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Enantiopure *trans*-3-Arylaziridine-2-carboxamides: Preparation by Bacterial Hydrolysis and Ring-Openings toward Enantiopure, Unnatural D-α-Amino Acids†

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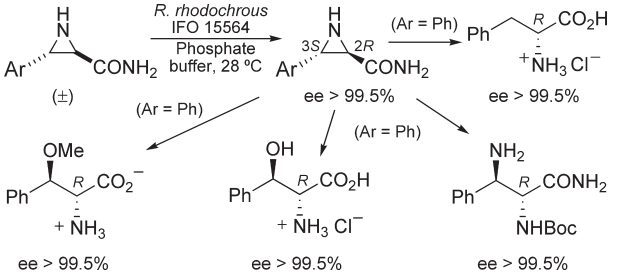

Several racemic *trans*-3-arylaziridine-2-carboxamides were prepared and then resolved by *Rhodococcus rhodochrous* IFO 15564-catalyzed hydrolysis. The resulting enantiopure (2*R*,3*S*)-3-arylaziridine-2-carboxamides are adequate substrates to undergo fully stereoselective nucleophilic ring-openings at the C-3 ring position to finally yield enantiopure, unnatural D-α-aminocarboxylic acids. Experimental evidence is provided that suggests the fate of the (2*S*,3*R*)-3-arylaziridine-2-carboxylic acids concomitantly formed during the resolution processes. In this context, the similar bacterial resolution of racemic 1-arylaziridine-2-carboxamides and -carbonitriles, previously investigated by our research group, has been partially re-examined.

Introduction

The current interest in aziridine chemistry is reflected in a series of reviews published during the past 16 years, ranging from those dealing with synthesis and/or reactivity[1] to other, more recent and specialized reviews covering areas such as nitrogen transfer reactions,[2] parallel- and solid-phase synthesis,[3] and C-heteroatom-substituted aziridines[4] (including the C-lithiated varieties[5]). Several aziridine compounds exhibit antibiotic, antitumor, or other biological activities.[1b,e] Moreover, enantiopure or enantioenriched aziridines have recently received a great deal of attention as precursors of bioactive nitrogen-containing molecules, mainly through their varied regio- and stereoselective ring-opening reactions,[1a,b,d,6] some of which meet most of the stringent criteria for a process to earn click chemistry status.[7]

As to their ring-opening processes, aziridines are usually classified as activated (those bearing an electron-withdrawing N-substituent such as acyl, sulfonyl, and phosphoryl) or unactivated (mainly the NH-, *N*-alkyl-, and *N*-arylaziridines). Ring-opening reactions of activated aziridines are easier and hence much more widely investigated than those of their unactivated counterparts, but suffer the drawback of requiring a further deprotection step of the amide or carbamate moiety present in their open products. Among recent examples of

† Dedicated to the memory of the late Professor José Manuel Concellón.

(1) (a) Tanner, D. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 599–619. (b) Osborn, H. M. I.; Sweeney, J. *Tetrahedron: Asymmetry* **1997**, *8*, 1693–1715. (c) Stamm, H. *J. Prakt. Chem.* **1999**, *341*, 319–331. (d) M°Coull, W.; Davis, F. A. *Synthesis* **2000**, 1347–1365. (e) Sweeney, J. B. *Chem. Soc. Rev.* **2002**, *31*, 247–258. (f) Müller, P.; Fruit, C. *Chem. Rev.* **2003**, *103*, 2905–2919. (g) Hu, X. E. *Tetrahedron* **2004**, *60*, 2701–2743. (h) Zhang, Y.; Lu, Z.; Wulff, W. D. *Synlett* **2009**, 2715–2739. (i) Sweeney, J. *Eur. J. Org. Chem.* **2009**, 4911–4919. (j) Lu, P. *Tetrahedron* **2010**, *66*, 2549–2560.

(2) Watson, I. D. G.; Lily, Y; Yudin, A. K. *Acc. Chem. Res.* **2006**, *39*, 194–206.

(3) Olsen, C. A.; Franzyk, H.; Jaroszewski, J. W. *Eur. J. Org. Chem.* **2007**, 1717–1724.

(4) Singh, G. S.; D'hooghe, M.; De Kimpe, N. *Chem. Rev.* **2007**, *107*, 2080–2131.

(5) Hodgson, D. M.; Bray, C. D.; Humphreys, P. G. *Synlett* **2006**, 1–22.

(6) (a) Cardillo, G.; Gentilucci, G.; Tolomelli, A. *Aldrich. Acta* **2003**, *36*, 39–50. (b) Lee, W. K.; Ha, H.-J. *Aldrich. Acta* **2003**, *36*, 57–63. (c) Pineschi, M. *Eur. J. Org. Chem.* **2006**, 4979–4988.

(7) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021.

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enantiopure unactivated aziridine's ring-openings, those of the following substrates are worth noting: a sizable number of *N*-[(*R* or *S*)-(1-phenylethyl)]-2-substituted aziridines, frequently converted into bioactive products by Ha, Lee, and co-workers;[6b,8] several (1′*S*,2*S*)-*N*-alkyl-2-[1-(dibenzylamino)-alkyl]aziridines opened with a set of heteronucleophiles by Concellón and co-workers;[9] a 2-(2-pyridyl)aziridine derived from (*S*)-valinol also opened with a number of heteronucleophiles;[10] and two 2-arylaziridines that underwent formal [3+2] cycloaddition processes.[11] Several groups have also investigated intramolecular ring-openings that are in fact aziridine expansion processes.[6a,12]

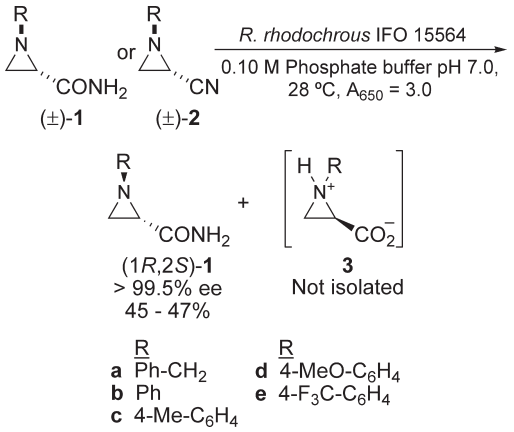
Our interest in this chemistry has recently been demonstrated by the preparation of a number of enantiopure *N*-alkyl- and *N*-arylaziridine-2-carboxamides by bacterial resolution and their further ring-openings, as well as those of some of their derivatives, with external[13] and internal[14] nucleophiles. Two weeks after our first paper, M.-X. Wang and coworkers also reported similar bacterial resolution of *N*-arylaziridine-2-carbonitriles and subsequent ring-openings of the resulting *N*-arylaziridine-2-carboxamides;[15] while about eight months later they extended their work mainly to *trans*-3-aryl-*N*-methylaziridine-2-carbonitriles and -carboxamides.[16] After comparing our work, carried out using the commercial bacterium *Rhodococcus rhodochrous* IFO 15564,[17] and that of Wang et al., who use the commercially unavailable strain *Rhodococcus erythropolis* AJ270,[18] several coincidences emerge, but also an important discrepancy (see first paragraph in the Results and Discussion section).

In this paper we shall extend our work to *trans*-3-arylaziridine-2-carboxamides, which, besides having similar sizes to our first substrates, lack the N-substituent, which would lend greater versatility to the possible enantiopure products resulting from their resolutions and further ring-openings. Moreover, with the aim of clarifying the cited discrepancy, some of our first substrates[13] will be again resolved and then worked up in an almost identical way to that of Wang et al.[16]

Results and Discussion

Bacterial Resolution of 1-Alkyl- and 1-Arylaziridine-2-carboxamides 1 and -carbonitriles 2. Scheme 1 shows our previous results.[13,14] The kinetic resolution of the amides **1** and the nitriles **2** by hydrolysis in the presence of *R. rhodochrous* IFO 15564 affords very good yields of the enantiopure amides **1**, although the corresponding aziridine-2-carboxylic acids, **3**, were never observed or isolated, in agreement with several prior observations.[19] However, besides the enantiopure or almost enantiopure amides **1**, Wang et al. claimed to obtain, in their *R. erythropolis* AJ270-mediated hydrolysis of a number of 1-arylaziridine-2-carbonitriles **2**,[20] very good yields of optically active (85 to >99.5% ee) methyl 1-arylaziridine-2-carboxylates, which proceed from the *in situ* treatment of the corresponding acids **3** with diazomethane.[15]

SCHEME 1. Our Previous Results in the Bacterial Resolution of Aziridines 1 and 2



As our experimental method and that of Wang and coworkers are not identical, we detected the following differences that might perhaps explain the previously mentioned divergent results: (1) Our substrate:whole cells wet weight ratio was approximately 2 mmol/1 g, whereas that of Wang et al. was 1 mmol/1 g. (2) In the isolation of the enantiopure amides **1**, we continuously extracted the supernatant liquids resulting from the biotransformations (CH_2Cl_2, 40 °C, 6 h), whereas Wang et al. extracted them manually (AcOEt, rt). (3) For access to the amino acids **3**, Wang et al. freeze-dried the remaining aqueous phases, whereas we concentrated them *in vacuo* with slight heating (<40 °C). Our slight heating in the above steps 2 and 3 could accelerate a possible decomposition of acids **3**. Additionally, our higher substrate: whole cells ratio, together with an apparent lower activity of our strain, implies longer biotransformation times in our case, which could favor a hypothetical metabolism of **3** by the bacterium.

We therefore decided to repeat the *R. rhodochrous* IFO 15564-mediated resolution of one of our common nitrile substrates, (±)-**2d**, in such a way that the incubation time was similar and the workup identical to those of Wang et al. Thus, 1.8 g wet weight of our bacterium added to 50 mL of potassium phosphate buffer (0.10 M, pH 7.0) produced a suspension (A_{650} = 15.1)[21] able to transform (28 °C, 45 min)

(8) (a) Yoon, H. J.; Kim, Y.-W.; Lee, B. K.; Lee, W. K.; Kim, Y.; Ha, H.-J. *Chem. Commun.* **2007**, 79–81. (b) Kim, Y.; Ha, H.-J.; Yun, S. Y.; Lee, W. K. *Chem. Commun.* **2008**, 4363–4365.

(9) Concellón, J. M.; Bernad, P. L.; Suárez, J. R.; García-Granda, S.; Díaz, M. R. *J. Org. Chem.* **2005**, *70*, 9411–9416, and their own references therein.

(10) Savoia, D.; Alvaro, G.; Di Fabio, R.; Gualandi, A. *J. Org. Chem.* **2007**, *72*, 3859–3862.

