Enantioselective One-pot Synthesis of Biaryl-substituted Amines by Combining Palladium and Enzyme Catalysis in Deep Eutectic Solvents

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ABSTRACT

The first application of *Deep Eutectic Solvents* (*DESs*) in asymmetric bioamination of ketones has been accomplished. The amine transaminases (ATAs) turned out to be particularly stable in *DES*-buffer mixtures at a percentage of up to 75% (w/w) neoteric solvent. Moreover, this reaction medium was used to perform a chemoenzymatic cascade toward biaryl amines by coupling a Suzuki reaction sequentially with an enantioselective bioamination catalyzed by the recently discovered ATA from *Exophiala xenobiotica* (EX-ωTA). The solubilizing properties of *DESs* enabled the metal-catalyzed step at 200 mM loading of substrate and the subsequent biotransformation at 25 mM.

KEYWORDS: Amines, asymmetric synthesis, biocatalysis, deep eutectic solvents, palladium catalysis, Suzuki cross-coupling reaction

INTRODUCTION

The biaryl moiety has emerged to a broadly used, valuable structural motif not only in the field of chiral ligands for asymmetric catalysis (as underlined by BINOL and BINAP as the presumably most prominent examples bearing such a biaryl structure),[1-3] but also in the field of natural products[4] and pharmaceuticals.[5,6] A commercialized product in this field is Valsartane (**1**) developed by Ciba-Geigy (now, Novartis), which is used as an angiotensin receptor blocker for treatment of, e.g. high blood pressure (Figure 1).[6] At the same time, chiral amine structural motifs can be widely found in today's marketed drugs.[4] Indeed, the FDA database reveals that 84% of the approved small molecule drugs bear at least one nitrogen atom.[5] Thus, "merging" these two structural motifs has raised interest in the field of drug development, and the related

biaryl-substituted amines have already turned out to represent versatile intermediates for promising drug candidates. For example, the biaryl-amine **2** is used for the synthesis of the cathepsin C inhibitor Odanacatib (**3**), which was evaluated in phase III for fracture prevention in postmenopausal women with osteoporosis by Merck & Co.[6]
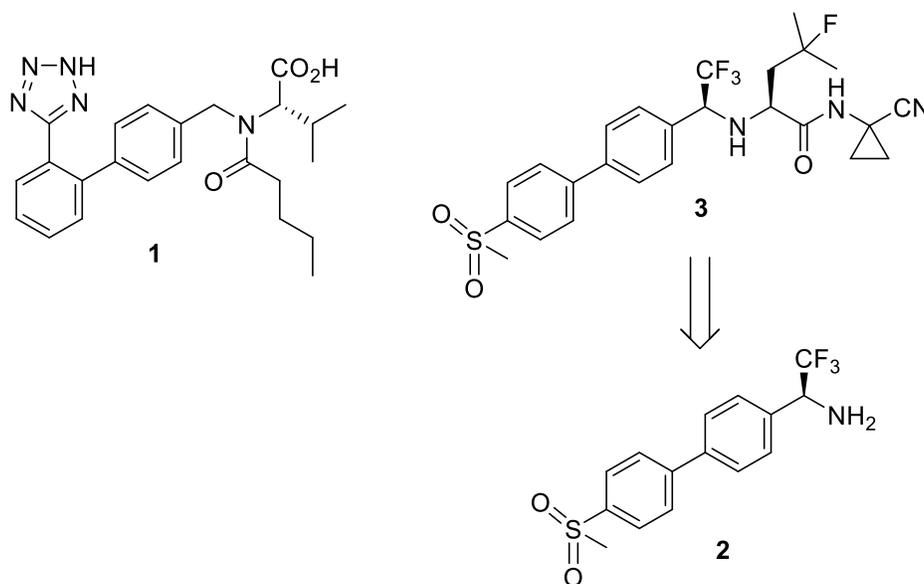
Figure 1. Examples of biaryl-containing drugs: Valsartane (**1**) and Odanacatib (**3**).

