UPDATES doi.org/10.1002/adsc.202101510 Advanced Synthesis & Catalysis

Amine Transaminase Mediated Synthesis of Optically Pure Piperazinones and 1,4-Diazepanones

Carlos Pérez-Martín,[a] Francisca Rebolledo,[a] and Rosario Brieva[a,*]

[a] Department of Organic and Inorganic Chemistry, University of Oviedo, 33006-Oviedo (Asturias), Spain
Phone: +34 98 5102994
E-mail: rbrieva@uniovi.es

Manuscript received: December 28, 2021; Revised manuscript received: February 15, 2022;
Version of record online: March 2, 2022

Supporting information for this article is available on the WWW under https://doi.org/10.1002/adsc.202101510

Abstract: A biocatalytic approach has been designed for the synthesis of optically active piperazinones and 1,4-diazepanones in aqueous medium under mild conditions. The method described herein is based on a biocatalytic transamination of an easily accessible *N*-(2-oxopropyl) amino acid ester and the subsequent spontaneous cyclization of the initially formed amine. Both enantiomers of the synthesized piperazinones can be prepared by the selection of amine transaminases of opposite selectivity. The reaction conditions were optimised and eight selected processes were performed on a preparative scale. Thus, seven optically active piperazinones were synthesized in high isolated yields (70–90%) and a 1,4-diazepanone in a moderate yield (51%), being the *ee* ≥99% in all the cases.

Keywords: Piperazinone; Piperazine; 1,4-Diazepanone; Cascade reaction; Stereoselective synthesis; Transaminase

Introduction

1,4-Diazo heterocycles such as piperazinones, piperazines or diazepanes are structural building blocks in an enormous number of drugs and bioactive molecules.[1] Piperazinones, as rigid templates in the construction of peptidomimetics,[2] have proven to be a valuable tool in drug discovery and they are susceptible of further transformations.[3] Moreover, these heterocycles are useful precursors of piperazines, which are privileged structures in medicinal chemistry and one of the most commonly present *N*-heterocycles in small molecule pharmaceuticals.[4] Not as widespread as piperazines, but also very significant examples of 1,4-diazepanes can be found as a part of structural skeletons in medicinally relevant compounds.[5]

The prevalence of these heterocycles in a vast number of proven bioactive compounds makes them excellent scaffolds upon which to introduce chirality and increase molecular complexity, as a powerful tool for the fine-tuning of their pharmacokinetic profile. It is noteworthy that, in most cases, particularly in piperazine-containing drugs, these moieties are used as linkers and have only substitutions on the nitrogen atoms.[6] Some significant examples of current or potential pharmaceuticals under study in which the core chiral is a piperazinone, piperazine or 1,4-diazepane ring are shown in figure 1.

Therefore, synthetic methods that efficiently provide these privileged structures with high regio-, stereo-, and enantioselectivities are of high and continuous interest.

Biocatalytic methods are especially attractive for this purpose because of their inherent characteristics as mildness, sustainability and high selectivity.[7] Nevertheless, the use of these methods for the synthesis of 1,4-diazo heterocycles has been scarcely reported. Recently, two interesting approaches involving imine reductases for the synthesis of chiral piperazines[8] and 1,4-diazepanes[9] have been described (Scheme 1A). Also, an evolving amine transaminase[10] (ATA) has been used in the enantioselective transamination of a mesylated hydroxy ketone. The subsequent intramolecular displacement of the mesylate group by the formed

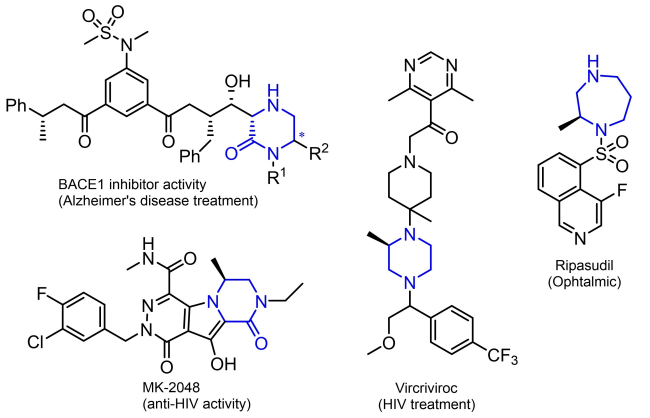


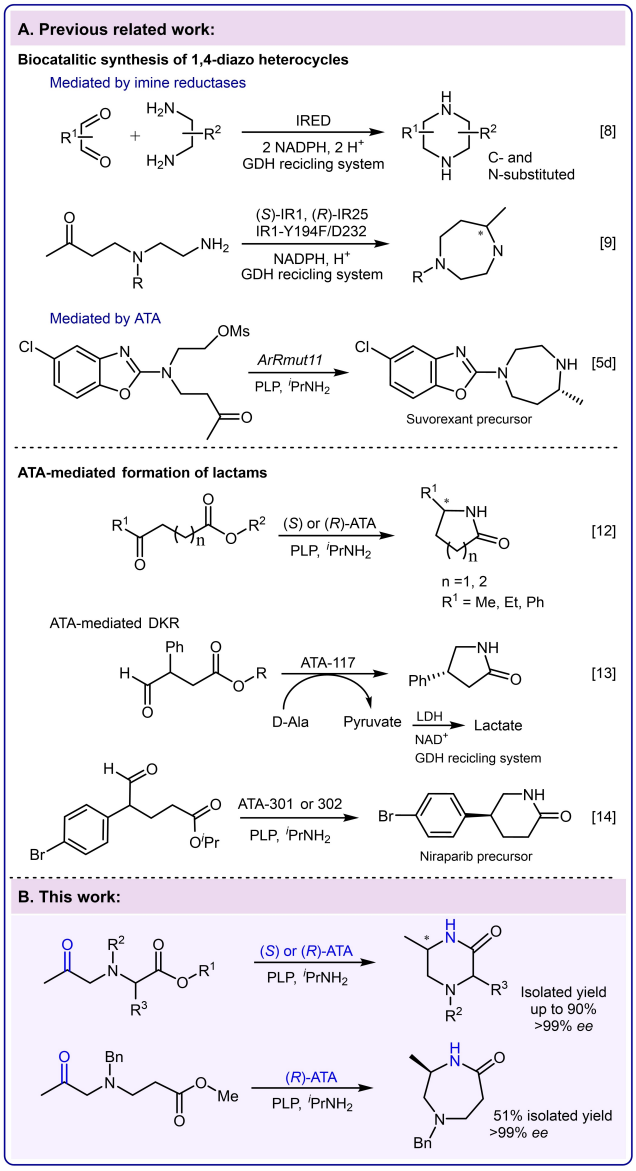
Figure 1. Representative drug molecules containing chiral piperazinone, piperazine, and 1,4-diazepane.

amine produced the corresponding 1,4-diazepane precursor of Suvorexant (Scheme 1A).[5d] This spontaneous cyclization circumvents unfavourable equilibrium towards the formation of the amine product, which otherwise could be a drawback of the enzymatic transamination.[11] Similar strategies, that relies upon the transformation of the initially formed amine to a secondary product, have been successfully applied to the preparation of (*S*)- and (*R*)-6-substituted piperidinones and 5-substituted pyrrolidinones from their corresponding keto esters (Scheme 1A).[12] In addition, the transaminase mediated DKR of racemic aldehydes has been used for the preparation of 4-phenylpyrrolidinone[13] and (*S*)-5-(4-bromophenyl)piperidin-2-one (Scheme 1A).[14]

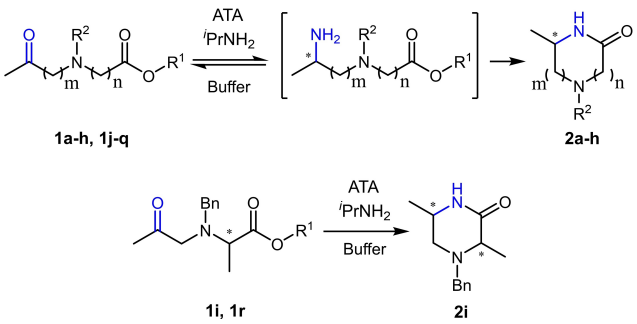
The method described herein is a general biocatalytic approach for the preparation of optically active piperazinones and diazepanones employing different amine transaminases (ATA) (Scheme 1B). Easily accessible *N*-(2-oxopropyl) or *N*-(3-oxobutyl) amino acid esters are used as substrates for the biotransamination to obtain the corresponding optically pure amine. The subsequent spontaneous cyclization of the formed amine drives the equilibrium towards the formation of the desired heterocycle in the reaction medium (Scheme 2).

