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SHORT COMMUNICATION

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Enzymatic Kinetic Resolution and Racemization of 2-(Tetramethylpiperidine-1-oxyl)ethanols

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The *Candida antarctica* lipase B catalyzed transesterification of 2-(tetramethylpiperidine-1-oxyl)ethanols provides acetates in useful yields with good to high enantiomeric excess values. By taking advantage of the persistent radical effect, the remaining alcohols can be cleanly racemized under mild conditions in the presence of TEMPO [2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl].

Introduction

In recent years, enzymatic kinetic resolutions have gained increasing importance as a versatile way to access enantiomerically enriched and pure compounds.[1,2] A large variety of enzymes is nowadays known to convert a multitude of functional groups enantioselectively, among which alcohols, esters, carboxylic acids, amines, and amino acids are common targets in the racemic substrates.[1] One principal drawback of such enzymatic kinetic processes, however, is that only a maximum product yield of 50% can be reached unless the substrate simultaneously racemizes under the chosen reaction conditions.[2] Although not as elegantly as through dynamic kinetic resolution,[3] improved efficacy of the process can alternatively be reached if the remaining substrate (with low enantiomeric excess) can be racemized in a simple way.[2,4] Regarding the commonly used strategies to achieve racemization, which include thermal racemization, base- or acid-catalyzed racemization, enzyme-catalyzed racemization, racemization through redox and radical reactions, as well as racemization by Schiff bases,[4] reactions proceeding through homolytic bond cleavage and recombination have so far only played a minor role. A beautiful example of dynamic kinetic resolution featuring the racemization of amines through reversible hydrogen-atom transfer to thiyl radicals was recently reported by Gastaldi, Gil, and Bertrand.[5,6] In this communication, we introduce 2-(tetramethylpiperidine-1-oxyl)ethanols as new substrates for kinetic enzymatic resolutions and their racemization under simple and mild conditions.

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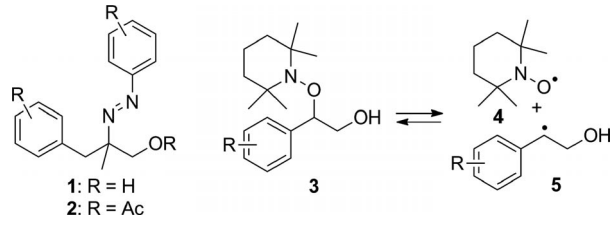
Results and Discussion

The interest in 2-(tetramethylpiperidine-1-oxyl)ethanols as potential substrates for enzymatic reactions arose from our recent observation that azo alcohols **1** and their corresponding acetates **2** are well accepted as substrates by enzymes such as *Candida antarctica* lipase B (CAL-B) but are difficult to racemize in a reasonable number of synthetic steps (Scheme 1).[7,8]

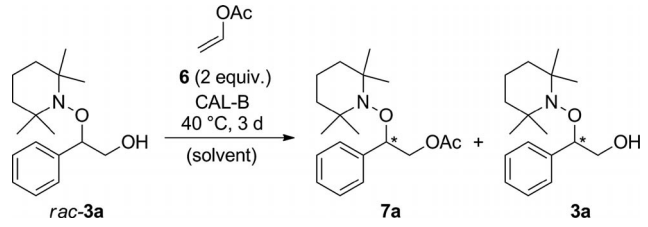


Scheme 1. Racemization of alcohols **3** through homolytic bond cleavage and recombination.

In contrast, alcohols **3** should undergo racemization at slightly elevated temperatures through homolytic cleavage of the carbon–oxygen bond and subsequent recombination of TEMPO [2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl, **4**][9] and stabilized benzylic radical **5**. The thermal generation of carbon-centered radicals from TEMPO adducts and their use for olefin functionalization and polymerization has intensively been studied by Studer.[10,11] Beneficially with regard to our studies, alcohol **3a** and ring-substituted derivatives can be accessed in a one-step synthesis from styrene, TEMPO (**4**), and hydrogen peroxide.[12,13] Initial screening with a variety of enzymes revealed that the transesterification of **3a** with vinyl acetate can be achieved under significantly milder conditions than the hydrolysis or aminolysis of the corresponding acetate. For the desired transesterification, CAL-B[14] again turned out to be the most suitable enzyme (see the Supporting Information). The results from

a screening of different organic solvents are summarized in Table 1.

