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Minor Enantiomer Recycling: Metal Catalyst, Organocatalyst and Biocatalyst Working in Concert

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Abstract: A minor enantiomer recycling one-pot procedure employing two reinforcing chiral catalysts has been developed. Continuous regeneration of the achiral starting material is effected via selective enzyme-catalyzed hydrolysis of the minor product enantiomer from Lewis acid–Lewis base catalyzed addition of acyl cyanides to prochiral aldehydes in a two-phase solvent system. The process provides O-acylated cyanohydrins in close to perfect enantioselectivities, higher than those obtained in the direct process, and in high yields. A combination of a (*S*,*S*)-salen Ti Lewis acid and *Candida antarctica* lipase B provides the products with *R* absolute configuration, whereas the opposite enantiomer is obtained from the (*R*,*R*)-salen Ti complex and *Candida rugosa* lipase.

Keywords: asymmetric catalysis · enantioselectivity · enzyme catalysis · Lewis acids · organocatalysis

Introduction

Major advances in asymmetric synthesis using metal catalysts,[1] organocatalysts[2] or biocatalysts[3] have resulted in a multitude of efficient methods for the preparation of chiral enantiomerically enriched compounds with large structural variations in both laboratory and industrial scale. The product enantiomeric ratio obtained in the catalytic reactions is determined by the difference in free energy of activation for the formation of the two enantiomers. Each reactant has one single chance to be transformed to the desired enantiomer, and reactions passing via the higher transition state barrier lead to formation of the "wrong" enantiomer. Such "mistakes" by the catalyst can be corrected by destroying the undesired enantiomer in the subsequent purification; this is often necessary in order to achieve the high enantiopurity, often $>99\,\%$ *ee*, which is required for many applications. Crystallization is most commonly used for this purpose, but selective chemical transformation of the minor, undesired enantiomer via a subsequent kinetic resolution to a compound which easily can be separated from the desired product has occasionally proven to be a useful option.[4] This additional step may lead to high enantiopurity, but inevitably results in loss of product, and consequently to lower yields.

A process allowing the minor product enantiomer to be recycled to the prochiral reactant, and thereby given another chance in the catalytic reaction, would circumvent this problem and would in principle allow quantitative conversion of the prochiral compound to a single enantiomer (Figure 1). However, for this to be realistic, a thermodynamic driving force, allowing for a unidirectional cycle, is required.[5]

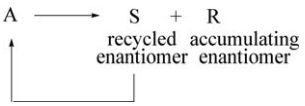

Figure 1. Minor enantiomer recycling.

From basic kinetic principles it follows that use of chiral catalysts for both the transformation and the regeneration of the reactant would lead to an enantioselectivity which is higher than that of each individual step. Thus, the resulting enantiomeric ratio e.r. of the product will benefit from the two selective steps:

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Generally, the enantiomeric ratio e.r. achieved in a reaction is defined as

$$\text{e.r.} = \frac{[R]}{[S]} \quad (1)$$

and is dependent on the enantioselectivity E of the catalyst used. In a simple transformation of an achiral substrate the enantiomeric ratio e.r. is constant and equal to E.

$$\text{e.r.} = E \quad (2)$$

When the selectivity of one catalyst is reinforced by the action of another, the resulting enantiomeric ratio will be a function of the enantioselectivities of both catalysts. Let E_{chem} and E_{enz} denote the enantioselectivities of a chemical catalyst and a reinforcing enzyme, respectively, defined as:

$$E_{chem} = \frac{k_{chem,R}}{k_{chem,S}} \quad (3)$$

$$E_{enz} = \frac{(k_{cat}/K_M)_S}{(k_{cat}/K_M)_R} \quad (4)$$

In a system utilizing both catalysts, the rate equations for R- and S-product formation become (assuming the metal-catalyzed reaction to follow second-order kinetics):

