L-Rhamnulose-1-phosphate Aldolase from *Thermotoga maritima* in Organic Synthesis: One-pot Multistep Reactions for the Preparation of Imino- and Nitrocyclitols

Isabel Oroz-Guinea,[a] Karel Hernández,[b] Flora Camps Bres,[c,d] Christine Guérard-Hélaine,[c,d] Marielle Lemaire,[c,d]* Pere Clapés[b]* and Eduardo García-Junceda[a]*

[a] Departmento de Química Bioorgánica, Instituto de Química Orgánica General (IQOG-CSIC). Juan de la Cierva 3. 28006, Madrid, Spain.
[Fax: (+34) 915644853, e-mail: eduardo.junceda@csic.es]
[b] Departmento de Química Biológica y Modelado Molecular, Instituto de Química Avanzada de Cataluña (IQAC–CSIC). Jordi Girona 18-26, 08034 Barcelona, Spain.
[Fax: (+34)-932045904; e-mail: pere.clapes@iqac.csic.es]
[c] Clermont Université, Université Blaise Pascal, ICCF, BP 10448, F-63000 Clermont-Ferrand, France
[d] CNRS, UMR 6296, ICCF, BP 80026, F-63171 Aubière, France.
[Fax: 33473407717; marielle.lemaire@univ-bpclermont.fr]

Abstract. Rhamnulose-1-phosphate aldolase from *Thermotoga maritima* (Rhu1PA*Tm*) has been recently cloned and characterized. This hyperthermophilic enzyme offers intriguing possibilities for practical catalysis. This is due to its high stability under extreme reaction conditions, such as high temperature or the presence of organic co-solvents.
The Rhu1PA*Tm* potentiality in organic synthesis has been explored focusing on i) the reaction stereocontrol, ii) the possibility of combining it with other mesophilic enzymes in multienzyme systems and iii) its application to the synthesis of imino- and nitrocyclitols.
In our study, Rhu1PA*Tm* diastereoselectivity was similar to the one reported by Rhu1PA from *Escherichia coli* (Rhu1PA*Ec*).
However, we observed significant differences for some aldehyde acceptors. Indeed, the diastereoselectivity control was not complete, since mixtures of L-*threo* (2*R*,3*S;* natural stereopreference) and D-*erythro* (2*R*,3*R*) diastereoisomers were obtained as described for Rhu1PA*Ec*. Rhu1PA*Tm* was able to catalyse aldol reactions using nitroaldehydes and *N*-Cbz-aminoaldehydes. Conversion of the selected nitroaldehydes was complete, leading to mixtures of L-*threo* and D-*erythro* diastereoisomers, in 95:5 and 75:25 ratios. When *N*-Cbz-aminoaldehydes were used, aldol reaction yields were lower and the stereoselectivity L-*threo*:D-*erythro* ranged between 32:68 to >95:5.

Keywords: aldol reaction; azasugars; biocatalysis; cascade reactions; lyases; thermophilic enzymes

Introduction

Carbon-carbon (C–C) bond formation is one of the cornerstone reactions in synthetic organic chemistry.[1] In this sense, the catalytic asymmetric C-C bond formation by the aldol addition reaction has long been recognized as one of the most useful tools for construction of new C−C bonds, since concomitant with the bond forming process is the formation of one or two new stereocentres.[2] The use of enzymes as catalysts is a good alternative to non-enzymatic synthesis. This is because they provide a number of advantages, such as their ability to carry out the reactions under moderate conditions of temperature, pH and pressure, high efficiency and regio-, chemo- and stereoselectivity.[3] Enzymes also represent an environmentally attractive option because they can catalyse reactions in water and are biodegradable.[4] The high stereoselectivity of aldolases in C-C bond formation gives them a wide range of synthetic applications. Among the different families of aldolases, dihydroxyacetone phosphate (DHAP)-dependent aldolases have particularly attracted the attention of chemists. Furthermore, they have been extensively used in chemoenzymatic syntheses.[5] DHAP-dependent aldolases lead to the formation of two new stereocentres with a high degree of stereochemical control. Despite this well proven synthetic utility of aldolases, there are still few processes that have been developed at an industrial scale and none of them —to the best of our knowledge— used DHAP-dependent aldolases.[6] This is unfortunate, considering the recent advances in understanding the mechanism of aldolase catalysis. This has allowed the successful application of many different engineering approaches to alter the catalytic properties of aldolases such as stability, substrate specificity and stereoselectivity.[7]

One of the main difficulties in applying these processes at an industrial scale is the lack of stability under reaction conditions. An approach to overcome this issue is the use of enzymes from extremophile microorganisms.[8] From a biocatalytical point of view, thermophilic and hyperthermophilic microorganisms have particularly attracted the attention, since they are sources of thermostable enzymes. Thermozymes display outstanding stability against high temperature, as well as against other denaturalization conditions, such as the presence of organic solvents, detergents or extreme pHs.[9] In this sense, we have recently described the heterologous expression of the rhamnulose-1-phosphate aldolase from *Thermotoga maritima*, its functional

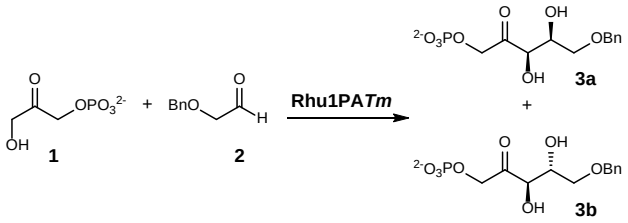
characterization and preliminary synthetic properties.[10] This enzyme presented an optimum temperature at 95 ºC and displayed a great stability to extreme reaction conditions, such as high temperatures (i.e., half-life of more than 3 hours at 115 ºC) and in the presence of different organic solvents. Furthermore, Rhu1PA*Tm* showed activity in a wide range of pH, with an optimum activity at pH at 8.0. A multienzyme system[11b] previously developed by the García-Junceda's group, coupling the *in situ* phosphorylation of DHA with the aldolase catalysed aldol reaction, was successfully assayed with Rhu1PA*Tm*.[11] Rhu1PA*Tm* was assayed using the one-pot/two-steps protocol with four different aldehydes: L-lactaldehyde, benzyloxyacetaldehyde, phenylacetaldehyde and ethyl-3-methyl-4-oxocrotonate. Rhu1PA*Tm* was able to react with these four aldehydes at 45 ºC and 80 °C in combination with other mesophilic enzymes, in a one-pot/two-steps approach. The reactions were significantly faster at the highest temperature.[10]

Herein, we describe the study of the synthetic utility of the Rhu1PA*Tm*, focusing on the stereocontrol of the aldol reaction, its ability to integrate with other mesophilic enzymes into multienzyme systems and, finally, its application to the synthesis of imino- and nitrocyclitols.

Results and Discussion

Study of the stereochemical outcome of Rhu1PA*Tm* catalysed reaction

We first performed a study on the stereochemical outcome of the reaction catalysed by Rhu1PA*Tm* as a function of the temperature. To this end, the percentage of each diastereoisomer formed in the aldol addition reaction of DHAP to benzyloxyacetaldehyde **2** was measured at different times. Reactions were conducted at 4 ºC, 25 ºC, 45 ºC and 80 ºC.



Scheme 1. Aldol addition reaction of DHAP (**1**) —*in situ* obtained by enzymatic phosphorylation of DHA— to benzyloxyacetaldehyde **2** catalysed by Rhu1PA*Tm*.

As expected, the diasteroisomer L-*threo* (3*R*,4*S*), which is the one of the natural preference of the enzyme, was synthesized faster (i.e. kinetic product) than the D-*erythro*. In particular, this difference was highlighted at 4 ºC due to the slowing down of the reaction rate (Figure 1A). In all temperatures assayed, the ratio *threo*:*erythro* decreased with the progress of the reaction, until reaching a plateau at 2:1 ratio (Figure 1).

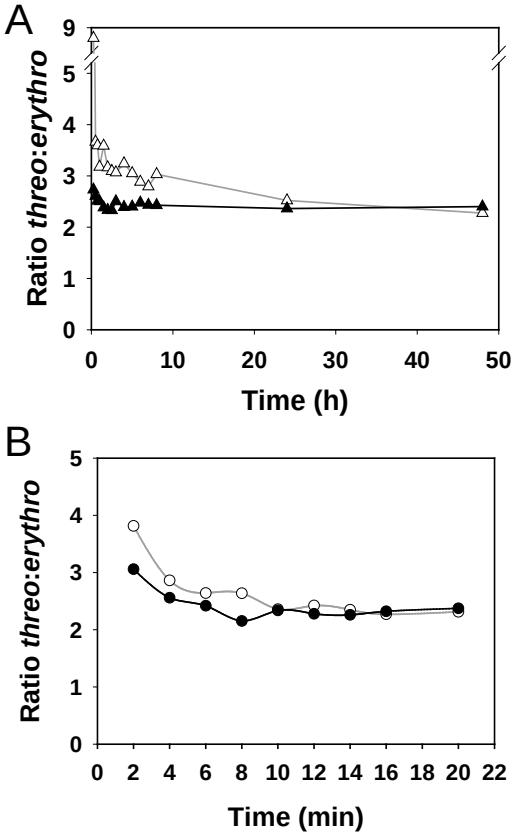


Figure 1. Diastereoisomer L-*threo*:D-*erythro* ratio obtained in the aldol reaction of DHAP to **2** catalysed by Rhu1PA*Tm* at 4 ºC (A; Δ), 25 ºC (A; ▲), 45 ºC (B; ○) and 80 ºC (B; ●).

