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Chemical and Enzymatic Synthesis of 2-(2-Carbamoylethyl)- and 2-(2-Carboxyethyl)aziridines and Their Conversion into δ-Lactams and γ-Lactones

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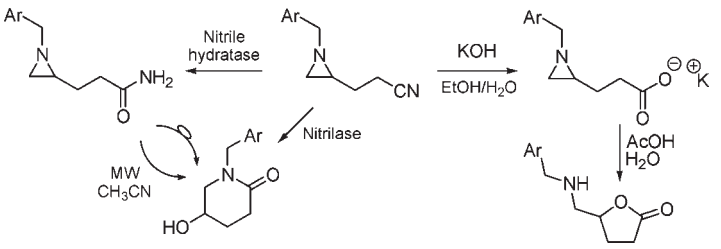
ABSTRACT



Treatment of 1-arylmethyl-2-(2-cyanoethyl)aziridines with a nitrile hydratase afforded the corresponding 2-(2-carbamoylethyl)aziridines, which underwent rearrangement into 5-hydroxypiperidin-2-ones upon heating under microwave irradiation. In addition, treatment of 2-(2-cyanoethyl)aziridines with a nitrilase selectively afforded 5-hydroxypiperidin-2-ones in good yields. On the other hand, chemical hydrolysis of 2-(2-cyanoethyl)aziridines using KOH in EtOH/H_2O furnished the corresponding potassium 3-(aziridin-2-yl)propanoates, which, upon acidification with acetic acid, smoothly rearranged into 4-(aminomethyl)butyrolactones.

. Amino nitriles comprise an interesting class of compounds, from both a chemical and a biological point of view. These compounds possess important biological properties, and several drugs containing an amino nitrile moiety are currently on the market or under study in advanced clinical trials.[1]

However, more importantly, amino nitriles are known to be excellent precursors for the synthesis of the corresponding amino acids and their derivatives, and therefore, they are mostly used to this end. In the past, extensive research has been conducted on the hydrolysis of amino nitriles applying both chemical and enzymatic methods.[2] Enzymes play a key role in the synthesis of amino acids, allowing the development of clean chemo-, regio-, and stereoselective processes under mild conditions in aqueous medium and neutral pH.[3] The enzyme-catalyzed hydrolysis of nitriles is known to occur via two different pathways to afford either amides or acids depending on the type of enzyme used. Nitrilases convert nitriles directly to the corresponding carboxylic acids without the formation of intermediate amides,

(1) Fleming, F. F.; Yao, L.; Ravikumar, P. C.; Funk, L.; Shook, B. C. *J. Med. Chem.* **2010**, *53*, 7902.

(2) For a few examples, see: (a) Klempier, N.; Winkler, M. In *Modern Biocatalysis: Stereoselective and Environmentally Friendly Reactions*; Fessner, W.-D., Anthonsen, T., Eds.; Wiley-VCH: Weinheim, Germany, 2009; p 247. (b) Sugai, T.; Yamazaki, T.; Yokoyama, M.; Ohta, H. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 1419. (c) Fitz, M.; Lundell, K.; Lindroos, M.; Fulop, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2005**, *16*, 3690. (d) Winkler, M.; Martinkova, L.; Knall, A. C.; Krahulec, S.; Klempier, N. *Tetrahedron* **2005**, *61*, 4249. (e) Weber, K.; Kuklinski, S.; Gmeiner, P. *Org. Lett.* **2000**, *2*, 647. (f) Thomas, C.; Orecher, F.; Gmeiner, P. *Synthesis* **1998**, 1491. (g) Gmeiner, P. *Tetrahedron Lett.* **1990**, *31*, 5717.

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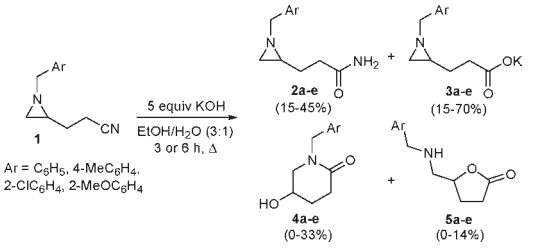
whereas the combination of nitrile hydratases (NHase) with amidases first transforms the nitriles toward amides, followed by the hydrolysis of these amides into carboxylic acids.[8a,b,9]

In this paper, both the chemical and enzymatic hydrolysis of 1-arylmethyl-2-(2-cyanoethyl)aziridines is investigated, providing the first synthesis of 3-(aziridin-2-yl)propionamides by using nitrile hydratases and the formation of potassium 3-(aziridin-2-yl)propanoates using potassium hydroxide in EtOH/H_2O. These functionalized aziridines were further elaborated into heterocyclic systems such as δ-lactams and γ-lactones.