$$\frac{d[R]}{dt} = k_{chem,R}[\text{AcCN}][\text{aldehyde}][\text{Ti}] - \left(\frac{k_{cat}}{K_M}\right)_R [R][\text{Enz}] \quad (5)$$

$$\frac{d[S]}{dt} = k_{chem,S}[\text{AcCN}][\text{aldehyde}][\text{Ti}] - \left(\frac{k_{cat}}{K_M}\right)_S [S][\text{Enz}] \quad (6)$$

where [Enz] and [Ti] denote the concentration of free enzyme and chemical catalyst, respectively. At steady state, where $\frac{d[R]}{dt} = \frac{d[S]}{dt} = 0$,

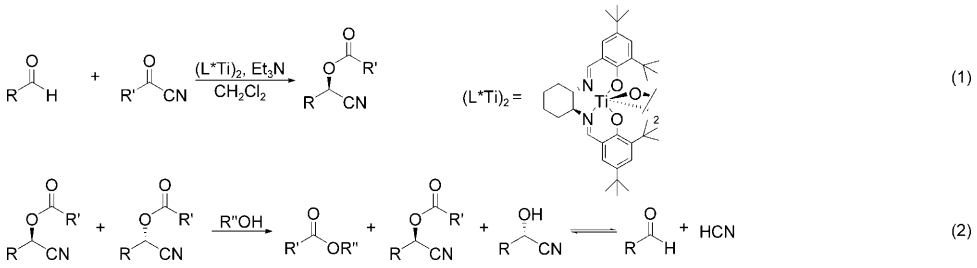
$$\frac{[R]}{[S]} = \frac{k_{chem,R}(k_{cat}/K_M)_S}{k_{chem,S}(k_{cat}/K_M)_R} \quad (7)$$

and thus

$$\text{e.r.} = E_{chem}E_{enz} \quad (8)$$

The selectivities of both catalysts will thus contribute to increased enantiopurity and yield.

In order to realize a minor enantiomer recycling process, catalytic reactions proceeding in both forward and backward directions are needed. We recently described a catalytic system consisting of a chiral Lewis acid and an achiral or chiral Lewis base for the addition of α-ketonitriles to prochiral aldehydes, producing highly enantioenriched O-acylated cyanohydrins [Scheme 1, Eq. (1)][6] which serve as both synthetic intermediates[7] and versatile target compounds.[8] Biocatalytic methods available for this transformation proceed by dynamic kinetic resolution of in situ formed cyanohydrins, often with moderate yields and selectivities.[9] In contrast, the reverse reaction, the hydrolysis or alcoholysis of acylated cyanohydrins catalyzed by *Candida antarctica* lipase B (CALB) is known to proceed with high selectivity for the S enantiomer [Scheme 1, Eq. (2)],[10] a circumstance that we exploited for the determination of enantiomeric purity of several acylated cyanohydrins[11] and which was subsequently used for the destruction of one enantiomer, resulting in an increased enantiomeric purity of the product.[12]



(1)

(2)

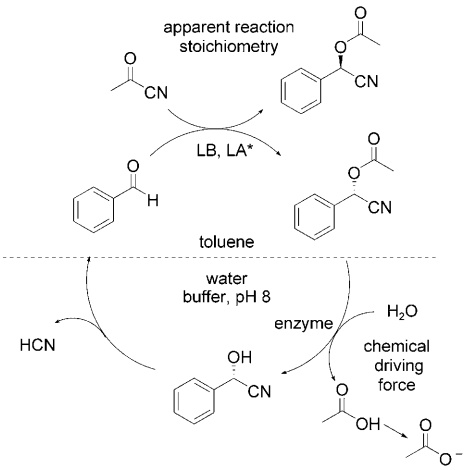
Scheme 1. Lewis acid-Lewis base catalyzed acylcyanation (1) and enzyme catalyzed hydrolysis (alcoholysis) of O-acylated cyanohydrin (2).

