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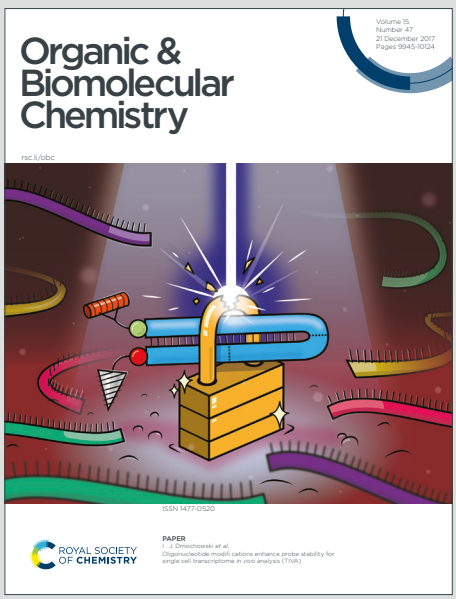

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Exploring the synthetic potential of a marine transaminase including discrimination at a remote stereocentre

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Abstract

The marine transaminase, *P*-ω-TA, can be employed for the transamination from 1-aminotetralins and 1-aminoindanes with differentiation of stereochemistry at both the site of reaction and at a remote stereocentre resulting in formation of ketone products with up to 93% *ee*. While 4-substituents are tolerated on the tetralin core, the presence of 3- or 8- substituents are not tolerated by the transaminase. In general *P*-ω-TA shows capacity for remote diastereoselectivity, although both the stereoselectivity and efficiency is dependent on the specific substrate structure. Optimum efficiency and selectivity are seen with 4-haloaryl-1-aminotetralins and 3-haloaryl-1-aminoindanes, which may be associated with the marine origin of this enzyme.

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Introduction

Chiral amines and related derivatives are common features found in many natural products (e.g. alkaloids), and the prevalence of similar motifs in pharmaceuticals and fine chemicals makes the availability of enantiopure amines as building blocks critically important to the pharmaceutical industry.[1, 2] For example, the aminotetralin motif (Figure 1) is present in a variety of pharmaceuticals, such as the anti-depressants sertraline and norsertraline with an α-aminotetralin core, and rotigotine, a treatment for Parkinson's disease containing a β-aminotetralin core unit (Figure 1).

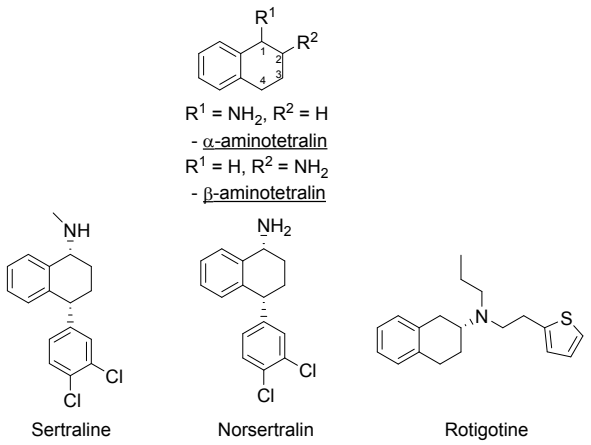

Figure 1: *Examples of pharmaceuticals with an α- or β-aminotetralin core*

To develop strategies for the enantioselective synthesis of complex amine building blocks, in addition to controlling the stereochemistry at the amine site, control of the stereochemistry at additional stereocentres in the molecule must also be addressed, and frequently this is more challenging. For example, in the synthesis of sertraline, further to controlling the stereochemistry at C-1 bearing the amino-substituent, the absolute stereochemistry at C-4 must also be controlled in the overall synthetic pathway.

The exquisite chemo-, regio- and stereoselectivity of biocatalysts can open the door to effective synthetic routes to enantiopure compounds, and in addition the use of biocatalysis often has positive benefits in relation to the environmental impact of a process, satisfying 10 of the 12 principles of green chemistry.[3] From a synthetic perspective transaminases can provide a route to enantiopure amines, *via* asymmetric transamination of ketones, or alternatively *via* kinetic resolution in deamination of racemic amines.[4] In the last decade transaminases have attracted considerable attention in synthetic biocatalysis, providing access to enantiopure amines in a synthetically versatile manner, and numerous reports have described their use for API synthesis in the pharmaceutical industry.[5-13] An excellent example of the implementation of a transaminase enzyme in the pharma industry is the use

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of an evolved and then immobilised transaminase for the synthesis of the API Sitagliptin (Januvia®), a Merck drug used in the treatment of diabetes mellitus type 2. This biocatalytic transformation surpasses its chemocatalytic counterpart in terms of selectivity, toxicity and efficiency.[14, 15]

While biocatalysis offers many advantages in enantioselective synthesis, in general, the focus is usually on the control of the absolute stereochemistry at, or near, the site of reaction. Resolution of a remote stereocentre is relatively unusual; however, as an example, the use of hydrolases to mediate the kinetic resolution of a remote stereocentre has been previously reported.[16-22] However in almost all the reports of the use of transaminases the focus is on controlling the absolute stereochemistry at the amine site,[8, 23, 24] with just a small number of reports of the resolution of an α-stereocentre using transaminases (e.g. Figure 2).[25, 26]

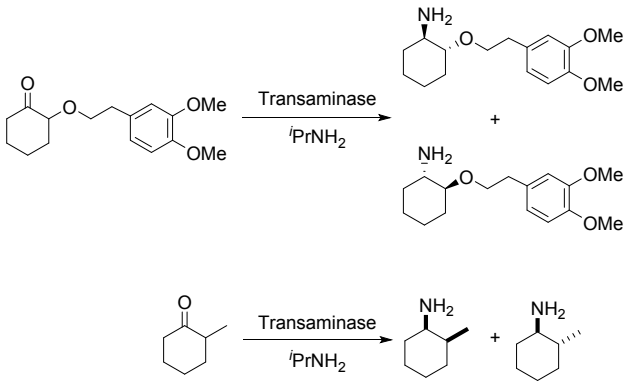


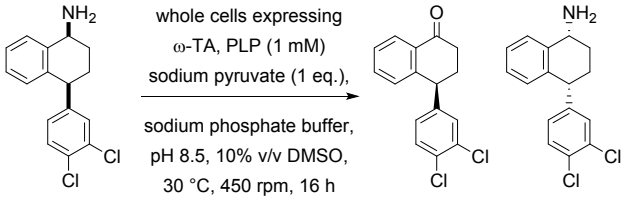
Figure 2: Examples of transaminase mediated asymmetric transamination of a compound with an α-stereocentre[25, 26]

