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Bacterial Preparation of Enantiopure Unactivated Aziridine-2-carboxamides and Their Transformation into Enantiopure Nonnatural Amino Acids and *vic*-Diamines

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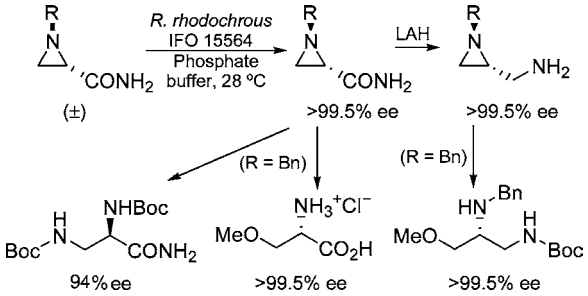
ABSTRACT



Enantiopure (1*R*,2*S*)-1-benzyl- and 1-arylaziridine-2-carboxamides were obtained by kinetic resolution of their racemates by *Rhodococcus rhodochrous* IFO 15564 catalyzed hydrolysis. Several regio- and enantioselective nucleophilic ring openings of (1*R*,2*S*)-1-benzylaziridine-2-carboxamide or its LAH-reduced product led to a series of enantiopure products, such as *O*-methyl-L-serine and some vicinal diamines.

Optically active aziridines have been widely employed in asymmetric synthesis.[1] In addition, they are now well established as valuable intermediates in the synthesis of interesting polyfunctionalized, biologically active compounds, mainly through their highly regio- and stereoselective nucleophilic ring openings (NROs).[1,2] These processes have been studied, by far, much more on activated aziridines (i.e., aziridines bearing an electron-withdrawing substituent at nitrogen) than on unactivated ones.[2d]

The main routes for the synthesis of enantioenriched aziridines start from optically active natural products,[1a,3] entail asymmetric aziridination processes,[4] or involve enzymic resolution of racemic functionalized aziridines by hydrolysis or transesterification reactions of carboxylic esters.[5] To the best of our knowledge, no enzymatic process involving hydration or/and hydrolysis of aziridinecarbonitriles or -carboxamides has been employed with this purpose. However, this methodology is worthy of consideration owing to the mildness of its reaction conditions and also in light of

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some recent bacterial resolutions of closely related molecules (cyclopropanes[6] and oxiranes[7]) bearing nitrile or amide functionalities.

We now report our first findings in this field, namely, the enantioselective hydrolysis of several unactivated 1-benzyl- [(±)-**1a**] or 1-arylaziridine-2-carboxamides [(±)-**1b**−**d**] by the amidase-containing, commercially available bacterium *Rhodococcus rhodochrous* IFO 15564,[8] leading to the easy preparation of enantiopure amides (1*R*,2*S*)-**1a**−**d**. Moreover, some regio- and stereoselective NROs of (1*R*,2*S*)-**1a** and its LAH reduction product are also described.

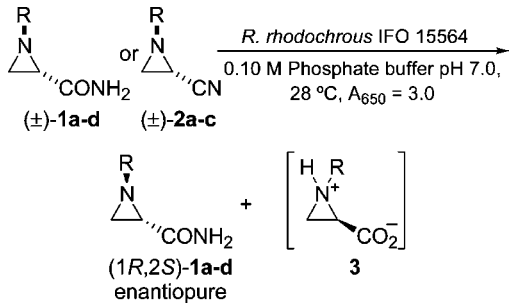
As the slow or blocked pyramidal inversion in aziridines introduces a stereogenic nitrogen,[1a] the possible existence of cis−trans diastereomers (invertomers) has to be taken into account for racemic and enantiopure compounds **1**. However, according to previous ^{1}H NMR studies,[9] our own ^{1}H and ^{13}C NMR spectra, including NOESY correlations, reveal the sole presence of the trans invertomers. DFT calculations (B3LYP method, 6-31G* basis set) also corroborate the existence of these structures.[10]

Biotransformations of racemic substrates *trans*-**1** were carried out with a standard cell concentration of *R. rhodochrous* IFO 15564[11] in the metabolic resting phase [approximately 2.7 mg/mL of aqueous 0.10 M, pH 7.0 potassium phosphate (equivalent to A_{650}[12] = 3.0)]. The reactions were stopped after HPLC analysis (Chiralcel OD) showed the presence of only one out of the two enantiomers of the corresponding aziridinecarboxamide **1**. All the enantiopure amides were obtained in this way with very good yields, considering that the upper limit for any kinetic resolution is 50% yield (Table 1).

