Pergamon

Tetrahedron: *Asymmetry* 12 (2001) 2447–2455

TETRAHEDRON: *ASYMMETRY*

Preparation of the enantiomers of 1-phenylethan-1,2-diol. Regio- and enantioselectivity of acylase I and *Candida antarctica* lipases A and B

Pauliina Virsu, Arto Liljeblad, Anu Kanerva and Liisa T. Kanerva*

Laboratory of Synthetic Drug Chemistry and Department of Chemistry, University of Turku, Lemminkäisenkatu 2, FIN-20520 Turku, Finland

Received 31 August 2001; accepted 2 October 2001

Abstract—Acylase I and *Candida antarctica* lipases A (CAL-A) and B (CAL-B) were evaluated for the preparation of the enantiomers of 1-phenylethan-1,2-diol. In the presence of CAL-B, the sequential one-pot methanolysis of the diacetate in acetonitrile allowed the preparation of (*S*)-diol (e.e. 97%) and (*R*)-1-acetoxy-1-phenylethanol (e.e. 94%). Base-catalyzed methanolysis of the monoacetate resulted in the corresponding (*R*)-diol. When one of the diol enantiomers was subjected to Mitsunobu esterification, inversion of configuration occurred, allowing transformation of the initially racemic mixture to one enantiomer. Acylase I-catalysis led to the chemo- and enantioselective formation of (*S*)-1-acetoxy-1-phenylethanol (e.e. 97%) in the presence of the primary hydroxyl function through acetylation of the secondary hydroxyl group. The low chemical yield (ca. 25%) was due to the moderate enzymatic regioselectivity. CAL-A behaved in a similar way to acylase I.

1. Introduction

1,2-Diols are important intermediates and building blocks for various synthetic applications. Thus, the enantiomers of 1-phenylethan-1,2-diol **1** are useful for the synthesis of chiral catalysts,[1,2] macrocyclic polyether–diester ligands,[3] pharmaceutically active compounds[4] and liquid crystals.[5] For the preparation of enantiomeric alcohols, the lipase (EC 3.1.1.3)-catalyzed kinetic resolution through transesterification in an appropriate organic solvent is among the most powerful procedures. Such resolution methods afford the two enantiomers in 50% conversion at best.

The biological function of lipases is to catalyze the hydrolysis of triacylglycerols at water–lipid interfaces. In these reactions, lipases are typically 1,3-regioselective. On this basis, it is hardly a surprise that the primary hydroxyl function reacts favourably in the lipase-catalyzed acylation of 1,2-diols in organic media.[6–9] Similarly, in the alcoholysis of the corresponding diesters a primary alcohol is first liberated in a highly regioselective manner.[7,10] These reactions, occurring far from the stereocentre, often proceed with very poor enantioselectivity although the substrate structure and the nature of a lipase can be critical in this respect. The role of a lipase is clearly seen for the methanolysis of dibutanoylated 1-*O*-trityl glycerol.[10] Thus, in the presence of *Pseudomonas cepacia* (lipase PS) and *fluorescens* lipases (lipase AK) the highly regio- and enantioselective (E>100; E is the enantiomer ratio[11]) reaction in diisopropyl ether gave the (*R*)-2-monoacylated product at 50% conversion and the reaction stopped. When *Candida antarctica* lipase B (CAL-B) was used as the catalyst, the initially formed 2-monoacylated product was racemic and further reaction resulted in (*R*)-1-*O*-tritylglycerol together with the less reactive (*S*)-2-monobutanoylated counterpart. The latter type of reaction was previously called one-pot sequential resolution and was successfully used for the lipase PS-catalyzed acylation of aryl-substituted 1,2-diols in organic solvents.[7–9,12]

The use of separately monoprotected 1,2-diols as substrates is a chemical means of forcing an enzymatic reaction to occur at a stereocentre rather than elsewhere in a molecule. Accordingly, highly enantioselective acylations of 1-phenoxy-2-alkanols with activated butanoates were previously performed in the presence of CAL-B.[13] On the other hand, the use of protecting

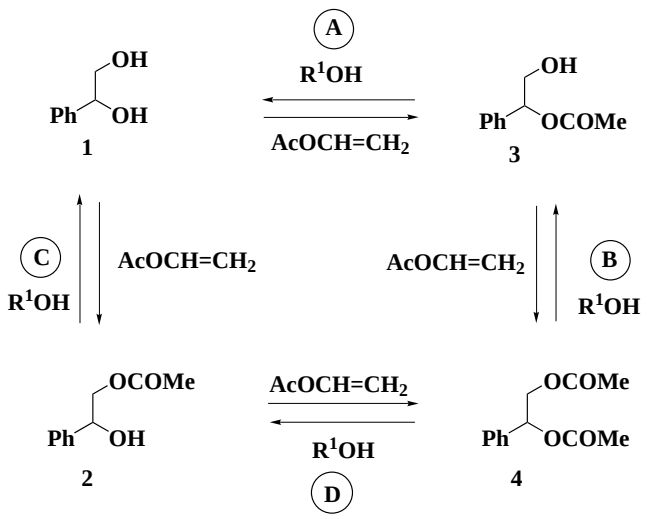
* Corresponding author. E-mail: lkanerva@utu.fi

0957-4166/01/$ - see front matter
PII: S0957-4166(01)00428-1

groups increases the number of synthetic steps and for that reason is usually avoided when other possibilities are at hand.