(11) Zhu, W.; Cai, G.; Ma, D. *Org. Lett.* **2005**, *7*, 5545–5548.

(12) (a) Hori, K.; Nishiguchi, T.; Nabeya, A. *J. Org. Chem.* **1997**, *62*, 3081–3088. (b) Ferraris, D.; Drury, W. J., III; Cox, C.; Lectka, T. *J. Org. Chem.* **1998**, *63*, 4568–4569. (c) Tomasini, C.; Vecchione, A. *Org. Lett.* **1999**, *1*, 2153–2156. (d) Lu, Z.; Zhang, Y.; Wulff, W. D. *J. Am. Chem. Soc.* **2007**, *129*, 7185–7194.

(13) Morán-Ramallal, R.; Liz, R.; Gotor, V. *Org. Lett.* **2007**, *9*, 521–524.

(14) Morán-Ramallal, R.; Liz, R.; Gotor, V. *Org. Lett.* **2008**, *10*, 1935–1938.

(15) Wang, J.-Y.; Wang, D.-X.; Zheng, Q.-Y.; Huang, Z.-T.; Wang, M.-X. *J. Org. Chem.* **2007**, *72*, 2040–2045.

(16) Wang, J.-Y.; Wang, D.-X.; Pan, J.; Huang, Z.-T.; Wang, M.-X. *J. Org. Chem.* **2007**, *72*, 9391–9394. The trans stereodescriptor refers to the substituents at the C-2 and C-3 positions.

(17) Available from: (a) Institute for Fermentation, Osaka, Japan, IFO 15564 strain; (b) Colección Española de Cultivos Tipo, Universidad de Valencia, Burjassot, Spain, CECT 5949 strain.

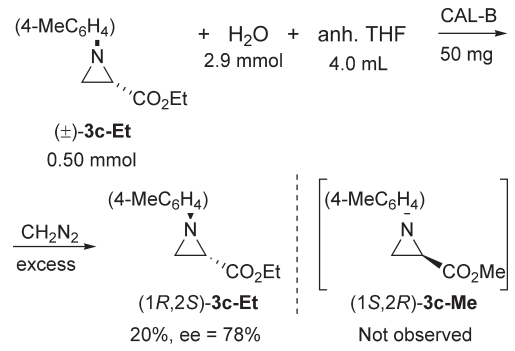
(18) Both strains show nitrile hydratase and amidase activities.

(19) (a) Lambert, C.; Viehe, H. G. *Tetrahedron Lett.* **1985**, *26*, 4439–4442. (b) Martres, M.; Gil, G.; Méou, A. *Tetrahedron Lett.* **1994**, *35*, 8787–8790. (c) Kumar, H. M. S.; Rao, M. S.; Chakravarthy, P. P.; Yadav, J. S. *Tetrahedron: Asymmetry* **2004**, *15*, 127–130.

(20) R = Ph, 4-F-C_6H_4, 4-Cl-C_6H_4, 4-Br-C_6H_4, 4-MeO-C_6H_4, 4-Me-C_6H_4, 3-Me-C_6H_4.

(21) A_{650}: absorbance or optical density measured at 650 nm.

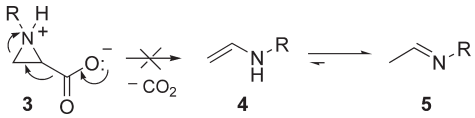
SCHEME 2. CAL-B-Catalyzed Hydrolysis of Ethyl (±)-*trans*-1-(*p*-Tolyl)aziridine-2-carboxylate



0.30 mmol of **2d** and to produce, after conventional room-temperature extraction of the supernatant liquid, the corresponding almost enantiopure amide (1*R*,2*S*)-**1d** (25% yield, 95% ee), virtually exactly as reported by Wang et al.[15] The remaining aqueous phase was lyophilized (−50 to −60 °C, 2×10^{-4} atm) and the resulting residue powdered and treated with a freshly prepared ethereal, yellow diazomethane solution (20 min, below −15 °C). No discoloring of this solution was observed and, finally, no residue was obtained after filtration and further solvent removal. A similar process starting from the amide (±)-**1c** produced (1*R*,2*S*)-**1c** (40% yield, 96% ee) and a very small amount [6% yield, probably (1*S*,2*R*)] of the enantiopure methyl ester derived from **3c**. Consequently, and given that no or very limited amounts of methyl esters were obtained, we conclude that amino acids **3**, which necessarily have to be formed due to bacterial amidase activity, are essentially unstable under the conditions of our biotransformations.

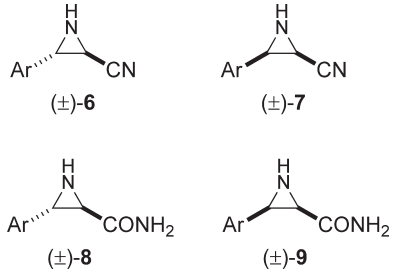
On the other hand, besides the data in the literature,[19] we can adduce clear evidence for the inherent instability of amino acids **3**. In fact, we carried out the *Candida antarctica* lipase catalyzed (CAL-B) hydrolysis (28 °C, 3 h) of the racemic ethyl ester **3c-Et** (0.50 mmol) in THF (4 mL) in the presence of a minimal amount of water (53 μL), thus avoiding the extraction and freeze-drying steps of bacterial biotransformations. The crude residue obtained by further *in vacuo* concentration at room temperature was treated with ethereal diazomethane without discoloring. After chromatographic purification, we were able to isolate the optically active ethyl ester (20% yield, 78% ee), but not the methyl ester **3c-Me** proceeding from the concomitantly formed amino acid **3c** (Scheme 2). Moreover, this methyl ester was not observed (^{1}H NMR) in the crude residue or in other chromatographic fractions.

Although we do not know the decomposition route of the amino acids **3**, it seems clear that it is different from the well-established pathway for some related oxirane-2-carboxylic (glycidic) acids.[22] Should it be so (eq 1), they would produce imines **5** or their hydrolysis products, which was not the case. To prove that these putative products would survive after our biotransformations, we prepared *N*-ethylidenebenzylamine, **5a** (R = PhCH$_2$), and exposed it to the same experimental conditions, and even reaction times, as our bacterial hydrolyses of (±)-**1a** and (±)-**2a**, both in the presence and in the absence (blank reaction) of the IFO 15564 strain. A conventional workup after the blank process leads to a 9:1 molar mixture of benzylamine and **5a**, whereas the workup after the former reaction yields benzylamine and several unidentified products, none of which were detected (NMR analysis) in the crude materials obtained after the standard biotransformations of **1a** and **2a**.



(1)

Synthesis and Bacterial Resolution of *trans*-3-Arylaziridine-2-carboxamides 8. Several failed attempts of resolving 1,2,3-trisubstituted aziridines [e.g., *trans*-1-benzyl-3-(trifluoromethyl)aziridine-2-carboxamide and *cis*-1-benzyl-3-phenylaziridine-2-carbonitrile],[23] which were recovered unchanged after more than one week of standard incubation with *R. rhodochrous* IFO 15564,[13] convinced us that they are too bulky to be accepted by the bacterial enzymes. Thus, we turned our attention to the 2,3-disubstituted NH-aziridines **6**−**9**. Besides their interesting features already pointed out for **8**, these substrates closely resemble 3-aryloxirane-2-carbonitriles and -carboxamides, which were successfully hydrolyzed in the presence of the *Rhodococcus* AJ270 strain.[24] Moreover, when our work was already in progress, the aforementioned resolution with this last strain of *trans*-3-aryl-1-methylaziridine-2-carbonitriles and -carboxamides was reported, being only slightly bulkier than our intended new substrates, as was that of *trans*-3-phenylaziridine-2-carboxamide, **8a**,[16] a set of processes that will allow us to establish new points of comparison between the behaviors of the two bacterial strains.



In principle, any of the racemic substrates **6**−**9** would have been adequate to begin the study. However, owing to the well-known low enantiodiscrimination displayed by the nitrile hydratase of *R. rhodochrous* IFO 15564,[13] we prepared only two nitrile substrates, (±)-**6a** and (±)-**7a** (Ar = Ph).[25] As expected, our attempts at resolving **6a** during the nitrile hydratase-catalyzed step (**6a** → **8a**) were disappointing, the reaction being so fast that it was necessary to employ a bacterial suspension in potassium phosphate buffer of A_{650} = 0.3 instead of the standard suspension (A_{650} = 3.0).

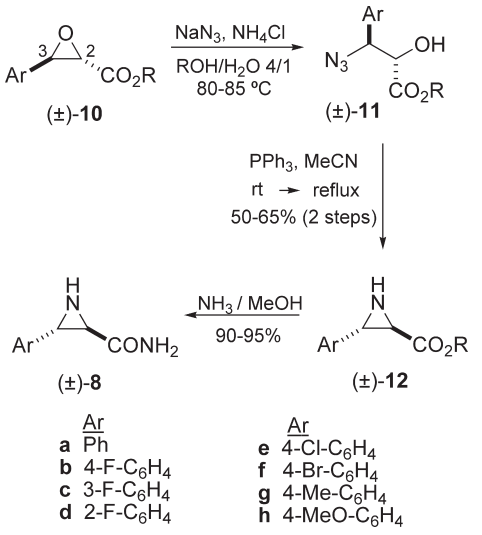
(22) Singh, S. P.; Kagan, J. *J. Org. Chem.* **1970**, *35*, 2203–2207, and references therein. However, this pathway does seem to work in the bacterial hydrolysis of 3-arylaziridine-2-carboxamides; see later.

(23) Stereodescriptors cis/trans describe here the relative positions of the C-2 and C-3 substituents.

(24) Wang, M.-X.; Lin, S.-J.; Liu, C.-S.; Zheng, Q.-Y.; Li, J.-S. *J. Org. Chem.* **2003**, *68*, 4570–4573.

(25) By addition of bromine to cinnamonitrile followed by Gabriel−Cromwell reaction between the obtained 2,3-dibromo-3-phenylpropanenitrile and ammonia (solution in methanol), diastereomers **6a** and **7a** are easily separable by flash column chromatography.

SCHEME 3. Preparation of (±)-*trans*-3-Arylaziridine-2-carboxamides 8

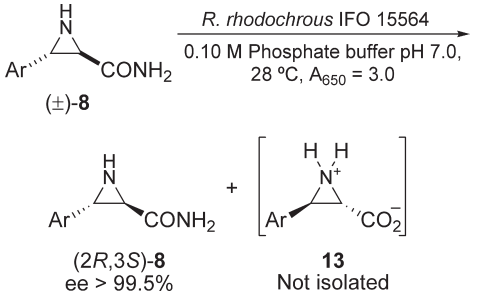


Under these conditions, and stopping the hydration step after 27% conversion of nitrile, the amide (2*S*,3*R*)-**8a** was obtained with an enantiomeric excess of only 56%, which is indicative of a poorly enantioselective process (enantiomeric ratio,[26] $E \cong 4$).[27] The hydration step of the *cis*-nitrile **7a** was much slower and required an A_{650} 6.0 bacterial suspension for it to progress adequately within a reasonable reaction time. Even so, its enantiomeric ratio was also very low ($E = 12$). These two disappointing experiments counseled us to finally discard the nitrile hydratase-catalyzed biotransformation of the nitrile substrates **6** and **7** as a valid resolution method.

