ORGANIC LETTERS

2007
Vol. 9, No. 13
2561−2563

Asymmetric Reduction of β-Ketonitriles with a Recombinant Carbonyl Reductase and Enzymatic Transformation to Optically Pure β-Hydroxy Carboxylic Acids

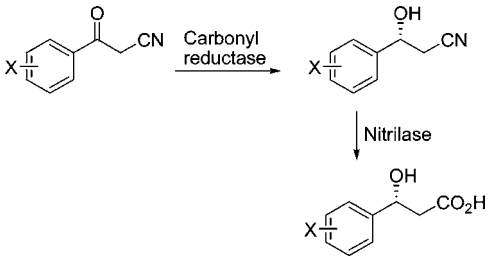
Dunming Zhu, Haribabu Ankati, Chandrani Mukherjee, Yan Yang, Edward R. Biehl, and Ling Hua*

Department of Chemistry, Southern Methodist University, Dallas, Texas 75275

lhua@smu.edu

Received April 24, 2007

ABSTRACT



α-Ethylation is concomitant with the reduction of aromatic β-ketonitriles catalyzed by whole-cell biocatalysts. Use of isolated carbonyl reductase has completely eliminated this competing reaction. (*R*)-β-Hydroxy nitriles were obtained via a reduction catalyzed by a recombinant carbonyl reductase with excellent optical purity and were further converted to (*R*)-β-hydroxy carboxylic acids via a nitrilase-catalyzed hydrolysis. The present study allows ready access to both chiral β-hydroxy nitriles and β-hydroxy carboxylic acids of pharmaceutical importance.

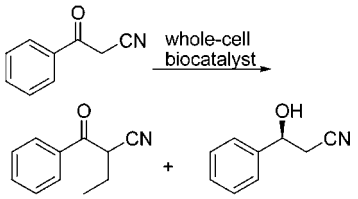
Optically active β-hydroxy nitriles are a class of important compounds because they can be converted to chiral 1,3-amino alcohols and β-hydroxy carboxylic acids. Optically active 1,3-amino alcohols have been widely used as precursors in the synthesis of β-blocker drugs and as chiral ligands in asymmetric reactions. Chiral β-hydroxy carboxylic acids are key intermediates for the synthesis of a variety of pharmaceutically important compounds. The prevalence of chiral β-hydroxy nitriles as key building blocks for the preparation of a variety of pharmacologically active compounds has stimulated the development of new methodologies for their construction, and many methods have been reported. These include asymmetric aldol-type reactions with acetonitrile,[1−5] β-boration of α, β-unsaturated nitriles followed by the oxidation,[6] borane reductions[7] and transfer hydrogenation[8−10] of β-ketonitriles, and lipase- or nitrilase-catalyzed resolution of racemic β-hydroxy nitriles.[11−14] However, these methods suffer from some drawbacks. For example, the aldol-type reactions must be carried out at much lower reaction temperature than 0 °C and give β-hydroxy nitriles of less than 80% ee. 3-Hydroxy-3-phenylpropanenitrile can be obtained with up to 98% ee by transfer hydrogenation of β-ketonitriles, but studies on the scope of this method has been limited. For the lipase- or nitrilase-catalyzed resolution, the intrinsic feature of kinetic resolution (the maximum yield of 50%) limits its application when only one enantiomer is desirable. Recently, Bäckvall et al. reported that chiral acetylated β-hydroxy nitriles could be obtained with 36−99% ee via the chemoenzymatic dynamic kinetic resolution of racemic β-hydroxynitriles using *Candida antarctica* lipase B and ruthenium catalyst.[15] Unfortunately, up to 26% of β-ketonitriles were generated as byproduct during the reaction. In addition, the residual metal in the products originated from the metal catalyst presents another challenge because of ever more stringent regulatory restrictions on the level of metals allowed in pharmaceutical products.[16]

Chiral β-hydroxy nitriles can also be prepared by the biocatalytic reduction of β-ketonitriles. However, bioreduc-

10.1021/ol070962b CCC: $37.00
Published on Web 05/24/2007

tion of β-ketonitriles has remained until now an unsolved problem. It has been scarcely reported that β-ketonitriles could be enantioselectively reduced to β-hydroxy nitriles by bakers' yeast and the fungus *Curvularia lunata*.[17,18] However, one of the most characteristic features of the bioreduction of β-ketonitriles by the reported whole-cell biocatalysts is the existence of a competing α-ethylation reaction (Scheme 1), resulting in low chemical yields of the desired β-hydroxy

Scheme 1. Whole-Cell Reduction of 3-Oxo-3-phenylpropanenitrile Concomitant with α-Ethylation[21]

