JOC Article

Asymmetric Synthesis of Both Antipodes of β-Hydroxy Nitriles and β-Hydroxy Carboxylic Acids via Enzymatic Reduction or Sequential Reduction/Hydrolysis

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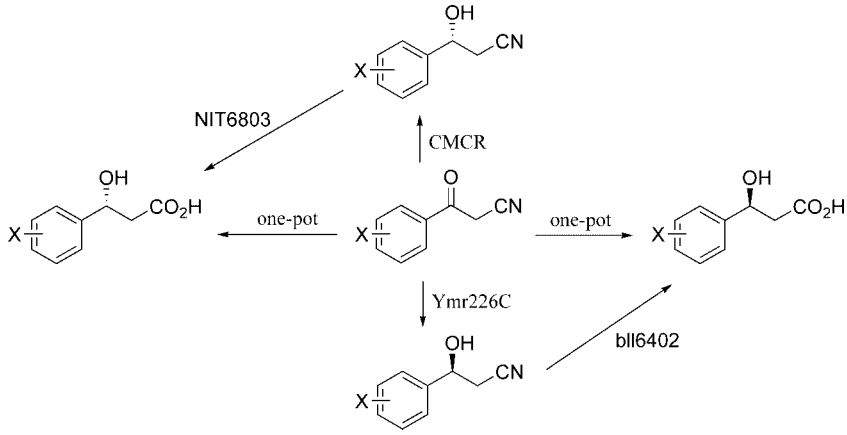

Use of isolated carbonyl reductases in the reduction of aromatic β-ketonitriles have completely eliminated the competing α-ethylation, which is often observed with whole cell biocatalysts. By choosing suitable recombinant carbonyl reductase, the reduction of β-ketonitriles afforded (*R*)- or (*S*)-β-hydroxy nitriles with excellent optical purity and yield. Subsequently, nitrilase-catalyzed hydrolysis of the obtained optically pure β-hydroxy nitriles led to the corresponding β-hydroxy carboxylic acids in high yields. More importantly, the sequential enzymatic reduction and hydrolysis could be carried out in "two-step-one-pot" fashion without the isolation of intermediates β-hydroxy nitriles, lowering the cost and minimizing the environmental impact. This allows ready access to both antipodes of chiral β-hydroxy nitriles and β-hydroxy carboxylic acids of pharmaceutical importance with excellent optical purity.

Introduction

Optically active β-hydroxy nitriles and β-hydroxy carboxylic acids are key building blocks for the synthesis of a variety of pharmaceutically important compounds. For example, many biologically active compounds such as β-blocker drugs contain 1,3-amino alcohol moieties, which are often prepared via reduction of β-hydroxy nitriles. In addition to utilization as anti-inflammatory drugs,[1] chiral β-hydroxy carboxylic acids can be converted to β-amino acids,[2] β-lactams,[3] and β-lactones[4] and have been used in the synthesis of pheromones.[5] The β-hydroxy carboxylic acid moiety has often been found in polyketide natural products such as amphotericin B,[6] tylosin, and rosaramicin[7] and the marine natural product hapalosin.[8] Therefore,

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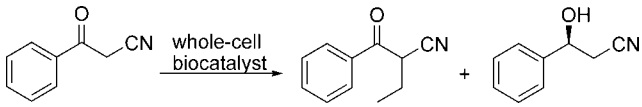
SCHEME 1. Whole-Cell Reduction of 3-Oxo-3-phenylpropanenitrile Concomitant with α-Ethylation



development of efficient and environmentally benign methodologies for the synthesis of enantiomerically pure β-hydroxy nitriles and β-hydroxy carboxylic acids is of practical importance.

