 1.61 (app dt, J = 5.9, 14.2, 1H), 1.24 (d, J = 6.3, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 67.9, 67.9, 41.8, 21.3, 20.8 (m), 20.0.

(2*R*,5*S*)-5-Acetoxy-2-(2,2,2-trideuterioacetoxy)hexane (18). Isolated as needle-shaped crystals. Yield 74%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.86 (m, 2H), 2.02 (s, 3H), 2.00 (m, 0.4H), 1.55 (m, 4H), 1.20 (d, J = 6.3, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 70.6, 70.6, 31.8, 21.3, 20.8 (m), 19.9.

(2*S*,5*R*)-5-Acetoxy-2-(2,2,2-trideuterioacetoxy)hexane (19). Isolated as needle-shaped crystals. Yield 77%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.87 (m, 2H), 2.03 (s, 3H), 2.00 (m, 0.3H), 1.55 (m, 4H), 1.20 (d, J = 6.3, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 70.7, 70.6, 31.8, 21.3, 20.9 (m), 19.9.

General Procedure for *R*-Selective Hydrolysis of Diacetates: (2*R*,4*S*)-4-(2,2,2-Trideuterioacetoxy)-2-pentanol (16). A mixture of diacetate **14** (182 mg, 0.95 mmol) and CALB (29 mg) in a 0.1 M phosphate buffer pH 7.5 (10 mL) was stirred at room temperature for 9 h. The solution was filtered and the enzyme was washed with EtOAc. Solid NaCl was added to saturate the water phase and the layers were separated. The aqueous phase was extracted with EtOAc (4 × 20 mL) and the combined extracts were washed with brine, dried ($MgSO_4$), and concentrated. Flash chromatography (pentane/Et_2O 1:2) gave **16** as a pale yellow oil (98 mg, 69%). $[\alpha]^{22}_D$ +2.0 (*c* 3.0, $CHCl_3$); ee 92%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.03 (app sextet, J = 6.4, 1H), 3.88 (m, 1H), 2.03 (s, 0.5H), 2.00 (m, 0.3H), 1.83 (app dt, J = 7.7, 14.3, 1H), 1.58 (app dt, J = 5.5, 14.2, 1H), 1.25 (d, J = 6.3, 3H), 1.20 (d, J = 6.2, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 69.4, 65.5, 45.1, 23.7, 20.9 (m), 20.3; MS (EI) *m*/*z* (rel) 150 (2.5, $M^+ + 1$), 71 (84), 64 (100), 63 (70), 61 (41).

(2*R*,4*S*)-4-Acetoxy-2-pentanol (17). A pale yellow oil. Yield 45%. ee 92%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.03 (m, 1H), 3.89 (m, 1H), 2.03 (s, 3H), 1.83 (app dt, J = 7.8, 14.3, 1H), 1.58 (app dt, J = 5.0, 14.3, 1H), 1.26 (d, J = 6.3, 3H), 1.20 (d,

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J = 6.2, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 69.5, 65.7, 45.2, 23.7, 21.4, 20.3; MS (EI) *m*/*z* (rel) 147 (0.7, $M^+ + 1$), 91 (13), 71 (45), 64 (13), 61 (100).

(2*R*,5*S*)-5-Acetoxy-2-hexanol (20). Isolated as a pale yellow oil (23 mg, 84%). $[\alpha]^{24}_D$ −8.8 (*c* 1.10, $CHCl_3$); ee >99% (chiral GC); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.90 (app sextet, J = 6.2, 1H), 3.79 (app sextet, J = 6.2, 1H), 2.03 (s, 3H), 1.63 (m, 2H), 1.46 (m, 2H), 1.21 (d, J = 6.2, 3H), 1.19 (d, J = 6.2, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 71.0, 67.8, 34.9, 32.2, 23.6, 21.4, 20.0; MS (EI) *m*/*z* (rel) 161 (62, $M^+ + 1$), 101 (100), 83 (48), 67 (80), 61 (47).

(2*R*,5*S*)-5-(2,2,2-Trideuterioacetoxy)-2-hexanol (21). Isolated as a colorless oil. Yield 55%. $[\alpha]^{29}_D$ −9.5 (*c* 1.30, $CHCl_3$); ee >99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.90 (app sextet, J = 6.3, 1H), 3.79 (m, 1H), 2.00 (m, 0.4H), 1.63 (m, 2H), 1.45 (m, 2H), 1.22 (d, J = 6.2, 3H), 1.19 (d, J = 6.2, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 70.9, 67.8, 34.9, 32.2, 23.6, 20.9 (m), 20.0; MS (EI) *m*/*z* (rel) 164 (36, $M^+ + 1$), 101 (100), 85 (38), 83 (43), 67 (71).

Enzymatic Acylation of Monoacetates for Kinetic Determinations. To a solution of **13** (34 mg, 0.21 mmol) and **4** (0.109 g, 0.64 mmol) in toluene-d_8 (0.64 mL) was added CALB (13 mg). The tube was evacuated and argon filled three times and was then rocked during the reaction, which was monitored by ^{1}H NMR spectroscopy.

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Supporting Information Available: Graphical presentation of the rates obtained from kinetic measurements of (*S*,*S*)-diol monoacetate **11** and (*S*,*S*)-diol **9** and ^{1}H NMR and ^{13}C NMR spectra of compounds **2**, **3**, **7**, **8**, and **13**−**21**. This material is available free of charge via the Internet at http://pubs.acs.org.

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