This potential for pharmaceutical applications also raised the question about efficient approaches to such chiral biaryl-substituted amine molecules. Retrosynthetically the biaryl unit can be constructed through a palladium-catalyzed Suzuki-cross coupling reaction,[10] a process which has been successfully implemented in eco-friendly media.[11-13] On the other hand, the asymmetric reductive amination enables to convert ketones into enantiomerically pure amines.[14] The combination of these two steps represents an elegant and straightforward approach toward these target molecules. The biocatalytic reductive amination can be conducted by amine transaminases.[15] Such an approach has been recently demonstrated for the enantioselective synthesis of biaryl amines by the Bornscheuer group for the first time. This was exemplified for

the conversion of a halogenated acetophenone with a boronic acid in a Suzuki reaction. Subsequent conversion of the resulting biaryl ketones in the presence of an amine transaminase led to the formation of amines with up to 84% overall conversion and >99% *ee* when conducting the two reactions sequentially at 2 mM and 1 mM substrate concentration, respectively.[16] In terms of a high overall process efficiency, the combination of these two steps within a one-pot process would be highly desirable as well as the increase of the substrate loading for achieving an improved volumetric productivity. Combinations of chemo- and biocatalysis have been identified as attractive process options in recent years. This is underlined by many successful examples.[17-21] Our groups have reported a related combination of a Suzuki-cross coupling reaction and subsequent enzymatic reduction in which biaryl-substituted alcohols were formed.[22-24] In these studies, *Deep Eutectic Solvents* (*DESs*)[25] were used as a well-known environmentally benign solvent class which turned out to be an attractive reaction medium.

In recent times, the pharmaceutical industry has become more receptive to the use of biocatalysis for the manufacture of active pharmaceutical ingredients in a sustainable manner.[26] Although the greenness of this technology is typically assumed, most biotransformations suffer from issues such as water consumption, wastewater production or unfavorable metrics due to the poor solubility of reagents in water.[27] To circumvent this drawback, organic solvents can be supplemented as co-solvents, to the expense of enzyme stability, generally limited in these media. On the other hand, a new awareness has arisen in today´s synthetic organic chemistry to replace toxic/carcinogenic petroleum based volatile organic compounds (VOCs) by new, greener and more sustainable solvents.[28,29] In this context, Abbot introduced *DESs*,[30] which consist of 2-3 compounds from renewable sources establishing an extensive H-bond network throughout the solvent with a melting point far below those of the individual components. Compared to the

related ionic liquids (ILs), *DESs* are cheaper, easier to make, tunable, highly biodegradable, virtually non-toxic and do not require further purification. As illustrated by the exponential growth of literature, *DES* technology has been applied to a wide-ranging area of research topics such as organic synthesis,[31,32] metal-catalysis[33-36] and organocatalysis,[37-40] energy technology,[41,42] material chemistry,[43] or separation processes.[44] In biocatalysis, since the 2008 proof-of-concept,[45] many examples have showcased *ad hoc* protocols for biotransformations in *DESs* and *DES*-buffer mixtures.[46] Interestingly, hitherto there is not any example involving ATAs. Recent advances in protein engineering have enabled the conversion of a variety of sterically demanding ketones by ATAs. For example, the rationally engineered (*S*)-selective ATA from *V. fluvialis* catalyzed an *ortho*-biaryl ketone to the corresponding (*S*)-biaryl amine.[47] Bornscheuer and coworkers,[16] in parallel to us,[48] have recently generated ATAs from *Aspergillus fumigatus* (4CHI-TA) and *Exophiala xenobiotica* (EX-ωTA) respectively, suitable for producing *meta*-and *para*-(*R*)-biaryl amines. Thus, we became interested in developing an efficient chemoenzymatic one-pot process route to biaryl-substituted amines based on the combination of a Suzuki-cross coupling reaction and an enzymatic transamination in *DESs* as sustainable reaction media. In the following, we report exactly such a process. It also represents the first example of an application of the enzyme class of amine transaminases in *DESs*.

EXPERIMENTAL SECTION

Reagents. D-(+)-Glucose was purchased from VWR. D-Alanine, PLP (Pyridoxal 5′-phosphate hydrate) and NAD^+ were purchased from Sigma Aldrich. The ligand TPPTS (Triphenylphosphine-3,3',3''-trisulfonic acid trisodium salt hydrate, tech. 85%) was purchased from Alfa Aesar. Palladium(II) chloride was purchased from TCI. Arylbromides and phenylboronic acids were purchased from Sigma Aldrich.

Enzymes. -LDH (Lactate dehydrogenase) from rabbit muscle (Type II, ammonium sulfate suspension, 800-1,200 U/mg protein); -GDH (Glucose dehydrogenase) from *Bacillus Megaterium* was expressed in *Escherichia coli*. The activity was established as 1884 U/ml;[49] -*Exophilia xenobiotica* ATA gene codon-optimized for expression in *Escherichia coli* was ordered from Geneart/LifeTech (Vienna, Austria). The activity determined spectrophotometrically according to the acetophenone assay was EX-wt: 16.9 U/ml and EX-STA: 23.35 U/ml; -Codex® Transaminase ATA Screening Kit (ATASK-000250) was purchased from Codexis; -Transaminases from *Chromobacterium violaceum* (Cv, internal plasmid number pET20) and *Arthrobacter* sp. [ArR (pEG23), ArS (pEG29) and ArRmut11 (pEG90)] were overexpressed in *E.coli* and used as lyophilized cells. The protein content of these four transaminases was established by the Pierce's method (mg protein/mg catalyst) according to the manufacturer's instructions: ArS (0.37), Cv (0.31), ArRmut11 (0.38), ArR (0.19).

