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Efficient synthesis of α-alkyl-β-amino amides by transaminase-mediated dynamic kinetic resolutions

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The biocatalytic stereocontrolled synthesis of various acyclic *anti-* or *syn-*α-alkyl-β-amino amides through a dynamic kinetic resolution strategy is demonstrated. A series of commercially available and in-house transaminases (TAs) was employed to perform the transamination of a series of chemically synthesized racemic α-alkyl-β-keto amides. Among them, commercial (*R*)-selective TAs showed the best activities and selectivities, usually giving access preferentially to the *anti-*diastereoisomers with low to high diastereomeric ratios (up to 96%) and excellent enantiomeric excess (>99%). The stereoselective biotransamination experiments were successfully demonstrated at semipreparative (25 mM, 100 mg substrate), leading the corresponding optically active α-alkyl-β-amino amides in 45-90% isolated yield after a simple liquid-liquid extraction protocol

Introduction

Chiral β-amino amides (also denoted as β³-amino amides) are valuable compounds due to their potential biological activities. Among them can be highlighted those that exhibit dipeptidyl peptidase IV (DPP-4) inhibitory activity such as sitagliptin[1] (**A**, Fig. 1), which are employed for type-II diabetes treatment. Since sitagliptin was discovered, other related β-amino amide analogs have been synthesized and biologically tested,[2] affording very promising results (*e.g.* compound **B**,[2e] Fig. 1). Moreover, if they are substituted at α-position (denoted as β²,³-amino amides), many interesting biological activities can be displayed, such as bestatin[3] (**C**, Fig. 1), an aminopeptidase inhibitor, or KNI-272 (**D**,[4] Fig. 1), an HIV protease inhibitor. Also, β-amino amides are synthetically useful precursors of valuable 1,3-diamines through reductive processes, and have been applied as ligands in metal-catalyzed reactions.[5]

Among the different synthetic approaches (Fig. 2) that have been described to get access to enantio- or diastereoenriched β³- or β²,³-amino amides, the Mannich transformation has been the most applied.[6] To induce chirality, enantiopure imines[6k] or amides[6a,b,i] have been utilized as precursors in the presence of a strong base at very low temperatures (<–55 °C). Also, the

employment of bases with sterically hindered ligands[6c,l] or metals such as samarium[6d] or cobalt[6e] gave straightforward access to β-amino amides with usually good diastereoselectivities. A very interesting methodology has been developed by Kumagai, Shibasaki and co-workers, where different β-amino amides bearing substitutions at α position such as trifluoromethyl,[6f] fluorine,[6g] methyl,[6h] chlorine,[6j] or benzyloxy[6m] were synthesized with high diastereomeric ratios (*dr*) and excellent enantiomeric excess (*ee*) starting from 7-azaindoline amides in the presence of a copper catalyst with a chiral ligand and using Barton's base. Other more specific synthetic methods involve the aza-Michael addition on α,β-unsaturated amides,[7] multicomponent transformations,[8] the hydrogenation[9a,b] or reduction[9c] of enamines, the reductive amination of β-keto amides,[10] or the reduction of oxime precursors.[11]

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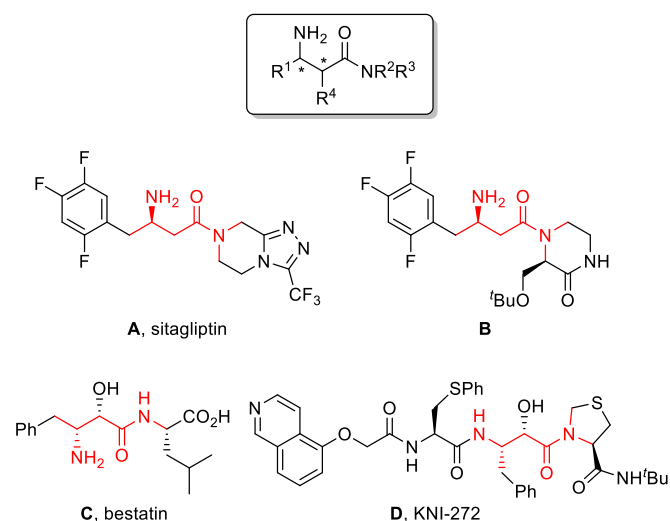
Fig. 1. General structure of β²,³-amino amides (top) and biologically active compounds **A**-**D** containing the β³- or β²,³-amino amide core (bottom).
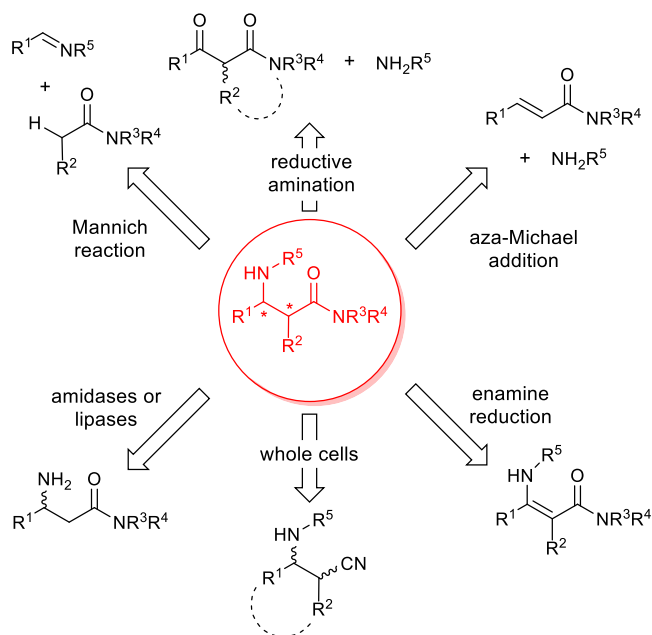
Fig. 2. Chemical and enzymatic approaches to obtain chiral (α-substituted) β-amino amides.

Remarkably, enzymatic methodologies (Fig. 2) have also led to β-amino amides with excellent selectivities under mild reaction conditions.[12] For instance, racemic β-amino nitriles have been selectively hydrolyzed *via* kinetic resolution (KR) using whole-cells displaying nitrile hydratase activity.[13] *Rhodococcus* species were found as the best candidates, and through this method the corresponding β-amino amides could be synthesized together with the corresponding β-amino acids due to the concomitant amidase activity found in the enzymatic preparation. Other enzymatic approaches to get access to these compounds have been the lipase-catalyzed aminolysis of racemic β-lactams[14] or the acylation of β-amino amides,[15] and the hydrolysis of racemic β-amino amides using aminopeptidases[16] or amidases.[17] All these protocols presented the limitation of a 50% conversion towards the final products, which is inherent to KRs. In order to overcome this drawback, desymmetrization of prochiral compounds[18] or dynamic kinetic resolutions (DKRs)[19] of racemic derivatives can be employed as efficient synthetic tools.