We considered a process combining the reactions in Scheme 1 to be a suitable candidate for the proof of concept of Figure 1. A process with the forward Lewis acid–Lewis base catalyzed reaction and backwards enzymatic hydrolysis proceeding simultaneously would take advantage of the atom efficiency and high yield of the former process to induce chirality, and the selectivity of the latter to enhance the enantiomeric ratio. It would constitute a dynamic kinetic resolution of the product (Figure 1).

Results

To allow the forward reaction, catalyzed by a chiral titanium salen complex[13] and a Lewis base, and the *Candida antarctica* lipase B catalyzed hydrolysis to proceed in concert, suitable experimental conditions were needed. The optimal conditions for the Lewis acid–Lewis base catalyzed reaction use dichloromethane as solvent and a reaction temperature of −40 °C,[6] whereas those for the enzymatic kinetic resolution use propanol/toluene and 60 °C.[10] None of these conditions is suitable for a cyclic process, since at −40 °C the enzyme does not have the required reactivity, and at 60 °C the acylcyanation is non-selective. Furthermore, in order to allow unidirectional recycling, a mass flow through the system providing a thermodynamic driving force is required, since a closed reversible reaction network would lead to racemic product. Replacement of propanol for water in the enzymatic process leads to acetic acid in place of propyl acetate and,

under neutral or basic conditions, to acetate; formation of acetate and HCN from acetyl cyanide and water should ensure sufficient gain in energy to allow the desired recycling (Scheme 2).



Scheme 2. Catalytic cycle for transformation and regeneration of reactant.

The reaction between benzaldehyde (**1a**) and acetyl cyanide (**2**) was used in attempts to find reaction conditions allowing the forward and backward reactions to proceed simultaneously (Scheme 2). By monitoring the ratio of product enantiomers, (*R*)-**3a** and (*S*)-**3a**, and conversion with gas chromatography, both processes could be followed over time. After having screened a variety of solvents and solvent mixtures, a two-phase system containing water and an organic solvent of lower density than water was found to allow both processes to occur simultaneously. Toluene/buffer pH 8 (aq) was finally found to be the best choice. A buffer was needed to neutralize the acetic acid formed in the hydrolysis. When simply mixing the reagents, the enzymatic hydrolysis proceeded well, as shown by a high ratio of product enantiomers, but chemical yields were low due to decomposition of acetyl cyanide to acetic acid. The problem was solved by continuous addition of the acyl cyanide. For the initial experiments, a reaction temperature of 40 °C was chosen in order to obtain a reasonable rate for the enzymatic reaction. Under these conditions, with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as base, the enantioselectivity of the metal-catalyzed process was low (62:38 e.r. with 10 mol % DBU and essentially racemic product, 53:47 e.r., with 20 mol %) but, due to the presence of the enzyme, the enantiomeric ratio of the product increased gradually as the minor enantiomer was recycled, at the same time as the total yield of the two enantiomers remained constant due to the balancing forward reaction. After complete addition of acetyl cyanide (a total of 3 equiv), the ratio of product enantiomers still increased (from 96.1:3.9 to 99.9:0.1), while product formation gradually ceased due to the decomposition of acetyl cyanide, resulting in a final yield of 80 %.

