HYBRID ORGANO- AND BIO-CATALYTIC PROCESS FOR THE ASYMMETRIC TRANSFORMATION OF ALCOHOLS INTO AMINES IN AQUEOUS MEDIUM

Elisa Liardo,[†,‡] *Nicolás Ríos-Lombardía,*[†] *Francisco Morís,*[†] *Francisca Rebolledo**[,‡] *and Javier González-Sabín**[,†]

[†] EntreChem SL, Edificio Científico Tecnológico, Campus El Cristo, 33006 Oviedo, Spain

[‡] Department of Organic and Inorganic Chemistry, University of Oviedo, Julián Clavería, 8, 33006 Oviedo, Spain

*Corresponding Authors:

e-mail: jgsabin@entrechem.com; frv@uniovi.es

ABSTRACT

A hybrid organo- and biocatalytic system for the asymmetric conversion of racemic alcohols into amines was developed. Combining an organocatalyst, AZADO, an oxidant, NaOCl, and an enzyme, ω-transaminase, we implemented a one-pot oxidation-transamination sequential process in aqueous medium. The method showed broad substrate scope and was successfully applied to conventional secondary alcohols and sterically hindered β-substituted cycloalkanols, where a highly stereoselective dynamic asymmetric bioamination enabled to set up both contiguous stereocenters with very high enantio- and diastereomeric ratio (>90% yield, >99% *ee* and up to 49:1 *dr*).

KEYWORDS: *One-pot reaction, organocatalysis, biocatalysis, asymmetric synthesis, amino alcohols, transaminase, AZADO.*

INTRODUCTION

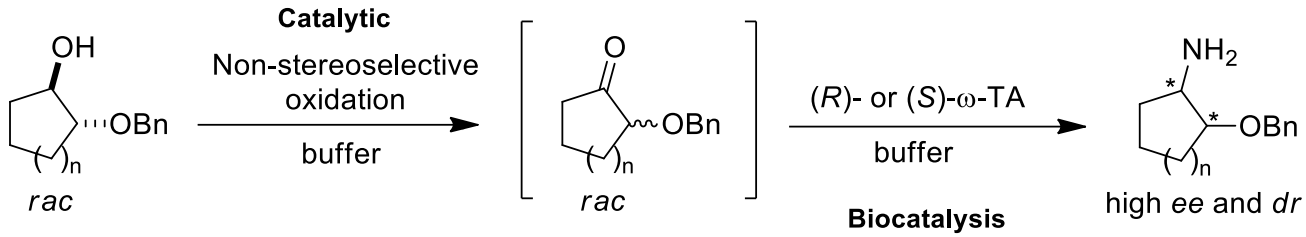
In recent years, chemists have tried to emulate *Mother Nature* by developing novel one-pot multi-step catalytic reaction networks to construct enantiomerically pure molecules in a new cleaner and more efficient manner.[1] From an environmental point of view, cascade processes are particularly attractive due to the avoidance of intermediate extraction and purification steps, resulting in simplified downstream operations with reduced waste and cost relative to traditional multi-step processes. On the other hand, the possibility of performing one-pot multi-step syntheses aided by enzymes is particularly appealing due to the intrinsic green features of biocatalysis.[2] Thus, multi-step enzymatic reactions in whole cell fashion were described in the late 1980's for the production of amino acids[3] and later on, several isolated enzyme classes have been successfully combined in one-pot fashion.[2] In addition, two different catalytic fields such as metal-catalysis and organocatalysis, developed rather independently from biocatalysis over the last few decades, have recently merged in a novel synthetic strategy that combines the advantages of both fields.[4] Thus, the discovery of water-compatible metal-catalysts for

well-established organic reactions has enabled their assembly with many biotransformations in aqueous media.[5,6] Combination of organocatalysts and enzymes, although scarce, has also been reported, for instance, a proline-derivative catalyzed aldol reaction and further bioreduction,[7a] or the complete oxidation of glycerol to CO_2 by the cooperative action of an oxalate oxidase and an organic oxidation catalyst.[7b]