However, thermodynamic equilibrium was reached faster with the increase of temperature. A similar result was obtained when Rhu1PA*Ec* was used with (*R*)-*N*-Cbz-2-aminohexanal as substrate. In this case, the diastereoisomer ratio obtained did not vary at different reaction temperatures. However, the L-*threo*:D-*erythro* ratio changed with the temperature when (*R*)-*N*-Cbz-2-aminopentanal or *N*-Cbz-2-amino-4-methylpentanal were used as acceptor substrates.[12] Thus, it cannot be excluded that the Rhu1PA*Tm* stereoselectivity could change with the temperature when using other aldehydes as substrates.

Synthesis of nitrocyclitols mediated by Rhu1PA*Tm*

Nitrocyclitols belong to the large family of cyclitols and are essentially found as synthetic precursors of aminocyclitols.[13] These amines are present in many pharmacologically significant compounds exhibiting antitumor, antibacterial, antifungal, antimalarial, and antiviral activities.[14] Consequently, in the last decade there has been an increasing demand for the development of appropriate synthetic procedures to prepare cyclitols as well as analogues with enhanced or more selective biological profiles.[15]

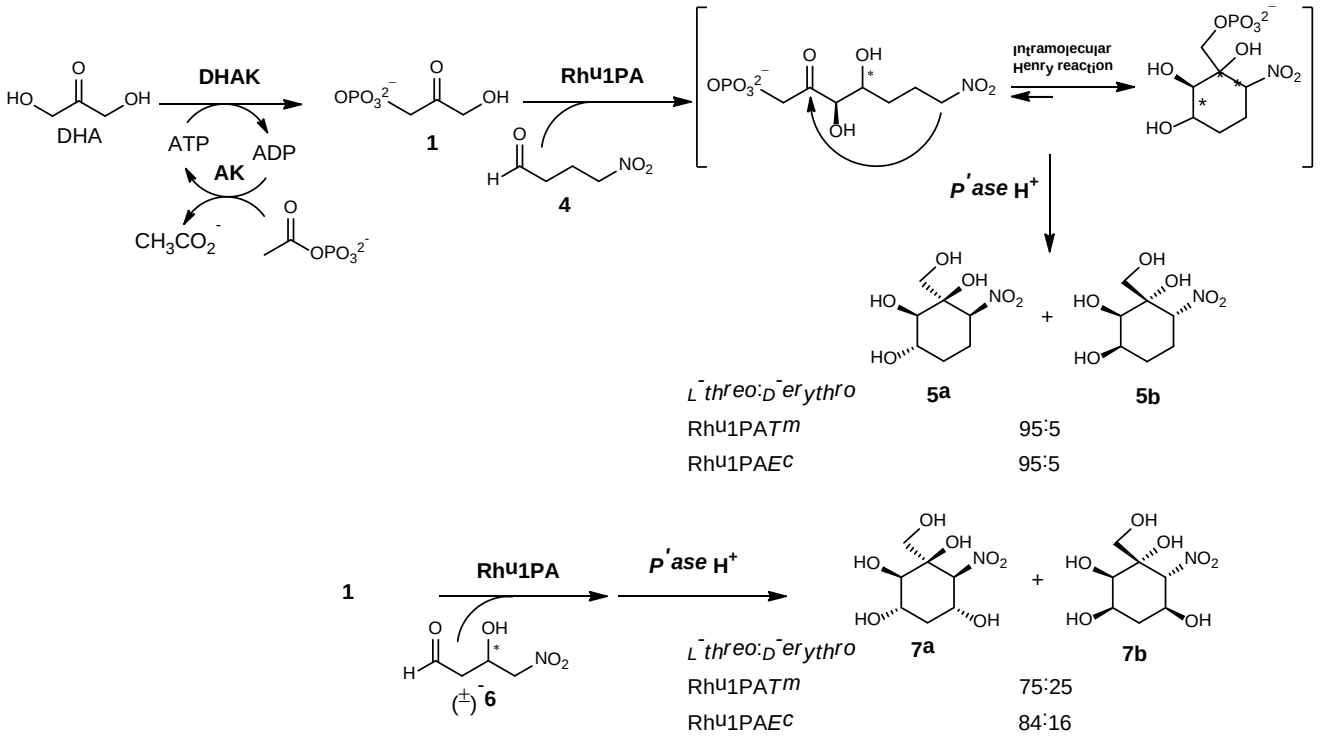
Lemaire's group reported a straightforward chemoenzymatic cascade to access nitro- and aminocyclitols. This process involved two stereoselective C-C bond formations. One was catalysed by an aldolase and the other was a spontaneous intramolecular Henry reaction.[16] Herein, we have studied the application of the Rhu1PA*Tm* in the context of one-pot/two-steps chemoenzymatic cascade to access to nitrocyclitols (Scheme 2).

Aldehydes 4-nitrobutanal (**4**) and (±)-3-hydroxy-4-nitrobutanal ((±)-**6**) were used as acceptor substrates in aldol addition to DHAP catalysed by Rhu1PA*Tm*. When DHAP concentration reached its maximum, the aldolase and aldehydes **4** or (±)-**6** were added.

In the first attempt, the aldol addition of DHAP to **4** at 0.1 mmol scale, with a DHAP/nitroaldehyde ratio of 2:1 at 45 °C failed. Unfortunately, nitroaldehyde **4** was labile at this temperature and induced DHAP degradation. Thus, reactions were carried out at 25 ºC. In spite of its hyperthermophile nature, Rhu1PA*Tm* retains between 5 and 10% of its maximum activity at 25 ºC.[10] In these conditions, aldehyde **4** led to nitrocyclitols **5a** and **5b** and aldehyde (±)-**6** led to nitrocyclitols **7a** and **7b** (Scheme 2). These compounds were isolated after purification in 70% and 71% yields for each couple of isomers respectively. These compounds are

identical to those previously described.[17] Their configurations were established on the basis of 3*R* stereoselectivity of the enzyme and ^{1}H NMR analysis.

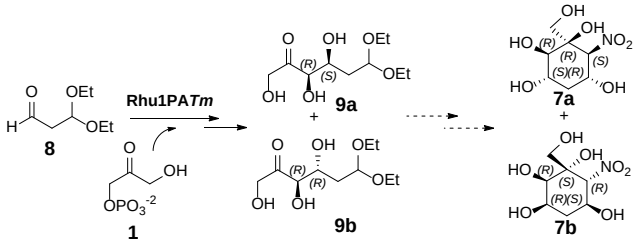
The stereoselectivity shown by the aldolase depended on the aldehyde acceptor (Table 1). Using the aldehyde **4** the major diastereoisomer obtained was the L-*threo*, which corresponds to the expected stereochemical preference for the Rhu1PA (Table 1).



Scheme 2. Cascade reactions for the synthesis of nitrocyclitols. First step phosphorylation of DHA catalysed by recombinant ATP-dependent DHAK from *Citrobacter freundii* CECT 4626 (AK=acetate kinase);[18] second step aldol reaction catalysed by Rhu1PA and *in situ* intramolecular Henry reaction; third step hydrolysis of the phosphate group using acid phosphatase. Data for Rhu1PA*Ec*, taken from reference [17]

The data presented in this work were consistent with the ones previously described using Rhu1PA*Ec* as catalyst.[17] Using (±)-**6** the actual stereoselectivity of Rhu1PA*Tm* could not be disclosed. The reaction produced **7a** and **7b** and the enzymatic aldol addition appeared to virtually produce exclusively the L-*threo* diastereoisomer from (*R*)-**6** and the D-*erythro* from (*S*)-**6** instead of the four possible diastereoisomers (SI Scheme S3). This may be explained because an all-equatorial arrangement of substituents (e.g. **7a** and almost all eq in **7b**) corresponds to the more stable product. The remaining mixture of diastereoisomers can then be re-subjected to further equilibration retroaldol-retro Henry and products **7a** and **7b** were accumulated.[19]

This study was continued using the 3,3-diethoxypropanal (**8**) as an acceptor (Scheme 3). The aldol reaction led to the linear compounds **9a** and **9b** with an isolated yield of 67%. Remarkably, both diastereoisomers L-*threo* and D-*erythro* were obtained in 70:30 ratio for the first time in this work using a rhamnulose-1-phosphate aldolase.



Scheme 3. Rhu1PA*Tm*-catalyzed synthesis of aldol products **9a** and **9b**, which are putative precursors of nitrocyclitols **7a** and **7b**.