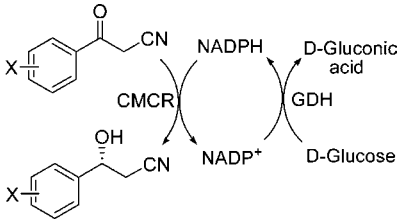


nitriles. Further studies suggested that the ethyl group may come from the ethanol produced by the yeast or fungal metabolism.[19,20] Delhi et al. surprisingly found that β-ketonitriles was reduced to (*S*)-β-hydroxy nitriles by fungus *Curvularia lunata* in 41−77% yield and 40−98% enantiopurity when methanol was used as the cosolvent.[20] Recently, an *E. coli* whole-cell system overexpressing yeast carbonyl reductases was used to improve the yield of the reduction product, but the ethylated product had not been completely eliminated.[21]

We thus reasoned that use of isolated carbonyl reductase should solve this problem because no ethanol is introduced or produced during the reaction. It has been found that a carbonyl reductase from *Candida magnoliae* (CMCR) catalyzed the reduction of a series of ketones, α- and β-ketoesters, to anti-Prelog configurated alcohols in excellent optical purity.[22] Therefore, this carbonyl reductase was applied to the reduction of a series of aromatic β-ketonitriles to test our hypothesis.

In this context, 3-oxo-3-phenylpropanenitrile (**1a**) was treated with a catalytic amount of CMCR and cofactor NADPH, which was regenerated with D-glucose and D-glucose dehydrogenase (GDH) systems (Scheme 2), in

Scheme 2. CMCR-Catalyzed Reduction of β-Keto Nitriles with D-Glucose and D-Glucose Dehydrogenase (GDH) Cofactor Recyling Systems



potassium phosphate buffer. The reaction mixture was extracted with methyl *tert*-butyl ether. Fortunately, HPLC analysis of the extract showed that 3-oxo-3-phenylpropanenitrile (**1a**) was completely converted to (*R*)-3-hydroxy-3-phenylpropanenitrile (**2a**), and no ethylated product 2-ethyl-3-oxo-3-phenylpropanenitrile was detected, indicating the competing ethylation reaction was completely eliminated. The reductions of 3-oxo-3-phenylpropanenitrile (**1a**) and other aromatic β-keton nitriles (**1b**−**j**) bearing various substituents on the phenyl ring with this carbonyl reductase were carried out at about 1 mmole scale. The reaction mixture was worked up as described in the experimental section. The product β-hydroxy nitriles were isolated and characterized by NMR analysis. The ee values were measured by chiral HPLC or GC analysis.[14] The results are presented in Table 1.

From Table 1 it can be seen that CMCR efficiently catalyzed the reduction of various aromatic β-ketonitriles bearing electron-withdrawing or electron-donating groups on the phenyl ring to the (*R*)-enantiomer of the corresponding β-hydroxy nitriles with excellent enantioselectivity. In all cases, no ethylated product was observed. As we proposed, therefore, use of isolated carbonyl reductase eliminates the competing ethylation reaction in the biocatalytic reduction of aromatic β-ketonitriles, and CMCR is a valuable enzyme for the preparation of (*R*)-β-hydroxy nitriles of pharmaceutical importance.

Chiral β-hydroxy carboxylic acids are widely used as precursors for the synthesis of a variety of pharmaceutically