The third strategy for the resolution of 1,2-diols (or their diesters) that has not been described in the literature is to use an enzyme that is 2- rather than 1,3-regioselective towards triacylglycerols, leading to a reaction at the stereocentre rather than remote from it. Interestingly, *C. antarctica* lipase A (CAL-A) was previously shown to exert clear preference for the 2-position when triacylglycerols were hydrolyzed.[14]

In the work described herein, CAL-A, CAL-B and acylase I were evaluated for the preparation of the enantiomers of 1-phenylethan-1,2-diol without the use of protecting groups. The purpose was to exploit sequential resolution in one-pot through the acetylation of **1** in vinyl acetate and through the alcoholysis of the corresponding diacetate **4** in butanol or in acetonitrile (or some other organic solvent) containing methanol (Scheme 1). Using this strategy, the aim was to obtain the two enantiomers of the diol simultaneously. The possibility of transforming one of the enantiomers to its antipode through the Mitsunobu reaction was also studied.[15] A different strategy was to study enzymes that allow an enantioselective reaction at the free or acetylated secondary hydroxyl group of **1** and **4**, respectively, in the presence of the primary one. Monoesters **2** and **3** were chemically prepared and separately subjected under enzymatic reaction conditions in order to study which of the enantiomers reacts faster to give **1** upon alcoholysis and **4** upon acetylation. Lipases were chosen on the basis of their previous behaviors.[10,14] On the other hand, acylase I (*N*-acylamino acid amidohydrolase, EC 3.5.1.14) represents an unexplored possibility in catalyzing the resolution of 1,2-diols. Acylase I was previously shown to be useful for the acylation of primary and secondary alcohols as well as for the alcoholysis of carboxylic acid esters as novel reactions.[16–21] Commercial enzymes from *Aspergillus* species were screened using 1-phenylethanol and the corresponding esters as model substrates.



Scheme 1.

2. Results and discussion

The enzymatic acetylation of **1** and alcoholysis of **4** were studied paying attention to regio- and enantioselectivities. The acetylation of **1** to product **4** [or alcoholysis of **4** to product **1**] can proceed through two different monoacetylated products **2** and **3** (Scheme 1). The reaction is said to be from low to highly regioselective when it proceeds from equal to different proportions of the monoacetylated products via steps A and C (or B and D). The progression curves with time for the various products **1**–**4** in the reaction mixture are shown in Fig. 1(a–c) for the acetylation of **1** and in Fig. 1(d–f) for the alcoholysis of **4**. Enantiodiscriminations (which enantiomer reacts faster) for the sequential steps A–D by different enzymes are shown in Table 1. It is notable that in the case of the present enzymes, the regioselectivity steps A and C for acetylation and B and D for alcoholysis proceed with opposite enantiodiscriminations except when one of the steps leads to a racemic product. Accordingly, the enantiomer that reacts faster depends on enzymatic regioselectivity.

2.1. Acylase I catalysis

Acylase I is well characterized as a Zn(II)-containing enzyme (1–3 zinc ions per subunit).[22,23] The exact structure of the active site is not known and the reaction mechanism is simply proposed on the basis of the mechanisms of Zn(II)-containing carboxypeptidases.[24] The reaction proceeds without the formation of a covalent acyl-enzyme intermediate (the formation of which is typical of lipases). The enzyme is relatively stable in polar and hydrophilic solvents such as alcohols.[20,21]

For acylase I-catalyzed transesterifications, the previous results indicated higher enantioselectivity for the acylation of secondary rather than primary alcohols.[16–19] On this basis, commercial acylase I enzymes were first screened for the acetylation of 1-phenylethanol in vinyl acetate and for the butanolysis of 1-phenylethyl acetate and butanoate in the neat alcohol as model reactions. The results are shown in Table 2. Acetylation proceeds considerably faster than butanolysis, the (*R*)-enantiomer always reacting faster than the (*S*)-isomer. Somewhat higher enantioselectivity is observed for the butanolysis of 1-phenylethyl acetate than for that of the butanoate. The enzyme on Eupergit C is practically ineffective. Clearly, acylase I from *Aspergillus melleus* is the best for acetylation, while the enzyme from *A. genus* is most usable for alcoholysis and the enzymes were, respectively, used in the present work.

The acylase I (*A. melleus*)-catalyzed acetylation of **1** with vinyl acetate favors the formation of 2 (▲) through step C over the formation of **3** (□) through step A (Scheme 1; Fig. 1(a)). Interestingly, step A is highly enantioselective, allowing the preparation of monoester (*S*)-**3** in 97% e.e. and ca. 25% chemical yield.