Transaminases (TAs, also known as aminotransferases),[20] belong to a group of enzymes that have been intensively applied in the last decade towards the stereoselective synthesis of amines. They catalyze the reversible transfer of an amino group between an amine donor (typically an α-amino acid) and an amine acceptor (keto acids, ketones or aldehydes), using pyridoxal 5'-phosphate (PLP) as a cofactor. Amongst the different transformations that TAs can mediate, the asymmetrization of carbonyl compounds can be emphasized. An outstanding example of the relevance of these biocatalysts at industrial scale is the development of a variant from *Arthrobacter* sp. TA to obtain sitagliptin from the ketone precursor at high substrate concentration.[21] Since Kroutil and co-workers showed the first example,[22a] TAs have been successfully applied in DKR processes.[22b-i] Researchers have usually taken advantage of the high acidity of the α-proton in

the carbonylic derivatives, so the low reacting enantiomer can be easily racemized even at neutral pH. In one of our groups a DKR protocol was designed for the synthesis of a wide panel of diastereo- and enantioenriched α-alkyl-β-amino esters starting from the corresponding racemic β-keto esters employing TAs.[23] In general, high conversion and *ee* values were found, however, moderate *dr* values (<60%) were observed. Following this study and due to the lack of biocatalytic methodologies to synthesize β[2,3]-amino amides, we decided to develop a TA-mediated protocol to produce different diastereo- and enantioenriched α-alkyl-β-amino amides through DKR transformations. Several reaction parameters including the source and amount of TA, pH and temperature have been studied in order to disclose an efficient and general asymmetric protocol.

Results and discussion

Initially, a wide panel of α-substituted β-keto amides was synthesized bearing different substitution patterns at the γ- (R^1) and α-position (R^4), as well as in the amide protecting group (R^2 and R^3).[24] As starting materials, commercially available β-keto esters **1a** and **1b** were used and a chemical[25] or an enzymatic[26] methodology was applied in order to obtain the corresponding β-keto amides **2a-g** (Scheme 1). The cyclic substrate **2h** was straightforwardly synthesized in moderate yield (48%) from the corresponding commercially available racemic methyl 2-oxocyclopentanecarboxylate (**1c**) using a CAL-B mediated aminolysis with benzylamine as nucleophile.

Once the substrates were obtained, it was necessary to synthesise the racemic amines **3a-h** that would serve as standards to develop GC and HPLC analysis methods (see ESI). With that purpose, the reductive aminations of the α-substituted β-keto amides **2a-h** were attempted using 2 equiv of NaBH₃CN as reduction agent and 10 equiv of ammonium acetate as nitrogen source in dry methanol (Scheme 1). Mixtures of *syn-* and *anti*-diastereoisomers of the α-substituted β-amino amides **3a-h** were obtained with low to high yields (36-70%).
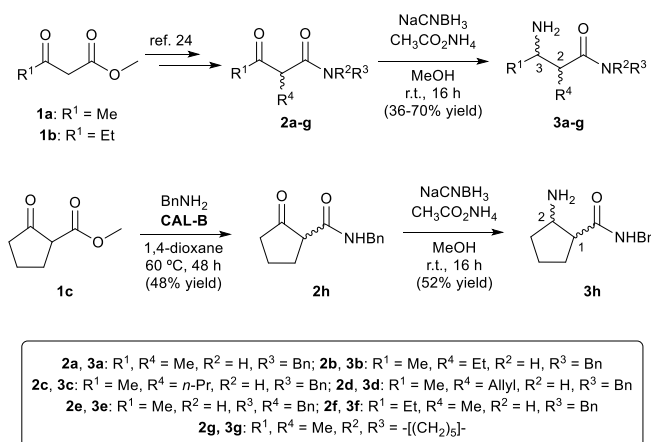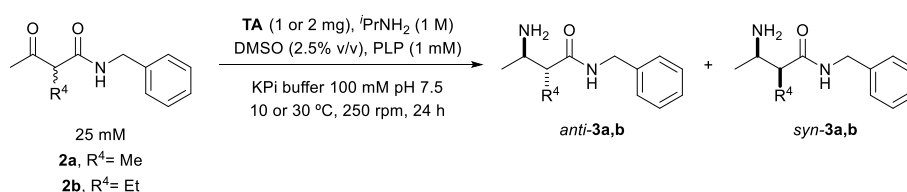

Scheme 1 Synthesis of racemic α-substituted β-keto amides and β-amino amides.

N-Benzyl-2-methyl-3-oxobutanamide (**2a**, 25 mM) was chosen as model substrate for our study and 28 commercial transaminases (2 mg) from Codexis Inc. were tested. Based on previous studies using transaminases,[27] 25 mM concentration of **2a** was used, selecting DMSO as cosolvent (2.5% v/v) for solubilizing the substrate, and phosphate buffer 100 mM pH 7.5 (final volume: 500 μL) containing PLP (1 mM) as reaction media. A large excess (1 M) of isopropylamine (^{i}PrNH$_2$) was added as amine donor in order to shift the equilibrium towards the amine formation. All biotransformations were incubated at 30 °C and 250 rpm for 24 hours finding good conversions, excellent enantioselectivities and low to moderate diastereoselectivities with some (*S*)- and (*R*)-transaminases (Table 1 and Section 4.1 in the ESI). As can be seen, in most cases the *anti* isomer of **3a** was obtained (an exception is shown in entry 1 with ATA-260). These usually poor diastereomeric ratios could be due to a slow racemization rate. In order to develop a more efficient DKR procedure, the possibility of slowing down the enzymatic transamination process was attempted by decreasing the temperature or adding less amount of enzyme with the (*S*)-selective ATA-251 (entries 4 and 5) and the (*R*)-selective ATA-013 (entries 7 and 8). On the one hand, a lower temperature (10 °C) led to diminished conversion values and similar

diastereomeric ratios with ATA-251 (entry 4) and a dramatic loss of activity with ATA-013 (entry 7). On the other hand, when a lower loading of the TA was used (1 mg), slightly lower conversions and similar diastereoselectivities were attained with both enzymes (entries 5 and 8).