We decided to optimize the reaction conditions using benzaldehyde as the substrate. We noted that the forward reaction resumed upon continued slow addition of acetyl cyanide; after addition of a total of four equivalents of acetyl cyanide the yield of product with enantiomeric ratio of 99.6:0.4 was 87 %. Somewhat higher initial enantioselectivity (79:21 e.r.) was observed when the forward reaction was run at room temperature in the presence of 1.1 equivalents of acetyl cyanide and 5 mol % each of the Ti dimer and 4-(dimethylamino)pyridine (DMAP) without CALB, which was added together with buffer only after 9 h. Further addition of acetyl cyanide (up to a total of 3 equiv) during 16.5 h at 40 °C again resulted in both increased yield (from an initial 83 to 95 %) and enantiomeric ratio (98:2). Stirring for an additional 5 h resulted in a yield of 92 % and 99.5:0.5 e.r. A final yield of 97 % of a product with 99.8:0.2 enantiomeric ratio was obtained after the addition of a total of 4.5 equivalents of acetyl cyanide and a total reaction time of 5.5 days (Figure 2, Scheme 3). This can be compared with previous results from the preparation of the same compound, 96 % yield and 94 % *ee* from the Lewis acid–Lewis base catalyzed acetylcyanation, 97 % yield and 98 % *ee* from enzymatic hydrolysis of enzyme catalyzed acetylation of the cyanohydrin,[9b] and 84 % yield and 97 % *ee* from enzymatic hydrolysis of a scalemic mixture of acetylated cyanohydrins.[12]

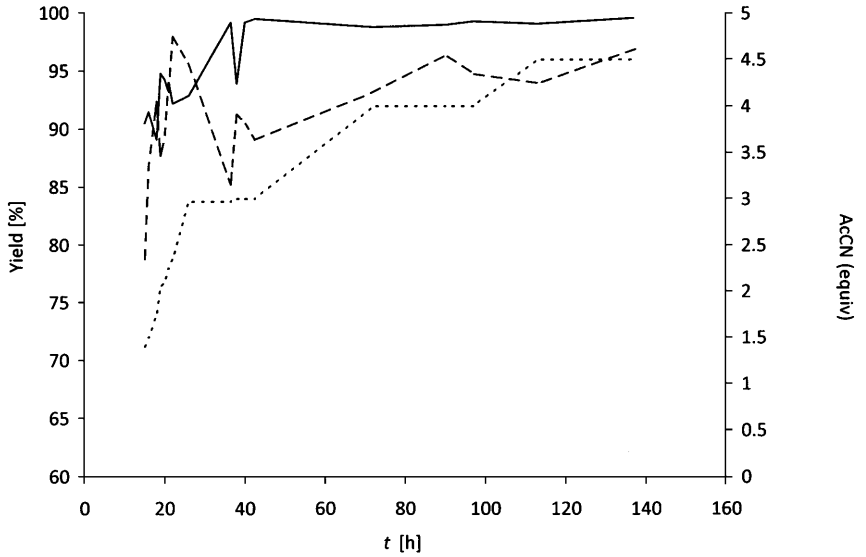
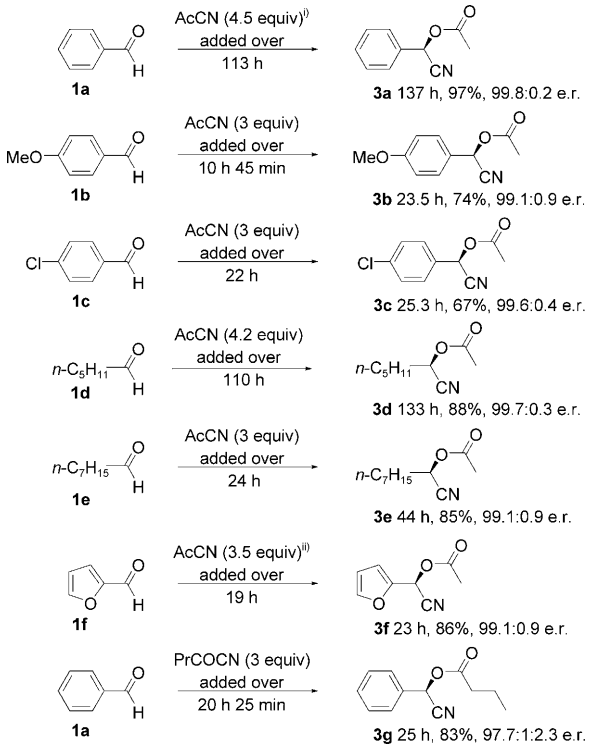


Figure 2. Enantiomeric excess (——) and total yield (-----) of **3a** as a function of time and amount of **2** (·····) added.