Table 1. CAL-B catalyzed transesterification of alcohol **3a** with vinyl acetate (**6**) in various solvents.[a]



Entry	Solvent	Conversion [%][b,c]	% ee_p (**7a**)[d]	% ee_s (**3a**)[d]	*E* value[e]
1	acetone	<1	n.d.[f]	n.d.[f]	n.d.[f]
2	CH_3CN	8	n.d.[f]	n.d.[f]	n.d.[f]
3	toluene	4	21	n.d.[f]	n.d.[f]
4	THF	<1	n.d.[f]	n.d.[f]	n.d.[f]
5	[TMBA][NTf_2]	19 (12)[g]	91	12	24
6	*n*-hexane	20 (18)	90	20	23
7	MTBE	34 (31)	91	40	31

[a] Reaction conditions: *rac*-**3a** (0.05 mmol), organic solvent (2 mL), vinyl acetate (**6**, 0.10 mmol), CAL-B (20 mg), 40 °C, 3 d. [b] Conversion determined by ^{1}H NMR spectroscopy. [c] The value in parentheses is the conversion as determined by the following equation: conv. = (ee_s)/(ee_s + ee_p). [d] Enantiomeric excess of **3a** and **7a** determined by chiral HPLC. [e] *E* value calculated from the *ee* data of alcohol **3a** and acetate **7a**. [f] n.d.: not determined. [g] This reaction was performed with 1 mL of [TMBA][NTf_2] as solvent.

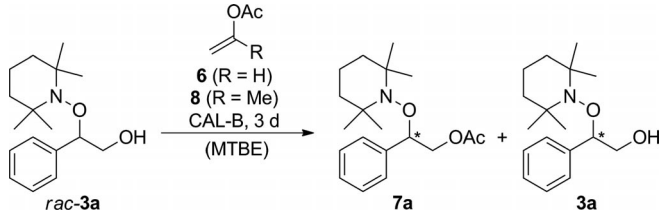
Although the use of acetone, acetonitrile, toluene, and tetrahydrofuran led to very low conversions after 3 d (Table 1, entries 1–4), *n*-hexane, methyl *tert*-butyl ether (MTBE), and the ionic liquid *N*-trimethyl-*N*-butylammonium–bis(trifluoromethylsulfonyl)imide ([TMBA][NTf_2])[15] gave acetate **7a** in reasonable yields and enantiomeric excess (Table 1, entries 5–7). Investigation of the reaction mixtures by ^{1}H NMR spectroscopy, which was used to determine the conversion of each experiment, did not indicate the formation of any other product than desired acetate **7a**. The enzymatic transesterification thus proceeds very cleanly under the chosen conditions.

Owing to the superior *E* value[16,17] of the related transformation (Table 1, entry 7), MTBE was selected as the solvent for further optimization experiments (Table 2). Variation of the amount of immobilized CAL-B from 400 mg mmol^{-1} to 200 and 800 mg mmol^{-1} led to a decreased *E* value (Table 2, entry 1) and no remarkable gain (Table 2, entry 3), respectively.

Given that it has been reported that increasing concentrations of the acyl transfer reagent can have a positive or negative influence on the *E* value of the transformation,[18] we then turned to investigating this parameter. Interestingly, a significant improvement was achieved by increasing the amount of vinyl acetate to 4 equiv. (Table 2, entry 4), but not further to 6 equiv. (Table 2, entry 5). A slightly higher reaction temperature (Table 2, entry 6) as well as an exchange of the acetyl source from **6** to isopropenyl acetate (**8**; Table 2, entry 7) did not give better results. With regard to the amount of enzyme that was necessary, we were pleased to find that the enzyme could be reused for further transformations, which showed comparable *E* values (see the Supporting Information).

With the optimized conditions in hand, we turned to the evaluation of the substrate scope (Table 3). Transformations with synthetically useful *E* values were observed for most substitution patterns on the aromatic core (Table 3, entries 1–6, 8) with the exception of 2-chloro- and 4-cyano-substituted derivatives **3g** and **3i** (Table 3, entries 7 and 9). For alcohol **3i**, we currently assign this to unfavorable interaction of the 4-cyano group with the catalytic site of the enzyme, as **3i** was not found to be less stable towards isomerization than the other alcohols. Again, and was evidenced by ^{1}H NMR spectroscopy, all enzymatic reactions proceeded cleanly without the formation of products other than desired acetates **7**. The unsuccessful reaction of **3j** (Table 3, entry 10), however, indicates that alcohols bearing

Table 2. Optimization experiments.[a]