The marine environment has provided a wealth of diversity in natural products, bioactive compounds and biocatalysts; developments in (meta)genomic-based technologies, that capture and decode the genetic blueprint of oceanic organisms, without the need to grow or sustain the source organism, have proven a vital tool in marine biodiscovery.[27-31] In the context of synthetic chemistry enzymes extracted from the marine environment often display a much higher tolerance when exposed to diverse substrates and reaction media, pressure and heat in comparison to their terrestrial counterparts.[32-34]

In the first example of transaminase-mediated resolution of a remote stereocentre, we recently reported a marine ω-transaminase *P*-ω-TA, from a *Pseudovibrio* species isolated from a marine sponge, identified using genome mining techniques.[35] *P*-ω-TA was used effectively for the resolution of amine **1a**, a potential intermediate in the synthesis of sertraline. Comparing the marine transaminase to the control transaminase *Chromobacterium violaceum* (*Cv*-ω-TA)[36] we showed that *P*-ω-TA discriminates at the remote stereocentre, selectively processing the *cis*-**1a** enantiomer over

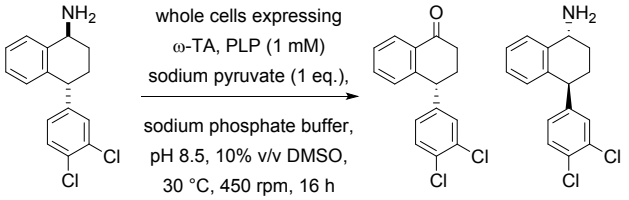
the *trans*-**1a** enantiomer (Table 1), unlike *Cv*-ω-TA which similarly processed both *cis*- and *trans*-**1a**, without noticeable discrimination. To the best of our knowledge, this was the first report of remote stereoselection using an ω-transaminase.[35]

*Table 1 Activity of P-ω-TA and Cv-ω-TA against cis- and trans-**1a**[35]*



cis-**1a** (*S*)-**2a** (1*S*,4*S*)-**1a**

Transaminase	Conversion (%)		enantiomeric excess substrate, **1a** ee_s (%)	enantiomeric excess product **2a**, ee_p (%)
	^{1}H NMR	E_{calc}[37]		
P-ω-TA	54	52	>99	93 (4*S*)
Cv-ω-TA	55	52	99	91 (4*S*)



trans-**1a** (*R*)-**2a** (1*R*,4*S*)-**1a**

Transaminase	Conversion		enantiomeric excess substrate, **1a** ee_s (%)	enantiomeric excess product **2a**, ee_p (%)
	^{1}H NMR	E_{calc}[37]		
P-ω-TA	5	-[1]	2	5 (4R)
Cv-ω-TA	44	54	95	80 (4*R*)

[1] Conversion too low to calculate

Herein, given the synthetic advantage associated with the remote stereoselection of *P*-ω-TA, we explore the expansion of the substrate scope beyond **1a** to determine the tolerance of *P*-ω-TA to

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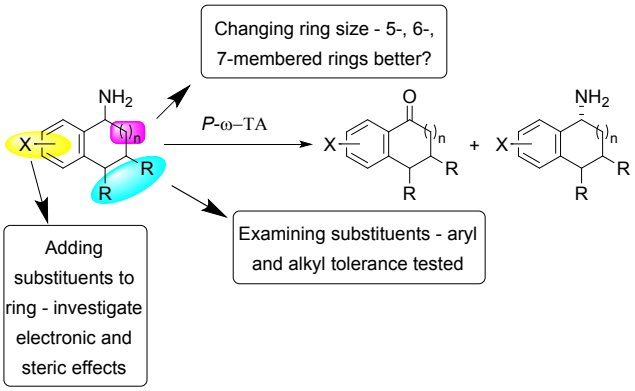
structural changes within the general framework of the sertraline intermediate **1a**, and to specifically explore the influence of variation of the substrate structure on efficiency, enantioselectivity and remote stereoselection. The overall objective was to establish the synthetic potential of *P*-ω-TA for enantioselective transformation with stereocontrol at the site of reaction in addition to distinguishing the stereochemistry at a remote stereocentre.

Results & Discussion

Substrates were designed to explore both steric and electronic effects of substituents on the tetralin core, the impact of ring size and the influence of different aryl and alkyl-substituents in the 3- and 4-position on the transaminase-mediated biotransformations, as summarised in Scheme 1.