Synthesis of Deep Eutectic Solvents. *ChCl-Gly* (1:2 w/w), *ChCl*-H_2O (1:2 w/w), *ChCl-Sorb* (1:1 w/w) and *ChCl*-Urea (1:2 w/w) were prepared by gently heating under stirring at 60-80 ºC for 1 hour the corresponding individual components until a clear solution was obtained.

General procedure for the bioamination of phenylacetone. Reactions were carried out in a 2.0 mL vial. The corresponding ATA (2.0 mg for Codexis' enzymes or 5.0 mg for Cv, ArR, ArS and ArRmut11) was added to 500 µL of the corresponding mixture of *DES* and buffer 100 mM phosphate buffer pH 7.5, containing propan-2-amine (1.0 M) and the cofactor PLP (1.0 mM). Then, a solution of **6** (2.0 mg) was added and the resulting mixture was shaken at 250 rpm and 30 °C for 24 h. After this time, a 50 µL aliquot was removed to determine the degree of conversion by HPLC (see Section 6.2 in the Supporting Information). The reaction mixture was finally quenched with aqueous 10 N NaOH (100 µL) and extracted with ethyl acetate (2 × 500

μL). The organic layers were separated by centrifugation (90 s, 13000 rpm), combined, and finally dried over anhydrous Na_2SO_4. The enantiomeric excess of the resulting amine was determined by chiral HPLC (see Section 6.3 the in Supporting Information) after conventional derivatization of the sample using acetic anhydride (2 μL / mg of substrate).

General procedure for the biarylamine synthesis in a one-pot process. At first, a suspension of $PdCl_2$ (0.086 mg; 1 mol %) and TPPTS (8.32 mg; 3 mol %) in degassed and deionized water was prepared (0.25 mL). After 30 min the resulting catalyst solution was added to a mixture consisting of aryl bromide (0.5 mmol), arylboronic acid (0.5 mmol), sodium carbonate (132 mg, 1.25 mmol), *DES* (2.0 mL) and degassed and deionized water (0.25 mL). The reaction mixture was heated to 100 ºC (homogeneous mixture) for 24 h. After cooling to room temperature, EX-STA lysate (11 mL, 257 U), KPi buffer 100 mM pH 7.5 (6.5 mL), PLP (1 mM), NAD^+ (0.1 mM), D-glucose (57 mM), D-alanine (130 mM), LDH (3600 U, 680 μL) and GDH (1200U, 400 mg) were added. The degree of conversion and *ee* were determined as described above and according to Sections 6,2 and 6.3 in the Supporting Information. Then, the reaction was quenched by addition of aqueous 10 N NaOH (10 mL) to adjust the pH to 14. The mixture was then extracted with ethyl acetate (2 × 25 mL) and the organic layers were separated by centrifugation (90s, 13000 rpm), combined and dried over anhydrous Na_2SO_4 to provide the crude product. Further filtration by flash chromatography (silica gel 60 Å, ethyl acetate) yielded the corresponding (*R*)-biaryl amines **5a-e**.

RESULTS AND DISCUSSION

In the recent report we revisited the synthesis of biaryl alcohols by means of a Suzuki cross-coupling and subsequent bioreduction of the transiently formed ketones with KREDs.[24] With

regard to previous research, the use of *DESs* enabled us to tackle the solubility hurdles and reach concentrations of 200 mM for the coupling step and 75 mM for the subsequent bioreduction. From a synthetic goal point of view, a first key challenge was to determine if EX-ωTA is active in these bio-based solvents. Accordingly, the bioamination of the biaryl ketone **4a** was investigated as a benchmark reaction with the variant EX-STA (amino acid exchange T273S). This variant leads to the highest conversions of biaryl ketones.[48] The biotransformation was conducted under the previously optimized reaction setup, namely based on the use of alanine as amino donor and the LDH/GDH recycling system, and supplemented with choline chloride (*ChCl*)/glycerol (*Gly*) (1:2). For the sake of comparison, other co-solvents such as DMSO, THF, and *i*-PrOH were also tested (5% and 15% v/v). As deduced from Table 1, the presence of THF and *i*-PrOH was detrimental for the enzyme activity, resulting in a very low conversion at 15% of co-solvent (Table 1, entries 2 and 4). Conversely, the ATA remained very active in both DMSO and 1*ChCl*/2*Gly* with almost quantitative conversions at 15% of co-solvent (Table 1, entries 5-8). An increase to 25% *DES* turned out to decrease the enzymatic performance (60% of conversion, Table 1, entry 9) while the activity of the ATA in *DES*:buffer 1:1 was negligible (Table 1, entry 10). EX-STA exhibited perfect enantioselectivity toward **4a** regardless of the co-solvent and its ratio, the resulting amine (*R*)-**5a** displaying >99% *ee* in all cases.