As to the racemic *cis*-amides **9**, we only prepared (±)-**9a** (Ar = Ph) by means of a somewhat unexpected method, namely, the exposure of an A_{650} 6.0 suspension of *R. rhodochrous* IFO 15564 to the *cis*-nitrile (±)-**7a** during five hours, after which the *cis*-amide **9a** was obtained with 89% yield as a racemate, as proven by chiral HPLC analysis. This experiment demonstrates that the *cis*-amide **9a** is not accepted as a substrate by the amidase of *R. rhodochrous* IFO 15564,[28] a feature that this microorganism shares with *R. erythropolis* AJ270.[16] We hence assumed that other *cis*-amides **9** would not be resolved by our bacterium.

The access to racemic *trans*-amides **8** is shown in Scheme 3. The methyl or ethyl *trans*-3-arylglycidates (±)-**10** are commercially available or easily accessible through a fully diastereoselective Darzens reaction between methyl chloroacetate and the corresponding substituted benzaldehyde.[29] The ring-opening of epoxides **10** with sodium azide[30] took place with complete regioselectivity at the C(3) benzylic position.[31]

TABLE 1. Bacterial Preparation of Enantiopure *trans*-3-Arylaziridine-2-carboxamides 8



product	Ar	time (h)	yield[a] (%)
(2*R*,3*S*)-**8a**	Ph	15	46
(2*R*,3*S*)-**8b**	4-F-C_6H_4	12	46
(2*R*,3*S*)-**8c**	3-F-C_6H_4	37	44
(2*R*,3*S*)-**8d**	2-F-C_6H_4	17.5	47
(2*R*,3*S*)-**8e**	4-Cl-C_6H_4	14.5	42
(2*R*,3*S*)-**8f**	4-Br-C_6H_4	18.5	40
(2*R*,3*S*)-**8g**	4-Me-C_6H_4	6.25	40

[a]Isolated yield after flash column chromatography.

A Staudinger reaction[32] of the β-azido alcohols **11** led to the *trans*-aziridine esters **12** through *cis*-1,3,2-oxazaphospholidine intermediates;[33] the retention of the trans geometry in compounds **12** relies on the fact that both chiral centers in **10** undergo inversion during the first (C-3) and the second step (C-2). Finally, a conventional ester ammonolysis gave rise to the amides **8**. The trans nature of all aziridinecarboxamides (±)-**8** was confirmed by means of the invaluable coupling constants between their aziridinic protons, the ^{1}H NMR signals of which are invariably broad singlets, whereas those of similar protons for *cis*-3-phenylaziridine-2-carboxamide, (±)-**9a**, are doublets with a coupling constant of 7.0 Hz.

Under conditions as particularly mild as those for substrates **1** and **2** (0.10 M phosphate buffer suspensions of *R. rhodochrous* IFO 15564, $A_{650} = 3.0$, 28 °C, pH 7.0),[13] *trans*-3-arylaziridine-2-carboxamides (±)-**8a**−**g** underwent efficient hydrolytic resolutions within hours (Table 1). The reactions were stopped after chiral HPLC analysis showed the presence of only one out of the two enantiomers of the corresponding aziridinecarboxamide, **8**. All the enantiopure amides were obtained in this way with good yields, given that the upper limit for any kinetic resolution is 50% yield. Only the reaction with the 3-(*p*-anisyl) substrate, (±)-**8h**, failed: in the presence of phosphate buffer, and even in the absence of the bacterium, it led to a complex mixture of products, from where only one product could be identified (see later).

Bearing in mind the results of our first study,[13] no efforts were made to resolve the corresponding *trans*-3-arylaziridine-2-carbonitriles (±)-**6** through the consecutive actions of the bacterial nitrile hydratase and amidase, as we were convinced that the final yields would not be improved. Comparing Scheme 1 and Table 1, it can seemingly be deduced that there was an inversion of the enantiopreferences shown by the *R. rhodochrous* IFO 15564 amidase toward amides **1** (2*R* preference) and

(26) (a) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299. (b) Faber K.; Hönig, H.; Kleewein, A. http://www.cis.TUGraz.at/orgc.

(27) This calculation is only approximate, as a small fraction of the obtained amide disappears as a result of the amidase activity of the bacterium.

(28) Of course, the direct exposure of *cis*-amide (±)-**9a** to *R. rhodochrous* IFO 15564 led to the quantitative recovery of the substrate after one week.

(29) Tranchant, M.-J.; Dalla, V. *Tetrahedron* **2006**, *62*, 10255–10270.

(30) Boruwa, J.; Borah, J. C.; Kalita, B.; Barua, N. C. *Tetrahedron Lett.* **2004**, *45*, 7355–7358.

(31) Legter, J.; Thijs, L.; Zwanenburg, B. *Tetrahedron Lett.* **1989**, *30*, 4881–4884.

(32) Lin, F. L.; Hoyt, H. M.; van Halbeek, H.; Bergman, R. G.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2005**, *127*, 2686–2695.

(33) Xiong, C.; Wang, W.; Cai, C.; Hruby, V. J. *J. Org. Chem.* **2002**, *67*, 1399–1402.

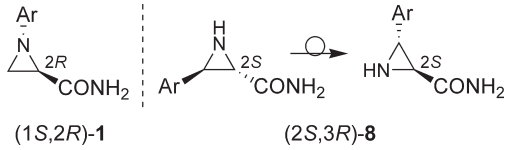
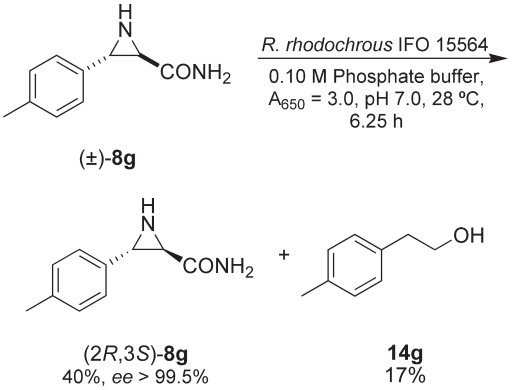


FIGURE 1. Coincidental geometries of the enantiomers of **1** and **8** transformed (not isolated) by the *R. rhodochrous* IFO 15564 amidase.

8 (2*S* preference). However, a 180° rotation around the axis that bisects the N1−C2−C3 angle in aziridines (2*S*,3*R*)-**8** clearly shows that the amidase actually selected enantiomers with an identical geometry in both cases (Figure 1), a feature that Wang et al. have also highlighted with respect to the behavior of their AJ270 amidase.[16] Although the topology of the active site of the IFO 15564 amidase remains unknown, this observation, together with its inability to accept *cis*-amide **9a** and *trans*-1-benzyl-3-(trifluoromethyl)aziridine-2-carboxamide, suggests that it contains an elongated rather than a globular pocket and that the factors that control the fixation of the substrates in it are mainly steric in nature.

As in the case of aziridinecarboxamides **1**, in the biotransformations of substrates **8** we were not able to isolate the expected amino acids **13** either. Nevertheless, on this occasion we did obtain some evidence regarding one of their possible decomposition pathways. In fact, the residue resulting from the fastest biotransformation, that of (±)-*trans*-3-(*p*-tolyl)aziridine-2-carboxamide, (±)-**8g**, consisted of a 71:29 molar mixture of (2*R*,3*S*)-**8g** and 2-(*p*-tolyl)ethanol, **14g**, which were finally isolated with 40% and 17% yields, respectively (Scheme 4).

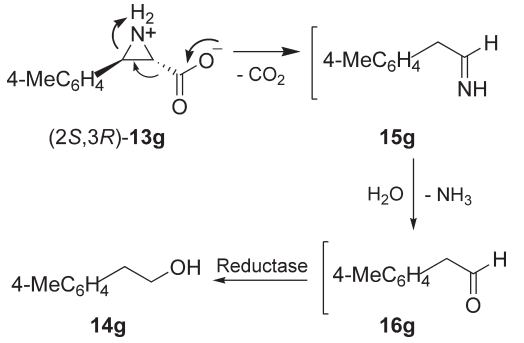
SCHEME 4. Bacterial Resolution of (±)-8g



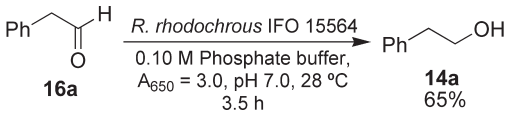
Seeing as the sum of both yields exceeds the 50% threshold, which is the maximum for the enantiopure amide, the unexpected alcohol must proceed from the elusive acid (2*S*,3*R*)-**13g**. This process can be rationalized assuming a decomposition pathway (Scheme 5) similar to that described for some glycidic acids,[22] a route found to be ineffective for the above-mentioned acids **3** (eq 1). The resulting unstable imine **15g** would hydrolyze in the phosphate buffer to *p*-tolylacetaldehyde, **16g**, which would in turn be converted into the alcohol **14g** by the action of a reductase from *R. rhodochrous* IFO 15564.

To the best of our knowledge, no reductase activity has been previously reported for the IFO 15564 cells, even in the presence of suitable substrates such as β-keto nitriles and β-keto amides.[34] However, it is well known that other species

SCHEME 5. Proposed Pathway for the Conversion of (2*S*,3*R*)-13g into 14g



of *Rhodococcus*,[35] and also *R. rhodochrous* different from the IFO 15564 strain,[36] catalyze the reduction of ketones. Moreover, *R. rhodochrous* IFO 15564 itself is able to enantioselectively oxidize secondary alcohols,[37] thus making its opposite reductase activity conceivable. To corroborate the last step proposed in Scheme 5, we therefore incubated 0.308 mmol of commercial phenylacetaldehyde **16a** [the maximum desirable amount according Scheme 5 after a standard biotransformation of 0.616 mmol of (±)-**8a**] with our bacterium under the same conditions as the cited standard biotransformations. In fact, the aldehyde disappeared after 3.5 h and 2-phenylethanol, **14a**, was finally isolated in 65% yield (eq 2).