Scheme 3. Minor enantiomer recycling of prochiral aldehydes. i) 14 h at RT before addition of buffer and CALB; ii) 2 h at −40 °C and 1 h at RT before addition of buffer and CALB.

Reactions with other aldehydes: In order to extend the scope of the recycling process, we decided to subject a few other aldehydes to the reaction conditions used for **1a**. Kinetic resolution of several aromatic aldehydes, with electron-donating as well as electron-attracting substituents, is known to proceed with high selectivity in the presence of CALB in a propanol/toluene mixture.[10b] Slow addition of acetyl cyanide to 4-methoxybenzene and 4-chlorobenzene in the presence of the Lewis acid–Lewis base–enzyme catalytic system under the non-optimized conditions initially used for benzaldehyde (3 equiv of acetyl cyanide) gave the expected products, (*R*)-**3b** and (*R*)-**3c**, with high selectivity, in enantiomeric ratios 99.1:0.9 and 99.6:0.4, respectively (Scheme 3).

In contrast, kinetic resolution of aliphatic analogues using the same enzyme has been reported to result in low selectivity,[10] and, probably as a consequence of this low selectivity, we had previously encountered problems with enzymatic determination of products from aliphatic aldehydes.[11] Treatment of a racemic mixture of **3d**, prepared by reaction of acetyl cyanide and hexanal at ambient temperature in the presence of triethylamine, with CALB in a toluene/buffer (aq, pH 8) system, that is, the same conditions as those used in the recycling process, verified the lower selectivity in the enzymatic hydrolysis of the aliphatic substrate; the hydrolysis of the *S* enantiomer proceeded rather slowly, at the same time as slow hydrolysis of the *R* enantiomer was observed ($E=15$). We were therefore pleased to find that the Lewis acid–Lewis base–enzyme catalyzed recycling process proceeded with excellent results; the addition of a total amount of 4.2 equivalents of acetyl cyanide over 110 h and a total reaction time of 133 h gave acetylated cyanohydrin **3d** in 88% yield and an enatiomeric ratio of 99.7:0.3. This can be compared to the Lewis acid–Lewis base catalyzed process which gave the same product in 89% yield but with an enantiomeric ratio of merely 95:5.[6b] Similar conditions for the minor enantiomer recycling reaction with octanal, which undergoes CALB-catalyzed hydrolysis with considerably higher selectivity than hexanal ($E > 1000$) gave **3e** in 84% yield and 99.3:0.7 e.r.

The acetylated cyanohydrin from furfural, **3f**, was prepared in racemic form in the same way as **3d** and exposed to CALB. The hydrolysis was only slightly more selective than that of aliphatic derivative **3d**, with hydrolysis of the *R* enantiomer occurring at an appreciable rate ($E=26$). Even in the absence of the enzyme, slow hydrolysis was observed for racemic **3f** in toluene/buffer (aq, pH 8) and, to mimic the reaction conditions, two equivalents of acetic acid; after 5 h at 40 °C 93% product remained, and after 60 h 84%.

The recycling procedure with **1f** provided the acylated cyanohydrin with *R* configuration with high enantiomeric ratios of >97:3, but with moderate yields under conditions employed for aldehyde **1c** (addition of 3 equiv of acetyl cyanide over 22–25 h). Moreover, the yield decreased over time, probably due to polymerization of furfural. Addition over a shorter period of time, 5 h 45 min, followed by stirring for an additional 2 h provided superior results, 81% yield and 98.6:1.4 e.r. The optimal conditions found consisted of 12 h reaction at −40 °C and 1 h at room temperature before addition of CALB and buffer followed by addition of acetyl cyanide over 6 h, and finally 4 h of stirring; under these conditions (*R*)-**3f** was obtained in 86% yield and 99.1:0.9 e.r. Addition of a larger excess of acetyl cyanide may require a stronger buffer in order to avoid decomposition of the aldehyde.

