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Research Article
doi.org/10.1002/cctc.202301199

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Insight into the Stereoselective Synthesis of (1*S*)-Nor(pseudo)ephedrine Analogues by a Two-Steps Biocatalytic Process

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Nor(pseudo)ephedrines (N(P)Es), vicinal amino alcohols possessing sympathomimetic biological activity, constitute valuable intermediates and chiral building blocks for the organic synthesis of several active pharmaceutical ingredients (APIs). Due to the presence of two chiral centers, their conventional chemical asymmetric syntheses often involve long, multi-step procedures, frequently with the aid of expensive and harmful metal catalysts, making it challenging to achieve high yields and optical purities overall. A two-steps biocatalytic synthetic sequence for the preparation of (1*S*)-N(P)E analogues was therefore designed and carried out, consisting of a benzoin-type condensation catalysed by the (*S*)-selective acetoin:dichlorophenolindophenol oxidoreductase (Ao:DCPIP OR) and a transamination mediated by either an (*S*)- or (*R*)-selective amine transaminase (ATA). A multistep chemical synthesis of racemic N(P)Es was also optimised in order to obtain reference material for evaluating the performance of the biocatalysed reactions. The novel bi-enzymatic synthesis provided the desired products with acceptable yields and good diastereo- and enantiomeric excesses, thereby paving the way for greener production of these important building blocks.

Introduction

The vicinal amino-alcohol motif is of utmost importance in organic chemistry as it is frequently found in natural products, nutraceuticals, and in both approved and experimental drugs (including antibiotics, anti-asthma drugs, hormones, alkaloids, enzyme inhibitors, and β-adrenergic blockers).[1] In particular, we dedicated our attention to the phenylpropanolamine (PPA) isomers and their analogues; due to the intrinsic presence of two stereocenters, in general, four PPA stereoisomers exist: (1*S*,2*S*)-norpseudoephedrine (L-NPE), known also as cathine, (1*R*,2*R*)-norpseudoephedrine (D-NPE), (1*S*,2*R*)-norephedrine (D-NE) and (1*R*,2*S*)-norephedrine (L-NE), as shown in Figure 1.

Nor(pseudo)ephedrines (N(P)Es) are vicinal amino alcohols belonging to the amphetamine and phenethylamine class of *Ephedra* alkaloids. They express a sympathomimetic function and can mimic adrenaline in the human body by acting both as non-selective adrenergic receptor agonists and norepinephrine reuptake inhibitors.[2–5] In the last century, they have been prescribed for mydriasis induction, blood pressure stabilization, nasal decongestion, appetite suppression, and cold or flu therapies.[6] However, in the first years of the 2000s, FDA warned against the extended use of these drugs which seemed to increase the risk of haemorrhagic stroke; therefore, phenylpropanolamine was removed from the over-the-counter drug list in most countries, but it is still available on prescription for

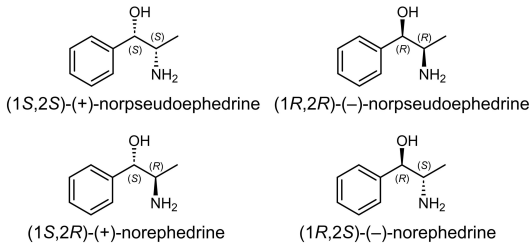

Figure 1. Possible phenylpropanolamine (PPA) isomers.

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Supporting information for this article is available on the WWW under https://doi.org/10.1002/cctc.202301199

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several applications.[7] In Germany, cathine, present in Alvalin® as the active ingredient, can be sold as a short-term appetite suppressant. Besides, these molecules can be also used as valuable intermediates and chiral building blocks for organic synthesis.[1] Indeed, the PPAs motif is actually contained in the structure of many approved drugs,[8] as shown in Figure 2.

Several chemical asymmetric syntheses of both NE and NPE stereoisomers can be found in the literature; however, they often involve long multi-step procedures difficult to scale up, in which high yields and optical purities at the same time are quite difficult to obtain. Of the four compounds, only cathine is produced naturally by extraction from the khat shrub (*Catha edulis*),[9] a plant growing in central Asia and eastern Africa. Therefore, the development of efficient and stereoselective methods for the production of each stereoisomer of N(P)Es is highly desirable.[10]

In particular, different synthetic enzyme cascades were considered valuable alternative routes for the stereoselective production of these compounds, since the isolation of by-products and reaction intermediates can be circumvented.[11–16]

For instance, a biocatalytic one-pot two-steps process was exploited to obtain (1*S*,2*S*)-NPE or (1*R*,2*S*)-NE by combining an (*S*)-selective amine transaminase (ATA) from *Chromobacterium violaceum* (Cv-ATA), with either an (*S*)- or (*R*)-selective alcohol dehydrogenase (ADH), starting from 1-phenylpropane-1,2-dione as substrate and obtaining high levels of optical purity.[11] Moreover, (1*S*,2*S*)-NPE and (1*R*,2*R*)-NPE were also synthesized through the conversion of *trans*- or *cis*-β-methylstyrene into 1-phenylpropane-1,2-diols, obtained by coupling a styrene monooxygenase with either an (*S*)- or (*R*)-selective epoxide hydrolase, followed by biocatalytic hydride-borrowing amination using an ADH along with an amine dehydrogenase.[17] In a recent example, all four N(P)E isomers were prepared through a bi-enzymatic one-pot reaction based on the ADH-catalysed conversion of 1-phenylpropane-1,2-diol to (*R*)- and (*S*)-phenylacetylcarbinol (PAC), followed by a transamination catalysed by

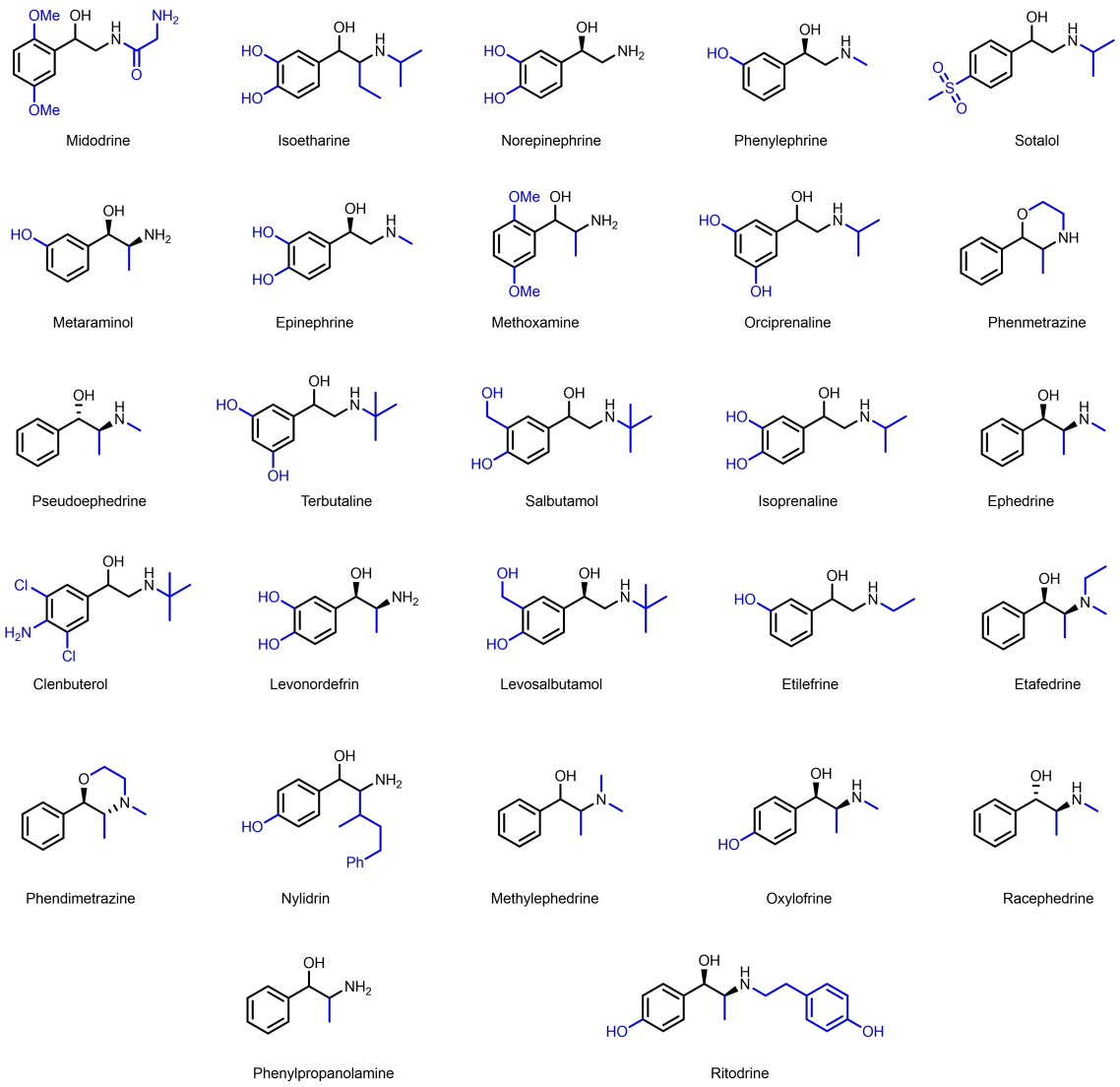


Figure 2. Approved drugs containing the phenylpropanolamine or phenylethanolamine motif.