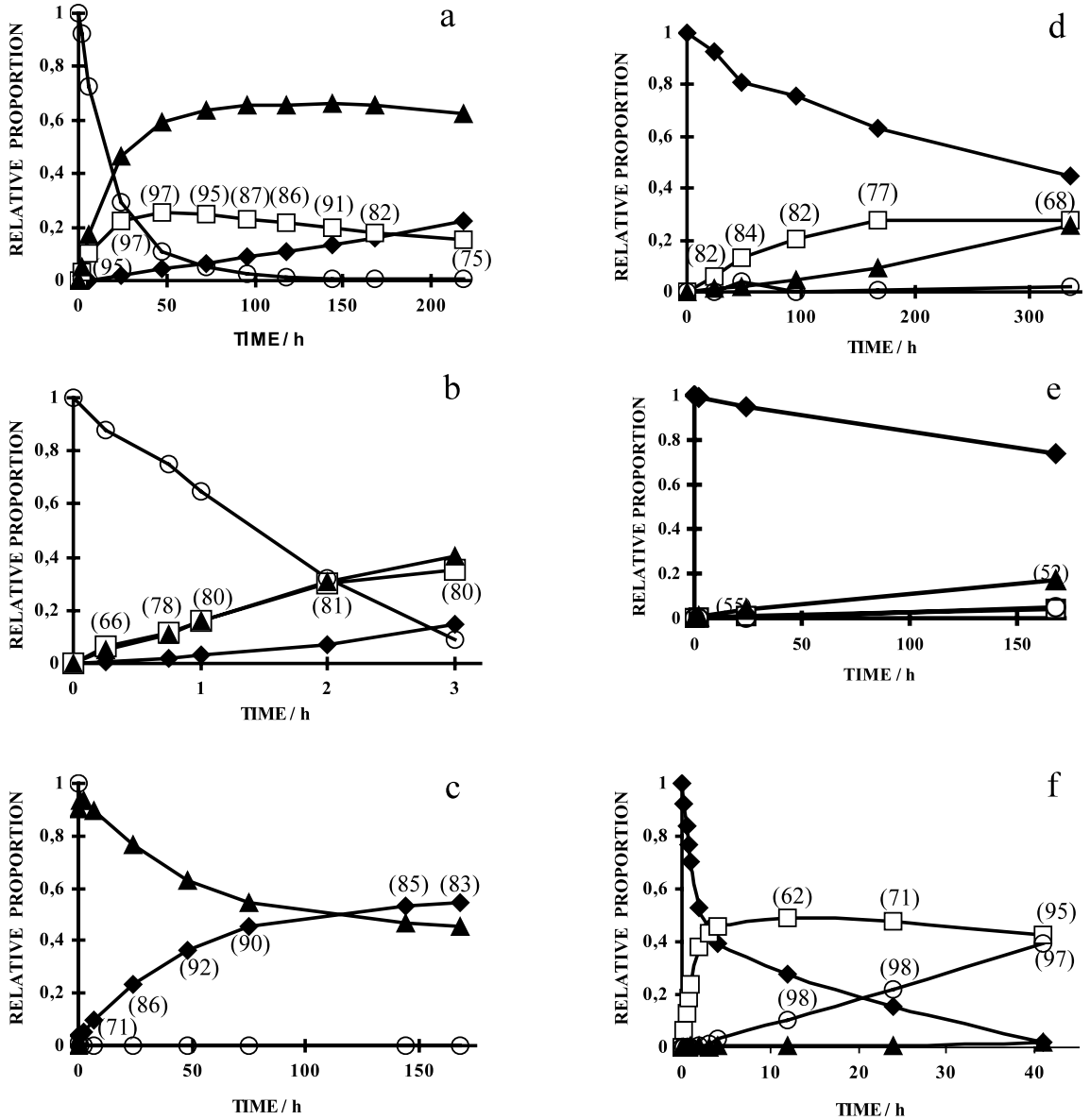


Figure 1. Progression curves with time for the acetylation of **1** in vinyl acetate in the presence of (a) acylase I (*A. melleus*), (b) CAL-A and (c) CAL-B and for the alcoholysis of **4** in butanol in the presence of (d) acylase I (*A. genus*) and with methanol (0.8 M) in acetonitrile (e) CAL-A and (f) CAL-B. (○) for **1**, (▲) for **2**, (□) for **3** and (◆) for **4**; enantiomeric excess (e.e./%) for highly enantioselective cases.

Table 1. Reactive enantiomer of steps A–D for the acetylation of **1** and for the alcoholysis of **4** by acylase I (*A. melleus* and *genus*), CAL-A and CAL-B

Step	Acetylation			Alcoholysis		
	Acylase I	CAL-A	CAL-B	Acylase I	CAL-A	CAL-B
A	*S*	*S*	*S*	*S* ($E=3$)[b]	*S* ($E=2$)[b]	*S* ($E\sim100$)[b]
B	*R* ($E=20$)[a]	*S* ($E=2$)[a]	*R* ($E=7$)[a]	*R*	*R*/*S*	*R*
C	*R*	*R*	*R*/*S*	*R*/*S* ($E=1$)[b]	*R* ($E=6$)[b]	*R* ($E=2$)
D	*R*/*S* ($E=1$)[a]	*R*/*S* ($E=1$)[a]	*S* ($E=35$)	*R*/*S*	*R*	(*R*/*S*)

[a] *E* for the enzymatic acetylation of chemically prepared **2** and **3** in vinyl acetate.
[b] *E* for the enzymatic alcoholysis of chemically prepared **2** in **3** in butanol or in acetonitrile containing methanol (0.8 M).

Table 2. Acylase I-catalyzed acetylation of 1-phenylethanol in vinyl acetate and butanolysis of 1-phenylethyl acetate/1-phenylethyl butanoate in butanol at room temperature (25°C)[a]

	Acetylation			Butanolysis		
	Time (h)	Conversion (%)	*E*	Time (h)	Conversion (%)	*E*
Aspergillus melleus	26	50	107	53/115	13/42	72/51
Aspergillus genus	26	51	61	53/115	27/52	146/129
Aspergillus on Eupergit C	26	5	3	20/115	0/18	–/15

[a] 75 mg/mL of the enzyme preparation.

The formation of the (*S*)-enantiomer is in accordance with the identical steric demands of (*S*)-**3** and (*R*)-1-phenylethanol. Step C is slightly (*R*)-enantioselective. The formation of (*S*)-**4** (◆) as the result of sequential reactions with e.e. between 20 and 50% (depending on the stage of the reaction) needs more careful consideration. Namely, the enzymatic acetylation of chemically prepared (±)-**2** proceeds slowly (10% conversion after 75 h) to (±)-**4**, while the acetylation of (±)-**3** (50% conversion at 24 h) leads to (*R*)-**4** with $E=20$ (Table 1). On this basis, the acetylation of **1** by acylase I is easily expected to lead to the formation of (*R*)-**4** rather than to the observed product with the (*S*) absolute configuration. As an explanation, the high concentration excess of (*S*)-**3** and the predominance of the sequential step B over D together result in the observed stereochemical outcome.

The acylase I (*A. genus*)-catalyzed sequential one-pot butanolysis of diacetate **4** to diol **1** in neat butanol proceeds too slowly to be of practical value (Fig. 1(d)). Regioselectivity is moderate, favoring the formation of **3** (□) through step B in an (*R*)-enantioselective reaction. The sequential step A was shown to be slightly (*S*) enantioselective (Table 1). Similar to the acetylation case above, the concentration effect of (*R*)-**3** evidently leads to the formation of slightly enantiomerically enriched (*R*)-**1** as the final product. For the butanolysis of **4**, the enzyme from *A. melleus* behaves in the same way, except that the reactions are even slower as expected according to the screening results in Table 2.

2.2. CAL-A catalysis

CAL-A is a calcium dependent, thermostable lipase that is reported to be highly active in a non-specific manner.[25,26] The enzyme is only rarely used in enantioselective reactions. In the present work it was studied because of its unique *sn*-2 preference towards triacylglycerols and of the highly enantioselective acylation of the amino groups in aliphatic β-substituted β-amino esters.[14,27]

The CAL-A-catalyzed acetylation of **1** in vinyl acetate proceeds smoothly, leading to the disappearance of the diol in 3–4 h (○, Fig. 1(b)). The enzyme is of low regioselectivity as product **2** (▲) is obtained in addition to the expected monoester **3** (□),[14] the progression curves being practically one on the other. Interestingly, the enzyme is highly (*S*) enantioselective for the reaction through step A (Table 1), enabling the formation of (*S*)-**3** with 80% e.e. in ca. 35% chemical yield. The formation of (*R*)-**2** in step C is less enantioselective, proceeding at an e.e. of around 50% at every stage of the reaction. In order to study the nature of the sequential steps B and D, chemically prepared monoesters **2** and **3** were separately subjected under the enzymatic reaction conditions, resulting in **4** with $E=1$ (77% conversion in 2 h) and $E=2$ (67% conversion in 2 hours), respectively. Thus, the formation of enantiomerically enriched diacetate (*S*)-**4** (◆) (e.e. 25% after 3 h reaction) is explained by the opposite enantiodiscriminations of steps A and C together with low enantioselectivities of the almost equally reactive sequential steps B and D, respectively.

