Organic Process Research & Development **2000**, *4*, 318–322

Stereoretentive Nitrile Hydratase-Catalysed Hydration of D-Phenylglycine Nitrile

M. A. Wegman,[†] U. Heinemann,[‡] A. Stolz,[‡] F. van Rantwijk,[†] and R. A. Sheldon*[,†]

Laboratory of Organic Chemistry and Catalysis, Delft University of Technology, Julianalaan 136, 2628 BL Delft, The Netherlands, and Institut für Mikrobiologie der Universität Stuttgart, Allmandring 31, 70569 Stuttgart, Germany

Abstract:

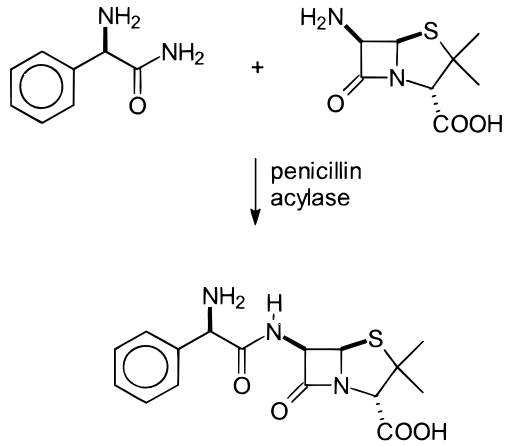
The hydration of D-phenylglycine nitrile to the corresponding amide, mediated by nitrile hydratase-containing microorganisms, was studied. Batch and fed-batch reactions were compared with regard to degradation and racemisation of the chemically labile substrate. A batch process gave satisfactory results and at up to 25 mM D-phenylglycine nitrile (D-1), D-phenylglycine amide was obtained in 94% yield with 92% ee using an immobilised *Rhodococcus* sp. (NOVO SP 361). The enzyme could be reused, although it slowly lost its activity. When the concentration of D-phenylglycine nitrile was increased to 100 mM in a batch reaction rapid decomposition of the substrate was observed and D-phenylglycine amide was obtained in only 37% yield. A fed-batch reaction afforded an improved yield, although the decomposition of the substrate could not be avoided completely. Lowering the temperature stabilised the substrate, and a fed-batch reaction at 5 °C resulted in a 96% yield of D-phenylglycine amide with 95% ee. A number of other whole-cell hydratase/amidase systems also hydrated D-1 in nearly quantitative yield and >94% ee. Moreover, the ee was further increased to >99% upon prolonged reaction times with minimal loss in yield due to the action of the L-specific amidase that is present in these biocatalysts.

Introduction

D-Phenylglycine amide is a key intermediate in the industrial enzymatic synthesis of semisynthetic β-lactam antibiotics[1] (Scheme 1).

Several routes to D-phenylglycine amide are known, all starting with a Strecker reaction on benzaldehyde (Scheme 2). A route that involves an aminopeptidase catalysed L-specific hydrolysis of D,L-phenylglycine amide[2−4] has been commercialised by DSM. In this kinetic resolution the yield of D-phenylglycine amide is limited to 50%, and the unwanted isomer, L-phenylglycine (L-PG), has to be racemised and recycled, which requires several steps. A more elegant approach is an asymmetric transformation of the diastereomeric salt of D,L-phenylglycine amide with L-mandelic acid (MA) in the presence of a catalytic amount of a carbonyl compound.[5,6] A 100% yield of enantiopure D-phenylglycine amide is—theoretically—possible, but the necessary recycle of L-mandelic acid complicates the process.

Scheme 1. **Enzyme-catalysed coupling of D-phenylglycine amide and 6-aminopenicillic acid**



We consider an alternative route to D-phenylglycine amide directly from the α-amino nitrile. Enantiopure D-phenylglycine nitrile tartaric acid salt is readily accessible via an asymmetric transformation with tartaric acid (TA) as resolving agent based on described procedures.[7−9] We investigated the stereoretentive nitrile hydratase-catalysed hydration of D-phenylglycine nitrile (D-**1**) into D-phenylglycine amide (D-**2**), (Scheme 3).

