Chemoenzymic Production of Lactams from Aliphatic α,ω-Dinitriles

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Received March 9, 1998

Five- and six-membered ring lactams have been prepared by first converting an aliphatic α,ω-dinitrile to an ω-cyanocarboxylic acid ammonium salt, using a microbial cell catalyst having an aliphatic nitrilase activity (*Acidovorax facilis* 72W, ATCC 55746) or a combination of nitrile hydratase and amidase activities (*Comamonas testosteroni* 5-MGAM-4D, ATCC 55744). The ω-cyanocarboxylic acid ammonium salt was then directly converted to the corresponding lactam by hydrogenation in aqueous solution, without isolation of the intermediate ω-cyanocarboxylic acid or ω-aminocarboxylic acid. Only one of two possible lactam products was produced from α-alkyl-substituted α,ω-dinitriles, where the nitrilase of *A. facilis* 72W regioselectively hydrolyzed only the ω-cyano group to produce a single cyanocarboxylic acid ammonium salt in greater than 98% yield.

Introduction

Nitriles are readily converted to the corresponding carboxylic acids by a variety of chemical methods, but these methods typically require strongly acidic or basic reaction conditions and high reaction temperatures, and also often generate inorganic salts as unwanted byproducts.[1] Enzyme-catalyzed hydrolysis of nitriles to the corresponding carboxylic acids can be preferable to some chemical methods, since the enzymatic reactions are often run at ambient temperature, do not require the use of strongly acidic or basic reaction conditions, and do not produce large amounts of undesirable byproducts. An additional advantage of enzyme-catalyzed hydrolysis of nitriles is that, for a variety of aliphatic or aromatic dinitriles, the hydrolysis reaction can be highly regioselective, where only one of the two nitrile groups is hydrolyzed to the corresponding carboxylic acid ammonium salt.

The use of aromatic nitrilases for the hydrolysis of aromatic nitriles to the corresponding carboxylic acid ammonium salts (with no formation of an amide intermediate) has been well-documented,[2] but it is only recently that aliphatic nitrilases (EC 3.5.5.7) from *Rhodococcus rhodochrous* K22,[3] *Comamonas testosteroni Ct*,[4] and *Rhodococcus rhodochrous* NCIMB 11216[5] have been reported. A combination of two enzymes, nitrile hydratase (NHase, EC 4.2.1.84) and amidase (EC 3.5.1.4), can also be used to convert aliphatic nitriles to the corresponding carboxylic acid ammonium salts in aqueous solution.[6] The nitrile is initially converted by the nitrile hydratase to an amide, which is subsequently converted by the amidase to the corresponding carboxylic acid ammonium salt. A wide variety of bacteria possess a diverse spectrum of nitrile hydratase and amidase activities, including *Rhodococcus*,[7] *Pseudomonas*,[8] *Corynebacterium*,[9] *Brevibacterium*,[10,11] *Bacillus*,[11] *Bacteridium*,[11] and *Micrococcus*.[11] The fungus *Fusarium merismoides* TG-1 has also been used as catalyst for the hydrolysis of aliphatic nitriles and dinitriles.[12] In addition to the production of carboxylic acids, the conversion of ω-hydroxynitriles by a microbial catalyst to lactones in one step has recently been reported.[13]

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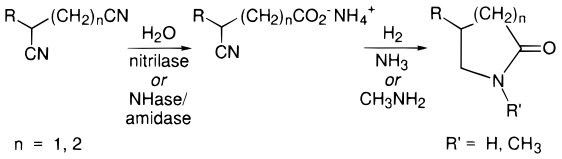
S0022-3263(98)00438-1 CCC: $15.00
Published on Web 06/25/1998

Scheme 1

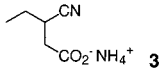
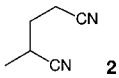
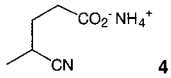
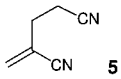
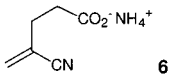
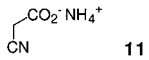
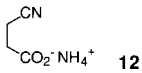
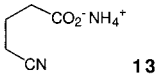
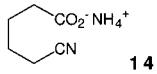


A method for the preparation of five- and six-membered ring lactams from aliphatic α,ω-dinitriles has now been developed (Scheme 1), whereby an aliphatic α,ω-dinitrile is first converted to an ammonium salt of an ω-cyanocarboxylic acid in aqueous solution using a microbial catalyst. The ammonium salt of the ω-cyanocarboxylic acid is then converted directly to the corresponding lactam or *N*-alkyl lactam by hydrogenation in aqueous solution, without isolation of the intermediate ω-cyanocarboxylic acid or ω-aminocarboxylic acid. Two new microbial catalysts have been isolated; the first, *Acidovorax facilis* 72W, contains a nitrilase which converts an α,ω-dinitrile directly to a corresponding ω-cyanocarboxylic acid ammonium salt. The second microbial catalyst, *Comamonas testosteroni* 5-MGAM-4D, contains a combination of two enzyme activities, nitrile hydratase and amidase, where an aliphatic α,ω-dinitrile is initially converted to an ω-cyanoalkylamide by the nitrile hydratase, and the ω-cyanoalkylamide is subsequently converted by the amidase to the corresponding ω-cyanocarboxylic acid ammonium salt. When the aliphatic α,ω-dinitrile is substituted at the α-carbon atom, the *A. facilis* 72W nitrilase produces the ω-cyanocarboxylic acid ammonium salt resulting from hydrolysis of the ω-cyano group with greater than 98% regioselectivity at 100% conversion, thereby producing only one of the two possible lactam products during the subsequent hydrogenation.

Results

Hydrolysis of Aliphatic α,ω-Dinitriles. *Acidovorax facilis* 72W (ATCC 55746) was isolated from soil samples using 2-ethylsuccinonitrile (**1**) as sole nitrogen source. When resting whole cells of *A. facilis* 72W were initially examined as catalyst for the hydrolysis of the α-alkyl-α,ω-dinitriles **1** or 2-methylglutaronitrile (**2**) at 27 °C, a mixture of products was obtained. Over the course of the hydrolysis reactions, the corresponding dicarboxylic acid monoamide ammonium salt and dicarboxylic acid diammonium salt were produced in addition to the desired ω-cyanocarboxylic acid ammonium salt. It was found that heating a suspension of *A. facilis* 72W in 0.10 M phosphate buffer (pH 7.0) at 50 °C for 1 h deactivated an endogenous nitrile hydratase activity which catalyzed the production of the dicarboxylic acid monoamides (which were subsequently converted by an amidase to the corresponding dicarboxylic acid), but did not affect the nitrilase activity present. Heat-treated microbial cells of *A. facilis* 72W converted α-alkyl-α,ω-dinitriles to the corresponding ω-cyanocarboxylic acid ammonium salts resulting from hydrolysis of the ω-cyano group with extremely high regioselectivity. The identification of the enzyme as a nitrilase was based in part on the absence of formation of any amide intermediates by the heat-treated cells, and the inability of the heat-treated cells to convert amides to carboxylic acids (which would normally be observed for a combination of nitrile hydratase and amidase enzymes).

Table 1. *A. facilis* 72W-Catalyzed Hydrolysis of Aliphatic α,ω-Dinitriles[a]

entry	α,ω-dinitrile	ω-cyano/acid ammonium salt	concn.[b] (M)	yield[c] (%)
1	 **1**	 **3**	1.25	100
2	 **2**	 **4**	1.98	99
3	 **5**	 **6**	2.00	100
4	 **7**	 **11**	0.83	83
5	 **8**	 **12**	1.24	99
6	 **9**	 **13**	1.38	92
7	 **10**	 **14**	< 0.05	< 50

[a] Reactions were run in phosphate buffer (20 mM, pH 7.0) at 27 °C, using 50 mg (wet cell weight) heat-treated *A. facilis* 72W/mL of total reaction mixture volume. [b] Concentration of ω-cyanocarboxylic acid ammonium salt in final product mixture. [c] Yields based on HPLC analysis of product mixtures.