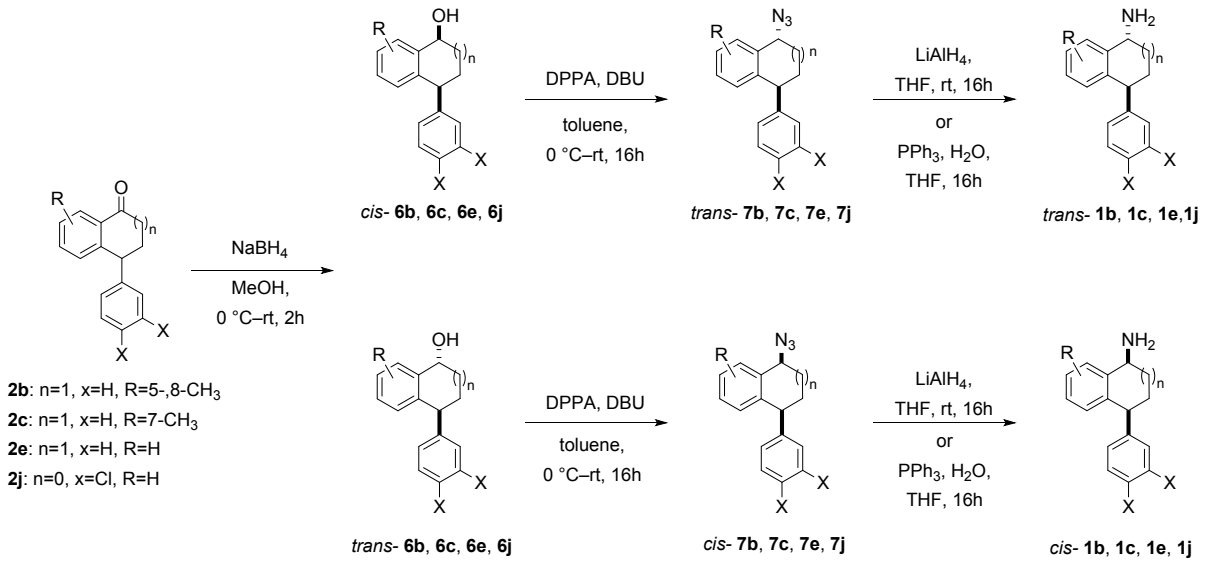


Scheme 1: Amine substrates investigated

Substrate synthesis

Racemic samples of each of the *cis*- and *trans*-amines were synthesised in addition to the racemic ketones as standards for chiral HPLC method development. The known ketones **2b-2m** were synthesised following literature procedures (details in the Supporting Information).[38-46] Subsequent transformation of each ketone **2b-2m** to both diastereomeric amines was undertaken as summarised below in Schemes 2–5, following literature precedent. While amines *cis*-**1e**,[47] **1h**,[48] **1j**[49] and **1l**[49] are known compounds with spectroscopic characteristics in agreement with literature data, amines **1b**, **1c**, **1d**, *trans*-**1e** **1f**, **1g**, **1k** and **1m** are novel and were fully characterised in this work. The hydrochloride salt of *cis*- and *trans*-**1i** has been reported before with only mass spectrometry reported.[50] Similarly, the intermediate alcohols **6b** and **6c**, azides **7b**, **7c**, **7e**, *cis*-**7f**, *trans*-**7h**, *trans*-**7i** and trans-**7k**, and Boc protected amines **8d** and **8m** are novel and were fully characterised in this work; other intermediates were known, with spectroscopic characteristics in agreement with reported data.

For the synthesis of each diastereomeric amine **1b**, **1c**, **1e** and **1j**, the corresponding ketone was reduced using sodium borohydride following a general procedure,[35, 51] to give a mixture of diastereomeric alcohols, which were separable by careful chromatography. The reductions generally proceeded cleanly with full conversion; however, in practice, the isolated yields of pure diastereomers was determined by the challenging chromatographic separation of the diastereomers. Each of the diastereomerically pure alcohols **6b**, **6c**, **6e**, **6j** were converted to their corresponding azides **7b**, **7c**, **7e**, **7j** with clean inversion of stereochemistry using diphenylphosphoryl azide and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) followed by a Staudinger reaction or reduction using $LiAlH_4$ (Scheme 2).[35, 52]



*Scheme 2: Synthesis of cis- and trans-**1b, 1c, 1e, 1j** from the corresponding ketones via reduction, azidation with inversion of stereochemistry and subsequent azide reduction*

Where the initial sodium borohydride reduction of the ketone afforded primarily one diastereomer and the separation/purification of the minor diastereomer proved difficult, the major diastereomer (*trans* for **1f** and *cis* for **1h**, **1i**, **1k** and **1l**) was subjected to azidation and reduction conditions to synthesise the inverted amine (*cis*-**1f** and *trans*-**1h**, **1i**, **1k** and **1l,** Scheme 3) by the same method as in Scheme 2.[52] The opposite diastereomers could be obtained by direct reductive amination of the ketone through treatment with ammonia in methanol and titanium(IV) isopropoxide or titanium(IV) ethoxide, followed by in-situ sodium borohydride reduction furnishing primarily the *trans*-amine **1f** and *cis*-amine **1h**, **1i**, **1k** and **1l** (Scheme 3).[35, 43] In general the crude amine was converted to the HCl salt, washed, precipitated, recrystallized if necessary and then a salt break furnished the pure amine without column chromatography.

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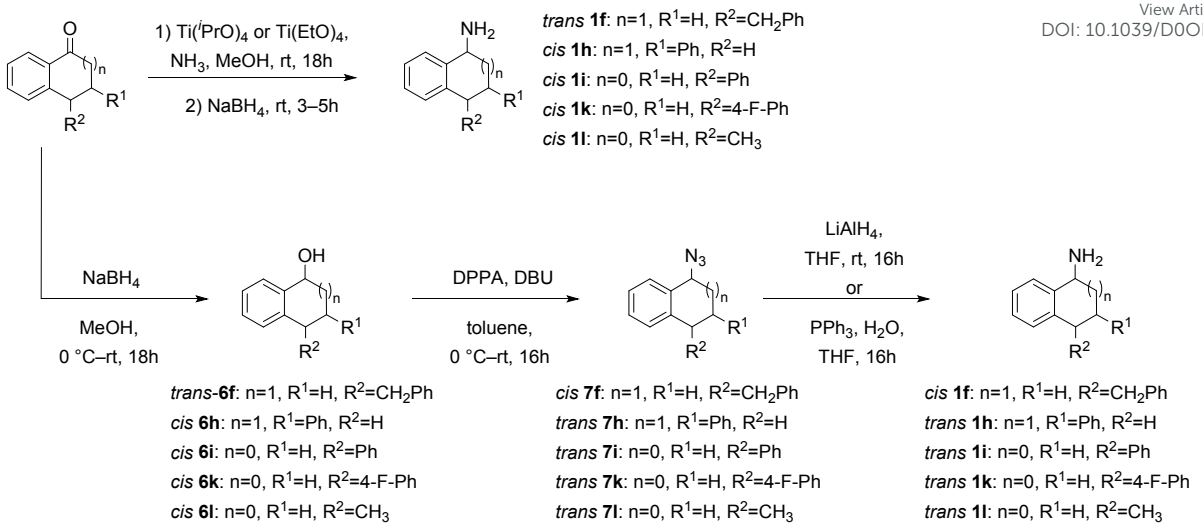
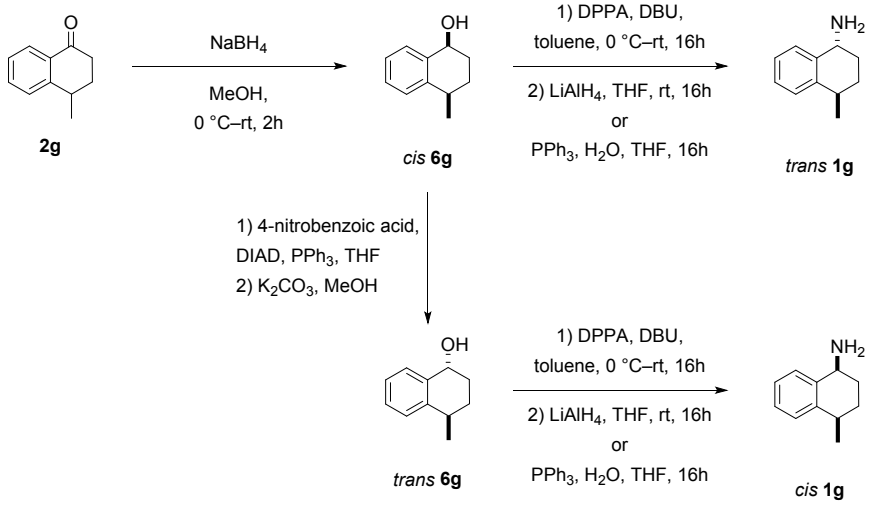


Scheme 3: Synthesis of amines via direct reductive amination or via reduction, azidation and azide reduction.