(2)

It should be emphasized that we observed only the formation of a 2-arylethanol, **14g**, at the end of the fastest (6.25 h) bacterial resolution (that of the aziridinecarboxamide **8g**, Scheme 4). From the complex mixture obtained after only six hours in the unsuccessful resolution of (±)-*trans*-3-(*p*-anisyl)-aziridine-2-carboxamide, (±)-**8h**, we were also consistently able to isolate the corresponding 2-(*p*-anisyl)ethanol, **14h**. All these data hint that 2-arylethanols **14** are metabolized by the IFO 15564 bacterium, a process that might ultimately be responsible for the usual elusiveness of the amino acids **13**.

Reality, however, seems to be somewhat more complex. In fact, the standard incubation during 24 h of 0.308 mmol of commercial 2-phenylethanol, **14a**, with *R. rhodochrous* IFO 15564 resulted in the recovery of 0.222 mmol of **14a** as the sole reaction product, which implies that approximately one-quarter of the alcohol was metabolized. This metabolic activity is very similar to that observed when **14a** was added in 14 successive portions to the bacterial suspension, an experiment designed to mimic the supposed progressive formation of **14a** during the bacterial hydrolysis of (±)-*trans*-3-phenylaziridine-2-carboxamide, **8a**. However, the apparent metabolic activity of the IFO 15564 strain versus **14a**

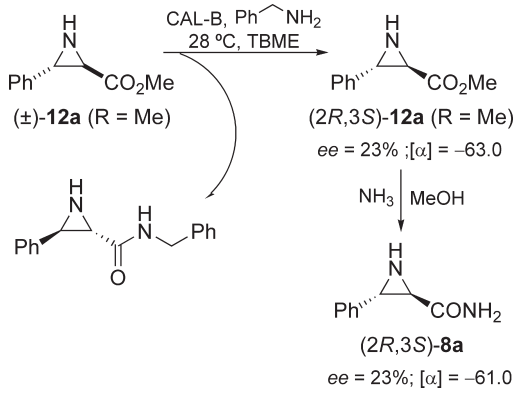
(34) Gotor, V.; Liz, R.; Testera, A. M. *Tetrahedron* **2004**, *60*, 607–618.

(35) (a) Stampfer, W.; Kosjek, B.; Moitzi, C.; Kroutil, W.; Faber, K. *Angew. Chem., Int. Ed.* **2002**, *41*, 1015–1017. (b) Stampfer, W.; Kosjek, B.; Faber, K.; Kroutil, W. *J. Org. Chem.* **2003**, *68*, 402–406. (c) Matsuda, T.; Yamanaka, R.; Nakamura, K. *Tetrahedron: Asymmetry* **2009**, *20*, 513–557.

(36) Patel, R. N.; Robison, R. S.; Szarka, L. J.; Kloss, J.; Thottathil, J. K.; Mueller, R. H. *Enzyme Microb. Technol.* **1991**, *13*, 906–912.

(37) Ohtsuka, Y.; Katoh, O.; Sugai, T.; Ohta, H. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 483–491.

SCHEME 6. Assignment of the Absolute Configuration of 8a



deduced from eq 2 would be much higher (35% of material disappearing after only 3.5 h). This observation implies that our bacterium not only reduces the aldehyde **16a** but also metabolizes it to a certain extent. Moreover, in the overall process of decomposition of amino acids **13**, it should be conceivable that the imines **15** and even the amino acids themselves were also partially metabolized. Thus, the exceptional observation of the alcohol **14g** after the standard bacterial hydrolysis of **8g** could be attributed to a combination of fast hydrolysis and (in all probability) slow metabolism.

Interestingly enough, another fact worth noting is that the amino acid **13a** was not observed after the *R. erythropolis* AJ270-catalyzed hydrolysis of (±)-**8a**, nor were the expected corresponding amino acids in the AJ270-mediated hydrolysis of a number of racemic 3-aryl-1-methylaziridine-2-carboxamides.[16] Therefore, after considering the aforementioned numerous similarities between our strain and the AJ270 bacterium, we lack a plausible explanation for the different fates of amino acids **3** in the resolutions of the amides **1** and nitriles **2** catalyzed by both bacteria.

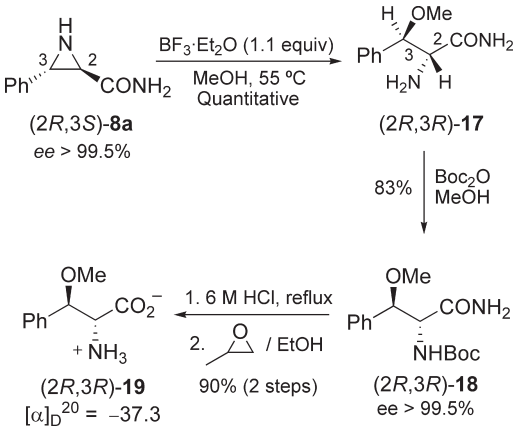
The (2*R*,3*S*)-absolute configuration of **8a** was assigned by its chemical correlation with methyl (2*R*,3*S*)-3-phenylaziridine-2-carboxylate, (2*R*,3*S*)-**12a**, whose $[\alpha]^{20}_D$ value is −290 (*c* 1.1, $CHCl_3$).[38] With this aim in view, (±)-**12a** (R = Me; prepared as shown in Scheme 3) was enzymatically resolved by CAL-B-catalyzed aminolysis with benzylamine, which affords an optically active sample of **12a** (R = Me) showing $[\alpha]^{20}_D$ = −63.0 (23% ee), i.e., (2*R*,3*S*)-configured (Scheme 6). A further conventional ammonolysis of the ester function in (2*R*,3*S*)-**12a** (R = Me) necessarily led, without racemization, to (2*R*,3*S*)-**8a**, with a $[\alpha]^{20}_D$ value of −61.0. Given that the amide **8a** obtained by bacterial hydrolysis was also levorotatory, its (2*R*,3*S*) configuration is thus confirmed. The *R* configuration of the C-2 chiral center in the enantiopure aziridine **8g** will be established later (Scheme 12); moreover, its trans geometry fixes its entire (2*R*,3*S*) configuration. In view of the close similarity between **8a**,**g** and the remaining *trans*-aziridinecarboxamides **8**, we also assume the same (2*R*,3*S*) configuration for all of them.

Ring-Openings of Enantiopure Aziridinecarboxamide 8a. Previous reports[13,15,16] have shown that ring-openings of aziridine-2-carboxamides constitute efficient ways to access unnatural amino acids,[39] a compound class with a wide profile

(38) Gentilucci, L.; Gijzen, Y.; Thijs, L.; Zwanenburg, B. *Tetrahedron Lett*. **1995**, *36*, 4665–4668.

(39) Boto, A.; Gallardo, J. A.; Hernández, D.; Hernández, R. *J. Org. Chem*. **2007**, *72*, 7260–7269, and references therein.

SCHEME 7. Ring-Opening of (2*R*,3*S*)-8a with Methanol



of interesting properties and applications such as, *inter alia*, their incorporation to semisynthetic proteins[40] and therapeutic peptides.[41] Although ring-openings of 3-unsubstituted aziridinecarboxamides **1** occur at the N−C(2) or N−C(3) bond depending on the used nucleophile and reaction conditions,[13,15] it is to be expected that those of (2*R*,3*S*)-**8a** mainly or exclusively take place with N−C(3) breaking owing to the benzylic nature of the C-3 aziridinic position and in close analogy with the ring-openings of 1-methyl-3-phenylaziridine-2-carboxamide.[16] Should this be so, the *R* configuration of the C-2 chiral center will be retained, which would smooth the way for access to (*R*)-2-aminocarboxylic acids, i.e., D-α-amino acids, as will be shown next. These increasingly attractive types of nearly unnatural amino acids, whose consistently low abundance in human tissues has been related to renal and neurodegenerative diseases,[42] have been used, for instance, in the construction of short all-D antimicrobial peptides with considerable resistance to enzymatic degradation, in the synthesis of anti-HIV drugs,[43] and in the replacement of L-amino acid residues in depsipeptides to enhance their activity against colon cancer.[44]

Ring-Opening of (2*R*,3*S*)-8a with Methanol. The use of this nucleophile at 55 °C, in the presence of 1.1 equiv of boron trifluoride−diethyl ether (1:1), resulted in the fully regioselective attack at the C-3 position, which led quantitatively to (2*R*,3*R*)-2-amino-3-methoxy-3-phenylpropanamide, (2*R*,3*R*)-**17** (Scheme 7).[45] The enantiopurity of (2*R*,3*R*)-**17** was confirmed by chiral HPLC analysis of its *N*-Boc derivative, (2*R*,3*R*)-**18**. To reach the corresponding D-α-amino acid,

(40) (a) Wang, L.; Schultz, P. G. *Angew. Chem., Int. Ed*. **2005**, *44*, 34–66. (b) Pellois, J.-P.; Muir, T. W. *Curr. Opin. Chem. Biol*. **2006**, *10*, 487–491. (c) Parrish, A. R.; Wang, W.; Wang, L. *Curr. Opin. Neurobiol*. **2006**, *16*, 585–592.

(41) Sato, A. K.; Viswanathan, M.; Kent, R. B.; Wood, C. R. *Curr. Opin. Biotechnol*. **2006**, *17*, 638–642.

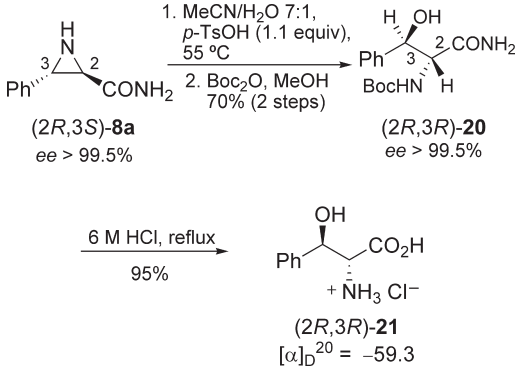
(42) Hamase, K.; Morikawa, A.; Zaitsu, K. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci*. **2002**, *781*, 73–91.

(43) (a) Wakayama, M.; Yoshimune, K.; Hirose, Y.; Moriguchi, M. *J. Mol. Catal. B: Enzym*. **2003**, *23*, 71–85. (b) Nakatani, S.; Hidaka, K.; Ami, E.; Nakahara, K.; Sato, A.; Nguyen, J.-T.; Hamada, Y.; Hori, Y.; Ohnishi, N.; Nagai, A.; Kimura, T.; Hayashi, Y.; Kiso, Y. *J. Med. Chem*. **2008**, *51*, 2992–3004.

(44) Otrubova, K.; Styers, T. J.; Pan, P.-S.; Rodríguez, R.; McGuire, K. L.; McAlpine, S. R. *Chem. Commun*. **2006**, 1033–1034.