Most published work on enzymatic conversions of α-amino nitriles has been directed at the production of optically pure amino acids via a nitrilase-catalysed conversion.[10−13] Only a few groups reported an α-aminonitrile hydrolysis via a nitrile hydratase/amidase system, yielding D-amide and L-acid.[14−17]

* Corresponding author. Fax: (+31) 152784700. E-mail: r.a.sheldon@tnw.tudelft.nl.

† Delft University of Technology.

‡ Institut für Mikrobiologie der Universität Stuttgart.

(1) Bruggink, A.; Roos, E. S.; de Vroom, E. *Org. Process. Res. Dev.* **1998**, *2*, 128.

(2) Elferink, V. H. M.; Breitgoff, D.; Kloosterman, M.; Kamphuis, J.; van den Tweel, W. J. J.; Meijer, E. M. *Recl. Trav. Chim. Pays-Bas.* **1991**, *110* 63

(3) Patel, R. N. *Stereoselective Biocatalysis*; Dekker: New York, 2000; p 23.

(4) Taillades, J.; Garrel, L.; Guillen, F.; Collet, H.; Commeyras, A. *Bull. Soc. Chim. Fr.* **1995**, *132*, 119.

(5) Boesten, W. H. J. Eur. Patent. Appl. EP 442584, 1991. [*Chem. Abstr.* **1992**, *116*: 42062r].

(6) Boesten, W. H. J. Eur. Patent. Appl. EP 442585, 1991. [*Chem. Abstr.* **1992**, *116*: 42063s].

(7) Clark, J. C.; Phillipps, G. H.; Steer, M. R. *J. Chem. Soc. Perkin. Trans. I* **1976**, 475.

(8) Hylton, T. A.; Shenton, F. L. U.S. Patent 4072698, 1978. [*Chem. Abstr.* **1978**, *88*: 152276x].

(9) Matthews, G. J. French Patent 2141354, 1971. [*Chem. Abstr.* **1973**, *79*: *P*42210e].

(10) Bhalla, T. C.; Miura, A.; Wakamoto, A.; Ohba, Y.; Furuhashi, K. *Appl. Microbiol. Biotechnol.* **1992**, *37*, 184.

(11) Fukuda, Y.; Fukui, M.; Harada, T.; Izumi, Y. *J. Ferment. Technol.* **1971**, *49*, 1011.

(12) Layh, N.; Hirrlinger, B.; Stolz, A.; Knackmuss, H.-J. *Appl. Microbiol. Biotechnol.* **1997**, *47*, 668.

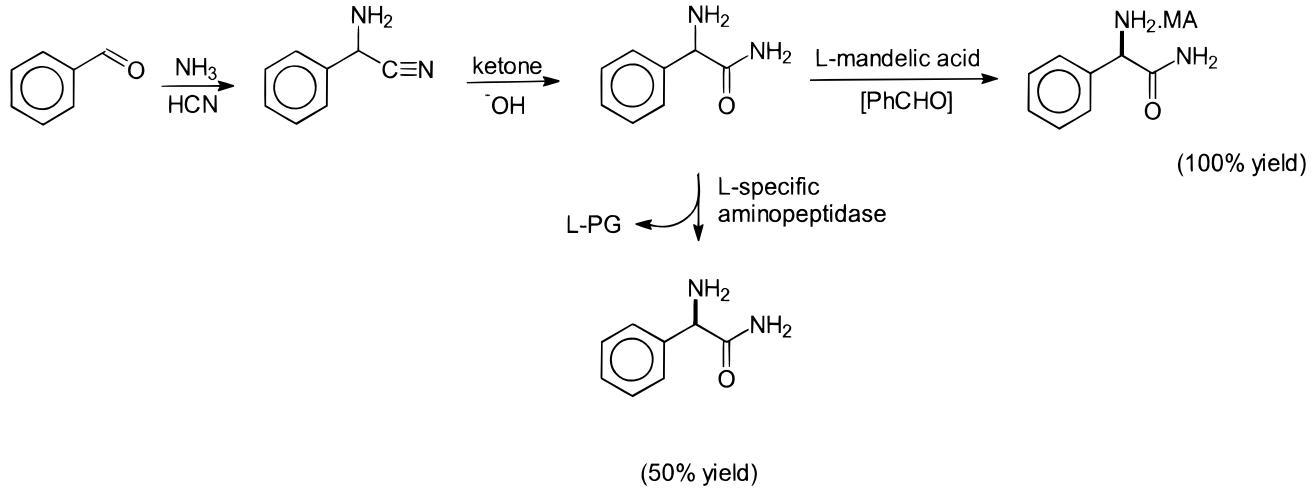
(13) Macadam, A. M.; Knowles, C. J. *Biotechnol. Lett.* **1985**, *7*, 865.