Heat-treated resting cells of *Acidovorax facilis* 72W were used as catalyst for the hydrolysis of 2-ethylsuccinonitrile (**1**) to 3-cyanopentanoic acid ammonium salt (**3**), 2-methylglutaronitrile (**2**) to 4-cyanopentanoic acid ammonium salt (**4**), and 2-methyleneglutaronitrile (**5**) to 4-cyano-4-pentenoic acid ammonium salt (**6**) in aqueous reaction mixtures. At complete conversion of these dinitriles, a 98−100% yield of only one of two possible ω-cyanocarboxylic acid ammonium salts was typically produced (Table 1). The corresponding dicarboxylic acid diammonium salts were the only observed byproducts. When the same catalyst was employed for the hydrolysis of the unsubstituted aliphatic α,ω-dinitriles malononitrile (**7**), succinonitrile (**8**), glutaronitrile (**9**), or adiponitrile (**10**), the corresponding ω-cyanocarboxylic acid ammonium salts of 2-cyanoacetic acid (**11**), 3-cyanopropionic acid (**12**), 4-cyanobutanoic acid (**13**) and 5-cyanopentanoic acid (**14**), respectively, were produced in yields ranging from less than 50% to 99.7%, depending on the chain length of the dinitrile (Table 1). The major byproducts from hydrolysis of **8**, **9**, and **10** were succinic, glutaric, and adipic acid diammonium salts, respectively, while the major byproduct from **7** was 2-cyanoacetamide.

Several of the unsubstituted or α-alkyl-substituted aliphatic α,ω-dinitriles used for the preparation of ω-cyanocarboxylic acid ammonium salts were only moderately water soluble, and their solubility was dependent on the temperature of the reaction mixture and the salt concentration (buffer and/or ω-cyanocarboxylic acid ammonium salt) in the aqueous phase. In phosphate buffer

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(20 mM, pH 7.0) at 25 °C, the solubility limits of **1**, **2**, **5**, and **10** were determined to be ca. 0.30 M (32 g/L), 0.52 M (56 g/L), 0.42 M (45 g/L), and 0.60 M (65 g/L), respectively. Production of aqueous solutions containing ω-cyanocarboxylic acid ammonium salts at a concentration greater than the solubilty limit of the starting α,ω-dinitrile was accomplished in batch reactions by preparing a mixture which was initially composed of two phases: an aqueous phase containing the microbial cell catalyst and dissolved α,ω-dinitrile, and the undissolved α,ω-dinitrile. As the reaction progressed, the dinitrile dissolved into the aqueous phase, and over the course of the reaction a single-phase aqueous product mixture was obtained. The highest final concentration of aliphatic ω-cyanocarboxylic acid ammonium salt generated in a product mixture at complete conversion of an α,ω-dinitrile was 2.0 M for 4-cyanopentanoic acid ammonium salt (254 g/L as the carboxylic acid).

The yields of ω-cyanocarboxylic acid ammonium salts **3**, **4**, **6**, and **12** (Table 1) were independent of the concentration of dinitrile initially present in the reaction mixture, and independent of the final product concentration over a range of concentrations from 0.10 to 2.0 M. The yields of ω-cyanocarboxylic acid ammonium salts **11** and **13** increased slightly with increasing initial dinitrile concentration or final product concentration over this same concentration range; at 0.40 M **7** or **9**, the yields of **11** and **13** were only 79% and 85%, respectively (compared to 83% at 0.83 M **11** and 92% at 1.38 M **13**; Table 1).

The wet cell weight of the microbial catalyst used in hydrolysis reactions typically ranged from 10 g/L to 50 g/L of total reaction mixture volume. The temperature of the hydrolysis reaction was chosen to optimize both the reaction rate and the stability of the microbial cell catalyst and was typically 27 °C, although reactions were run between 15 °C and 35 °C. Catalyst suspensions were prepared by dispersing the cells in an aqueous phosphate buffer which maintained the initial pH of the reaction between 7.0 and 8.0. After addition of the aliphatic dinitrile, the pH of the reaction mixture changed only slightly over the course of the reaction due to the formation of an ammonium salt of the carboxylic acid; reactions were run to complete conversion of dinitrile with no additional pH control.

Two mutants of the *A. facilis* 72W strain were prepared (by chemical mutagenesis) which produced only very low levels of the undesirable nitrile hydratase activity responsible for the formation of the byproducts obtained when using unheated *A. facilis* 72W. These mutant strains, *A. facilis* 72 PF-15 (ATCC 55747) and *A. facilis* 72 PF-17 (ATCC 55745), did not require heat-treatment of the cells prior to use as catalyst for the hydrolysis of an aliphatic α,ω-dinitrile to the corresponding ammonium salt of an ω-cyanocarboxylic acid (Figure 1). The yields of **4** and byproduct 2-methylglutaric acid diammonium salt (**15**) which were produced by the hydrolysis of **2** with unheated and heat-treated *A. facilis* 72W, and with the unheated *A. facilis* 72 PF-15 mutant strain, are compared in Table 2; a slight increase in the yield of **4** was observed with increasing substrate/product concentration when using the PF-15 catalyst.

A second novel microbial catalyst which was capable of producing higher yields of **14** from **10** than *A. facilis* 72W was isolated from soil samples using 2-methylglutaramide as sole nitrogen source. This microbial catalyst,

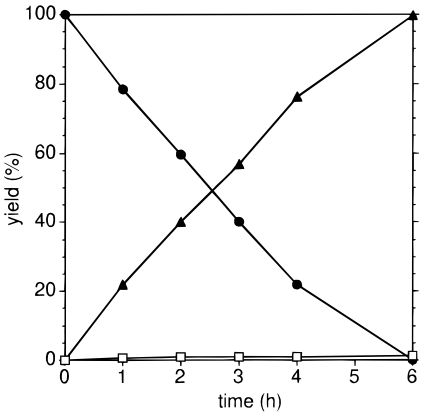


Figure 1. Time course for the hydrolysis of 2-methylglutaronitrile (**2**, 0.40 M) in aqueous phosphate buffer (20 mM, pH 7.0) containing *A. facilis* 72 PF-15 (50 mg wet cell weight/mL) at 27 °C: 2-methylglutaronitrile (●), 4-cyanopentanoic acid ammonium salt (**4**) (▲), 2-methylglutaric acid diammonium salt (□).

Table 2. Comparison of *A. facilis* 72W and *A. facilis* 72W PF-15 Microbial Cell Catalysts for Hydrolysis of 2-Methylglutaronitrile (2)[a]

catalyst	**4** + **15** (M)	**4** (% yield)[b]	**15** (% yield)[b]
A. facilis 72W, not heated	0.10	63	35
A. facilis 72W, heat-treated	0.10	99	1.0
A. facilis 72 PF-15, not heated	0.10	96	4.0
A. facilis 72W, heat-treated	1.00	99	1.0
A. facilis 72 PF-15, not heated	1.00	99	1.0

[a] Reactions were run in phosphate buffer (20 mM, pH 7.0) at 27 °C, using 50 mg (wet cell weight) heat-treated *A. facilis* 72W/mL of total reaction mixture volume. [b] Yields based on HPLC analysis of product mixtures.

Comamonas testosteroni 5-MGAM-4D (ATCC 55744), contained several nitrile hydratase and amidase activities, and when initially employed as a microbial catalyst for the hydrolysis of **10**, produced adipic acid diammonium salt (**16**), adipamide (**17**) and adipamic acid ammonium salt (**18**) as the major reaction products, and only a minor amount of **14**. Heating a suspension of the cells in phosphate buffer (20 mM, pH 7.0) at 50 °C for 1 h deactivated an undesirable nitrile hydratase activity to a significant extent, leaving the microbial catalyst with a second nitrile hydratase activity which converted **10** to 5-cyanovaleramide (**19**), and an amidase which converted **19** to **14** (Figure 2). At 100% conversion of **10**, heat-treated *C. testosteroni* 5-MGAM-4D produced **14** in yields of from 97% (0.10 M) to 88% (1.25 M), where the yield of **14** decreased and byproduct **18** increased with increasing substrate/product concentration.