(45) The regiochemistry of all the open products in this paper was elucidated by NMR-HMBC experiments (see the Supporting Information). For instance, a three-bond correlation was observed between the ortho carbon atoms and the C-3 proton in **17**, but not between the ortho carbon atoms and the C-2 proton.

SCHEME 8. Ring-Opening of (2*R*,3*S*)-8a with Water

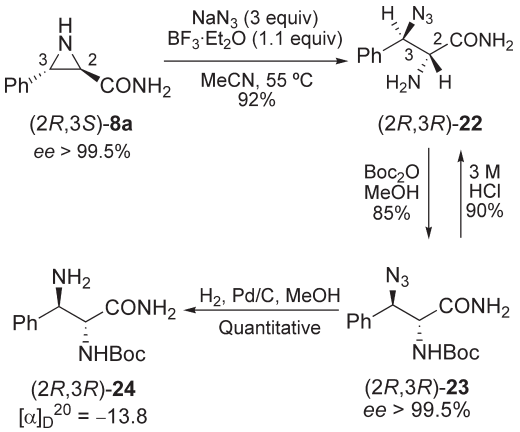


(3*R*)-β-methoxy-D-phenylalanine, (2*R*,3*R*)-**19** (75% overall yield), the amide and carbamate functions in (2*R*,3*R*)-**18** were hydrolyzed, with further treatment with propylene oxide. The optical rotation of the obtained **19**, $[\alpha]^{20}_D$ = −37.3 (*c* 0.60, H_2O), compared with that reported for (2*S*,3*S*)-**19**, $[\alpha]^{20}_D$ = +34.5 (*c* 0.6, H_2O),[46] together with the obligatory retention of configuration of the C-2 aziridinic chiral center, allows us to assign the 2*R*,3*R* configuration to **19**, which is consistent with an inversion of configuration at C-3 as a result of an S_N2 attack.

Ring-Opening of (2*R*,3*S*)-8a with Water. The reaction of compounds **8** with water constitutes an evident way for accessing β-amino alcohol derivatives. The β-amino alcohol (also known as 1,2-amino alcohol) moiety is a common structural component in a vast group of natural products, synthetic pharmacologically active molecules, and ligands or chiral auxiliaries.[47] Moreover, β-amino alcohols are suitable intermediates for the synthesis of unnatural amino acids, β-blockers, insecticidal agents, and antibiotics.[48]

The ring-opening of (2*R*,3*S*)-**8a** with water failed in the presence of boron trifluoride−diethyl ether (1:1) or trifluoroacetic acid, but was successful when employing *p*-toluenesulfonic acid (1.1 equiv) as activating agent at 55 °C (Scheme 8). To facilitate the purification process, the crude material was treated *in situ* with di-*tert*-butyl dicarbonate, after which only the product proceeding from an initial nucleophilic attack at the C-3 ring position, (2*R*,3*R*)-2-(*tert*-butoxycarbonylamino)-3-hydroxy-3-phenylpropanamide, (2*R*,3*R*)-**20**, was isolated with a 70% overall yield. The enantiopurity of this carbamate (HPLC) demonstrates that the ring-opening was completely stereoselective. Finally, simultaneous hydrolysis of the amide and carbamate moieties in (2*R*,3*R*)-**20** led to the (3*R*)-β-hydroxy-D-phenylalanine hydrochloride, (2*R*,3*R*)-**21**, whose absolute configuration was assigned by comparison of the sign of its specific rotation, $[\alpha]^{20}_D$ = −59.3 (*c* 1.0, 6 M HCl), with a recently reported value in the literature.[49]

SCHEME 9. Ring-Opening of (2*R*,3*S*)-8a with Sodium Azide



Ring-Opening of (2*R*,3*S*)-8a with Sodium Azide. The ring-opening of enantioenriched aziridines with the azide anion is a useful way for obtaining optically active vicinal diamines. The preparation of these compounds is currently a goal of outstanding importance because of their biological properties, medicinal interest, and versatility in organic synthesis.[50]

Owing to the lack of N-substituent, the azide attack to (2*R*,3*S*)-**8a** will generate, after reduction of the azide group, the same open structure (2,3-diamino-3-phenylpropanamide) irrespective of the C-2 or C-3 point of attack. However, the formation of D-α-amino acid derivatives requires the configuration of the C-2 chiral center to be retained, i.e., the attack at C-3. Our previous reported methodology for the ring-opening of the 3-unsubstituted aziridinecarboxamide (1*R*,2*S*)-**1a** with azide anion afforded, in the presence of aluminum trichloride, a 2.5:1 mixture of the C-2 and C-3 opening products,[13] which would be inappropriate for our current interest. Then, although the existence of a 3-phenyl substituent in (2*R*,3*S*)-**8a** could favor the C-3 attack, we decided to assay other reaction conditions for enhancing C-3 regioselectivity in (1*R*,2*S*)-**1a**. The treatment of this aziridine with sodium azide (3 equiv) in refluxing acetonitrile and in the presence of 1.1 equiv of boron trifluoride−diethyl ether (1:1) thus led exclusively to the C-3 opening product.[51] By applying this methodology to (2*R*,3*S*)-**8a**,[52] we obtained (2*R*,3*R*)-2-amino-3-azido-3-phenylpropanamide, (2*R*,3*R*)-**22**, with a very good yield (Scheme 9). A further reaction of this intermediate product with di-*tert*-butyl dicarbonate afforded the azido(carbamoyl)carbamate (2*R*,3*R*)-**23**, a synthetic equivalent of an orthogonally protected *vic*-diamine, as proven by its reversion into (2*R*,3*R*)-**22** via acid hydrolysis and its hydrogenolysis to (2*R*,3*R*)-3-amino-2-(*tert*-butoxycarbonylamino)-3-phenylpropanamide, (2*R*,3*R*)-**24**, which was finally obtained with an overall yield of 78%. The enantiopurity of the carbamate (2*R*,3*R*)-**23** (HPLC) proved that the initial ring-opening took place fully stereoselectively, which is consistent with an S_N2 attack of the azide anion.

(46) Hansen, D. B.; Joullié, M. M. *Tetrahedron: Asymmetry* **2005**, *16*, 3963–3969. These authors also describe the diastereomer (2*S*,3*R*)-**19**, whose $[\alpha]^{20}_D$ = −18.4 (*c* 0.4, H_2O), considered in its absolute value, reinforces our assignment of configuration.

(47) (a) Ager, D. J.; Prakash, I.; Schaad, D. R. *Chem. Rev.* **1996**, *96*, 835–875. (b) Bergmeier, S. C. *Tetrahedron* **2000**, *56*, 2561–2576.

(48) (a) Corey, E. J.; Zhang, F.-Y. *Angew. Chem., Int. Ed.* **1999**, *38*, 1931–1934. (b) Bose, D. S.; Narsaiah, A. V. *Bioorg. Med. Chem.* **2005**, *3*, 627–630. (c) Yadav, J. S.; Reddy, A. R.; Narsaiah, A. V.; Reddy, B. V. S. *J. Mol. Catal. A: Chem.* **2007**, *261*, 207–212. (d) Clerici, A.; Ghilardi, A.; Pastori, N.; Punta, C.; Porta, O. *Org. Lett.* **2008**, *10*, 5063–5066.

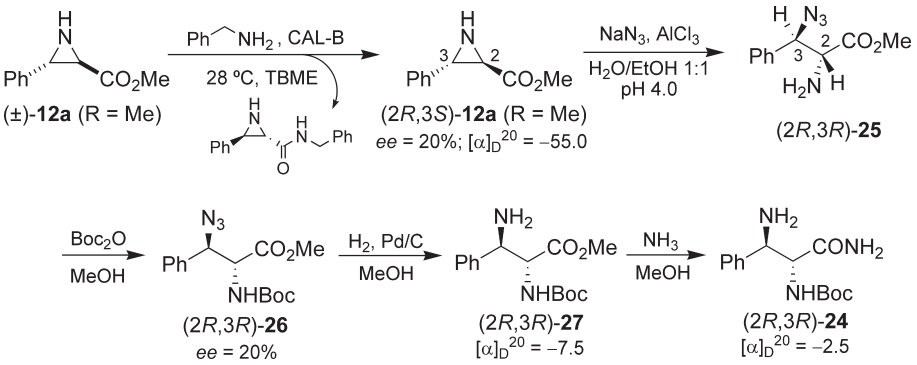
(49) Davies, F. A.; Srirajan, V.; Fanelli, D. L.; Portonovo, P. *J. Org. Chem.* **2000**, *65*, 7663–7666. $[\alpha]^{20}_D$ = −63.2 (*c* 0.65, 6 M HCl).

(50) (a) Lucet, D.; Le Gall, T.; Mioskowski, C. *Angew. Chem., Int. Ed.* **1998**, *37*, 2580–2627. (b) Kim., H.; So, S. M.; Kim, B. M.; Chin, J. *Aldrich. Acta* **2008**, *41*, 77–88.

(51) Morán Ramallal, R. Ph.D. dissertation, University of Oviedo, 2009.

(52) If NaN_3 is previously dissolved in the minimum amount of water, the process is much faster, with no competition of water as nucleophile. The reaction was carried out at 55 °C, seeing as it was very slow at room temperature; on the other hand, it afforded a complex mixture of products in refluxing MeCN.

SCHEME 10. Assignment of the Absolute Configuration of 22



The (2*R*,3*R*)-configuration of the open product **22** was assigned by chemical correlation between the monoprotected diamino amide (2*R*,3*R*)-**24** and its corresponding methyl ester, (2*R*,3*R*)-**27** (Scheme 10), assuming that, as in other similar transformations, the configurations of the chiral centers not involved in the successive reactions in Schemes 9 and 10 are retained. The first step in Scheme 10 is the same as in Scheme 6, although the resulting ester showed a slightly lower ee value in this case. The azide ring-opening of the nonracemic aziridinic methyl ester, accomplished this time in the presence of aluminum trichloride, occurred exclusively at the N−C(3) bond to generate (2*R*,3*R*)-**25**; the inversion of configuration at the C-3 chiral center will be made evident forthwith. The protection of this amino ester led to carbamate **26**, whose retained ee value demonstrated that no racemization took place until this point. Given that the following azide hydrogenolysis cannot affect the chiral center's configurations either, it must produce the monoprotected diamino ester (2*R*,3*R*)-**27**. This hypothesis was confirmed by its specific rotation, $[\alpha]^{20}_D = -7.5$ (*c* 0.6, $CHCl_3$), the sign of which is opposite that previously reported for its enantiomer.[53] Finally, conventional ammonolysis of the ester function in (2*R*,3*R*)-**27** has to necessarily produce the amide (2*R*,3*R*)-**24**, whose levorotatory nature coincides with that of the same amide in Scheme 9, thus completing the initial objective of this paragraph.