Optically active amines are among the most valuable compounds in chemistry, not only by their presence in a variety of natural products, but also as intermediates in the synthesis of pharmaceuticals and agrochemicals.[8] Currently, biocatalysis offers a variety of enzymes for amine synthesis,[9] transaminases (ω-TAs) being one of the most effective catalysts. The most straightforward approach based on these transferases consists of the asymmetric reductive-amination of prochiral or chiral carbonyl compounds (ketones or aldehydes). However, the easier availability of some alcohols in comparison with their oxidized carbonyl derivatives has encouraged the development of multi-step approaches for the one-pot conversion of alcohols into amines.[10] Thus, the prior state-of-the-art includes a selective biocatalytic two-step sequence consisting of an alcohol dehydrogenase (ADH)-catalyzed oxidation of an alcohol and further bioamination mediated by ω-TAs or amine dehydrogenases (AmDHs).[11] However, due to the excellent selectivity generally offered by ADHs, when a racemic alcohol is used, two enzymes with opposite stereopreference became compulsory. Alternatively, alcohol oxidases were also combined with ω-TAs for transforming cinnamic and benzylic alcohols (two-enzyme cascade)[12a] and also non-activated aliphatic alcohols (five-enzyme cascade).[12b] More recently, a non-stereoselective oxidation catalyzed by laccases has been coupled with ω-TAs, but requiring a high amount of the chemical mediator TEMPO (2,2,6,6-tetramethylpiperidine *N*-oxyl, 30 mol%) for the transformation of benzylic alcohols.[13]

With all these precedents in mind, and keeping in mind the aqueous natural environment for ω-TAs, we focused on the search of an efficient and water-compatible alcohol-oxidation method to orchestrate a cascade process in aqueous media. Ideally, the method should enable to convert, among

other substrates, the easily available racemic *trans*-2-(benzyloxy)cycloalkanols into optically active *cis*- or *trans*-2-(benzyloxy)cycloalkanamines (Scheme 1). Actually, these are highly valuable compounds since the β-amino alcohol moiety is ubiquitous in nature and the subfamily of β-aminocycloalkanols can be found in many natural products and synthetic bioactive molecules such as the antiarrhythmic vernakalant[14a] or the inhibitor of HIV indinavir.[14b] Furthermore, they have also found great application in asymmetric catalysis as auxiliaries or ligands.[15]



Scheme 1. Proposal of cascade for the asymmetric synthesis of 2-(benzyloxy)cycloalkanamines in aqueous medium.

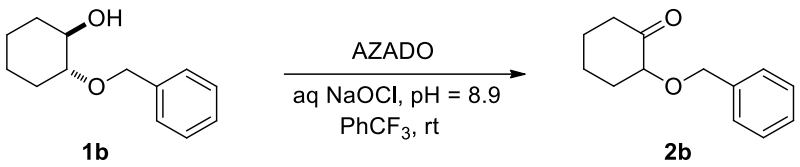
RESULTS AND DISCUSSION

The oxidation of 2-(benzyloxy)cyclohexanol (**1b**) was selected as a benchmark reaction for testing different oxidation systems. Initially, from the plethora of reported water-compatible oxidants, we turned our attention to the family of nitroxyl radicals,[16] and its best known member TEMPO, based on its efficient performance in the pharmaceutical industry.[17] Aimed at developing a fully-biocatalytic process, we tested the methodology pointed out above based on a laccase/TEMPO catalytic system.[13] However, upon the experimental conditions described for the oxidation of benzylic alcohols, that is, laccase from *Trametes versicolor*, O_2, and TEMPO (33 mol%) in citrate buffer 50 mM, pH 5.0 and 30 ºC, the starting **1b** was recovered unaltered.[18] Interestingly, it has been reported that 2-azaadamantane *N*-oxyl (AZADO) displays a superior catalytic proficiency to TEMPO enabling also to oxidize structurally hindered secondary alcohols in a variety of conditions.[19] In fact, a laccase-AZADO catalytic system was used by Ying *et al*. for the aerobic oxidation of hindered alcohols in water under mild

reaction conditions.[20] However, after some attempts based on these reaction conditions, the highest percentage (58%) of 2-(benzyloxy)cyclohexanone (**2b**) was achieved when *Trametes versicolor* laccase, 20 mol% of AZADO, O_2, acetate buffer pH 4.5, and α,α,α-trifluorotoluene ($PhCF_3$) as a co-solvent were used.