Table 1. Effect of co-solvent on the conversion of the EX-STA-catalyzed bioamination of biaryl ketone **4a**.[a]
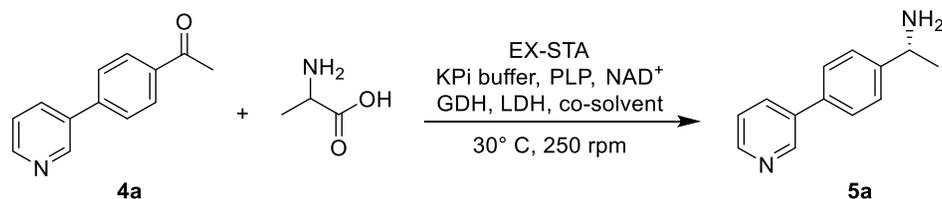
Entry	Co-solvent (%)	Conv. (%)[b]	Ee (%)[c]
1	THF (5%)	8	>99 (R)
2	THF (15%)	-	n.d.[d]
3	i-PrOH (5%)	64	>99 (R)
4	i-PrOH (15%)	<5	n.d.[d]
5	DMSO (5%)	>99	>99 (R)
6	DMSO (15%)	>99	>99 (R)
7	1ChCl/2Gly (5%)	>99	>99 (R)
8	1ChCl/2Gly (15%)	95	>99 (R)
9	1ChCl/2Gly (25%)	60	>99 (R)
10	1ChCl/2Gly (50%)	<5	n.d.[d]

[a] Reaction conditions: **4a** (20 mM) was dissolved in the co-solvent (variable ratio) and then KPi buffer 100 mM, pH 7.5 (1 mM PLP, 0.1 mM NAD+), EX-STA (20 U), D-alanine (130 mM), glucose (60 mM), GDH (30 U) and LDH (90 U) were added and the mixture shaken for 24 h at 250 rpm and 30 °C. [b] Measured by HPLC. [c] Measured by chiral HPLC. [d] Not detected.

As the next step, we sought to get more insight in the unveiled stability of ATAs in *DES*-buffer mixtures by extending the study to enzymes from a commercial kit (Codexis),[50] and also the *S*-selective ATAs from *Chromobacterium violaceum* (Cv)[51] and (*S*)-*Arthrobacter* (ArS)[52] and the *R*-selective ATAs from (*R*)-*Arthrobacter* (ArR)[53] and its evolved variant ArRmut11.[54] For this

study, phenylacetone (**6**) was selected as a substrate, which had been efficiently converted by these ATAs in conventional aqueous medium.[55] Four choline chloride-based eutectic mixtures, namely 1*ChCl*/2*Gly*, 1*ChCl*/2H_2O, 1*ChCl*/1*Sorb* (*Sorb* = sorbitol) and 1*ChCl*/2*Urea* were screened at variable water content (Table 2). In a typical experiment aimed at evaluating the enzymatic performance, **6** (30 mM) was incubated in a mixture of *DES* and potassium phosphate buffer (KPi) 100 mM (1 mM PLP and 1 M *i*PrNH$_2$) at pH 7.0, 30 ºC and 250 rpm during 24 h. An initial conclusion extracted from Table 2 is that the *DES*-buffer mixtures resulted in highly suitable reaction media for the ATAs at 25% or 50% (w/w) *DES*. On the one hand, the commercial enzymes led to very high conversions in the four tested media (Table 2, entries 1-4). Indeed, the conversion values were almost identical to those reported in buffer solution,[55] and in the case of ATA-256 the conversion even increased from 57% to 90-95% in the neoteric mixtures (Table 2, entry 3). Cv and ArS were less active in 1*ChCl*/2H_2O, 1*ChCl*/1*Sorb* and 1*ChCl*/2*Urea,* while they exhibited comparable activities in 1*ChCl*/2*Gly* as in buffer solution (Table 2, entries 5-6).[55] ArR and ArRmut11 led to good conversion in all the *DES*-buffer mixtures, especially in the case of ArRmut11. For the particular case of 1*ChCl*/2*Gly*, a further increase to 75% (w/w) *DES* proved to be harmless for ATAs with changes in the conversion rate lower than 5%. The excellent tolerance of ATAs towards *DESs* by ATAs is noteworthy since it is far greater than toward organic solvents.[56] According to a recent study, the *DES* nanostructure is maintained to a remarkably high level of water (ca. 42 wt % H_2O) because of solvophobic sequestration of water into nanostructured domains around cholinium cations.[57] Therefore, it can be assumed that the bioamination proceeds in a choline chloride/glycerol/water deep eutectic solvent mixture. Likewise, the enantioselectivity of the bioamination catalyzed by ATA-237 was enhanced from 96% to >99% *ee* in the four *DES*-buffer mixtures (see details in SI). This feature