(1) Suto, Y.; Tsuji, R.; Kanai, M.; Shibasaki, M. *Org. Lett.* **2005**, *7*, 3757−3760.
(2) Suto, Y.; Kumagai, N.; Matsunaga, S.; Kanai, M.; Shibasaki, M. *Org. Lett.* **2003**, *5*, 3147−3150.
(3) Sott, R.; Granander, J.; Hilmersson, G. *Chem.—Eur. J.* **2002**, *8*, 2081−2087.
(4) Granander, J.; Eriksson, J.; Hilmersson, G. *Tetrahedron: Asymmetry* **2006**, *17*, 2021−2027.
(5) Soai, K.; Hirose, Y.; Sakata, S. *Tetrahedron: Asymmetry* **1992**, *3*, 677−680.
(6) Mun, S.; Lee, J.-E.; Yun, J. *Org. Lett.* **2006**, *8*, 4887−4889.
(7) Wang, G.; Liu, X.; Zhao, G. *Synlett* **2006**, 1150−1154.
(8) Li, Y.; Li, Z.; Li, F.; Wang, Q.; Tao, F. *Org. Biomol. Chem.* **2005**, *3*, 2513−2518.
(9) Liu, P. N.; Gu, P. M.; Wang, F.; Tu, Y. Q. *Org. Lett.* **2004**, *6*, 169−172.
(10) Watanabe, M.; Murata, K.; Ikariya, T. *J. Org. Chem.* **2002**, *67*, 1712−1715.
(11) Kamal, A.; Khanna, G. B. R.; Ramu, R. *Tetrahedron: Asymmetry* **2002**, *13*, 2039−2051.
(12) Garcia-Urdiales, E.; Rebolledo, F.; Gotor, V. *Tetrahedron: Asymmetry* **2001**, *12*, 3047−3052.
(13) Itoh, T.; Mitsukura, K.; Kanphai, W.; Takagi, Y.; Kihara, H.; Tsukube, H. *J. Org. Chem.* **1997**, *62*, 9165−9172.
(14) Kamila, S.; Zhu, D.; Biehl, E. R.; Hua, L. *Org. Lett.* **2006**, *8*, 4429−4431.
(15) Pamies, O.; Backvall, J.-E. *Adv. Synth. Catal.* **2002**, *344*, 947−952.
(16) Thayer, A. *Chem. Eng. News* **2005**, *83*, 55−58.
(17) Smallridge, A. J.; Ten, A.; Trewhella, M. A. *Tetrahedron Lett.* **1998**, *39*, 5121−5124.
(18) Gotor, V.; Dehli, J. R.; Rebolledo, F. *J. Chem. Soc., Perkin Trans. 1* **2000**, 307−309.
(19) Florey, P.; Smallridge, A. J.; Ten, A.; Trewhella, M. A. *Org. Lett.* **1999**, *1*, 1879−1880.
(20) Dehli, J. R.; Gotor, V. *Tetrahedron: Asymmetry* **2000**, *11*, 3693−3700.
(21) Hammond, R. J.; Poston, B. W.; Ghiviriga, I.; Feske, B. D. *Tetrahedron Lett.* **2007**, *48*, 1217−1219.
(22) Zhu, D.; Yang, Y.; Hua, L. *J. Org. Chem.* **2006**, *71*, 4202−4205.

Table 1. CMCR-Catalyzed Reduction of β-Keto Nitriles

X	time (h)	β-hydroxy nitrile	yield (%)[a]	ee (%)[b]
4-H (**1a**)	60	**2a**	85	98 (*R*)
4-F (**1b**)	24	**2b**	90	99 (*R*)[c]
2,4-F_2 (**1c**)	24	**2c**	92	98 (*R*)[c]
4-Cl (**1d**)	24	**2d**	90	99 (*R*)
4-Br (**1e**)	36	**2e**	88	99 (*R*)
4-CH_3 (**1f**)	48	**2f**	90	98 (*R*)
4-CN (**1g**)	72	**2g**	90	99 (*R*)
4-NO_2 (**1h**)	24	**2h**	86	99 (*R*)
3-NO_2 (**1i**)	24	**2i**	87	99 (*R*)
3-CH_3O (**1j**)	24	**2j**	89	97 (*R*)

[a] Isolated yield. [b] The ee value was measured by chiral HPLC analysis.[14] [c] The ee value was measured by chiral GC analysis.

Table 2. Nitrilase-Catalyzed Hydrolysis of β-Hydroxy Nitriles[a]

X	time (h)	β-hydroxy acid	yield (%)[b]	ee (%)[c]
4-H (**2a**)	24	**3a**	85	98 (*R*)
4-F (**2b**)	24	**3b**	86	99 (*R*)
2,4-F_2 (**2c**)	24	**3c**	89	98 (*R*)
4-Cl (**2d**)	24	**3d**	86	99 (*R*)
4-Br (**2e**)	24	**3e**	85	99 (*R*)
4-CH_3 (**2f**)	24	**3f**	85	98 (*R*)
4-CN (**2g**)	24	**4g**	80	99 (*R*)[d]
4-CN (**2g**)[e]	24	**3g**	90	99 (*R*)
4-NO_2 (**2h**)	24	**3h**	88	99 (*R*)
3-NO_2 (**2i**)	24	**3i**	87	99 (*R*)
3-CH_3O (**2j**)	24	**3j**	90	97 (*R*)

[a] The nitrilase was a nitrilase from cyanobacterium *Synechocystis* sp. strain PCC 6803 (NIT6803), except indicated otherwise. [b] Isolated yield. [c] The ee value was determined by chiral HPLC analysis.[14] [d] The product was 3-(4′-carboxyphenyl)-3-hydroxypropanoic acid (**4g**). [e] A nitrilase (bll6402) from *Bradyrhizobium japonicum* strain USDA110 was used.