Products **9a** and **9b** were previously synthesized using the L-fuculose-1-phosphate aldolase from *E. coli* (Fuc1PA*Ec*).[20] As expected, the percentage obtained from D-*erythro* diastereoisomer was higher in the reactions catalysed by Fuc1PA*Ec* (L-*threo*:D-*erythro* ratio 3:97) than when the catalyst was the Rhu1PA*Tm*. These compounds could be considered as nitrocyclitol precursors. Indeed, deprotection of the aldehyde followed by a Henry reaction with nitromethane would lead to nitrocyclitols **7a** and **7b**.

Synthesis of iminocyclitols mediated by Rhu1PA*Tm*

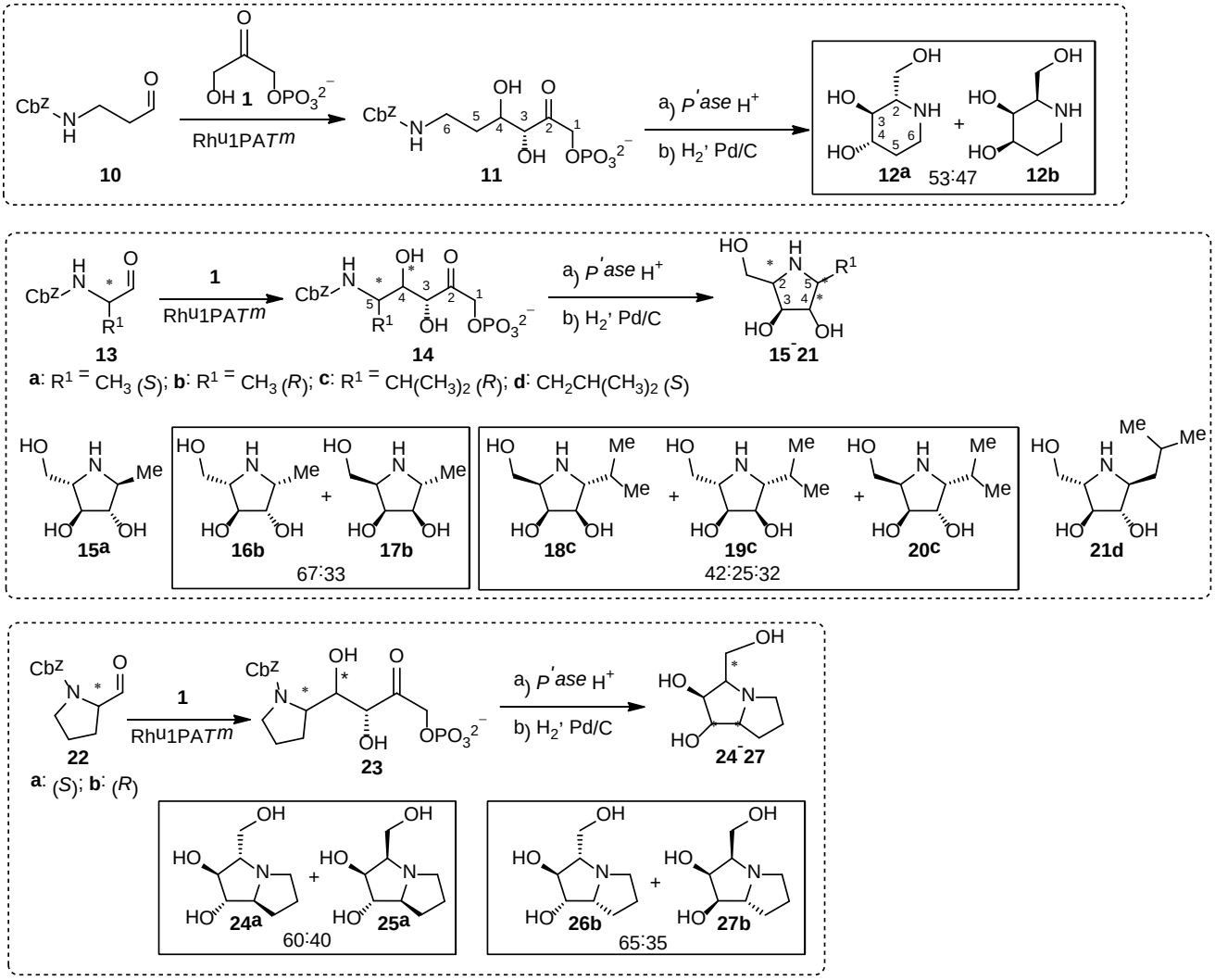
The second cascade reaction of the Rhu1PA*Tm*, with the DHAK-mediating the DHAP formation was the one developed by Clapés's group for obtaining iminocyclitols (Scheme 4). The strategy consists of the aldol addition of DHAP to either (*R*)- or (*S*)-*N*-Cbz-aminoaldehydes. This is followed by an acid phosphatase-catalysed hydrolysis of the phosphate group and subsequent intramolecular reductive amination by treatment with H_2 in presence of Pd/C. This strategy allows the formation of three new stereogenic centres for each aldehyde tested (Scheme 4):[12a, 21] two of them controlled by the aldolase and one generated during the reductive amination.

First, the capability of the Rhu1PA*Tm* to catalyse the aldol addition reaction of DHAP to *N*-Cbz-aminoaldehydes was tested on an analytical scale using an *N*-Cbz-aminoaldehyde:DHAP ratio of 1:1.7. DMF (30% v/v) was added to the reaction medium to improve the solubility of the aldehydes. Reaction temperature was set up at 45 ºC to retain a good activity of the aldolase, while preventing a high degradation rate of the DHAP.[10]

Reactions were monitored by HPLC and stopped when the formation of aldol adduct was maximal (Table 2), thus preventing the spontaneous retroaldol reaction as it has been reported for similar reactions.[21a] In these conditions, all the *N*-Cbz-aminoaldehydes tested were accepted as substrates by Rhu1PA*Tm*, which was able to use aldehydes with both linear and branched substituent at Cα (Table 2).

Good conversions (61-74%) were obtained with *N*-Cbz-aminoaldehydes with small Cα substituents (e.g., **10**, **13a** and **13b**) (Table 3) and moderate (37-49%) with Cα branched substituents including prolinal derivatives. Using the same aldehyde acceptors, Rhu1PA*Ec* at 25 ºC gave similar yields for **10** and **13a**,**b** but required longer reaction times (4-5 h).[21a] For **13c, 13d, 22a** and **22b** Rhu1PA*Ec* gave superior conversions (58-69% conversion).[12a,21c]

While this ability to accept a wide variety of substituents on the Cα is shared with its mesophilic counterpart from *E. coli*, studies with other DHAP-dependent aldolases demonstrated that not all of them exhibit the same degree of tolerance towards structurally diverse *N*-Cbz-aminoaldehydes.[12a,22,23]



Scheme 4. Cascade reaction for the synthesis of iminocyclitols employing Rhu1PA*Tm*.

Table 2. Percentage of aldol adducts (**11**, **14a-d**, and **23a-b**) formed at analytical scale reactions catalysed by Rhu1PA*Tm*.

Aldehyde	Aldol product (%)[a)]	Optimum reaction time (min)[b)]
10	**11**(51)	15
13a	**14a** (61)	20
13b	**14b** (74)	30
13c	**14c** (39)	60
13d	**14d** (49)	10
22a	**23a** (37)	10
22b	**23b** (38)	30

[a)] Percentage of aldol adduct formed determined by HPLC using an external standard method. [b)] Time at which maximum accumulation of aldol adduct was reached.

After these preliminary studies, reactions for obtaining the corresponding iminocyclitols were scaled up to mmol scale (i.e., 2 mmol DHAP; 1.2 mmol aldehyde). Once the reaction reached the maximum aldol adduct formed, the unreacted aldehyde was removed and aldol adducts **11**, **14a-d, 23a** and **23b** were treated with acid phosphatase. Afterwards, aldols were purified by reverse-phase HPLC, paying special attention to prevent the losses of possible diastereoisomers formed during the reaction. Then, aldol adducts were submitted to Cbz deprotection/intramolecular reductive amination to afford the corresponding iminocyclitols, following the method previously described.[21b] This procedure provided overall yields ranging from 9 to 47% (Table 3). The structure and relative stereochemistry of the iminocyclitols were determined by NMR and in all cases were consistent with those previously described in the literature.[12a, 21a, 21c, 23] As in previous examples of aldol

additions catalysed by DHAP-dependent aldolases, it can be assumed from mechanistic considerations that the absolute configuration at C-3 (Scheme 4) (i.e. the stereogenic centre generated from the DHAP donor) will be conserved throughout with any DHAP-aldolases and with no exceptions known so far.[5,21,22] Moreover, the specific absolute configuration introduced by some *N*-Cbz-aminoaldehyde chiral center (e.g., **13a-d**, **22a** and **22b**, Scheme 4) allowed the unequivocal assessment of the absolute stereochemistry of the iminocyclitols. Thus, from the stereochemical configuration of the iminocyclitols it was possible to infer the stereochemical outcome of both the enzymatic aldol addition and the reductive amination.

