

Highly enantioselective synthesis of α,α -disubstituted malonic 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 malonic 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 decades¹ 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 limited² 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 malonic acids. We found that the results were improved remarkably when the reaction temperature decreased from 30 to 20 °C. A number of malonic 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 -methylmalonic 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**

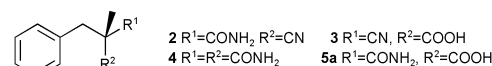
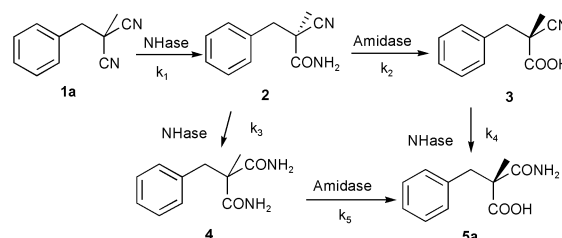


Fig. 1 Hydrolysis products of α -benzyl- α -methylmalononitrile **1a**.

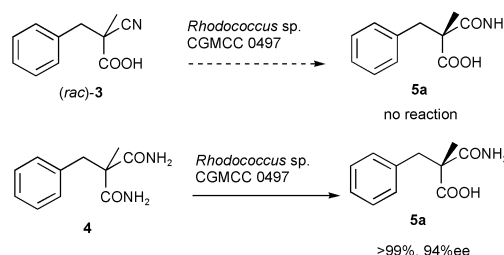
(52%, 95% ee) and **3** (44%, 1.2% ee). The high enantiomeric excess of **5a** encouraged us to better explore reaction conditions to improve its chemical yield.

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 -methylmalonic 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 .



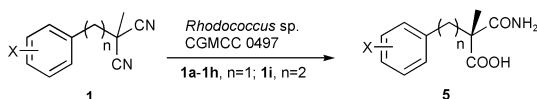
Scheme 2 Hydrolysis of racemic **3** and **4**.

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/>

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

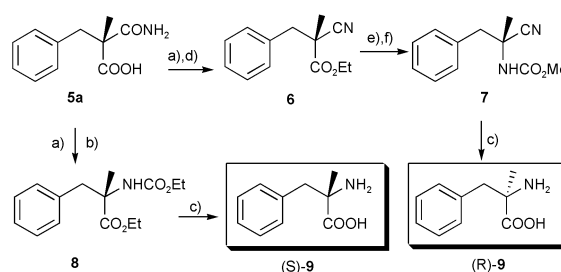
Entry ^a	Substrate	X	T/°C	Yield (%)	ee (%) ^b
1	1b	<i>p</i> -CH ₃	30	31	> 99
2	1b	<i>p</i> -CH ₃	20	58	> 99
3	1c	<i>p</i> -F	30	43	> 99
4	1c	<i>p</i> -F	20	80	> 99
5	1d	<i>p</i> -Cl	30	42	> 99
6	1d	<i>p</i> -Cl	20	83	> 99
7	1e	<i>p</i> -Br	30	40	> 99
8	1e	<i>p</i> -Br	20	81	> 99
9	1f	<i>p</i> -MeO	30	30	> 99 ^c
10	1f	<i>p</i> -MeO	20	58	> 99 ^c
11	1g	<i>m</i> -Cl	30	40	97
12	1g	<i>m</i> -Cl	20	85	98
13	1h	<i>o</i> -Cl	30	34	98 ^c
14	1h	<i>o</i> -Cl	20	65	99 ^c
15	1i	H	30	90	99 ^d
16	1i	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, K₂CO₃, rt; (b) DMF, Hg(OAc)₂, NBS, EtOH, rt; (c) 20% HCl, reflux; (d) P₂O₅, toluene; (e) 3 N NaOH, THF, rt; (f) SOCl₂, NaN₃, MeOH.

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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 μ l acetone). The reaction was quenched by centrifugation. The resulting supernatant was acidified and extracted with ethyl acetate and dried over Na₂SO₄. After concentration, the residue was purified by flash chromatography on silica gel (elute: petroleum ether–EtOAc–AcOH 150:100:1).

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