C. testosteroni 5-MGAM-4D was also examined as catalyst for the hydrolysis of the unsubstituted aliphatic dinitriles **7**, **8**, and **9**, and the α-alkyl-substituted dinitriles **1** and **2**. Maximum yields of ω-cyanocarboxylic acids from **7**, **8**, and **9** were only 6.1%, 70%, and 2.6%, respectively, with amides and diamides being produced as major reaction byproducts from **7** and **8**. Very little (<5%) conversion of **9** was obtained under any reaction conditions. Hydrolysis of **1** produced **3** in at least a 99% yield at either 0.10 or 1.0 M final product concentrations. Hydrolysis of **2** (0.40 M) by *C. testosteroni* 5-MGAM-4D produced a mixture of **4** (68% yield) and 2-methyl-4-cyanobutanoic acid (**20**, 22.3% yield) as the major reaction products. Reaction rates slowed significantly at all

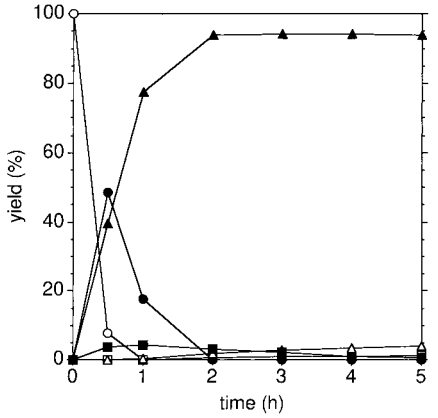


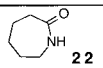
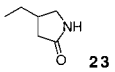
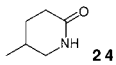
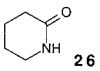
Figure 2. Time course for the hydrolysis of adiponitrile (**10**, 0.40 M) in aqueous phosphate buffer (20 mM, pH 7.0) containing *C. testosteroni* 5-MGAM-4D (20 mg wet cell weight/mL) at 27 °C: adiponitrile (○), 5-cyanopentanoic acid ammonium salt (**14**) (▲), 5-cyanopentanamide (●), adipamide (■), adipamic acid ammonium salt (△), adipic acid diammonium salt (□).

substrate/product concentrations above 0.40 M when using *C. testosteroni* 5-MGAM-4D as catalyst, whereas reaction rates with *A. facilis* 72 W were unchanged at concentrations of up to 2.0 M substrate/product in single batch reactions; this difference may indicate that the nitrile hydratase of *C. testosteroni* may be more susceptible to substrate and/or product inhibition than the nitrilase of *A. facilis*.

The aqueous product mixtures containing ω-cyanocarboxylic acid ammonium salts were used directly for the production of lactams in subsequent hydrogenation reactions after recovery of the microbial cell catalyst by centrifugation and/or filtration. The recovered catalyst still maintained significant activity, and could be reused in subsequent hydrolysis reactions; in one series of catalyst recycles, 30 g (dry cell weight) of *A. facilis* 72W was used in six consecutive batch reactions to produce at total of 2.59 kg of **4** at concentrations of up to 2.0 M. The ω-cyanocarboxylic acids were also readily isolated from these aqueous product mixtures by acidification of the product mixture and extraction of the resulting ω-cyanocarboxylic acid with a suitable organic solvent, followed by crystallization or distillation (see Experimental Section).

Preparation of Lactams from ω-Cyanocarboxylic Acid Ammonium Salts. Hydrogenations of ω-cyanocarboxylic acid ammonium salts in aqueous solution were performed using a Raney nickel catalyst at temperatures of 70 °C to 180 °C. Ammonium hydroxide was added to reaction mixtures at a concentration of up to 3.0 M (added in addition to the ammonium ion concentration already present as the ammonium salt of the carboxylic acid) to limit reductive alkylation of the imine intermediate produced during the hydrogenation by the product ω-aminocarboxylic acid.[14] Hydrogenation of **14** under these reaction conditions proceeded to at least 95% conversion and produced 6-aminohexanoic acid ammonium salt (**21**) as the major product, with less than a 3% yield of caprolactam (**22**) being produced (Table 3). Yields of **22** of less than 3% were also obtained when aqueous solutions prepared by mixing authentic **21** and ammonium hydroxide were treated under these same hydrogenation conditions. The cyclization of **21** (prepared by the hydrogenation of **14** at 70 °C) in aqueous solution was also attempted at temperatures greater than 200 °C by first removing the hydrogenation catalyst, then heating the ca. 1.0 M solution of **21** at 280 °C for 2 h. An 18% yield of **22** was produced with no added ammonia, and at 1.0 and 2.0 M added ammonia the yields were 17% and 9.0%, respectively.

Table 3. Production of Lactams by Hydrogenation of ω-Cyanocarboxylic Acid Ammonium Salts[a]

entry	ω-nitrile/acid NH_4^+ salt	temp. (°C)	$[NH_4OH]$ (M)	catalyst (wt. %)[b]	convn. (%)	lactam	yield[c] (%)
1	**14**	160	2.0	5	95	 **22**	2.7
2	**3**	160	0	5	99	 **23**	80
3	**3**	160	1.0	5	99	**23**	88
4	**3**	160	2.0	10	100	**23**	91
5	**3**	160	3.0	5	100	**23**	85
6	**4**	160	2.0	5	100[d]	 **24**	96
7	**6**	160	2.0	5	100	**24**	85
8	**12**	180	3.0	10	100	 **25**	91
9	**13**	180	3.0	10	100	 **26**	94

[a] 1.0 M ω-cyanocarboxylic acid ammonium salt, 500 psig hydrogen, 2 h reaction time. [b] Chromium-promoted Raney-nickel catalyst, wt % based on wt of acid. [c] Yields based on GC analysis of product mixtures. [d] 3 h reaction time.

Using the same reaction conditions described above for the hydrogenation of **14**, aqueous mixtures of **3** were directly converted to the corresponding lactam 4-ethylpyrrolidin-2-one (**23**) in yields of up to 91%, and **4** was directly converted to 5-methyl-2-piperidone (**24**) in yields as high as 96% (Table 3). The yields of **23** and **24** both increased with increasing concentration of added ammonium hydroxide. Hydrogenation of aqueous solutions of **6** resulted in hydrogenation of both the nitrile and carbon−carbon double bond to produce **24** in up to 85% yield. Aqueous mixtures of **12** were directly converted to the corresponding lactam 2-pyrrolidinone (**25**) in yields of up to 91%, and **13** was directly converted to 2-piperidone (**26**) in yields as high as 94% (Table 3). Hydrogenation of **11** to 2-azetidinone (a four-membered ring lactam) was not attempted, as β-alanine (the expected hydrogenation product) could not be converted to azetidinone in the presence of a 3-fold excess of added ammonia at 160 °C, and authentic 2-azetidinone was completely converted to unidentified products under the hydrogenation conditions tested.

Five-membered ring and six-membered ring lactams were also produced in high yields when aqueous solutions of ω-aminocarboxylic acids, the expected initial hydrogenation products of ω-cyanocarboxylic acids, were heated with a 3-fold molar excess of added ammonia under the hydrogenation conditions typically employed (500 psig hydrogen, 180 °C); quantitative conversion of 4-aminobutanoic acid to **25**, and 5-aminopentanoic acid to **26**, was

(14) De Bellefon, C.; Fouilloux, P. *Catal. Rev. Sci. Eng.* **1994**, *36*, 459−506.

obtained. In contrast, cyclization of 6-aminohexanoic acid in aqueous solution with 2-fold molar excess of added ammonium hydroxide at 170 °C produced **22** in 39% yield; the significantly lower yields of **22** obtained upon hydrogenation of **14** may have resulted from a low selectivity for production of **21** from **14** under the reaction conditions employed.

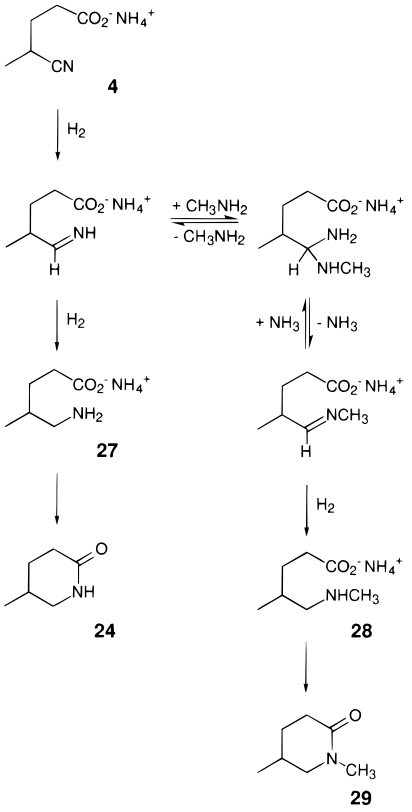
The addition of an excess of ammonia (as ammonium hydroxide) to the hydrogenations of ω-cyanocarboxylic acid ammonium salts in aqueous solution resulted in some conversion of the starting material to the dicarboxylic acid monoamide ammonium salt and/or the dicarboxylic acid diammonium salt under the reaction conditions employed, but in very low yields relative to the production of five-membered ring and six-membered ring lactams. A second byproduct-forming reaction between excess ammonia and the product lactam could have produced an equilibrium mixture of the lactam with its expected ammonolysis product, an ω-aminocarboxamide, but the high yields of five-membered and six-membered ring lactams attained under the reaction conditions employed suggest that ammonolysis (or base-catalyzed hydrolysis) of the lactams was not significant.