For chiral β-hydroxy nitriles, asymmetric aldol-type reactions with acetonitrile,[9−13] β-boration of α,β-unsaturated nitriles followed by the oxidation,[14] borane reductions,[15,16] and transfer hydrogenation[17−19] of β-ketonitriles and lipase- or nitrilase-catalyzed resolution of racemic β-hydroxy nitriles have been reported.[20−23] Although these methods have been successful in some cases, they suffer from some drawbacks such as requirement of low reaction temperature, hazardous reagents, unsatisfactory enantiomeric purity, or maximum yield of 50%. For example, chemoenzymatic dynamic kinetic resolution of racemic β-hydroxynitriles using *Candida antarctica* lipase B and ruthenium catalyst resulted in acetylated β-hydroxy nitriles with 36−99% ee with concomitant formation of up to 26% of β-ketonitriles, limiting its application.[24] An alternative approach to access of chiral β-hydroxy nitriles is the biocatalytic reduction of β-ketonitriles. However, a competing α-ethylation reaction (Scheme 1) has been often observed in the bioreduction of aromatic β-ketonitriles by whole cell biocatalysts such as bakers' yeast and fungus *Curvularia lunata*,[25,26] resulting in low chemical yields of the desired β-hydroxy nitriles. The ethylated product has not been completely eliminated even in an *E. coli* whole cell system overexpressing yeast carbonyl reductases.[27] The ethyl group is proposed to come from the ethanol produced by the whole cell metabolism.[28,29]

Optically active β-hydroxy carboxylic acids are usually obtained from their ester or amide derivatives via carefully controlled hydrolysis. Optically active β-hydroxy carboxylic acid esters or amides in turn are prepared by asymmetric acetate aldol reactions,[30−33] Reformatsky reactions,[34−37] metal-catalyzed hydrogenation/transfer hydrogenation,[16,38−46] and enzymatic[47−50] reductions of β-keto esters or enzymatic resolution of racemic acylated β-hydroxy esters.[51] Reduction of β-keto acids has been achieved with (−)-diisopinocampheylborane affording enantiomerically enriched β-hydroxy carboxylic acids.[52,53] Optically active β-hydroxy carboxylic acids have also been prepared by the kinetic resolution of racemic β-hydroxy carboxylic acid esters.[54,55] Although these methods proved successful to some extent, improvement in the methodology for the synthesis of optically pure β-hydroxy carboxylic acids is still sought after.

In the course of exploring the application of enzyme catalysts in green chemical synthesis, we embraced an approach in which both antipodes of chiral β-hydroxy nitriles and β-hydroxy carboxylic acids could be obtained with excellent optical purity and yield via enzymatic reduction or sequential reduction/

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SCHEME 2. Bioreduction of β-Ketonitriles with Isolated Enzymes

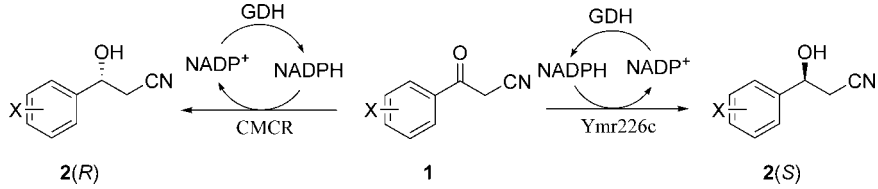


TABLE 1. Enzymatic Reduction of β-Keto Nitriles with Ymr226c

X	time (h)	yield[a] (%)	ee[b] (%)
4-H (**1a**)	48	83	99 (*S*)
4-F (**1b**)	24	88	99 (*S*)[c]
2,4-F_2 (**1c**)	24	85	99 (*S*)[c]
4-Cl (**1d**)	24	81	99 (*S*)
4-Br (**1e**)	24	83	99 (*S*)
4-CH_3 (**1f**)	48	85	99 (*S*)
4-CN (**1g**)	36	90	95 (*S*)
4-NO_2 (**1h**)	36	80	99 (*S*)
3-NO_2 (**1i**)	36	78	99 (*S*)
3-CH_3O (**1j**)	36	75	95 (*S*)
4-CH_3O (**1k**)	24	90	99 (*S*)

[a] Isolated yield. [b] The ee value was measured by chiral HPLC analysis unless indicated otherwise.[23] [c] The ee value was measured by chiral GC analysis.