important compounds such as β-amino acids,[23] β-lactams,[24] β-lactones,[25] and pheromones,[26] and are the key components of polyketide natural products such as amphotericin B,[27] tylosin, and rosaramicin,[28] and the marine natural product hapalosin.[29] In addition, β-hydroxy carboxylic acids and their derivatives are well-known drugs for their anti-inflammatory activity.[30] Therefore, the (*R*)-β-hydroxy nitriles (**2a**−**j**) obtained from CMCR-catalyzed reduction were treated with a nitrilase from cyanobacterium *Synechocystis* sp. strain PCC 6803 (NIT6803) in potassium phosphate buffer (pH 7.2).[31] 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.[14] The results are summarized in Table 2. As shown in the table, all aromatic (*R*)-β-hydroxy nitriles (**2a**−**j**) were converted to the chiral (*R*)-β- hydroxy carboxylic acids (**3a**−**j**) in high yields. 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 the β-hydroxy group, yielding unsaturated byproducts.[32] It was worthy noting that 3-(4′-cyanophenyl)-3-hydroxypropanenitrile (**2g**) was hydrolyzed to 3-(4′-carboxyphenyl)-3-hydroxypropanoic acid (**4g**) when nitrilase NIT6803 was used as the catalyst. Recently, we have found that a nitrilase bll6402 from *Bradyrhizobium japonicum* strain USDA110 selectively hydrolyzed α,ω-dinitriles to give ω-cyanocarboxylic acids exclusively and showed much lower activity toward the hydrolysis of benzonitrile than for the aliphatic nitriles. Nitrilase bll6402 was thus tested for the hydrolysis of (*R*)-3-(4′-cyanophenyl)-3-hydroxypropanenitrile (**2g**), and (*R*)-3-(4′-cyanophenyl)-3-hydroxypropanoic acid (**3g**) was obtained as the sole product.

In conclusion, effective asymmetric reduction of aromatic β-ketonitriles has been successfully achieved with CMCR, and the competing ethylation reaction has been completely eliminated with this isolated recombinant enzyme. The obtained (*R*)-β-hydroxy nitriles have been converted to (*R*)-β-hydroxy carboxylic acids in high yields. This novel two-step enzymatic process has been demonstrated to be an effective and environmentally benign methodology for preparation of optically pure β-hydroxy carboxylic acids. Therefore, the present study allows ready access to both chiral β-hydroxy nitriles and β-hydroxy carboxylic acids with excellent enantiomeric purity, which are important pharmaceutical intermediates and chiral building blocks in organic synthesis. Further studies are needed to search for suitable enzymes for the synthesis of (*S*)-enantiomer of these two categories of compounds and aliphatic counterparts, as is currently being investigated in our laboratory.

Acknowledgment. This work is financially supported by DARPA (Grant HR0011-06-1-0032) and Robert A. Welch Foundation (Grant N-118).

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

OL070962B

(23) Thaisrivongs, S.; Schostarez, H. J.; Pals, D. T.; Turner, S. R. *J. Med. Chem.* **1987**, *30*, 1837−1842.
(24) Schostarez, H. J. *J. Org. Chem.* **1988**, *53*, 3628−3631.
(25) Capozzi, G.; Roelens, S.; Talami, S. *J. Org. Chem.* **1993**, *58*, 7932−7936.
(26) Oertle, K.; Beyeler, H.; Duthaler, R. O.; Lottenbach, W.; Riediker, M.; Steiner, E. *Helv. Chim. Acta* **1990**, *73*, 353−358.
(27) Nicolaou, K. C.; Chakraborty, T. K.; Ogawa, Y.; Daines, R. A.; Simpkins, N. S.; Furst, G. T. *J. Am. Chem. Soc.* **1988**, *110*, 4660−4672.
(28) Schlessinger, R. H.; Poss, M. A.; Richardson, S. *J. Am. Chem. Soc.* **1986**, *108*, 3112−3114.
(29) Palomo, C.; Oiarbide, M.; Garcia, J. M.; Gonzalez, A.; Pazos, R.; Odriozola, J. M.; Banuelos, P.; Tello, M.; Linden, A. *J. Org. Chem.* **2004**, *69*, 4126−4134.
(30) Abdel-Rahman, H. M.; Hussein, M. A. *Arch. Pharm. (Weinheim, Ger.)* **2006**, *339*, 378−387.
(31) Mukherjee, C.; Zhu, D.; Biehl, E. R.; Hua, L. *Eur. J. Org. Chem.* **2006**, 5238−5242.
(32) Hann, E. C.; Sigmund, A. E.; Fager, S. K.; Cooling, F. B.; Gavagan, J. E.; Ben-Bassat, A.; Chauhan, S.; Payne, M. S.; Hennessey, S. M.; DiCosimo, R. *Adv. Synth. Catal.* **2003**, *345*, 775−782.