The screening for the transaminase selection and optimisation of the reaction conditions will be disclosed in order to give access to both enantiomers of a series of protected piperazinones (**2a–f**) from easily accessible glycinates (Table 1, **1a–f** and **1j–o**). The substrate scope of this approach has been exemplified by increasing in one carbon the chain to give the corresponding diazepanones **2g**,**h** and by using the D- or L-alanine ester derivatives as starting material to obtain the four nearly pure stereoisomers of piperazinone **2i**.

In addition, the usefulness of the obtained optically pure piperazinones is tested in their further trans-



Scheme 1. A. Previous related work: biocatalytic synthesis of piperidines and 1,4-diazepanes and ATA-mediated formation of lactams. B. This work: synthesis of piperazinones and 1,4-diazepanones.



Scheme 2. General formation of optically active piperazinones or 1,4-diazepanones mediated by amine transaminases (ATAs).

Table 1. Ethyl esters **1a–i** and methyl esters **1j–r** used as substrates in this work.

R^1 Et	Me	R^2	m	n
a	**j**	Benzyl (Bn)	1	1
b	**k**	*tert*-Butoxycarbonyl (Boc)	1	1
c	**l**	Benzyloxycarbonyl (Cbz)	1	1
d	**m**	Benzoyl (Bz)	1	1
e	**n**	(4-Methylbenzene)sulfonyl (Ts)	1	1
f	**o**	(4-Methoxybenzene)sulfonyl (Mbs)	1	1
g	**p**	Bn	1	2
h	**q**	Bn	2	1
i	**r**	Bn	1	1

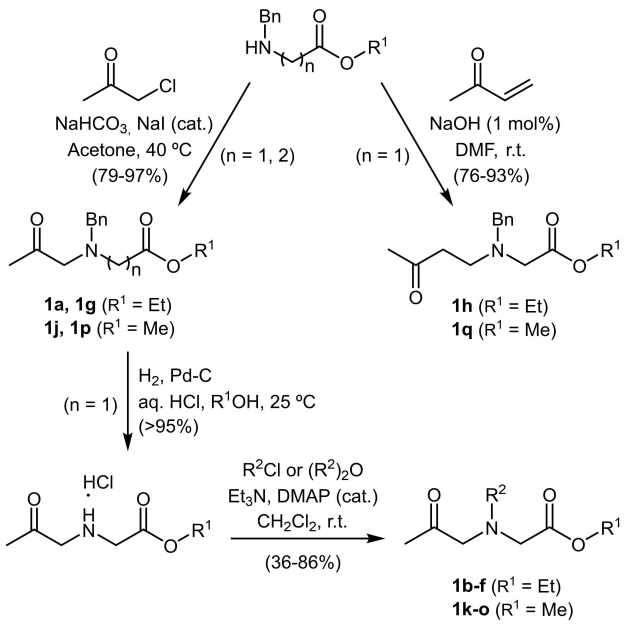
formations, such as in the preparation of an orthogonally protected piperazine derivative.

Results and Discussion

Synthesis of the Substrates

Scheme 3 summarizes the synthesis of substrates **1a–q** from *N*-benzyl α- or β-amino acid esters.

N-Benzylglycine ethyl ester is commercially available. The other analogues were prepared from the α- or β-amino acids or obtained from their commercial esters as described in the Supporting information (SI). The *N*-benzylation of the amino acid esters was carried out through the standard procedure of $NaBH_4$ reduction of the corresponding formed imine with benzaldehyde.



Scheme 3. Synthesis of substrates.

Substrates **1a**,**g** (R^1 = Et) and **1j**,**p** (R^1 = Me), as well as their alanine derivative analogues **1i** and **1r** (not showed in Scheme 3), have been prepared by the reaction of the corresponding *N*-benzyl amino ester with chloroacetone using NaI as a catalyst. The *N*-(3-oxobutyl) substrates **1h** and **1q** have been synthesized in high yields by a Michael-type reaction between the *N*-benzyl glycinate and methyl vinyl ketone.

The substrates **1b–f** and **1k–o** have been prepared from **1a** and **1j**, respectively, by the removal of the benzyl group via Pd–C catalysed hydrogenolysis in an acid medium and the subsequent introduction of the new protecting group without the purification of the intermediate hydrochloride salt.

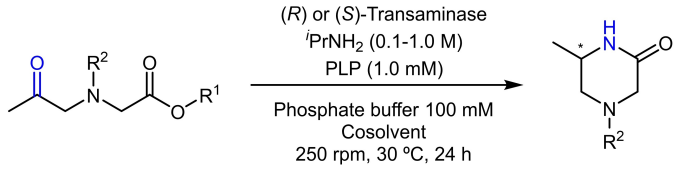
Biocatalytic Transamination

In the first set of experiments, the model substrate *N*-benzyl-*N*-(2-oxopropyl)glycinate (**1a**) was screened against 28 commercial transaminases and other four lyophilized whole-cell biocatalysts overexpressed in *E. coli*: the (*S*)-selective ATAs from *Chromobacterium violaceum* (*Cv*TA)[15] and *Arthrobacter citreus* (*Ar*STA),[16] and the (*R*)-selective wild-type transaminase from *Arthrobacter sp.* (*Ar*RTA)[17] and its engineered variant *ArRmut11*.[10] Substrate **1a** concentration was 25 mM, isopropylamine was used as an amine donor (1.0 M, 40 eq.) and DMSO (2.5% v/v) was employed as a cosolvent in combination with phosphate buffer 100 mM, pH 7.5 (final volume 500 µL). The reactions were incubated for 24 h at 30 °C.

Several of the tested ATAs provided the desired piperazin-2-one (*R*)-**2a** and (*S*)-**2a** with excellent enantioselectivities (>99% *ee*) and moderate conversions (Table 2, entries 1 and 2; a wide selection of results can be found in Table S1 in the SI). Among the (*R*)-selective transaminases, the best result was obtained with the *ArRmut11*, the HPLC-measured yield for the resulting enantiopure (*R*)-**2a** being 46% (entry 1), while the best (*S*)-selective biocatalyst was ATA-237 that led to the formation of enantiopure (*S*)-**2a** with a 40% yield (entry 2).

When each reaction was analysed at the end of the experiment, the remaining substrate **1a** concentration was below 5% in all the cases. This fact contrasts with the moderate-low analytical yield measured by HPLC for the produced **2a**, which was attributed to a competing background ester hydrolysis also occurring in the absence of enzyme. Thus, selecting the best (*S*)- and (*R*)-selective biocatalysts, the influence of some reaction conditions such as pH, temperature, cosolvent, alkyl group of the ester, and isopropylamine concentration were examined in order to circumvent this competitive hydrolysis reaction. Table 2 collects the best results obtained for the two enantio-complemen-

Table 2. Stereoselective transformations of substrates **1 a–f** into piperazinones **2 a–j**.[a]



1 **2** (*ee* > 99%)[b]