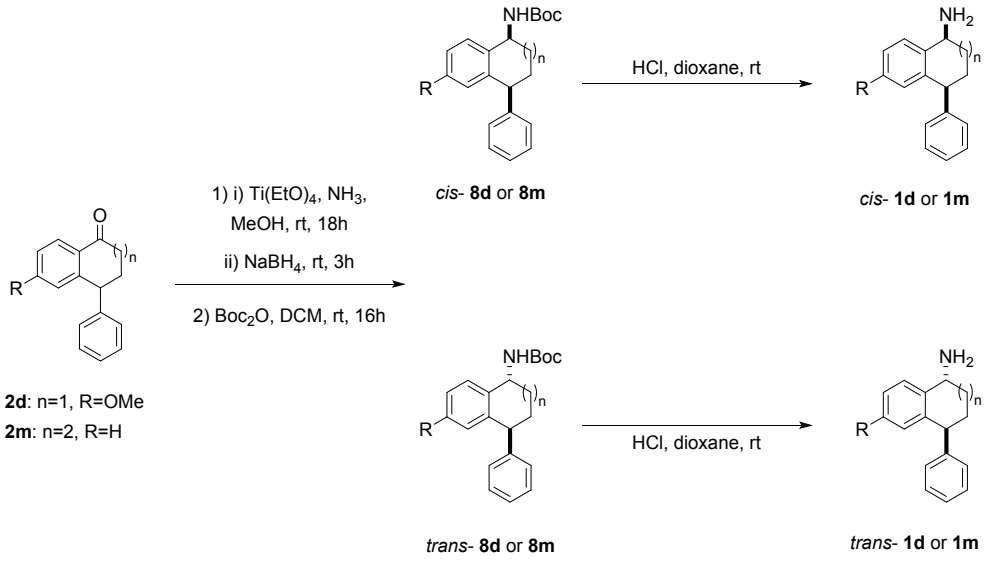
Reduction of the 4-methyl-1-tetralone **2g** substrate furnished a mixture of *cis*- and *trans*-**1g** alcohols in a diastereomeric ratio of 55:45 respectively, which could not be separated through flash column chromatography.[51] However, the *cis*-alcohol **6g** could be obtained through recrystallization from hexane. The *trans*-alcohol **6g** was accessed by inversion of the stereochemistry *via* an ester intermediate following a literature procedure.[53] The two novel amines *cis*- and *trans*-**1g** were each obtained by azidation and reduction as described above with clean inversion in each case (Scheme 4).[52]



*Scheme 4: Synthesis of cis- and trans- aminotetralin **1g***

When the synthesis of amines **1d** (*p*-OMe) and novel **1m** (7-membered ring) was attempted following the synthetic sequence outlined in Scheme 2, the corresponding amines were not isolated as single diastereomers. To overcome this problem, the ketones **2d** and **2m** were subjected instead to reductive

amination and the diastereomeric amines in the crude mixtures were protected using Boc-anhydride (Scheme 5).[54] The Boc amines **8d** and **8m** were purified and the diastereomers separated by flash column chromatography on silica gel. The rather low yields can again be attributed to the challenging separation. In addition to making the compounds easier to separate, this approach had the advantage that the Boc protected amines could be used for HPLC method development. The free amines **1d** and **1m** were readily accessed by deprotection of the Boc amines under acidic conditions. Characterisation of the benzosuberone derived amines **1m** and Boc amines **8m** is complicated by conformational effects in the ^{1}H and ^{13}C NMR spectra.[55]



*Scheme 5 – Reductive amination of ketone **2d** and **2m** with diastereomer separation of the N-Boc derivatives to yield diastereomerically pure amines **1d** and **1m***

Substrate scope

As our primary objective was to establish a substrate scope for the remote stereoselection, we tested a range of substrates against the biocatalysts *P*-ω-TA and the control *Cv*-ω-TA for activity in the thermodynamically favoured deamination reaction using the *cis*- and *trans*-amine substrates separately (Table 2 and Table 3 respectively). All the biotransformations reported in Tables 2 and 3 below were conducted under the same conditions to enable comparison and insight into the impact of substituents. For each substrate, optimisation of the efficiency and stereoselectivity by adjusting the reaction conditions (e.g. the reaction time, scale up, etc) could be envisaged. The efficiencies of conversion are recorded in Tables 2 and 3 below in two ways, firstly based on integration of ^{1}H NMR spectra of the crude product mixtures, and secondly by use of E_{calc} based on the chiral HPLC data.[37] While in most instances there is reasonable alignment, differences may be explained by the challenges

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in accurately integrating NMR spectra where one of the components is present in a very low level, e.g. Table 2, entry 16 in which case E_{calc} perhaps is a better indicator of biocatalyst efficiency.

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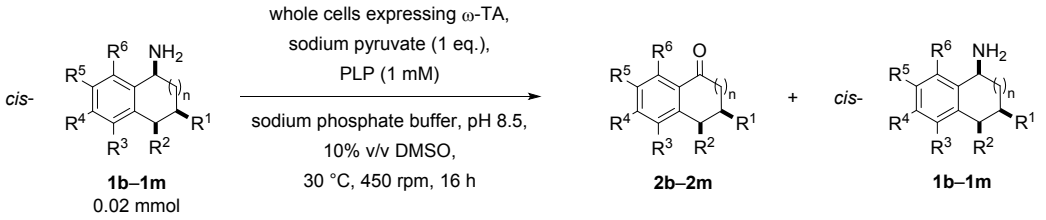
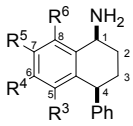
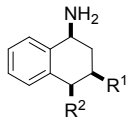
Table 2 Activity of P-ω-TA and Cv-ω-TA against a range of amine substrates with cis relative stereochemistry





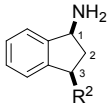
cis-**1b–1d**

cis-**1b** R^3, R^6 = CH_3, R^4, R^5 = H
cis-**1c** R^3, R^4, R^6 = H, R^5 = CH_3
cis-**1d** R^3, R^5, R^6 = H, R^4 = OMe