In continuation of our interest in 2-(ω-cyanoalkyl)-aziridines as building blocks for the preparation of a wide variety of amino nitriles[4] and amino acid derivatives,[5] a new strategy was explored in this work. All previously described methodologies were based on the initial ring opening of the aziridine moiety, followed by transformation of the nitrile functionality into amides, acids, or amines. In this paper, the reverse strategy is contemplated, involving initial hydrolysis of the cyano group without affecting the strained three-membered ring system. This approach would thus provide a useful entry into functionalized aziridines such as 2-(ω-carbamoylalkyl)- and 2-(ω-carboxyalkyl)aziridines as suitable substrates for further elaboration.

Since aziridines are sensitive to ring opening upon treatment with acids,[6,10] no acid-catalyzed hydrolysis of the nitrile moiety in 2-(ω-cyanoalkyl)aziridines can be employed to provide an entry into the corresponding amides, leaving basic or enzymatic hydrolysis as the only options. At first, 1-arylmethyl-2-(2-cyanoethyl)aziridines **1**, prepared from 2-(bromomethyl)aziridines[7] by treatment with α-lithiated trimethylsilylacetonitrile in THF according to a literature protocol,[10] were treated with 5 equiv of KOH in EtOH/H_2O (3/1) and heated under reflux for 3−6 h. These experiments resulted in complex reaction mixtures, in which the target compounds 3-(aziridin-2-yl)propionamides **2** were present in low yields (15−45%), due to their further hydrolysis toward 3-(aziridin-2-yl)propanoates **3** (15−70%), 5-hydroxypiperidin-2-ones **4** (0−33%), and 4-(aminomethyl)butyrolactones **5** (0−14%) (Table 1). To conclude, treatment of 2-(2-cyanoethyl)aziridines **1** with KOH in EtOH/H_2O for 3 or 6 h results in the formation of a set of reaction products, rendering this an inefficient route for the synthesis of **2**.

Table 1. Hydrolysis of 2-(2-Cyanoethyl)aziridines **1**



1 (Ar)	time (h)	**2** (%)	**3** (%)[a]	**4** (%)[a]	**5** (%)[a]
1a (C_6H_5)	6	33	56	12	0
1b (4-MeC_6H_4)	6	16	70	0	14
1c (2-ClC_6H_4)	3	45	15	33	7
1d (2-$MeOC_6H_4$)	6	42	50	0	8

[a] Based on ^{1}H NMR and/or LC of the crude reaction mixture.

Nitrile-hydrolyzing enzymes have proven to be excellent tools in organic synthesis and industrial processes and have been reviewed extensively in the past.[8] The main advantages of enzymatic approaches over chemical transformations are related to the fact that no harsh conditions, such as the use of concentrated base or acid and elevated temperatures, are required and the fact that enzymes often display high levels of chemo- and enantioselectivity. For instance, nitrile hydratases can selectively transform nitriles to the corresponding primary amides at neutral pH and ambient temperature, whereas chemical approaches often rely on the use of acid conditions and heating under reflux.

Only a few studies have been conducted on the enzymatic hydrolysis of nitriles containing an aziridine moiety, all related to the use of 2-cyanoaziridines.[9] These reports showed a good nitrile hydratase activity, and due to the presence of a highly enantioselective amidase in the whole cells, one enantiomer was further converted to the corresponding acid, thus lowering the yield of the primary amide.

Up to now, no reports are available dealing with the selective hydrolysis of aziridinyl nitriles toward the

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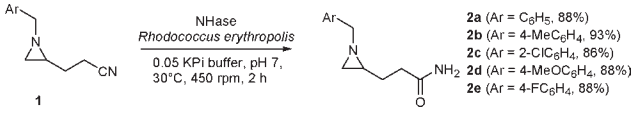
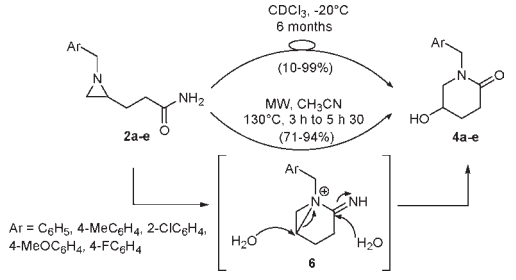
corresponding amides, and also the enzymatic hydrolysis of 2-(ω-cyanoalkyl)aziridines has not been described in the literature so far.