The preparation of *N*-methyllactams from **3** or **4** by the substitution of methylamine for ammonia in the hydrogenation reactions was also examined. Addition of from 1 to 4 equiv of methylamine (pK_a = 10.62 for the protonated amine)[15] to an aqueous solution of **4** containing 1 equiv of ammonium ion (pK_a 9.25)[16] was expected to convert a significant amount of ammonium ion to ammonia (due to the relative differences in pK_as of protonated methylamine and ammonium ions in water). This ammonia could compete with unprotonated methylamine for reaction with the intermediate imine produced during hydrogenation of the nitrile, and in the case of **4**, produce a mixture of 5-amino-4-methylpentanoic acid (**27**) and 5-(*N*-methylamino)-4-methylpentanoic acid (**28**) ammonium salts (Scheme 2). These intermediates would subsequently cyclize to produce **24** and 1,5-dimethyl-2-piperidone (**29**), respectively.

The relative yields of **24** and **29** produced by hydrogenation of **4** (1.0 M) in aqueous solution with added methylamine were dependent on the choice of catalyst. Raney nickel and ruthenium/alumina each produced **24** as the major lactam product, even in the presence of 3 equiv of methylamine, while **29** was the major product when using 5% Pd/carbon, or 4.5% Pd/0.5% Pt/carbon, as catalyst at the same methylamine concentration (Table 4). When using 5% Pd/carbon as catalyst, the yield of **29** increased with increasing concentration of methylamine. The substitution of 2.0 M methylamine for ammonia in the hydrogenation of an aqueous solutions of **3** (1.0 M) at 140 °C and using 5% Pd/carbon catalyst produced 4-ethyl-1-methylpyrrolidin-2-one (**30**) and **23** in 69.8% and 20.4% yields, respectively, at 96% conversion.

Lysis of the microbial cell catalysts during the hydrolysis reaction, or contaminants present from the microbial cell catalyst preparation, could have introduced compounds into the hydrogenation reaction mixture (e.g., thiols) which could have poisoned the hydrogenation catalyst activity. No poisoning or deactivation of the hydrogenation catalysts was observed when the hydrogenation of ω-cyanocarboxylic acid ammonium salt mixtures produced via microbial hydrolysis were compared with the hydrogenation of aqueous solutions of the same ω-cyanocarboxylic acid which were first isolated from the hydrolysis product mixtures and purified prior to hydrogenation as the corresponding ammonium salt. The lactams or *N*-methyllactams were readily isolated from the hydrogenation product mixtures by first filtering the mixture to recover the hydrogenation catalyst and then distilling the product directly from the resulting aqueous filtrate. The ammonia produced during the cyclization reaction, or added to the reaction mixture, could also be recovered for recycling by this distillation process, thus avoiding the generation of undesirable inorganic salts as waste products. The lactams or *N*-methyllactams were alternately recovered by filtering the product mixture to recover the catalyst, adjusting the filtrate to a pH of ca. 7.0 with concentrated HCl and saturation with sodium chloride, and extraction of the lactam or *N*-methyllactam (batch or continuous extraction) with an appropriate organic solvent.

Scheme 2



Discussion

There are currently no nonenzymatic catalysts which hydrolyze only one nitrile group of an aliphatic α,ω-dinitrile to a carboxylic acid ammonium salt with high regioselectively at complete conversion of the dinitrile. Nonenzymatic hydrolysis of a dinitrile can be run to incomplete conversion (typically less than 20% conversion) to obtain regioselective production of a monoacid, but the product must then be separated from unreacted dinitrile and the dinitrile recycled. Nonenzymatic nitrile hydrolysis also typically employs a strong acid or base catalyst at elevated temperatures, and the steps required

(15) *Lange's Handbook of Chemistry*, 14th ed.; Dean, J. A., Ed.; McGraw-Hill: NY, 1992; p 8.55.
(16) Ibid., p 8.13.

Table 4. Relative Yields of Lactam and *N*-Methyllactam from Hydrogenation of 4-Cyanopentanoic Acid Ammonium Salt (4)[a]

entry	temp (°C)	$[CH_3NH_2]$ (M)	catalyst[b]	conv (%)	**29** (% yield)[c]	**24** (% yield)[c]
1	160	3.0	Raney-Ni	100	19.2	68.5
2	160	3.0	5% Ru/Al_2O_3	100	19.0	63.5
3	140	3.0	5% Pd/C	99	83.4	0
4	160	1.0	5% Pd/C	100	53.5	0
5	160	1.25	5% Pd/C	100	68.3	0
6	160	1.5	5% Pd/C	100	76.4	0
7	160	2.0	5% Pd/C	100	81.5	0
8	160	3.0	5% Pd/C	100	81.7	7.8
9	160	4.0	5% Pd/C	100	88.4	1.9
10	180	3.0	5% Pd/C	97	73.0	6.6
11[d]	160	2.3	4.5% Pd/0.5% Pt/C	99	94.0	3.1

[a] 1.0 M **4**, 500 psig hydrogen, 2 h reaction time. [b] 5 wt %, based on wt of **4** as acid. [c] Yields based on GC analysis of product mixtures. [d] 1.4 M **4**, 3 h reaction time, 2.5 wt % catalyst, based on wt of **4** as acid.

to separate and purify the product from this reaction mixture can be complicated and costly. The enzyme-catalyzed hydrolysis of dinitriles described herein can be run to complete conversion in aqueous solution at ambient temperature and at neutral pH with no added acid or base. High yields (83−100%) and product concentrations of up to 25% (weight/volume) have been demonstrated, and product recovery and catalyst recycle were simple to perform.

The nitrilase activity of *A. facilis* 72 W and 72W PF-15 microbial cell catalysts was particularly effective at completely converting several α-alkyl-substituted- and unsubstituted aliphatic α,*ω*-dinitriles to the corresponding *ω*-cyanocarboxylic acids (as their ammonium salts) at concentrations of up to 2.0 M. When the α,*ω*-dinitrile was substituted at the α-position (e.g., **1** and **2**, byproducts of a commercial process for the production of adiponitrile from butadiene and HCN),[17] a *ω*-cyanocarboxylic acid was produced with 99−100% regioselectivity at 100% conversion. When the α,*ω*-dinitriles were unsubstituted, the regioselectivity of the *A. facilis* 72W nitrilase was dependent on the chain length, with an optimum regioselectivity observed for hydrolysis of **8**. In the case of the dinitrile **10**, where *A. facilis* 72W produced the dicarboxylic acid diammonium salt **16** as the major hydrolysis product, *C. testosteroni* 5-MGAM-4D utilized a combination of nitrile hydratase and amidase activities to produce the desired *ω*-cyanocarboxylic acid ammonium salt **14** with high regioselectivity. The nitrile hydratase of heat-treated *C. testosteroni* 5-MGAM-4D did not show the same regioselectivity for the hydrolysis of α-alkyl α,*ω*-dinitriles to *ω*-cyanocarboxylic acid ammonium salts as was found for *A. facilis* 72W nitrilase.

Both the *A. facilis* 72 W and *C. testosteroni* 5-MGAM-4D microbial cell catalysts contained more than one enzyme capable of nitrile hydrolysis, which initially resulted in poor selectivities to the desired *ω*-cyanocarboxylic acids. A simple heat treatment of an aqueous suspension of the microbial cells prior to their use as catalyst eliminated the undesirable nitrile hydratase activity, while leaving the relatively heat-stable nitrilase of *A. facilis* 72W, or both a nitrile hydratase and amidase of *C. testosteroni* 5-MGAM-4D, unaffected. The stability of the *C. testosteroni* 5-MGAM-4D nitrile hydratase to heating at 50 °C for periods of 1−2 h was unexpected; mesophilic nitrile hydratases have typically been used only at ambient temperature or lower because of their thermal instability, and several nitrile hydratases which have been employed in the commercial production of acrylamide from acrylonitrile are relatively unstable in use at temperatures above 5−15 °C.[18] Recently, both nitrilase and nitrile hydratase activities having improved thermal stability been identified in the thermophile *Bacillus pallidus* Dac521.[19]

Hydrogenations of *ω*-cyanocarboxylic acid ammonium salts in aqueous solution were typically run using an excess of added ammonia (as ammonium hydroxide) to prevent reductive alkylation of the nitrile, and the pH of the reaction mixture was typically between 9 and 10. Under these reaction conditions, the initial hydrogenation product was an ammonium salt of an *ω*-aminocarboxylic acid. The pK_as of the carboxylic acid and the protonated amine of **21** are 4.373 and 10.804, respectively.[20] At a pH between 9 and 10, greater than 99.9% of the carboxylic acid functionality of **21** would be present as the ammonium salt, and a significant fraction of the *ω*-amino group would also be protonated. Although the pK_as of the carboxylic acid and protonated amine of 4-amino-3-ethylbutanoic acid (from hydrogenation of **3**) or **27** have not been reported, they are most likely similar to those of the corresponding acid and amine groups of **21**. Intramolecular cyclization to produce a lactam by reaction of the *ω*-amino group with the ammonium carboxylate in excess aqueous ammonia was not expected under these reaction conditions.