Due to the fact that the modification of the enzyme quantity and temperature did not lead to any improvement, the application of typical TA reaction conditions to afford the DKR on other substrates was next envisaged. This way, the influence of the alkyl group at the α-position could be studied. For this purpose, *N*-benzyl-2-ethyl-3-oxobutanamide (**2b**) was employed as starting material for the transamination using all the transaminases working on the model substrate (Table 1 and Section 4.2 in the ESI). After analyzing the reaction mixtures, we found out that (*S*)-TAs were not selective with this substrate except in the case of ATA-234, which afforded *anti*-(2*S*,3*S*)-**3b** with very high *dr* and *ee* values (entry 9). Nevertheless, (*R*)-transaminases still provided good results in terms of conversion and enantioselectivity values (entry 10). Furthermore, higher diastereomeric ratios (up to 80:20 *anti:syn*) were obtained in comparison with those achieved with the model substrate **2a**.

Table 1 Transamination of substrates **2a** and **2b** under dynamic conditions using commercial TAs^a

25 mM
2a, R^4= Me
2b, R^4= Et

TA (1 or 2 mg), ^{i}PrNH$_2$ (1 M)
DMSO (2.5% v/v), PLP (1 mM)

KPi buffer 100 mM pH 7.5
10 or 30 ºC, 250 rpm, 24 h

anti-**3a,b**   +   *syn*-**3a,b**

Entry	Substrate	Transaminase	T (°C)	c (%)^b	Ratio *anti:syn*^{c}	ee anti (%)^c	ee syn (%)^c
1	**2a**	ATA-260 (*S*)	30	96	31:69	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
2	**2a**	ATA-254 (*S*)	30	89	85:15	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
3	**2a**	ATA-251 (*S*)	30	98	65:35	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
4	**2a**	ATA-251 (*S*)	10	65	63:37	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
5	**2a**	ATA-251 (*S*)^d	30	94	65:35	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
6	**2a**	ATA-013 (*R*)	30	98	62:38	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
7	**2a**	ATA-013 (*R*)	10	11	n.d.	n.d.	n.d
8	**2a**	ATA-013 (*R*)^d	30	74	70:30	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
9	**2b**	ATA-234 (*S*)	30	64	97:3	95 (2*S*,3*S*)	n.d.
10	**2b**	ATA-013 (*R*)	30	96	80:20	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)

^{a} Reaction conditions: Substrates **2a** or **2b** (25 mM), transaminase (2 mg), isopropylamine (1 M), PLP (1 mM), DMSO (2.5% v/v, 12.5 μL), phosphate buffer 100 mM pH 7.5 (final volume: 500 μL), 10 or 30 °C, 24 h and 250 rpm. ^{b} Conversion values were determined by GC analysis. ^{c} Selectivities (*dr* and *ee*) were determined by HPLC analysis using chiral columns. The major diastereoisomer is shown in parentheses. ^{d} Only 1 mg of transaminase was used. n.d. not determined.
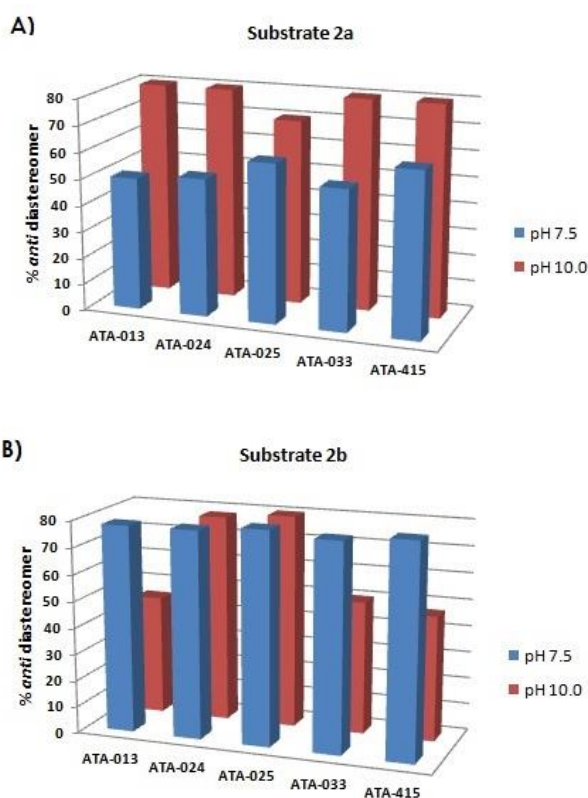
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Fig. 3. Influence of the pH in the DKR of α-alkylated β-keto amides **2a** and **2b** mediated by (*R*)-transaminases. Blue color is used to indicate the percentage of *anti*-(2*R*,3*R*)-**3a,b** (>95% conversion and >99% *ee* in all cases) at pH 7.5, while red bars denotes the percentage of *anti*-(2*R*,3*R*)-**3a,b** (>97% conversion and >99% *ee* in all cases) at pH 10.0. A) Biotransaminations with **2a**. B) Biotransaminations with **2b**.

Later on, the influence of the pH was analyzed in order to speed up the racemization process by increasing the pH up to 10 (Sections 6.1 and 6.2 in the ESI). After 24 hours, no improvement was found with the (*S*)-TAs for substrate **2a** but significantly better results were achieved in this DKR process using the (*R*)-TAs (Fig. 3A). Thus, at pH 10, these transaminases were capable of producing *anti*-(2*R*,3*R*)-**3a** with full conversions, excellent *ee* values and improved *dr* going from ratios close to 50:50 *anti*:*syn* up to 80:20 *anti*:*syn*. Encouraged by these results, the biotransaminations of **2b** into **3b** were set up at pH 10 (Fig. 3B). However, even though the DKR led to full conversions and excellent *ee* values, no better diastereomeric ratios towards the formation of *anti*-(2*R*,3*R*)-**3b** were found, as they remained untouched in some cases (ATA-024 and ATA-025) and were much lower in other cases (ATA-013, ATA-033 and ATA-415). Therefore, it can be concluded that pH can largely affect the selectivity of these processes, but that it must be checked carefully for each substrate as a clear trend was not observed.

Afterwards, additional substrates **2c-e** bearing different substitution patterns at the α-position to the amide (R⁴= *n*-Pr, allyl, and benzyl, respectively) were tested at pH 7.5 and pH 10 (Table 2 and Sections 4.3-4.5 and 6.3-6.5 in the ESI). The best results were provided by (*R*)-transaminases ATA-013, ATA-024 and ATA-033. Substrates **2c** and **2d**, bearing a *n*-propyl or an allyl moiety at the α-position, respectively, led to similar results than those obtained in the biotransamination of **2b** (R⁴= Et). At pH 7.5 good conversions, moderate diastereomeric ratios towards the formation of the *anti*-amines (**3c** and **3d**) and excellent *ee* values for both diastereoisomers were found (entries 1 and 3). Furthermore, when the pH was basified up to pH 10, the biotransformations led to the formation of the amines with full conversions and excellent enantioselectivities and similar *dr* (entries 2 and 4). Next, the reactivity of **2e** bearing the bulkiest substituent (a benzyl group) was studied, finding an inversion in the diastereoselectivity as the *syn*-diastereoisomer was the major one. At pH 7.5, high conversions, moderate diastereomeric ratios and excellent *ee* values were observed (*e.g.*, ATA-033, entry 5). At pH 10, a significant improvement in the diastereoselectivity was observed for this biocatalyst, as can be seen in entry 6.