At this point AZADO emerged as the only effective mediator but in order to reach complete oxidation of **1b**, we decided to substitute the uneffective laccase/O_2 system by other oxidant reagents. Based on the pioneering research of Iwabuchi *et al*. on the catalytic potential of AZADO combined with oxidants such as NaOCl/KBr/Bu_4NBr or $PhI(OAc)_2$ for recycling the catalyst,[19] we selected inexpensive aqueous NaOCl to check the regeneration of the catalyst in presence of $PhCF_3$. Moreover, NaOCl, which is the active agent in bleach and swimming pool chlorine, offers several benefits over traditional oxidants such as low material cost, mild reaction conditions and no metallic waste. Consequently, in a first experiment the oxidation of **1b** was tested at room temperature in an aqueous solution of NaOCl (2.0 equiv) employing 5 mol% of AZADO and $PhCF_3$ (entry 1, Table 1). Pleasantly, this oxidation system worked very efficiently, affording the ketone **2b** in quantitative yield in just 1 h at room temperature under basic pH. Further parametrization included amount of NaOCl and organocatalyst loading. Thus, a decrease to equimolar amounts of NaOCl was detrimental and the conversion halted in 95% after 13 h, being 1.2 equivalents the minimum required (entries 2 and 3). On the other hand, we were delighted to find that AZADO retained indeed the catalytic activity at levels as low as 1 mol% (entry 4). The conversion of the alcohol to ketone was dependent on the catalyst loading, leading to conversions of 94 and 76% with 0.5 and 0.1% of AZADO, respectively, after longer reaction times (entries 6,7). Besides, when AZADO was replaced by TEMPO under the optimal conditions found in entry 4, the conversion dropped to 51% after 24 h and did not evolve further. Lastly, other co-solvents (entries 8-11) were also checked but $PhCF_3$ remained the optimal choice.

Table 1. AZADO-catalyzed oxidation of *trans*-(±)-2-(benzyloxy)cyclohexanol (**1b**) with aq. NaOCl.[a]



1b **2b**

Entry	AZADO (equiv)	aq NaOCl (equiv)[b]	Co-solvent (% v/v)[c]	Reaction time (h)	*C* (%)[d]
1	0.05	2.0	$PhCF_3$ (15)	1	>95[e]
2	0.05	1.0	$PhCF_3$ (30)	13	95
3	0.05	1.2	$PhCF_3$ (25)	1	>95
4	**0.01**	**1.2**	**$PhCF_3$ (25)**	**1**	**>95**
5	-	1.2	$PhCF_3$ (25)	24	-
6	0.001	1.2	$PhCF_3$ (25)	14	76
7	0.005	1.2	$PhCF_3$ (25)	1.5	94[f]
8	0.01	1.2	CH_2Cl_2 (40)	1	94
9	0.01	1.2	DMSO (25)	1	0
10	0.01	1.2	AcOEt (25)	1	91
11	0.01	1.2	CH_3CN (25)	1	92

[a] 50 μmol (11 mg) of alcohol was used. [b] A 0.40 M aq solution of NaOCl (pH = 8.9) was used. [c] 50 μL of $PhCF_3$ was added as co-solvent. [d] Degree of conversion determined by analysis of the ^{1}H-NMR spectrum of the crude material. Values >95% means that no starting material is observed in the spectrum. [e] Formation of an unknown over-oxidation product was observed. [f] Conversion did not evolve with a longer reaction time.