(14) Arnaud, A,; Galzy, P.; Jallageas, J.-C. *Bull. Soc. Chim. Fr. II* **1980**, 87.

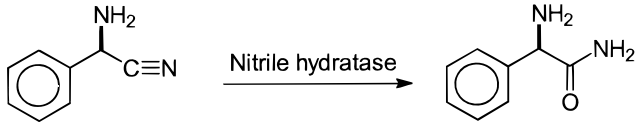
(15) Bauer, R.; Hirrlinger, B.; Layh, N.; Stolz, A.; Knackmuss, H.-J. *Appl. Microbiol. Biotechnol.* **1998**, *42*, 1.

 10.1021/op000055l CCC: $19.00
Published on Web 08/26/2000

Scheme 2. **Several routes to D-phenylglycine amide**



Scheme 3. **Hydration of D-phenylglycine nitrile**



D-**1** D-**2**

The bioconversion of α-amino nitriles is hampered by their spontaneous degradation into aldehyde and ammonia via a retro-Strecker reaction. This reaction takes place readily under the conditions that are optimal for the enzymatic reaction, that is, pH 7,[13,18] with low yields of amides as a result. Decomposition of the nitrile could partially be avoided by performing the reaction at concentrations of 0.5−1.5 mM, which have low synthetic significance.[15−17]

We have undertaken to improve the nitrile hydratase-catalysed conversion of D-**1** into D-**2** by reaction engineering. As biocatalyst we selected Novo SP 361, an immobilised *Rhodococcus* sp. that has been widely used. Herein we report two different concepts for the SP 361-catalysed hydration of D-**1** with the aim of achieving high yields of D-phenylglycine amide (D-**2**) by minimising competing racemisation and degradation of D-**1**.

Experimental Section

Chemicals and Catalysts. Immobilised *Rhodococcus* sp. SP 361 was a gift from Novo Nordisk A/S (Bagsværd, Denmark). Enantiomerically pure D-phenylglycine, D-phenylglycine amide, and D-phenylglycine nitrile tartaric acid salt (Alpha Drug, India LTD) were kindly donated by DSM (Geleen, The Netherlands). Racemic phenylglycine nitrile hydrochloride (technical grade) and racemic phenylglycine were obtained from Acros. The nitrile was purified by neutralisation of the α-aminonitrile hydrochloric acid salt with base, followed by an extraction with dichloromethane. The combined organic layers were acidified with 1 N HCl, and the nitrile hydrochloric acid salt was re-extracted in the aqueous phase, which was concentrated in vacuo, to give racemic phenylglycine nitrile hydrochloride as a white solid. Racemic phenylglycine amide was chemically synthesised via ammonolysis of the corresponding methyl ester.[19]

D-Phenylglycine nitrile was used as the free base. Therefore the pH of a 25 mM solution of D-phenylglycine nitrile tartaric acid in water was adjusted to pH 5 with 2 N sodium hydroxide. The aqueous phase was extracted with CH_2Cl_2, dried over $MgSO_4$, and concentrated in vacuo to give D-**1** as a yellowish oil, which was used directly.

