Lipase-catalyzed kinetic resolution with in-situ racemization: one-pot synthesis of optically active cyanohydrin acetates from aldehydes, J. Amer. Chem. Soc., 113, 9360-9361

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at will. Although the capacity shown here compares more than well with, for example, a biological alternative method using the protein cellulase[6d] [the amounts of timolol with optimal base-line separation were 18.9 and 19.9 μg/g of dry CSP, respectively (Figure 2), and in connection with the aforementioned polarimetric study of propranolol, with fair resolution but no base-line separation, 400 μg/g of dry CSP], work aimed at increasing the capacity of analogous imprinted polymers is in progress and will further increase the applicability of the technique for (semi)-preparative isolation of pure enantiomers of compounds such as β-blockers. However, we feel that already today the removal of contaminating small amounts of the nondesired enantiomer may be feasible on a technical scale with the described technique.

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Lipase-Catalyzed Kinetic Resolution with in Situ Racemization: One-Pot Synthesis of Optically Active Cyanohydrin Acetates from Aldehydes

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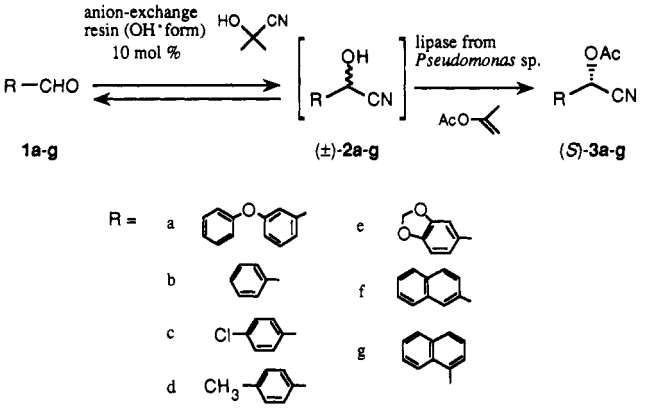
Lipase-catalyzed kinetic resolution of racemic compounds has been recognized as an effective tool for the preparation of optically active compounds.[1] Like conventional resolution processes, however, the maximum yield of one enantiomer cannot exceed 50%. Therefore, the development of a method which includes in situ racemization of the substrate is a challenging objective, because it would allow for the quantitative conversion of the racemic substrate into a single enantiomer. This type of enzymatic second-order asymmetric transformation has only been achieved in a limited number of cases.[2,3]

Cyanohydrins are promising candidates for such a second-order asymmetric transformation, because they can be reversibly decomposed into achiral aldehydes under basic conditions; moreover, they are easily converted into various useful chiral synthons. Although numerous examples of the enzyme-catalyzed synthesis of optically active cyanohydrins have been reported,[4] many of them have been based on simple kinetic resolution, and, in some cases, one of the enantiomers was decomposed or racemized.[4a,b,d-g]

Scheme I



We now report a novel one-pot synthesis of optically active cyanohydrin acetates from aldehydes, through the combination of in situ cyanohydrin formation with a lipase-catalyzed kinetic resolution of cyanohydrins in an organic solvent (Scheme I). Aromatic aldehydes **1a-g** were reversibly converted into the corresponding cyanohydrins **2a-g** through transhydrocyanation with acetone cyanohydrin, catalyzed by an anion-exchange resin (OH^- form). The resulting cyanohydrins were enantioselectively acetylated by subsequent lipase-catalyzed transesterification using isopropenyl acetate as an acyl donor[5] to yield optically active cyanohydrin acetates **3a-g** having high enantiomeric excesses. Due to the reversible nature of the base-catalyzed transhydrocyanation,[6] cyanohydrin **2** is subject to rapid racemization. When the acidic hydroxy group in **2** is protected by acetylation,[7] racemization is greatly reduced and hence cyanohydrins **2** formed in situ from aldehydes **1** are converted into a single stereoisomer of the cyanohydrin acetate **3**. Acetone cyanohydrin, which is easier to handle than HCN, was used as the hydrogen cyanide source for this reaction.[8] It produces acetone as the only byproduct after the release of HCN. Importantly, acetone cyanohydrin is not subject to acetylation by lipase probably due to its steric bulk.[9] Therefore, it remains an effective HCN donor throughout the reaction.

