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Highly enantioselective synthesis of α,α-disubstituted malonamic acids through asymmetric hydrolysis of dinitriles with *Rhodococcus* sp. CGMCC 0497†

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Highly enantioselective hydrolysis of α,α-disubstituted malononitriles by the strain *Rhodococcus* sp. CGMCC 0497 expressing both nitrile hydratase and amidase activity to give (*R*)-α,α-disubstituted malonamic acids which could be converted to valuable (*R*)- or (*S*)-α-alkylated amino acids are reported and the yields of the products are improved remarkably at a lower reaction temperature.

Nitrile-converting enzymes have been known for several decades[1] and demonstrated great potential in organic synthesis. Moreover, organonitriles are versatile intermediates in organic synthesis and can be readily prepared by a number of methods. However, the substrates studied for nitrile-converting enzymes are still very limited[2] and the ability of the enzymes to catalyze stereoselective conversion remains largely unexploited. So far, most studies focus on the enantioselective conversion of racemic nitriles, such as α-alkyl nitriles, α-hydroxy nitriles, α-amino nitriles and β-acetoxy nitriles,[3] while only a few on prochiral nitriles[4,5] especially malononitrile derivatives.[6]

The products of asymmetric hydrolysis of α,α-disubstituted malononitriles catalyzed by nitrile-converting enzymes could serve as precursors of α-alkylated α-amino acids. This class of non-proteinogenic amino acids play an important role in the design of conformationally modified bioactive peptides and in the inhibition of enzyme activities.[7] Their extensive use is only limited by the availability of enantiopure compounds in large scale.[8] As a result, the synthesis of optically pure α-alkylated α-amino acids has attracted considerable attention in recent years.

We have screened and optimized the culture condition of the strain *Rhodococcus* sp. CGMCC 0497, which was isolated by our group and proved to have high nitrile-converting activity and enantioselectivity.[9] We report here a highly efficient enantioselective hydrolysis of α,α-disubstituted malononitriles using *Rhodococcus* sp. CGMCC 0497 to afford (*R*)-α,α-disubstituted malonamic acids. We found that the results were improved remarkably when the reaction temperature decreased from 30 to 20 °C. A number of malonamic acid derivatives were obtained with excellent enantiomeric excesses and high yields.

The reactions were initially carried out at 30 °C, the conventional incubation temperature in asymmetric hydrolysis catalyzed by nitrile-converting enzymes. In accordance with the literature,[6] α-butyl-α-methylmalononitrile can be converted to (*R*)-α-butyl-α-methylmalonamic acid neatly by the strain *Rhodococcus* sp. CGMCC 0497. However, when α-benzyl-α-methylmalononitrile **1a** was used as substrate, most probably due to the steric hindrance, the product isolated was a complex mixture of hydrolysis intermediates. After 24 h, the reaction gave a mixture of (*S*)-**2** (70%, 48% ee), (*R*)-**3** (19%, 72% ee) and **4** (8%). After 90 h, the reaction gave a mixture of (*S*)-**2** (22%, 99% ee), (*R*)-**3** (35%, 6% ee) and **5a** (41%, 88% ee)(Fig. 1). By prolonging reaction time to 112 h, the mixture converted to **5a**

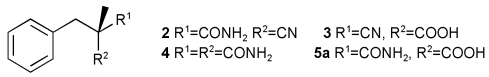
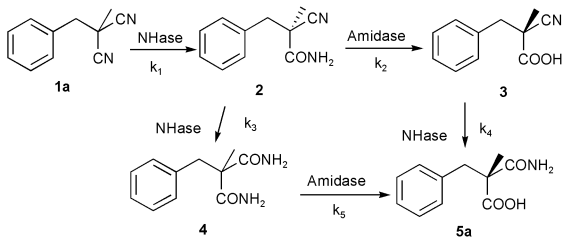


Fig. 1 Hydrolysis products of α-benzyl-α-methylmalononitrile **1a**.

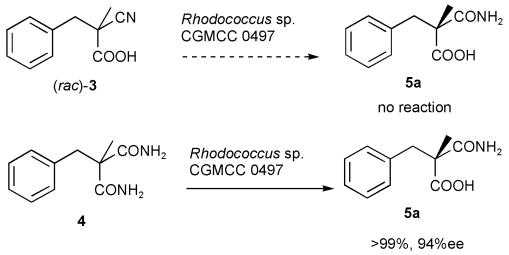
(52%, 95% ee) and **3** (44%, 1.2% ee). The high enantiomeric excess of **5a** encouraged us to better explore reaction conditions to improve its chemical yield.

It is well known that enzyme-catalyzed hydrolysis of nitriles proceeds by two routes: by nitrilase or by a combination of nitrile hydratase (NHase) and amidase through an intermediate amide.[10] *Rhodococcus* sp. CGMCC 0497 acts mainly by the latter route.[9b] The possible pathway of the hydrolysis of **1a** was illustrated as Scheme 1. It is clear that α-benzyl-α-methylmalonamic acid **5a** may derive from two ways: one involved 2-cyano-2-methyl-3-phenylpropionic acid **3** and the other involved α-benzyl-α-methylmalonamide **4**.