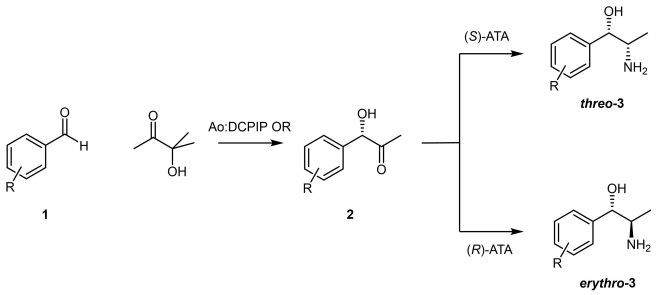
either (*S*)- or (*R*)-ATAs.[13] The cofactor and alanine are internally recycled by an alanine dehydrogenase.

An alternative strategy proposed some years ago by Rother's group involves the use of a thiamine diphosphate (ThDP)-dependent acetohydroxyacid synthase I (AHAS-I) in combination with either an (*S*)- or (*R*)-selective ATA.[14] The first enzyme catalyses the condensation of pyruvate with benzaldehyde accompanied by the release of CO_2 and formation of (*R*)-phenylacetylcarbinol ((*R*)-PAC) with high stereoselectivity (e.e. > 98%), which can be in turn converted to (1*R*,2*R*)-NPE or (1*R*,2*S*)-NE by a transamination catalysed by transaminases. Since alanine is employed as the amine donor for the transamination reaction, pyruvate is obtained as a by-product and can be recycled for the first reaction step. From the combination of AHAS-I with the (*S*)-selective enzyme Cv-ATA, conversions exceeding 80% were achieved, whereas by coupling AHAS-I with the (*R*)-selective ATA from *Aspergillus terreus* (At-ATA), conversions higher than 96% were obtained, in both cases with excellent stereoselectivity (e.e. > 99%). Furthermore, in a later work, the AHAS/ATA cascade was exploited (using isopropyl amine as amine donor instead of alanine) to synthesize the (NE) analogue 2-hydroxy-(NE) and obtain the stereoisomers of 1,2,3,4-tetrahydroisoquinoline-4,6-diol through an additional (bio)catalysed step.[18]

To access to (1*S*,2*R*)-NE and (1*S*,2*S*)-NPE, the combination of a carboligation step, starting from benzaldehyde and pyruvate yielding (*S*)-PAC, with a transamination reaction was previously performed using an engineered variant of the *Acetobacter pasteurianus* pyruvate decarboxylase (ApPDC-E469G) and either (*S*)-selective Cv-ATA or (*R*)-selective At-ATA, but because of the low stereoselectivity showed by the carboligating enzyme, N(P)E products were obtained with low d.e. (70%).[11] Subsequently, further protein engineering of ApPDC lead to a triple variant (ApPDC-E469G/I468A/W543F) with significantly improved stereoselectivity in the synthesis of (*S*)-PAC.[16] This rationally designed biocatalyst was also recently applied to the synthesis of methoxamine (2,5-dimethoxy-N(P)E) stereoisomers in 1-pot 2-step cascade reactions. Specifically, overall conversions up to 80% and excellent selectivity were obtained starting from pyruvate and 2,5-dimethoxybenzaldehyde, and using either AHAS-I or the ApPDC variant to get the intermediates (*R*)- or (*S*)-2,5-dimethoxy-PAC, then used as substrates by either a (*R*)-selective or a (*S*)-selective ATA in the following transamination reaction with isopropylamine as amine donor.[19]

Inspired by these approaches, in this work, we aimed to synthesize a series of (1*S*)-N(P)E analogues through their corresponding (*S*)-PAC derivatives by a sequential bi-enzymatic reaction: in the first step, a biocatalysed benzoin-type condensation mediated by the ThDP-dependent acetoin:dichlorophenolindophenol oxidoreductase (Ao:DCPIP OR) transforms an aromatic aldehyde (**1**) into the corresponding (*S*)-arylacetylcarbinol (**2**); then, an (*S*)- or (*R*)-ATA converts the intermediate **2** into the corresponding N(P)E analogue (*threo*-**3** or *erythro*-**3**) depending on the enzyme stereospecificity (Scheme 1).

The first biocatalyst involved in the proposed synthesis, i.e. Ao:DCPIP OR (EC 2.3.1.190), is an interesting ThDP-dependent enzyme discovered some years ago from *Bacillus*



Scheme 1. Biocatalysed stereoselective synthesis of (1*S*)-nor(pseudo)ephedrine analogues.

licheniformis,[20,21] that plays a key role in the bacterial degradation of acetoin in the acetoin dehydrogenase enzyme system.[22] In fact, this protein catalyses *in vivo* the oxidative cleavage of acetoin releasing acetaldehyde and transferring the acetyl anion equivalent to the lipoamide cofactor of the second enzyme of the system.

However, most interestingly, this oxidoreductase can also employ the activated acetyl group for *in vitro* carboligation with a further carbonyl compound introduced as a cosubstrate, yielding the corresponding α-hydroxyketone (see Scheme S1, Supporting Information, for a comparison of physiological and biocatalytic routes). Previous studies showed that Ao:DCPIP OR is a broad scope (*S*)-specific wild-type enzyme promoting this type of asymmetric cross-carboligation, with good stereoselectivity in the case of various chiral products.[20]

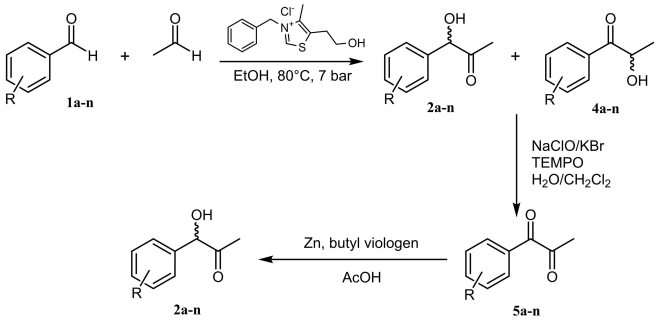
In particular, this enzyme can accept different acetyl anion precursors, among which methylacetoin (3-hydroxy-3-methylbutan-2-one) proved to be particularly advantageous in terms of minimization of the homocoupling byproduct.[23] The enzyme selectivity is quite relaxed also for what concerns the acyl acceptor, often showing remarkable results in terms of yields and enantiomeric excess, even on substrates that are sterically demanding such as 1-naphthaldehyde and 4-(*tert*-butyl)benzaldehyde. Recently, this enzyme has been also employed in two-step cascade reactions with a NADH-dependent reductase affording enantiomerically enriched 1,2-diols in good yields, thus showing good compatibility in multienzymatic systems.[24] For these reasons, we believed it to be particularly suitable for the synthesis of variously substituted (*S*)-PAC analogues and further multistep biocatalytic applications.

The second step of the proposed synthesis, i.e. the conversion of the α-hydroxyketone into the 1,2-aminoalcohol, is mediated by an amine transaminase (ATA) of chosen selectivity. These pyridoxal-5′-phosphate (PLP)-dependent enzymes, especially in the last decade, have attracted a lot of attention due to their effectiveness and flexibility in the transamination of ketones.[25,26] So far, many ATAs have been discovered, characterised and efficiently applied to organic synthesis often showing excellent stereoselectivity and tolerance to organic cosolvents.[26–28] Hence, ATAs are a natural choice for promoting the conversion of our intermediates into the target products, also in consideration of their diverse stereoselectivity. Seven

ATAs (three (*R*)-selective and four (*S*)-selective) from our in-house collection, including highly thermostable ATAs,[29–31] were screened on the (*S*)-PAC analogues coming from the first step and the resulting mixture was analysed to determine conversion and diastereoselectivity.