Cyclization of *ω*−aminoaliphatic carboxylic acids or *ω*-(*N*-alkylamino) aliphatic carboxylic acids to produce lactams have typically been run under reaction conditions which remove water and/or ammonia.[21−23] Five-membered ring lactams have been reported to be produced in

(17) (a) Tolman, C. A.; Seidel, W. C.; Druliner, J. D.; Domaille, P. J. *Organometallics* **1984**, *3*, 33−38. (b) Seidel, W. C.; Tolman, C. A. *Ann. N. Y. Acad. Sci.* **1983**, *415*, 201−221.

(18) (a) Yamada, H.; Kobayashi, M. *Biosci. Biotech. Biochem.* **1996**, *60*, 1391−1400. (b) Nagasawa, T.; Shimizu, H.; Yamada, H. *Microbiol. Biotechnol.* **1993**, *40*, 189−195. (c) Hwang, J.-S.; Chang, H.-N. *Kor. J. Appl. Microbiol. Biotech.* **1990**, *18*, 56−60. (d) Ashina, Y.; Suto, M. In *Industrial Application of Immobilized Biocatalysts*; Tanaka, A., Tosa, T., Kobayashi, T., Eds.; Marcel Dekker: New York, 1993; Chapter 7. (e) Asano, Y.; Fujishiro, Y. T.; Tani, Y.; Yamada, H. *Agric. Biol. Chem.* **1982**, *46*, 1165−1174.

(19) Cramp, R.; Gilmour, M.; Cowan, D. A., *Microbiology* **1997**, *143*, 2313−2320.

(20) *Lange's Handbook of Chemistry*, Dean, 14th ed., J. A., Ed.; McGraw-Hill: NY, 1992; p 8.22.

(21) (a) Taylor, E. C.; McKillop, A. *J. Am. Chem. Soc.* **1965**, *87*, 1984−1990. (b) Taylor, E. C.; McKillop, A.; Ross, R. E. *J. Am. Chem. Soc.* **1965**, *87*, 1990−1995. (c) Bladé-Font, A. *Tetrahedron Lett.* **1980**, *21*, 2443−2446. (c) Demmin, T. R.; Rogic, M. M. US Patent 4,329,498, 1982.

(22) Frank, R. L.; Schmitz, W. R.; Zeidman, B. *Org. Synth.* **1954**, *3*, 328−329.

(23) Mares, F.; Sheehan, D. *Ind. Eng. Chem. Process Des. Dev.* **1978**, *17*, 9−16.

aqueous solution only under acidic conditions,[21c] requiring the presence of the protonated carboxylic acid and not the carboxylate salt. The cyclization of 6-aminohexanoic acid to **22** using water or ethanol as solvent has been extensively studied.[23] In water, the cyclization reaction was reversible, and the percentage of **22** was reported to increase from 38.7% at 180 °C to 92.2% at 250 °C; higher yields of **22** were obtained in ethanol, which was attributed to a shift in the equilibrium which favors the free-acid/free-amine form of 6-aminohexanoic acid in ethanol, rather than the intramolecular alkylammonium carboxylate form which predominates in water.

The processes described above for production of lactams or *N*-alkyllactams suffer from one or more of the following disadvantages: the use of temperatures in excess of 250 °C to obtain high yields of lactams when using water as a solvent, the removal of water from the reaction mixture to drive the equilibrium toward lactam formation, the adjustment of the pH of the reaction mixture to an acidic value to favor lactam formation, or the use of an organic solvent in which the starting material is sparingly soluble. Many of these processes generate undesirable waste streams, or mixtures of products which are not easily separated. *N*-alkyllactams have also been produced by the direct hydrogenation of aqueous mixtures containing **2**, a primary alkylamine, and a hydrogenation catalyst;[24] a mixture of 1,3- and 1,5-dialkyl-2-piperidones are produced at lower yields than for the corresponding process described here.

The preparation of five- and six-membered ring lactams in two steps by the initial enzyme-catalyzed hydrolysis of aliphatic α,ω-dinitriles to ω-cyanocarboxylic acid ammonium salts, followed by the direct hydrogenation of the resulting aqueous product mixture, proceeded in high yield, with little byproduct or waste stream production, and with a facile method of product recovery. This method did not require the isolation of the ω-cyanocarboxylic acid ammonium salt from the product mixture of the hydrolysis reaction prior to the hydrogenation step, nor did it require the conversion of the ω-cyanocarboxylic acid ammonium salt to the free acid prior to hydrogenation, or isolation of the resulting ω-aminocarboxylic acid ammonium salt from the hydrogenation product mixture and conversion of the ammonium salt to the free carboxylic acid prior to cyclization. The inability to produce four-membered ring lactams, and the very low yields of the seven-membered ring lactam **22** produced by these reaction conditions, may be due in part to the relatively greater ring strain of four- and seven-membered rings,[25] such that intermolecular polymerization effectively competes with intramolecular lactamization.

Experimental Section

Materials and Methods. All chemicals and hydrogenation catalysts were obtained from commercial sources and used as received unless otherwise noted. Hydrogenations were performed in a 300-mL stirred autoclave or in 20-mL glass vial shaker tubes. The percent recovery of aliphatic α,ω-dinitriles and the percent yields of the hydrolysis products formed in nitrile hydrolysis reactions were based on the initial amount of α,ω-dinitrile present in the reaction mixture, and determined by HPLC using a refractive index detector and either a Supelcosil LC-18 DB column (25 cm × 4.6 mm diam) and 10 mM acetic acid/10 mM sodium acetate in 2.5% methanol/water as mobile phase (for hydrolysis of **1**, **2**, **5**, **9**, and **10**), or a Bio-Rad HPX-87H column (30 cm × 7.8 mm diam) and 0.001 N sulfuric acid as mobile phase at 50 °C (for hydrolysis of **7** and **8**). The yields of lactams and *N*-methyllactams produced by the hydrogenation of aqueous solutions of ω-cyanocarboxylic acid ammonium salts were based on the initial concentration of ω-cyanocarboxylic acid ammonium salt present in the reaction mixture and determined by gas chromatography using a DB-1701 capillary column (30 m × 0.53 mm ID, 1 μm film thickness). Isolated yields of ω-cyanocarboxylic acids and lactams are unoptimized, and melting points are uncorrected. Chemical shifts for ^{1}H and ^{13}C NMR spectra are expressed in parts per million positive values downfield from internal TMS. Identification of lactam products **25** and **26** was made by comparison of ^{1}H and ^{13}C NMR spectra to commercially available samples.

Acidovorax facilis 72W (ATCC 55746), *Acidovorax facilis* 72 PF-15 (ATCC 55747), *Acidovorax facilis* 72 PF-17 (ATCC 55745), and *Comamonas testosteroni* 5-MGAM-4D (ATCC 55744) were stored frozen at −80 °C. Wet cell weights of whole cell catalysts employed in reactions or assays were obtained from cell pellets prepared by centrifugation of fermentation broth or cell suspensions in buffer. Dry cell weights were determined by lyophilization of wet cells, and the ratio of dry cell weight to wet cell weight for all cells was typically 0.25. Assays of the nitrilase activity of *A. facilis* 72 W (heated as a 10% wet cell weight suspension in 0.10 M phosphate buffer (pH 7.0) for 1 h at 50 °C), *A. facilis* 72 PF-15, and *A. facilis* 72 PF-17 were performed by stirring a suspension of 12.5 mg dry cell weight/mL in 25 mM phosphate buffer (pH 7.0) and 0.30 M dinitrile substrate at 25 °C and analyzing aliquots removed at 1, 5, 10 and 15 min for the rate of production of ω-cyanocarboxylic acid ammonium salt. A unit of nitrilase activity (IU) was equivalent to 1 μmol/min of production of ω-cyanocarboxylic acid ammonium salt.