of *DESs* had been previously observed in KRED-catalyzed bioreductions, including an intriguing enantioselectivity switch by tuning the ratio of the *DES*-buffer system.[58-60]

Table 2. Effect of different *DES*-buffer media on the conversion of the ATA-catalyzed bioamination of phenylacetone (**6**).[a,b,c]
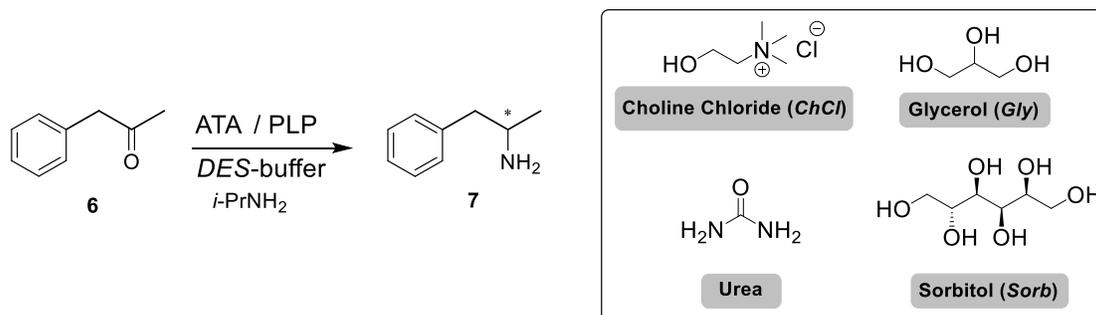
Entry	ATA	Buffer[d]	1*ChCl*/2*Gly* 25% DES	50% DES	75% DES	1*ChCl*/2H$_2$O 25% DES	50% DES	1*ChCl*/1*Sorb* 25% DES	50% DES	1*ChCl*/2*Urea* 25% DES	50% DES
1	ATA-237	95	98	93	93	90	90	93	88	91	88
2	ATA-251	95	97	92	92	93	90	>99	91	95	86
3	ATA-256	57	95	95	95	91	92	85	88	90	85
4	ATA-P1-G06	95	95	95	92	95	90	96	91	95	90
5	Cv	91	94	90	85	5	30	12	15	<5	<5
6	ArS	64	45	42	40	10	25	10	35	<5	<5
7	ArR	91	55	60	72	50	80	85	90	70	65
8	ArRmut11	>99	95	95	95	90	80	73	95	85	90

[a] Reaction conditions: **6** (30 mM) in a *DES*-KPi buffer mixture (500 µL) with PLP (1 mM) and *i*PrNH$_2$ (1 M), ATA (2 mg for the ATAs of Codexis and 5 mg for ArS, ArRmut11 and ArR), for 24 h at 250 rpm and 30 ºC. [b] Conversion measured by HPLC. [c] The *ee* was >99% in all cases with measurable conversion. [d] Results extracted from ref. 29. Aqueous buffer includes 5% v/v of DMSO. *Ee* in the aqueous buffer was >99% except for ATA-237 (96%).