Butanoyl cyanide: Due to the importance of acylated cyanohydrins with different acyl groups it was considered to be of interest to study whether the recycling process could be performed with some additional ketonitrile. Exchange of acetyl cyanide for butanoyl cyanide did indeed give butanoyl ester **3g** in good yield with high enantioselectivity (Scheme 3).

Opposite enantiomer: For a catalytic process to work satisfactory, it should provide access to both enantiomers of the product. Two enzyme preparations, known as *Candida rugosa* lipase (CRL) and *Candida cylindracea* lipase (CCL), were found to exhibit the opposite selectivity in the hydrolysis of **3a**, thus preferentially transforming the *R* enantiomer to the aldehyde, albeit with moderate selectivity ($E=29$ and 23 for CRL and CCL, respectively).[14] Having identified these two enzymes, the recycling procedure was studied using the Ti complex with opposite, that is, (*R*,*R*), absolute configuration. Due to slow enzymatic hydrolysis, longer re-

action times were needed than in reactions using CALB. Under non-optimized conditions 92% yield of *O*-acetyl-(*S*)-2-hydroxy-2-phenylacetonitrile with 95.2:4.8 e.r. was obtained using CRL and 93% yield of the same product with 94.0:6.0 e.r. using CCL, in both cases after 61 h. The enantiomeric ratios were improved by continued stirring without further addition of acetyl cyanide, but at the same time gradually decreasing yields were observed; after stirring for a further five days, the yields were 71 and 72% and enantiomeric ratios 98.2:1.8 and 98.1:1.9, respectively.

Reactions with one chiral catalyst: At the high temperature used (40°C), the Lewis base can serve as the sole catalyst for the acetylcyanation. Although racemic product is formed initially (and the selectivity thus determined only by the enzyme), the recycling process results in high final enantioselectivities (99:1 e.r. in reaction with benzaldehyde), albeit in somewhat lower yield (77%) than that observed in the presence of Lewis acid. A higher number of cycles and more acyl cyanide are also required; a reaction run under conditions where the metal catalyzed reaction results in an e.r. of 80:20 and the enzymatic reaction proceeds with perfect selectivity gives a 99.8:0.2 ratio of the product enantiomers after four cycles and consumes 1.25 equivalents of acyl cyanide, while nine cycles and 1.99 equivalents of acyl cyanide are required to achieve the same enantiomeric purity using only the achiral Lewis base.

Discussion

Reinforced enantioselectivity by using two chiral catalysts: In the present study, the selectivity of a chiral metal catalyst is reinforced by an enantioselective enzyme, which recycles the minor product enantiomer to starting material. The combination of two catalysts in this recycling system results in an enantiomeric ratio of the product that is the product of the individual selectivities of the two catalysts. Further, the recycling reaches a kinetic equilibrium. Without recycling, the enzymatic resolution step inevitably consumes both the major and the minor product. The recycling of product to starting material therefore contributes to an increased yield.

Comparison to other processes: The process developed here (Figure 3A) is different from established procedures in which biocatalysts and metal catalysts or synthetic reagents have been used in combination. In those cases an enzyme catalyzes a chemical transformation of one of the enantiomers of a racemic mixture, whereas in the present case a minor product enantiomer is recycled to achiral reactant. In addition, in our minor enantiomer recycling procedure, two chiral catalysts are employed. In dynamic kinetic resolutions, an enzyme catalyzes a transformation of one enantiomer and the role of the metal complex is to racemize the remaining substrate (Figure 3B).[15] In contrast to our minor enantiomer recycling, the selectivity in this process relies only on the selectivity of the biocatalyst. In a different approach, one of the enantiomers of a racemic mixture can selectively be reacted by an enzyme to an achiral compound, which is then transformed to a racemic mixture of the original compound by an achiral stoichiometric organic reagent. This process, called cyclic deracemization,[16] results in deracemization of the original racemic mixture (Figure 3C).[17] In contrast to our procedure, which constitutes a chemical transformation, in cyclic deracemization, as expressed by its pioneer, "there is no product, but simply an optical enrichment of the substrate".[18] Even if the cycle in principle could be entered via the prochiral intermediate, this possibility has not been demonstrated and would, at least in the examples reported so far, be hampered by the instability of this intermediate. In this case, the selectivity relies on a single chiral catalyst.