During the enzymatic reactions there is a potential risk of producing cyanide via retro-Strecker reaction of D-phenylglycine nitrile. Care must be taken by performing all reactions in a fume cupboard provided with an efficient draught.

Microorganisms, Culture Conditions and Growth Media. Bacterial cultures were isolated by enrichment from soil taken from the area of Stuttgart (Germany). Nitrogen-free media were prepared in a mineral medium containing per liter: 11.2 g of Na_2HPO_4, 4 g of KH_2PO_4, 0.05 g of $CaCl_2 \cdot 2H_2O$, 0.03 g of Fe^{3+}-citrate, 0.02 g of $MgSO_4 \cdot 7\ H_2O$, and 1 mL of a standard trace element solution[20] without iron salts and EDTA. With *R. globerulus* MAWA fructose (18 $g \cdot L^{-1}$) was added as the sole source of carbon and energy, and phenylacetonitrile (1 $g \cdot L^{-1}$) was added as the sole source of nitrogen. The other strains were cultured with glycerol (10 $g \cdot L^{-1}$) as the sole source of carbon and energy. Additionally, urea[21] (1 $g \cdot L^{-1}$) and 1 mL of a vitamin solution were added.[22] In all cases phenylacetonitrile (1 $g \cdot L^{-1}$) was added as the source of nitrogen.

Cells were grown in 300-ml flasks with baffles with 50 mL of medium at 25 °C with shaking (125 rpm). After 24 h incubation cultures were transferred (1:20 v/v) to fresh medium with the same composition. After 24−48 h, the cells

(16) Choi, S. Y.; Goo, Y. M. *Arch. Pharmacal Res.* **1986**, *9*, 45.
(17) Stolz, A.; Trott, S.; Binder, M.; Bauer, R.; Hirrlinger, B.; Layh, N.; Knackmuss, H.-J. *J. Mol. Catal. B: Enzymatic* **1998**, *5*, 137.
(18) Ospiran, I.; Jarret, C.; Strauss, U.; Kroutil, W.; Orru, R. V. A.; Felfer, U.; Willetts, A. J.; Faber, K. *J. Mol. Catal. B: Enzymatic* **1999**, *6*, 555.
(19) Reilen, H.; Knöpfe, J. *Liebigs Ann. Chem*. **1936**, *523*, 199.
(20) Pfennig, M.; Lippert, K. D. *Arch. Mikrobiol*. **1966**, *55*, 245.
(21) Kobayashi, M.; Fujita, T.; Shimizu, S. *Appl. Microbiol. Biotechnol*. **1996**, *45*, 176.
(22) Hunter-Cevera, J. C.; Belt, A. *Manual of Industrial Microbiology and Biotechnology;* ASM Press: Washington, 1999; p 3.

Table 1. **Nitrile hydratase activity of several strains**

strain	culture time (h)	wet biomass (g/L)	total activity (U)	specific activity (U/g)
R. globerulus MAWA	48	3.0	213	71
R. rhodochrous MAWB	16	6.0	228	38
R. rhodochrous MAWE	16	7.2	403	56
R. erythropolis MAWF	24	2.7	59	22

were, in the late exponential growth phase, harvested by centrifugation (Table 1).

Initial Taxonomic Characterisation and Classification of the Bacterial Strains. For the taxonomic description the almost complete 16S rDNA was amplified by PCR; the resulting fragment was cloned and partially sequenced using the universal primers 27f and 519r.[23] The nucleotide sequences obtained were compared with the NCBI database using the program BLASTN. Strains MAWB and MAWE showed the highest degree of sequence identity (more than 99% sequence identity) with strain *R. rhodochrous* NCIMB 13064 (this strain is listed in the NCBI database as *R. erythropolis*). A BLAST search with the sequence obtained for the strain MAWF showed the highest degree of similarity (>99%) with a sequence (X80618) deposited for *Nocardia calcarea*. Because *N. calcarea* is generally considered as a synonym for *R. erythropolis*,[24] this strain was designated as *R. erythropolis* MAWF. The strains are deposited at the Deutsche Sammlung von Mikroorganismen (DSM) (Braunsweig, Germany).