Scheme 1 Possible procedure of the hydrolysis of **1a**.

Further experiments were carried out using racemic **3** and **4** as substrates respectively (Scheme 2). The transformation of racemic **3** did not occur at all after one day and **3** was recovered quantitatively, while the diamide **4** was converted to **5a** in 94% ee and > 99% yield, which demonstrated that **5a** derived mostly from diamide **4** and the ratio of products **5a** to **3** depends on the value of k_3/k_2.



Scheme 2 Hydrolysis of racemic **3** and **4**.

In the successful application of NHase to the industrial production of acryamide, the reaction is performed at an especially low temperature (2–4 °C),[11,12] which, as M. Kobayashi *et al*. explained, reduces the amidase activity and exerts little effect on the NHase activity.[11] This phenomenon promoted us to explore the possibility of enhancing the value of k_3/k_2 by decreasing reaction temperature. Expectedly, decreas-

† Electronic supplementary information (ESI) available: full experimental details. See http://www.rsc.org/suppdata/cc/b2/b210743k/

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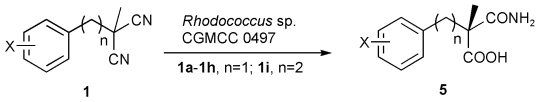
ing the reaction temperature to 20 °C increased the yield of **5a** to 84% and the enantiomeric excess was 96% ee.

The results of enantioselective hydrolysis of various α,α-disubstituted malononitriles by *Rhodococcus* sp. CGMCC 0497 (Scheme 3) at 20 °C or 30 °C are summarized in Table 1.‡ As shown, in all cases, the chemical yields of α,α-disubstituted malonamic acids **5** were greatly improved at 20 °C compared to that at 30 °C and all the products were achieved in excellent enantioselectivity. The strain tolerates aromatic ring substituents in the *ortho*-, *meta*-, and *para*-positions and all *para*-substituted substrates gave slightly high enantiomeric excesses than *ortho*- and *meta*- ones. 2-Phenylethyl-2-methylmalononitrile **1i** gave product 2-phenylethyl-2-methylmalonamic acid **5i** with 96% yield and > 99% ee as the exclusive product at 20 °C.



Scheme 3 Asymmetric hydrolysis of α,α-disubstituted malononitriles.

Table 1 Enantioselective hydrolysis of various α,α-disubstituted malononitriles by *Rhodococcus* sp. CGMCC 0497

Entry[a]	Substrate	X	*T*/°C	Yield (%)	ee (%)[b]
1	**1b**	*p*-CH_3	30	31	> 99
2	**1b**	*p*-CH_3	20	58	> 99
3	**1c**	*p*-F	30	43	> 99
4	**1c**	*p*-F	20	80	> 99
5	**1d**	*p*-Cl	30	42	> 99
6	**1d**	*p*-Cl	20	83	> 99
7	**1e**	*p*-Br	30	40	> 99
8	**1e**	*p*-Br	20	81	> 99
9	**1f**	*p*-MeO	30	30	> 99[c]
10	**1f**	*p*-MeO	20	58	> 99[c]
11	**1g**	*m*-Cl	30	40	97
12	**1g**	*m*-Cl	20	85	98
13	**1h**	*o*-Cl	30	34	98[c]
14	**1h**	*o*-Cl	20	65	99[c]
15	**1i**	H	30	90	99[d]
16	**Ii**	H	20	96	> 99[e]

[a] All the reactions were carried out for 6 days at 30 °C or 7 days at 20 °C unless stated otherwise. [b] Determined by HPLC on a Chiralpak AS column with hexane–propan-2-ol mixtures unless stated otherwise. [c] Determined by HPLC on a Chiralcel OJ column. [d] The reactions were carried out for 90 h. [e] The reactions were carried out for 98 h.

The products of the enantioselective hydrolysis of α,α-disubstituted malononitriles, (*R*)-α,α-disubstituted malonamic acids **5**, could afford either (*R*)- or (*S*)-α-alkylated amino acids after routine conversion. For example, (*R*)-α-benzyl-α-methylmalonamic acid **5a** was transferred to (*R*)-**9** or (*S*)-**9** in a yield of 81% or 89%. (Scheme 4). α-Methylphenylalanine **9** is an efficient β-turn and helix former, much stronger than its non-methylated parent compound phenylalanine.[8]

In conclusion, we have demonstrated a successful application of the strain *Rhodococcus* sp. CGMCC 0497 in the asymmetric hydrolysis of α,α-disubstituted malononitriles to afford enantiopure (*R*)-α,α-disubstituted malonamic acids, which can be converted to either (*R*)- or (*S*)-α-alkylated amino acids. The new strategy to carry out the reaction at a lower temperature greatly improved the efficacy of the reaction.