Mixtures of L-*threo* and D-*erythro* diastereoisomers were obtained, which depended upon the structure of the acceptor aldehyde (Table 3). Rhu1PA*Tm* provided a good stereoselectivity for the (*S*)- configured *N*-Cbz-aminoaldehydes furnishing always the L-*threo* configured aldol adducts while L-*threo*:D-*erythro* mixtures were formed with the (*R*)- configured ones (Table 3). This behaviour was similar to that observed for Rhu1PA*Ec* using both DHAP or DHA in the presence of borate buffer as donors.[12a, 21a, 22, 23] The aldol addition of DHAP to (*R*)-*N*-Cbz-valinal (**13c**) gave a mixture of L-*threo*:D-*erythro* (32:68) being the D-*erythro* the major one (Table 3). Interestingly, the same reaction catalysed by Rhu1PA*Ec* was fully D-*erythro* stereoselective.[12a] For **13c** both enzymes have a preference for the D-*erythro* stereochemistry, which is typically obtained with Fuc1PA*Ec* catalyst.

Table 3. Overall yield and stereochemical outcome obtained in the synthesis of iminocyclitols catalysed by Rhu1PA*Tm*.

Aldehyde	Iminocyclitol	Iminocyclitol Overall yield (%)[a)]	L-*threo*:D-*erythro* (%)[b)]
10	**12a**+**12b**	32	53:47
13a	**15a**	19	>95:5
13b	**16b**+**17b**	47	67:33
13c	**18c**+**19c**[c)]+**20c**	18	32:68
13d	**21**	12	>95:5
22a	**24a**+**24b**	9	>95:5
22b	**24c**+**24d**	12	65:35

[a)] Purification procedures were not optimized. [b)] Ratio corresponding to the aldol adducts inferred from the iminocyclitol compounds and determined within the limits of NMR detection. [c)] **18c** and **19c** are epimers at C-2 formed during reductive amination.

In another example, the aldol addition of DHAP to (*R*)-*N*-Cbz-prolinal (**22b**), Rhu1PA*Tm* gave a mixture of L-*threo*:D-*erythro* 65:35, whereas using Rhu1PA*Ec* the reaction was reported to be fully stereoselective.[23] Given the high sequence identity and structural homology between the Rhu1PA from *T. maritima* and *E. coli*, it could be argued that the lack of selectivity in the Rhu1PA*Tm* enzyme is determined for similar reasons to those already determined for Rhu1PA*Ec*. Thus, according to Kroemer *et al*.,[24] L-lactaldehyde binds to the catalytic centre of the Rhu1PA*Ec* through two hydrogen bonds, directing the methyl group to the hydrophobic surroundings. As a result, the substrate is anchored to the active site of the enzyme favouring the nucleophilic attack by just one enantiotopic face. Restrictions imposed by the structure of the different *N*-Cbz-aminoaldehydes, as well as the lack of the hydroxyl group, may induce an incorrect orientation of the aldehyde in the catalytic pocket.[21a] Moreover, some important differences may occur in the active site of both aldolases since Rhu1PA*Tm* was not able to use DHA in presence of borate buffer, an ability so far restricted to Rhu1PA*Ec*.[22]

As previous studies have reported, stereochemistry of the iminocyclitols at C-2 (C-3 in pyrrolizidines **24-27**) revealed that the reductive amination with H_2 in presence of Pd/C depended on the structure of the aldol adduct precursor. For most of the examples, e.g. iminocyclitols **12a-b**, **15a**, **16b, 17b**, **21d**, **26b** and **27b** reductive amination was highly diastereoselective. Here, the hydrogenation took place from the face opposite to the C-4 hydroxyl group (C-1 hydroxyl group for the pyrrolizidine derivatives), regardless of the relative stereochemistry of the other substituents. Exceptions were observed in the iminocyclitols **18c, 19c, 24a** and **25a**.

Conclusion

The work presented herein describes the synthetic scope of the Rhu1PA*Tm*. Since this aldolase maintains a significant percentage of activity at temperatures much lower than its optimum (95 °C), it is compatible with multienzyme systems for C-C bond formation involving mesophilic enzymes. This enzyme was successfully tested in two different chemo-enzymatic cascades for the synthesis of nitrocyclitols and iminocyclitols. In both cases, Rhu1PA*Tm* was able to catalyse the aldol addition using the corresponding aldehydes to provide the desired products with similar or higher yields than Rhu1PA*Ec*. Additionally, the stereoselectivity shown by the aldolase from *T. maritima* was, in general, similar to the one of the Rhu1PA*Ec*, even though there were

significant differences on specific substrates. Our data indicated that the formation of the L-*threo* isomer was faster than that of the D-*erythro* isomer. No effect of the temperature was observed in this process.

Aldol adduct precursors of iminocyclitols **14a**, **14d** and **23a** were obtained with full L-*threo* (3*R*,4*S*) stereoselectivity. The rest aldol adducts from nitroaldehydes and *N*-Cbz-aminoaldehydes were obtained as mixtures of L-*threo* (3*R*,4*S*) and D-*erythro* (3*R*,4*R*) diastereoisomers. The major diasteromer formed was the one with the natural stereopreference of the enzyme (L-*threo*), with the exception of aldehyde **13c**.

Experimental Section

Materials

All solvents and reagents were purchased from Sigma-Aldrich or Acros. Triosephosphate isomerase (TIM), α-glycerophosphate dehydrogenase (αGDH), phosphatase acid from potato and acetate kinase (AK) was purchased from Sigma-Aldrich. Rhu1PA from *T. maritima* and DHAK from *C. freundii* were expressed and purified as previously described.[10, 18] DHAP was enzymatically prepared from DHA as previously described by Oroz-Guinea et al.[11c] with slight modifications. Acetyl phosphate (7.15 mmol), $MgSO_4$ (1.25 mmol) and DHA (5 mmol) were added to phosphate buffer (30 mL; 60 mM; pH=7.5) and water to a final reaction volume of 90 mL. DHAK (431 U) and AK (225 U) were suspended in ultra-pure water (pH ≈ 7.0) and added to the reaction. To start the reaction ATP (163 μmol) was added. The phosphorylation reaction was completed after 30 minutes. For the storage of the mixture the pH was adjusted to 5.0 to avoid the degradation of DHAP and was subsequently frozen. Nickel/iminodiacetic acid (Ni^{2+}/IDA) agarose was supplied by Agarose Bead Technologies (Spain). Dialysis was performed in SPECTRA/Por® membranes (Spectrum) with 12,000-14,000 Da cut-off.

Rhu1PA*Tm* activity assay

The retro-aldol activity of Rhu1PA*Tm* was spectrophotometrically measured using rhamnulose-1-phosphate (Rhu-1P) as substrate. Rhu-1P was synthesized using the multienzyme system described by Sánchez-Moreno et al.[11a] at 1 mmol of DHA scale. The reaction was carried out in 30 mL of HEPES buffer (20 mM; pH 7.5) containing DHA (1 mmol), L-lactaldehyde (1.5 mmol), acetyl phosphate (2 mmol), ATP (68 pmol), $MgSO_4$ (250 pmol), $ZnCl_2$ (0.3 pmol), DHAK (15 U), AK (30 U) and Rhu1PA from *E. coli*. Activity assays were run at 45 ºC following the decrease in absorbance at λ=340 nm (ε^{NADH}=6220 $M^{-1}cm^{-1}$) for 10 min in a reaction mixture (1 mL) containing Tris-HCl buffer (40 mM; pH 8.0), NADH (0.2 mmol), αGDH/TIM (2.4 U of αGDH and 24.8 U of TIM), Rhu-1P (10 μmol), and Rhu1PA*Tm*. 1 unit aldolase activity was defined as the amount of enzyme that converts 1 μmol of Rhu-1P in DHAP and L-lactaldehyde per minute in the above described conditions.

Stereochemistry study of the Rhu1PA*Tm* catalysed reaction

Stereochemistry of the Rhu1PA*Tm* was evaluated by measuring the percentage of each diasteroisomer formed depending on the reaction time and temperature. Benzyloxyacetaldehyde **2** (100 μmol) was dissolved into 600 μL of DMSO (10% final volume) and, then, added to 200 μmol of DHAP. Afterwards, the reaction pH was adjusted to 6.9. Finally, aldolase (0.4 U) was added to start the reaction. Four different temperatures were chosen to carry out the study: 4 ºC, r.t, 45 ºC and 80 ºC. Aliquots of each reaction were taken at different times and formation of the diasteroisomer was monitored by HPLC, using a RP-HPLC XBridge® C18, 5 μm, 4.6 x 250 mm column (Waters). The elution system used was: solvent (A); 0.1% trifluoroacetic acid (TFA) in H_2O (v/v) and solvent (B); 0.095% (v/v) TFA in CH_3CN/H_2O 4:1; gradient elution from 10% to 70% B over 30 min. Flow rate was 1 mL min^{-1}, detection was at 215 nm. The amount of aldol adduct produced was quantified from the peak areas using an external standard.