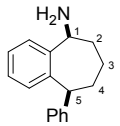
cis-**1e–1h**

cis-**1e** R^1 = H, R^2 = Ph
cis-**1f** R^1 = H, R^2 = CH_2Ph
cis-**1g** R^1 = H, R^2 = Me
cis-**1h** R^1 = Ph, R^2 = H



cis-**1i–1l**

cis-**1i** R^2 = Ph
cis-**1j** R^2 = 3,4-Cl-Ph
cis-**1k** R^2 = 4-F-Ph
cis-**1l** R^2 = Me



cis-**1m**

Entry	Substrate	TA	Conversion (%)		*ee* %		E^{37}
			^{1}H NMR	E_{calc}[37]	ee_S	ee_P	
1	***cis*-1b**	*P*-ω-TA	0	-	-	-	-
2	***cis*-1b**	*Cv*-ω-TA	6	3	1	33	2
3	***cis*-1c**	*P*-ω-TA	7	6	5	82	10
4	***cis*-1c**	*Cv*-ω-TA	50	51	>99	94	170
5	***cis*-1d**	*P*-ω-TA	28	31	42	93	42
6	***cis*-1d**	*Cv*-ω-TA	51	55	>99	82	52
7	***cis*-1e**	*P*-ω-TA	-[1]	8	8	93	30
8	***cis*-1e**	*Cv*-ω-TA	-[1]	16	11	56	4
9	***cis*-1f**	*P*-ω-TA	4	12	7	50	3
10	***cis*-1f**	*Cv*-ω-TA	61	60	>99	65	23
11	***cis*-1g**	*P*-ω-TA	14	16	18	93	33
12	***cis*-1g**	*Cv*-ω-TA	53	55	>99	82	52
13	***cis*-1h**	*P*-ω-TA	0	-	-	-	-
14	***cis*-1h**	*Cv*-ω-TA	0	-	-	-	-
15	***cis*-1i**	*P*-ω-TA	4	14	7	43	3
16	***cis*-1i**	*Cv*-ω-TA	100	71	10	4	1
17	***cis*-1j**	*P*-ω-TA	46	45	72	87	31
18	***cis*-1j**	*Cv*-ω-TA	51	52	82	77	19
19	***cis*-1k**	*P*-ω-TA	51	51	84	82	27
20	***cis*-1k**	*Cv*-ω-TA	76	78	>99	28	7
21	***cis*-1l**	*P*-ω-TA	63	42	57	79	3
22	***cis*-1l**	*Cv*-ω-TA	96	89	88	11	15
23	***cis*-1m**	*P*-ω-TA	27	17	20	95	47
24	***cis*-1m**	*Cv*-ω-TA	53	49	93	96	168

[1] It was not possible to accurately estimate the % conversion using ^{1}H NMR spectroscopy.

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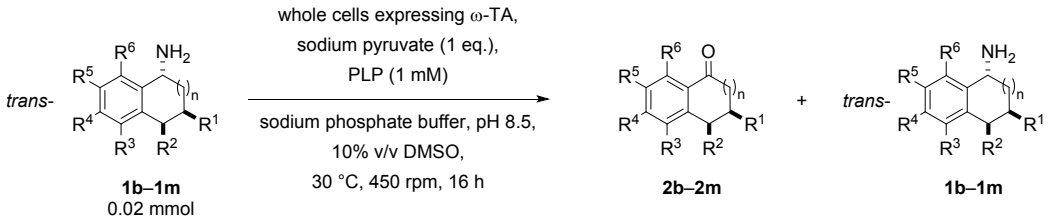
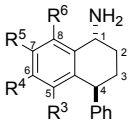
*Table 3 Activity of P-ω-*TA *and Cv-ω-*TA *against a range of amine substrates with trans* relative stereochemistry



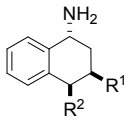
1b–1m
0.02 mmol

2b–2m

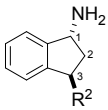
1b–1m



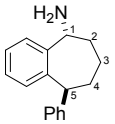
trans-**1b–1d**



trans-**1e–1h**



trans-**1i–1l**



trans-**1m**

trans-**1b** R^3, R^6 = CH_3, R^4, R^5 = H
trans-**1c** R^3, R^4, R^6 = H, R^5 = CH_3
trans-**1d** R^3, R^5, R^6 = H, R^4 = OMe

trans-**1e** R^1 = H, R^2 = Ph
trans-**1f** R^1 = H, R^2 = CH_2Ph
trans-**1g** R^1 = H, R^2 = Me
trans-**1h** R^1 = Ph, R^2 = H

trans-**1i** R^2 = Ph
trans-**1j** R^2 = 3,4-Cl-Ph
trans-**1k** R^2 = 4-F-Ph
trans-**1l** R^2 = Me

Entry	Substrate	TA	Conversion (%)		*ee* %		E^{37}
			^{1}H NMR	$E_{calc}{}^{37}$	ee_S	ee_P	
1	***trans*-1b**	*P*-ω-TA	0	-	-	-	-
2	***trans*-1b**	*Cv*-ω-TA	0	-	-	-	-
3	***trans*-1c**	*P*-ω-TA	0	-	-	-	-
4[1]	***trans*-1c**	*Cv*-ω-TA	10	-	-	-	-
5[1]	***trans*-1d**	*P*-ω-TA	<7	-	-	-	-
6	***trans*-1d**	*Cv*-ω-TA	52	54	94	82	36
7	***trans*-1e**	*P*-ω-TA	0	-	-	-	
8	***trans*-1e**	*Cv*-ω-TA	-[2]	22	24	87	18
9	***trans*-1f**	*P*-ω-TA	0	-	-	-	-
10	***trans*-1f**	*Cv*-ω-TA	53	54	>99	84	60
11	***trans*-1g**	*P*-ω-TA	18	20	23	94	40
12	***trans*-1g**	*Cv*-ω-TA	51	53	>99	89	90
13	***trans*-1h**	*P*-ω-TA	0	-	-	-	-
14	***trans*-1h**	*Cv*-ω-TA	0	-	-	-	-
15	***trans*-1i**	*P*-ω-TA	<2	3	1	28	2
16	***trans*-1i**	*Cv*-ω-TA	<2	2	1	63	4
17	***trans*-1j**	*P*-ω-TA	4	6	1	16	1
18	***trans*-1j**	*Cv*-ω-TA	8	11	7	58	4
19	***trans*-1k**	*P*-ω-TA	0	-	-	-	-
20	***trans*-1k**	*Cv*-ω-TA	4	6	4	62	4
21	***trans*-1l**	*P*-ω-TA	<2	5	3	61	4
22	***trans*-1l**	*Cv*-ω-TA	29	30	32	76	10
23	***trans*-1m**	*P*-ω-TA	0	-	-	-	-
24	***trans*-1m**	*Cv*-ω-TA	49	-	-	92	-

[1] It was not possible to estimate enantiopurity accurately from the HPLC data due to the limited extent of conversion.
[2] It was not possible to accurately estimate the % conversion using ^{1}H NMR spectroscopy.