The results are summarized in Table I. A lipase from *Pseudomonas* sp. M-12-33 (Amano)[10] and three types of strongly basic, macroporous anion-exchange resins (OH^- form, 10 mol % equiv) were used as catalysts. The reaction was monitored by ^1H NMR, and the degree of cyanohydrin formation and enzymatic acetylation were calculated from the ratio of each component **1**, **2**, and **3** found in the reaction mixture. 3-Phenoxybenzaldehyde (**1a**) was transformed into the corresponding acetate **3a** in 3 days via a 95% conversion in the transhydrocyanation (from **1a** to **2a**) and an 88% conversion in the enzymatic acetylation (from **2a** to **3a**).

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(7) The dissociation of HCN from cyanohydrins is initiated by the abstraction of a proton from the OH group of the cyanohydrin: see ref 6a.

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(9) Acetone cyanohydrin was not acetylated by the lipase (from *Pseudomonas* sp.) after incubation (40 °C, 6.7 days) with isopropenyl acetate in diisopropyl ether.

(10) The lipase from *Pseudomonas* sp. M-12-33 (Amano Pharm. Co., Ltd.) was obtained as lyophilized powder and used after immobilization as follows: the lipase powder was dissolved in 20 mM Tris-HCl buffer (pH 8.0) and mixed with Hyflo Super-Cel (10 mg lipase/g Hyflo Super-Cel). The resulting paste was dried in a desiccator over P_2O_5 (Inagaki, M.; Hiratake, J.; Nishioka, T.; Oda, J. *Agric. Biol. Chem.* **1989**, *53*, 1879-1884).

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Table I. One-Pot Synthesis of (*S*)-Cyanohydrin Acetates **3a–g** from Aldehydes **1a–g** Catalyzed by Lipase and Anion-Exchange Resin (OH^- Form)[a]

aldehydes	anion-exchange resin	reaction time (days)	conversion[b]		(*S*)-acetate **3**	
			1 to **2** (%)	**2** to **3** (%)	yield[c] (%)	ee[d] (%)
1a	Amberlite IRA-904	2.9	95	88	80	89
1a	Amberlyst A-27	2.9	90	77	68	88
1a	Duolite A-162	2.1	97	93	82	80
1b	Amberlite IRA-904	6.3	100	100	96	84
1c	Amberlite IRA-904	3.8	97	93	83	84
1d	Amberlite IRA-904	2.5	88	82	64	91
1e	Amberlite IRA-904	6.5	89	96	81	91
1f	Amberlite IRA-904	6.1	98	98	88	85
1g	Amberlite IRA-904	6.1	91	79	70	70

[a] Typical conditions: 3-phenoxybenzaldehyde (**1a**) (991 mg, 5.0 mmol), acetone cyanohydrin (851 mg, 10.0 mmol), isopropenyl acetate (1502 mg, 15.0 mmol), lipase (500 mg), anion-exchange resin Amberlite IRA-904 (OH^- form, 192 mg, 10 mol % equiv to **1a**), 3 Å ground molecular sieves (200 mg), dry diisopropyl ether (40 mL), 40 °C. The addition of 3 Å molecular sieves accelerated the reaction although 3 Å MS catalyzes neither the transhydrocyanation nor the acetylation. [b] Determined by ^1H NMR. [c] Isolated yield from aldehyde **1**. [d] Determined by ^1H NMR in the presence of the chiral shift reagent, Eu(hfc)$_3$.

By comparing the sign of the optical rotation with that reported,[11] the absolute configuration of **3a** was found to be *S*. The ee was determined to be 89% by ^1H NMR in the presence of a chiral shift reagent.

An initial approach using quinidine or quinine as a catalyst for transhydrocyanation resulted in a long reaction time as well as a low optical yield of **3a**,[12] presumably due to slow racemization of the cyanohydrins. Consequently, a stronger base was required as a catalyst for the present process. The OH^- form of anion exchange resins, such as Amberlite IRA-904, is a strong base and was found to be an efficient catalyst for rapid transhydrocyanation between aromatic aldehyde and acetone cyanohydrin in an organic solvent.[13] Moreover, such resins are insoluble in the reaction solvent and are insulated completely from the lipase, causing no unfavorable effects on lipase activity. All of the three resin types listed in Table I effectively catalyzed the transhydrocyanation (racemization) to produce high chemical yields of (*S*)-**3a**. The somewhat low ee for **3a** in the reaction using Duolite A-162 may be attributable to the nonenzymatic acetylation of racemic **2a** catalyzed by this resin.[14]