Results and Discussion

As stated in the Introduction, the aim of the present work was to synthesize a series of analogues of norpseudoephedrines/norephedrines (N(P)Es) through a sequential bi-enzymatic reaction. In particular, the first part of this study was focused on the enzymatic synthesis of (*S*)-phenylacetyl carbinol ((*S*)-PAC) analogues starting from substituted benzaldehydes. To provide reference material for evaluating the performances of the carboligation reactions, the chemical synthesis of racemic PAC and analogues was preliminarily studied. Access to the desired products resulted to be not trivial and optimized procedures were defined by exploiting a pressurized and thermostated continuous-flow reactor. In the second part of this study, a screening of different (*S*)- and (*R*)-selective amine transaminases against the obtained intermediates was carried out in order to obtain variously substituted N(P)Es, possibly with high stereoselectivity.



Scheme 2. Chemical synthesis of racemic **2a–n**.

Chemical synthesis of racemic PAC and analogues

The synthetic sequence for racemic PAC and analogues (**2a–n**, see structures in the following paragraph) is based on the condensation between the opportune aromatic aldehyde and acetaldehyde and consists of three steps (Scheme 2). The first step is an acyloin condensation promoted by a *N*-heterocyclic carbene (*N*HC) catalyst that is formed *in situ* from a thiazolium salt (3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride) and a base (triethylamine, TEA).[32]

Since the *N*HC catalyst is not chiral, the products obtained were racemic. In addition, the chemoselectivity was often low and the resulting mixture generally included both homocoupling (acetoin and benzoin derivatives) and cross-coupling products.

Hence, a stoichiometric excess of acetaldehyde was employed to suppress the formation of benzoin derivatives at the cost of the increased presence of acetoin in the final mixture. Nevertheless, this product is relatively volatile and subject to removal under high-vacuum. Therefore, the final mixture - after workup - is simply a combination of the racemates of the two regioisomers obtained by the cross-coupling of the substrates (**2a–n** and **4a–n**, Scheme 2). In order to achieve an acceptable reaction rate, the reaction temperature should be 80 °C or higher; in these conditions, under atmospheric pressure, acetaldehyde would quickly evaporate and escape the reaction environment. While in the original work a sealed tube was employed to address this issue, here we exploited instead a pressurized and thermostated continuous-flow reactor, which ensures simplicity of use, easy scalability and the possibility to continuously monitor the reaction (Figure 3).

Since the reactivity of the employed aldehydes towards the benzoin-type condensation was heavily dependent on the nature of the aldehyde itself, a three-way valve was installed after the backpressure regulator to switch between *recirculation mode* if the reaction was not complete and *collection mode* when all the aromatic aldehyde **1a–n** was converted. This synthetic step worked for all the substrates with the exception of hydroxy compounds **4f** and **2g**, which gave several degradation products and needed a different strategy (see later in the text). Even if the hydroxyketones **2a–e**, **h–n** generally

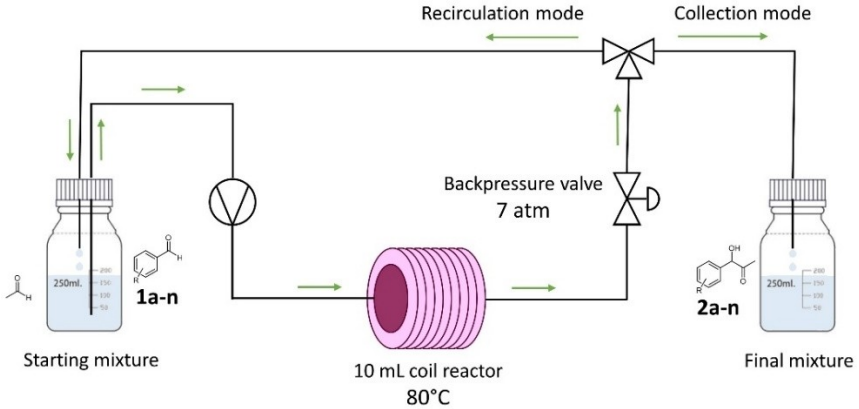


Figure 3. Diagram of the setup employed for the chemical benzoin-type condensation.

constituted the major components of the final mixture, their separation from the other components, in particular from their homocoupling isomers **4a**–**e**, **h**–**n**, was sometimes too difficult to afford an analytical standard of sufficient purity. Therefore, two further passages were introduced to create a regioconvergent synthesis leading to the exclusive formation of the desired **2a**–**e**, **h**–**n** products.

In the second step, the product mixture was oxidized employing sodium hypochlorite and TEMPO [(2,2,6,6-tetramethylpiperidin-1-yl)oxyl] in a vigorously stirred water/dichloromethane biphasic mixture,[33] resulting in the production of the corresponding arylpropanedione **4a**–**e**,**h**–**n** as the sole product.

The last step is the regioselective reduction of the intermediate to the racemic arylacetyl carbinol **2a**–**e**, **h**–**n** by means of an excess of zinc dust in glacial acetic acid at room temperature.[34] In the case of particularly recalcitrant substrates, a catalytic amount of butyl viologen has been added to the reaction mixture to promote the selective formation of the product.[35]

The hydroxy compounds **2f** and **2g** proved to be particularly challenging substrates. After many tries, **2f** was successfully synthesised by employing the Rovis triazolium catalyst,[36] whereas for racemic **2g** it was necessary to racemise the enantiopure compound obtained from the enzymatic synthesis (see Supporting Information for the procedures).

Enzymatic synthesis of (*S*)-PAC analogues on analytical scale

The performances of Ao:DCPIP OR in the enzymatic carboligation reactions with a series of *ortho*-, *meta*-, and *para*-substituted benzaldehydes (**1b**–**m**) and a thiophene analogue (**1n**) (Figure 4) were compared to those obtained with the reference substrate benzaldehyde (**1a**).

Preliminary experiments using a slight excess (1.3 equiv.) of methylacetoin were monitored on analytical scale (reaction volume: 2 mL) to investigate if and how selectively the substrates were converted into the corresponding (*S*)-PAC analogues (**2a**–**n**, Scheme 3).

The results of the biotransformations, obtained from GC-MS analyses, are shown in Figure 4. Both the electronic properties and the position of the substituent on the aromatic ring seem to affect the performances of the cross-benzoin process. In general, the most common byproduct was identified as the corresponding diketone **5a**–**n**, whose production is responsible for the difference between conversions and yields. Chlorobenzaldehydes (**1b** and **1c**), tolualdehydes (**1h**–**j**), 2-hydroxybenzaldehyde (**1f**) and trifluoromethylbenzaldehydes (**1k**–**m**) displayed good performances with high conversions (88–99%) and yields (80–97%). 4-Hydroxybenzaldehyde **1g** displayed a slightly lower performance with both conversion and yield around 60%. The other compounds resulted to be poorer substrates: aldehyde **1d** showed high conversion but low yield, while the full consumption of substrate **1e** led to the formation of a complex reaction mixture not containing the expected PAC derivative. Finally, the reaction on substrate **1n** resulted in neither high conversion nor selectivity. For these reasons, substrates **1e** and **1n** were excluded from the reaction scale-up performed in view of the second enzymatic step.

Preparative synthesis of (*S*)-PAC and analogues

At this point, the hydroxyketones (*S*)-**2a**–**d**, **f**–**m** were prepared in higher amounts to provide material for the transamination reactions. Conversions and yields were similar to those obtained on a smaller scale, thus confirming the previously obtained data. It is worthwhile to highlight that hydroxy compounds (*S*)-**2f** and (*S*)-**2g** were synthesised and isolated without any