Entry	Substrate	R^1	R^2	[mM]	^{i}PrNH$_2$ [M]	pH	Cosolvent	ATA	Product Yield [%][b]	
1	**1 a**	Et	Bn	25	1.0	7.5	DMSO	*ArRmut11*	(*R*)-**2 a**	46
2	**1 a**	Et	Bn	25	1.0	7.5	DMSO	ATA-237	(*S*)-**2 a**	40
3	**1 a**	Et	Bn	25	0.1	8.5	TBME	*ArRmut11*	(*R*)-**2 a**	84
4	**1 a**	Et	Bn	25	0.1	8.5	*n*-Heptane	ATA-237	(*S*)-**2 a**	84
5	**1 j**	Me	Bn	25	0.1	8.5	TBME	*ArRmut11*	(*R*)-**2 a**	70
6	**1 j**	Me	Bn	25	0.1	8.5	*n*-Heptane	ATA-237	(*S*)-**2 a**	76
7	**1 b**	**Et**	**Boc**	**25**	**1.0**	**8.5**	**TBME**	***ArRmut11***	**(*R*)-2 b**	**83**
8	**1 b**	Et	Boc	25	1.0	8.5	TBME	*Cv*TA	(*S*)-**2 b**	54
9	**1 b**	**Et**	**Boc**	**25**	**1.0**	**8.5**	***n*-Heptane**	***Cv*TA**	**(*S*)-2 b**	**89**
10	**1 c**	**Et**	**Cbz**	**25**	**1.0**	**8.5**	**TBME**	***ArRmut11***	**(*R*)-2 c**	**90**
11	**1 c**	Et	Cbz	25	1.0	8.5	TBME	ATA-237	(*S*)-**2 c**	76
12	**1 d**	Et	Bz	25	1.0	8.5	TBME	ATA-412	(*R*)-**2 d**	32
13	**1 d**	Et	Bz	25	1.0	8.5	TBME	ATA-237	(*S*)-**2 d**	26
14	**1 e**	**Et**	**Ts**	**25**	**1.0**	**8.5**	**MeCN**	***ArRmut11***	**(*R*)-2 e**	**91**
15	**1 e**	Et	Ts	25	1.0	8.5	MeCN	ATA-237	(*S*)-**2 e**	73
16	**1 f**	**Et**	**Mbs**	**25**	**1.0**	**8.5**	**MeCN**	***ArRmut11***	**(*R*)-2 f**	**78**
17	**1 f**	Et	Mbs	25	1.0	8.5	MeCN	ATA-237	(*S*)-**2 f**	45
18	**1 b**	Et	Boc	50	1.0	8.5	*n*-Heptane	*Cv*TA	(*S*)-**2 b**	86
19	**1 c**	Et	Cbz	50	1.0	8.5	TBME	*ArRmut11*	(*R*)-**2 c**	82
20	**1 c**	Et	Cbz	50	1.0	8.5	TBME	Cell-free *ArRmut11*[c]	(*R*)-**2 c**	92
21	**1 e**	Et	Ts	50	1.0	8.5	MeCN	*ArRmut11*	(*R*)-**2 e**	87
22	**1 e**	Et	Ts	50	1.0	8.5	MeCN	Cell-free *ArRmut11*[c]	(*R*)-**2 e**	88

[a] General reaction conditions. Substrate (25 or 50 mM). ATA: 10 mg (for *Cv*TA or *ArRmut11*) or 2.0 mg (for Codexis' enzymes or lyophilized cell-free *ArRmut11*). Cosolvent: 2.5% v/v (10% v/v in the case of *n*-heptane). ^{i}PrNH$_2$ (0.1 or 1.0 M). Assay final volume 500 µL.

[b] Analytical yield and *ee* values were determined by HPLC analysis on chiral stationary phase. The analytical yield was calculated using a calibration line (see SI).

[c] The reaction was run at 45 °C.

tary ATAs (entries 3 and 4). Other selected results are summarized in Table S2 of the SI.

Raising the pH at 8.5 resulted beneficial in all the processes while, on the contrary, increasing the temperature up to 45 °C did not appreciably affect the yields.

The effect of the cosolvent was evaluated using acetonitrile and ethanol, commonly used in ATA-catalysed processes, and also the less frequently used *t*-butyl methyl ether (TBME) or *n*-heptane.[18] We theorize that the use of a two-phase system would minimise the undesired side reaction. In order to better evaluate the effect of cosolvent in the process, the concentration of isopropylamine was reduced to 0.1 M. Effectively, the best results were obtained using the water-immiscible cosolvents. The yield of (*R*)-**2 a** increased to 84% when TBME (2.5% v/v) was used for the *ArRmut11* catalysed process at pH 8.5 and 30 °C (Table 2, entry 3). On the other hand, the yield of (*S*)-**2 a** raised to 84% when *n*-heptane (10% v/v) was the cosolvent in the ATA-237 catalysed transamination, under the same reaction conditions (Table 2, entry 4).

In addition, the use of acetonitrile (2.5% v/v) also improved significantly the results obtained in DMSO, 72–76% yields were obtained depending on the catalyst and pH (Table S2).

Regarding the alkyl group of the ester, we tested the methyl ester derivative **1 j** as substrate but, under the optimized conditions, lower assay yield values were achieved (see entries 5 and 6, Table 2) in comparison with the ethyl ester analogue **1 a** (entries 3 and 4).

With the aim of explore the influence of the substrate structure and, at the same time, obtain valuable optically pure piperazinones protected with different groups, the compounds **1 b–f** were evaluated as substrates in the biocatalytic transamination.

In general, the presence of carbamoyl or sulfonyl groups increased the stability of the substrates in the reaction medium. In all the cases, the competitive ester hydrolysis happened in a lesser extent than for substrate **1a**. Based on the results obtained with substrate **1a**, pH 8.5 was fixed for the general screening against the transaminases panel and TBME (2.5% v/v) was selected as cosolvent for substrates **1b–d**; in the case of sulfonyl derivatives **1e** and **1f**, poorly soluble in TBME, acetonitrile (2.5% v/v) was used as cosolvent. An additional advantage of these solvents in relation to DMSO is their low boiling point, very convenient in order to perform the transamination on a preparative scale. All the remainder conditions were the same as in the initial screening for substrate **1a**. After 24 h of incubation, several tested transaminases provided good conversions and excellent enantioselectivities (>99% *ee*). The best biocatalyst for the synthesis of both piperazinone antipodes for each substrate is shown in Table 2, entries 7–17.

Excellent activity and selectivity for *ArRmut11* was notably achieved towards the formation of (*R*)-**2b** (83% yield, entry 7), (*R*)-**2c** (90% yield, entry 10), (*R*)-**2e** (91% yield, entry 14), and (*R*)-**2f** (78% yield, entry 16), with >99% *ee* in all cases. ATA-415 and ATA-412 also provided good results in terms of enantioselectivity towards the (*R*)-isomers and good yields depending on the substrate (Table S3, SI).

Among the (*S*)-selective transaminases, moderate activity for *Cv*TA was achieved towards the formation of the Boc derivative (*S*)-**2b** (54% yield, Table 2, entry 8) with excellent enantioselectivity (>99% *ee*), while ATA-237 allowed the formation of (*S*)-**2c** (76% yield, Table 2, entry 11) and (*S*)-**2e** (73% yield, Table 2, entry 15).

The change of TBME by *n*-heptane (10% v/v) as a cosolvent had a positive effect on the *Cv*TA catalysed transamination of the Boc-derivative **1b**. The yield of the piperazinone (*S*)-**2b** was increased from 54 to 89% (Table 2, entry 9).

Similar to the study carried out with substrate **1a**, the effect of the leaving group of the esters was also examined, performing the biocatalytic transamination experiments over the methyl esters **1k–o**. In general, these reactions led to the corresponding piperazinone with similar or lower yields than the ethyl ester analogues, with some non-significant exceptions. The best results obtained for these substrates are shown in Table S3 (SI).

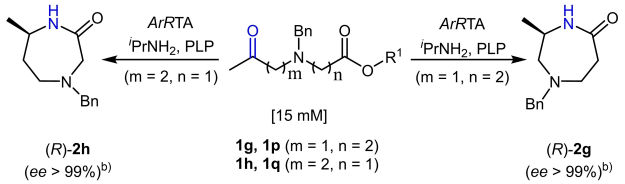
Before performing these biotransformations on a preparative scale, the substrate concentration was increased until 50 mM for those substrates with which the highest yields had been obtained. Among all the reactions studied, those using the substrates **1b**, **1c**, and **1e** showed conversions only slightly lower than at 25 mM, but the stereoselectivity was still unchanged (>99% *ee*), (Table 2, entries 18, 19, and 21). Therefore, a 50 mM substrate concentration was later used to carry out these biotransformations on a preparative scale.