Biotransformations can also start from the N-substituted aziridine-2-carbonitriles (±)-**2a**−**c**, due to the concomitant presence in the bacterium of a nitrile hydratase. In such cases, yields (42−43%) were slightly lower than those obtained from (±)-**1**. The nitrile hydratase step was found to be too fast and poorly enantioselective, as has been generally reported for *Rhodococci* strains.[13,14]

It should be noted that *N*-aryl racemic substrates **1b**−**d** (and also **2b**,**c**) produce the corresponding enantiopure amides at least ten times faster than *N*-benzyl substrates (**1a** and also **2a**). A possible reason for such a great difference in reaction rates may lie in the fact that aryl substituents are stiffer than the benzyl group, thus fitting more efficiently into the active site of the amidase.

Table 1. Bacterial Preparation of Enantiopure[a] N-Substituted Aziridine-2-carboxamides **1a**−**d**



product	R	time (h)	yield[b] (%)
(1*R*,2*S*)-**1a**	$PhCH_2$	67	45
(1*R*,2*S*)-**1b**	Ph	4.5	47
(1*R*,2*S*)-**1c**	4-$MeOC_6H_4$	4.1	46
(1*R*,2*S*)-**1d**	4-$F_3CC_6H_4$	6.7	45

[a] ee >99.5% (HPLC). [b]Isolated yield after column chromatography (from *rac*-**1a**−**d** as starting materials).

None of the expected optically active aziridinecarboxylic acids **3** could be isolated after biotransformations. The instability of some aziridine-2-carboxylic acids [including that of 1-(α-methylbenzyl)aziridine-2-carboxylic acid, closely related to **3a**] has been explicitly or implicitly established several times,[15] though without any indication as to their decomposition pathways. By means of complementary experiments, however, we were able to discard a concerted decarboxylation mechanism similar to that well-known for oxiranecarboxylic acids.[16]

To rule out possible incompatibility between amino acids **3** and aqueous media, we carried out a *Candida antarctica* lipase (CAL-B) catalyzed hydrolysis of the ethyl ester of **3a** (0.5 mmol) in anhydrous THF (4 mL), adding a minimal amount of water (54 μL). Although ^{1}H and ^{13}C NMR spectra of the crude material were not conclusive as to the presence of the amino acid **3a**, it can be discarded from the mass spectrum (ESI). After silica gel column chromatography, the remaining ester showed a modest enantiomeric excess (ee = 20%), an indication that enzymatic hydrolysis proceeded, but the tail fractions consisted of a very complex mixture of

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(10) *trans*-**1** compounds are 2.7−4.8 kcal/mol more stable than *cis*-**1**. In *trans*-**1**, an intramolecular hydrogen bond (amide N−H as a proton donor, cyclic N as a proton acceptor) accounts for their extra stabilization in relation to *cis*-**1** compounds.

(11) Gotor, V.; Liz, R.; Testera, A. M. *Tetrahedron* **2004**, *60*, 607−618.

(12) Absorbance or optical density at 650 nm.

(13) Beard, T.; Cohen, M. A.; Parratt, J. S.; Turner, N. J. *Tetrahedron: Asymmetry* **1993**, *4*, 1085−1104.

(14) In a complementary experiment, *R. rhodochrous* was grown in the presence of diethyl phosphoramidate (an amidase inhibitor[11]), suspended in the usual phosphate buffer with an A_{650} of only 0.30, and then exposed to (±)-1-benzylaziridine-2-carbonitrile (**2a**) during 34 min. From the crude product (a 56:44 mixture of **2a**/**1a**; crude yield, 90%), we were able to isolate the amide (1*S*,2*R*)-**1a** with 8% ee. Therefore, the nitrile hydratase has a poor 2*R*-selectivity, the same as the amidase's high 2*R*-selectivity. This observation accounts for the fact that the yields in (1*R*,2*S*)-**1** obtained from (±)-**2** were slightly lower than those obtained from (±)-**1**.

(15) See ref 5c and also: Lambert, C.; Viehe, H. G. *Tetrahedron Lett.* **1985**, *26*, 4439−4442. In ref 5e, such acids have to be formed, but no comment is made about them.

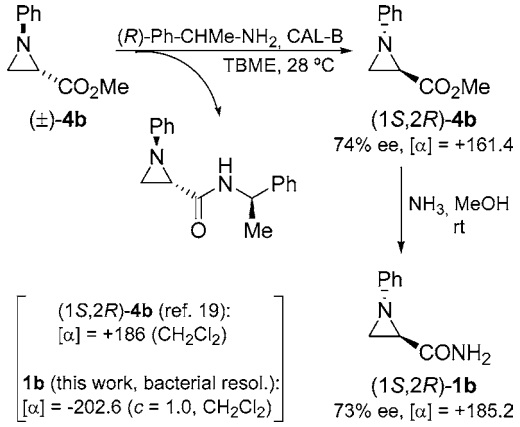
(16) Singh, S. P.; Kagan, J. *J. Org. Chem.* **1970**, *35*, 2203−2207 and references therein.

unidentifiable decomposition compounds. Thus, the above results are also indicative of the instability of the aziridinecarboxylic acids **3**.