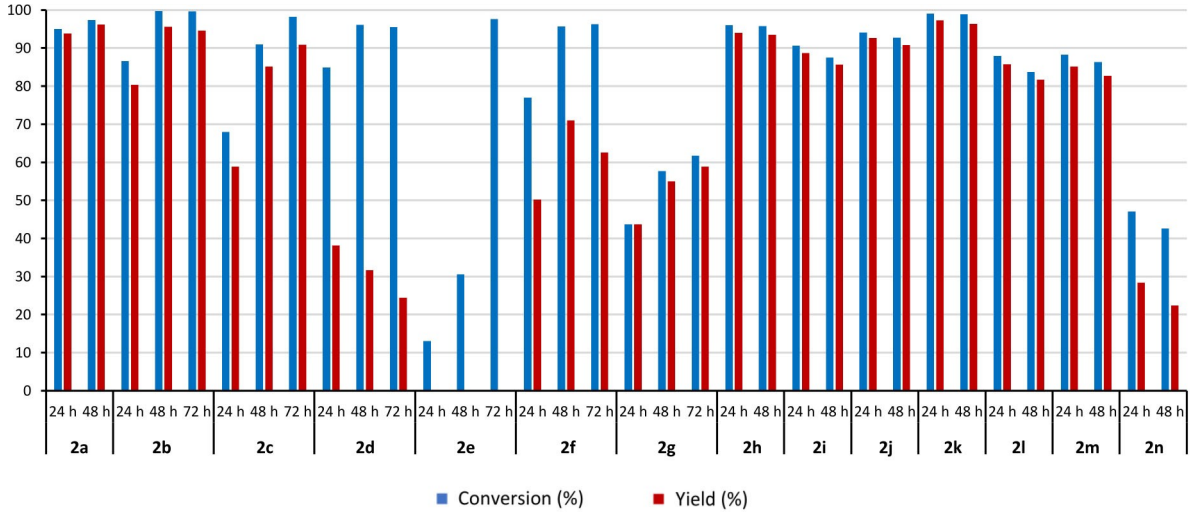
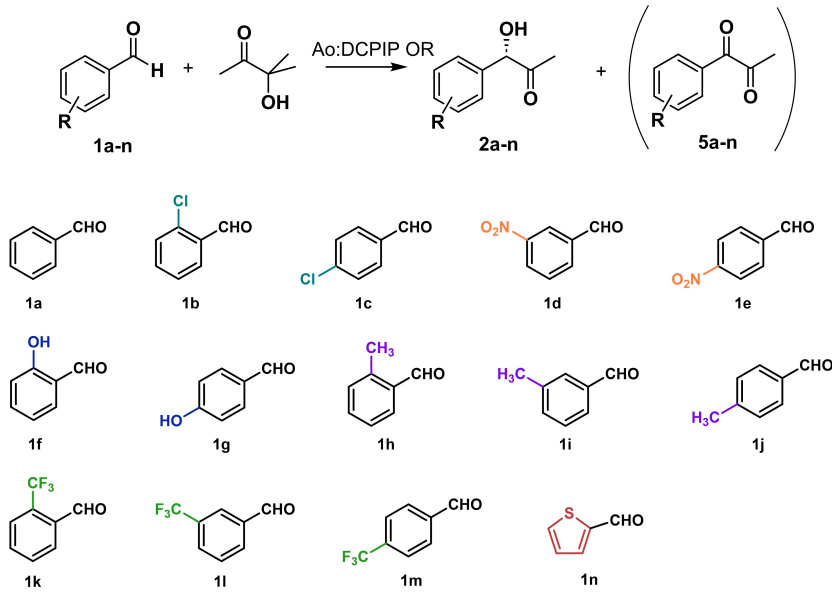


Figure 4. Results obtained in the cross-acyloin condensation between methylacetoin and aldehydes **1a**–**n** in function of reaction time. Reaction conditions: 30 °C, 50 mM phosphate buffer, pH 6.5, 10% (*v*/*v*) DMSO, 20 mM aldehyde, 26 mM methylacetoin, 0.4 mM ThDP, 0.9 mM $MgSO_4$, 0.25 mg mL^{-1} Ao:DCPIP OR, final volume: 2 mL. The conversion has been calculated as [products]/([products] + [limiting reagent]), whereas the yield has been evaluated as [expected product]/([limiting reagent] + [expected product]).



Scheme 3. The biocatalysed carboligation reaction with the possible products and the employed aldehydes.

particular issue, in contrast with the attempted chemical syntheses (see before). Therefore, the obtained compounds were purified by column chromatography in order to get rid of the byproducts which would act as alternative substrates for the ATAs in the successive step, thus complicating the reaction outcome. The results are summarised in Table 1. The absolute configuration was assigned to the obtained hydroxyketones based on the literature data for products **2a**–**j**,[20] whereas products **2k**–**m** (trifluoromethyl-substituted PACs) were analogously labelled as (*S*) given their high similarity with the other substrates. Moreover, the results obtained after the introduction of an additional stereogenic centre in the subsequent step were adequately coherent with the proposed assignment.

Table 1. Results of preparative carboligation step. Reaction conditions: 30 °C, 50 mM phosphate buffer, pH 6.5, 10 % (*v*/*v*) DMSO, 20 mM aldehyde, 26 mM methylacetoin, 0.4 mM ThDP, 0.9 mM $MgSO_4$, 24 h, 0.25 mg mL^{-1} Ao:DCPIP OR, final volume: 25 mL. Reaction parameters determined by GC-MS. Conversion = [products]/([products] + [limiting reagent]); Yield = isolated yield; e.e. determined by HPLC.

Product	Conversion (%)	Yield (%)	e.e. (*S*) (%)
(*S*)-**2a**	95	84	96
(*S*)-**2b**	>99	96	86
(*S*)-**2c**	98	91	65
(*S*)-**2d**	96	31	82
(*S*)-**2f**	77	69	94
(*S*)-**2g**	44	35	99
(*S*)-**2h**	96	91	90
(*S*)-**2i**	95	75	96
(*S*)-**2j**	94	76	95
(*S*)-**2k**	>99	97	87
(*S*)-**2l**	94	83	92
(*S*)-**2m**	98	86	87

Enzymatic production of (1*S*,2*S*)-NPE, (1*S*,2*R*)-NE and analogues

In this phase, the hydroxyketones (*S*)-**2a**–**d**, **f**–**m** were aminated using (*S*)/(*R*)-ATAs from different origins and either (*S*)- or (*R*)-phenylethylamine (PEA) as amine donor according to ATAs selectivity. The reactions were monitored by GC-MS. First of all, a preliminary screening was carried out to identify ATAs from our in-house collection (see Supplementary Material, Table S2) able to catalyse the biotransformation of (S)-**2a** and find suitable reaction conditions.

The reactions were performed using different buffers (borate, phosphate and HEPES), different pH values (according to ATAs' optimum pH), as well as different reaction temperatures (according to ATA thermophilicity). The ATAs from our library that have been already described as active toward (*S*)-**2a**, (i.e., Cv-ATA and At-ATA) were not included in this first screening and were directly used for the conversion of (*S*)-**2a**–**d** and (*S*)-**2f**–**m** under the same reaction conditions described in [11].

As shown in Figure 5, among the (*S*)-selective ATAs, Sbv333-ATA showed the best reaction conversions (Table S4), while the two (*R*)-selective ATAs, i.e., ATA117 and Nf-ATA, were comparably highly active in the biotransformation of **2a**. In general, the effect of the buffers on reaction conversions was modest with slightly better outcomes when using HEPES, while a detrimental effect of borate buffer on Sbv333-ATA activity was observed. Moreover, in agreement with the previously described thermostability,[30,31] higher temperatures did not affect B3-ATA and Is3-ATA performances.

Vf-ATA reaction, showing promising results, was further optimized and, surprisingly, performing the reaction in HEPES buffer at pH 7.5 significantly improved the Vf-ATA-catalysed biotransformation with conversions around 90 %. As a result of this study, ATA117, Nf-ATA, Vf-ATA and Sbv333-ATA, together

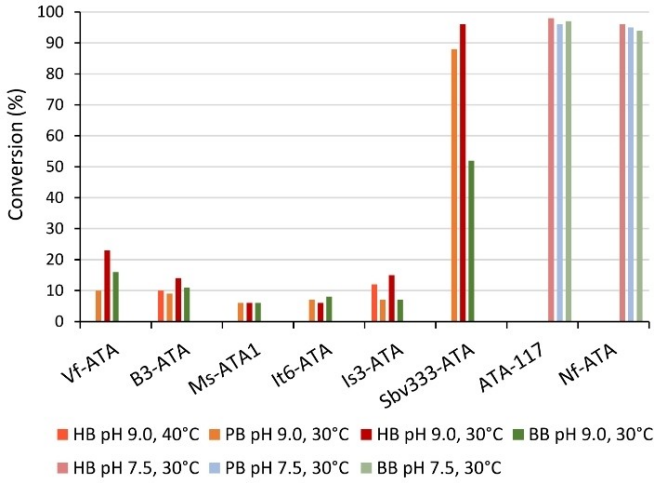


Figure 5. Synthesis of (1*S*,2*S*)-NPE and (1*S*,2*R*)-NE starting from (*S*)-PAC and (*S*)/(*R*)-PEA: effect of medium and temperature on the reaction conversion. PB: 0.1 M phosphate buffer, HB: 0.1 M HEPES buffer, BB: 0.1 M borate buffer. Reaction time: 24 h.

with Cv-ATA and At-ATA previously described as active toward (*S*)-**2a**, (Table 2) were selected and screened on each substrate (**2a**–**d** and **2f**–**m**) and the products were analysed by GC-MS in order to characterise the reaction outcome (Figure 6 and Table S5).

In particular, the conversions were calculated based on (*R*)- or (*S*)-PEA transformed into acetophenone, whereas the diastereomeric excess (d.e.) was evaluated from the peak areas of the product in GC-MS.