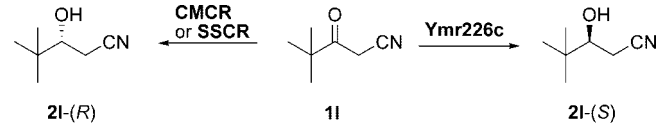
hydrolysis, respectively, and the preliminary results on the (*R*)-enantiomer were communicated previously.[56]

Results and Discussion

Bioreduction of β-ketonitriles has remained until now an unsolved problem because of a competing α-ethylation reaction (Scheme 1) observed in this bioreduction with whole cell biocatalysts, resulting in lower chemical yields of the desired β-hydroxy nitriles. As reported in our preliminary communication, reduction of aromatic β-ketonitriles (**1a**−**j**) with an isolated carbonyl reductase from *Candida magnoliae* (**CMCR**) showed the absence of the competing α-ethylation (Scheme 2).[56] Having screened several carbonyl reductases obeying Prelog's rule, which became available in our laboratory, it has been found that the isolated alcohol dehydrogenase (**Ymr226c**) from *Saccharomyces cerevisiae* efficiently catalyzed the reduction of various aromatic β-ketonitriles bearing an electron-withdrawing or electron-donating group on the phenyl ring to afford (*S*)-enantiomer of the corresponding β-hydroxy nitriles with high optical purity (Scheme 2 and Table 1). In all cases, no ethylated product was observed. As such, use of isolated carbonyl reductase eliminates the competing ethylation reaction in the biocatalytic reduction of aromatic β-ketonitriles, and **CMCR** and **Ymr226c** are valuable enzymes for the preparation of both enantiomers of β-hydroxy nitriles.

The reduction of an aliphatic β-ketonitrile, 4,4-dimethyl-3-oxo-pentanenitrile, was also achieved by enzymes **CMCR** and **Ymr226c** to give (*R*)- or (*S*)-4,4-dimethyl-3-hydroxypentanenitrile, respectively (Scheme 3). However, the reduction with **CMCR** as catalyst showed moderate enantioselectivity (55% ee as determined by chiral GC analysis). (*R*)-4,4-Dimethyl-3-hydroxypentanenitrile could be obtained with excellent enantiomeric purity via the reduction catalyzed by **SSCR**, a carbonyl reductase from red yeast *Sporobolomyces salmonicolor* AKU 4429.

SCHEME 3. Bioreduction of 4,4-Dimethyl-3-oxopentanenitrile



CMCR: 85% yield, 55% ee;
SSCR: 81% yield, 99% ee.

Ymr226c: 75% Yield, 99% ee.

The hydrolysis of β-hydroxy nitriles to β-hydroxy carboxylic acids still presents a challenging task in synthetic chemistry, and careful control of the reaction is required.[24] Recently, we have found that nitrilases from cyanobacterium *Synechocystis* sp. strain PCC 6803 (**NIT6803**) and *Bradyrhizobium japonicum* strain USDA110 (**bll6402**) effectively catalyzed the hydrolysis of β-hydroxy nitriles to β-hydroxy carboxylic acids under mild conditions.[23,57] (*R*)-β-Hydroxy nitriles (**2a**−**j**) obtained from **CMCR**-catalyzed reductions were hydrolyzed with nitrilase **NIT6803** to give (*R*)-β-hydroxy carboxylic acids without racemization.[56] Compared to chemical hydrolysis of nitriles, biocatalytic hydrolysis avoids the strong basic or acidic conditions and elevated reaction temperature that often result in the undesirable elimination of OH for nitriles with β-hydroxy group, yielding unsaturated byproduct.[58] In order to obtain (*S*)-β-hydroxy carboxylic acids, the (*S*)-β-hydroxy nitriles (**2a**−**k**) obtained from **Ymr226c**-catalyzed reductions were treated with nitrilase **bll6402** in potassium phosphate buffer (pH 7.2).[23] After the reaction was complete as monitored by TLC, the reaction mixture was worked up and the product β-hydroxy carboxylic acids were isolated and characterized by NMR analysis. The ee values were measured by chiral HPLC analysis.[23] The results are summarized in Table 2. As shown in Table 2, all aromatic (*S*)-β-hydroxy nitriles (**2a**−**k**) were converted to the corresponding (*S*)-β- hydroxy carboxylic acids (**3a**−**k**) in high yields. Unfortunately, (*R*)- and (*S*)-4,4-dimethyl-3-hydroxypentanenitrile could not be hydrolyzed by either nitrilase **NIT6803** or **bll6402**. Further research for an active nitrilase toward these nitriles is needed.