Then, *N*-benzyl-2-methyl-3-oxopentanamide (**2f**) bearing an ethyl group in R¹ position was used as starting material for the biotransamination process (Table 2 and Sections 4.6 and 6.6 in the ESI). In this case, only one (*S*)-TA displayed significant activity with this substrate (ATA-234, entry 7) and, even though, it revealed very good diastereoselectivity and excellent *ee* values towards the synthesis of the *anti*-(2*S*,3*S*)-diastereoisomer, the conversion was very low (15%). Furthermore, several (*R*)-transaminases were capable of synthesizing the corresponding amine **3f**, being ATA-024 especially good providing high conversions, moderate diastereomeric ratios and excellent enantioselectivity (entries 8 and 9). The influence of the amide moiety was studied by introducing a piperidine group (Table 2 and Sections 4.7 and 6.7 in the ESI). The biotransamination of **2g** was revealed to be efficient with (*S*)-TAs as occurred with the model substrate **2a**, obtaining very promising results with ATA-256 (entry 10), which afforded selectively *syn*-(2*R*,3*S*)-**3g**. On the other hand, several (*R*)-TAs led to conversion values and diastereomeric ratios comparable to those achieved with substrates **2b-d**. Excellent enantioselectivities were observed in all cases. Furthermore, an increase in the pH up to 10 led to better *dr*, especially when ATA-025 was the TA of choice (entries 11 and 12).

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Table 2 Transaminase-mediated DKR of α-substituted β-keto amides **2c-h** at pH 7.5 and pH 10[a]
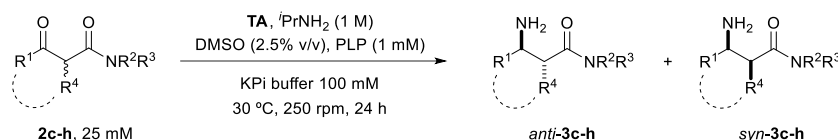
Entry	Substrate	TA	pH	c (%)[b]	Ratio *anti*:*syn*[c]	*ee anti* (%)[c]	*ee syn* (%)[c]
1	**2c**	ATA-024	7.5	97	89:11	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
2	**2c**	ATA-024	10	>99	88:12	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
3	**2d**	ATA-013	7.5	97	74:26	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
4	**2d**	ATA-013	10	>99	80:20	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
5	**2e**	ATA-033	7.5	99	28:72	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
6	**2e**	ATA-033	10	99	13:87	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
7	**2f**	ATA-234	7.5	15	99:1	>99 (2*S*,3*S*)	n.d.
8	**2f**	ATA-024	7.5	84	79:21	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
9	**2f**	ATA-024	10	97	72:28	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
10	**2g**	ATA-256	7.5	85	2:98	n.d.	>99 (2*R*,3*S*)
11	**2g**	ATA-025	7.5	95	64:36	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
12	**2g**	ATA-025	10	>99	91:9	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
13[d]	**2h**	ATA-415	7.5	82	90:10	>99 (1*R*,2*R*)	>99 (1*S*,2*R*)
14[d]	**2h**	ATA-415	10	97	92:8	>99 (1*R*,2*R*)	>99 (1*S*,2*R*)

[a] Reaction conditions: Substrates **2c-h** (25 mM), transaminase (2 mg), isopropylamine (1 M), PLP (1 mM), DMSO (2.5% v/v, 12.5 μL), phosphate buffer 100 mM pH 7.5 or 10 (final volume: 500 μL), 30 °C, 24 h, 250 rpm. [b] Conversion values were determined by GC analysis. [c] Selectivities (*dr* and *ee*) were determined by HPLC analysis using chiral columns. The major diastereoisomer is shown in parentheses. [d] Note that for this substrate the chiral centers are in positions 1 and 2, as shown in Scheme 1. n.d. not determined.

Finally, encouraged by previous studies where excellent diastereomeric ratios were attained by using a cyclic β-keto ester as starting material,[23] the biotransamination of cyclic β-keto amide **2h** was envisaged through this DKR strategy (Table 2 and Sections 4.8 and 6.8 in the ESI). In this case, only (*R*)-TAs seemed to be effective biocatalysts, leading ATA-415 to the best results (entries 13 and 14). Especially at pH 10, excellent conversions, *ee* values and high *dr* (92:8) towards the formation of (1*R*,2*R*)-**3h** was attained. It was interesting to observe that ATA-013 could catalyze at pH 10 the formation of a side-product corresponding to the enamine intermediate obtained after the

addition of one molecule of isopropylamine to the β-keto amide (65%).

All the amines were fully characterized by ^{1}H, ^{13}C and DEPT-NMR experiments, IR spectroscopy and high resolution mass spectrometry (HR-MS). Furthermore, homodecoupling NMR experiments were performed and the coupling constants between H_2 (H_1 for **3h**) and H_3 (H_2 for **3h**) calculated in order to determine which diastereoisomer was the major one (Section 8 in the ESI). In all cases, with the exception of the α-benzylated β-amino amide **3e**, the *anti*-diastereoisomer was found to be preferentially formed, which is consistent with the previous results obtained with the commercial enzymes when α-alkyl-β-

amino esters were synthesized.[23] The absolute configuration was also assigned based on the known stereopreference shown by these TAs with structurally similar α-alkyl-β-keto esters.[23]

Table 3 Synthesis of α-substituted β-amino amides **2a-h** through a DKR process catalyzed by (S)-selective transaminases *Bm*TA and *Bm*TA S119G[a]
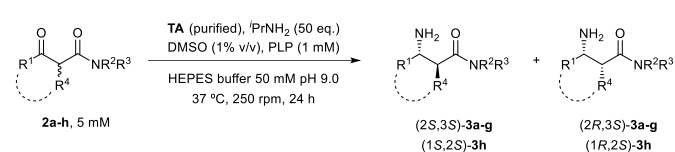