(*R*)-ATAs display in most cases both high conversions and diastereoisomeric excesses, whereas (*S*)-ATAs show a broader range of results: Sbv333-ATA is usually the most active, albeit often not the most selective, while Cv-ATA and Vf-ATA show similar behaviours with the same substrate with practically absolute diastereoselectivity in the case of **3b** and **3h**, and higher than 91% for products **3h**–**j**. This excellent diastereoisomeric excesses in the presence of incomplete conversions may be explained by a potential supplementary enantioselectivity of the enzyme which preferentially accepts the (*S*)-PAC leaving the (*R*)-PAC mostly unreacted.

Table 2. Amine transaminases employed for the biotransformation of hydroxyketones **2a**–**d** and **2f**–**m**.

Abbreviation	Origin	Selectivity	Employed pH
At-ATA	*Aspergillus terreus*	*R*	7.5
Nf-ATA	*Neosartorya fischeri*	*R*	7.5
ATA117	*Arthrobacter* sp. KNK168	*R*	7.5
Cv-ATA	*Chromobacterium violaceum*	*S*	7.5
Vf-ATA	*Vibrio fluvialis*	*S*	7.5
Sbv333-ATA	*Streptomyces* sp.	*S*	9.0

Preparative synthesis of (1*S*,2*S*)-NPE, (1*S*,2*R*)-NE and analogues

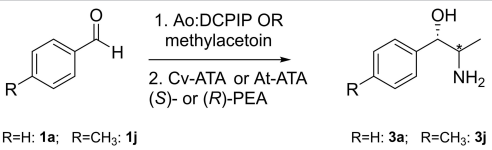
Given the good results obtained, we decided to conduct some preparative synthesis in order to test the bi-enzimatic sequence on a larger scale. In particular, we were able to transform the substrates **1a** and **1j** into the corresponding (*S*)-PACs **2a** and **2j**. We then employed two enzymes with opposite selectivity (Cv-ATA and At-ATA) to convert the intermediates either into the relative NPEs (*threo*-**3a** and *threo*-**3j**) or the respective NEs (*erythro*-**3j** and *erythro*-**3j**) with excellent stereoselectivity (Table 3). In these cases, the absolute configurations of the final products were confirmed using ^{1}H NMR, as the two possible products, being diastereomers, have distinct spectra. Furthermore, also the overall diastereomeric excesses were measured by ^{1}H NMR (see the Supporting Information). Since the final d.e. values are practically identical to the e.e. of the intermediates, it is possible to state that the employed ATAs showed a very high stereoselectivity in creating the new chiral center.

Conclusions

In this work, a series of analogues of nor(pseudo)ephedrines with specific stereochemistry, i.e., (1*S*,2*S*)- and (1*S*,2*R*)-N(P)Es, was synthesized through a two-step carboligation/transamination procedure catalysed by the (*S*)-selective enzyme Ao:DCPIP OR and by an amine transaminase (ATA) with (*S*)- or (*R*)-selectivity, respectively.

At first, in order to provide reference material for the stereochemical analysis of the products obtained through the biocatalysed carboligation, we developed a novel protocol for the synthesis of the corresponding racemates where the key step is an organocatalysed benzoin condensation. In particular, a thiazolylidene catalyst directly obtained from the deprotonation of an inexpensive and commercially available thiazolium salt (3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride) was chosen due to its high activity, stability, negligible toxicity and easiness of use.[37] Moreover, its structural similarity with the ThDP cofactor provides a suggestive comparison between

Table 3. Results of preparative bi-enzymatic sequence. Y1: isolated yield of the carboligation step. The e.e.(*S*) are related to the intermediate phenylacetylcarbinol and were measured by HPLC. Y2: isolated yield of the transamination step. The d.e. values were measured by ^{1}H NMR.



R=H: **1a**; R=CH_3: **1j** R=H: **3a**; R=CH_3: **3j**

Product	Y1 (%)	e.e. (*S*) (%)	ATA	Y2 (%)	d.e. (%)
threo-**3a**	84	96	Cv-ATA	46	97
erythro-**3a**			At-ATA	54	98
threo-**3j**	76	95	Cv-ATA	72	95
erythro-**3j**			At-ATA	50	95

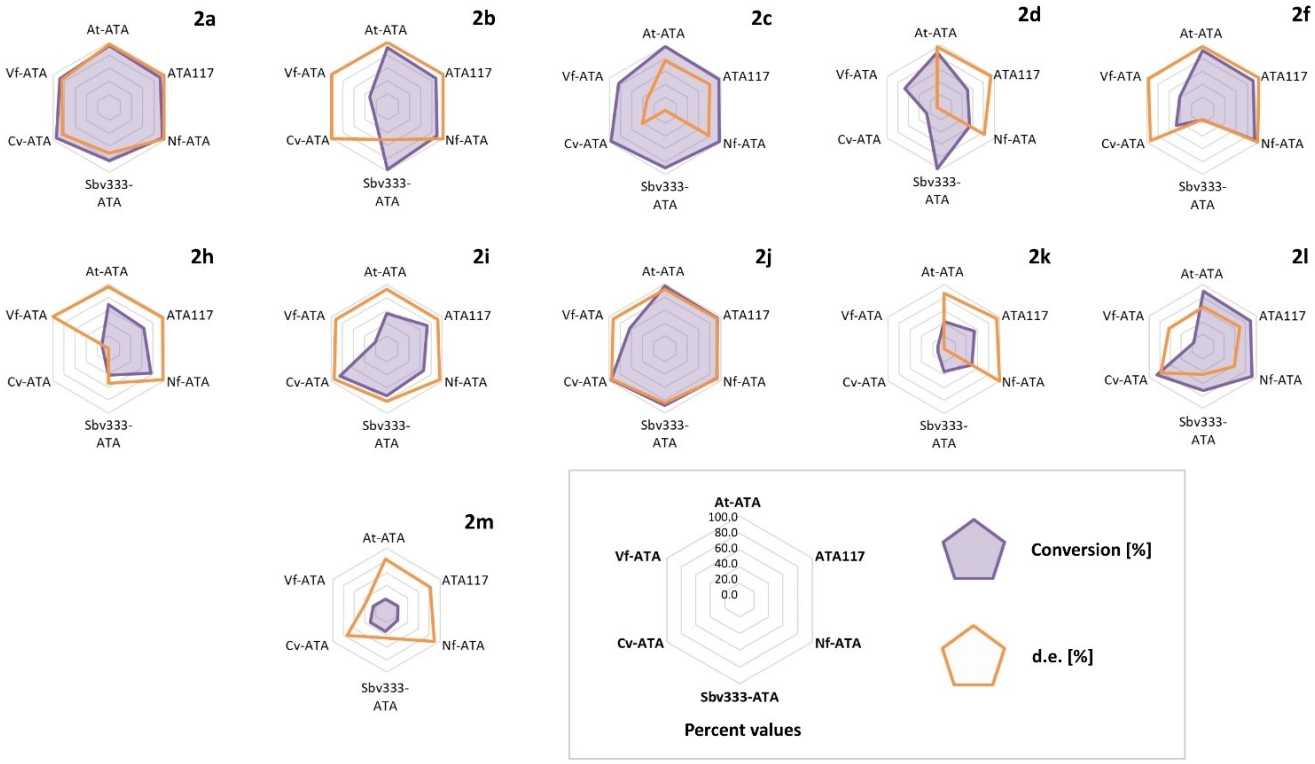


Figure 6. Outcome of ATAs-catalyzed transaminations after 24 h. Reaction conditions: 100 mM HEPES buffer, pH 7.5 or pH 9.0, 2% (*v*/*v*) DMSO, 10 mM hydroxyketone, 10 mM (*R*)- or (*S*)-PEA, 0.25 mM PLP, 1 mg mL^{-1} enzyme, final volume: 0.5 mL. The d.e. values were in all cases calculated as ([major diastereomer]-[minor diastereomer])/([major diastereomer] + [minor diastereomer]) regardless of the different enzyme stereoselectivity. If the conversion was < 10%, the d.e. was not calculated.

the organocatalysed and the biocatalysed reactions. The main element of novelty is constituted by a pressurized continuous-flow setup that was employed to overcome the limitations due to the acetaldehyde volatility in the reaction conditions. In the present work, benzaldehyde (**1a**), *ortho*-, *meta*-, and *para*-substituted benzaldehydes (**1b**–**m**) and a thiophene analogue (**1n**) were employed as substrates in biocatalysed carboligation reactions with methylacetoin yielding the PAC analogues. Reactions were first performed on analytical scale and incubated for up to 72 h, a reaction conversion plateau being generally reached in 24–48 h in agreement with previous studies.[20] The results confirmed the broad substrate scope of this biocatalyst, which yielded, among others, products **2k**–**n** with conversions ranging from 30–50% in the case of **2n** to 80–99% for **2k**–**m**. Remarkably, to the best of our knowledge, substituted benzaldehydes **1k**–**m** and thiophene analogue **1n** have been never tested before as substrates of Ao:DCPIP OR or other (*S*)-selective carboligases. On the contrary, products **2a**–**c**,**h**–**j** were already obtained using variants of the *Acetobacter pasteurianus* pyruvate decarboxylase with high conversion, but with e.e. not always satisfactory (e.e > 95% for **2a** and **2h**–**i**, around 50% for **2b** and **2j**, < 5% for (S)-**2c**.[16]

On the basis of this preliminary screening, reactions aimed at the formation of **2a**–**d**, **f**–**m** were scaled up and products were obtained with yields up to 97%.