Substrates **1b–d** illustrate the effects of substituents on the benzene ring of the tetralin core.

- ***cis*- and *trans*-1b (Table 2 and Table 3, entries 1&2 respectively)**

Examining the outcome of the reactions of *cis*- and *trans*-**1b**, with methyl groups in the 5- and 8-positions, it is clear that the increased steric demand close to the site of reaction completely shuts down any enzyme activity towards *cis*- and *trans*-**1b** (Table 2 and Table 3, entries 1&2 respectively).

- ***cis*- and *trans*-1c (Table 2 and Table 3, entries 3&4 respectively)**

With a methyl substituent at the 7-position on the aromatic ring of the tetralin core, the kinetic resolution of *cis*-**1c** using *P*-ω-TA (Table 2, entry 3) displayed diminished activity when compared to the conversion observed for *cis*-**1a**, the control *Cv*-ω-TA showed high activity (50% conversion) and enantioselectivity [>99% enantiomeric excess of the product (ee_p)] against *cis*-**1c** (Table 2, entry 4). Interestingly, in contrast to the conversion of *trans*-**1a**, in this case the control enzyme *Cv*-ω-TA did not react to any significant extent with *trans*-**1c** (Table 3, entry 4).

- ***cis*- and *trans*-1d (Table 2 and Table 3, entries 5&6 respectively)**

To investigate the effect of electron-donating groups on the activity of the novel transaminase, a substrate with a methoxy group in the 6-position **1d** was synthesised. The *P*-ω-TA mediated kinetic resolution of *cis*-**1d** (Table 2, entry 5) led to a slightly reduced activity and decreased selectivity, while the activity of *Cv*-ω-TA (Table 2, entry 6) was unaffected by the presence of the 6-methoxy substituent when compared to *cis*-**1a** (Table 1). The activity and selectivity of the known control *Cv*-ω-TA towards *trans*-**1d** (Table 3 entry 6) was comparable to that observed for *cis*-**1d**, but for *P*-ω-TA the activity for *trans*-substituted substrate **1d** (Table 3, entry 5) was much lower than *cis*-**1d**.

Substrates **1e–h** illustrates the effects of substituents at the remote stereocentre of the tetralin core.

- ***cis*- and *trans*-1e (Table 2 and table 3, entries 7&8 respectively)**

In contrast to **1a**, use of the unsubstituted C4 phenyl substituent in **1e** has an impact on the activity and stereochemical outcome using both transaminases. Focusing initially on *Cv*-ω-TA it is clear that once again both the *cis* (Table 2, entry 8) and *trans* (Table 3, entry 8) isomers are processed with similar efficiencies but with decreased enantioselectivity for *cis*-**1e** (56% ee_p from *cis*-**1e** and 87% ee_p from *trans*-**1e**). The activity and selectivity of *Cv*-ω-TA towards *cis*-**1e** is decreased relative to the results observed with the chlorinated derivative (Table 1) *cis*-**1a** [with 56% ee_p **2e** (Table 2, entry 8) relative to 91% ee_p **2a** (Table 1)]. Interestingly *Cv*-ω-TA processes *trans*-**1e** (Table 3) with lower efficiency but

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slightly higher enantioselectivity when compared to *trans*-**1a** (Table 1). With transaminase *P*-ω-TA, the selectivity for *cis*-**1e** (Table 2, entry 7) is similar to that seen with the chlorinated derivative *cis*-**1a** [with 93% ee_p **2e** (Table 2, entry 7) relative to 96% ee_p **2a** (Table 1)] but intriguingly the activity is notably decreased for the formation of **2e** (8% E_{calc}) from *cis*-**1e** compared to **2a** (52% E_{calc}) from *cis*-**1a**. There is no activity observed for *P*-ω-TA towards *trans*-**1e**.

Clearly removing the chloro substituents has an effect on the activity and selectivity of the transaminases. As observed for the previous five substrates a trend is evident whereby the *P*-ω-TA exhibits selectivity toward the *cis*-substituted substrates relative to the *trans*-substituted substrates to a much greater extent than seen with the control transaminase *Cv*-ω-TA. The impact of substituents on the stereoselectivity patterns is very interesting with both steric and electronic effects evident; the effect of removal of the chloro-substituents from **1a** to **1e** is particularly notable.

- ***cis*- and *trans*-1f (Table 2 and table 3, entries 9&10 respectively)**

To investigate the effect of altered steric demand at the remote stereocentre, the phenyl ring was replaced with a benzyl ring (**1f**). For the control *Cv*-ω-TA the activity and selectivity for both *cis*-**1f** (Table 2, entry 10) and *trans*-**1f** (Table 3, entry 10) were comparable to each other and to *cis*- and *trans*-**1a** (Table 1). Transaminase *P*-ω-TA displayed no activity towards *trans*-**1f** with 0% conversion seen by ^{1}H NMR spectroscopy, and very limited activity toward *cis*-**1f** (Table 3 and Table 2, entry 9 respectively). Interestingly, the enantiodiscrimination in the transformation of the *cis*-substituted benzyl derivative is notably lower across both enzymes than that seen with *cis*-**1a** (Table 1) but is somewhat comparable to that seen with the phenyl derivative *cis*-**1e** (Table 2 entries 7&8).

- ***cis*- and *trans*-1g (Table 2 and Table 3, entries 11&12respectively)**

Replacing the C-4-aryl group with a less sterically demanding methyl substituent at the C-4 position results in lower activity displayed by *P*-ω-TA towards *cis*-**1g** (Table 2, entry 11) when compared to *cis*-**1a** (Table 1). The transaminase *P*-ω-TA shows low activity towards *cis*-**1g** but displays high enantioselectivity 93% *ee* for **2g** (Table 2, entry 11). Notably, the *trans*-**1g** isomer is processed by *P*-ω-TA with increased conversion and selectivity (Table 3, entry 11) when compared to *trans*-**1a** (Table 1). Evidently due to the smaller C-4-methyl substituent, both the *cis* and *trans* isomers can be accommodated in the active site of the enzyme although *cis*-**1g** is processed less efficiently than the C-4-aryl derivatives, *cis*-**1a**. Again *Cv*-ω-TA is very active and enantioselective for both *cis*-**1g** and *trans*-**1g** (Table 2 and Table 3, entry 12 respectively).