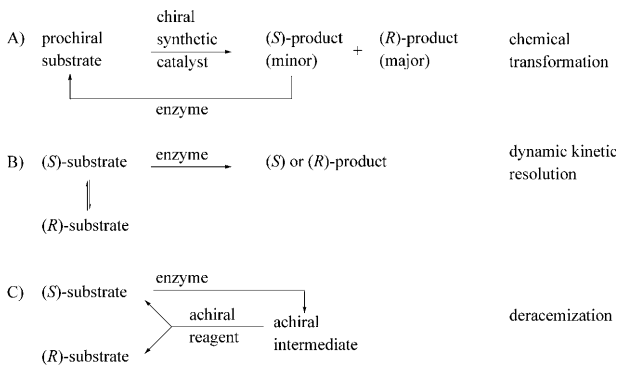


Figure 3. Enantioselective processes by combined use of synthetic reagents or catalysts and biocatalysts.

Previous methodologies describe the quantitative transformation of racemic mixtures to enantioenriched products using one enantioselective catalyst, while the present method is a dynamic transformation using two reinforcing enantioselective catalysts and starting from an achiral substrate. We describe a conceptually new procedure in which two reinforcing chiral catalysts are used. In the present example the enantioselectivity of one catalyst is low ($E=1.4$) whereas that of the other is high ($E>500$). The reinforcing effect is therefore difficult to demonstrate experimentally in this particular case, which however serves as a proof-of-concept and shows that minor enantiomer recycling is experimentally feasible.

Conclusions

In conclusion, we have found a one-pot minor enantiomer recycling procedure in which the enantioselectivity of a metal-catalyzed reaction is improved by a biocatalyst with concomitant increase in yield of the desired enantiomer. The combined use of the two reinforcing catalysts results in formation of close to enantiopure products in high yields, even in reactions where the selectivity in both the Lewis acid–Lewis base and the enzyme catalyzed reactions is not ideal. By proper choice of the combination of metal catalyst and biocatalyst both product enantiomers are accessible.

Future studies will be devoted to further applications of this new procedure, whereby mistakes by one catalyst are corrected by a second catalyst.

Experimental Section

General: All aldehydes were distilled (benzaldehyde from CaH_2) prior to use. Solvents were collected from a Glass-contour solvent dispensing system. Internal standard, *Candida antarctica* lipase B (CALB, Novozyme 435), *Candida rugosa* lipase, and *Candida cylindracea* were purchased and used without further purification. (*S*,*S*)- and (*R*,*R*)-[(salen)Ti(μ-O)]$_2$,[13,19] acetyl cyanide[20] and butanoyl cyanide[20] were prepared following published procedures. Yields and enantiomeric ratios were determined by GC/MS using a chiral column (Chiraldex, G-TA (gamma cyclodextrin trifluoroacetyl, 30 m × 0.25 mm) and *n*-undecane as internal standard.