Enzyme Assay and Definition of Units. An appropriate amount of whole cells was incubated with 2% (v/v) Triton X-100 for 30 min. After washing with 0.9% NaCl, 2 mL of 54 mM sodium phosphate buffer pH 7.4 and 4 μmol phenylpropionitrile (400 mM stock solution in ethanol) were added and shaken at room temperature. Samples were periodically withdrawn, the cells were removed by centrifugation, and the supernatant was analysed by HPLC. One unit (U) of nitrile hydratase activity is defined as the amount of enzyme (g wet biomass) that converts 1 μmol phenylpropionitrile per min.

Batch-Wise Hydration of D-Phenylglycine Nitrile. D-Phenylglycine nitrile (0.25 mmol, 33 mg) and 0.5 g of SP 361 in 10 mL of sodium phosphate buffer (10 mM) were shaken in 50-mL reaction vessels at room temperature at a selected pH, which was kept constant using an automatic titrator (Metrohm 665 Dosimat and a Metrohm 614 Impulsomat). The reactions were monitored by periodically withdrawing 0.1-mL samples from the reaction mixture. To stop the reaction in the sample 0.5 mL of 0.1 N $HClO_4$ was added, followed by HPLC analysis. The experiments were performed in duplo and were reproducible within the margin of error. The symbols in Figures 1 and 2 indicate the error margin.

Fed-Batch Hydration of D-Phenylglycine Nitrile. D-Phenylglycine nitrile (1.0 mmol, 132 mg) in 5 mL of 0.2 N H_2SO_4 was added in 1−5 h to—unless mentioned otherwise—0.5 g of SP 361 in 5 mL of sodium phosphate buffer (10 mM). The reaction mixture was shaken at the selected temperature at pH 7. The pH was kept constant using an automatic titrator (Metrohm 665 Dosimat and a Metrohm 614 Impulsomat). The reactions were monitored by periodically withdrawing 0.1-mL samples from the reaction mixture. To stop the reaction in the sample 0.5 mL of 0.1 N $HClO_4$ was added, followed by HPLC analysis. The experiments were performed in duplo and were reproducible within the margin of error. The symbols in Figure 3 indicate the error margin.

(23) Gerhardt, P.; Murray, R. G. E.; Wood, W. A.; Krieg, N. R. *Methods for General and Molecular Bacteriology*; A.S.M Press: Washington, 1994.
(24) Rainey, F. A.; Burghardt, J.; Kroppenstedt, R. M.; Klatte, S.; Stackebrandt, E. *Microbiology* **1995**, *141*, 523.

Analysis and Equipment

The reaction mixtures of the stereoretentive hydration were analysed by chiral HPLC on a Daicel Chemical Industries Ltd. 4.6 × 150 mm 5 μ Crownpak CR (+) column using a Waters 625 LC pump and a Waters 486 UV detector. The eluent was aqueous $HClO_4$, pH 1.0 at a flow of 0.6 mL·min^{-1}, the column temperature was 18 °C.

The reaction mixtures of the nitrile hydratase activity assays were analysed by HPLC on a custom-packed Symmetry C_{18} cartridge (Waters Radial-Pak, 8 × 100 mm, 5 μm) contained in a Waters RCM 8 × 10 compressing unit. A Waters 590 pump and a Waters 486 UV detector were used. The eluent was acetonitrile/water 40/60 with 0.1% trifluoroacidic acid at a flow of 1.0 mL·min^{-1}.