Synthesis of nitrocyclitols 5a, 5b, 7a and 7b and nitrocyclitol precursors 9a and 9b.

Nitroaldehyde **4** was prepared mixing acrolein with a excess of nitromethane with KF as a mild base at -35 ºC, as described by El Blidi et al.[16a] Nitroaldehyde **(±)6** was obtained starting from aldehyde **8**, as reported by El Blidi et al.[16b] Finally, aldehyde **8** was prepared by ozonolysis of the corresponding alkene,[25] previously obtained using the procedure of Hoaglin et al.[26]

0.5 mmol of the corresponding aldehyde —**4** (58.5 mg), **(±)6** (66.5 mg) or **8** (73.1 mg)— and 3 U of Rhu1PA*Tm* were added to 1 mmol of DHAP, previously adjusted to pH 6.9. Distilled water was added to a final volume of 30 mL. DMSO (10% in the final volume) was added to the reaction media to dissolve aldehyde **4**. The reaction was kept at room temperature for 24 h with stirring. The pH was then adjusted to 4.7 with HCl (1M) and Rhu1PA*Tm* was eliminated by centrifugation. Subsequently, acid phosphatase from potato (200 U) was added and the reaction was carried out during 24 h. After this time, the phosphatase was removed and the resulting solution was concentrated under vacuum. Reactions were monitored spectrophotometrically measuring the disappearance of DHAP[11b] and by TLC (Merck 60 F254 silica gel TLC plates). The residue was purified by column chromatography with Merck 60/230-400 and 60/40-63 mesh silica gel, using a solvent gradient from 9:1 to 8:2 of CH_2Cl_2/MeOH to afford the nitrocyclitols **5a-b**, and **7a-b** and 95:5 of CH_2Cl_2/MeOH for the ketone diacetal **9a-b**. Overall yields of the desired products were: **5a**+**5b** (72 mg, 70%), **7a**+**7b** (79.2 mg, 71%) and **9a**+**9b** (77.1 mg, 67%). ^1H and ^{13}C NMR spectra of these products were recorded on a Bruker Avance 400 spectrometer in $CDCl_3$, D_2O, and CD_3OD. In all cases, resonance spectra were consistent with those previously described for these compounds.[17, 19]

1-(Hydroxymethyl)-6-nitrocyclohexane-1,2,3-triol: 2 isomers. (2*R*,3*S*):(2*R*,3*R*) ratio **5a**:**5b**, 95:5.

Isomer (1*R*,2*R*,3*S*,6*S*) **5a**: ^{1}H NMR (400 MHz, MeOD, 298 K): δ 4.79 (dd, *J*= 13.0 Hz, *J*=4.0 Hz, 1H, H-6), 3.82 (d, *J*=11 Hz, 1H, H-7_B), 3.73 (ddd, *J*=9 Hz, *J*=11.5 Hz, *J*=4.6 Hz, 1H, H-3), 3.37 (d, *J*=9.3 Hz, 1H, H-2), 3.33 (d, *J*=11.0 Hz, 1H, H-7_A), 2.43 (m, 1H, H-5), 2.01 (m, 1H, H-5), 1.97 (m, 1H, H-4_B), 1.32 (m, 1H, H-4_A).

Isomer (1*R*,2*R*,3*R*,6*S*) **5b**: ^{1}H NMR (400 MHz, MeOD, 298 K): δ 4.75 (dd, *J*=12.4 Hz, *J*=4.0 Hz, 1H, H-6), 4.00 (m, 1H, H-3), 3.88 (d, *J*=2.6 Hz, 1H, H-2), 3.83 (d, *J*=11.5 Hz, 1H, H-7_B), 3.55 (d, *J*=11.5 Hz, 1H, H-7_A), 2.41 (m, 1H, H-5), 1.93 (m, 2H, H-5, H-4_B), 1.76 (m, 1H, H-4_A).

1-(Hydroxymethyl)-6-nitrocyclohexane-1,2,3,5-tetraol: 2 isomers. (2*R*,3*S*):(2*R*,3*R*) ratio **7a**:**7b**, 75:25.

Isomer (1*R*,2*R*,3*S*,5*R*,6*S*) **7a**: ^{1}H NMR (400 MHz, MeOD, 298 K): δ 4.67 (d, *J*=10.6 Hz, 1H, H-6), 4.50 (ddd, *J*=11.0 Hz, , *J*=11.0 Hz, , *J*=4.9 Hz, 1H, H-5), 3.86 (m, 1H, H-3), 3.77 (d, , *J*=11.2 Hz, 1H, H-7_B), 3.41 (d, *J*=9.5 Hz, 1H, H-2), 3.24 (d, *J*=10.8 Hz, 1H, H-7_A), 2.26 (m, 1H, H-4_B), 1.41 (pq, *J*=12.1 Hz, *J*=12.1 Hz, *J*=12.1 Hz, 1H, H-4_A).

Isomer (1*R*,2*R*,3*R*,5*R*,6*S*) **7b**: ^{1}H NMR (400 MHz, MeOD, 298 K): δ 4.55 (d, *J*=10.6 Hz, 1H, H-6), 4.43 (ddd, *J*=11.0 Hz, *J*=11.0 Hz, , *J*=4.9 Hz, 1H, H-5), 4.06 (m, 1H, H-3), 3.82 (d, *J*=2.4 Hz, 1H, H-2), 3.79 (d, *J*=11.3 Hz, 1H, H-7_B), 3.50 (d, *J*=11.3 Hz, 1H, H-7_A), 2.02 (m, 1H, H-4_B), 1.82 (ddd, *J*=11.9 Hz, *J*=11.9 Hz, *J*=10.8 Hz, 1H, H-4_A).

6,6-Diethoxy-1,3,4-trihydroxyhexan-2-one: 2 isomers. (3*R*,4*S*):(3*R*,4*R*) ratio **9a**:**9b**, 70:30.

Isomer (3*R*,4*S*) **9a**: ^{1}H NMR (400 MHz, CD_3OD, 298 K): δ 4.66 (dd, 1H, *J*=4.5 Hz y *J*=6.8 Hz, H-6), 4.50 (d, 1H, *J*=19.4 Hz, H-1_A) 4.40 (d, 1H, *J*=19.4 Hz, H-1_B), 3.88 (m, 1H, H-3), 3.59 (m, 4H, OCH_2-7, OCH_2-9), 3.27 (m, 1H, H-4), 1.82 (m, 2H, CH_2-H5), 1.15 (m, 6H, CH_3-8, CH_3-10); ^{13}C NMR (100 MHz, CD_3OD, 298 K): δ 213.47 (C-2), 102.38 (C-6), 79.67 (C-3), 70.29, 67.95, 63.29, 62.72 (C-1, C-4, C-7, C-9), 38.57 (C-5), 15.66 (C-8, C-10).

Isomer (3*R*,4*R*) **9b**: ^{1}H NMR (400 MHz, CD_3OD, 298 K): δ 4.69 (dd, 1H, *J*=4.1 Hz y *J*=7.5 Hz, H-6), 4.48 (d, 1H, *J*=19.4 Hz, H-1_A) 4.37 (d, 1H, *J*=19.4 Hz, H-1_B), 4.05 (m, 6H, H-3, H-4, OCH_2-7, OCH_2-9), 1.77 (m, 2H, CH_2-H5), 1.15 (m, 6H, CH_3-8 CH_3-10); ^{13}C NMR (100 MHz, CD_3OD, 298 K): δ 212.94 (C-2), 102.25 (C-6), 79.77 (C-3), 70.89, 68.12, 63.02, 62.80 (C-1, C-4, C-7, C-9), 37.79 (C-5), 15.66 (C-8 , C-10).

Analytical scale synthesis of iminocyclitols

Analytical scale reactions (1 mL total volume) were conducted in 2 mL test tubes with screw cap incubated at 45 ºC. 15 µmol of aldehyde —**10**, **13a** and **13b** (3.1 mg each); **13c** (3.5 mg); **13d** (3.74 mg); **22a** and **22b** (3.49 mg each)— was dissolved in DMF (300 µL) and then mixed with freshly prepared DHAP neutralized at pH 6.9. Reaction was started by adding the aldolase (between 0.75 and 1.6 U measured at 45 ºC). Reactions were monitored by HPLC, as described above. To monitor those reactions with aldehydes **13c and d**, **22a** and **22b** the gradient elution was changed to 30% to 100% B over 30 min.

Preparative scale synthesis of iminocyclitols

The general procedure for the production of the aldols **11**, **14** and, **23** was as described above, with only slight variations on the stoichiometry: 1.2 mmol of the corresponding aldehyde—**10**, **13a** and **13b** (249 mg each); **13c** (282 mg), **13d** (299 mg); **22a** and **22b** (278 mg each)— was dissolved in 24 mL of DMF (35% of final volume), followed by the addition of distilled water (until a final volume of 70 mL), 2 mmol of DHAP (previously synthesized and adjusted to pH 6.9) and 10 U of Rhu1PA*Tm*. The reaction progress was followed spectrophotometrically, measuring the aldol formed by retro-aldol reaction catalysed by Rhu1PA from *E. coli*. When a maximum of phosphorylated aldol conversion was achieved, 2 volumes of MeOH were added to stop the reaction and precipitate the enzyme (**10**: 105 min; **13a**: 90 min; **13b**: 75 min; **13c**: 150 min; **13d**: 150 min; **22a**: 60 min; **22b**: 90 min). Then, the mixture was filtered through a 0.45 mm cellulose membrane filter and MeOH was removed under vacuum. The filtrate was washed with AcOEt (3x100 mL) to remove the aldehyde that did not react.