Finally, a library of derivatives of (1*S*,2*S*)- and (1*S*,2*R*)-nor(pseudo)ephedrines was synthesized starting from the PAC analogues and using either (*S*)- or (*R*)-ATAs. A preliminary screening was carried out in the search of ATAs from our in-house collection able to catalyse the biotrasformation of (*S*)-**2a** into the corresponding vicinal amino-alcohols under suitable reaction conditions. Different buffers and different pHs were tested as well as different reaction temperatures. HEPES buffer was chosen for further experiments as it allowed in most cases the best enzyme performances. Curiously, despite Vf-ATA displays an alkaline pH optimum,[38] this enzyme showed best performances at pH 7.5. In general, high conversions and high d.e. values were obtained with both the screened (*R*)-selective-ATAs and the (*S*)-selective transaminases Cv-ATA and Vf-ATA. Instead, Sbv333-ATA gave high conversions, but d.e. values were not fully satisfactory (80%).

The ATAs screening allowed the selection of a set of biocatalysts to be used in the synthesis of compounds **3b**–**d, 3f** and **3h**–**m**. The results heavily depend on the specific substrate but, as a general trend, (*R*)-ATAs display better performances, whereas (*S*)-ATAs are strongly influenced by the presence of substituents on the aromatic ring. Nonetheless, high conversions were achieved with both Sbv333-ATA and Cv-ATA when testing 4-Cl-PAC (**2c**) and 2- and 3-methyl-PAC (**2i, 2j**), while the best diastereoselectivity was indeed obtained with the same two (*S*)-ATAs and 3-methyl-PAC (**2j**) as substrate.

In light of the overall results on the tested PAC derivatives, Sbv333-ATA is, among the novel ATAs investigated in this work, the most promising biocatalyst considering the high conver-

sions obtained with various substrates, thus confirming the broad substrate scope of this recently discovered transaminase.[31] However, further efforts are necessary to improve its performances in terms of stereoselectivity to practically useful levels and investigations are currently running in our labs to this aim.

Therefore, this work paves the way to the development of tailored multienzymatic processes for the preparation of much more sterically hindered N(P)E derivatives, as well as to the enhancement of biocatalysts regio- and stereoselectivity by the rational design of engineered enzyme variants.

Experimental Section

Equipment

All chemicals and solvents were purchased from Fisher Scientific Italia (Rodano, Italy) or Merck Life Science (Milan, Italy) and used without further purification. TLC analyses were performed on Merck Kieselgel 60F_{254} plates.

For the production, purification and characterization of the enzymes, the following instruments were used: Thermoshaker INNOVA 42 (New Brunswick); Sorvall RC6 Plus (Thermo) centrifuge, A.L.C. 4226 centrifuge, Omni-Ruptor 250 sonicator, UV-VIS V-530 (Jasco) spectrophotometer. Ni Sepharose 6 Fast Flow (GE Healthcare) resin was used for enzyme purification (≈4 mL). Washing buffer (500 mM NaCl, 20 mM imidazole, 20 mM KH_2PO_4, pH 7.0), elution buffer (500 mM NaCl, 300 mM imidazole, 20 mM KH_2PO_4, pH 7.0), 20% (*v/v*) ethanol solution were used in the purification step. A peristaltic pump (flow rate: 0.9 mL min^{-1}) and a porous baffle glass column (ø 1 cm, height 10 cm) were used in the purification step.

GC-MS analyses were performed employing a Finnigan TRACE DSQ GC-MS instrument (ThermoQuest, San Jose, CA) equipped with a HP-5MS column (30 m×0.25 mm×0.25 μm, Agilent Technologies Italia S.p.A., Cernusco sul Naviglio, Italy), ion source 250 °C. The employed temperature programs are illustrated in the Supporting Information.

HPLC analyses were performed using an Agilent 1100 device equipped with a thermostated oven and UV detection. Stationary phases: Chiralcel OD-H and Chiralpak IA (250 mm×4.6 mm, 5 μm, Daicel Chiral Technologies, Illkirch, France); Chiral Art amylose-SA (250 mm×4.6 mm, 5 μm, YMC Europe, Dinslaken, Germany). Analytical conditions: see Supporting Information.

^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 MHz spectrometer (Bruker, Billerica, USA) in $CDCl_3$ at room temperature. The chemical shift scale is based on tetramethylsilane.

The continuous-flow reactions were performed using an E-Series Integrated Flow Chemistry system from Vapourtec (Alfatech S.p.A., Genova, Italy) equipped with a 10 mL tubular coil reactor. The chromatographic purifications were carried out on a PuriFlash XS-420+ apparatus (Interchim, Montluçon, France) using Purezza-Daily Standard Flash cartridges (Sepachrom, Rho, Italy).

All the spectrophotometric assays were carried out with a UV-VIS V-730 spectrophotometer (Jasco, Mary's Court Easton, USA).

Preparation of Ao:DCPIP OR and ATAs

Ao:DCPIP OR and amine transaminases were expressed in *Escherichia coli*. *E. coli* strains and plasmids used for enzyme expression are reported in Table S3 (Supporting Information).

Cells from glycerol stocks were streaked on LB-Agar plates containing the appropriate antibiotics (Supporting Information, Table S3) and incubated overnight at 37 °C (30 °C for the growth of Ao:DCPIP OR). The following day some colonies were inoculated in 100 mL of LB containing the required antibiotics. Cells were grown overnight at 37 °C (30 °C for the growth of Ao:DCPIP OR) and 220 rpm.

The pre-cultures were then used for the inoculation of 1 L of LB media, with the necessary antibiotics, and grown at 37 °C (or 30 °C for Ao:DCPIP OR) and 220 rpm.

At scheduled times, the culture optical density at 600 nm (OD_{600}) was spectrophotometrically measured and, when this value was between 0.5 and 1, the proper inducer was added to activate enzyme expression.

Subsequently, cell cultures were incubated at the appropriate temperature of growth at 220 rpm for the appropriate time, as summarized in Table S3 (Supporting Information). Cells were recovered by centrifugation at 5000 rpm and 4 °C for 30 min and resuspended in 20 mL of washing buffer (500 mM NaCl, 20 mM imidazole, 20 mM potassium phosphate buffer, pH 7.0). Cell lysis was performed by 5 cycles of 30 s of sonication on ice followed by a 15 s rest period to avoid sample overheating.

All the enzymes were purified by Immobilized Metal Affinity Chromatography (IMAC) using Ni-NTA resin as the stationary phase. Specifically, the lysates were collected and incubated on ice with Ni-NTA Sepharose resin for 90 min, then the resin was loaded into a glass column and protein elution was carried out by increasing the concentration of imidazole up to 300 mM.

Protein fractions were quantified by using the Bradford method, collected, dialysed overnight in a suitable buffer at 4 °C. The presence of the target proteins in the eluted fractions was verified by SDS-PAGE (10% T, 2.6% C).

Ao:DCPIP OR activity assay

The oxidative cleavage of methylacetoin in the presence of 2,6-dichlorophenolindophenol (DCPIP) was employed as the reference reaction for evaluating the enzyme activity of Ao:DCPIP OR.[21] The decrease in absorbance was followed at 595 nm at 20 °C and the variation of absorbance over time was correlated to the enzyme activity. The assay solution contains 0.2 mM ThDP, 0.4 mM $MgSO_4$, 0.2 mM DCPIP, and 0.1 mM methylacetoin in 50 mM phosphate buffer at pH 7.0. 10 μL of enzyme solution were added to 990 μL of the assay solution in a plastic cuvette (1 mL). Then the cuvette was rapidly shaken, inserted into the instrument and the absorbance was recorded for 200 s. The amount of oxidised substrate was determined considering a molar absorptivity for DCPIP equal to 17,000 $mM^{-1}cm^{-1}$ and the activity was expressed in International Units (1 U = 1 μmol min^{-1}).