Encouraged by the potential of the AZADO/NaOCl oxidation system to operate in a basic-pH aqueous medium compatible with ω-TAs, we assessed the scope of this methodology with a variety of cyclic and acyclic secondary alcohols. Thus, all the substrates included in Figure 1 underwent efficient oxidation to afford the corresponding ketones in excellent yields (>92%). Reaction conditions (220-290 mM, 1 h) were only slightly modified in some cases to avoid over-oxidation products. Thus, when **1d** was allowed to react at room temperature with 1.2 equiv of NaOCl, the conversion was only 79% and besides product **2d**, a mixture of 1-chloro- and 1,1-dichloro-1-phenylpropan-2-ones was also observed. However, >95% of conversion was achieved by setting the temperature to 5 ºC and using 1.4 equiv of oxidant. In the other cases, no noticeable over-oxidation products were detected under the optimized conditions. Some features of this AZADO/NaOCl system worth highlighting are: i) the oxidation of a

variety of secondary alcohols takes place under mild reaction conditions, ii) the product ketones are isolated in excellent yield without need for further purification, iii) 1 mol% of AZADO and 1.0-1.4 equiv. of NaOCl are sufficient for quantitative oxidation of alcohols in just 1 h, showing excellent atom economy and total mass transfer from substrates to products.

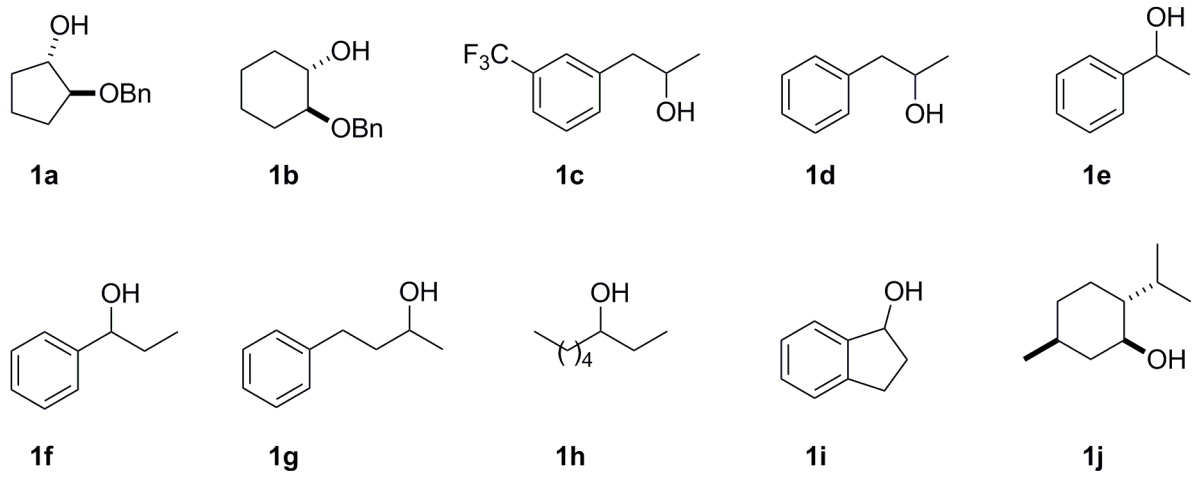


Figure 1. Substrate scope of the organocatalytic AZADO/NaOCl oxidation system.

Next, we studied the bioamination of the product ketones **2,** to complete a potential cascade process.[21] Initially, we used both the 2-(benzyloxy)cyclohexanone (**2b**) obtained above and its cyclopentylic homologue **2a**. The main feature of these compounds relies on the lability of its chiral centre which is in α-position to both carbonyl and benzyloxy groups. Thus, they are potentially amenable to racemise at slightly basic pH and trigger a dynamic kinetic resolution (DKR) leading to 100% theoretical yield. Likewise, the bioamination process would deliver two contiguous stereocenters, with four possible stereoisomers. In contrast to dynamic reductive kinetic resolutions (DYRKR),[22] reports of dynamic asymmetric-transaminations involving two chiral centres are very scarce.[23] In an example published by Merck,[23b] the highly enantio- and diastereoselective bioamination of a 2-(2-arylethoxy)cyclohexanone was the key asymmetric step towards vernakalant, an antiarrhythmic drug for atrial fibrillation.[16a] Inspired by this report, we envisaged a dynamic asymmetric-transamination with the cyclic α-benzyloxyketones **2a**,**b**. Selection of the benzyloxy group obeys to practical reasons: i) the