Afterwards, the aqueous phase was acidified (pH 4.7) and phosphatase acid from potato was added (5.3 U per mmol of phosphorylated product). Dephosphorylation reaction was followed by HPLC using the same methodology as described above. When the product was completely dephosphorylated the reaction was stopped by adding two volumes of MeOH. After removal of the enzyme by filtration, the filtrate was adjusted to pH 3.0 and loaded onto a semi-preparative HPLC column X-Terra® (19/250 mm). Products were eluted using CH_3CN gradient. The flow rate was 10 mL min^{-1} and the products were detected at 215 nm. Fractions containing the pure product were pooled and lyophilized. Overall yields of the desired products were: **12a**+**12b** (112.8 mg, 32 %), **15a** (68.9 mg, 19%), **16b**+**17b** (168.2 mg, 47%), **18c**+**19c**+**20c** (70.0 mg, 18%), **21d** (48.1 mg, 12%), **24a**+**25a** (36.5 mg, 9%), **26b**+**27b** (48.0 mg, 12%).

The product obtained was dissolved in H_2O/MeOH (1:1 v/v) and treated with H_2 (50 psi) in the presence of Pd/C (2 eq. w/w) at room temperature during 24 h. After that, the catalyst was removed and the filtrate was adjusted to pH 6.4 with formic acid and lyophilized obtaining a solid material.

Separation of the isomers of iminocyclitols **16b**+**17b**, **18c**+**19c**+**20c** and **26b**+**27b** was performed in a FPLC system using CM-Sepharose CL-6B (Amersham Pharmacia) packed in a glass column (45 x 25 cm). The column was washed with four volumes (1vol ~ 220 mL) of a 100 mM solution of NH_3 and then equilibrated with four additional volumes of distilled H_2O (pH ~ 7.0). Iminocyclitols were dissolved in 10 ml of H_2O and the pH of the solution adjusted to 6.0 with formic acid

to be loaded in the column. The column was washed with three volumes of distilled H_2O (pH ~ 7.0) and the products were eluted with a 10 mM solution of NH_3 at a flow of 1 ml min^{-1}.

Fractions containing the compounds were analysed by NMR. ^1H and ^{13}C NMR iminocyclitols spectra were recorded on a Varian System 400 spectrometer. The compounds described were fully characterized using typical gradient-enhanced 2D experiments: COSY, HSQC, recorded under routine conditions (see the Supporting Information for spectra).

2-(Hydroxymethyl)piperidine-3,4-diol: 2 isomers. (2*S*,3*S*,4*S*):(2*S*,3*S*,4*R*) ratio **12a**+**12b** 53:47.

Isomer (3*S*,4*S*) **12a**: ^1H NMR (400 MHz, D_2O, 298 K): δ 3.87 (dd, J=3.2, *J*=12.6 Hz, 1H, H-7_A), 3.73 (dd, *J*=5.7 Hz, *J*=12.7 Hz, 1H, H-7_B), 3.6 (dt, *J*=4.9 Hz, J=9.5 Hz, *J*=11.6 Hz, 1H, H-4), 3.13 (t, 1H, H-3), 3.0 (br, 1H, H-6_A), 3.0 (m, 1H, H-2), 2.95 (dt, *J*=3.2 Hz, *J*=13.1 Hz, 1H, H-6_B), 1.91 (br, 1H, H-5_A), 1.38 (dq, *J*=4.3 Hz, 1H, H-5_B). ^{13}C NMR (125 MHz, D_2O, 298 K): δ 72.38 (C-4), 72.11 (C-3), 60.57 (C-7), 60.45 (C-2), 44.38 (C-6), 31.44 (C-5).

Isomer (3*S*,4*R*) **12b**: ^1H NMR (400 MHz, D_2O, 298 K): δ 4.0 (m, 1H, H-3), 3.80 (m, 1H, H-4), 3.2 (m, 1H, H-2), 1.85 (m, 1H, H-5_A), 1.75 (m, 1H, H-5_B), 3.76 (dd, 1H, H-7_A), 3.72 (dd, 1H, H-7_B), 3.18 (m, 1H, H-6_A), 2.95 (m, 1H, H-6_B). ^{13}C NMR (125 MHz, D_2O, 298 K): δ 68.26 (C-4), 66.45 (C-3), 60.17 (C-7), 59.65 (C-2), 42.40 (C-6), 25.13 (C-5).

(5*S*)-2-(Hydroxymethyl)-5-methylpyrrolidine-3,4-diol (15a).

^1H NMR (400 MHz, D_2O, 298 K): δ 3.77 (t, *J*=7.0 Hz, 1H, H-3), 3.66 (dd, *J*=4.2 Hz, *J*=12.0 Hz, 1H, H-7_A), 3.60 (t, *J*=7.5 Hz, 1H, H-4), 3.58 (dd, *J*=6.4 Hz, *J*=12.0 Hz, 1H, H-7_B), 3.13 (dt, *J*=4.2 Hz, *J*=6.4 Hz, *J*=7.0 Hz, 1H, H-2), 3.06 (dq, *J*=7.0 Hz, 1H, H-5), 1.16 (d, *J*=7.0 Hz, 3H, H-6). ^{13}C NMR (125 MHz, D_2O, 298 K): δ 81.15 (C-4), 76.53 (C-3), 61.67 (C-2), 60.65 (C-7), 56.28 (C-5), 15.95 (C-6).

(5*R*)-2-(Hydroxymethyl)-5-methylpyrrolidine-3,4-diol 2 isomers. (2*S*,3*S*,4*S*,5*R*):(2*S*,3*S*,4*R*,5*R*) ratio **16b**:**17b** 67:33.

Isomer (2*S*,3*S*,4*S*,5*R*) **16b**: ^1H NMR (400 MHz, D_2O, 298 K): δ 4.01 (dd, *J*=1.4 Hz, *J*=3.2 Hz, 1H, H-4), 3.95 (dd, *J*=1.8 Hz, *J*=3.2 Hz, 1H, H-3), 3.84 (dd, *J*=4.9 Hz, *J*=11.9 Hz, 1H, H-7_A), 3.48 (dq, *J*=3.5 Hz, *J*=6.5 Hz, 1H, H-5), 3.71 (dd, *J*=8.8 Hz, *J*=12.3 Hz, 1H, H-7_B), 3.16 (dt, *J*=3.3 Hz, *J*=5.1 Hz, *J*=8.2 Hz, 1H, H-2), 1.24 (d, *J*=6.7 Hz, 3H, H-6). ^{13}C NMR (125 MHz, D_2O, 298 K): δ 76.3 (C-4), 76.3 (C-3), 67.5 (C-2), 59.6 (C-7), 56.51 (C-5), 11.8 (C-6).

Isomer (2*S*,3*S*,4*R*,5*R*) **17b**: ^1H NMR (400 MHz, D_2O, 298 K): δ 4.28 (t, *J*=3.7 Hz, 1H, H-3), 3.95 (dd, *J*=3.7 Hz, *J*=9.0 Hz, 1H, H-4), 3.9 (dd, *J*=4.7 Hz, *J*=11.8 Hz, 1H, H-7_A), 3.8 (dd, *J*=8.4 Hz, *J*=11.8 Hz, 1H, H-7_B), 3.58 (m, *J*=3.7 Hz, *J*=4.7 Hz, *J*=8.4 Hz, 1H, H-2), 3.31 (m, 1H, H-5), 1.32 (d, *J*=6.7 Hz, 3H, H-6). ^{13}C NMR (125 MHz, D_2O, 298 K): δ 76.7 (C-4), 70.4 (C-3), 61.5 (C-2), 58.4 (C-7), 55.5 (C-5), 16.06 (C-6).

2-(Hydroxymethyl)-5-isopropylpyrrolidine-3,4-diol 3 isomers. (2*R*,3*R*,4*S*,5*R*):(2*R*,3*R*,4*S*,5*S*):(2*R*,3*S*,4*S*,5*R*) ratio **18c**:**19c**:**20c**, 42:25:32.

Isomer (2*R*,3*R*,4*S*,5*R*) **18c**: ^1H NMR (400 MHz, D_2O) δ 4.22 (t, *J* = 3.9 Hz, 1H), 4.13 (dd, *J* = 8.5, 4.3 Hz, 1H), 3.88 (dd, *J* = 11.5, 6.3 Hz, 1H), 3.75 (dd, *J* = 11.4, 7.1 Hz, 1H), 3.47 (td, *J* = 6.7, 3.6 Hz, 1H), 3.01 (m, 1H), 1.89 (dq, *J* = 13.7, 6.8 Hz, 1H), 1.03 (d, *J* = 6.8 Hz, 3H), 1.01 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, D_2O): δ 71.4 (C-3), 74.2 (C-4), 65.8 (C-5), 60.4 (C-2), 59.9 (C-6), 29.9 (C-7), 19.6 (C-8), 18.3 (C-9).