Ring-Opening of (2*R*,3*S*)-8a,g by Catalytic Hydrogenolysis. Among the reported catalytic hydrogenolyses of aziridines, those of compounds **28** and **29** (Figure 2), by Ha, Lee, and coworkers,[6b] are worthy of note. These researchers ascribe their observed differences to the presence of an electron-withdrawing group at the C-2 position [N−C(2) breaking in **28**] or to the absence of such a substituent [N−C(3) breaking in **29**]. Our aziridines **8** bear a weaker electron-withdrawing group at C-2,

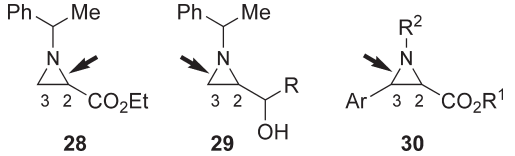


FIGURE 2. Catalytic hydrogenolysis of several aziridines, arrows denoting the broken bonds. (The stereochemistry of the chiral centers is not shown.)

and moreover, their C-3 position is benzylic in nature. It is therefore not surprising that Wang et al. have reported that the hydrogenolysis of (2*R*,3*S*)-**8a** takes place exclusively with N−C(3) breaking.[16] Quite logically, the catalytic hydrogenolyses of 2-arylaziridines[1g] and, specifically, those of 3-arylaziridine-2-carboxylic acid esters **30**[54] occur at their corresponding benzylic positions.

In our hands, however, under virtually the same reaction conditions (balloon pressure of hydrogen, 10% Pd/C, room temperature) as those employed by Wang and co-workers, the crude material obtained starting from (2*R*,3*S*)-**8a** consisted mainly of the expected product, (*R*)-2-amino-3-phenylpropanamide, (*R*)-**31a**, though accompanied by a slight amount of 3-phenylpropanamide, **33a**[55] (25:1 molar ratio, respectively; Scheme 11). As it is not conceivable that **33a** might proceed from (*R*)-**31a**, it is necessary to accept its formation via the unexpected and not observed open product (*R*)-**32a**, the substituted benzylamine nature of which means that it may be easily hydrogenolyzed under our reaction conditions.

Two further steps (amine protection and double hydrolysis) successively converted (*R*)-**31a** into the carbamate (*R*)-**34a** (which proved to be enantiopure by chiral HPLC analysis) and then into D-phenylalanine hydrochloride, (*R*)-**35a**. The *R* configuration of the latter product, established by means of its specific rotation, $[\alpha]^{20}_D = +8.8$ (*c* 1.1, H_2O),[56] together with the enantiopurity of (*R*)-**34a**, proves that the majority ring-opening of (2*R*,3*S*)-**8a** took place with full retention of configuration at C-2, a final point of divergence with Wang et al., who reported obtaining (*R*)-**31a** with only 90% ee.[16] This observation suggests that we tentatively assign the *R* configuration to the non-observed direct product of the minority ring-opening, **32a**.

A methodology identical to that contained in the above scheme was applied to the (2*R*,3*S*)-*trans*-3-(*p*-tolyl)aziridine-2-carboxamide, (2*R*,3*S*)-**8g** (Scheme 12). On this occasion, 3-(*p*-tolyl)propanamide, **33g**, proceeding from the minority N−C(2) breaking was observed only as nonquantifiable trace amounts in the crude material after hydrogenolysis and has, subsequently, been excluded from Scheme 12. The overall process allows us to obtain another D-α-amino acid, *β*-(*p*-tolyl)-D-alanine hydrochloride, (*R*)-**35g**, $[\alpha]^{20}_D = +10.0$ (*c* 1.0, H_2O),[57]

(53) Capone, S.; Guaragna, A.; Palumbo, G.; Pedatella, S. *Tetrahedron* **2005**, *61*, 6575–6579. $[\alpha]^{20}_D = +29.0$ (*c* 0.9, $CHCl_3$) for (2*S*,3*S*)-**27**.

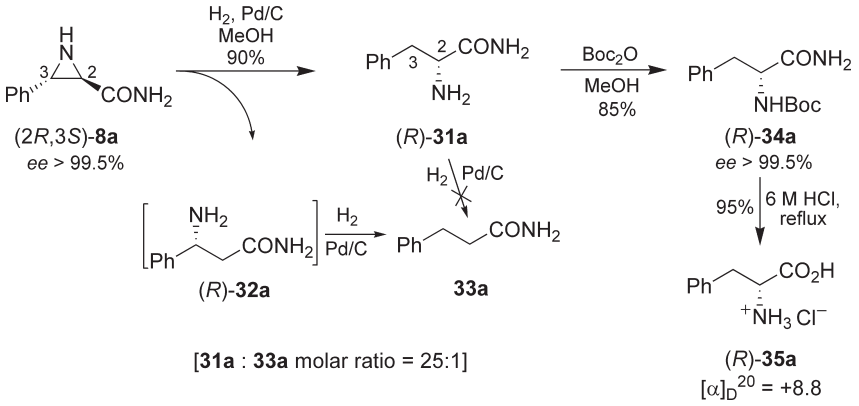
(54) Davis, F. A.; Deng, J.; Zhang, Y.; Haltiwanger, R. C. *Tetrahedron* **2002**, *58*, 7135–7143, and references therein.

(55) The identity of this byproduct was established by comparison with an authentic sample prepared by (1) catalytic hydrogenation of methyl cinnamate and (2) conventional ammonolysis of the ester function.

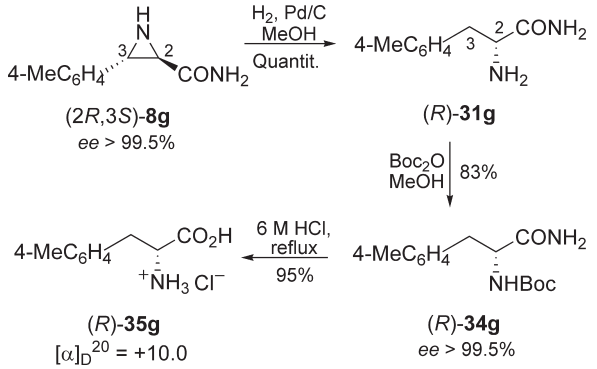
(56) The value $[\alpha]^{20}_D = -8.1$ (*c* 0.91, H_2O) has been previously reported for (*S*)-**35a**: Barfoot, C. W.; Harvey, J. E.; Kenworthy, M. N.; Kilburn, J. P.; Ahmed, M.; Taylor, R. J. K. *Tetrahedron* **2005**, *61*, 3403–3417.

(57) The value $[\alpha]^{20}_D = -6.5$ (*c* 0.1, 0.5 M HCl) has been previously reported for (*S*)-**35g**: Zhuze, A.; Lost, K.; Kasafirek, E.; Rudinger *J. Collect. Czech. Chem. Commun.* **1964**, *29*, 2648–2662.

SCHEME 11. Catalytic Hydrogenolysis of (2*R*,3*S*)-8a



SCHEME 12. Catalytic Hydrogenolysis of (2*R*,3*S*)-8g



in 79% overall yield. As the initial configuration of the C-2 aziridinic chiral center remains unchanged throughout the process, this result, together with the trans geometry of the starting **8g**, proves the (2*R*,3*S*) configuration of this aziridine, as was already anticipated elsewhere in this paper.

In summary, a mild bacterial resolution method of a series of N-unsubstituted *trans*-3-arylaziridine-2-carboxamides has been developed that produces good yields of the slower reacting enantiomers in enantiopure form. The faster reacting enantiomers were converted into unstable aziridine carboxylic acids, the decomposition pathway of which, elucidated in some cases, implies a previously unknown reductase activity of the used bacterial strain, *Rhodococcus rhodochrous* IFO 15564. The ring-opening reactions of the enantiopure aziridine amides with methanol, water, and azide anion, as well as by catalytic hydrogenolysis, not only proceed with full stereoselectivity and practically complete regioselectivity at their C-3 position but also lead to enantiopure high added value products, unnatural D-α-amino acids. The absolute configurations of the hydrolysis and ring-opening reaction products have been unequivocally assigned by means of chemical correlations and complementary experiments. Moreover, a retrospective experiment, the enzymatic hydrolysis of a 1-arylaziridine-2-carboxylic acid ethyl ester, demonstrates that 1-arylaziridine-2-carboxylic acids also are inherently unstable.

(58) The keys for NMR assignments are included in the Supporting Information, General.

Experimental Section[58]

Biotransformations of (±)-8a−g with *Rhodococcus rhodochrous* IFO 15564. A solution of the corresponding substrate (±)-**8a−g** (100 mg; 0.42−0.62 mmol) in 2.0 mL of EtOH (some substrates required a slight heating for total solution) was added to a bacterial suspension (A_{650} = 3.0) in fresh 0.10 M potassium phosphate buffer pH 7.0 (100 mL). Incubation (rotary shaker, 200 rpm, 28 °C) was started, 500 μL aliquots being extracted (AcOEt) from time to time and analyzed by chiral HPLC until one of the two enantiomers of the corresponding amide **8** disappeared. The cells were then discarded by centrifugation (5000 rpm, 3 min). The supernatant liquid was filtered through a pad of diatomaceous earth and then continuously extracted with CH_2Cl_2 (6 h). After drying the organic phase, low-pressure removal of the solvent yielded the corresponding, essentially pure white solid (2*R*,3*S*)-**8**, which was further purified by silica gel column chromatography (1:3 hexane−AcOEt as eluent). To the best of our knowledge, only the amide (2*R*,3*S*)-**8a** has been previously prepared.[16]

(2*R*,3*S*)-3-(*p*-Chlorophenyl)aziridine-2-carboxamide [(2*R*,3*S*)-8e]: yield 42%; white solid; mp 178.8−180.5 °C. $[\alpha]^{20}_D$ = −212.2 (*c* 0.6, MeOH). ee > 99.5%. IR (CH_2Cl_2): 3450, 3367, 1665 cm^{-1}. ^1H NMR (CD_3OD, 400.13 MHz): 7.36 (d, $H^c + H^e$, ^3J = 8.5 Hz), 7.31 (d, $H^b + H^f$, ^3J = 8.5 Hz), 3.19 (br s, H^3), 2.63 (br s, H^2). ^{13}C NMR (CD_3OD, 100.63 MHz): 173.5 (CO), 138.5 (C^d), 134.4 (C^a), 129.5, 128.9 ($C^b + C^f$, $C^c + C^e$), 40.8 (C^2), 39.5 (C^3). ESI-MS (*m*/*z*, %): 197.0 [$(M + H)^+$, 100]. EI-HRMS: calcd for $C_9H_9ClN_2O$, 196.0403; found, 196.0403.