Taking into account that the evolved variant *ArRmut11* has been revealed to be the best transaminase for the synthesis of most of the (*R*)-piperazinones, this variant was subjected to a thermal semipurification through a modification of the procedure previously described by López-Iglesias and col.[19] The purification procedure and the results of the transamination reactions using the lyophilized cell-free *ArRmut11* are summarized in Table S4 (SI). For some of the substrates, the use of the lyophilized cell-free *ArRmut11* improved the performance of the transamination. As it is shown in Table 2, at a 50 mM concentration and 45 °C, the yield of (*R*)-**2c** increased until 92% (entry 20) and (*R*)-**2e** (entry 22) was also obtained in very high yield (88%). It is also remarkable that a less amount of transaminase was employed in these assays.

In order to test this synthetic strategy for the preparation of higher size heterocyclic compounds, ethyl esters **1g** and **1h**, and methyl esters **1p** and **1q** were subjected to the action of ATAs. If the enzyme catalyses the amination of the ketone moiety and the resulting optically active amine experiments the cyclization, 1,4-diazepanes could be obtained. In the initial screening carried out in the reaction conditions optimized for substrate **1a**, all the tested ATAs led to very low conversions (<10%) of the heterocycle. Furthermore, in the case of glycinate derivatives **1h** and **1q**, as occurred with their analogue **1a**, a great tendency to hydrolysis was evidenced, making the cyclization step even more difficult. Nevertheless, by reducing the substrate concentration at 15 mM and carrying the reactions at pH 10.0, enantiopure 1,4-diazepane (*R*)-**2h** was obtained with 15 and 20% yield from *Ar*RTA catalysed transamination of **1h** and **1q**, respectively (Table 3, entries 1 and 2). On the other hand, using *Ar*RTA and the less hydrolyzable β-amino esters **1g** and **1p**, improved results were obtained (Table 3, entries 3 and 4), the yields for the resulting enantiopure (*R*)-**2g** being 45 and 63%, respectively. Interestingly, in these reactions methyl esters were more efficient than ethyl esters, in contrast to that generally observed in the formation of piperazinones. Other selected results of this are summarized in Table S5 (SI).

The use of optically active α-amino acids as starting materials could be a simple strategy to increase the structural complexity of the substrates here investigated, and so of the achieved piperazinone. Taking this into account, in order to extend the scope of the biocatalytic transamination, the substrates (*S*)-**1i**, (*S*)-**1r**, and (*R*)-**1r** were synthesized from commercially available D- or L-alanine ethyl or methyl esters

Table 3. Stereoselective transformations of substrates **1 g**, **1 p**, **1 h**, and **1 q** into 1,4-diazepanones **2 g** and **2 h**.[a]



Entry	Substrate	R^1	Product	Yield [%][c]
1	**1 h**	Et	(*R*)-**2 h**	15
2	**1 q**	Me	(*R*)-**2 h**	20
3	**1 g**	Et	(*R*)-**2 g**	45
4	**1 p**	Me	(*R*)-**2 g**	63

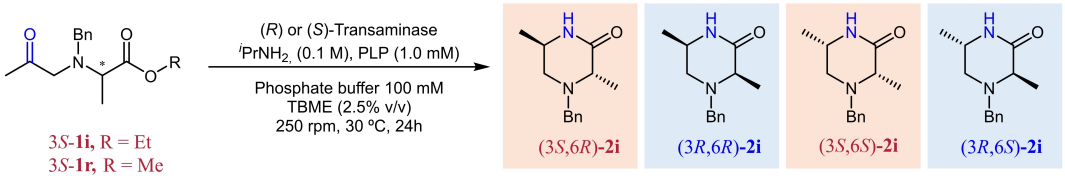
[a] General reaction conditions: Substrate (15 mM). *ArR*TA (10 mg), TBME (2.5% v/v), ^{i}PrNH$_2$ (0.5 M), PLP (1.0 mM), phosphate buffer (100 mM), pH 10.0, assay final volume 500 µL; 250 rpm, 30 °C, 24 h.
[b] Absolute configurations were assigned according to the stereochemical preference of the transaminase.
[c] Analytical yield and *ee* values were determined by HPLC analysis on chiral stationary phase (SI).

following the same procedure than for the glycine derivatives (see SI).

The biotransaminations of optically active **1 i** and **1 r** were performed in the same reaction conditions optimized for their analogue **1 a**. Nevertheless, as indicated in the experimental procedure, the pH must be carefully controlled during the work-up of the reactions in order to avoid the racemization of the prefixed chiral α-carbon.

Several of the tested transaminases provided a moderate or good conversion to the corresponding piperazin-2-one **2 i**. The HPLC analysis of the stereoisomeric composition of **2 i** revealed that the reductive amination catalysed by the transaminase took place with very high (*R*)- or (*S*)-selectivity depending on the ATA used (Table 4). Thus, using (*R*)-selective transaminase *ArRmut11*, substrates (*S*)-**1 i** and (*S*)-**1 r** were transformed into (3*S*,6*R*)-**2 i** (entries 1 and 7), and (*R*)-**1 r** was transformed into (3*R*,6*R*)-**2 i** (entry 9) in high yields (80–89%). Lyophilized cell-free *ArRmut11* was also tested against substrate (*S*)-**1 i** but the conversion was lower than with the crude variant (Table 4, entry 2). In addition, high conversions (85–88%) were also achieved in the biotransamination of the substrate (*R*)-**1 r** with the (*R*)-selective ATA-412 and ATA-024 (Table 4, entries 11 and 12). Among the (*S*)-selective transaminases, the best results were obtained with ATA-237. Thus, piperazinone (3*S*,6*S*)-**2 i** was prepared from (*S*)-**1 i** and (*S*)-**1 r**, and the stereoisomer (3*R*,6*S*)-

Table 4. Stereoselective transformations of substrates **1 i**, and **1 r** into piperazinones **2 i**.[a]



Entry	Substrate (*ee*, %)[b]	R	[mM]	ATA	Yield [%][b]	Stereoisomeric composition of **2 i**[b] (3*S*,6*R*)	(3*R*,6*R*)	(3*S*,6*S*)	(3*R*,6*S*)
1	(*S*)-**1 i** (99)	Et	25	*ArRmut11*	89	**99**	1	< 1	< 1
2	(*S*)-**1 i** (99)	Et	25	Cell-free *ArRmut11*	78	**99**	1	< 1	< 1
3	(*S*)-**1 i** (99)	Et	50	*ArRmut11*	65	**98**	1	1	< 1
4	(*S*)-**1 i** (99)	Et	50	*ArRmut11*	74[c]	**98**	1	< 1	< 1
5	(*S*)-**1 i** (99)	Et	25	ATA-237	92	< 1	< 1	> **99**	< 1
6	(*S*)-**1 i** (99)	Et	50	ATA-237	83	< 1	< 1	> **99**	< 1
7	(*S*)-**1 r** (97)	Me	25	*ArRmut11*	80	**97**	2	1	< 1
8	(*S*)-**1 r** (97)	Me	25	ATA-237	82	< 1	< 1	**98**	2
9	(*R*)-**1 r** (96)	Me	25	*ArRmut11*	82	3	**96**	1	< 1
10	(*R*)-**1 r** (96)	Me	25	ATA-237	82	< 1	< 1	3	**97**
11	(*R*)-**1 r** (96)	Me	25	ATA-412	88	3	**96**	1	< 1
12	(*R*)-**1 r** (96)	Me	25	ATA-024	85	3	**96**	1	< 1
13	(±)-**1 r**	Me	25	ATA–P2-A01	25	**78**	18	4	< 1
14	(±)-**1 r**	Me	25	ATA-260	40	< 1	< 1	**90**	10

[a] General reaction conditions. Substrate (25 or 50 mM). ATA: 10 mg (for *Cv*TA or *ArRmut11*) or 2.0 mg (for Codexis' enzymes or lyophilized cell-free *ArRmut11*). ^{i}PrNH$_2$ (0.1 M). Assay final volume 500 µL.
[b] Analytical yield and *ee* values were determined by HPLC analysis on chiral stationary phase (SI).
[c] The reaction was carried out at 45 °C.

2 i from (*R*)-**1 r** in high yields (Table 4, entries 5, 8, and 10, yields 82–92%).