The CAL-A-catalyzed sequential one-pot methanolysis of diacetate **4** to diol **1** in acetonitrile proceeds slowly and with enantioselectivities which are too low to be of practical value (Fig. 1(e)). It is interesting to note that the reaction at the stereocentre in the formation of monoester **2** (▲; step D) is favored over the formation of **3** (□; step B). The CAL-A-catalyzed methanolysis of chemically prepared monoesters **2** and **3** reveal that diol **1** is mainly obtained when product **2** reacts further through the slightly (*R*)-enantioselective step C (Table 1).

Noteworthy of the CAL-A-catalyzed reactions is that the same enantiodiscrimination is observed in the sequential steps A and B as well as C and D (or a product is racemic), although the regiodiscrimination steps A and C as well as B and D are of the opposite enantiodiscrimination (Table 1).

2.3. CAL-B catalysis

The properties and the wide application area of CAL-B as a regio- and enantioselective catalyst in organic synthesis are well described.[25,26,28,29] The enzyme is calcium independent and works without interfacial activation. It is also active in many hydrophilic solvents and tolerates high concentrations of various alkan-1-ols including methanol.[10]

The CAL-B-catalyzed acetylation of **1** in vinyl acetate leads to the rapid consumption of the diol (○, step C) and to the formation of practically racemic monoacetate 2 (▲) in a highly regioselective manner through step C (Fig. 1(c)). This reaction is followed by sequential acetylation through step D, allowing the formation of (*S*)-**4** (◆) at around 90% e.e. in over 40% yield. The

enantioselectivity of step D is in accordance with $E=35$, which was calculated for the CAL-B-catalyzed acetylation of chemically prepared **2** in vinyl acetate (Table 1). In spite of the high regioselectivity, traces [5% relative proportion for (*S*)-**3** (e.e. 40%) after 15 min] of monoacetate **3** were detected in the very beginning of the reaction before **1** was consumed in the fast step C. Evidently, the traces smoothly disappear through the sequential step B, explaining the initially moderate e.e. values (71 and 86%) for (*S*)-**4**. This conclusion is supported by the fact that chemically prepared monoester **3** was totally acetylated with vinyl acetate and CAL-B in an (*R*)-enantioselective reaction ($E=7$) in 1 day (Table 1).

The CAL-B-catalyzed methanolysis of **4** (◆) in acetonitrile first proceeds smoothly to ca. 60% conversion. The reaction slows down when the (*R*)-enantiomer has more or less reacted to monoacetate **3** (□) through step B (Fig. 1(f)). $E=15$ can be approximated for this reaction using the GLC data that are collected before the first signs of **1** appear. The highly (*S*)-enantioselective methanolysis of **3** in step A finally drives the sequential resolution to the end in the formation of (*S*)-**1** (○) at 97% e.e. and (*R*)-**3** (□) at 95% e.e. both close to 50% chemical yields (Table 3, row 1). The CAL-B-catalyzed methanolysis of chemically prepared monoesters **3** and **2** in acetonitrile led to (*S*)-**1** with $E\sim100$ (conversion 15% after 2 h) through step A and (*R*)-**1** with $E=$**2** (conversion 80% after 2 h) through step C (Table 1), respectively. These results further evidence the favor of highly regioselective formation of **3** over **2** in the methanolysis of **4**.

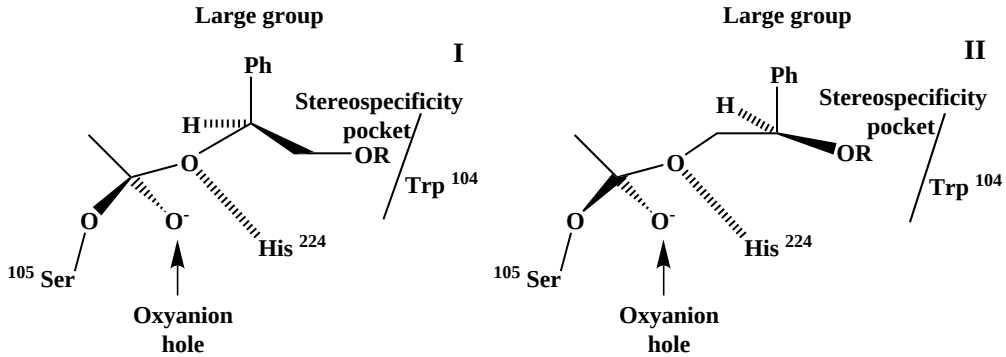
Lipase-catalyzed reactions proceed through an acyl-enzyme intermediate. In this intermediate, an ester bond covalently binds the serine residue of the catalytic triad to the carbonyl carbon of an acyl donor at the active site of the enzyme.[28] Typical of neutral and basic ester hydrolysis mechanisms, an anionic tetrahedral intermediate precedes the formation and follows the decomposition of the ester bond. Substrate/active site models for the anionic tetrahedral intermediates of the faster reacting enantiomers where the secondary (I) and primary (II) hydroxyl groups of the present substrates are bound to the carbonyl carbon during acetylation and methanolysis are given in Scheme 2 (R = H or Ac). The opposite enantiodiscriminations for the sequential steps A and B as well as for C and D can now be attributed to steric requirements for correct orientation of an alcohol (or an ester) in the three-dimensional space of the active site of CAL-B. Scheme 2 is a modification according to the previous presentation for 1-*O*-alkyl-2-alkanols in the active site cleft of CAL-B.[13]