The impossibility of isolate amino acids **3** precludes combining their ee values with those of the amides **1** to determine the enantioselectivity (enantiomeric ratio, E[17]) of the processes. However, it is possible to make an estimation of E values taking into account that, at the most, the conversion values range from 0.55 (**1a**, **1d**) to 0.53 (**1b**). From these data, and assuming the ee of enantiopure amides **1** to be at least 99.5%, the lower limits for E values are very good,[18] ranging from 57 to 94, respectively.

All the *R. rhodochrous* mediated obtained amides **1** are (1*R*,2*S*)-configured. To assign this configuration to amide **1b** (Scheme 1), the racemic ester **4b** was kinetically resolved

Scheme 1. Assignment of the Configuration for **1b**



by CAL-B catalyzed aminolysis[19] with (*R*)-α-methylbenzylamine, which left the remaining dextrorotatory ester with moderate ee. Thus, a (2*R*)-configuration could be established for optically active **4b**,[20] i.e., the (1*S*,2*R*)-configuration given its trans structure.[21] Further conventional ammonolysis led without apparent racemization to the amide (1*S*,2*R*)-**1b**, which proved to be dextrorotatory, the opposite to the amide **1b** resulting from *R. rhodochrous* catalyzed hydrolysis. A similar pathway was followed for amide **1a** although after aminolysis the remaining ester **4a** proved to be (1*R*,2*S*)-configured.[22] In the case of amide **1c**, the first step from the racemic ester **4c** was hydrolysis catalyzed by the lipase from *Candida rugosa* (CRL) leading to (−)-**4c**, the configuration of which has been reported to be (1*R*,2*S*).[5e] In view of the close structural similitude between **1b**,**c** and the remaining amide **1d**, we also assume the same (1*R*,2*S*)-configuration for the latter.

As the NRO of unactivated aziridines usually occurs only after protonation, quaternization, or formation of a Lewis acid adduct, we refluxed the aziridineamide (1*R*,2*S*)-**1a** in methanol in the presence of 1 equiv of a diethyl ether−boron trifluoride complex[23] (Scheme 2). This reaction led to an approximately 3:1 mixture of the C-3 and C-2 opening products, respectively, from which the former, enantiopure N^2-benzyl-*O*-methyl-L-serinamide [(*S*)-**5**], was isolated in 61% yield. Subsequent hydrogenolysis followed by reaction with di-*tert*-butyl dicarbonate afforded (85% yield) N^2-Boc-*O*-methyl-L-serinamide, (*S*)-**6**, the careful hydrolysis of which led to the non-proteinogenic amino acid *O*-methyl-L-serine hydrochloride, (*S*)-**7**, in 50% overall yield from (1*R*,2*S*)-**1a**. This latter product[24] proves that the configuration of the C-2 chiral center of the starting (1*R*,2*S*)-**1a** was retained, as it had to be because it was not involved in the process.

The access to homochiral vicinal diamines is currently a goal of outstanding importance because of their biological properties, medicinal interest, and versatility in organic synthesis.[25] Thus, we envisaged that, after reduction of the amide function of (1*R*,2*S*)-**1a**, a further NRO of the resulting product might lead to such a target.

LAH reduction of (1*R*,2*S*)-**1a** did not alter, as expected, the configurations of its stereogenic centers and produced enantiopure (1*R*,2*R*)-1-benzyl-2-aziridinemethanamine, (1*R*,2*R*)-**8**, whose trans structure was established by NOESY correlations. When this compound was submitted to a reaction similar to that described recently for (1*R*,2*S*)-**1a** (in the presence of 2 equiv of $Et_2O{\cdot}BF_3$, as it bears two amino groups), the nucleophilic attack of methanol was fully regioselective at the nonstereogenic C-3 position, thus leading almost quantitatively to enantiopure (*R*)-N^2-benzyl-3-methoxypropane-1,2-diamine, (*R*)-**9**. By means of fine adjustment of the reaction conditions with Boc_2O, we were able to transform (*R*)-**9** into (*R*)-**10**, a *vic*-diamine bearing two orthogonally stable protecting groups,[26] which was shown to be enantiopure by HPLC (Chiralcel OD). As proof of its orthogonality, (*R*)-**10** was easily hydrogenolyzed to (*R*)-N^1-Boc-3-methoxypropane-1,2-diamine, (*R*)-**11**, and, of course, reverted to (*R*)-**9** by acid hydrolysis.

Another obvious way to obtain *vic*-diamines from (1*R*,2*S*)-**1a** is its opening with sodium azide. In fact, this goal was attained by appropriate control of the reaction conditions previously used for the opening of aziridine-2-carboxylic esters and 2-acylaziridines.[27]

Thus, (1*R*,2*S*)-**1a** was reacted at 50 °C with sodium azide (2 equiv) and aluminum trichloride (0.6 equiv) in 50%

(17) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299.