Screening Reactions for Microbial Hydrolysis of Aliphatic α,ω-Dinitriles. In a typical procedure, 0.60 g (wet cell weight) of frozen *A. facilis* 72W cells (previously heat-treated at 50 °C for 1 h) and 12 mL of potassium phosphate buffer (20 mM, pH 7.0) were added to a 15-mL polypropylene centrifuge tube. After thawing and suspending the cells, the resulting suspension was centrifuged and the supernatant discarded. The resulting cell pellet was resuspended in a total volume of 12 mL of this same phosphate buffer. Into a second 15-mL centrifuge tube was weighed 0.1081 g (1.00 mmol) of **2**, and then 9.89 mL of the *A. facilis* 72W cell suspension (containing 0.494 g wet cells) was added and the resulting suspension mixed on a rotating platform at 27 °C. Samples (0.300 mL) were withdrawn and centrifuged, and then 0.180 mL of the supernatant was placed in a Millipore Ultrafree-MC filter unit (10 K MWCO) and mixed with 0.020 mL of an aqueous solution of 0.750 M *N*-methylpropionamide (HPLC internal standard). Sufficient 1.0 M HCl was added to lower the pH of the sample to ca. 2.5, and the resulting solution was filtered and analyzed by HPLC. After 1.0 h, the HPLC yields of **4** and 2-methylglutaric acid were 99.3% and 0.7%, respectively, with 100% conversion of **2**.

4-Cyanopentanoic Acid.[26] Into a 4-L Erlenmeyer flask equipped with a magnetic stir bar was placed 150 g (wet cell weight) of frozen *A. facilis* 72W (previously heat-treated in 20 mM phosphate buffer at pH 7.0 at 50 °C for 1 h) and suspended in a total volume of 2.5 L of potassium phosphate buffer (20 mM, pH 7.0). With stirring, 129.6 g (136.4 mL, 1.20 mol, 0.400 M) of **2** was added, and the final volume adjusted to 3.00 L with phosphate buffer. The mixture was stirred at 25 °C, and samples were withdrawn at regular intervals and analyzed by HPLC. After 21.5 h, the HPLC yields of **4** and 2-methylglutaric acid diammonium salt were 99.5% and 0.5%, respec-

(24) Kosak, J. R. US Patent 5,449,780, 1995.

(25) (a) Gutman, A. L.; Meyer, E.; Yue, X.; Abell, C. *Tetrahedron Lett.* **1992**, *33*, 3943−3946. (b) Patterson, K. H.; Depree, G. J.; Zender, J. A.; Morris, P. J. *Tetrahedron Lett.* **1994**, *35*, 281−284. (c) Sawada, H. *J. Macromol. Sci., Rev. Macromol. Chem.* **1970**, *51*, 151−173. (d) Wan, P.; Modro, T. A.; Yates, K. *Can. J. Chem.* **1980**, *58*, 2423−2432. (c) Mandolini, L. *J. Am. Chem. Soc.* **1978**, *100*, 550−554. (d) DeTar, D.; Luthra, N. P. *J. Am. Chem. Soc.* **1980**, *102*, 4505−4512.

tively, with 100% conversion of **2**. The reaction mixture was centrifuged and the supernatant decanted and filtered using an Amicon 2.5 L Filter Unit equipped with a YM-10 filter (10K MWCO). The filtrate was placed in a 4.0 L flask, and the pH of the solution adjusted to 2.5 with 6 N HCl. The resulting solution was saturated with sodium chloride, and then 1.0 L portions of the resulting solution were extracted with 4 × 500 mL of ethyl ether. The combined ether extracts were dried ($MgSO_4$) and filtered, and the filtrate was concentrated to 1.0 L by rotary evaporation at reduced pressure. To the concentrate were then added 1.2 L of hexane and 200 mL of ethyl ether, and the resulting solution was cooled to −78 °C. The white crystals which formed were isolated by rapid vacuum filtration and washed with 300 mL of cold (5 °C) hexane. Residual solvent was removed under high vacuum (150 millitorr) to yield 120.3 g (79% isolated yield) of 4-cyanopentanoic acid: mp 31.9−32.6 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.75 (s, 1 H), 2.84−2.72 (m, 1 H), 2.68−2.50 (m, 2 H), 1.99−1.85 (m, 2 H), 1.36 (d, J = 7.1 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.1, 122.0, 31.1, 28.7, 24.8, 17.8; IR (melt) 3700−2800 (br), 3118, 2986, 2673, 2242, 1713, 1387 cm^{-1}; MS (EI) m/z 128 (MH^+, 12), 110 (78), 109 (40), 100 (11), 81 (82), 68 (100); HRMS calcd for $C_6H_{10}NO_2$ (MH^+) 128.0712, found 128.0704.

3-Cyanopentanoic Acid. The procedure for the preparation of 4-cyanopentanoic acid was repeated using 161 g of heat-treated *A. facilis* 72W cells and 325 g (3.00 mol) of **1** in at total volume of 2.40 L of potassium phosphate buffer (20 mM, pH 7.0). After 183 h, the HPLC yield of **3** was 100%, with 100% conversion of **1**. The concentration of **3** in the filtered product mixture was 1.26 M. A 300-mL portion of the filtrate was adjusted to pH 2.5 with ca. 60 mL of 6 N HCl and then saturated with sodium chloride and extracted with 4 × 200 mL of ethyl ether. The combined organic extracts were dried ($MgSO_4$) and filtered, and the solvent was removed by rotary evaporation at reduced pressure at 28 °C. The resulting slightly yellow viscous oil was stirred under high vacuum (50 millitorr) to remove residual solvent at 28 °C and then cooled to −20 °C to produce 3-cyanopentanoic acid as a crystalline white solid (45.9 g, 96% yield): mp 33.0−34.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.53 (s, 1 H), 3.02−2.93 (m, 1 H), 2.81−2.61 (m, 2 H), 1.77−1.65 (m, 2 H), 1.11 (t, J = 7.5 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 120.5, 35.8, 28.6, 24.8, 11.1; IR (neat) 3600−2800 (br), 3200, 2974, 2940, 2882, 2244, 1737, 1463, 1411 cm^{-1}; MS (EI) m/z 128 (MH^+, 5), 99 (24), 82 (48), 68 (67), 54 (100); HRMS calcd for $C_6H_{10}NO_2$ (MH^+) 128.0712, found 128.0708.

4-Cyano-4-pentenoic Acid. The procedure for the preparation of 4-cyanopentanoic acid was repeated using 50.0 g of heat-treated *A. facilis* 72W cells and 133 g (1.25 mol) of **5** in a total volume of 1.0 L of potassium phosphate buffer (20 mM, pH 7.0). After 26 h, the HPLC yield of **6** was 100%, with 100% conversion of **5**. The final concentration of **6** in the filtered product mixture was 1.298 M. A 100-mL portion of the filtrate was adjusted to pH 2.7 with 6 N HCl and then saturated with sodium chloride and extracted with 4 × 100 mL of ethyl ether. The combined organic extracts were dried ($MgSO_4$) and filtered and the volume of the combined extracts reduced to 100 mL by rotary evaporation at reduced pressure at 28 °C. To the concentrate was added 200 mL of hexane, and the resulting solution was cooled to −78 °C. The resulting white solid which crystallized was isolated by rapid vacuum filtration and washed with 100 mL of cold (5 °C) hexane. Residual solvent was removed under high vacuum (150 millitorr) to yield 9.80 g (60% isolated yield) of 4-cyano-4-pentenoic acid (stored at −20 °C): mp 26.5−27.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.69 (s, 1 H), 5.94 (s, 1 H), 5.85 (s, 1 H), 2.69−2.59 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.4, 131.7, 121.0, 117.9, 31.8, 29.3; IR (neat) 3700−2800 (br), 3150, 2925, 2225, 1740, 1714, 1624, 1435, 1414 cm^{-1}; MS (EI) m/z 126 (MH^+, 8), 108 (48), 79 (100), 66 (8), 53 (32); HRMS calcd for $C_6H_8NO_2$ (MH^+) 126.0555, found 126.0537.