Entry	Substrate	TA	c (%)[b]	Ratio anti:syn[c]	ee anti (%)[c]	ee syn (%)[c]
1	**2a**	*Bm*	91	35:65	>99 (2S,3S)	>99 (2R,3S)
2	**2a**	*Bm* S119G	60	30:70	>99 (2S,3S)	>99 (2R,3S)
3	**2b**	*Bm*	70	60:40	>99 (2S,3S)	>99 (2R,3S)
4	**2b**	*Bm* S119G	57	45:55	>99 (2S,3S)	>99 (2R,3S)
5	**2c**	*Bm*	76	70:30	>99 (2S,3S)	>99 (2R,3S)
6	**2c**	*Bm* S119G	49	41:59	>99 (2S,3S)	>99 (2R,3S)
7	**2d**	*Bm*	78	60:40	>99 (2S,3S)	>99 (2R,3S)
8	**2d**	*Bm* S119G	58	42:58	>99 (2S,3S)	>99 (2R,3S)
9	**2e**	*Bm*	15	75:25	>99 (2S,3S)	>99 (2R,3S)
10	**2e**	*Bm* S119G	<1	n.d.	n.d.	n.d.
11	**2f**	*Bm*	<1	n.d.	n.d.	n.d.
12	**2f**	*Bm* S119G	<1	n.d.	n.d.	n.d.
13	**2g**	*Bm*	30	45:55	>99 (2S,3S)	>99 (2R,3S)
14	**2g**	*Bm* S119G	<1	n.d.	n.d.	n.d.
15[d]	**2h**	*Bm*	92	80:20	>99 (1S,2S)	>99 (1R,2S)
16[d]	**2h**	*Bm* S119G	79	30:70	>99 (1S,2S)	>99 (1R,2S)

[a] Reaction conditions: Substrates **2a-h** (5 mM), transaminase (2 mg/mL), isopropylamine (50 equiv), PLP (1 mM), DMSO (1% v/v, 5 μL), HEPES 50 mM pH 9.0 (500 μL final volume), 37 °C, 24 h, 250 rpm. [b] Conversion values were determined by GC analysis. [c] Selectivities (*dr* and *ee*) were determined by HPLC analysis using chiral columns. The major diastereoisomer is shown in parentheses. [d] Note that for this substrate the chiral centers are in positions 1 and 2, as shown in Scheme 1. n.d. not determined.

At this point, it seemed evident that only (R)-TAs were capable of accepting all this panel of substrates to perform efficient DKRs. For this reason, we decided to explore the activity of some in-house transaminases. TAs containing the *spuC* gene from *Pseudomonas* species, in particular *P. putida* (*Pp* spuC), *P. chlororaphis* subsp. *aureofaciens* (*Pc* spuC) and *P. fluorescens* (*Pf* spuC),[28] and transaminases from *Vibrio fluvialis* (*Vf*),[29] *Chromobacterium violaceum* (*Cv*),[30] *Alcaligenes denitrificans* (*Ad*),[31] *Bacillus megaterium* (*Bm*)[32] and our high overproducing transaminase variant *Bm* S119G[33] were tested towards α-substituted β-keto amides **2a-h** finding that only *Bm*TA and *Bm*TA S119G were capable of reacting with the selected substrates (Table 3).

The desired enantiopure amines **3a-d,h** were successfully synthesized by both (S)-transaminases. In all cases, moderate to high conversions and excellent *ee* values were found, leading *Bm*TA and *Bm*TA S119G to the formation of *syn*-(2R,3S)-**3a** with low diastereomeric ratios (entries 1 and 2). However, with the rest of the substrates, *Bm*TA led to the preferential formation of the *anti*-diastereoisomer as the major product with low to moderate *dr* (entries 3, 5, 7 and 15), while *Bm*TA S119G afforded the opposite diastereoisomer, leading to the formation of *syn*-**3b-d,h** with low to moderate diastereoselectivities (entries 4, 6, 8 and 16). Unfortunately, the α-benzylated keto amide (**2e**, entries 9 and 10) was not a good substrate for these enzymes. Meanwhile the derivative **2f** was not accepted by either *Bm*TA or *Bm*TA S119G (entries 11 and 12). *Bm*TA was still capable of producing *anti*-(2S,3S)-**3e** with 15% conversion and moderate *dr* but *Bm*TA S119G completely lost its activity. Similar results were found with the substrate bearing a piperidine group in the amide moiety (**2g**), the *Bm*TA displaying low activity (entry 13), while *Bm*TA S119G was not capable of producing **3g** in any extension (entry 14). The absolute configuration of the final products was determined due to the well-known (S)-selectivity of these two enzymes.[32,33]
In order to demonstrate the applicability and reproducibility of this procedure, semipreparative biotransformations were set up. Hence, this would allow us to isolate and fully characterized the new diastereoenriched and enantiopure α-substituted β-amino amides **3a-h**. For this purpose, the robust ATA-025 was chosen as biocatalyst and DMSO was replaced with acetonitrile (MeCN) as cosolvent in order to facilitate the work-up protocols. DKRs of all substrates were carried out under the optimized reaction conditions at pH 7.5 (Scheme 2A).[34] The final products were obtained after acid-base extractions in moderate to high isolated yields (45-86%) starting from 100 mg of the corresponding racemic α-substituted β-keto amides **2a-h**. The *anti*-diastereoisomer was always obtained as the major product with the exception of α-benzylated β-amino amide **3e**, finding low to moderate diastereomeric ratios (from 60:40 to 85:15) and excellent enantioselectivities in all cases (>99% *ee* for both diastereoisomers). Additionally, the biotransformation of **2a** into the corresponding α-methylated β-amino amide **3a** was performed at pH 10 (Scheme 2B). In this case, the *anti*-amino amide was obtained as the major diastereoisomer in moderate *dr* (80:20 *anti*:*syn*), high isolated yield (90%) and excellent *ee* values for both diastereoisomers (>99% *ee*).
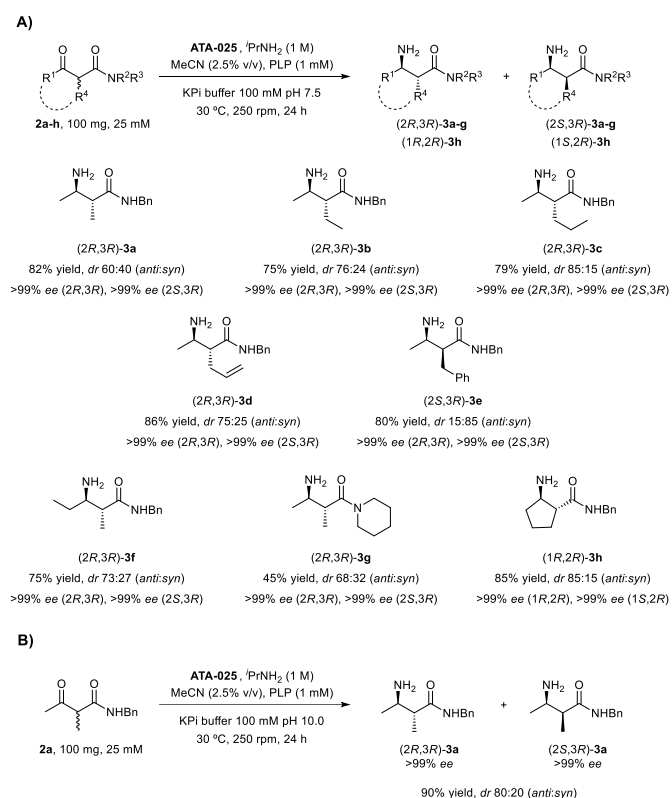
Scheme 2 Semipreparative scale biotransformations of α-substituted β-keto amides **2a-h** into the corresponding diastereoenriched and enantiopure amines **3a-h**. A) At pH 7.5. B) Synthesis of α-methylated β-amino amide **3a** at pH 10. The major diastereoisomer is drawn.