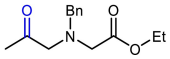
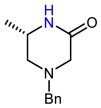
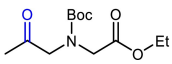
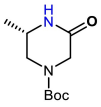
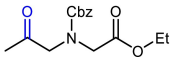
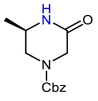
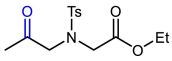
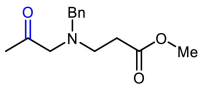
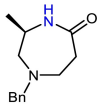
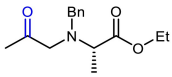
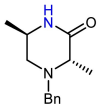
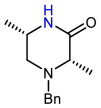
As shown in Table 4, these piperazinones were obtained in a steroisomeric ratio ≥96%. The presence of the other stereoisomers (≤3%) was mainly a consequence of the *ee* (99–96%) of the starting alanine derivative used as the substrate.

Selecting the biocatalysts that showed the best results, *ArRmut11* and ATA-237, the concentration of substrate (*S*)-**1 i** was increased until 50 mM (Table 4, entries 3 and 6). These processes showed perfect stereoselectivity but the conversion decreased in these conditions, especially in the case of *ArRmut11*, and only a 65% yield was measured. Nonetheless, this yield could be improved to 74% by increasing the temperature of the process to 45 °C (Table 4, entry 4).

As the enatiospecificity of transaminases has been scarcely reported,[13,14] we consider interesting to study this issue using the racemic substrate (±)-**1 r**. This substrate was screened against the transaminases panel in the same reaction conditions as their optically active analogues. The best results toward the two optical antipodes were shown in Table 4, entries 13 and 14. The (*S*)-selective ATA-260 catalysed the transamination of the carbonyl group with total selectivity, only the stereoisomer 6*S* was observed, and, at the same time, it showed a moderate enantiopreference towards the (*S*)-enantiomer of the ester (±)-**1 r**, giving piperazinone (3*S*,6*S*)-**2 i** in a 40% of conversion and a 90% of stereoisomeric ratio.

In order to illustrate the usefulness of the biotransformations, some representative processes have been carried out on a preparative scale. For this purpose, the best reaction conditions found for each substrate were selected, and the obtained results are summarized in Table 5. In almost all the cases the products were isolated in enantiopure form and their isolated yield after purification was in accordance with that obtained in the corresponding analytical assay.

Table 5. Stereoselective transformations at preparative scale.

Substrate		[mM]	ATA	Product		Yield [%][a]	*ee* [%][b]
1 a		25	*ArRmut11*	(*R*)-**2 a**		83	>99
			ATA-237	(*S*)-**2 a**		81	>99
1 b		50	*Cv*TA	(*S*)-**2 b**		82	>99
1 c		50	*ArRmut11*[c]	(*R*)-**2 c**		90	>99
1 e		50	*ArRmut11*[c]	(*R*)-**2 e**		73	>99
1 p		15	*Ar*RTA	(*R*)-**2 g**		51	>99
(*S*)-**1 i**[d]		50	*ArRmut11*	(3*S*,6*R*)-**2 i**		70	99
		50	ATA-237	(3*S*,6*S*)-**2 i**		78	>99

[a] Isolated yield after purification by flash column chromatography.
[b] Determined by HPLC analysis on chiral stationary phase.
[c] Lyophilized cell-free *ArRmut11*. The reaction was run at 45 °C.
[d] *ee* = 99%.

The absolute configuration of each product was initially established according to the known stereospecificity of the corresponding transaminase. Nevertheless, this aspect was confirmed in some cases by chemical correlation with compounds of known configuration (Scheme 4).

Thus, the (*R*)-configuration of optically pure piperazinone (−)-**2 a**, obtained from the process catalysed by the (*R*)-selective *ArRmutt11*, was confirmed after its reduction with $LiAlH_4$ to the corresponding piperazine (+)-**3 a**. Comparison of the optical rotation of (+)-**3 a** with that reported for the (*R*)-(+)-**3 a**[20] corroborated the (*R*)-configuration previously assigned. Additionally, (+)-**3 a** was treated with di-*tert*-butyl pyrocarbonate to obtain the orthogonally protected piperazine (−)-**4 a**, whose optical rotation sign was also in accordance with that reported for the (*R*)-enantiomer.[20b] On the other hand, the (*R*)-configuration of piperazinone (−)-**2 c**, prepared using the same (*R*)-selective *ArRmutt11* transaminase, was confirmed by the transformation of (−)-**2 c** into the known (*R*)-(−)-**2 a** (Scheme 4).

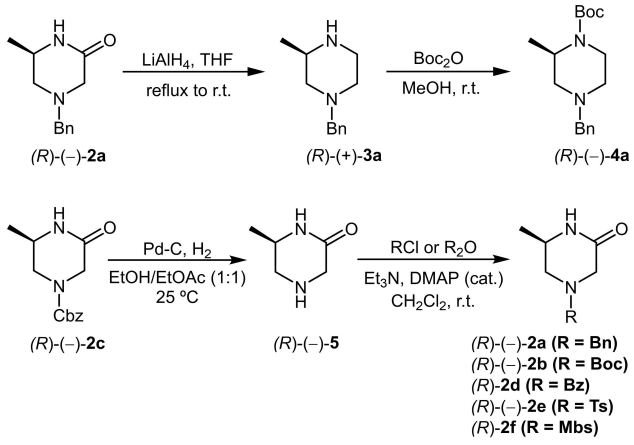
Removal of the Cbz group of (−)-**2 c** by hydrogenolysis yielded (−)-**5**, which was subjected to benzylation of the secondary amino group to yield (*R*)-(−)-**2 a**. Once established (*R*)-configuration for (−)-**5**, this compound was also converted by simple processes into the piperazinones (*R*)-**2 b**, **d**, **e** and **f**. Comparison of the HPLC chromatograms of these compounds with those of the corresponding products obtained by the transamination reactions allowed us to confirm the absolute configuration initially assigned.

Conclusion

A biocatalytic method based on the use of transaminases has been developed to obtain valuable enantiopure piperazinones in excellent yields and perfect enantioselectivity starting from readily accessible *N*-(2-oxopropyl) amino acid esters, and under mild reaction conditions. The two-step cascade initiated by the biocatalytic reductive amination of the ketone moiety, and followed by the spontaneous cyclization of the formed amine, gave access to both antipodes of the piperazinones by the selection of a (*R*)- or (*S*)-selective transaminase. The method was applied to glycine and alanine derivatives with different substitution patterns, and its synthetic utility has been demonstrated by performing several of the processes on a preparative scale. As an example, *N*-benzyloxycarbonyl substituted piperazinone (*R*)-**2 c** has been obtained in 90% isolated yield and *ee* > 99%.

The method was also applicable to the preparation of the enantiopure 1,4-diazepanone (*R*)-**2 g**, with moderate isolated yield (51%), starting from methyl *N*-(2-oxopropyl)-3-aminopropanoate.

The transformations performed in order to confirm the absolute configuration assigned to the optically pure piperazinones, including the preparation of the orthogonally protected piperazine (*R*)-(−)-**4 a**, evidenced the versatility of these key structural units for the synthesis of a number of bioactive compounds.



Scheme 4. Chemical transformations of optically pure piperazinones for the assignment of their absolute configuration.

Experimental Section

Lyophilized *E. coli* cells containing the overexpressed transaminases *Cv*TA, *Ars*TA, *Arr*TA and its evolved mutant *ArRmut11* obtained as previously described[10,15–17] were provided by Prof. Wolfgang Kroutil (University of Graz), while commercial ATAs were acquired from Codexis Inc. All other reagents were obtained from commercial sources (Sigma-Aldrich) and used as received except anhydrous solvents that were previously distilled under an argon atmosphere according to standard procedures.

Thin layer chromatography (TLC) was conducted with silica gel pre-coated plates and visualized with UV and potassium permanganate stain. Column chromatography was performed using silica gel (230–400 mesh).

^{1}H-NMR and ^{13}C-NMR spectra were obtained using a Bruker AV300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) spectrometer. Chemical shifts (δ) are reported in ppm and referenced to the residual solvent signal as an internal standard. Coupling constants (*J*) are reported in Hz to the nearest 0.1 Hz. High-resolution mass spectra (HRMS) were obtained in a Q-ToF Micro spectrometer using ESI^+. Measurement of the optical rotation values was carried out at 590 nm on a PerkinElmer 343 polarimeter.