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- ***cis*- and *trans*-1h (Table 2 and Table 3, entries 13&14 respectively)**

Moving the phenyl ring to the C-3-postion in **1h** is a significant structural change to the substrate. This change allowed us to investigate if the wild-type transaminase active site could accommodate a phenyl substituent at this position in the way it was accommodated at the C-4 position; to the best of our knowledge there are currently no wild type transaminases that can accommodate such substrates. This structural change was not tolerated by either of the ω-transaminases, with 0% conversion across the screen, indicating that the active site of both enzymes (*P*-ω-TA and *Cv*-ω-TA) could not accommodate a C-3-aryl substituent irrespective of whether it was *cis* or *trans* to the amino substituent. (Table 2 and Table 3, entries 13&14 respectively).

Substrates **1i–l** illustrates the effects of substituents on the C3-position of the indane core.

- ***cis*- and *trans*-1i (Table 2 and Table 3, entries 15&16 respectively)**

Moving from the tetralin core to the indane core structure, the first substrate of this series tested was **1i**, which had a phenyl ring at the C3-position on the indane moiety. *P*-ω-TA exhibited low activity for *cis*-**1i** with 4% conversion (Table 2, entry 15); when compared to *cis*-**1e** for which a percentage conversion of 51% (Table 2, entry 7) was achieved. The enantiopurity of the ketone formed **2i** from *cis*-**1i** with *P*-ω-TA was 43% ee_p, lower than the enantioselectivity observed for *cis*-**1e**, the corresponding tetralin derivative. Notably *Cv*-ω-TA processed 100% of *cis*-**1i** to the ketone **2i**, with no enantiodiscrimination, again an unusual outcome (Table 2, entry 16).

The activity of *P*-ω-TA towards *trans*-**1i** (Table 3, entry 15) was very low, as seen with *trans*-**1e**, and for the limited amounts processed, the enantioselectivity was very low. The most substantive difference seen between the tetralin **1e** and the indane **1i** was with the *Cv*-ω-TA, which processed *trans*-**1e** effectively but showed very little catalytic activity towards *trans*-**1i**. This is the only example in the study where *Cv*-ω-TA distinguished between the *cis*- and *trans*-substituted substrates, differentiating on the basis of the stereochemistry at the remote site; the observation of both enantiomers of *cis*-**1i** being processed without discrimination is notable.

- ***cis*- and *trans*-1j (Table 2 and Table 3, entries 17&18 respectively)**

Adding two chloro-substituents in the 3- and 4-position of the phenyl ring (**1j**), analogous to **1a**, re-established the remote stereoselection of *cis*-**1j** for *P*-ω-TA and *Cv*-ω-TA (Table 2, entries 17&18), when compared to *cis*-**1i**. This result is significant as it is clear that having the dichloro-substituted phenyl ring at the C-4 position of the tetralin core and at the C-3 position of the indane ring is critical

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for how the substrate binds to the active site of the transaminase but becomes even more evidently important for the indane core structure binding capacity.

In contrast, *P*-ω-TA showed no activity towards *trans*-**1j** (Table 3, entry 17) and *Cv*-ω-TA displayed very low activity (8% conversion) and moderate enantioselectivity towards *trans*-**1j** (58% ee_p) (Table 3, entry 18). This result for *Cv*-ω-TA is notable because for *trans*-**1a** this control transaminase displays a high level of activity (44% conversion) and enantioselectivity (88% ee_p). Accordingly, while both enzymes display selectivity for transformation of *cis*-**1j** over *trans*-**1j** the distinction between the transaminase *P*-ω-TA and the benchmark *Cv*-ω-TA is less clear cut with the indane derivative than seen with the original sertraline amine **1a**. These results indicate that both enzymes display enantiodiscrimination towards *cis*-**1j** over *trans*-**1j**.

- ***cis*- and *trans*-1k (Table 2 and Table 3, entries 19&20 respectively)**

To further probe the effect of substituents in the indane series, a fluoro-substituent was added in the 4-position on the C3-phenyl ring (**1k**). The *P*-ω-TA displays good activity and enantioselectivity towards *cis*-substrate-**1k** (amine, 84% ee_s) and product-**2k** (ketone, 82% ee_p), giving access to both enantioenriched products (Table 2, entry 19). Adding an electronegative fluorine atom to the phenyl ring at the C-3 position positively impacts both the activity and enantioselectivity of the transaminase towards *cis*-**1k** when compared to *cis*-**1i,** with the unsubstituted phenyl substituent. Having the fluorine atom on the phenyl ring at the C-3 position allows *cis*-**1k** to interact with the active site of *P*-ω-TA and display similar conversion and enantioselectivity to that of *cis*-**1a** and *cis*-**1j**. Control transaminase, *Cv*-ω-TA displays good activity towards *cis*-**1k** (78%) with a high degree of enantiopurity 99% ee_s, but poor enantioselectivity for **2k** (ketone, 28% ee_p) . For *trans*-**1k** *Cv*-ω-TA displays very low activity with 6% conversion (Table 3, entry 20) and *P*-ω-TA showed no activity for the *trans*-substituted substrate **1k** (Table 3, entry 19).

Clearly the introduction of the halo-substituents on the C-3 phenyl ring enhances the efficiency of discrimination of *cis* and *trans* isomers in addition to the enantiodiscrimination, mirroring the trend seen when comparing **1a** to **1e** in the tetralin series.

- ***cis*- and *trans*-1l (Table 2 and Table 3, entries 21&22 respectively)**

The replacement of the phenyl substituent at the C-3 position of the indane ring with a less sterically demanding methyl group has a significant impact on both the activity and selectivity of *P*-ω-TA toward *cis*-**1l** with an increase in activity (Table 2, entry 21) when compared to *cis*-**1i**. Clearly the smaller *cis*-methyl substituent can be accommodated more easily in the active site of the enzyme. Comparing the

outcome to that seen for the analogous tetralin derivative *cis*-**1g**, it is clear that the enantiodiscrimination is more effective in **1g** but less so in **1l** highlighting the difference between the way in which the indane and tetralin core structures are accommodated in the active site of the transaminase.