Conclusions

β-Amino amides are structures that can be found in many biologically active compounds. Various efficient and selective chemical methodologies have been described to synthesize β[3]-amino amides in enantioenriched form, but the introduction of a chiral center at α-position (β[2,3]-amino amides) hampers the stereoselective synthesis of these derivatives due the existence of four possible product diastereoisomers. This is especially challenging for the case of acyclic α-alkyl-β-amino amides, where effective chemical or enzymatic methods have still not been found.

After reaction optimization, various commercial (*R*)-transaminases displayed interesting activities as they recognized the different keto amides **2a**-**h** working at a remarkable substrate concentration (25 mM), attaining in general high conversions, moderate diastereomeric ratios and excellent *ee* values by choosing the appropriate pH for each particular case. In general, *anti*-isomers were preferentially obtained except for the case of the α-benzylated compound, where the *syn*-(2*S*,3*R*)-**3e** isomer was preferentially achieved. Remarkably, in-house (*S*)-selective TAs from *Bacillus megaterium* (*Bm*TA and a mutant *Bm*TA S119G) were also able to transform some of these substrates, affording the opposite enantiomers *anti*-(2*S*,3*S*)- and *syn*-(2*R*,3*S*)-**3a**-**d** or *trans*-(1*S*,2*S*)- and *cis*-(1*R*,2*S*)-**3h** with low diastereomeric ratios.

Furthermore, semipreparative biotransformations using commercially available ATA-025 were set up, obtaining results comparable to those obtained at small-scale. This fact proved the reproducibility and applicability of the method, facilitating the characterization of a wide panel of new diastereoenriched α-substituted β-amino amides.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Notes and references

1   (*a*) D. Kim, L. Wang, M. Beconi, G. J. Eiermann, M. H. Fisher, H. He, G. J. Hickey, J. E. Kowalchick, B. Leiting, K. Lyons, F. Marsilio, M. E. McCann, R. A. Patel, A. Petrov, G. Scapin, S. B. Patel, R. Sinha Roy, J. K. Wu, M. J. Wyvratt, B. B. Zhang, L. Zhu, N. A. Thornberry and A. E. Weber, *J. Med. Chem.*, 2005, **48**, 141; (*b*) S. D. Edmondson, M. H. Fisher, D. Kim, M. Maccoss, E. R. Parmee, A. E. Weber and J. Xu, US Pat., 6699871, 2004.

2   Selected bibliography: (*a*) J. E. Kowalchick, B. Leiting, K. D. Pryor, F. Marsilio, J. K. Wu, H. He, K. A. Lyons, G. J. Eiermann, A. Petrov, G. Scapin, R. A. Patel, N. A. Thornberry, A. E. Weber and D. Kim, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 5934; (*b*) S. P. O'Connor, US Pat., 20070099913, 2007; (*c*) D. Kim, J. E. Kowalchick, L. L. Brockunier, E. R. Parmee, G. J. Eiermann, M. H. Fisher, H. He, B. Leiting, K. Lyons, G. Scapin, S. B. Patel, A. Petrov, K. D. Pryor, R. Sinha Roy, J. K. Wu, X. Zhang, M. J. Wyvratt, B. B. Zhang, L. Zhu, N. A. Thornberry and A. E. Weber, *J. Med. Chem.*, 2008, **51**, 589; (*d*) Z. Shan, M. Peng, H. Fan, Q. Lu, P. Lu, C. Zhao and Y. Chen, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 1731; (*e*) H. J. Kim, W. Y. Kwak, J. P. Min, J. Y. Lee, T. H. Yoon, H. D. Kim, C. Y. Shin, M. K. Kim, S. H. Choi, H. S. Kim, E. K. Yang, Y. H. Cheong, Y. N. Chae, K. J. Park, J. M. Jang, S. J. Choi, M. H. Son, S. H. Kim, M. Yoo and B. J. Lee, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 3809; (*f*) T. Jiang, Y. Zhou, Z. Chen, P. Sun, J. Zhu, Q. Zhang, Z. Wang, Q. Shao, X. Jiang, B. Li, K. Chen, H. Jiang, H. Wang, W. Zhu and J. Shen, *ACS Med. Chem. Lett.*, 2015, **6**, 602.

3   H. Umezawa, T. Aoyagi, H. Suda, M. Hamada and T. Takeuchi, *J. Antibiot.*, 1976, **29**, 97.

4   T. Mimoto, J. Imai, S. Kisanuki, H. Enomoto, N. Hattori, K. Akaji and Y. Kiso, *Chem. Pharm. Bull.*, 1992, **40**, 2251.

5   K. Csillag, Z. Szakonyi and F. Fülöp, *Tetrahedron: Asymmetry*, 2013, **24**, 553.

6   (*a*) J. L. Vicario, D. Badía and L. Carrillo, *J. Org. Chem.*, 2001, **66**, 9030; (*b*) A. Iza, J. L. Vicario, L. Carrillo and D. Badía, *Synthesis*, 2006, 4065; (*c*) S. Saito, T. Tsubogo and S. Kobayashi, *Chem. Commun.*, 2007, 1236; (*d*) J. M. Concellón, H. Rodríguez-Solla, C. Concellón, C. Simal and N. Alvaredo, *Synlett*, 2010, 2119; (*e*) O. Prieto and H. W. Lam, *Org. Biomol. Chem.*, 2008, **6**, 55; (*f*) L. Yin, L. Brewitz, N. Kumagai and M. Shibasaki, *J. Am. Chem. Soc.*, 2014, **136**, 17958; (*g*) L. Brewitz, F. Arteaga Arteaga, L. Yin, K. Alagiri, N. Kumagai and M. Shibasaki, *J. Am. Chem. Soc.*, 2015, **137**, 15929; (*h*) F. Arteaga Arteaga, Z. Liu, L. Brewitz, J. Chen, B. Sun, N. Kumagai and M.