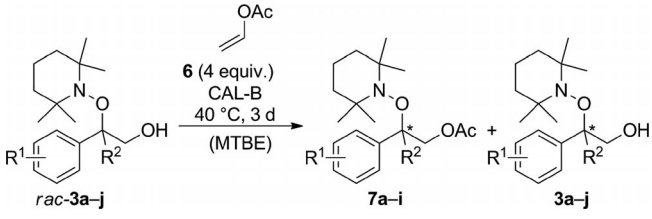


Entry	CAL-B [mg mmol^{-1}]	**6**/**8** (equiv.)	*T* [°C]	Conversion [%][b,c]	% ee_p (**7a**)[d]	% ee_s (**3a**)[d]	E[e]
1	200	**6** (2)	40	19 (15)	83	15	13
2	400	**6** (2)	40	34 (31)	91	40	31
3	800	**6** (2)	40	52 (46)	87	74	32
4	400	**6** (4)	40	39 (40)	93	62	52
5	400	**6** (6)	40	35 (33)	91	45	33
6	400	**6** (4)	50	50 (48)	85	80	30
7	400	**8** (4)	40	25 (23)	92	28	31

[a] Reaction conditions: *rac*-**3a** (0.05 mmol), MTBE (2 mL), CAL-B (20 mg), 3 d. [b] Conversion determined by ^{1}H NMR spectroscopy. [c] The value in parentheses is the conversion as determined by the following equation: conv. = (ee_s)/(ee_s + ee_p). [d] Enantiomeric excess of **3a** and **7a** determined by chiral HPLC. [e] *E* value calculated from the *ee* data of alcohol **3a** and acetate **7a**.

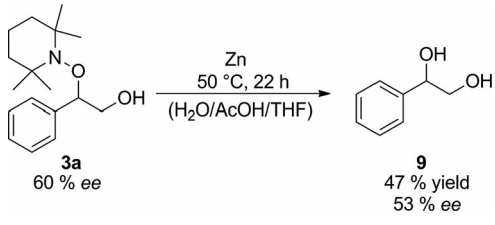
quaternary centers are most probably not tolerated. The absolute stereochemistry of acetates **7** was determined by reducing the low-enantioenriched, nonconverted alcohol **3a** (Table 3, entry 1) into corresponding diol **9** (Scheme 2).[19,20]

Table 3. Substrate scope.[a]



Entry	Alcohol (R^1, R^2)	Conv. [%][b,c]	% ee_p (**7a–i**)[d]	% ee_s (**3a–j**)[d]	E[e]
1	**3a** (H, H)	40 (39)	92	60	44
2	**3b** (4-OMe, H)	42 (40)	92	62	45
3	**3c** (4-Me, H)	31 (27)	>97[f]	36[f]	93
4	**3d** (4-F, H)	42 (39)	84[g]	54	20
5	**3e** (4-Cl, H)	47 (46)	90	77	44
6	**3f** (4-Br, H)	25 (28)	91	35	30
7	**3g** (2-Cl, H)	13 (13)	82[f]	12[f]	11
8	**3h** (3-Cl, H)	49 (48)	80[g]	75	20
9	**3i** (4-CN, H)	42 (42)	77[g]	56	13
10	**3j** (H, Me)	<1	n.d.[h]	n.d.[h]	n.d.[h]

[a] Reaction conditions: *rac*-**3a**–**j** (0.20 mmol), MTBE (8 mL), vinyl acetate (**6**, 0.80 mmol), CAL-B (80 mg), 40 °C, 3 d. [b] Conversion determined by ^{1}H NMR spectroscopy. [c] The value in parentheses is the conversion as determined by the following equation: conv. = $(ee_s)/(ee_s + ee_p)$. [d] Unless otherwise stated, the enantiomeric excess values of alcohols **3** and acetates **7** were determined by chiral HPLC. [e] E value calculated from the *ee* data of alcohols **3** and acetates **7**. See also ref.[23] [f] Enantiomeric excess determined after esterification with methyl 4-chloro-4-oxobutanoate. [g] Enantiomeric excess determined after conversion to the corresponding alcohol. [h] n.d.: not determined.

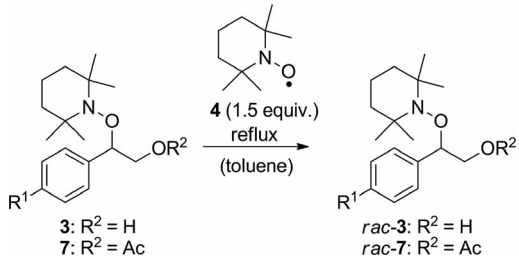


Scheme 2. Reduction of alcohol **3a** to diol **9**.