High-performance liquid chromatography (HPLC) analyses were carried out in a Hewlett Packard 1100 chromatograph UV detector at different wavelengths, using the chiral columns Chiralcel OJ–H, Chiralcel OD, Chiralpak AD–H, Chiralpak IB, and Chiralpak IC (see SI for details).

General protocol for the analytical transformation of keto esters 1 a–r into optically active piperazinones or diazepanones. The corresponding keto ester **1 a–r** (15, 25 or 50 mM)

was dissolved in TBME (12.5 µL), acetonitrile (12.5 µL) or *n*-heptane (50 µL) inside a 1.5 mL Eppendorf tube. Then, phosphate buffer (100 mM, pH 8.5–10.0, final volume 500 µL) containing PLP (final concentration 1.0 mM) and $^{i}PrNH_2$ (final concentration 0.05–1.0 M) was added, followed by the addition of the corresponding transaminase (2.0 mg for Codexis' enzymes or lyophilized cell-free *ArRmut11*, and 10 mg for the lyophilized cells of *E. coli* containing overexpressed transaminases from: *Cv, ArS*, *ArR* and *ArRmut11*). The reaction was shaken at 250 rpm and 30 °C or 45 °C for 24 h. After this time, the reaction was quenched by adding aq. NaOH 10 M (100 µL) and extracted with ethyl acetate (2 × 500 µL), except in the case of **1 i** and **1 r**, that were extracted directly with ethyl acetate (2 × 500 µL) without the previous addition of the base. In the case of **1 e**, **1 f**, **1 n**, and **1 o**, poorly soluble in AcOEt, the extraction was done with dichloromethane. The organic layers were separated by centrifugation (90 s, 13000 rpm), combined, and dried over Na_2SO_4. The solvent was removed in a SpeedVac, and the resulting reaction crude was analyzed through HPLC to determine analytical yield and enantiomeric excess values.

General protocol for the preparative transformation of keto esters 1 a, b, c, e, p, and (*S*)-1 i into optically active products. The corresponding keto ester (15, 25 or 50 mM) was dissolved in the corresponding cosolvent (2.5% or 10%) inside a Falcon conical tube. Then, phosphate buffer (100 mM, pH 8.5 or 10.0) containing $^{i}PrNH_2$ (final concentration 0.1, 0.5 or 1.0 M) and PLP (final concentration 1.0 mM) was added, followed by the addition of the corresponding transaminase. The resulting mixture was shaken at 30 or 45 °C and 250 rpm for 24 h. The reaction was quenched by adding aq. NaOH 10 M until pH ≈10, extracted with ethyl acetate or dichloromethane (for **1 e**) and centrifuged (5 min, 4900 rpm), except in de case of alanine derivatives that were directly extracted with EtOAc at pH 8.5 in order to avoid possible racemization. This extraction and centrifugation protocol was performed three times and, finally, the organic layers were combined and dried over Na_2SO_4. The solvent was removed under reduced pressure to give the crude residue that was purified by flash column chromatography on silica gel (EtOAc, or EtOAc/MeOH mixtures as the eluent), yielding the corresponding enantiopure piperazinone or 1,4-diazepanone.

(*R*)-4-Benzyl-6-methylpiperazin-2-one, (*R*)-2 a. Substrate: **1 a** (125 mg, 0.50 mmol, final concentration 25 mM). Cosolvent: TBME (500 µL, 2.5% v/v). Phosphate buffer (100 mM, pH 8.5, 19.5 mL) containing $^{i}PrNH_2$ (0.1 M) and PLP (1.0 mM). Transaminase: *ArRmut11* (403 mg). *T*: 30 °C. Product (*R*)-**2 a**: white solid (83.2 mg, 83% yield). R_f 0.60 (EtOAc/MeOH 8:1). Mp: 94–95 °C. $[\alpha]_D^{20} = -4.4$ (*c* 1.0, $CHCl_3$, >99% *ee*). ^1H NMR (300.13 MHz, $CDCl_3$): δ 7.40–7.19 (m, 5H, Ph), 6.51 (br s, 1H, NH), 3.70–3.49 (m, 1H, H-6), 3.58 (d, 1H, $|^2J| = 13.1$ Hz, C*H*H–Ph), 3.53, (d, 1H, $|^2J| = 13.1$ Hz, CH*H*-Ph), 3.28 (dd, 1H, $^4J = 1.4$, $|^2J| = 16.5$ Hz, *H*H-3), 2.95 (d, 1H, $|^2J| = 16.5$ Hz, H*H*-3), 2.81 (dd, 1H, $^3J = 4.1$, $|^2J| = 11.6$ Hz, *H*H-5), 2.12 (dd, 1H, $^3J = 8.3$, $|^2J| = 11.6$ Hz, H*H*-5) 1.15 (d, 3H, $^3J = 6.4$ Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 170.0 (CO), 137.1 (C), 129.1 (2 CH), 128.5 (2 CH), 127.6 (CH), 61.7 (CH_2), 56.6 (CH_2), 55.9 (CH_2), 47.6 (CH), 20.1 (CH_3). HRMS (ESI^+, *m/z*): calcd for $(C_{12}H_{17}N_2O)^+$ $(M+H)^+$205.1335; found 205.1338.

(*S*)-4-Benzyl-6-methylpiperazin-2-one, (*S*)-2 a. Substrate: **1 a** (18.3 mg, 0.073 mmol, final concentration 25 mM). Cosolvent: *n*-heptane (300 µL, 10% v/v). Phosphate buffer (100 mM, pH 8.5, 2.7 mL) containing $^{i}PrNH_2$ (0.1 M) and PLP (1.0 mM). Transaminase: ATA-237 (12 mg). *T*: 30 °C. Product (*S*)-**2 a**: white solid (12.1 mg, 81% yield). $[\alpha]_D^{20} = +4.6$ (*c* 1.1, $CHCl_3$, >99% *ee*).

(*S*)-4-*tert*-Butoxycarbonyl-6-methylpiperazin-2-one, (*S*)-2 b. Substrate: **1 b** (61.0 mg, 0.24 mmol, final concentration 50 mM). Cosolvent: *n*-heptane (460 µL, 10% v/v). Phosphate buffer (100 mM, pH 8.5, 4.2 mL) containing $^{i}PrNH_2$ (1.0 M) and PLP (1.0 mM). Transaminase: *Cv*TA (95 mg). *T*: 30 °C. Product (*S*)-**2 b**: white solid (41.1 mg, 82% yield). R_f 0.41 (EtOAc). Mp: 109–110 °C. $[\alpha]_D^{20} = +20.7$ (*c* 0.5, $CHCl_3$, >99% *ee*). ^1H NMR (300.13 MHz, $CDCl_3$): δ 8.02 (br s, 1H, NH), 4.04 (d, 1H, $|^2J| = 18.4$ Hz, *H*H-3), 3.88–3.75 (m, 2H, *H*H-5 and H*H*-3), 3.55–3.40 (m, 1H, H-6), 2.98–2.74 (m, 1H, H*H*-5), 1.33 (s, 9H, ^{t}Bu), 1.08 (d, 3H, $^3J = 6.5$ Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 168.6 (CO), 153.8 (CO), 80.5 (C), 47.1 (CH), 46.2 (CH_2), 45.8 (CH_2), 28.2 (3 CH_3), 18.9 (CH_3). HRMS (ESI^+, *m/z*): calcd for $(C_{10}H_{18}N_2NaO_3)^+$ $(M+Na)^+$ 237.1210; found 237.1213.