Benzaldehyde **1b** was completely (100% yield) converted into acetate (*S*)-**3b** with an 84% ee. Similarly, optically active cyanohydrin acetates **3c–g** having a high optical purity (up to 91%) were obtained from the corresponding aldehydes **1c–g** in 64–88% isolated yields. All the acetates **3a–g** were of *S* configuration,[15] indicating that the lipase preferentially converted the *S* cyanohydrin into the corresponding acetate irrespective of the substituents on the aromatic ring. The *S* isomer of the cyanohydrin **2a** is the desired enantiomer for the preparation of pyrethroids which are characterized by high insecticidal activity.[16]

In order to effectively convert racemic **2** into optically active **3**, the rate of racemization must be faster than that of lipase-catalyzed acetylation. The half-life ($t_{1/2}$) of the racemization of (*R*)-**2a** was 74 min when 10 mol % of Amberlite IRA-904 (OH^- form) was used as a catalyst in the presence of acetone cyanohydrin in anhydrous diisopropyl ether at 25 °C,[17] which confirms that racemization is much faster than lipase-catalyzed acetylation.

Supplementary Material Available: Experimental details and analysis data (including ^1H NMR, IR, ^{13}C NMR, and MS), schemes showing the preparation of optically active **2c**, **2f**, and **2g**, and ^1H NMR spectra of **3a** (15 pages). Ordering information is given on any current masthead page.

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(12) When quinidine was used as a transhydrocyanation catalyst in the present reaction, (*S*)-**3a** with 62% ee was obtained in 71% conversion yield after 13.7 days. Cinchona alkaloids such as quinine and quinidine were used as catalysts for enantioselective addition of HCN to benzaldehyde. Prelog, V.; Wilhelm, M. *Helv. Chim. Acta* **1954**, *37*, 1634–1660.

(13) The strongly basic macroporous resin, Amberlite IRA-904 (OH^- form, 10 mol %), catalyzed the formation of cyanohydrin **2a** from 3-phenoxybenzaldehyde (**1a**) in the presence of 2 equiv mol of acetone cyanohydrin in anhydrous diisopropyl ether (82% conversion, 80 min, 40 °C). On the other hand, neither a weakly basic macroporous resin such as Amberlite IRA-35 (free base form) nor a strongly basic but gel-type resin such as Amberlite IRA-400 (OH^- form) catalyzed the reaction under the same reaction conditions.

(14) The nonenzymatic acetylation of (±)-**2a** catalyzed by anion-exchange resins was estimated in the absence of the lipase: Duolite A-162 (39%), Amberlite IRA-904 (8%), and Amberlyst A-27 (0%) after 2.9 days.

(15) The optical rotation values have been reported as follows: for **3d** and **3e**, ref **4d**; for **2c**, **3f**, and **2g**, Mattews, B. R.; Jackson, W. R.; Jayatilake, G. S.; Wilshire, C.; Jacobs, H. A. *Aust. J. Chem.* **1988**, *41*, 1697–1709.

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(17) Optically active cyanohydrin **2a** (87% ee) was racemized completely in 6.2 h in the presence of 10 mol % of Amberlite IRA-904 and acetone cyanohydrin (10 equiv) in dry diisopropyl ether at 25 °C. Only 7% of **2a** was decomposed into the aldehyde **1a**. In the absence of acetone cyanohydrin, however, decomposition of cyanohydrin was observed (**2a**/**1a** = 75:25, equilibrium). The addition of acetone cyanohydrin prevented decomposition of **2a** and promoted clean racemization of **2a**.

Conversion of D-Glucose into Catechol: The Not-So-Common Pathway of Aromatic Biosynthesis

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Synthesis of catechol by *Escherichia coli* has led to the discovery of a pathway which can siphon away most of the D-glucose equivalents initially directed into the common pathway of aromatic biosynthesis (Scheme I). Induction of the discovered pathway constitutes an important variable to consider in the design of biocatalytic syntheses of aromatic amino acids and related secondary metabolites. The induced pathway may also be a useful route for converting D-glucose into catechol, a molecule from which a variety of pharmaceuticals, pesticides, flavors, and polymerization inhibitors are industrially derived.[1]

The percentage of D-glucose consumed by *E. coli* which is siphoned into aromatic biosynthesis is greatly increased when transketolase (*tkt*) and an isozyme of DAHP synthase (*aroF*) are amplified upon transformation with plasmid pKD136.[2] In addition to *tkt* and *aroF*, pKD136 carries an *aroB* locus which prevents accumulation of 3-deoxy-D-*arabino*-heptulosonic acid (DAH). Expression of pKD136 by *E. coli aroE* results in synthesis of a 30 mM concentration of 3-dehydroshikimate.[2b] By contrast,

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