increased synthetic value of the resulting protected cyclic amino alcohol; ii) easy cleavage of the benzyl group; iii) its hydrophobicity, to facilitate extraction from aqueous media in downstream process; iv) the inductive effect that exalts the acidity of the α-proton, facilitating stereocenter epimerization, a pre-requisite for a viable DKR. Thus, we screened **2a**,**b** with a series of 28 commercially available ω-TAs (Codex® Transaminase Screening Kit) and also with four ω-TAs overexpressed in *E. coli*: the (*S*)-selective TAs from *Chromobacterium violaceum* (Cv)[24] and (*S*)-*Arthrobacter* (ArS),[25] or the (*R*)-selective TAs from (*R*)-*Arthrobacter* (ArR),[26] and its evolved variant ArRmut11[21d] (see the Supporting Information Available). Initially, under the standard conditions at pH 7.5 and using isopropylamine in molar excess as amino donor, most ω-TAs were very active and reached high conversions after 24 h. More interestingly, most of them displayed perfect asymmetry in the amination of the carbonyl group of **2a**,**b**, such it is deduced analyzing the C-1 configuration and the *ee* >99% obtained for both *cis*- and *trans*-amines in every reaction.[27] In addition, the diastereomeric ratios (*dr*) obtained for reactions with *C* >90% were low, meanwhile moderate or very high *dr* (up to >99:<1) was associated to reactions with lower conversion degree (*C* ≤65%). A selection of results illustrating these aspects are collected in Table 2. This means that, even though ω-TAs exhibited enantioselectivity towards the ketone (see, for instance, entries 2, 4, and 6), the low conversion achieved could be consequence of a slow epimerization rate of its chiral centre.

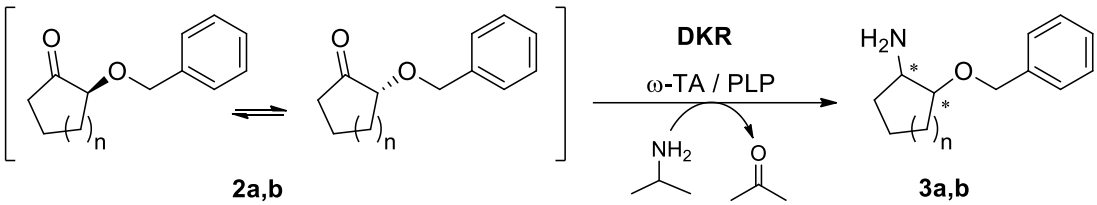
With the aim of accelerating the racemization and optimizing the diastereoselectivity outcome, the bioamination of **2b** was checked at higher pH values (8.5, 10 and 11.5). In general, although a basic pH such as 10 or 11.5 was detrimental for the stability of ω-TAs, a noteworthy increase in the *dr* was observed in some cases (see Table 2, entries 9-16). Thus, changing pH from 8.5 to 10, the *dr* was increased until 6:1 and 7:1 in the reactions mediated by ATA-254 and ATA-P2-A07, respectively (see entries 9,10 and 15,16). Similarly, ATA-412 displayed an enhanced selectivity at pH 11.5 (see entries 13 and 14) despite a lower catalytic activity. More interestingly, the ATA-303 catalyzed amination experienced the most significant improvement, with the enzyme retaining the activity and exhibiting the

highest selectivity of the series to yield enantiopure (1*R*,2*R*)-**3b** with 24:1 *dr* (entry 12). Because of the enantiocomplementarity of ATA-254 and ATA-P2-A07, both enantiomers of *cis*-**3b** can be obtained from these processes with high *ee* and yield.