3-Cyanopropanoic Acid.[27] The procedure for the preparation of 4-cyanopentanoic acid was repeated using 20.0 g of heat-treated *A. facilis* 72W cells and 101.1 g (1.25 mol) **8** in a total volume of 1.00 L of potassium phosphate buffer (20 mM, pH 7.0). After 1.0 h, the HPLC yield of **12** and succinic acid diammonium salt were 99.7% and 0.3%, respectively, with 100% conversion of **8**. The final concentration of **12** in the filtered product mixture was 1.31 M. A 200-mL portion of the filtrate was adjusted to pH 2.5 with 6 N HCl and then saturated with sodium chloride and extracted with 4 × 200 mL of ethyl ether. The combined organic extracts were dried ($MgSO_4$) and filtered, and the solvent was removed by rotary evaporation at reduced pressure. The resulting colorless oil was dissolved in 150 mL of ethyl ether, 100 mL of hexane was added, and then the resulting solution cooled to −78 °C. The resulting white solid which crystallized was isolated by vacuum filtration, and residual solvent was removed under high vacuum (150 millitorr) to yield 14.32 g (55% isolated yield) of 3-cyanopropanoic acid: mp 49.5−51.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.49 (s, 1H), 2.78 (t, J = 6.8 Hz, 2 H), 2.67 (t, J = 6.8, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.7, 118.2, 29.5, 12.5; IR (KBr) 3418−2883 (b), 2981, 2954, 2683, 2251, 1713, 1420, 1345 cm^{-1}; MS (EI) m/z 99 (M^+, 11), 82 (15), 54 (100), 45 (24); HRMS calcd for $C_4H_5NO_2$ (M^+) 99.0320, found 99.0320.

4-Cyanobutanoic Acid.[26] The procedure for the preparation of 4-cyanopentanoic acid was repeated using 15.0 g of heat-treated *A. facilis* 72W cells and 42.78 g (0.450 mol) of **9** in a total volume of 300 mL of potassium phosphate buffer (20 mM, pH 7.0). After 4.0 h, the HPLC yield of **13** and glutaric acid diammonium salt were 92.3% and 7.7%, respectively, with 100% conversion of **9**. The filtered product mixture was adjusted to pH 3.5 with 6 N HCl and then saturated with sodium chloride and extracted with 4 × 300 mL of ethyl ether. The combined organic extracts were dried ($MgSO_4$) and filtered, and the solvent was removed by rotary evaporation at reduced pressure followed by stirring under vacuum (100 millitorr) to yield 35.3 g (62% yield) of 4-cyanobutanoic acid as a pale yellow oil which solidified upon standing. The solid was recrystallized from 1:1 ethyl acetate/hexane at 5 °C; mp 39.6−40.2 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.70 (s, 1 H), 2.56 (t, J = 7.0 Hz, 2 H), 2.50 (t, J = 7.0 Hz, 2 H), 2.00 (quintet, J = 7.0 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.8, 118.7, 32.0, 20.3, 16.3; IR ($CHCl_3$) 3600−2800 (b), 3022, 2949, 2671, 2250, 1713, 1428, 1417 cm^{-1}; MS (EI) m/z 114 (MH^+, 14), 96 (45), 67 (34), 60 (45), 54 (55), 41 (100); HRMS calcd for $C_5H_8NO_2$ (MH^+) 114.0555, found 114.0528.

5-Cyanopentanoic Acid.[28] The procedure for the preparation of 4-cyanopentanoic acid was repeated using 4.0 g of *C. testosteroni* 5-MGAM-4D cells (previously heat-treated at 50 °C for 1 h) and 270 g (2.50 mol) of **10** in a total volume of 2.0 L of potassium phosphate buffer (20 mM, pH 7.0) at 27 °C. After 63 h, the reaction rate had slowed considerably, so an additional 10.0 g of the microbial cell catalyst was added to the mixture. After 86 h, the HPLC yields of **14**, adipamic acid ammonium salt, adipamide, and adipic acid diammonium salt were 88.2%, 4.7%, 6.6%, and 0%, with 100% conversion of **10**. A 200-mL portion of the filtered product mixture was adjusted to pH 2.5 with 6 N HCl and then saturated with sodium chloride and extracted with 4 × 200 mL of ethyl ether. The combined ether extracts were dried ($MgSO_4$) and filtered, and the solvent was removed by rotary evaporation at reduced pressure. Remaining ether was removed by stirring the colorless liquid at room temperature under high vacuum (60 millitorr) for 5 h to yield 27.32 g (95% isolated yield) of 5-cyanopentanoic acid. The 5-cyanopentanoic acid was then redistilled under vacuum at 110−112 °C (75 millitorr) without decomposition: ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.58 (s, 1 H), 2.44−2.35 (m, 4 H), 1.81−1.66 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.9, 119.0, 32.2, 23.9, 22.9, 16.0; IR (neat) 3600−2800 (b), 2949, 2878, 2248, 1737, 1709, 1459, 1424 cm^{-1}; MS (EI) m/z 128 (MH^+, 18), 110 (100), 82 (25), 81 (40), 68 (48), 54 (60); HRMS calcd for $C_6H_{10}NO_2$ (MH^+) 128.0712, found 128.0713.

5-Methyl-2-piperidone (24).[29] Into a 100 mL graduated cylinder was placed 54.4 mL of an aqueous solution of **4** (1.85 M, 0.100 mol, produced by microbial hydrolysis of **2** and filtered as described above), 12.9 mL of concentrated ammonium hydroxide (29.3% NH_3, 0.20 mol NH_3) was added, and then the final volume was adjusted to 100 mL with distilled

water. To the resulting solution was added 0.631 g of chromium-promoted Raney nickel (Grace Davison Raney 2400 Active Metal Catalyst), and the resulting mixture was charged to a 300-mL stirred autoclave. After flushing the reactor with nitrogen, the contents of the reactor were stirred at 1000 rpm and heated at 160 °C under 500 psig of hydrogen for 3 h. After cooling to room temperature, analysis of the product mixture by gas chromatography and HPLC indicated a 96.4% yield of **24**, with 100% conversion of **4**. The product mixture was filtered to remove the catalyst, adjusted to pH 6.0 with 6 N HCl, and saturated with sodium chloride. The resulting solution was extracted with 5 × 100 mL of dichloromethane, the combined organic extracts were dried ($MgSO_4$), filtered, and the solvent was removed by rotary evaporation under reduced pressure to yield a colorless oil. Removal of remaining solvent under vacuum (100 millitorr) produced a white solid which was recrystallized from 150 mL of ethyl ether at −78 °C to yield 6.69 g (59% isolated yield) of **24**: mp 55.5−56.2 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17 (bs, 1 H), 3.33−3.28 (m, 1 H), 2.94−2.89 (m, 1 H), 2.44−2.31 (m, 2 H), 1.95−1.82 (m, 2 H), 1.52−1.43 (m, 1 H), 1.01 (d, J = 6.6 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 49.0, 30.7, 29.0, 28.2, 18.4; IR (KBr) 3247, 3208, 3084, 2953, 2932, 2875, 1674, 1651, 1499, 1466 cm^{-1}; MS (EI) *m/z* 113 (M^+, 100), 98 (6), 84 (8), 70 (31), 56 (40), 42 (25); HRMS calcd for $C_6H_{11}NO$ (M^+) 113.0841, found 113.0840.

4-Ethylpyrrolidin-2-one (23).[30] The procedure for the preparation of **24** was repeated using 12.71 g (0.100 mol) of 3-cyanopentanoic acid and 19.34 mL of concentrated ammonium hydroxide (29.3% NH_3, 0.30 mol of NH_3) in a total volume of 100 mL. To the resulting solution was added 0.636 g of chromium-promoted Raney nickel catalyst, and the resulting mixture was hydrogenated at 160 °C and 500 psig hydrogen for 2 h. Analysis of the product mixture by gas chromatography and HPLC indicated a 92.1% yield of **23**, with 100% conversion of 3-cyanopentanoic acid. The product mixture was filtered to remove the catalyst, adjusted to pH 7.0 with 6 N HCl, and saturated with sodium chloride. The resulting solution was extracted with 4 × 100 mL of dichloromethane, the combined organic extracts were dried ($MgSO_4$) and filtered, and the solvent was removed by rotary evaporation under reduced pressure to yield a colorless oil. After removal of the remaining solvent under vacuum (100 millitorr), the oil was dissolved in 150 mL of ethyl ether, which was then cooled to −78 °C. After 1 h, a white crystalline solid was collected by vacuum filtration to yield a total of 8.96 g (79% isolated yield) of **23**: mp 40.5−41.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (bs, 1 H), 3.51−3.47 (m, 1 H), 3.03−3.00 (dd, J = 6.2 and 9.5 Hz, 1 H), 2.45−2.33 (m, 2 H), 2.02−1,97 (dd, J = 7.3 and 16 Hz, 1 H), 1.52−1.46 (quintet, J = 7.3 Hz, 2 H), 0.93 (t, J = 7.3 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.8, 47.9, 36.6, 36.3, 27.4, 11.7; IR (KBr) 3241, 3122, 2958, 2931, 2900, 2875, 1683, 1664, 1493, 1462 cm^{-1}; MS (EI) *m/z* 113 (M^+, 100), 98 (5), 83 (26), 70 (16), 56 (44), 41 (83); HRMS calcd for $C_6H_{11}NO$ (M^+) 113.0840, found 113.0841.