A comparison of the experimental optical rotation values with values reported in the literature[21] revealed that the remaining alcohols are of the (*S*) configuration and that the transesterification is therefore (*R*) selective. Moreover, this reaction shows that the N–O bond can be cleaved under mild conditions to access the corresponding diols without a major loss of enantiomeric excess.[22]

The racemization of the nonconverted alcohols possessing low enantiomeric excess values was studied by increasing the temperature of their solutions in toluene (Table 4). Under reflux, alcohols **3a**, **3b**, **3e**, and **3i** showed complete and clean racemization after 6–8 h, so that the starting materials could be recovered in racemic form and in quantitative yield after the given reaction times. The racemization of the acetates, as exemplified by **7f** and **7i**, was achieved under similar conditions. These reactions (Table 4, entries 5 and 6) did, however, lead to small amounts of byproducts.

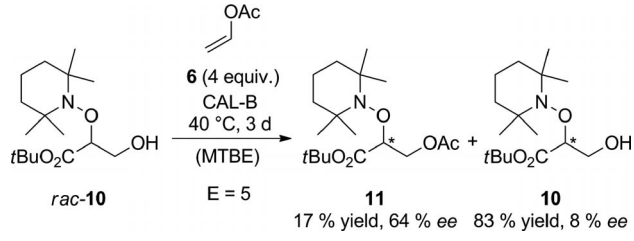
Table 4. Racemization of alcohols **3a**, **3b**, **3e**, and **3i** and acetates **7f** and **7i**.[a]



Entry	Alcohol/acetate (R^1)	Time [h]	% ee_{init}[b]	% ee_{final}[d]
1	**3a** (H)	7.5	60	0[b]
2	**3b** (OMe)	5.5	62	0[b]
3	**3e** (Cl)	6.0	77	0[b]
4	**3i** (CN)	6.0	56	0[b]
5	**7f** (Br)	10.0	91[c]	0[c]
6	**7i** (CN)	10.0	77[c]	0[c]

[a] Reaction conditions: **3** (30.0 μmol), TEMPO (**4**, 1.5–2.5 equiv.), toluene (1 mL), argon atmosphere, reflux. [b] Enantiomeric excess was determined by chiral HPLC. [c] Racemization monitored by optical rotation. [d] No byproducts were detected by ^{1}H NMR spectroscopy for experiments in entries 1–4. Small amounts of byproducts (5–15%) were found in the experiments with acetates **7f** and **7i**.

The addition of TEMPO (**4**) to the alcohols or acetates was necessary to avoid the formation of decomposition products. In that way, benzylic radicals **5** (Scheme 1) were reliably trapped by **4** at the beginning of the racemization process. The reaction thus takes advantage of the persistent radical effect (PRE) without having to initially build up an excess amount of free **4** through partial decomposition of the alcohols or acetates.[24] Attempts to characterize the products arising from the thermal decomposition were not successful owing to the multitude of compounds and the at same time the small quantities in which they were formed. To explore the potential of expanding the methodology to aliphatic substrates, we finally investigated the kinetic resolution of alcohol **10**, which was prepared in one step from *tert*-butyl acrylate under conditions similar to those used for the synthesis of alcohols **3** from styrenes (Scheme 3).[12] Although only a comparably low E value of 5 was determined for this initial experiment under unoptimized condi-



Scheme 3. Kinetic enzymatic resolution of aliphatic TEMPO alcohol **10** to acetate **11**.

tions, the reaction nevertheless shows that aliphatic alcohols are also tolerated by the enzyme. Comparable to styrene-derived alcohols **3** reported in Table 4, remaining low-enantioenriched aliphatic alcohol **10** underwent clean racemization in boiling toluene.

Conclusions

In summary, we have shown that 2-(tetramethylpiperidine-1-oxyl)ethanols are well-suited substrates for CAL-B-catalyzed transesterification with vinyl acetate. Beneficially, the remaining alcohols can be cleanly racemized under mild conditions in the presence of TEMPO by exploiting the persistent radical effect (PRE). An initial experiment with an ester functionality at the place of the radical-stabilizing phenyl group showed that expansion of the scope towards aliphatic substrates is possible, although further optimization is required for this type of substrate. As a result of the importance of water activity in many enzymatic reactions, our current studies are also directed towards evaluation of this parameter.[25]

Supporting Information (see footnote on the first page of this article): Detailed experimental procedures; ^{1}H NMR and ^{13}C NMR spectra for compounds **3b**–**j**, **7b**–**i**, **10**, and **11**; and ^{1}H NMR spectra for compounds **3a** and **7a**.

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