Shibasaki, *Org. Lett.*, 2016, **18**, 2391; (*i*) M. J. Houghton, C. J. Huck, S. W. Wright and D. B. Collum, *J. Am. Chem. Soc.*, 2016, **138**, 10276; (*j*) B. Sun, P. V. Balaji, N. Kumagai and M. Shibasaki, *J. Am. Chem. Soc.*, 2017, **139**, 8295; (*k*) X. Chen, Y. Li, J. Zhao, B. Zheng, Q. Lu and X. Ren, *Adv. Synth. Catal.*, 2017, **359**, 3057; (*l*) S. Paladhi, S. Y. Park, J. W. Yang and C. E. Song, *Org. Lett.*, 2017, **19**, 5336; (*m*) B. Sun, R. Pluta, N. Kumagai and M. Shibasaki, *Org. Lett.*, 2018, **20**, 526.

7   (*a*) S. G. Davies, A. J. Edwards and I. A. S. Walters, *Recl. Trav. Chim. Pays-Bas*, 1995, **114**, 175; (*b*) J. Etxebarria, J. L. Vicario, D. Badia, L. Carrillo and N. Ruiz, *J. Org. Chem.*, 2005, **70**, 8790.

8   (*a*) J. E. Semple, T. D. Owens, K. Nguyen and O. E. Levy, *Org. Lett.*, 2000, **2**, 2769; (*b*) B. Huang, L. Zeng, Y. Shen and S. Cui, *Angew. Chem., Int. Ed.*, 2017, **56**, 4565.

9   (*a*) K. B. Hansen, Y. Hsiao, F. Xu, N. Rivera, A. Clausen, M. Kubryk, S. Krska, T. Rosner, B. Simmons, J. Balsells, N. Ikemoto, Y. Sun, F. Spindler, C. Malan, E. J. J. Grabowski and J. D. Armstrong III, *J. Am. Chem. Soc.*, 2009, **131**, 8798; (*b*) S. Mallik, V. Bhajammanavar, I. Ramakrishna and M. Baidya, *Org. Lett.*, 2017, **19**, 3843; (*c*) S. Qiao, J. Wu, J. Mo, P. T. Spigener, B. N. Zhao, B. Jiang and G. Li, *Synlett*, 2017, **28**, 2483.

10   (*a*) D. Steinhuebel, Y. Sun, K. Matsumura, N. Sayo and T. Saito, *J. Am. Chem. Soc.*, 2009, **131**, 11316; (*b*) D. Mtat, R. Touati and B. B. Hassine, *Tetrahedron Lett.*, 2014, **55**, 6354; (*c*) C. G. Goodman and J. S. Johnson, *J. Am. Chem. Soc.*, 2015, **137**, 14574; (*d*) Y. Lou, Y. Hu, J. Lu, F. Guan, G. Gong, Q. Yin and X. Zhang, *Angew. Chem., Int. Ed.*, 2018, **57**, 14193.

11   M. Vishe and J. N. Johnston, *Chem. Sci.*, 2019, **10**, 1138.

12   V. Chhiba, M. Bode, K. Mathiba and D. Brady, in *Cascade Biocatalysis: Integrating Stereoselective and Environmentally Friendly Reactions*, eds. S. Riva and W.-D. Fessner, Wiley-VCH Verlag, Weinheim, Germany, 2014, p. 297.

13   (a) M. Preiml, H. Hönig and N. Klempier, *J. Mol. Catal. B: Enzym.*, 2004, **29**, 115; (*b*) M. Winkler, L. Martínková, A. C. Knall, S. Krahulec and N. Klempier, *Tetrahedron*, 2005, **61**, 4249; (*c*) D.-Y. Ma, D.-X. Wang, J. Pan, Z.-T. Huang and M.-X. Wang, *J. Org. Chem.*, 2008, **73**, 4087; (*d*) V. Chhiba, M. L. Bode, K. Mathiba, W. Kwezi and D. Brady, *J. Mol. Catal. B: Enzym.*, 2012, **76**, 68; (*e*) O. K. L. Rapheeha, M. P. Roux-van der Merwe, J. Badenhorst, V. Chhiba, M. L. Bode, K. Mathiba and D. Brady, *J. Appl. Microbiol.*, 2017, **122**, 686.

14   X.-G. Li, M. Lähitie and L. T. Kanerva, *Tetrahedron: Asymmetry*, 2008, **19**, 1857.

15   E. Choi, Y. Kim, Y. Ahn, J. Park and M.-J. Kim, *Tetrahedron: Asymmetry*, 2013, **24**, 1449.

16   T. Heck, D. Seebach, S. Osswald, M. K. J. ter Wiel, H.-P. E. Kohler and B. Geueke, *ChemBioChem*, 2009, **10**, 1558.

17   Z. Xue, Y. Chao, D. Wang, M. Wang and S. Qian, *J. Ind. Microbiol. Biotechnol.*, 2011, **38**, 1931.

18   E. García-Urdiales, I. Alfonso and V. Gotor, *Chem. Rev.*, 2011, **111**, PR110.

19   (*a*) B. Martín-Matute and J.-E. Bäckvall, *Curr. Opin. Chem. Biol.*, 2007, **11**, 226; (*b*) J. H. Lee, K. Han, M.-J. Kim, and J. Park, *Eur. J. Org. Chem.*, 2010, 999; (*c*) H. Pellissier, *Tetrahedron*, 2011, **67**, 3769; (*d*) A. S. de Miranda, L. S. M. Miranda and R. O. M. A. de Souza, *Biotechnol. Adv.*, 2015, **33**, 372; (*e*) A. Díaz-Rodríguez, I. Lavandera and V. Gotor, *Curr. Green Chem.*, 2015, **2**, 192.