Ring-Opening of (2*R*,3*S*)-8a with MeOH−$BF_3\cdot Et_2O$. $BF_3\cdot Et_2O$ (0.34 mmol, 43 μL) was added under nitrogen atmosphere at 0 °C to a stirred solution of (2*R*,3*S*)-**8a** (0.31 mmol, 50 mg) in anhydrous MeOH (2.0 mL). The mixture was heated at 55 °C for 8 h and then cooled in an ice bath. Aqueous 3 M NaOH was added until basic, and the mixture was extracted with AcOEt (3 × 10 mL). The combined organic layers were dried and the solvents removed under reduced pressure to quantitatively obtain (3*R*)-*β*-methoxy-D-phenylalaninamide [(2*R*,3*R*)-2-amino-3-methoxy-3-phenylpropanamide], (2*R*,3*R*)-**17** (60 mg).

(3*R*)-*β*-Methoxy-D-phenylalaninamide [(2*R*,3*R*)-17]: white solid; mp 97.0−98.5 °C. $[\alpha]^{20}_D$ = −71.2 (*c* 1.0, MeOH). IR (CH_2Cl_2): 3405, 3300, 1676 cm^{-1}. ^1H NMR (CD_3OD, 400.13 MHz): 7.50−7.30 (m, Ph), 4.42 (d, H^3, ^3J = 6.6 Hz), 3.68 (br s, H^2), 3.26 (s, OCH_3). ^{13}C NMR (CD_3OD, 100.63 MHz): 177.0 (CO), 138.7 (C_{ipso}), 129.5, 128.9 ($2C_{ortho}$, $2C_{meta}$, C_{para}), 86.2 (C^3), 60.6 (C^2), 57.2 (OCH_3). ESI-MS (*m*/*z*, %): 389.1 [$(2M + H)^+$, 60], 357.1 [(2M + H − MeOH), 20], 195.1 [$(M + H)^+$, 100].

Ring-Opening of (2*R*,3*S*)-8a with H_2O−*p*-TsOH and Subsequent *N*-*tert*-Butoxycarbonylation. A mixture of (2*R*,3*S*)-**8a** (0.31 mmol, 50 mg), *p*-TsOH·H_2O (0.34 mmol, 65 mg), and 7:1 MeCN−H_2O (1.7 mL) was reacted at 55 °C for 8 h. After

removal of MeCN under reduced pressure, aqueous 3 M NaOH was added until basic, subsequently extracting the mixture with AcOEt (3 × 10 mL). The combined organic layers were dried and concentrated *in vacuo*, and the obtained residue was then treated overnight at room temperature with Boc_2O (0.32 mmol, 70 mg) in MeOH (4.0 mL). After removal of MeOH under reduced pressure, the resulting crude material was purified by flash column chromatography [eluent, hexane−AcOEt: (1) 3:1; (2) 1:3] to yield (3*R*)-N^2-Boc-*β*-hydroxy-D-phenylalaninamide {*tert*-butyl *N*-[(1*R*,2*R*)-1-carbamoyl-2-hydroxyethyl]carbamate}, (2*R*,3*R*)-**20** (60 mg, 70% overall yield).

(3*R*)-N^2-Boc-*β*-Hydroxy-D-phenylalaninamide [(2*R*,3*R*)-20]: white solid; mp 183.6−184.9 °C. $[\alpha]^{20}_D = -29.5$ (*c* 1.0, MeOH). ee > 99.5%. IR (CH_2Cl_2): 3503, 3393, 3346, 1685, 1663 cm^{-1}. ^1H NMR (CD_3OD, 300.13 MHz): 7.49−7.28 (m, Ph), 4.89 (superimposed d, H^3), 4.35 (d, H^2, ^3J = 7.7 Hz), 1.34 [s, $(CH_3)_3C$]. ^{13}C NMR (CD_3OD, 75.48 MHz): 175.9 ($CONH_2$), 157.2 ($NHCOOBu^t$), 142.2 (C_{ipso}), 129.1, 128.8, 128.2 ($2C_{ortho}$, $2C_{meta}$, C_{para}), 80.6 ($OCMe_3$), 75.2 (C^3), 60.6 (C^2), 28.6 [$C(CH_3)_3$]. ESI-MS (*m*/*z*, %): 303.1 [$(M + Na)^+$, 100], 203.1 [$(M + Na - C_4H_8 - CO_2)^+$, 50].

Ring-Opening of (2*R*,3*S*)-8a with $NaN_3-BF_3\cdot Et_2O$. $BF_3\cdot Et_2O$ (0.34 mmol, 43 *μ*L) was added under nitrogen atmosphere at 0 °C to a solution of (2*R*,3*S*)-**8a** (0.31 mmol, 50 mg) in anhydrous MeCN (1.5 mL), and the mixture was stirred at 0 °C during 10 min. A solution of NaN_3 (0.93 mmol, 60 mg) in the smallest amount of water was then added, and the mixture was heated to 55 °C for 12 h. After cooling (ice bath), aqueous 3 M NaOH was added until basic, and the mixture was extracted with AcOEt (3 × 10 mL). The combined organic layers were dried and concentrated *in vacuo* to obtain (3*R*)-*β*-azido-D-phenylalaninamide [(2*R*,3*R*)-2-amino-3-azido-3-phenylpropanamide], (2*R*,3*R*)-**22** (58 mg, 92%).

(3*R*)-*β*-Azido-D-phenylalaninamide [(2*R*,3*R*)-22]: white solid; mp 127.0−128.0 °C. $[\alpha]^{20}_D = -172.5$ (*c* 1.0, MeOH). IR (CH_2Cl_2): 3377, 3055, 1689 cm^{-1}. ^1H NMR (CD_3OD, 400.13 MHz): 7.52−7.30 (m, Ph), 4.86 (partially superimposed d, H^3, ^3J = 7.0 Hz), 3.66 (d, H^2, ^3J = 7.0 Hz). ^{13}C NMR (CD_3OD, 100.63 MHz): 176.7 (CO), 137.1 (C_{ipso}), 129.9, 129.2 ($2C_{ortho}$, $2C_{meta}$, C_{para}), 69.7 (C^3), 60.0 (C^2). ESI-MS (*m*/*z*, %): 206.1 [$(M + H)^+$, 100], 163.1 [$(M + H - CONH_2)^+$, 23].

Ring-Opening of (2*R*,3*S*)-8a by Catalytic Hydrogenolysis. (2*R*,3*S*)-**8a** (0.31 mmol, 50 mg) and 10% Pd/C (48 mg) were placed in a 50 mL round-bottomed flask. The flask was stoppered with a rubber septum and evacuated (rotary pump). A hydrogen-filled balloon was then connected via a needle, and MeOH (6.0 mL) was added via a syringe. The mixture was stirred at room temperature for 2.5 h and then filtered through a pad of diatomaceous earth. After washing the pad with MeOH, the combined methanol solutions were concentrated *in vacuo* to yield a crude material, which was finally purified by flash column chromatography (MeOH as eluent) to obtain D-phenylalaninamide [(*R*)-2-amino-3-phenylpropanamide], (*R*)-**31a** (46 mg, 90%). The aziridineamide (*R*)-**8g** (0.284 mmol, 50 mg) was hydrogenolyzed in a similar way, except that no chromatographic purification was required, to yield almost quantitatively *β*-(*p*-tolyl)-D-alaninamide [(*R*)-2-amino-3-(4-methylphenyl)propanamide], (*R*)-**31g**.

D-Phenylalaninamide [(*R*)-31a]: white solid; mp 131.0−132.5 °C (lit.:[59] 135−137 °C). $[\alpha]^{20}_D = -12.5$ (*c* 1.0, MeOH) [lit.: −9.0 (*c* 1.0, MeOH), ee 77%]. IR (CH_2Cl_2): 3295, 3200, 3054, 1681 cm^{-1}. ^1H NMR (CD_3OD, 400.13 MHz): 7.37−7.20 (m, Ph), 3.60 (dd, H^2, ^3J = 7.6, ^3J = 6.0 Hz), 3.06 [dd, C(3)*H*H, ^2J = 13.4, ^3J = 6.0 Hz], 2.83 [dd, C(3)H*H*, ^2J = 13.4, ^3J = 7.6 Hz]. ^{13}C NMR (CD_3OD, 100.63 MHz): 179.5 (CO), 138.9 (C_{ipso}), 130.4 ($2C_{ortho}$), 129.5 ($2C_{meta}$), 127.7 (C_{para}), 57.4 (C^2), 42.5 (C^3). ESI-MS (*m*/*z*, %): 329.1 [$(2M + H)^+$, 15], 165.0 [$(M + H)^+$, 100].

(59) Wang, M.-X.; Lin, S.-J. *J. Org. Chem.* **2002**, *67*, 6542–6545.

General Procedure for *N*-*tert*-Butoxycarbonylation of α-Aminoamides (2*R*,3*R*)-17, (2*R*,3*R*)-22, and (*R*)-31a,g. Each α-aminoamide (0.31 mmol) was treated overnight at room temperature with Boc_2O (0.39 mmol, 84 mg) in MeOH (5.0 mL). After removal of MeOH under reduced pressure, the resulting crude material was purified by flash column chromatography [eluent, hexane−AcOEt: (1) 3:1; (2) 1:1], yielding the following *tert*-butyl carbamates:

(3*R*)-N^2-Boc-*β*-methoxy-D-phenylalaninamide [(2*R*,3*R*)-18]: yield 83%; white solid; mp 200.2−201.4 °C. $[\alpha]^{20}_D = -61.9$ (*c* 0.8, MeOH). ee > 99.5%. IR (CH_2Cl_2): 3391, 3354, 1685, 1660 cm^{-1}. ^1H NMR (DMSO, 300.13 MHz): 7.41 (br s, CON*H*H), 7.35−7.21 (m, Ph), 7.01 (br s, CONH*H*), 6.57 (d, N*H*Boc, ^3J = 9.4 Hz), 4.27 (d, H^3, ^3J = 9.0 Hz), 4.13 (t, H^2, J = 9.2 Hz), 3.06 (s, OCH_3), 1.19 [s, $(CH_3)_3C$]. ^{13}C NMR (DMSO, 75.48 MHz): 172.3 ($CONH_2$), 154.7 ($NHCOOBu^t$), 138.6 (C_{ipso}), 128.0, 127.9 ($2C_{ortho}$, $2C_{meta}$, C_{para}), 82.9 (C^3), 78.1 ($OCMe_3$), 58.5 (C^2), 56.6 (OCH_3), 28.2 [$C(CH_3)_3$]. ESI-MS (*m*/*z*, %): 489.2 [$(2M+H-C_4H_8-CO_2)^+$, 60], 295.1 [$(M+H)^+$, 18], 195.1 [$(M + H - C_4H_8 - CO_2)^+$, 100]. ESI-TOF-HRMS: calcd for $C_{15}H_{22}N_2O_4$, 295.1652; found, 295.1644.