Moving back to the chiral biaryl amines and keeping in mind both the reported Suzuki cross-coupling reaction in *DESs* and the unveiled good tolerance of EX-STA toward these solvents, we envisaged to set up a cascade combining metal catalysis and biocatalysis in such reaction media. Accordingly, we focused on the first step of the cascade, namely the Suzuki cross-coupling reaction. Equimolar amounts of *p*-Br-acetophenone (**8**) and phenylboronic acid (**9**) were reacted at 40 mM in a mixture of water and different co-solvents at room temperature. As depicted in Figure 2, the measured conversions toward 4'-phenylacetophenone (**4b**) were very high (≥85%) at 50% of *i*-PrOH, THF and 1*ChCl*/2*Gly,* while the reaction did not work at the same percentage of DMSO. As stated above, the negative impact of THF and *i*-PrOH on the catalytic performance of the EX-STA precluded the use of these solvents in the chemoenzymatic cascade. As a result, 1*ChCl*/2*Gly* emerged as the only co-solvent addressing the requirements of the two-step process. Based on the knowledge gained in our previous work, the reaction was accomplished at 200 mM substrate concentration in a *DES*:water 4:1 mixture and 100 ºC.
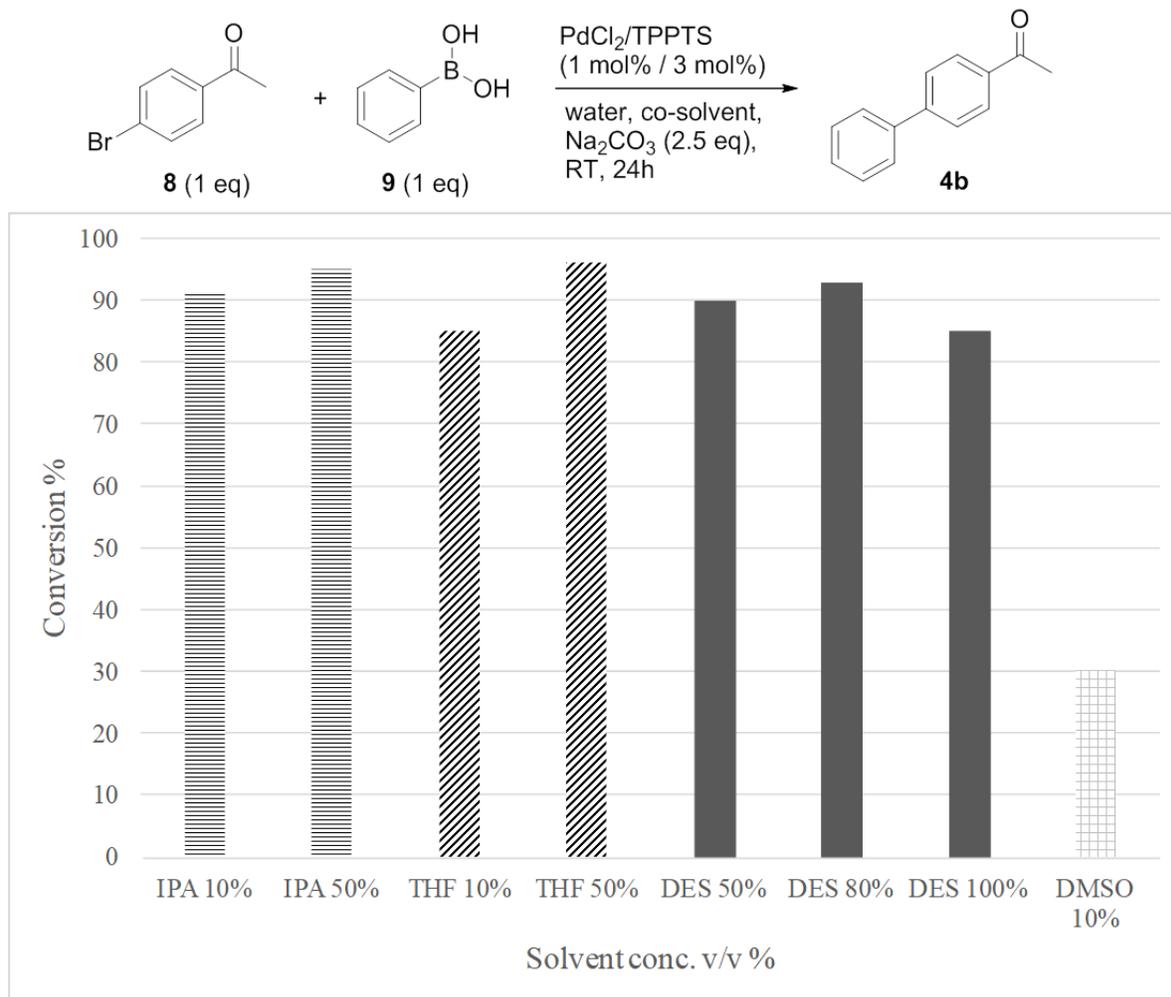
Figure 2. Effect of co-solvent on the Suzuki cross-coupling reaction of *p*-Br-acetophenone (**8**) and phenylboronic acid (**9**); *DES*: 1*ChCl*/2*Gly*.