In order to enhance the efficiency for the synthesis of optically active β-hydroxy carboxylic acids from β-ketonitriles, the possibility of carrying out the reduction and hydrolysis in a "two-step-one-pot" fashion without the isolation of intermediates β-hydroxy nitriles was explored. A β-ketonitrile was reduced using a carbonyl reductase; after the reduction was complete as monitored by TLC analysis, a nitrilase was added to the resulting reaction mixture. As expected, the reduction product, β-hydroxy nitrile, was hydrolyzed smoothly. Table 3 summarizes the results with the catalyst combination of reductase **CMCR** and nitrilase **NIT6803**. Four tested β-ketonitriles were

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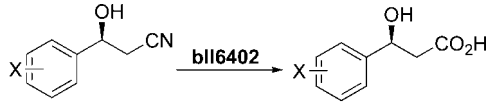
TABLE 2. Nitrilase-Catalyzed Hydrolysis of β-Hydroxy Nitriles

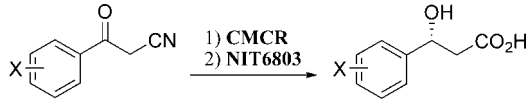


2-(*S*) **3**-(*S*)

X	time (h)	yield[a] (%)	ee[b] (%)
4-H (**2a**)	24	90	99
4-F (**2b**)	24	93	99
2,4-F_2 (**2c**)	24	91	99
4-Cl (**2d**)	24	92	99
4-Br (**2e**)	24	89	99
4-CH_3 (**2f**)	24	91	99
4-CN (**2g**)	24	92	95
4-NO_2 (**2h**)	24	87	97
3-NO_2 (**2i**)	24	88	99
3-CH_3O (**2j**)	24	88	95
4-CH_3O (**2k**)	24	91	99

[a] Isolated yield. [b] The ee value was determined by chiral HPLC analysis.[23]

TABLE 3. "Two-Step-One-Pot" Process Using Reductase CMCR and Nitrilase NIT6803

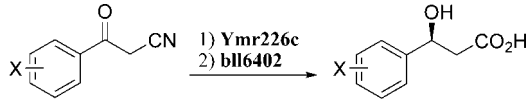


1 **3**-(*R*)

X	time[a] (h)	yield[b] (%)	ee[c] (%)	yield[d] (%)
H (**1a**)	60/24	90	99	72
4-F (**1b**)	24/24	90	99	77
4-Cl (**1d**)	24/24	93	99	77
3-CH_3O (**1j**)	24/24	92	98	80

[a] Reduction time/hydrolysis time. [b] Isolated yield. [c] The ee value was determined by chiral HPLC analysis.[23] [d] Combined yield for sequential process with isolation of β-hydroxy nitrile.

TABLE 4. "Two-Step-One-Pot" Process Using Reductase Ymr226c and Nitrilase bll6402



1 **3**-(*S*)

X	time[a] (h)	yield[b] (%)	ee[c] (%)	yield[d] (%)
H (**1a**)	48/24	92	99	75
2,4-F_2 (**1c**)	24/24	93	99	77
4-Cl (**1d**)	24/24	95	99	75
4-CH_3O (**1k**)	24/24	95	99	82

[a] Reduction time/hydrolysis time. [b] Isolated yield. [c] The ee value was determined by chiral HPLC analysis.[23] [d] Combined yield for sequential process with isolation of β-hydroxy nitrile.

converted to the corresponding (*R*)-β-hydroxy carboxylic acids with excellent yields and optical purity.

Four β-ketonitriles were examined by using reductase **Ymr226c** and nitrilase **bll6402**. As shown in Table 4, these β-ketonitriles were reduced and then hydrolyzed in a "two-step-one-pot" process to afford (*S*)-β-hydroxy carboxylic acids with great than 92% yields and in essentially optically pure form. As shown in Tables 3 and 4, the product yields in the "two-step-one-pot" process were higher than the combined yields of the sequential process with isolation of β-hydroxy nitrile. The results demonstrate that suitable combination of reductase and nitrilase offers an efficient "two-step-one-pot" process to synthesize both antipodes of β-hydroxy carboxylic acids from the readily available β-ketonitriles.