Results and Discussion

SP 361-Catalysed Batch Reaction at Low pH. Although pH 7 is optimal for SP 361, initial experiments were conducted at pH 5 in an attempt to minimise racemisation and degradation of D-**1**. Even at such a low pH D-**1** was hydrated to D-**2** by the non-selective nitrile hydratase, and racemisation as well as decomposition of D-**1** were negligible. The amidase appeared to be slow and highly L-specific, and no formation of phenylglycine was observed. However, when the enzyme was reused in a second cycle, its performance had decreased considerably. This effect was more pronounced when the substrate was used as its tartaric acid salt rather than as the free base (Table 2). We tentatively concluded that tartaric acid deactivated SP 361; hence, further experiments were performed with D-**1** as the free base.

Table 2. **Hydration of D-phenylglycine nitrile at pH 5**[a]

		cycle 1		cycle 2	
substrate	time (h)	yield $_{D-2}$ (%)	ee $_{D-2}$ (%)	yield $_{D-2}$ (%)	ee $_{D-2}$ (%)
tartaric acid salt	3	65	95	11	86
free base	3	70	99	31	94
	5	77	99	38	92

[a] **Reaction conditions**: 0.25 mmol α-aminonitrile D-**1** and 500 mg SP 361 in 10 mL of 10 mM phosphate buffer pH 5 were shaken at room temperature.

To gain more insight into the deactivation of SP 361, the effect of the buffer on the enzymatic hydration of D-**1** was briefly investigated. A 10 mM phosphate buffer had a slight accelerating effect compared with reaction in the absence

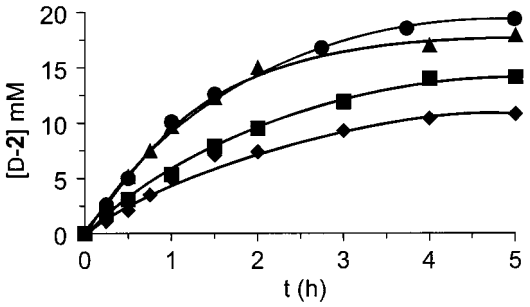


Figure 1. **SP 361-catalysed hydration of D-1 (25 mM) at pH 5 in various buffers: (▲) no buffer, (●) 10 mM phosphate, (■) 100 mM phosphate, (◆) 100 mM acetate.**

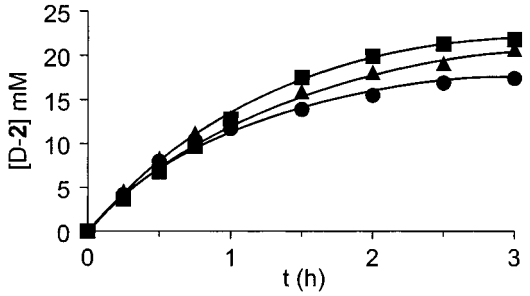


Figure 2. **SP 361-catalysed batch-wise hydration of D-1 (25 mM) at pH 7 (■) cycle 1, (▲) cycle 2, (●) cycle 3.**

of buffer, presumably because of the unavoidable pH excursions in the latter case. Phosphate (100 mM) caused a decrease in rate as well as in yield; acetate (100 mM) had an even more pronounced effect (Figure 1).

In an attempt to reactivate the catalyst $FeCl_3$ (1 mM) as well as $CoCl_2$ (1 mM) were added to the reaction mixture, since it is known that some nitrile hydratases contain iron or cobalt as cofactor,[25,26] but no increase in enzyme activity was observed. We conclude that D-**1** can be hydrated to D-**2** at pH 5 without racemisation or decompostion, but recycling of the catalyst is not possible. In view of these results all further experiments were carried out at pH 7.

SP 361-Catalysed Batch Reaction at pH 7. At pH 7 D-**1** spontaneously racemises and decomposes, but this can be minimised by keeping its concentration low. Surprisingly we found that up to 25 mM D-**1** was converted to D-**2** in high yield with negligible decomposition or racemisation. Again no formation of acid was observed. The enzyme could be recycled several times, although its activity slowly declined (Figure 2).