2-Pyrrolidinone (25).[31] The procedure for the preparation of **24** was repeated using 75.8 mL of an aqueous solution of **12** (1.31 M, 0.100 mol, produced by the enzymatic hydrolysis of **8** and filtered as described above) and 19.4 mL of concentrated ammonium hydroxide (29.3% NH_3, 0.30 mol of NH_3) in a total volume of 100 mL. To the resulting solution was added 0.99 g of chromium-promoted Raney nickel, and the resulting mixture was hydrogenated at 500 psig of hydrogen at 70 °C for 4.5 h and an additional 5 h at 180 °C. Analysis of the product mixture by HPLC and gas chromatography indicated a 91.0% yield of **25**, with 100% conversion of **12**. An 81 mL portion of the product mixture was filtered to remove the catalyst, adjusted to pH 7.0 with 6 N HCl, and saturated with sodium chloride. The resulting solution was extracted with 4 × 150 mL of dichloromethane, the combined organic extracts were dried ($MgSO_4$) and filtered, and the solvent was removed by rotary evaporation under reduced pressure to yield a colorless liquid. This liquid was distilled and the fraction boiling at 83.0 °C (125 millitorr) collected to yield 3.31 g of **25** (48% isolated yield).

2-Piperidone (26).[32] The procedure for the preparation of **24** was repeated using 70.6 mL of an aqueous solution of **13** (1.42 M, 0.100 mol, produced by the enzymatic hydrolysis of **9** and filtered as described above) and 19.4 mL of concentrated ammonium hydroxide (29.3% NH_3, 0.30 mol of NH_3) in a total volume of 100 mL. To the resulting solution was added 1.13 g of chromium-promoted Raney Nickel, and the resulting mixture was hydrogenated at 500 psig of hydrogen and 70 °C for 3.5 h. Analysis by gas chromatography indicated a 29.7% yield of **26**, with complete conversion of **13**. The temperature was increased to 180 °C for an additional 2 h to give a 93.5% yield of **26**. An 86 mL portion of the product mixture was filtered to remove the catalyst, adjusted to pH 7.0 with 6 N HCl, and saturated with sodium chloride. The resulting solution was extracted with 4 × 150 mL of dichloromethane, the combined organic extracts were dried ($MgSO_4$) and filtered, and the solvent was removed by rotary evaporation under reduced pressure to yield a colorless liquid. This liquid was distilled and the fraction boiling at 78.0 °C (120 millitorr) collected to yield 6.28 g of **26** (74% isolated yield) as a white crystalline solid: mp 37.7−39.1 °C.

1,5-Dimethyl-2-piperidone (29).[33] The procedure for the preparation of **24** was repeated using 54.4 mL of an aqueous solution of **4** (1.85 M, 0.100 mol, produced by microbial hydrolysis of **2** and filtered as described above) and 25.8 mL of 40 wt % methylamine (9.31 g methylamine, 0.30 mol) in a 100 mL final volume. To the resulting solution was added 0.636 g of 5% Pd on carbon powder, and the resulting mixture was hydrogenated for 25.5 h at 70 °C and 500 psig hydrogen. Analysis by gas chromatography indicated a 38.2% yield of **26**, with 54% of unreacted **4**. The temperature was increased to 160 °C and 500 psig hydrogen for 4 h, and analysis of the product mixture by gas chromatography and HPLC indicated yields of 72.8% yield of **29**, 3.5% of **24**, 19.9% of 2-methylglutaric acid diammonium salt, and 1.6% **4** remaining. A 61 mL portion of the product mixture was filtered to remove the catalyst, adjusted to pH 7.0 with 6 N HCl, and saturated with sodium chloride. The resulting solution was extracted with 4 × 100 mL of ethyl ether, the combined organic extracts were dried ($MgSO_4$), and filtered, and the solvent was removed by rotary evaporation under reduced pressure to yield a colorless liquid. This liquid was distilled and the fraction boiling at 70.0−71.5 °C (3.5 torr) collected to yield 4.65 g (60% isolated yield) of **29**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.28−3.24 (m, 1 H), 2.98−2.94 (m, 1 H), 2.92 (s, 3 H), 2.43−2.28 (m, 2 H), 2.02−1.95 (m, 1 H), 1.87−1.82 (m, 1 H), 1.51−1.43 (m, 1 H), 1.02 (d, J = 6.7 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 56.3, 34.0, 31.0, 29.0, 28.4, 17.9; IR (neat) 2954, 2929, 2875, 1649, 1504, 1462, 1422 cm^{-1}; MS (EI) *m/z* 128 (MH^+, 76), 127 (M^+, 90), 112 (11), 85 (25), 84 (27), 70 (15), 57 (43), 44 (100); HRMS calcd for $C_7H_{14}NO$ (MH^+) 128.1076, found 128.1075.

4-Ethyl-1-methylpyrrolidin-2-one (30). The procedure for the preparation of **29** was repeated using 79.4 mL of aqueous **3** (1.26 M, 0.100 mol, produced by the enzymatic hydrolysis of **1** and filtered as described above) and 17.2 mL of 40 wt % methylamine (6.21 g methylamine, 0.200 mol) in a 100 mL final volume. To the resulting solution was added 0.636 g of 5% Pd on carbon powder, and the resulting mixture

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was hydrogenated for 4 h at 140 °C and 500 psig hydrogen. Analysis of the product mixture by gas chromatography and HPLC indicated a 69.8% yield of **30** and a 20.4% yield of **23**, with 100% conversion of **3**. An 80 mL portion of the product mixture was filtered to remove the catalyst, adjusted to pH 7.0 with 6 N HCl, and saturated with sodium chloride. The resulting solution was extracted with 4 × 100 mL of dichloromethane, the combined organic extracts were dried ($MgSO_4$) and filtered, and the solvent was removed by rotary evaporation under reduced pressure to yield a colorless liquid. This liquid was fractionally distilled and the fraction boiling at 100 °C (16 torr) collected (5.15 g, 51% yield). The resulting **30** contained <5% **23** as impurity, and redistillation and collection of the fraction boiling at 128 °C (40 torr) yielded 3.71 g (37% isolated yield) of pure **30**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.52−3.48 (dd, J = 9.7 and 7.9 Hz, 1 H), δ 3.05−3.02 (dd, J = 9.7 and 6.4 Hz, 1 H), 2.80 (s, 3 H), 2.47−2.42 (dd, J = 8.8 and 16.5 Hz, 1 H), 2.30−2.23 (m, 1 H), 2.00−1.96 (dd, J = 7.7 and 16.5 Hz, 1 H), 1.51−1.45 (m, 2 H), 0.93 (t, J = 7.3 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 53.4, 35.7, 31.7, 27.8, 26.3, 10.3; IR (neat) 2960, 2929, 2876, 2859, 1694, 1501, 1462, 1426 cm^{-1}; MS (EI) m/z 127 (M^+, 55), 112 (3), 99 (8), 85 (55), 70 (8), 56 (12), 44 (100); HRMS calcd for $C_7H_{13}NO$ (M^+) 127.0997, found 127.0998.

Acknowledgment. L. Winnie Wagner and co-workers of the DuPont Fermentation Resource Facility provided supplies of microbial catalysts used in this work.

Supporting Information Available: Experimental section describing the isolation and growth of *Acidovorax facilis* 72W (ATCC 55746), *Acidovorax facilis* 72 PF-15 (ATCC 55747), *Acidovorax facilis* 72 PF-17 (ATCC 55745), and *Comamonas testosteroni* 5-MGAM-4D (ATCC 55744) (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO9804386