Conclusion

Effective asymmetric reduction of β-ketonitriles has been successfully achieved with isolated recombinant reductases **CMCR** and **Ymr226c**, affording (*R*)- and (*S*)-β-hydroxy nitriles with high yield and optical purity. The obtained β-hydroxy nitriles have been converted to optically active β-hydroxy carboxylic acids via nitrilase-catalyzed hydrolysis in high yields. This novel two-step enzymatic process can be carried out in a "two-step-one-pot" fashion without the isolation of intermediates β-hydroxy nitriles, offering an effective and environmentally benign methodology for preparation of optically active β-hydroxy carboxylic acids from readily available β-ketonitriles. Therefore, the present study allows ready access to both enantiomers of chiral β-hydroxy nitriles and β-hydroxy carboxylic acids with excellent enantiomerically purity, which are important pharmaceutical intermediates and chiral building blocks in organic synthesis.

Experimental Section

Carbonyl Reductase-Catalyzed Reduction of β-Ketonitriles. The reaction was carried out as follows: D-glucose (400 mg), D-glucose dehydrogenase (10 mg), NADPH (10 mg), and carbonyl reductase **Ymr226c** (10 mg) were mixed in a potassium phosphate buffer (50 mL, 100 mM, pH 7.0). To the mixture was added a 3-oxo-3-phenylpropanenitrile (**1a**) solution (170 mg 1.17 mmol) in 5.0 mL of DMSO. The mixture was stirred at room temperature and monitored by TLC until conversion was complete. The mixture was extracted with methyl *tert*-butyl ether. The organic extract was dried over anhydrous sodium sulfate, and removal of the solvent gave (*S*)-3-hydroxy-3-phenylpropanenitrile (**2a**-(*S*), 143 mg, 83% yield), which was identified by comparison of ^{1}H and ^{13}C NMR with literature data.[23,59−61] The absolute configurations of the product β-hydroxy nitriles were determined by comparison of the sign of specific rotation data with those in the literature.[18,23]

Nitrilase-Catalyzed Hydrolysis of β-Hydroxy Nitriles. The reaction was carried out as follows: (*S*)-3-Hydroxy-3-phenylpropanenitrile (**2a**-(*S*), 70 mg, 0.48 mmol) was suspended in 50 mL of potassium phosphate buffer (100 mM, pH 7.2). Nitrilase **bll6402** (3 mg) was added to the mixture, which was stirred at 30 °C. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was acidified with 2 N HCl, saturated with NaCl, and extracted with ethyl acetate. The organic extract was dried over sodium sulfate and evaporated under reduced pressure to give crude product, which was purified by preparative TLC. The product (*S*)-3-hydroxy-3-phenylpropanoic acid (**3a**-(*S*)) was obtained as a white crystalline solid (71 mg, 90% yield), which was identified by comparison of ^{1}H and ^{13}C NMR with literature data.[23,62,63] The absolute configurations of the product β-hydroxy carboxylic acids were determined by comparison of the sign of specific rotation data with those in the literature.[23,64]

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"Two-Step-One-Pot" Process. The reaction was carried out as follows: D-glucose (400 mg), D-glucose dehydrogenase (10 mg), NADPH (10 mg), and carbonyl reductase **CMCR** (10 mg) were mixed in a potassium phosphate buffer (50 mL, 100 mM, pH 7.0). To the mixture was added a 3-oxo-3-phenylpropanenitrile (**1a**) solution (150 mg 1.03 mmol) in 5.0 mL of DMSO. The mixture was stirred at room temperature and monitored by TLC until conversion was complete. The pH was adjusted to 7.2. Nitrilase NIT6803 (10 mg) was added to the mixture, which was stirred at 30 °C. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was acidified with 2 N HCl, saturated with NaCl, and extracted with ethylacetate. The organic extract was dried over sodium sulfate and evaporated under reduced pressure to give crude product, which was purified by preparative TLC using hexane/ethyl acetate (80: 20). The product (*R*)-3-hydroxy-3-phenylpropanoic acid (**3a**-(*R*)) was obtained as white crystalline solid (149 mg, 90% yield).

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Supporting Information Available: General experimental procedures, ^{1}H, ^{13}C NMR, and specific rotation data, and ^{1}H and ^{13}C NMR spectra of β-hydroxy nitriles and β-hydroxy carboxylic acid. This material is available free of charge via the Internet at http://pubs.acs.org.

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