General procedure for Lewis acid–Lewis base–CALB catalyzed synthesis of O-acylated cyanohydrins: Immobilized CALB (10 mg) and 1 M phosphate buffer pH 8 (0.5 mL) were added to a solution of (*S*,*S*)-[(salen)Ti-(μ-O)]$_2$ (6.7 mg, 0.0055 mmol), DBU (3.4 μL, 0.024 mmol), internal standard $C_{11}H_{24}$ (10 μL, 0.047 mmol), aldehyde (0.12 mmol), and acyl cyanide (0.014 mmol) in toluene (0.25 mL) Acyl cyanide (0.35 mmol) diluted to 0.25 mL with toluene was then added over 5–22 h to the vigorously stirred reaction mixture at 40 °C using a syringe pump. The reaction was monitored by taking aliquots from the organic phase (ca 20 μL, which were filtered through a plug of silica and eluted with diethyl ether), which were analyzed by GC. The structure and absolute configuration of the O-acylated cyanohydrins were verified by comparison with previously reported NMR spectral data and with optical rotations.[6b,21]

Alternative procedure for Lewis acid–Lewis base–CALB catalyzed synthesis of O-acylated cyanohydrins: A solution of (*S*,*S*)-[(salen)Ti(μ-O)]$_2$ (6.7 mg, 0.0055 mmol), DMAP (6.7 mg, 0.006 mmol), internal standard $C_{11}H_{24}$ (10 μL, 0.047 mmol), benzaldehyde (12 μL, 0.12 mmol) and acetyl cyanide (9.4 μL, 0.13 mmol) in toluene (0.3 mL) was stirred at room temperature for 9 h. Immobilized CALB (10 mg) was then added followed by 1 M phosphate buffer pH 8 (0.5 mL). More acetyl cyanide (16.2 μL, 0.23 mmol) diluted to 0.2 mL with toluene was then added over 16.5 h to the vigorously stirred reaction mixture at 40 °C using a syringe pump. The reaction was monitored by GC while stirring was continued.

Preparative scale Lewis acid–Lewis base–CALB catalyzed synthesis and purification of *O*-acetyl-(*R*)-2-hydroxy-2-phenylacetonitrile: Immobilized CALB (100 mg) was added to a solution of (*S*,*S*)-[(salen)Ti(μ-O)]$_2$ (70 mg, 0.06 mmol), DBU (34 μL, 0.24 mmol), benzaldehyde (122 μL, 1.2 mmol) and acetyl cyanide (5 μL, 0.071 mmol) in toluene (4.25 mL), followed by 1 M phosphate buffer pH 8 (5 mL). Acetyl cyanide (250 μL, 3.5 mmol) diluted to 1 mL with toluene was then added over 18 h to the vigorously stirred reaction mixture at 40 °C using a syringe pump. Stirring was continued for 3 h. After dilution with Et_2O, the reaction mixture was filtered through silica, the silica rinsed with ether, and the solvent was evaporated under vacuum. The residue was purified with flash chromatography on silica gel (hexane/ethyl acetate 7:1) to give *O*-acetyl-(*R*)-2-hydroxy-2-phenylacetonitrile (180 mg, 86 %, 99.67:0.33 e.r.).

Lewis acid–Lewis base–CRL (or CCL) catalyzed synthesis of O-acylated cyanohydrins: Acetyl cyanide (9.4 μL, 0.132 mmol) was added to a solution of (*S*,*S*)-[(salen)Ti(μ-O)]$_2$ (6.7 mg, 0.0055 mmol), DMAP (0.73 mg, 0.006 mmol), internal standard $C_{11}H_{24}$ (10 μL, 0.047 mmol) and benzaldehyde (12 μL, 0.12 mmol) in toluene (0.3 mL). The reaction mixture was stirred at room temperature overnight. Enzyme (CRL or CCL) was added to 1 M phosphate buffer pH 8 (0.5 mL) until the solution was saturated (ca 5 mg). The slurry was stirred for 10 min and then centrifuged. Insoluble material was filtered off and the solution was added to the organic reaction mixture. Acetyl cyanide (17 μL, 0.228 mmol) diluted to 0.25 mL with toluene was then added over 20 h to the vigorously stirred reaction mixture at 40 °C using a syringe pump. Additional acetyl cyanide (8.5 μL, 0.12 mmol) diluted to 0.1 mL with toluene, was then added over 10 h. The reaction was monitored by GC.

Acknowledgements

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