(3*R*)-*β*-Azido-N^2-Boc-D-phenylalaninamide [(2*R*,3*R*)-23]: yield 85%; white solid; mp 183.3−184.7 °C. $[\alpha]^{20}_D = -126.3$ (*c* 1.0, MeOH). ee > 99.5%. IR (CH_2Cl_2): 3388, 3346, 1686, 1660 cm^{-1}. ^1H NMR (DMSO, 300.13 MHz): 7.63 (br s, CON*H*H), 7.42−7.25 (m, Ph), 7.23 (br s, CONH*H*), 6.87 (d, N*H*Boc, ^3J = 9.3 Hz), 4.77 (d, H^3, ^3J = 9.3 Hz), 4.30 (t, H^2, ^3J = 9.3 Hz), 1.20 [s, $(CH_3)_3C$]. ^{13}C NMR (DMSO, 75.48 MHz): 171.5 ($CONH_2$), 154.7 ($NHCOOBu^t$), 136.4 (C_{ipso}), 128.6, 128.5, 128.3 ($2C_{ortho}$, $2C_{meta}$, C_{para}), 78.4 ($OCMe_3$), 65.5 (C^3), 57.0 (C^2), 28.1 [$C(CH_3)_3$]. ESI-MS (*m*/*z*, %): 511.2 [$(2M + H - C_4H_8 - CO_2)^+$, 100], 427.1 (85), 306.1 [$(M + H)^+$, 10], 279.0 [$(M + H - CHN)^+$, 38], 206.0 [$(M + H - C_4H_8 - CO_2)^+$, 70].

***N*²-Boc-D-Phenylalaninamide [(*R*)-34a]:** yield 85%; white solid; mp 153.1−154.3 °C (lit.:[60] 141−143 °C). $[\alpha]^{20}_D = -12.8$ (*c* 1.0, MeOH) [lit.: −14.5 (*c* 1.0, EtOH)]. ee > 99.5%. IR (CH_2Cl_2): 3392, 3346, 1678, 1657 cm^{-1}. ^1H NMR ($CDCl_3$, 400.13 MHz): 7.35−7.17 (m, Ph), 6.24 (br s, CON*H*H), 6.03 (br s, CONH*H*), 5.31 (br s, N*H*Boc), 4.44 (br s, H^2), 3.04 [br s, C(3)H_2], 1.38 [s, $(CH_3)_3C$]. ^{13}C NMR ($CDCl_3$, 100.63 MHz): 174.0 ($CONH_2$), 155.4 ($NHCOOBu^t$), 136.6 (C_{ipso}), 129.2, 128.5 ($2C_{ortho}$, $2C_{meta}$), 126.8 (C_{para}), 80.0 ($OCMe_3$), 55.2 (C^2), 38.5 (C^3), 28.2 [$C(CH_3)_3$]. ESI-MS (*m*/*z*, %): 429.2 [$(2M + H - C_4H_8 - CO_2)^+$, 100], 165.0 [$(M + H - C_4H_8 - CO_2)^+$, 37]. EI-HRMS: calcd for $C_{14}H_{20}N_2O_3$, 220.1338 $(M - CONH_2)^+$; found, 220.1339. In a similar way N^2-Boc-*β*-(*p*-tolyl)-D-alaninamide [(*R*)-**34g**] (83% yield) was prepared.

Hydrolysis of the Carbamoylcarbamates (2*R*,3*R*)-18, (2*R*,3*R*)-20, and (*R*)-34a,g. A suspension of the corresponding carbamoylcarbamate (0.14 mmol) in 6 M HCl (2.6 mL) was refluxed for 3 h and then cooled at room temperature. After concentrating to nearly dryness under reduced pressure, the obtained residue was washed with AcOEt (2 × 7.5 mL), with *in vacuo* removal of the solvent after each washing. The resulting solid was dried *in vacuo* over P_2O_5 (10 h, room temperature) to yield the corresponding D-α-amino acid hydrochloride. The D-α-amino acid hydrochloride (0.13 mmol, 30 mg) proceeding from (2*R*,3*R*)-**18** was further transformed (for identification purposes) into the free amino acid by treatment with propylene oxide (31 mmol, 2.2 mL) in EtOH (10 mL) at 90 °C for 1 h.[61] Finally, the solvent and volatile compounds were removed under reduced pressure to yield (3*R*)-*β*-methoxy-D-phenylalanine [(2*R*,3*R*)-**19**].

(3*R*)-*β*-Methoxy-D-phenylalanine [(2*R*,3*R*)-19]: yield 90%; white solid; mp 190.0−192.0 °C. $[\alpha]^{20}_D = -37.3$ (*c* 0.6, H_2O) [lit.:[46] +34.5 (*c* 0.6, H_2O) for (2*S*,3*S*)-**19**]. IR (Nujol): 3131, 3048, 1614 cm^{-1}. ^1H NMR (D_2O, 300.13 MHz): 7.40−7.17 (m, Ph), 4.79 (d, H^3, ^3J = 3.5 Hz), 4.05 (d, H^2, ^3J = 3.5 Hz), 3.26 (s, OCH_3). ^{13}C

(60) Pozdnev, V. F. *Tetrahedron Lett.* **1995**, *36*, 7115–7118.
(61) Jefford, C. W.; McNulty, J. *Helv. Chim. Acta* **1994**, *77*, 2142–2146.

NMR (D_2O, 100.63 MHz): 174.3 (CO), 135.5 (C_{ipso}), 128.8, 128.7, 127.2 ($2C_{ortho}$, $2C_{meta}$, C_{para}), 82.4 (C^3), 59.5 (C^2), 56.6 (OCH_3). ESI-MS (*m*/*z*, %): 196.1 (M^+, 100).

(3*R*)-β-Hydroxy-D-phenylalanine hydrochloride [(2*R*,3*R*)-21]: yield 95%, white solid; mp 171.5−172.6 °C (dec) [lit.:[49] 174 °C (dec)]. $[\alpha]^{20}_D = -59.3$ (*c* 1.0, 6 M HCl) [lit.: −63.2 (*c* 0.65, 6 M HCl)]. IR (Nujol): 3424, 3315, 3123, 3032, 1735 cm^{-1}. ^1H NMR (D_2O, 400.13 MHz): 7.42−7.27 (m, Ph), 5.29 (d, H^3, $^3J = 3.8$ Hz), 4.29 (d, H^2, $^3J = 3.8$ Hz). ^{13}C NMR (D_2O, 100.63 MHz): 169.4 (CO), 136.9 (C_{ipso}), 129.0, 128.9 ($2C_{ortho}$ or $2C_{meta}$, C_{para}), 126.1 ($2C_{meta}$ or $2C_{ortho}$), 70.8 (C^3), 58.9 (C^2). ESI-MS (*m*/*z*, %): 182.1 (M^+, 100).

β-(*p*-Tolyl)-D-alanine hydrochloride [(*R*)-35g]: yield 95%; white solid; mp 264.0−265.1 °C. $[\alpha]^{20}_D = +10.0$ (*c* 1.0, H_2O) [lit.:[57] −6.5 (*c* 0.1, 0.5 M HCl) for (*S*)-**35g**]. IR (Nujol): 3399, 3142, 2923, 1738 cm^{-1}. ^1H NMR (D_2O, 300.13 MHz): 7.16 (d, $2H_{ortho}$ or $2H_{meta}$, $^3J = 8.1$ Hz), 7.11 (d, $2H_{meta}$ or $2H_{ortho}$, $^3J = 8.1$ Hz), 4.18 (dd, H^2, $^3J = 7.6$, $^3J = 5.5$ Hz), 3.19 [dd, C(3)*H*H, $^2J = 14.5$, $^3J = 5.5$ Hz], 3.07 [dd, C(3)H*H*, $^2J = 14.5$, $^3J = 7.6$ Hz], 2.22 (s, CH_3). ^{13}C NMR (D_2O, 75.48 MHz): 172.1 (CO), 138.4, 131.1 (C_{ipso}, C_{para}), 130.0, 129.6 ($2C_{ortho}$, $2C_{meta}$), 54.7 (C^2), 35.5 (C^3), 20.4 (CH_3). ESI-MS (*m*/*z*, %): 180.0 (M^+, 100). In a similar way D-phenylalanine hydrochloride [(*R*)-**35a**] (95% yield) was prepared.

Hydrogenolysis of the Azido Group in the Azido(carbamoyl)-carbamate (2*R*,3*R*)-23. (2*R*,3*R*)-**23** (0.115 mmol, 35 mg) and 10% Pd/C (18 mg) were placed in a 50 mL round-bottomed flask. The flask was stoppered with a rubber septum and evacuated (rotary pump). A hydrogen-filled balloon was then connected via a needle, and MeOH (2.2 mL) was added via a syringe. The mixture was stirred at room temperature for 12 h and then filtered through a pad of diatomaceous earth. After washing the pad with MeOH, the combined methanol solutions were concentrated *in vacuo* to quantitatively obtain (3*R*)-β-amino-N^2-Boc-D-phenylalaninamide {*tert*-butyl *N*-[(2*R*,3*R*)-2-amino-1-carbamoyl-2-phenylethyl]carbamate}, (2*R*,3*R*)-**24** (32 mg).

(3*R*)-β-Amino-N^2-Boc-D-phenylalaninamide [(2*R*,3*R*)-24]: colorless oil. $[\alpha]^{20}_D = -13.8$ (*c* 1.0, MeOH). IR (film): 3400, 3362, 1680, 1656 cm^{-1}. ^1H NMR (CD_3OD, 400.13 MHz): 7.45−7.22 (m, Ph), 4.35 (poorly resolved d, H^2, $^3J = 6.4$ Hz), 4.20 (poorly resolved d, H^3), 1.36 [s, $(CH_3)_3C$]. ^{13}C NMR (CD_3OD, 100.63 MHz): 175.8 ($CONH_2$), 157.4 ($NHCOOBu^t$), 142.4 (C_{ipso}), 129.4, 128.6, 128.5 ($2C_{ortho}$, $2C_{meta}$, C_{para}), 80.6 ($OCMe_3$), 60.9 (C^3 or C^2), 58.5 (C^2 or C^3), 28.6 [$C(CH_3)_3$]. ESI-MS (*m*/*z*, %): 280.1 [$(M + H)^+$, 100], 180.1 [$(M + H - C_4H_8 - CO_2)^+$, 5].

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