Amine transaminases activity assay

The assay solution consisted in 2.5 mM sodium pyruvate (amino acceptor), 2.5 mM (*S*)- or (*R*)-phenylethylamine (PEA, amino donor), depending on the enzyme selectivity, 50 mM phosphate buffer, pH 8.0 and 0.25% (*v/v*) DMSO. The pH was adjusted to either 9.0 or 7.5 (see Table 2). The assay was started by adding 3–5 μL of enzyme

solution into 800 μL of assay solution in a quartz cuvette (1 mL). The cuvette was rapidly shaken and the absorbance at 245 nm was measured for 60 s. The activity was determined considering for the reaction product (acetophenone) a molar absorptivity equal to 12,000 $mM^{-1}\,cm^{-1}$.

Enzymatic synthesis of PAC and analogues

Analytical scale: the aromatic aldehyde (**1a–m**, 40 μmol) and methylacetoin (5.2 μL, 1.3 equiv.) were solubilized in 200 μL DMSO. The mixture was poured into a solution of 50 mM phosphate buffer at pH 6.5 (total reaction volume 2 mL) with ThDP (0.36 mg, 0.83 μmol) and $MgSO_4$ (0.23 mg, 1.6 μmol). Then, lyophilized Ao:DCPIP OR (0.5 mg) was added. Final concentrations of the reagents were as follows: 0.4 mM ThDP, 0.9 mM $MgSO_4$, 20 mM substituted benzaldehyde, 26 mM methylacetoin, 10% (*v/v*) DMSO. The reaction mixture was gently shaken (100 rpm) at 30 °C, and after 24, 48 or 72 h samples (0.2 mL) were withdrawn and extracted with AcOEt. Organic phases were then dried and analysed by GC-MS.

Preparative scale: the substituted benzaldehyde (**1a–d**, **1h–m**, 0.5 mmol) and methylacetoin (66.4 μL, 1.3 equiv.) were solubilized in 2.5 mL DMSO. The mixture was poured into 50 mM phosphate buffer at pH 6.5 (total reaction volume: 25 mL) with ThDP (4.5 mg, 10 μmol) and $MgSO_4$ (2.7 mg, 20 μmol). Then, Ao:DCPIP OR (0.5 U) was added. Final concentrations of the reagents were as follows: 0.4 mM ThDP, 0.9 mM $MgSO_4$, 20 mM substituted benzaldehyde, 26 mM methylacetoin, 10% (*v/v*) DMSO. The reaction mixture was gently shaken (100 rpm) at 30 °C until a conversion > 90% was reached (24–96 h depending on the substrate), then the reaction mixture was extracted three times with AcOEt. Organic phases were recovered and dried over Na_2SO_4, evaporated, and purified by column chromatography (hexane:AcOEt) on silica.

The GC-MS retention times are reported in the Supporting Information together with the isolated yields.

Chemical synthesis of PAC (2a)

Step 1: ligation. After dissolving the pre-catalyst 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride (0.51 g, 0.1 equiv.) in EtOH (5 mL), the aromatic aldehyde (**1a**, 2 g, 18.9 mmol), acetaldehyde (5.26 mL, 5 equiv.) and triethylamine (1.57 mL, 0.6 equiv.) were added in an ice bath at 0 °C. In the meanwhile, the flow-chemistry apparatus was conditioned using EtOH as a solvent and brought to the reaction conditions (flow rate: 0.17 mL min^{-1}; counter pressure: 7 atm; reactor temperature: 80 °C) until no formation of bubbles was observed along the tubes. The homogeneous reagent mixture (still kept in an ice bath) was then pumped in using a 0.17 mL min^{-1} flow rate for 1 h, at 80 °C and 7 atm.

Most of the solvent was then evaporated, the residue was redissolved in a AcOEt:HCl (0.1 M) mixture and an extraction was performed. The aqueous acidic solution was further extracted with AcOEt, the organic fractions were brought together, washed against a NaCl saturated solution, then separated, dried over Na_2SO_4, and evaporated. A mixture of regio-isomeric racemates (**2a/4a**) was obtained.

Step 2: oxidation. A solution of 1 M NaOCl was prepared and the pH was adjusted to 8.9 with $NaHCO_3$. The mixture (**2a/4a**, 1 g, 6.7 mmol) was solubilized in 10 mL of CH_2Cl_2, TEMPO ((2,2,6,6-tetramethyl- piperidin-1-yl) oxyl, 11 mg, 0.01 equiv.) and KBr (80 mg, 0.1 equiv.) were added followed by 1.5 mL of distilled water at 0 °C in an ice bath under vigorous stirring. The NaOCl solution (8.4 mL, 1.25 equiv.) was slowly added to the reaction mixture through a dropping funnel until the complete disappearance of the reagents (TLC on silica, hexane:AcOEt = 8:2). If needed, extra NaOCl solution was added. The solution was then separated and extracted three times with CH_2Cl_2; the reunited organic phases were washed against a $NaHSO_3$ solution, dried over Na_2SO_4 and evaporated. The crude mixture was finally purified by column chromatography on silica gel (hexane:AcOEt), yielding pure phenylpropanedione (**5a**).

Step 3: reduction. Isolated product **5a** (0.626 g, 4.2 mmol) dissolved in 2 mL of acetic acid was added to a well-stirred mixture of freshly activated zinc dust (1.38 g, 5 equiv.) in 6 mL of acetic acid at rt and left to react for at least 12 h. The reaction was monitored by TLC (hexane:AcOEt = 8:2) and GC-MS. If needed, extra zinc was added, till all the reagent was consumed. After the reaction, the solid zinc acetate and excess zinc dust were removed by filtration through a celite pad. The filtrate was then extracted three times with AcOEt, the reunited organic phases were washed against a $NaHCO_3$ saturated solution until the aqueous washings were basic, then were dried over Na_2SO_4, evaporated and purified by column chromatography on silica gel (hexane:AcOEt), yielding product **2a**.

Chemical synthesis of PAC analogues (2b–d, 2h–m)

The procedure is the same as illustrated in the previous paragraph with only small variations depending on the substrate, as shown in the Supporting Information.

1-Hydroxy-1-phenylpropane-2-one, 2a. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.28 (m, 5H), 5.09 (s, 1H), 4.29 (s, 1H), 2.08 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.2, 138.1, 129.1, 128.9, 127.5, 80.3, 25.4. Overall isolated yield = 14.7%.

1-(2-Chlorophenyl)-1-hydroxy-2-propanone, 2b. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.37 (m, 1H), 7.27 (dd, *J* = 3.2, 1.3 Hz, 3H), 5.58 (d, *J* = 4.1 Hz, 1H), 4.34 (d, *J* = 4.2 Hz, 1H), 2.12 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.4, 135.9, 133.6, 130.2, 130.0, 129.1, 127.7, 76.6, 25.4. Overall isolated yield = 40.7%.

1-(4-Chlorophenyl)-1-hydroxy-2-propanone, 2c. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.22 (m, 4H), 5.06 (s, 1H), 4.28 (s, 1H), 2.08 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.6, 136.6, 134.9, 129.4, 128.8, 79.6, 25.3. Overall isolated yield = 4.7%.

1-(2-Methylphenyl)-1-hydroxy-2-propanone, 2h. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.12 (m, 4H), 5.26 (d, *J* = 3.7 Hz, 1H), 4.15 (d, *J* = 3.8 Hz, 1H), 2.41 (s, 3H), 2.05 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.7, 136.6, 136.1, 131.5, 128.9, 128.5, 126.8, 78.3, 25.5, 19.4. Overall isolated yield = 5.9%.

1-(3-Methylphenyl)-1-hydroxy-2-propanone, 2i. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30–7.12 (m, 4H), 5.06 (d, *J* = 4.2 Hz, 1H), 4.26 (d, *J* = 4.3 Hz, 1H), 2.37 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.3, 139.0, 138.0, 129.6, 129.0, 128.0, 124.7, 80.3, 25.4, 21.5. Overall isolated yield = 6.1%

1-(4-Methylphenyl)-1-hydroxy-2-propanone, 2j. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30–7.15 (m, 4H), 5.07 (d, *J* = 3.9 Hz, 1H), 4.26 (d, *J* = 4.2 Hz, 1H), 2.37 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.4, 138.7, 135.2, 129.8, 127.4, 80.1, 25.3, 21.3. Overall isolated yield = 2.8%.

1-(2-Trifluoromethylphenyl)-1-hydroxy-2-propanone, 2k. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75–7.25 (m, 4H), 5.48 (d, *J* = 4.0 Hz, 1H), 4.38 (d, *J* = 4.0 Hz, 1H), 2.05 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.2, 136.8, 132.6, 128.9, 128.7 (q, *J* = 30.3 Hz), 128.7, 126.1 (q, *J* = 5.7 Hz), 124.2 (q, *J* = 273.8 Hz), 74.9, 25.2. Overall isolated yield = 52.4%.