Once having assessed both steps of the cascade separately and, taking into account that the metal-catalyzed reaction occurs first, the focus was on studying the potential inhibitory effects of the remaining reagents from the first step on the biocatalytic system. Thus, the bioamination of **4a** catalyzed by EX-STA under the optimized setup (Table 1, entry 8) was subjected to a reaction parameter investigation including the impact of boronic acid, aryl halide TPPTS (in excess with respect to PdCl$_2$) and the catalyst [Pd(TPPTS)$_2$Cl$_2$] on this enzymatic step (see SI for details). First, both boronic acid and aryl halide were innocuous in working conditions since the reported

Suzuki cross-coupling occurs quantitatively from equimolar amounts of these species, leading to their full consumption prior to the enzymatic second step. The presence of TPPTS in an extended amount of up to 10 mol% was perfectly tolerated by EX-STA. Finally, 1 mol% of [Pd(TPPTS)$_2$Cl$_2$] inhibited the enzyme slightly, resulting in a conversion of 81%. These findings revealed a compatibility window for the one-pot chemoenzymatic cascade performed in a sequential mode. Indeed, the Suzuki cross-coupling is accomplished at 100 ºC and 200 mM of substrate concentration while the bioamination works efficiently up to 50 ºC and 75 mM substrate concentration. Likewise, although the first step is accomplished at 80% (w/w) *DES*, the EX-STA enzyme only accepts 15% of this solvent. With these premises, the coupling of equimolar amounts of *p*-Br-acetophenone and phenylboronic acid (200 mM) was efficiently conducted at 100 ºC in *DES*-water 4:1. Once the reaction was completed (as judged by HPLC analysis), the reaction mixture containing 4'-phenylacetophenone (**4b**) was diluted to 75 mM with the aqueous buffer for the bioamination and supplemented with EX-STA, LDH, GDH, glucose, related cofactors and the amino donor. After 24 h of incubation at 30 ºC and 250 rpm, the resulting 1-([1,1'-biphenyl]-4-yl)ethanamine (**5b**) was produced with a conversion of 15%. A plausible explanation for this low conversion could arise from the high content of *DES* during the bioamination. Actually, the dilution of the *DES*-water 4:1 mixture from 200 mM to 75 mM results in a medium containing ~30% *DES*, while Table 1 displays that a percent higher than 15% impacts negatively on the enzyme activity. Accordingly, the reaction mixture containing the biaryl ketone was diluted to 50 mM, resulting in a final 20% of *DES* for the biotransformation. As expected, the conversion for **4b** increased up to 30%. Further dilution to 25 mM, which results in 10% of *DES*, led to an optimized conversion of 45% (Table 3, entry 3). It should be noted that this conversion was identical to that reported in the bioamination of **4b** in aqueous

buffer (c = 35% at 20 mM substrate concentration).[23] Next, we decided to test the cascade with the wild-type enzyme, called EX-wt, which had exhibited the highest conversion in the single bioamination of **4b** (83%).[23] However, upon the optimal cascade setup described above (25 mM in the biotransformation) the conversion dropped to 35% (Table 3, entry 4). Encouraged by the success of this dual catalytic system, the methodology was extended to other biaryl amines with both EX-STA and EX-wt (wild type). The metal-catalyzed step proceeded quantitatively in all cases, leading to 3-acetyl-biphenyl **4c** and methyl pyridylphenyl ketones **4a,d,e**. EX-STA converted the resulting ketones quantitatively (c >95%) into the corresponding (*R*)-biaryl amines **5a,c-e** with >99% *ee* (Table 3, entries 1, 5, 7, 9). Conversely, EX-wt showed poor stability in the *DES*-buffer medium which resulted in very low (Table 3, entries 6, 8, 10) or even no conversion (Table 3, entry 6). Although originally conceived for an enlarged small binding pocket compared to the wild-type enzyme, the resulting variant EX-STA turned out to be a more stable enzyme in *DES*-buffer mixtures.