The bacterial strain of *Rhodococcus erythropolis* NCIMB 11540 has already been shown to possess a good NHase activity for the synthesis of a variety of aliphatic primary amides.[10] In this paper, the cell-free extract of *R. erythropolis* NCIMB 11540 nitrile hydratase was used for the first synthesis of stable **2** starting from **1**. The latter amino nitriles **1** were dissolved in DMSO and added to a KH_2PO_4/K_2HPO_4 buffered NHase solution, after which the reaction mixture was incubated at 30 °C and 450 rpm for 2 h, affording **2** in excellent yields as the sole reaction products (Scheme 1). The only report describing primary 3-(aziridin-2-yl)propionamides comprised the synthesis of an unstable *N*-Boc-protected aziridine which was not characterized due to insufficient data.[11] In this paper, the first and highly efficient conversion of aziridinyl nitriles toward the corresponding aziridinyl amides using a NHase is presented. Surprisingly, **2** slowly underwent a spontaneous rearrangement into **4** upon standing at room temperature or at −20 °C over a period of several months in a solution of deuterated chloroform (Scheme 2).

Scheme 1. Synthesis of 3-(Aziridin-2-yl)propionamides **2**



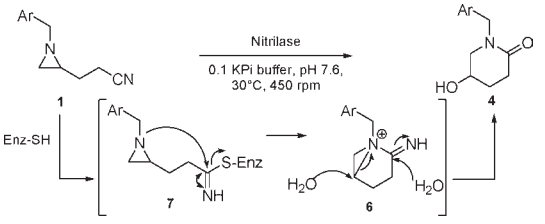
Scheme 2. Rearrangement of Aziridines **2** into Piperidinones **4**



The substituent on the aromatic ring could have an influence on the rate of this transformation, as for example the benzyl (Ar = C_6H_5) and 2-chlorobenzyl (Ar = 2-ClC_6H_4) derivatives **2a** and **2c** were completely converted into the ring-expanded piperidinones **4a** and **4c** over a period of 6 months, whereas for the other derivatives only 10−15% of piperidinones **4** were formed over the same period of time. However, this rearrangement is probably catalyzed by traces of acid formed from the slow decomposition of $CDCl_3$, and the rearrangement rate can be ascribed to different concentrations of acid present in the solvent. The synthesis of **4** proceeds via 5-*exo*-*trig* ring closure of the aziridine nitrogen atom across the amido group, thus creating a bicyclic aziridinium intermediate **6** which subsequently undergoes a regiospecific ring opening at the more substituted carbon atom by means of water. The spectral data of piperidinone **4a** were compared to those reported in the literature,[12] which unambiguously assigned 1-arylmethyl-5-hydroxypiperidin-2-ones **4** as the sole reaction products in this transformation. In order to provide a synthetically useful process, the applicability of microwave irradiation was investigated. Thus, aziridinyl amides **2b**,**d**,**e** were subjected to microwave irradiation in acetonitrile for 3 h to 5 h 30 min at 130 °C, resulting in the formation of piperidinones **4b**,**d**,**e** in 71−94% yield.

In addition, the enzymatic hydrolysis of 2-(2-cyanoethyl)aziridines **1** was investigated using nitrilases as the hydrolyzing tools. Thus, **1** was dissolved in DMSO and added to a KH_2PO_4/K_2HPO_4 buffered nitrilase solution, after which the reaction mixture was incubated at 30 °C and 450 rpm for 24−48 h, affording **4** in 80−93% yield as the sole reaction products (Table 2). A nitrilase testing kit from Biocatalytics containing 12 nitrilases was used, and the best results are summarized in Table 2. Compounds **4a** (Ar = 2-MeC_6H_4) and **4b** (Ar = 2-ClC_6H_4) were synthesized on a larger scale (1.5 mmol), allowing their isolation in analytically pure form by means of column chromatography on silica gel. The fact that no 3-(aziridin-2-yl)propanoates **3** were obtained might be explained by the mechanism by which these enzymes operate. Nitrilases possess a conserved Glu-Lys-Cys sequence in the active site,[13] where the cysteine forms a covalent bond with the nitrile to form a thioimidate intermediate **7**.[14] Normally, water hydrolyzes the thiolimidate toward a thioester, which is then further hydrolyzed to the acid.[14] In this case, however, an intramolecular attack of the nucleophilic aziridine nitrogen atom takes place, forming a bicyclic aziridinium intermediate **6**, which is