Control transaminase *Cv*-ω-TA displays very high level of activity for *cis*-**1l** coupled with a high degree of enantiopurity for the substrate *cis*-**1l** (88% ee_s) but poor enantiopurity for the product **2l** (11% ee_p). This difference in enantiodiscrimination between the marine *P*-ω-TA (Table 2, entry 21) and the control *Cv*-ω-TA (Table 2, entry 22) again highlights the difference in the active sites of each enzyme.

P-ω-TA processes *trans*-**1l** with very limited activity and moderate selectivity (Table 3, entry 21). This result, when compared to the results for *cis*-**1l**, highlights that the remote stereodiscrimination of the transaminase is retained even though the enantiodiscrimination is decreased with the methyl substituent.

Interestingly, variation of the C-3 substituent had a much greater impact on the enzyme activity and selectivity towards the indane substrates **1i–k** relative to the tetralin substrates **1b–h**. For the indane series of substrates, the marine transaminase follows a similar trend to that seen with the tetralin series, where it displays activity and enantioselectivity preferences towards the *cis*-substituted substrates over the *trans*-substituted substrates while the detailed selectivity patterns vary somewhat. The halogenated aryl substituents offer clear advantages both in terms of efficiency and stereoselectivity, perhaps related to marine origin of the transaminase.[30, 56]

- ***cis*- and *trans*-1m (Table 2, entries 23-24 and Table 3, 23-24 respectively)**

The final substrate screened was **1m**, with the 6-membered ring increased to a 7-membered ring while retaining a phenyl substituent at the C5-position. Gratifyingly, *P*-ω-TA exhibited remote stereoselection for benzosuberan **1m** with moderate activity and excellent enantioselectivity towards the *cis*-substrate (Table 2, entry 23) but no activity toward *trans*-**1m** (Table 3, entry 23), which is an interesting observation as benzosuberone-based natural products are an important class of medicinal and pharmaceutical compounds.[57] For the control transaminase, *Cv*-ω-TA, it was seen that it could process both *cis*-**1m** and *trans*-**1m** with high levels of conversion 53% and 49% respectively and also displayed a high degree of enantioselectivity (Table 2 and Table 3, entry 24 respectively). Once again, the marine transaminase demonstrates the synthetically useful discrimination of the remote stereocentre which is not possible with *Cv*-ω-TA.

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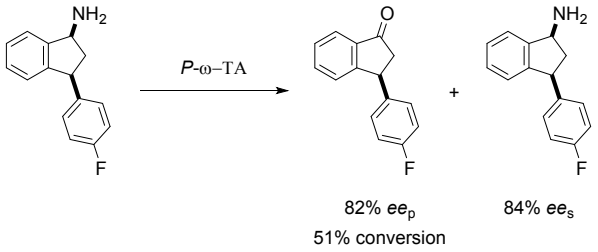
Conclusion for substrate scope:

Overall, the use of *P*-ω-TA for the deamination of a series of 1-aminotetralins and 1-aminoindanes indicates that this enzyme can be successfully employed across a broader substrate range compared to our initial study using aminotetralin **1a,** leading to products with enantiopurity up to 94% *ee*. It is clear that while 4- substituents are tolerated on the tetralin core, the presence of 3- or 8- substituents inhibits enzyme activity. The substrate scope can be expanded to include 1-indanones and 1-benzosuberones. In general *P*-ω-TA shows capacity for remote diastereoselection to a much greater extent than the control *Cv*-ω-TA, although both the stereoselectivity and efficiency is dependent on the precise substrate structure. Given the importance of the 1-aminotetralin and 1-aminoindane core structures the potential of this biocatalyst in enantioselective synthesis is clear. Interestingly, optimum results were obtained with 4-haloaryl 1-aminotetralin, cis-**1a** and 3-haloaryl 1-aminoindanes, *cis*-**1j** and *cis*-**1k** which may be linked to the marine origin of this enzyme. In some instances, both the ketone and the amine can be obtained in high enantiopurity highlighting the synthetic utility of this marine biocatalyst (Scheme 6).



Scheme 6: Synthetic utility of P-ω-TA which yielded good enantiopurity in both recovered substrate and product

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Materials and Methods

Heterologous expression of *P*-ω-TA and *Cv*-ω-TA

Protein expression was preformed following previous protocols.[35] In brief, gene specific primers were used to generate a gene amplicon of the transaminases, *P*-ω-TA and *Cv*-ω-TA. Directional cloning produced in-frame His-Tag gene fusions in the pET28a expression plasmid. Consensus gene sequence fidelity was analysed by sequencing the insert (Eurofins Genomics) and using the bioinformatic tool, Clustal Omega for local sequence alignment agreement.[58] The His-Tag gene fusions in pET28a were introduced into the heterologous host, *E. coli* BL21 DE RIPL. Cells were cultured with shaking at 180 rpm for 4 h in 37°C in LB supplemented with 50 µg/mL chloramphenicol and 50 µg/mL kanamycin. Following this growth phase, the plasmids were induced with 0.5 mM IPTG and incubated for a further 4 h with shaking (180 rpm) in 23°C. Cells were pelleted using a Sorvall RC series (Thermo Scientific) at 12,000 rpm for 10 min, maintained at a temperature of 4°C.

General Procedure for the deamination of amines

E. coli BL21 DE RIPL cells containing the expressed transaminase (30 mg) were suspended in sodium phosphate buffer (50 mM, pH 8.5) in a 15 mL centrifuge tube. The suspension was sonicated (30% intensity) using a probe for 10 s, followed by 30 s on ice. This process was repeated 5 times to lyse the cells. PLP solution (in 50 µL of buffer, final conc. 1 mM) and sodium pyruvate solution (in 50 µL of buffer, overall 1 eq.) were added, followed by the amine substrate (0.02 mmol) dissolved in 100 µL DMSO made up to a total volume of 1 ml, so that the final amine concentration is 20 mM. The solution was shaken at 30 °C, 400 rpm for 16 h. The reaction was stopped through the addition of NaOH (400 µL, 5 M aq. solution). Ethyl acetate (≈ 4 mL) was added and the tubes were centrifuged to pellet the cells. The organic phase was passed through a silica plug containing Na_2SO_4 and the solvent was removed *in vacuo*. The crude products were analysed by ^{1}H NMR and chiral HPLC (detailed methodology is provided in Supplementary Data). All experiments were replicated to ensure reproducibility.

Conflicts of interest

There are no conflicts to declare

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