As shown in Fig. 1(f) and Table 3, small amounts (ca. 2%) of **2** (▲) are detected throughout the course of the sequential methanolysis of **4** although the reaction is highly regioselective and as stated before the methanolysis of **2** produces diol **1** smoothly (80% conversion in 2 h). Acyl migration is proposed as an explanation. Intramolecular acyl migration in monoacylated products can take place while heating and in the presence of acids and bases, the transformation from **3** to **2** being energetically more favorable.[30,31] For GLC analysis, the derivatization of free hydroxyl groups in a sample with

Table 3. Effect of methanol concentration on the CAL-B-catalyzed methanolysis of **4** in acetonitrile at room temperature: relative proportion/e.e./% for compounds **1**–**4**

Row	MeOH (M)	Time (h)	**1**	**2**	**3**	**4**
1	0.8[a]	41	44/97	2/34	53/95	1/17
2	0.8[b]	89	44/96	2/26	53/94	1/8
3	0.8[c]	79	43/96	3/7	51/90	3/4
4	1.2[a]	79	46/96	2/24	51/96	1/10
5	1.2[b]	110	44/97	2/13	53/94	1/39

[a] Small-scale.
[b] Gram-scale.
[c] BuOH in the place of MeOH.



Scheme 2.

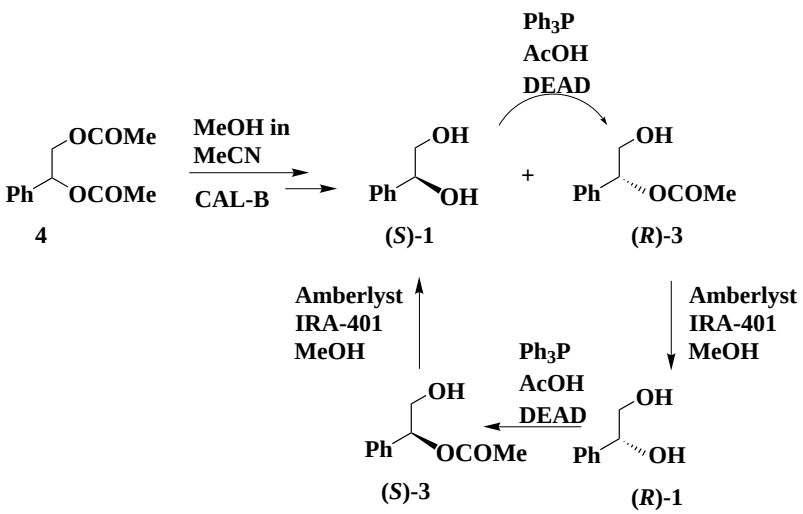
trifluoroacetic anhydride (liberates trifluoroacetic acid) can expose **3** to acyl migration. Accordingly, it was possible to show that the sample of (*R*)-**3** (0.1 M, 200 μL, e.e. 94%) was transformed to the 3/2 mixture of (*R*)-**3** (e.e. 45%)/(*R*)-**2** (e.e. 18%) when treated with trifluoroacetic acid (20 μL) for 5 h before the anhydride (50 μL) was added for derivatization. This result shows that considerable racemization is accompanied by acyl migration. When trifluoroacetic anhydride was added before the addition of trifluoroacetic acid, normal 2% of **2** was detected by GLC and the racemization of derivatized (*R*)-**3** was not observed after acid treatment overnight. Separation on silica may also expose **3** to slightly acidic conditions. Accordingly, only 2% of **2** in the resolution mixture (1.2 M MeOH; Table 3, row 5) increased to 5% after separation of the products by column chromatography (as described in Section 4.3).

2.3.1. Optimization for gram-scale resolution. The CAL-B-catalyzed methanolysis of **4** in acetonitrile allows the preparation of both enantiomers of **1** simultaneously through sequential resolution in one-pot (Schemes 1 and 3). In this reaction, the first step B is moderately (*R*)-enantioselective (approximated $E=15$) and thus the wrong enantiomer is initially in excess for the reaction through step A. Thus, the time needed for the transformation of **4** to the mixture of (*S*)-**1** and (*R*)-**3** would be shorter if step B were (*S*)-selective or non-enantioselective. Solvent effects were studied as a means of decreasing the reaction time. Tetrahydrofuran or toluene in the place of acetonitrile retarded the formation of diol (*S*)-**1** through step A (only 10% formed in 41 h) without affecting the reaction through step B. In diisopropyl ether, the reaction behaved almost identically to that in acetonitrile. The use of butanol in the place of methanol as a nucleophile in acetonitrile caused a drop in *E* from 15 to 10 for the reaction through step B without the desired effect on reaction time (Table 3, row 3). On this basis, methanolysis in acetonitrile was chosen as a method for the present work.

Ester alcoholysis in organic solvents most probably stops at an equilibrium conversion, leading to the gradual racemization of the less reactive enantiomer when the product enantiomer converts back to the starting material. The reaction conditions for the methanolysis of **4** were chosen according to the previous results, where 0.8 M methanol in acetonitrile was enough to prevent the effects of equilibria.[10] It seems that the reaction of (*S*)-**3** to (*S*)-**1** does not quite go to completion with 0.8 M alcohol solutions of the present work (Table 3, rows 1–3). Thus, the methanolysis of **4** was repeated using 1.2 M methanol in acetonitrile (rows 4 and 5). Although the reaction takes more time with increased methanol concentration and there is no clear difference in the results, the gram-scale resolution of **4** was performed using both methanol concentrations and the resolution in the presence of 1.2 M methanol is described in Section 4.3. For unknown reasons, more time was needed for the completion of the reaction when a small-scale reaction (rows 1 and 4) was changed to a gram-scale volume (rows 2 and 5).

2.4. Preparation of (*R*)-3 and (*S*)-3; inversion of configuration via Mitsunobu esterification

The disadvantage of conventional resolution is the 50% limit for the chemical yield of one enantiomer without special arrangements. Resolution followed by Mitsunobu esterification of a free hydroxyl group in one of the enantiomers was previously shown to lead to inversion at the stereocentre and accordingly to give yield enhancement for the other enantiomer.[15] Under Mitsunobu conditions, 1,2-diols undergo predominant ester formation at the secondary centre due to the formation of a phosphorane intermediate, which opens via protonation of the least hindered oxygen.[32] When commercial (*R*)-**1** (e.e. 98%) was acetylated under Mitsunobu conditions the product was the corresponding monoester (*S*)-**3** with 98% e.e., indicating that the resolution–inversion method is excellent for the transformation of (*S*)-**1** to (*R*)-**3**. To this end, (*S*)-**1** with 97% e.e. was subjected to Mitsunobu conditions resulting in (*R*)-**3** with 95% e.e. as was expected.