When the substrate concentration was increased to 100 mM, the above procedure yielded only 37 mM D-**2.** Racemisation of D-**1** was negligible (5.5%), but its decomposition into benzaldehyde and ammonia amounted to 43%. In conclusion, this simple methodology is not feasible at practically relevant concentrations.

SP 361-Catalysed Fed-Batch Reactions at pH 7. A fed-batch procedure would present an option to combine a low actual concentration of D-**1**—to retard its decomposition—with a high over-all throughput.

(25) Sugiura, Y.; Kuwahara, J.; Nagasawa, T.; Yamada, H. *J. Am. Chem. Soc.* **1987**, *109*, 5848.
(26) Nagasawa, T.; Takeuchi, K.; Yamada, H. *Eur. J. Biochem.* **1991**, *196*, 581.

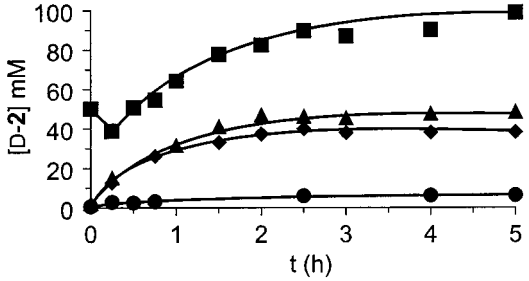


Figure 3. **Study of inhibition effect on SP 361 in a (▲) fed-batch reaction (feeding in 1 h) of D-1 (100 mM) at pH 7 in the presence of (■) 50 mM D-phenylglycine amide, (◆) 100 mM sulfate, (●) 0.5 mM benzaldehyde.**

Accordingly, we carried out fed-batch reactions by adding a 200 mM solution of D-**1**, resulting in a final concentration of 100 mM reaction mixture. When the feedstock was added in 1 h, 50 mM D-**2** was obtained (whereas the batch reaction yielded only 37 mM D-**2**). However, attempts to increase the yield by prolonging the addition time from 1 to 5 h failed; in all cases the reaction stopped at 50 mM D-**2**.

A turnover effect would be a possible explanation, but when the amount of catalyst was doubled, only 79 mM D-**2** was obtained instead of the expected 100 mM D-**2**. This prompted us to investigate inhibition effects on SP 361 (Figure 3). First, product inhibition was examined. When the reaction was performed in the presence of 50 mM D-**2**, no decrease in reaction rate was observed; therefore, we concluded that SP 361 is not inhibited by the product. The effect considered next was ionic strength, because in all fed-batch processes D-**1** had been added to the reaction mixture as its sulphuric acid salt, whereas in the batch reactions at pH 5 the enzyme activity decreased at high buffer concentrations. Possible inhibition by sulfate was examined by performing the reaction in the presence of 100 mM Na_2SO_4, but only a small decrease in enzyme activity was observed. Moreover, when D-**1** was added as the free base, the reaction also stopped at 50 mM D-**2**. Finally, the possible inhibition by the retro-Strecker product, benzaldehyde, was investigated. We found that benzaldehyde caused a drastic decrease in enzyme activity at concentrations as low as 0.5 mM. Hence, we conclude that the decomposition of D-**1** is the main cause of deactivation of the nitrile hydratase, besides being detrimental to the yield.

SP 361-Catalysed Reactions at Low Temperature. On the basis of the above findings it is clear that to increase the yield of D-**2**, the stability of D-**1** towards benzaldehyde formation via a retro-Strecker reaction must be increased. We found that decomposition—and racemisation—of D-**1** is less at low temperatures. After 24 h at 5 °C and pH 7 spontaneous degradation of D-**1** (100 mM) to benzaldehyde was 13%, and the ee of the remaining D-**1** was 82% compared to 75% and complete racemisation at room temperature.