1-(4-Trifluoromethylphenyl)-1-hydroxy-2-propanone, 2m. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70–7.44 (m, 4H), 5.15 (s, 1H), 4.36 (s, 1H), 2.10 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.2, 139.2, 131.6 (q, *J* =

32.6 Hz), 130.8, 129.7, 125.8 (q, J = 3.8 Hz), 124.3 (q, J = 3.8 Hz), 124.0 (q, J = 272.5 Hz), 79.7, 25.3. Overall isolated yield = 0.5 %.

Enzymatic synthesis of (1*S*,2*S*)-NPE, (1*S*,2*R*)-NE and analogues (3 a–d,f–m)

For the ATA screening, the reaction mixtures were prepared by adding 5 μL of 25 mM PLP solution (6.1 mg, 25 μmol, in 1 mL 0.1 M phosphate buffer, pH 8.0), 5 μL of 1 M hydroxyketone **2 a** solution in DMSO, and 5 μL of 1 M (*S*)/(*R*)-PEA solution (12 mg, 0.1 mmol, in 100 μL DMSO) into a 0.1 M HEPES buffer of the appropriate pH (Table 2). Then, the enzyme (0.5 mg) was added and the tube was inserted in a thermomixer at 30 °C. Syntheses of **3 a**–**d**, **f**–**m** were then carried out under best reaction conditions, i.e. HEPES buffer, pH 9.0 or 7.5, at 30 °C using **2 a**–**d**, **f**–**m** as substrate. Final concentrations of the reagents were as follows: 10 mM (*S*)/(*R*)-PEA (donor), 10 mM (*S*)-PAC or analogue (acceptor), 0.25 mM PLP, 2 % (*v*/*v*) DMSO. The final volume of the reaction mixture was 0.5 mL. After 24 h, the reactions were stopped and submitted to work-up: 50 μL of 5 M NaOH were added and the solutions were extracted with 350 μL AcOEt. The organic phases were then dried with Na_2SO_4. Finally, 100 μL of each organic phase were transferred into a GC vial to be derivatised by treatment with acetic anhydride (15 μL) and pyridine (15 μL). The samples were left overnight at room temperature and analysed by GC-MS the morning after.

Preparative enzymatic synthesis of (1*S*,2*S*)-NPE (*erythro*-3 a) and (1*S*,2*R*)-NE (*threo*-3 a)

The reaction mixtures were prepared by adding 133 μL of 25 mM PLP solution (6.1 mg, 25 μmol, in 1 mL 0.1 M phosphate buffer, pH 8.0), 133 μL of 1 M PAC solution in DMSO, and 133 μL of 1 M (*S*)/(*R*)-PEA solution in DMSO into a 0.1 M HEPES buffer of the appropriate pH (Table 2). Then, the enzyme (1 mg mL^{-1}) was added and the vial was incubated in a thermomixer at 30 °C for 24 h. Final concentrations of the reagents were as follows: 10 mM (*S*)/(*R*)-PEA (donor), 10 mM (*S*)-PAC (acceptor), 0.25 mM PLP, 2 % (*v*/*v*) DMSO. The final volume of the reaction mixture was 13.32 mL. After 24 h, the reactions were stopped and submitted to work-up: after basifying with 5 M NaOH, the solution was extracted three times with AcOEt. The organic phases were recovered and dried over Na_2SO_4, evaporated, and purified by column chromatography (DCM:MeOH:NH_3 (30 % aq.) = 9 : 1 : 0.1) on silica gel.

(1*S*,2*S*)-2-amino-1-phenylpropan-1-ol, *erythro*-3 a. (R_f = 0.22, 9.3 mg, 61.6 μmol, 46 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.20 (m, 5H), 4.25 (d, J = 6.6 Hz, 1H), 3.03 (p, J = 6.5 Hz, 1H), 1.03 (d, J = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.6, 128.3, 127.6, 126.6, 78.6, 53.0, 20.5. $[\alpha]_D^{25\,°C} = +40.9$ (c = 0.5 in $CHCl_3$).

(1*S*,2*R*)-2-amino-1-phenylpropan-1-ol, *threo*-3 a. (R_f = 0.22, 10.8 mg, 71.8 μmol, 54 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53–7.01 (m, 5H), 4.56 (d, J = 4.6 Hz, 1H), 3.42–3.02 (m, 1H), 0.97 (d, J = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.2, 128.2, 127.5, 126.5, 77.3, 52.0, 17.9. $[\alpha]_D^{25\,°C} = +25.1$ (c = 0.5 in $CHCl_3$).

Preparative enzymatic synthesis of *erythro*-3 j and *threo*-3 j

The reaction mixtures were prepared by adding 120 μL of 25 mM PLP solution (6.1 mg, 25 μmol, in 1 mL 0.1 M phosphate buffer, pH 8.0), 120 μL of 1 M **1 j** solution in DMSO, and 120 μL of 1 M (*S*)/(*R*)-PEA solution in DMSO into a 0.1 M HEPES buffer of the appropriate pH (Table 2). Then, the enzyme (1 mg mL^{-1}) was added and the vial was incubated in a thermomixer at 30 °C for 24 h. Final concentrations of the reagents were as follows: 10 mM (*S*)/(*R*)-PEA (donor), 10 mM **1 j** (acceptor), 0.25 mM PLP, 2 % (*v*/*v*) DMSO. The final volume of the reaction mixture was 12 mL. After 24 h, the reactions were stopped and submitted to work-up: after basifying with 5 M NaOH, the solution was extracted three times with AcOEt. The organic phases were recovered and dried over Na_2SO_4, evaporated, and purified by column chromatography (DCM:MeOH:NH_3 (30 % aq.) = 9 : 1 : 0.1) on silica gel.

(1*S*,2*S*)-2-amino-1-(p-tolyl)propan-1-ol, *erythro*-3 j. (R_f = 0.23, 14.3 mg, 86 μmol, 74 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, J = 8.1 Hz, 2H), 7.14 (d, J = 7.9 Hz, 2H), 4.21 (d, J = 6.8 Hz, 1H), 3.01 (p, J = 6.6 Hz, 1H), 2.33 (s, 3H), 1.00 (d, J = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.6, 137.2, 129.0, 126.5, 78.6, 53.0, 21.1, 20.3. $[\alpha]_D^{25\,°C} = +85.4$ (c = 0.5 in $CHCl_3$).

(1*S*,2*R*)-2-amino-1-(p-tolyl)propan-1-ol, *threo*-3 j. (R_f = 0.24, 9.9 mg, 60 μmol, 50 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, J = 8.1 Hz, 2H), 7.15 (d, J = 7.9 Hz, 2H), 4.51 (d, J = 4.8 Hz, 1H), 3.17 (dt, J = 11.3, 5.6 Hz, 1H), 2.34 (s, 3H), 0.98 (d, J = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.4, 137.3, 129.0, 126.6, 77.6, 52.1, 21.2, 18.2. $[\alpha]_D^{25\,°C} = +27.6$ (c = 0.5 in $CHCl_3$).

Acknowledgements

We would like to kindly acknowledge Susanna Bertuletti for her technical support.

Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: biocatalysis · chiral drugs · green chemistry · stereoselective synthesis · transaminases

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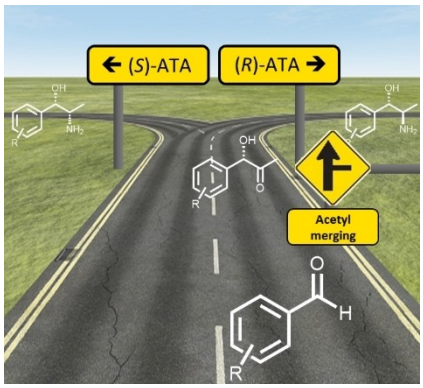
Manuscript received: September 25, 2023
Revised manuscript received: November 17, 2023
Accepted manuscript online: November 20, 2023
Version of record online: ■■, ■■

RESEARCH ARTICLE

The stereodivergent synthesis of variously substituted norephedrines has been attained starting from the cheap and commercially available corresponding aromatic aldehydes through a bi-enzymatic synthetic sequence. In the first step, a benzoin-type condensation is mediated by the (*S*)-selective acetoin:dichlorophenolindophenol oxidoreductase (Ao:DCPIP OR), while in the second step either a (*R*)- or a (*S*)-amino transaminase (ATA) is employed to carry out a transamination and obtain the final products. An array of 14 substrates has been combined with 6 different ATAs, and the products have been characterised in terms of conversion and enantiomeric excess.



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