Scheme 3.

In order to exploit the Mitsunobu reaction to give the (*R*)-enantiomer, (*R*)-**3** must be first transformed to the diol (*R*)-**1** (Scheme 3). The base-catalyzed deacylation of 1,2-diol esters has proved its applicability in various connections.[6–9] As described in Section 4.4, the method was also successfully used in the present work.

3. Conclusions

The regio- and enantioselectivities of acylase I, CAL-A and CAL-B were evaluated in order to study their applicability to the preparation of the enantiomers of 1-phenylethan-1,2-diol **1**. To this end, **1** was subjected to acetylation in vinyl acetate and the corresponding diacetate **4** for alcoholysis in butanol or in acetonitrile containing methanol (Scheme 1). For the present enzymes, the enantiomer that reacts faster was shown to depend on enzymatic regioselectivity, i.e. the formation of monoester **2** was shown to proceed with opposite enantiodiscrimination to the formation of monoester **3** (Table 1). In the case of CAL-B catalysis, the substrate/active site models for the anionic tetrahedral intermediates that precede the formation and follow the decomposition of an acyl-enzyme intermediate were used to explain the opposite enantiodiscriminations (if not one of the steps is racemic) for the sequential steps.

Excellent regioselectivity towards the reaction at the primary alcohol (acetylation, step C) or at its ester (methanolysis, step B) functions is obvious for CAL-B catalysis, the respective sequential steps D and A being highly enantioselective (Scheme 1, Fig. 1(c and f)). Accordingly, the sequential one-pot resolution of **4** with methanol in acetonitrile allowed the preparation of (*S*)-**1** (e.e. 97%) and (*R*)-**3** (e.e. 94%) on gram-scale (Scheme 3, Table 3). (*R*)-**3** was further transformed to (*R*)-**1** via base-catalyzed methanolysis. When only one of the enantiomers of **1** is needed, the Mitsunobu acetylation at the secondary hydroxyl group of (*R*)-**1** or (*S*)-**1** was shown to allow the transformation of (±)-**1** to one enantiomer.

Due to low regioselectivities, CAL-A and acylase I catalyze the formation of both monoesters **2** and **3**, the formation of **3** proceeding in a highly enantioselective manner (Fig. 1(a, b, d and e)). Thus, for the acetylation of **1**, the preparation of (*S*)-**3** with 97% e.e. (25% theoretical yield) in the case of acylase I and with 80% e.e. (35% theoretical yield) in the case of CAL-A are possible (Fig. 1(a and b)). The reactions are unique in that the secondary alcohol function is enantioselectively acetylated in the presence of the primary one. More work is needed in order to increase the amounts of the fraction of (*S*)-**3** over that of **2**.

4. Experimental

4.1. Materials

Acylase I from *A. melleus* (0.49 U/mg) and *genus* (35 U/mg) were obtained from Sigma (Deisenhofen, Germany) and Tokyo Kasei (Tokyo, Japan), respectively. Acylase I from *Aspergillus* on Eupergit C (388.4 U/g) was a product from Fluka (Buchs, Switzerland). The origin of the immobilized enzyme is not reported more accurately by the producer. Immobilized *C. antarctica* lipase B (CAL-B, Chirazyme L5) and *C. antarctica* lipase A (CAL-A, Chirazyme L5) were the products of Boehringer Mannheim. Before use, CAL-A was immobilized on Celite in the presence of sucrose (20% lipase on Celite).[33] 1-Phenylethanol, 1-phenylethan-1,2-diol 1 (racemic and the pure (*R*)-enantiomer), vinyl acetate and trifluoroacetic anhydride (used as derivatization reagent) were products of Aldrich. 1-Phenylethyl acetate and butanoate were from the previous work.[15] The solvents were of the highest analytical grade and obtained from Lab Scan Ltd., Riedel-de Haën and J.T. Baker.

4.1.1. Preparation of (±)-1,2-diacetoxy-1-phenylethane 4. 1-Phenylethan-1,2-diol **1** (1.43 g, 10.3 mmol) was dissolved in dichloromethane (25 mL) with triethylamine (4.30 mL, 31.0 mmol), DMAP (4-*N*,*N*-dimethylaminopyridine; 63.2 mg, 0.52 mmol) and acetic anhydride (2.94 mL, 31.0 mmol). The mixture was stirred at room temperature (25°C) for 24 h. The addition of methanol (10 mL) stopped the reaction. After evaporation in vacuo the product was purified by column chromatography on silica using acetone:petroleum ether (3:7) as an eluent, yielding **4** (2.21 g, 9.9 mmol). ^{1}H NMR: δ (ppm) 2.03 (s, 3H, CH_3CO$_2$CH$_2$), 2.09 (s, 3H, CH_3CO$_2$CH), 4.29 (m, 2H, CH_2), 5.99 (dd, 1H, CH), 7.27–7.38 (m, 5H, Ar-H). ^{13}C NMR δ (ppm) 20.7 (*C*H$_3$CO$_2$CH$_2$), 21.0 (*C*H$_3$CO$_2$CH), 66.0 (*C*H$_2$), 73.2 (*C*H), 126.6, 128.6 and 136.4 (6C, Ar-*C*), 170.0 (*C*O$_2$CH$_2$), 170.6 (*C*O$_2$CH). Mass spectrum: M$^+$ = 222.