Table 2. Synthesis of 5-Hydroxypiperidin-2-ones **4**



4 (Ar)	nitrilase	time (h)	conversion (%)[a]
4a (4-MeC_6H_4)	N108	48	88
4b (2-ClC_6H_4)	N107	24	93
4c (4-$MeOC_6H_4$)	N107	24	92
4d (4-FC_6H_4)	N101	48	80

[a] Based on LC of the crude reaction mixture.

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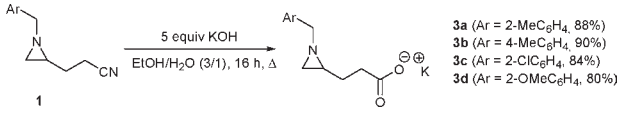
regiospecifically ring opened at the more substituted carbon atom and further hydrolyzed toward **4** (Table 2).

A second objective of this study involved the hydrolysis of aziridinyl nitriles toward the corresponding carboxylic acids as valuable templates in organic and medicinal chemistry.

Thus, 1-arylmethyl-2-(2-cyanoethyl)aziridines **1** were treated with 5 equiv of KOH in a solvent mixture of Et OH/H_2O (3/1) and heated under reflux for 16 h, affording the corresponding potassium 3-(aziridin-2-yl)propanoates **3** in 80−90% yield (Scheme 3). 3-(Aziridin-2-yl)-propionamides **2** were observed as minor constituents (10−20%), as well, and could be easily removed from the potassium carboxylates via extraction with CH_2Cl_2. These novel 3-(aziridin-2-yl)propanoates **3** can be considered as interesting constrained γ-amino acid building blocks for the synthesis of new heterocyclic compounds, β-amino alcohols, and γ- and δ-amino acids,[15] the latter being important for their applications in peptidomimetics,[16] PNA chains,[17] and as GABA analogues.[18]

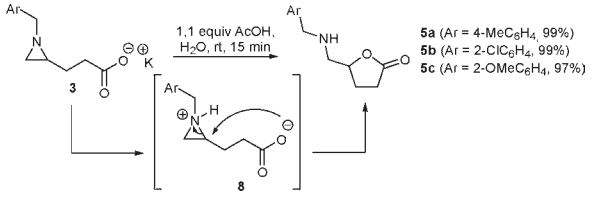
Scheme 3. Synthesis of 3-(Aziridin-2-yl)propanoates **3**



In order to assess their aptitude toward ring rearrangements, **3** was treated with 1.1 equiv of acetic acid in water for 15 min at room temperature, affording **5** in almost quantitative yields (Scheme 4). Neutralization of the alkaline medium transformed potassium salts **3** into the corresponding amino acids, which exist as zwitterionic structures **8** at neutral pH.

The latter intermediates **8** are highly susceptible to ring transformation because of the combination of an electrophilic aziridinium moiety and the nucleophilic carboxylate at a remote position.[19] Intramolecular ring opening

Scheme 4. Rearrangement of Propanoates **3** into γ-Lactones **5**



then furnishes γ-lactones **5** as ring-expanded products. No δ-lactone formation was observed, probably due to a disfavored 6-*endo-tet* ring closure according to Baldwin's rules as compared to a favored 5-*exo-tet* cyclization. γ-Lactones are a widespread entity in natural products, and many of them display important biological activities such as germination stimulation, anticancer, and anti-HIV activity, while others are often used as food additives (flavorants) and represent valuable building blocks in organic synthesis.[20]

In conclusion, enzymatic and chemical hydrolysis have been used in a complementary way for the synthesis of novel 3-(aziridin-2-yl)propionamides and potassium 3-(aziridin-2-yl)propanoates, respectively, starting from 1-arylmethyl-2-(2-cyanoethyl)aziridines without affecting the sensitive aziridine ring. Furthermore, 3-(aziridin-2-yl)propionamides were rearranged into δ-lactams, and 3-(aziridin-2-yl)propanoates were shown to be good precursors for the synthesis of γ-lactones.

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Supporting Information Available. Spectroscopic data of compounds **2a**−**e**, **3a**−**d**, **4a**,**b**,**e**, and **5a**−**c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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