At 5 °C a 100 mM batch reaction yielded 63 mM D-**2**, compared with 37 mM at room temperature. This prompted us to carry out a fed-batch reaction at low temperature. The feedstock (200 mM D-**1** in 0.2 N H_2SO_4) was added over 3 h. The reaction was followed in time, and after 7 h we obtained 96% yield of D-**2** (102 mM) with 95% ee. Hence,

Table 3. **Fed-batch hydration of D-phenylglycine nitrile by different strains[a]**

entry	enzyme (U)	time (h)	yield (%)	ee (%)
1	SP 361 (−)	7	96.0	95.0
2	*R. globerules* MAWA (69)	4	98.7	94.6
3	*R. erythropolis* MAWF (44)	7	95.6	97.4
4	*R. rhodochrous* MAWE (56)	7	98.6	96.8
5	*R. rhodochrous* MAWB (38)	6	95.4	98.3
		22	95.1	99.5

[a] **Reaction conditions:** 1 mmol α-amino nitrile D-**1** in 5 mL of 0.2 N H_2SO_4 was added in 3 h to the microorganism in 5 mL of sodium phosphate buffer (10 mM). The reaction mixture was shaken at 5 °C.

by performing the reaction in a fed-batch mode at low temperatures decomposition is avoided, and D-**2** can be obtained in nearly quantitative yield.

Other Nitrile Hydratase/Amidase Systems. Because of uncertainty regarding the commercial availability of SP 361 other nitrile hydratase/amidase systems were also tested in our set-up in order to demonstrate the general applicability of our fed-batch procedure at low temperature for the conversion of chemically labile D-**1**. We selected several microorganisms which hydrated D,L-phenylglycine nitrile **1** efficiently.[27] The bacterial cultures were grown on defined media; the whole cells were permeabilised by incubation with Triton X-100 and were used without further treatment in the fed-batch hydration of D-**1**.

In all cases D-**1** was hydrated to D-**2** with high yield and ee (Table 3). Moreover, the enantiomeric purity of D-**2** could be increased—with a minimum loss in yield—by running the reaction for a longer time (Table 3, entry 5). The amidase present in the microorganism converted the unwanted L-amide slowly into the corresponding L-acid, resulting in 95% yield of D-**2** with >99% ee.

(27) Wegman, M. A.; Heinemann, U.; van Rantwijk, F.; Stolz, A.; Sheldon, R. A. *J. Mol. Catal. B: Enzymatic* **2000**, *in press*.

Conclusions

Notwithstanding the instability of D-phenylglycine nitrile (D-**1**), its hydration to the corresponding amide (D-**2**) is feasible in high yield. Up to 25 mM D-**1**, a batch reaction at room temperature gives D-**2** in 94% yield and 92% ee. At higher substrate concentrations a fed-batch mode of operation at lower temperatures is necessary to achieve high yields and enantiopurities; for example, at 5 °C and a final concentration of 100 mM, D-**2** was obtained in 95% yield and 94% ee. This concept appeared to be a generally applicable method, as other nitrile hydratase/amidase systems gave similar results. Moreover, the L-amidase yielded an increased ee up to 99.5% with minimal loss in yield.

Acknowledgment

Generous donations of nitrile hydratase/amidase SP 361 by Novo Nordisk A/S (Bagsværd, Denmark) are gratefully acknowledged. DSM Life-Sciences is acknowledged for a gift of D-phenylglycine nitrile tartaric acid salt. We thank Dr. P. Porskamp of DSM Deretil (Almeria, Spain) for his encouragement and Professor Dr. L. Fischer (University of Stuttgart-Hohenheim, Germany) for his help with cultivating the several nitrile hydratase/amidase systems. This work was financially supported by The Netherlands Ministry of Economic Affairs and coordinated by DSM Life Sciences (Geleen, The Netherlands).

Received for review May 19, 2000.

OP000055L