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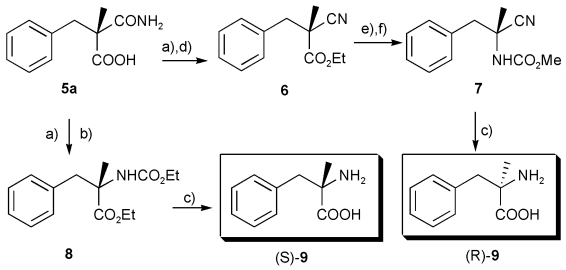

Scheme 4 Synthesis of either (*R*)- or (*S*)-α-alkylated amino acid: (a) DMF, EtBr, K_2CO_3, rt; (b)DMF, $Hg(OAc)_2$, NBS, EtOH, rt; (c) 20% HCl, reflux; (d) P_2O_5, toluene; (e) 3 N NaOH, THF, rt; (f) $SOCl_2$, NaN_3, MeOH.

Notes and references

‡ A suspension of 10 g washed wet cells and 80 ml 0.1 mM potassium phosphate buffer (pH = 7.0) was incubated at 30 or 20 °C for 30 min with continuously magnetic stirring before the addition of **1** (100 mg in 100 μl acetone). The reaction was quenched by centrifugation. The resulting supernatant was acidified and extracted with ethyl acetate and dried over Na_2SO_4. After concentration, the residue was purified by flash chromatography on silica gel (elute : petroleum ether–EtOAc–AcOH 150 : 100 : 1).

1 K. V. Thimann and S. Mahadervan, *Arch. Biochem. Biophys*, 1964, **105**, 133; Y. Asano, K. Fujishiro, Y. Tani and H. Yamada, *Agri. Biol. Chem.*, 1982, **46**, 1165.

2 J. Crosby, J. Moilliet, J. S. Parratt and and N. J. Turner, *J. Chem. Soc., Perkin Trans. 1*, 1994, 1679; M. Wieser and T. Nagasawa, in *Stereoselective Biocatalysis*, ed. R. N. Patel, Dekker, New York, 2000, pp. 461–486.

3 M.-X. Wang, G. Lu, G.-J. Ji, Z.-T. Huang, O. Meth-Cohn and J. Colby, *Tetrahedron: Asymmetry*, 2000, **11**, 1123; S. Payne, S. Wu, R. D. Fallon, G. Tudor, B. Stieglitz, J. M. Turner and M. J. Nelson, *Biochemistry*, 1997, **36**, 5447; K. Yamamoto, K. Oishi, I. Fujimatsu and K. I. Komatus, *Appl. Environ. Microbiol.*, 1991, **57**, 3028; N. Layh, A. Stolz, S. Forster, F. Effenberger and H.-J. Knackmuss, *Arch. Microbiol.*, 1992, **158**, 405; A. M. Macadam and C. J. Knowles, *Biotechnol. Lett.*, 1985, **7**, 865; M.-X. Wang and S.-J. Lin, *J. Org. Chem.*, 2002, **67**, 6542; H. Idogaki, N. Kasai, M. Takeuchi, M. Hatada and T. Suzuki, *Tetrahedron: Asymmetry*, 2001, **12**, 369.

4 G. DeSantis, Z. Zhu, W. A. Greenberg, K. Wong, J. Chaplin, S. R. Hanson, B. Farwell, L. W. Nicholson, C. L. Rand, D. P. Weiner, D. E. Robertson and M. J. Burk, *J. Am. Chem. Soc.*, 2002, **124**, 9024.

5 F. Effenberger and S. Osswald, *Synthesis*, 2001, **12**, 1866; T. Beard, M. A. Cohen, J. S. Parratt, N. J. Turner, J. Crosby and J. Moilliet, *Tetrahedron: Asymmetry*, 1993, **4**, 1085; H. Kakeya, N. Sakai, A. Sano, M. Yokoyama, T. Sugai and H. Ohta, *Chem. Lett.*, 1991, 1823.

6 M. Yokoyama, T. Sugai and H. Ohta, *Tetrahedron: Asymmetry*, 1993, **4**, 1081.

7 B. M. Trost and X. Ariza, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 2635 and references therein; D. Ma and K. Ding, *Org. Lett.*, 2000, **2**, 2515; J. S. Clark and M. D. Middleton, *Org. Lett.*, 2002, **4**, 765; T. Ooi, M. Takeuchi, M. Kameda and K. Maruoka, *J. Am. Chem. Soc.*, 2000, **122**, 5228.

8 C. Cativiela and M. D. Diaz-de-Villegas, *Tetrahedron: Asymmetry*, 1998, **9**, 3517 and references therein.

9 (*a*) Z.-L. Wu and Z.-Y. Li, *Tetrahedron: Asymmetry*, 2001, **12**, 3305; (*b*) Z.-L. Wu and Z.-Y. Li, *Biotechnol. Appl. Biochem.*, 2002, **35**, 61.

10 K. Faber, *Biotransformations in organic chemistry: a textbook*, 4th ed., Spring-Verlag, Berlin, 2000.

11 M. Kobayashi, T. Nagasawa and H. Yamada, *TIBTECH*, 1992, **10**, 402.

12 M. Kobayashi and S. Shimizu, *Curr. Opin. Chem. Biol.*, 2000, **4**, 95.