Isomer (2*R*,3*S*,4*S*,5*R*) **19c**: ^1H NMR (400 MHz, D_2O) δ 4.13 (d, *J* = 4.7 Hz, 1H), 4.00 (dd, *J* = 2.8, 0.9 Hz, 1H), 3.82 (dd, *J* = 11.6, 5.6 Hz, 1H), 3.74 (m, 1H), 3.23 (ddd, *J* = 7.0, 5.6, 2.8 Hz, 1H), 3.01 (m, 1H), 1.97 (m, 1H), 1.01 (d, *J* = 3.1 Hz, 3H), 1.00 (d, *J* = 3.1 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ 74.7 (C-3), 74.6 (C-4) , 68.2 (C-5), 62.3 (C-2), 58.0 (C-6), 25.7 (C-7), 18.7 (C-8), 18.4 (C-8).

Isomer (2*R*,3*S*,4*S*,5*R*) **20c**: ^1H NMR (400 MHz, D_2O, 298 K): δ 4.21 (t, *J*=4.0 Hz, 1H, H-3), 4.12 (m, 1H, H-4), 3.95 (dd, *J*=11.5 Hz, *J*=5.0 Hz, 1H, H-6_A), 3.74 (dd, *J*=11.2 Hz, *J*=7.4 Hz, 1H, H-6_B), 3.46 (dt, *J*=3.6 Hz, *J*=3.1 Hz, 1H, H-2), 3.00 (t, *J*=8.0 Hz, 1H, H-5), 1.88 (dt, *J*=6.8, 1H, H-7), 1.00 (m, 6H, H-8, H-9). ^{13}C NMR (100 MHz, D_2O): δ 76.2 (C-4), 73.4 (C-3), 68.7 (C-5), 64.1 (C-2), 60.2 (C-6), 32.0 (C-7), 21.4 (C-8), 20.5 (C-9).

2-(Hydroxymethyl)-5-isobutylpyrrolidine-3,4-diol (21d).

^1H NMR (400 MHz, D_2O, 298 K): δ 3.93 (t, *J*=6.9 Hz, 1H, H-3), 3.83 (m, 1H, H-4), 3.78 (m, *J*=12.6, 3.9 Hz, 1H, H-6_A), 3.83 (m, *J*=12.7, 6.0 Hz, 1H, H-6_B), 3.25 (m, 1H, H-2), 3.20 (m, 1H, H-5), 1.71 (m, 1H, H-8), 1.59 (m, 1H, H-7_A), 1.56 (m, 1H, H-7_B), 0.94 (m, 6H, H-9, H-10). ^{13}C NMR (100 MHz, D_2O): δ 80.4 (C-4), 76.3 (C-3), 61.5 (C-2), 60.5 (C-5), 58.5 (C-6), 41.4 (C-7), 24.4 (C-8), 22.4 (C-9), 20.9 (C-10).

(7a*S*)-3-(Hydroxymethyl)hexahydro-1*H*-pyrrolizine-1,2-diol: 2 isomers. (1*S*,2*S*,3*S*,7a*S*):(1*R*,2*S*,3*S*,7a*S*) ratio **24a**:**25a**, 60:40.

Isomer (1*S*,2*S*,3*S*,7a*S*) **24a**: ^1H NMR (400 MHz, D_2O, 298 K): δ 3.79 (m, *J*=12.2 Hz, *J*=8.2 Hz, 1H, H-8_A), 3.78 (bs, 1H, H-2), 3.74 (t, *J*=8.0 Hz, 1H, H1), 3.65 (m, *J*=11.8, 6.5 Hz, 1H, H-8_B), 3.20 (m, 1H, H-7a), 2.94 (bs, 1H, H-5_A), 2.80 (m, 1H, H-5_B), 2.75 (m, 1H, H-3), 1.96 (m, 1H, H-7_A), 1.88 (m, 1H, H-6_A), 1.79 (m, 1H, H-6_B), 1.76 (m, 1H, H-7_B). ^{13}C NMR (100 MHz, D_2O): δ 79.5 (C-1), 77.5 (C-2), 69.2 (C-3), 66.6 (C-7a), 55.8 (C-8), 54.8 (C-5), 29.3 (C-7), 24.3 (C-6).

Isomer (1*R*,2*S*,3*S*,7a*S*) **25a**: ^1H NMR (400 MHz, D_2O, 298 K): δ 4.33 (dd, *J*=3.8 Hz, *J*=1.5 Hz, 1H, H-2), 4.26 (bt, *J*=1.9 Hz, 1H, H-1), 4.08 (m, 2H, H-8_A, H-8_B), 4.02 (m, 1H, H-3), 3.93 (m, 1H, H-7a). 3.50 (m, 2H, H-5_A, H-5_B), 2.41 (m, 1H, H-7_A), 2.23 (m, 1H, H-6_A), 1.87 (m, 2H, H-7_B, H-6_B). ^{13}C NMR (100 MHz, D_2O): δ 78.5 (C-1), 77.5 (C-2), 74.8 (C-3), 65.8 (C-7a), 55.8 (C-8), 50.1 (C-5), 28.9 (C-7), 25.8 (C-6).

(7a*R*)-3-(Hydroxymethyl)hexahydro-1*H*-pyrrolizine-1,2-diol: 2 isomers. (1*S*,2*S*,3*S*,7a*R*):(1*R*,2*S*,3*R*,7a*R*) ratio **26b**:**27b**, 65:35.

Isomer (1*S*,2*S*,3*S*,7a*R*) **26b**: ^{1}H NMR (400 MHz, D_2O, 298 K): δ 4.04 (dd, *J*=6.3, 1.4 Hz, 1H, H-1), 3.83-3.73 (m, 4H, H-2, H-7a, H-8_A, H-8_B), 3.13 (m, 1H, H-3), 3.04 (m, 1H, H-5_A), 2.83 (dt, *J*=6.2 Hz, *J*=4. 3 Hz, 1H, H-5_B), 1.89 (bs, 1H, H-6_A), 1.79 (m, 2H, H-7_A, H-7_B), 1.65 (m, 1H, H-6_B). ^{13}C NMR (100 MHz, D_2O): δ 75.9 (C-2), 72.9 (C-1), 65.8 (C-7a), 64.6 (C-3), 58.8 (C-8), 54.6 (C-5), 26.9 (C-6), 22.8 (C-7).

Isomer (1*R*,2*S*,3*R*,7a*R*) **27b**: ^{1}H NMR (400 MHz, D_2O, 298 K): δ 4.27 (dd, *J*=3.6, 1.6 Hz, 1H, H-1), 3.94 (dd, *J*=4.4 Hz, *J*=1.5 Hz, 1H, H-2), 3.87 (m, 1H, H-7a), 3.70 (m, *J*=11.2 Hz, *J*=6.5 Hz, 1H, H-8_A), 3.45 (m, 1H, H-8_B), 3.00 (m, 1H, H-5_A), 2.91 (m, 1H, H-3), 2.78 (m, 1H, H-5_B), 1.97 (m, 1H, H-6_A), 1.87 (m, 1H, H-7_A), 1.82 (m, 2H, H-6_B, H-7_B). ^{13}C NMR (100 MHz, D_2O): δ 77.9 (C-2), 74.2 (C-1), 70.3 (C-7a), 69.4 (C-3), 58.8 (C-8), 56.6 (C-5), 29.5 (C-6), 25.4 (C-7).

Acknowledgements

Work at CSIC has been supported by the Spanish Ministerio de Economia y Competitividad (Grant CTQ2010-19073/BQU, CTQ2012-31605), Generalitat de Catalunya (2009 SGR 00281) ERA-IB MICINN, PIM2010EEI-00607 (EIB.10.012. MicroTechEnz-EIB., www.fkit.unizg.hr/miten). COST Action: CM1303 "Systems Biocatalysis" is acknowledged. K. Hernandez and I. Oroz acknowledge the CSIC for a JAE Predoctoral contract program.

References

[1] E. J. Corey, X. M. Cheng, *The Logic of Chemical Synthesis*; John Wiley & Sons, New York **1989**.

[2] a) C. J. Li, *Chem. Rev*. **2005**, *105*, 3095-3166; b) C. Palomo, M. Oiarbide, J. M. García, *Chem. Soc. Rev*. **2004**, *33*, 65-75.

[3]E. García-Junceda, J. F. García-García, A. Bastida, A. Fernández-Mayoralas, *Bioorg. Med. Chem*. **2004**, *12*, 1817-1834.

[4] P. Anastas, N. Eghbali, *Chem. Soc. Rev*., **2010**, *39*, 301-312.