20   Recent reviews: (a) M. Fuchs, J. E. Farnberger and W. Kroutil, *Eur. J. Org. Chem.*, 2015, 6965; (b) I. Slabu, J. L. Galman, R. C. Lloyd and N. J. Turner, *ACS Catal.*, 2017, **7**, 8263; (c) F. Guo and P. Berglund, *Green Chem.*, 2017, **19**, 333; (d) S. A. Kelly, S. Pohle, S. Wharry, S. Mix, C. C. R. Allen, T. S. Moody and B. F. Gilmore, *Chem. Rev.*, 2018, **118**, 349; (e) E. E. Ferrandi and D. Monti, *World J. Microbiol. Biotechnol.*, 2018, **34**, 13; (f) M. D. Patil, G. Grogan, A. Bommarius and H. Yun, *Catalysts*, 2018, **8**, 254; (g) A. Gomm and E. O'Reilly, *Curr. Opin. Chem. Biol.*,

2018, **43**, 106; (*h*) P. Kelefiotis-Stratidakis, T. Tyrikos-Ergas and I. V. Pavlidis, *Org. Biomol. Chem.*, 2019, **17**, 1634.

21   C. K. Savile, J. M. Janey, E. C. 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 and G. J. Hughes, *Science*, 2010, **329**, 305.

22   (*a*) D. Koszelewski, D. Clay, K. Faber and W. Kroutil, *J. Mol. Catal. B: Enzym.*, 2009, **60**, 191; (*b*) C. K. Chung, P. G. Bulger, B. Kosjek, K. M. Belyk, N. Rivera, M. E. Scott, G. R. Humphrey, J. Limanto, D. C. Bachert and K. M. Emerson, *Org. Process Res. Dev.*, 2014, **18**, 215; (*c*) C. S. Fuchs, M. Hollauf, M. Meissner, R. C. Simon, T. Besset, J. N. H. Reek, W. Riethorst, F. Zepeck and W. Kroutil, *Adv. Synth. Catal.*, 2014, **356**, 2257; (*d*) Z. Peng, J. W. Wong, E. C. Hansen, A. L. A. Puchlopek-Dermenci and H. J. Clarke, *Org. Lett.*, 2014, **16**, 860; (*e*) J. Limanto, E. R. Ashley, J. Yin, G. L. Beutner, B. T. Grau, A. M. Kassim, M. M. Kim, A. Klapars, Z. Liu, H. R. Strotman and M. D. Truppo, *Org. Lett.*, 2014, **16**, 2716; (*f*) S. Pedragosa-Moreau, A. Le Flohic, V. Thienpondt, F. Lefoulon, A.-M. Petit, N. Ríos-Lombardía, F. Morís and J. González-Sabín, *Adv. Synth. Catal.*, 2017, **359**, 485; (*g*) E. Liardo, N. Ríos-Lombardía, F. Morís, F. Rebolledo and J. González-Sabín, *ACS Catal.*, 2017, **7**, 4768; (*h*) N. Yasuda, E. Cleator, B. Kosjek, J. Yin, B. Xiang, F. Chen, S.-C. Kuo, K. Belyk, P. R. Mullens, A. Goodyear, J. S. Edwards, B. Bishop, S. Ceglia, J. Belardi, L. Tan, Z. J. Song, L. DiMichele, R. Reamer, F. L. Cabirol, W. L. Tang and G. Liu, *Org. Process Res. Dev.*, 2017, **21**, 1851; (*i*) C. S. Fuchs, J. E. Farnberger, G. Steinkellner, J. H. Sattler, M. Pickl, R. C. Simon, F. Zepeck, K. Gruber and W. Kroutil, *Adv. Synth. Catal.*, 2018, **360**, 768.

23   A. Cuetos, I. Lavandera and V. Gotor, *Chem. Commun.*, 2013, **49**, 10688.

24   D. Méndez-Sánchez, Á. Mourelle-Insua, V. Gotor-Fernández and I. Lavandera, *Adv. Synth. Catal.*, 2019, **361**, 2706.

25   B. Štefane and S. Polanc, *Tetrahedron*, 2007, **63**, 10902.

26   M. J. García, F. Rebolledo and V. Gotor, *Tetrahedron Lett.*, 1993, **38**, 6141.

27   Á. Mourelle-Insua, L. A. Zampieri, I. Lavandera and V. Gotor-Fernández, *Adv. Synth. Catal.*, 2018, **360**, 686.

28   J. L. Galman, I. Slabu, N. J. Weise, C. Iglesias, F. Parmeggiani, R. C. Lloyd and N. J. Turner, *Green Chem.*, 2017, **19**, 361.

29   J.-S. Shin and B.-G. Kim, *Biotechnol. Bioeng.*, 1999, **65**, 206.

30   U. Kaulmann, K. Smithies, M. E. B. Smith, H. C. Hailes and J. M. Ward, *Enzyme Microb. Technol.*, 2007, **41**, 628.

31   H. Yun, S. Lim, B.-K. Cho and B.-G. Kim, *Appl. Environ. Microbiol.*, 2004, **70**, 2529.

32   (*a*) D. Koszelewski, M. Göritzer, D. Clay, B. Seisser and W. Kroutil, *ChemCatChem*, 2010, **2**, 73; (*b*) N. van Oosterwijk, S. Willies, J. Hekelaar, A. C. Terwissscha van Scheltinga, N. J. Turner and B. W. Dijkstra, *Biochemistry*, 2016, **55**, 4422.

33   B. Z. Costa, J. L. Galman, I. Slabu, S. P. France, A. J. Marsaioli and N. J. Turner, *ChemCatChem*, 2018, **10**, 4733.

34   General protocol for the semipreparative biotransamination of α-substituted β-keto amides **2a-h** using the commercial transaminase ATA-025. In an Erlenmeyer, ATA-025 (75 mg) and α-substituted β-keto amide (100 mg, 25 mM) were added in phosphate buffer 100 mM (1 mM PLP, 250 mM isopropylamine, pH 7.5 for keto amides **2a-h** and pH 10.0 for keto amide **2a**) and MeCN (2.5% v/v). The reaction was shaken at 30 °C and 250 rpm for 24 h and then stopped by adding a saturated aqueous solution of Na_2CO_3 until pH 10-11. Then, the mixture was extracted with EtOAc (3 x 15 mL), the organic layer separated by centrifugation (5 min, 4900 rpm), combined and finally dried over Na_2SO_4 and conversions were measured by GC analysis. In order to obtain the pure amines, the crude reaction was acidified using HCl up to pH ~ 3 and extracted with Et_2O (4 x 10 mL). The aqueous layer was basified by adding 2-3 pellets of NaOH up to pH ~ 13 and extracted with Et_2O (4 x 10 mL). The organic layers were

combined and dried over Na_2SO_4 and the solvent was eliminated under reduced pressure, obtaining the amines **3a-h** in moderate to high yields (45-90%).