Table 3. One-pot synthesis of enantiopure biaryl amines **5a-e** by palladium-catalyzed Suzuki cross-coupling followed by enzymatic transamination in a *DES*-buffer mixture.[a]
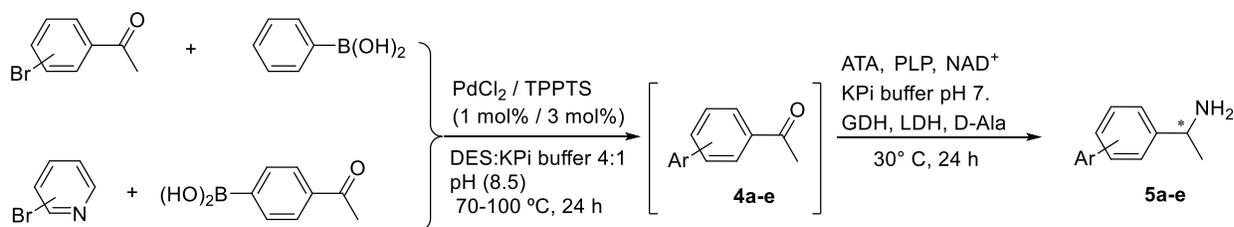
Entry	ATA	Product	Overall conv. (%)[b]	Isolated yield (%)	Ee (%)[c]
1	EX-STA	**5a**	>99	88	>99 (R)
2	EX-wt		10	n.d.	n.d.
3	EX-STA	**5b**	45	32	>99 (R)
4	EX-wt		35	n.d.	>99 (R)
5	EX-STA	**5c**	95	85	>99 (R)
6	EX-wt		-	n.d.	n.d.
7	EX-STA	**5d**	>99	85	>99 (R)
8	EX-wt		20	n.d.	>99 (R)
9	EX-STA	**5e**	>99	90	>99 (R)
10	EX-wt		5	n.d.	n.d.

[a] Reaction conditions: A solution of PdCl$_2$ (1 mol%) and TPPTS (3 mol%), previously stirred in 0.25 mL of degassed water during 30 min, was added to a mixture of boronic acid (0.5 mmol, 200 mM), aryl halide (0.5 mmol), 1*ChCl*:2*Gly* (2.0 mL), Na$_2$CO$_3$ (1.25 mmol) and deionized water (0.25 mL). Then, the mixture was stirred at 100 ºC during 24 h. After cooling to rt, KPi buffer 100 mM, pH 7.5, EX-STA (11 mL, 257 U) or EX-wt (11 mL, 186 U), PLP (1 mM), NAD$^+$ (0.1 mM), D-alanine (130 mM), glucose (57 mM), GDH (1200 U) and LDH (3600 U) were added and the mixture shaken for 24 h at 250 rpm and 30 ºC. [b] Determined by HPLC. [c] Determined by chiral HPLC.

In conclusion, the bioamination of ketones was demonstrated in *ad hoc* mixtures of *Deep Eutectic Solvents* and aqueous buffer. The ATAs exhibited good stability and catalytic performance at very high percentages of *DES* (up to 75%). Owing to the unique properties of

DESs, a chemoenzymatic cascade toward enantiopure biaryl amines was efficiently established. This unprecedented enzymatic activity of ATAs is an excellent proof of concept of the practical value of biorenewable solvents for synthetic chemists. Although immense advances are being made in areas such as protein engineering, it is no less true that a much simpler technique like medium engineering can be a valuable solution for optimizing a given biotransformation.

ASSOCIATED CONTENT

Supporting Information.

Additional information obtained from this study regarding the characterization of biaryl amines (NMR spectra), inhibition studies and analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

BINOL, 1,1'-Bi-2-naphthol; BINAP, (2,2′-bis(diphenylphosphino)-1,1′-binaphthyl); $PdCl_2$, Palladium chloride; TPPTS, [tris(3-sulfonatophenyl)phosphine hydrate, sodium salt; KPi, Potassium phosphate buffer; IL, Ionic liquid; DES, Deep Eutectic Solvent; ChCl, Choline chloride; Gly, Glycerol; THF, Tetrahydrofuran; DMSO, dimethyl sulfoxide; *i*-PrOH, Propan-2-ol; Na_2CO_3, Sodium carbonate; ATA, Amine transaminase; EX-ωTA, ω-Transaminase from *Exophiala xenobiotica*; EX-wt, wild type EX-ωTA; EX-STA, variant of EX-ωTA with the amino acid exchange T273S; Cv, *Chromobacterium violaceum*; and ArS, (*S*)-*Arthrobacter*; ArR, (*R*)-*Arthrobacter*; PLP, Pyridoxal 5′-phosphate; D-Ala, D-Alanine; LDH/GDH, Lactate dehydrogenase/Glutamate dehydrogenase; NAD^+, Nicotinamide adenine dinucleotide; ADH, Alcohol dehydrogenase; *Ee*, Enantiomeric excess; *C*, conversion.

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SYNOPSIS

This paper demonstrates how the unique properties of deep eutectic solvents enable a chemoenzymatic cascade consisting of a Suzuki cross-coupling followed by the unprecedented enzymatic bioamination in these bio-based solvents.