4.1.2. Preparation of (±)-1-acetoxy-1-phenylethanol 3. Prepared by Mitsunobu reaction:[15] diol **1** (2.16 g, 15.6 mmol) was dissolved in tetrahydrofuran (30 mL) and triphenylphosphine (4.09 g, 15.6 mmol) and acetic acid (0.89 mL, 15.6 mmol) were added followed by the dropwise addition of DEAD (diethyl azodicarboxylate; 2.43 mL, 15.6 mmol). The mixture was stirred at room temperature (25°C) for 21 h before the work-up as above, yielding **3** (2.08 g, 11.5 mmol) containing 10% of (±)-**2** according to the ^{1}H NMR spectra. A small amount of diacetate **4** was also produced in the reaction mixture in accordance with the previous Mitsunobu acylation of **1** but it was easily separated during the workup.[32] ^{1}H NMR: δ (ppm) 2.04 (s, 3H, CH_3CO$_2$), 3.75 (qd, 2H, CH_2), 5.75 (dd, 1H, CH), 7.14–7.31 (m, 5H, Ar-H). ^{13}C NMR δ (ppm) 21.1 (*C*H$_3$CO$_2$), 65.8 (*C*H$_2$OH), 76.8 (*C*H), 126.6, 128.6 and 137.0 (6C, Ar-*C*), 170.7 (*C*O$_2$). Mass spectrum: M$^+$ = 180.

4.1.3. Preparation of (±)-2-acetoxy-1-phenylethanol 2. Diol **1** (2.30 g, 16.6 mmol) was dissolved in dichloromethane (20 mL) and triethylamine (3.46 mL, 25.0 mmol) and DMAP (0.10 g, 0.8 mmol) were added in an ice/ethanol bath. Acetic anhydride (1.57 mL 16.6 mmol) was added dropwise to the reaction mixture. The solution was stirred for 1.5 h. The addition of methanol (15 mL) stopped the reaction, work-up yielding **2** (0.85 g, 4.7 mmol) containing 7% of (±)-**3** according to GLC

analysis. ^{1}H NMR: δ (ppm) 2.09 (s, 3H, CH_3CO$_2$), 2.80 (s, 1H, CHOH), 4.19 (qd, 2H, CH_2), 4.93 (dd, 1H, CH), 7.26–7.40 (m, 5H, Ar-H). ^{13}C NMR δ (ppm) 20.8 (CH$_3$CO$_2$), 69.2 (CHOH), 72.3 (CH$_2$), 126.1, 128.5 and 139.8 (6C, Ar-C) 171.1 (CO$_2$). Mass spectrum: M$^+$= 180.

4.2. Methods

The progress of the reactions was followed by taking samples (200 μL for acetylation and 500 μL for butanolysis) at intervals, filtering off the enzyme and analyzing the sample by GLC on Astec Chiraldex G-TA column. Free alcohol groups in the samples were derivatized with 50 μL of trifluoroacetic anhydride before the GLC analysis. For alcoholysis reactions, the unreacted alcohol was evaporated and the residue dissolved in 200 μL of dichloromethane before the derivatization. Enantiomeric excess values (e.e.) and relative proportions of the components in the reaction mixture at a given time were determined according to the peak areas in the chromatograms and to the calibration mixture of **1**–**4** being 0.1 M for each.

^{1}H and ^{13}C NMR spectra were measured in CDCl$_3$ on a Jeol Lambda 400 or Bruker 200 Spectrometer with tetramethylsilane as an internal standard. MS spectra were recorded on a VG Analytical 7070E instrument equipped with a ZAXstation 3100 M76 computer. Optical rotations were measured using a Jasco DIP-360 polarimeter. Elemental analyses were performed using a Perkin–Elmer CHNS-240 Ser II Elemental Analyzer. The melting point of compound **1** was measured using BÜCHI 510 Melting Point instrument.

4.3. Enzymatic resolution

The reactions were typically performed as small scale experiments where one of the substrates **1**–**4**, 1-phenylethanol and 1-phenylethyl acetate or butanoate (0.1 M) was dissolved in vinyl acetate, 1-butanol or acetonitrile containing methanol or butanol (0.8 or 1.2 M). Diisopropyl ether, toluene and tetrahydrofuran were also used as solvents for the CAL-B-catalyzed methanolysis of **4**. The enzyme preparation (75 mg/mL) was added in order to start the reaction. The reaction mixture was shaken at room temperature (25°C).

4.3.1. Gram-scale resolution of 1,2-diacetoxy-1-phenylethane 4. 1,2-Diacetoxy-1-phenylethane **4** (2.00 g, 9.0 mmol) was dissolved in acetonitrile (90.0 mL). Methanol (4.38 mL, 108 mmol) and CAL-B (6.7 g) were added. After 110 h the enzyme was removed by filtration and washed with acetonitrile. Purification by column chromatography (acetone:petroleum ether, 3:7) yielded (*S*)-**1** [0.58 g, 4.2 mmol, e.e. 97%, $[\alpha]_D^{20}$ +63 (*c* 1, CHCl$_3$); $[\alpha]_D^{20}$ (lit.)[34] +66 (*c* 1, CHCl$_3$)] and (*R*)-**3** [0.51 g, 2.8 mmol, e.e. 94%, $[\alpha]_D^{20}$ −80 (*c* 1, CHCl$_3$)] containing 5% of **2** according to GLC analysis. It is possible that the real amount of **2** is less than 5% in the separated (*R*)-**3** (see acyl migration during derivatization as described in Section 2.3). (*S*)-**1**: ^{1}H NMR: δ (ppm) 2.43, (s, 1H, CH$_2$OH), 2.86 (s, 1H, CHOH), 3.70 (m, 2H, CH_2), 4.79, (m, 1H, CH), 7.21–7.37 (m, 5H, Ar-H). ^{13}C NMR δ (ppm) 68.0 (CH$_2$), 74.7 (CH), 126.0, 128.0, 128.5 and 140.4 (6C, Ar-C). Mass spectrum: M$^+$=138. Melting point: 64°C; (lit.)[34] 67–69°C. Elemental analysis: obs. C, 69.36, H; 7.51%. Calcd C, 69.54; H, 7.30% for the C$_8$H$_{10}$O$_2$. The spectroscopic data for (*R*)-**3** are in accordance with those for (±)-**3**.

4.4. Inversion of configuration; transformation to the diol

A solution of (*S*)-**1** (0.150 g, 1.1 mmol) in tetrahydrofuran (5 mL) was treated with triphenylphosphine (0.285 g, 1.1 mmol) and acetic acid (62 μL, 1.1 mmol), followed by the addition of DEAD (169 μL, 1.1 mmol). The work-up was as above. The product was isolated yielding (*R*)-**3** [82 mg, 0.46 mmol, e.e. 95%, $[\alpha]_D^{20}$ −80 (*c* 1, CHCl$_3$)] and containing 6% of (±)-**2** according to the GLC method.