(*R*)-4-Benzyloxycarbonyl-6-methylpiperazin-2-one, (*R*)-2 c. Substrate: **1 c** (142 mg, 0.48 mmol, final concentration 50 mM). Cosolvent: TBME (240 µL, 10% v/v). Phosphate buffer (100 mM, pH 8.5, 9.4 mL) containing $^{i}PrNH_2$ (1.0 M) and PLP (1.0 mM). Transaminase: lyophilized cell-free *ArRmut11* (40 mg). *T*: 45 °C. Product (*R*)-**2 c**: white solid (108 mg, 90% yield). R_f 0.50 (EtOAc). Mp: 114–116 °C. $[\alpha]_D^{20} = -17.8$ (*c* 1.0, $CHCl_3$, >99% *ee*). ^1H NMR (300.13 MHz, $CDCl_3$): δ 7.85–7.67 (br s, 1H, NH), 7.39–7.22 (m, 5H, Ph), 5.14 (AB system, 2H, $|^2J| = 12.5$ Hz, CH_2–Ph), 4.26 (d, 1H, $|^2J| = 18.4$ Hz, *H*H-3), 4.04–3.97 (m, 2H, H*H*-3 and *H*H-5), 3.68–3.51 (m, 1H, H-6), 3.11–2.87 (m, 1H, H*H*-5), 1.18 (d, 3H, $^3J = 6.4$ Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 168.0 (CO), 154.7 (CO), 136.1 (C), 128.6 (2 CH), 128.3 (2 CH), 128.1 (CH), 67.7 (CH_2), 47.2 (CH), 46.8 (CH_2), 46.5 (CH_2), 19.1 (CH_3). HRMS (ESI^+, *m/z*): calcd for $(C_{13}H_{17}N_2O_3)^+$ $(M+H)^+$249.1234; found 249.1234.

(*R*)-4-[(4-Methylbenzene)sulfonyl]-6-methylpiperazin-2-one, (*R*)-2 e. Substrate: **1 e** (70 mg, 0.22 mmol, final concentration 50 mM). Cosolvent: MeCN (111 µL, 2.5% v/v). Phosphate buffer (100 mM, pH 8.5, 4.4 mL) containing $^{i}PrNH_2$ (1.0 M) and PLP (1.0 mM). Transaminase: lyophilized cell-free *ArRmut11* (18 mg). *T*: 45 °C. Product (*R*)-**2 e**: white solid (43.5 mg, 90% yield). R_f 0.27 (EtOAc). Mp: 190–194 °C. $[\alpha]_D^{20} = -3.5$ (*c* 0.8, $CHCl_3$, >99% *ee*). ^1H NMR (300.13 MHz, $CDCl_3$): δ 7.66 (d, 2H, $^3J = 8.2$ Hz, Ar), 7.36 (d, 2H, $^3J = 8.2$ Hz, Ar), 6.71 (br s, 1H, NH), 3.88 (dd, 1H, $^4J = 1.3$, $|^2J| = 16.7$, *H*H-3), 3.76–3.65 (m, 1H, H-6), 3.58 (dd, 1H, $^3J = 3.9$, $|^2J| = 12.0$ Hz, *H*H-5), 3.39 (d, 1H, $|^2J| = 16.7$ Hz, H*H*-3), 2.55 (dd, 1H, $^3J = 8.2$, $|^2J| = 12.0$ Hz, H*H*-5), 2.44 (s, 3H, CH_3), 1.20 (d, $^3J = 6.4$ Hz, 3H, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 166.4 (CO), 144.6 (C), 132.2 (C), 130.2 (2 CH), 127.9 (2 CH), 49.0 (CH_2), 48.2 (CH_2), 47.6 (CH), 21.7 (CH_3), 19.6 (CH_3). HRMS (ESI^+, *m/z*): calcd for $(C_{12}H_{17}N_2O_3S)^+$ $(M+H)^+$269.0954; found 269.0953.

(*R*)-1-Benzyl-3-methyl-1,4-diazepan-5-one, (*R*)-2 g. Substrate: **1 p** (57.0 mg, 0.23 mmol, final concentration 15 mM). Cosolvent: TBME (380 µL, 2.5% v/v). Phosphate buffer (100 mM, pH 10.0, 14.9 mL) containing $^{i}PrNH_2$ (0.5 M) and PLP

(1.0 mM). Transaminase: *Ar*RTA (305 mg). *T*: 30 °C. Product (*R*)-**2 g**: white solid (25.6 mg, 51% yield). R_f 0.33 (EtOAc). Mp: 104–106 °C. $[\alpha]_D^{20} = +6.9$ (*c* 0.8, $CHCl_3$, >99% *ee*). ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 7.48–7.05 (m, 5H, Ph), 5.88 (br s, 1H, NH), 3.79–3.62 (m, 1H, H-3), 3.59 (AB system, 2H, $|^2J| =$ 13.5 Hz, CH_2–Ph), 2.96–2.71 (m, 3H, *H*H-2, *H*H-6, *H*H-7), 2.53–2.30 (m, 2H, H*H*-6, H*H*-7), 2.19 (dd, 1H, $^3J = 8.8$, $|^2J| =$ 12.6 Hz, H*H*-2), 1.15 (d, 3H, $^3J = 6.9$ Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 176.9 (CO), 138.2 (C), 130.0 (2 CH), 128.5 (2 CH), 127.4 (CH), 64.0 (CH_2), 63.2 (CH_2), 50.4 (CH_2), 48.8 (CH), 38.2 (CH_2), 20.1 (CH_3). HRMS (ESI^+, *m/z*): calcd for $(C_{13}H_{18}N_2NaO)^+$ $(M+Na)^+$241.1311; found 241.1311.

(3*S*,6*R*)-4-Benzyl-3,6-dimethylpiperazin-2-one, (3*S*,6*R*)-2 i. Substrate: (*S*)-**1 i** (56 mg, 0.21.0 mMol, final concentration 50 mM). Cosolvent: TBME (106 µL, 2.5% v/v). Phosphate buffer (100 mM, pH 8.5, 4.0 mL) containing ^{i}PrNH$_2$ (0.1 M) and PLP (1.0 mM). Transaminase: *ArRmut11* (85 mg). *T*: 30 °C. Product (3*S*,6*R*)-**2 i**: white solid (32.4 mg, 83% yield). R_f 0.44 (EtOAc). Mp: 91–93 °C. $[\alpha]_D^{20} = +49.4$ (*c* 1.0, $CHCl_3$, 99% *ee*). ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 7.42–7.24 (m, 5H, Ph); 5.96 (br s, 1H, NH), 4.01 (d, 1H, $|^2J| = 13.6$ Hz, C*H*H–Ph), 3.66–3.51 (m, 1H, H-6), 3.26 (d, 1H, $|^2J| = 13.6$ Hz, CH*H*-Ph), 3.09 (q, 1H, $^3J = 6.7$ Hz, H-3), 2.85 (dd, 1H, $^3J = 3.8$, $|^2J| =$ 11.8 Hz, *H*H-5), 2.01 (dd, 1H, $^3J = 9.2$, $|^2J| = 11.8$ Hz, H*H*-5), 1.51 (d, 3H, $^3J = 6.7$ Hz, CH_3), 1.07 (d, 3H, $^3J = 6.4$ Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 172.9 (CO), 138.1 (C), 129.0 (2 CH), 128.5 (2 CH), 127.4 (CH), 60.2 (CH), 58.4 (CH_2), 54.6 (CH_2), 47.3 (CH), 20.0 (CH_3), 16.1 (CH_3).

(3*S*,6*S*)-4-benzyl-3,6-dimethylpiperazin-2-one, (3*S*,6*S*)-2 i. Substrate (*S*)-**1 i** (61 mg, 0.23 mmol, final concentration 50 mM). Cosolvent: TBME (115 µL, 2.5% v/v). Phosphate buffer (100 mM, pH 8.5, 4.5 mL) containing ^{i}PrNH$_2$ (0.1 M) and PLP (1.0 mM). Transaminase: ATA-237 (19 mg). *T*: 30 °C. Product (3*S*,6*S*)-**2 i**: white solid (32.4 mg, 83% yield). R_f 0.43 (EtOAc). Mp: 99–101 °C. $[\alpha]_D^{20} = +10.4$ (*c* 1.0, $CHCl_3$, >99% *ee*). ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 7.41–7.22 (m, 5H, Ph), 6.89 (br s, 1H, NH), 3.80 (d, 1H, $|^2J| = 13.5$ Hz, C*H*H–Ph), 3.67–3.55 (m, 1H, H-6), 3.55 (d, 1H, $|^2J| = 13.5$ Hz, CH*H*-Ph), 3.31 (q, 1H, $^3J = 7.0$ Hz, H-3), 2.58 (d, 2H, $|^3J| = 5.9$ Hz, CH_2-5), 1.39 (d, 3H, $^3J = 7.0$ Hz, CH_3), 1.13 (d, 3H, $^3J = 6.4$ Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 174.0 (CO), 138.4 (C), 128.7 (2 CH), 128.5 (2 CH), 127.3 (CH), 58.8 (CH), 57.9 (CH_2), 50.2 (CH_2), 46.6 (CH), 20.4 (CH_3), 14.3 (CH_3).