[5] a) P. Clapés, J. Joglar, in *Modern Methods in Stereoselective Aldol Reactions* (Ed.: R. Mahrwald), Wiley-VCH Weinheim, **2013**, pp. 475-527; b) K. Fesko, M. Gruber-Khadjawi, *ChemCatChem* **2013**, *5*, 1248-1272; c) M. Müller, *Adv. Synth. Catal.* **2012**, *354*, 3161-3174; d) M. Brovetto, D. Gamenara, P. Saenz Méndez, G. A. Seoane, *Chem. Rev.* **2011**, *111*, 4346-4403; e) P. Clapés, W.-D. Fessner, In *Science of Synthesis. Stereoselective Synthesis 2. Stereoselective Reactions of Carbonyl and Imino Groups*, Vol. 2, (Ed.: G. A. Molander), Georg Thieme Verlag KG: Suttgart (Germany), **2011**, p 677-734; f) P. Clapés, W.-D. Fessner, G. A. Sprenger, A. K. Samland, *Curr. Opin. Chem. Biol.* **2010**, *14*, 154-167.

[6] a) R. N. Patel, *ACS Catalysis* **2011**, *1*, 1056-1074; b) S.-H. Baik, H. Yoshioka, *Biotechnol. Lett*. **2009**, *31*, 443-448; c) M. Wolberg, B. H. N. Dassen, M. Schürmann, S. Jennewein, M. G. Wubbolts, H. E. Schoemaker, D. Min, *Adv. Synth. Catal*. **2008**, *350*, 1751-1759; d) J. A. Castillo, J. Calveras, J. Casas, M. Mitjans, M. P. Vinardell, T. Parella, T. Inoue, G. A. Sprenger, J. Joglar, P. Clapes, *Org. Lett.* **2006**, *8*, 6067-6070; e) M. Müller, *Angew. Chem*. **2005**, *117*, 366-369; *Angew. Chem. Int. Ed*. **2005**, 44, 362-365; f) W. A. Greenberg, A. Varvak, S. R. Hanson, K. Wong, H. J. Huang, P. Chen, M. J. Burk, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 5788-5793.

[7] For a recent review on engineering of aldolases for use as industrial biocatalysts, see: C. L. Windle, M. Müller, A. Nelson, A. Berry, *Curr. Opin. Chem. Biol.* **2014**, *19*, 25-33.

[8] a) P. Falcicchio, S. Wolterink-Van Loo, M. C. R. Franssen, J. van der Oost, *Extremophiles* **2014**, *18*, 1-13; b) F. Canganella, J. Wiegel, *Naturwissenschaften*, **2011**, *98*, 253-279; c) L. D. Unsworth, J. van der Oost, S. Koutsopoulos, *FEBS J*., **2007**, *274*, 4044-4056.

[9]a) S. Elleuche, C. Schröder, K. Sahm, G. Antranikian, *Curr. Opin. Biotechnol*. **2014**, *29*, 116-123; b) H. Atomi, T. Sato, T. Kanai, *Curr. Opin. Biotechnol.*, **2011**, *22*, 618-626; c) C. Bertoldo, G. Antranikian, In *Enzyme Catalysis in Organic Synthesis: A Comprehensive Handbook, Second Edition* (Eds K. Drauz and H. Waldmann), Wiley-VCH Weinheim, **2008**, pp. 313-334; d) H. Atomi, *Curr. Opin. Biotechnol.*, **2005**, *9*, 166-173.

[10] I. Oroz-Guinea, I. Sánchez-Moreno, M. Mena, E. García-Junceda, *Appl. Microbiol. Biotechnol.*, **2015**, *99*, 3057-3068.

[11] a) I. Sánchez-Moreno, J. F. García-García, A. Bastida, E. García-Junceda, *Chem. Commun*. **2004**, 1634-1635; b) I. Sanchez-Moreno, L. Iturrate, E. G. Doyagüez, J. A. Martinez, A. Fernandez-Mayoralas, E. Garcia-Junceda, *Adv. Synth. Catal.* **2009**, *351*, 2967-2975; c) I. Oroz-Guinea, I. Sánchez-Moreno, E. García-Junceda, in *Practical Methods for Biocatalysis and Biotransformations 2* (Eds.: J. Whittall, P. Sutton), John Wiley & Sons, Ltd, **2012**, pp. 323-331.

[12] a) J. Calveras, M. Egido-Gabás, L. Gómez, J. Casas, T. Parella, J. Joglar, J. Bujons, P. Clapés, *Chem. Eur. J.*, **2009**, *15*, 7310-7328; b) A. T. Carmona, J. Fuentes, I. Robina, E. R. Garcia, R. Demange, P. Vogel, A. L. Winters, *J. Org. Chem.*, **2003**, *68*, 3874-3883.

[13] X. Chen, Y. Fan, Y. Zheng, Y. Shen, *Chem. Rev.*, **2003**, *103*, 1955–1977.

[14] a) J. Duchek, D. R. Adams, T. Hudlicky, *Chem. Rev*. **2011**, *111*, 4223-4258; b) F. Cagide-Fagín, O. Nieto-García, H. Lago-Santomé, R. Alonso, *J. Org. Chem*. **2012**, *77*, 11377-11382.

[15] a) R. S. Babu, Q. Chen, S.-W. Kang, M. Zhou, G. A. O'Doherty, *J. Am. Chem. Soc*., **2012**, *134*, 11952-11955; b) Y. Leshch, A. Jacobsen, J. Thimm, J. Thiem, *Org. Lett*., **2013**, *15*, 4948-4951; c) R. Mohanrao, A. Asokan, K. M. Sureshan, *Chem. Commun*. **2014**, *50*, 6707-6710; d) K. P. Stockton, B. W. Greatrex, D. K. Taylor, *J. Org. Chem*. **2014**, 79, 5088-5096; e) A. Vidyasagar, K. M. Sureshan, *Eur. J. Org. Chem*. **2014**, 2349-2356.

[16] a) L. El Blidi, D. Crestia, E. Gallienne, C. Demuynck, J. Bolte, M. Lemaire, *Tetrahedron: Asymmetry* **2004**, *15*, 2951-2954; b) L. El Blidi, M. Ahbala, J. Bolte, M. Lemaire, *Tetrahedron: Asymmetry* **2006**, *17*, 2684-2688; c) L. El Blidi, Z. Assaf, F. Camps Bres, H. Veschambre, V. Théry, J. Bolte, M. Lemaire, *ChemCatChem*, **2009**, *1*, 463-471.

[17] F. Camps Bres, C. Guérard-Hélaine, V. Hélaine, C. Fernandes, I. Sánchez-Moreno, M. Traïkia, E. García-Junceda, M. Lemaire, *J. Mol. Catal. B: Enzym*., **2015**, *114*, 50-57.

[18] I. Sánchez-Moreno, L. Iturrate, R. Martín-Hoyos, M.L. Jimeno, M. Mena, A. Bastida, E. García-Junceda, *ChemBioChem*, **2009**, *10*, 225-229.

[19] a) R. Ballini, L. Barboni, F. Fringuelli, A. Palmieri, F. Pizzo, L. Vaccaro, *Green Chem.*, **2007**, *9*, 823-838; b) F. Camps Bres, C. Guérard-Hélaine, C. Fernandes, J. A. Castillo, M. Lemaire, *Tetrahedron: Asymmetr*y, **2013**, *24*, 1075-1081; c) W.-D. Fessner, C. Walter, *Top. Curr. Chem.*, **1997**, *184*, 97-194.

[20] W.-D. Fessner, G. Sinerius, A. Schneider, M. Dreyer, G. E. Schulz, J. Badia, J. Aguilar, *Angew. Chem. Int. Ed. Engl.*, **1991**, *30*, 555-558.

[21] a) L. Espelt, T. Parella, J. Bujons, C. Solans, J. Joglar, A. Delgado, P. Clapés, *Chem. Eur. J.*, **2003**, *9*, 4887-4899; b) L. Espelt, J. Bujons, T. Parella, J. Calveras, J. Joglar, A. Delgado, P. Clapés, *Chem. Eur. J.*, **2005**, *11*, 1392-1401; c) J. Calveras, J. Casas, T. Parella, J. Joglar, P. Clapés, *Adv. Synth. Catal.*, **2007**, *349*, 1661-1666.

[22] X. Garrabou, J. Calveras, J. Joglar, T. Parella, J. Bujons, P. Clapés, *Org. Biomol. Chem.*, **2011**, *9*, 8430-8436.

[23] X. Garrabou, L. Gomez, J. Joglar, S. Gil, T. Parella, J. Bujons, P. Clapés, *Chem. Eur. J.*, **2010**, *16*, 10691-10706.

[24] M. Kroemer, I. Merkel, G. E. Schulz, *Biochemistry*, **2003**, 42, 10560-10568.

[25] B. M. Trost, J. Dumas, M. Villa, *J. Am. Chem. Soc*., **1992**, *114*, 9836-9845.

[26] R. I. Hoaglin, D. G. Kubler, A. E. Montagna, *J. Am. Chem. Soc*., **1958**, *80*, 5460-5463.