(*R*)-**3** (0.266 g, 1.5 mmol, e.e. 94%) was dissolved in methanol (10 mL) and basic ion-exchange resin Amberlite IRA-401 (ca 1 g) was added. Deacetylation was complete in 1 h, as detected by TLC. The ion-exchange resin was removed by filtration and the filtrate was dried with Na$_2$SO$_4$. Evaporation of the solvent yielded (*R*)-**1** (0.184 g, 1.3 mmol, e.e. 94%).

4.5. Determination of absolute configurations

The absolute configurations for the enantiomers of **1** were determined by using commercial (*R*)-**1**. For that purpose (*R*)-**1** in CH$_2$Cl$_2$ (0.1 M, 100 μL) was added into the solution of (±)-**1** (0.1 M, 100 μL). The mixture was derivatized with trifluoroacetic anhydride and analyzed by the GLC method. The absolute configurations for the enantiomers of **4** were determined in the same way by acetylating the mixture of commercial (*R*)-**1** (0.1 M, 100 μL) and (±)-**1** (0.1 M, 100 μL) with acetic anhydride (10 μL) in the presence of DMAP (10 μL, 5% solution in pyridine). In the case of **2**, the absolute configurations were obtained by preparing (*R*)-**2** from commercial (*R*)-**1**, as described above. In the case of **3**, absolute configurations were decided from the results for the CAL-B-catalyzed alcoholysis of **4** leading to the formation of (*S*)-**1** and accordingly leaving (*R*)-**3** unreacted. The sample was analyzed by GLC. Absolute configurations were defined according to the enantiomeric peak areas in the chromatogram.

References

1. Brown, J. M.; Murrer, B. A. *J. Chem. Soc., Perkin Trans. 2* **1982**, 489–497.
2. King, R. B.; Bakos, J.; Hoff, C. D.; Markó, L. *J. Org. Chem.* **1979**, *44*, 1729–1931.
3. Bradshaw, J. S.; Jolley, S. T.; Izatt, R. M. *J. Org. Chem.* **1982**, *47*, 1229–1232.
4. Märki, H. P.; Crameri, Y.; Eigenmann, R.; Krasso, A.; Ramuz, H.; Bernauer, K.; Goodman, M.; Melmon, K. L. *Helv. Chim. Acta* **1988**, *71*, 320–336.

5. Pauluth, D.; Wächtler, E. F. In *Chirality in Industry II*; Collins, A. N.; Sheldrake, G. N.; Crosby, J., Eds.; John Wiley & Sons: Chichester, 1997; pp. 280–281.
6. Janssen, A. J. M.; Klunder, A. J. H.; Zwanenburg, B. *Tetrahedron* **1991**, *47*, 7409–7416.
7. Bosetti, A.; Bianchi, D.; Cesti, P.; Golini, P.; Spezia, S. *J. Chem. Soc., Perkin Trans. 1* **1992**, 239–242.
8. Lemke, K.; Theil, F.; Kunath, A.; Schick, H. *Tetrahedron: Asymmetry* **1996**, *7*, 971–974.
9. Theil, F.; Weidner, J.; Ballschuh, S.; Kunath, A.; Schick, H. *J. Org. Chem.* **1994**, *59*, 388–393.
10. Liljeblad, A.; Vänttinen, E.; Kanerva, L. T. *Chirality* **1999**, *11*, 432–439.
11. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7298.
12. Lundell, K.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1995**, *6*, 2281–2286.
13. Engholm Jacobsen, E.; Hoff, B. H.; Anthonsen, T. *Chirality* **2000**, *12*, 654–659.
14. Rogalska, E.; Cudrey, C.; Ferrato, F.; Verger, R. *Chirality* **1993**, *5*, 24–30.
15. Vänttinen, E.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1995**, *6*, 1779–1786.
16. Herradón, B.; Valverde, S. *Synlett* **1995**, 599–602.
17. Ors, M.; Morcuende, A.; Jiménez-Vacas, M. I.; Valverde, S.; Herradón, B. *Synlett* **1996**, 449–451.
18. Faraldos, J.; Arroyo, E.; Herradón, B. *Synlett* **1997**, 367–370.
19. Bakker, M.; Spruijt, A. S.; van Rantwijk, F.; Sheldon, R. A. *Tetrahedron: Asymmetry* **2000**, *11*, 1801–1808.
20. Liljeblad, A.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1999**, *10*, 4405–4415.
21. Liljeblad, A.; Lindborg, J.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2000**, *11*, 3957–3966.
22. Chenault, H. K.; Dahmer, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 6354–6364.
23. Anders, M. W.; Dekant, W. *Adv. Pharm.* **1994**, *59*, 431–448.
24. Silverman, R. B. In *The Organic Chemistry of Enzyme-Catalyzed Reactions*; Academic Press: San Diego, 2000; pp. 55–60.
25. Nielsen, T. B.; Ishii, M.; Kirk, O. *Biotechnol. Appl. Cold-Adapted Org.* **1999**, 49–61.
26. Anderson, E. M.; Larsson, K. M.; Kirk, O. *Biocat. Biotrans.* **1998**, *16*, 181–204.
27. Gedey, S.; Liljeblad, A.; Lázár, L.; Fülöp, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2001**, *12*, 105–110.
28. Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis. Regio- and Stereoselective Biotransformations*; Wiley: Weinheim, 1999.
29. Orrenius, C.; Hæffner, F.; Rotticci, D.; Öhrner, N.; Norin, T.; Hult, K. *Biocat. Biotrans.* **1998**, *16*, 1–15.
30. van Lohuizen, O. E.; Verkade, P. E. *Recl. Trav. Chim. Pays-Bas* **1960**, *79*, 133–159.
31. Lok, C. M.; Ward, J. P. *Chem. Phys. Lipids* **1986**, *39*, 19–29.
32. Pautard, A. M.; Evans, S. A. *J. Org. Chem.* **1988**, *53*, 2300–2303.
33. Kanerva, L. T.; Sundholm, O. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2407–2410.
34. Handbook of Fine Chemicals and Laboratory Equipment, Aldrich, 2000–2001, p. 1346.