Acknowledgements

Financial support of this work by the Regional Government of Asturias (FICYT) SV-PA-21-AYUD/2021/51542 is gratefully acknowledged. C. P.-M. also thanks University of Oviedo for a predoctoral fellowship. We also thank Prof. Wolfgang Kroutil (University of Graz, Austria) for the donation of overexpressed amine transaminases and Prof. Ivan Lavandera (University of Oviedo, Spain) for the assistance in the purification of ArRmut11.

References

[1] Z. Ye, S. Adhikari, Y. Xia, M. Dai, *Nat. Commun.* **2018**, *9*, 1–11.

[2] a) C. De Risi, M. Pelà, G. P. Pollini, C. Trapella, V. Zanirato, *Tetrahedron: Asymmetry* **2010**, *21*, 255–274; b) L. R. Whitby, A. M. Lee, S. Kunz, M. L. B. A. Oldstone, D. L. Boger, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3771–3774; c) A. K. Ghosh, M. Brindisi, Y.-C. Yen, E. L. Cárdenas, J.-R. Ella-Menye, N. Kumaragurubaran, X. Huang, J. Tang, A. D. Mesecar, *Bioorg. Med. Chem. Lett.* **2017**, *27*, 2432–2438.

[3] a) C. J. Dinsmore, D. C. Beshore, *Org. Prep. Proced. Int.* **2002**, *34*, 367–404; b) C. Sandoval, N.-K. Lim, H. Zhang, *Org. Lett.* **2018**, *20*, 1252–1255; c) T. Maeba, K. Suzawa, M. Kotoku, R. Masuo, D. Motoda, N. Yamaoka, US 2020/0017505 Al, **2020**.

[4] a) M. Shaquiquzzaman, G. Verma, A. Marella, M. Akhter, W. Akhtar, M. Faraz Khan, S. Tasneem, M. M. Alam, *Eur. J. Med. Chem.* **2015**, *102*, 487–529; b) Z. Ye, K. E. Gettys, M. Dai, *Beilstein J. Org. Chem.* **2016**, *12*, 702–715; c) K. E. Gettys, Z. Ye, M. Dai, *Synthesis* **2017**, *49*, 2589–2604.

[5] a) Y. Feng, P. V. LoGrasso, O. Defert, R. Li, *J. Med. Chem.* **2016**, *59*, 2269–2300; b) C. D. Cox, M. J. Breslin, D. B. Whitman, J. D. Schreier, G. B. McGaughey, M. J. Bogusky, A. J. Roecker, S. P. Mercer, R. A. Bednar, W. Lemaire, J. G. Bruno, D. R. Reiss, C. M. Harrell, K. L. Murphy, S. L. Garson, S. M. Doran, T. Prueksaritanont, W. B. Anderson, C. Tang, S. Roller, T. D. Cabalu, D. Cui, G. D. Hartman, S. D. Young, K. S. Koblan, C. J. Winrow, J. J. Renger, P. J. Coleman, *J. Med. Chem.* **2010**, *53*, 5320–5332; c) K. P. Garnock-Jones, *Drugs* **2014**, *74*, 2211–2215; d) I. K. Mangion, B. D. Sherry, J. Yin, F. J. Fleitz, *Org. Lett.* **2012**, *14*, 3458–3461.

[6] a) M. Yu, M. Lizarzaburu, H. Beckmann, R. Connors, K. Dai, K. Haller, C. Li, L. Liang, M. Lindstrom, J. Maa, A. Motani, M. Wanska, A. Zhang, L. Li, J. C. Medina, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1758–1762; b) S. Hu, Z. Wang, T. Hou, X. Maa, J. Li, T. Liu, X. Xie, Y. Hua, *Bioorg. Med. Chem.* **2015**, *23*, 1157–1168; c) J. H. Ryu, J. A. Lee, S. Kim, Y. A. Shin, J. Yang, H. Y. Han, H. J. Son, Y. H. Kim, J. H. Sa, J.-S. Kim, J. Lee, J. Lee, H.-G. Park, *J. Med. Chem.* **2016**, *59*, 10176–10189.

[7] a) J. P. Adams, M. J. B. Brown, A. Díaz-Rodríguez, R. C. Lloyd, G.-D. Roibanb, *Adv. Synth. Catal.* **2019**, *361*, 2421–2432; b) J. I. Ramsden, S. C. Cosgrove, N. J. Turner, *Chem. Sci.* **2020**, *11*, 11104–11112; c) S. Wu, R. Snajdrova, J. C. Moore, K. Baldenius, U. T. Bornscheuer, *Angew. Chem. Int. Ed.* **2021**, *60*, 88–119; *Angew. Chem.* **2021**, *133*, 89–123; d) S. Slagman, W. D. Fessner, *Chem. Soc. Rev.* **2021**, *50*, 1968–2009; e) M. Hall, *RSC Chem. Biol.* **2021**, *2*, 958–989.

[8] N. Borlinghaus, S. Gergel, B. M. Nestl, *ACS Catal.* **2018**, *8*, 3727–3732.

[9] Z. Xu, P. Yao, X. Sheng, J. Li, J. Li, S. Yu, J. Feng, Q. Wu, D. Zhu, *ACS Catal.* **2020**, *10*, 8780–8787.

[10] C. K. Savile, J. M. Janey, E. M. Mundorff, J. C. Moore, S. Tam, W. R. Jarvis, J. C. Colbeck, A. Krebber, F. J. Fleitz, J. Brands, P. N. Devine, G. W. Huisman, G. J. Georges, *Science* **2010**, *329*, 305–309.
[11] F. Guo, P. Berglund, *Green Chem.* **2017**, *19*, 333–360 and references therein.
[12] a) M. D. Truppo, J. D. Rozzell, N. J. Turner, *Org. Process Res. Dev.* **2010**, *14*, 234–237; b) Á. Mourelle-Insua, L. A. Zampieri, I. Lavandera, V. Gotor-Fernández, *Adv. Synth. Catal.* **2018**, *360*, 686–695.
[13] D. Koszelewski, D. Clay, K. Faber, W. Kroutil, *J. Mol. Catal. B* **2009**, *60*, 191–194.
[14] C. K. Chung, P. G. Bulger, B. Kosjek, K. M. Belyk, N. Rivera, M. E. Scott, G. R. Humphrey, J. Limanto, D. C. Bachert, K. M. Emerson, *Org. Process Res. Dev.* **2014**, *18*, 215–227.
[15] U. Kaulman, K. Smithies, M. E. B. Smith, H. C. Hailes, J. M. Ward, *Enzyme Microb. Technol.* **2007**, *41*, 628–637.
[16] S. Pannuri, S. V. Kamat, A. R. M. Garcia, PCT Int. Appl. WO 2006063336 A2 20060615, **2006**.
[17] A. Iwasaki, Y. Yamada, N. Kizaki, Y. Ikenaka, J. Hasegawa, *Appl. Microbiol. Biotechnol.* **2006**, *69*, 499–505.
[18] A. Fiorati, P. Berglund, M. S. Humble, D. Tessaro, *Adv. Synth. Catal.* **2020**, *362*, 1156–1166 and references therein.
[19] M. López-Iglesias, D. González-Martínez, V. Gotor, E. Busto, W. Kroutil, V. Gotor-Fernández, *ACS Catal.* **2016**, *6*, 4003–4009.
[20] a) P. Maity, B. König, *Org. Lett.* **2008**, *10*, 1473–1476; b) J. W. Mickelson, K. L. Belongs, E. J. Jacobsen, *J. Org. Chem.* **1995**, *60*, 4177–4183.