(18) Faber, K.; Hönig, H.; Kleewein, A. http://www.cis.TUGraz.at/orgc.

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(20) Aires-de-Sousa, J.; Prabhakar, S.; Lobo, A. M.; Rosa, A. M.; Gomes, M. J. S.; Corvo, M. C.; Williams, D. J.; White, A. J. P. *Tetrahedron: Asymmetry* **2001**, *12*, 3349−3365.

(21) All the aziridinecarboxylic esters **4** have trans structures as deduced from comparison of their ^{1}H NMR spectra with those of the corresponding aziridineamides **1**.

(22) Häner, R.; Olano, B.; Seebach, D. *Helv. Chim. Acta* **1987**, *70*, 1676−1693.

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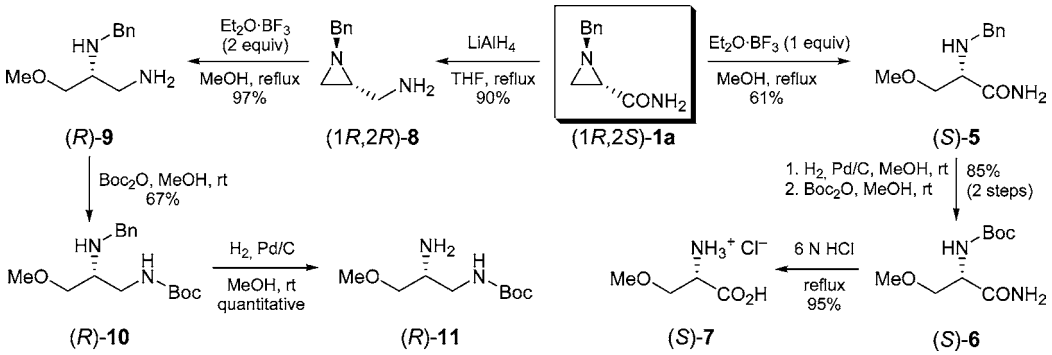
(25) For a review, see: Lucet, D.; Le Gall, T.; Mioskowski, C. *Angew. Chem., Int. Ed.* **1998**, *37*, 2580−2627.

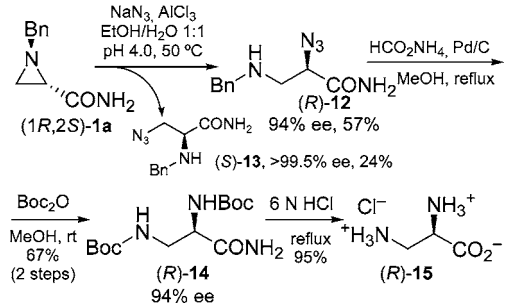
(26) For a review, see: Schelhaas, M.; Waldmann, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2056−2083.

Scheme 2. Ring Openings with Methanol



aqueous ethanol at pH 4.0, to bring the process to completion and to minimize side reactions (Scheme 3). We thereby

Scheme 3. NRO of (1*R*,2*S*)-**1a** with an Azide Anion



observed the formation of an approximately 2.5:1 mixture of the C-2 and C-3 opening products, respectively, and a small amount of the *β*-elimination product proceeding from the former product, namely, 2-azidoacrylamide. The major product, (*R*)-2-azido-3-(benzylamino)propanamide, (*R*)-**12**, was isolated with 94% ee and 57% chemical yield, whereas the minor product, 3-azido-2-(benzylamino)propanamide, (*S*)-**13**, isolated in 24% chemical yield, was shown to be enantiopure. The enantiopurity of (*S*)-**13** proves that starting aziridine did not racemize under the reaction conditions, but the azide attack on C-2 leading to **12** was probably approximately 94% S_N2 and 6% S_N1.[28]

The (*R*)-configuration of product **12** was assigned (Scheme 3) by double hydrogenolysis (ammonium formate, Pd/C) leading to the corresponding, nonisolated diaminoamide, which reacted in situ with Boc_2O to enable isolation of the doubly protected *vic*-diaminoamide (*R*)-**14**. Further hydrolysis of (*R*)-**14** afforded (−)-2,3-diaminopropionic acid hydrochloride, (*R*)-**15**, whose configuration was established by comparison with the literature.[29]

In conclusion, we have accomplished the first bacterial preparation of enantiopure, unactivated aziridine-2-carboxamides and have shown that these compounds can be regio- and stereoselectively opened to several synthetically interesting products such as non-proteinogenic α-amino acids and vicinal diamines. Studies to further extend these methodologies are now in progress.

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Supporting Information Available: Experimental procedures, characterization data, and copies of the ^1H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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