Once demonstrated the efficiency of the high pH value, bioaminations of ketone **2a** were carried out at pH 10 and 11.5 (Table 2, entries 20-24). Thus, we identified biocatalysts which preserved activity and gave rise to three of the four possible stereoisomers in high *dr* (up to 49:1) and >99% *ee*. ATA-303 was the most efficient catalyst towards the *trans*-diastereomer and (1*R*,2*R*)-**3a** was obtained with improved disatereoselectivity at pH 11.5 (entry 24, 7:1 *dr*, and >99% *ee*). Regarding the *cis*-diastereomer, significant conversion enhancement was achieved by raising the pH from 7.5 to 10 in the reactions with ATA-251 and ATA-P2-A01 (entries 2 and 4 *vs* 21 and 22, respectively). Although the improvement in the *dr* was noteworthy with ATA-P2-A01 (19:1), even more remarkable was the excellent *dr* (49:1) exhibited by ATA-117 at pH 10 (entry 20). With respect to the Cv, ArR, ArS and ArRmut11 ω-TAs, they remained active at pH (except Cv-ω-TA), and displayed excellent enantioselectivity for both *cis* and *trans* isomers (entries 17-19 and 25-27). Interestingly, ArR showed an excellent *dr* of 49:1 and 19:1 (and *ee* >99%) for **2a** and **2b** respectively (entries 19 and 27).

From these results we would like highlight the high *cis*-diastereoselectivity exhibited by some ω-TAs which enabled enantiopure *cis*-β-aminocycloalkanol derivatives, complementing the existing methodologies, mostly aimed at *trans*-diastereoisomers, readily available from epoxides and aziridines. Actually, most of the existing approaches towards *cis*-β-aminocycloalkanols are rather difficult and tedious.[28]

Table 2. A selection of ω-TA-catalyzed transamination of (±)-2-(benzyloxy)cycloalkanones **2a,b**.[a]



2a,b

(**a**: n = 1; **b**: n = 2)

3a,b

Entry	Ketone	ω-TA[c]	pH	*C* (%)[b]	*cis*:*trans*[b]	*ee* (%)[b]	
						cis	*trans*
1	**2a**	ATA-237	7.5	96	60:40	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
2	**2a**	ATA-251	7.5	63	82:18	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
3	**2a**	ATA-303	7.5	>99	48:52	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
4	**2a**	ATA-P2-A01	7.5	59	>99:<1	>99 (1*R*,2*S*)	
5	**2b**	ATA-254	7.5	69	77:23	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
6	**2b**	ATA-256	7.5	50	88:12	>99 (1*S*,2*R*)	-
7	**2b**	ATA-303	7.5	>99	45:55	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
8	**2b**	ATA-P1-G06	7.5	>99	67:33	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
9	**2b**	ATA-254	8.5	>99	74:26	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
10	**2b**	ATA-254	10	90	85:15	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
11	**2b**	ATA-303	10	>99	32:68	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
12	**2b**	ATA-303	11.5	>99	4:96	-	>99 (1*R*,2*R*)
13	**2b**	ATA-412	10	>99	50:50	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
14	**2b**	ATA-412	11.5	62	91:9	>99 (1*R*,2*S*)	-
15	**2b**	ATA-P2-A07	8.5	77	77:23	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
16	**2b**	ATA-P2-A07	10	90	87:13	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
17	**2b**	ArS	10	>99	46:54	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
18	**2b**	ArRmut11	10	>99	50:50	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
19	**2b**	ArR	10	58	95:5	>99 (1*R*,2*S*)	-
20	**2a**	ATA-117	10	90	98:2	>99 (1*R*,2*S*)	-
21	**2a**	ATA-251	10	>99	86:14	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
22	**2a**	ATA-P2-A01	10	>99	95:5	>99 (1*R*,2*S*)	-
23	**2a**	ATA-025	11.5	>99	81:19	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
24	**2a**	ATA-303	11.5	>99	12:88	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
25	**2a**	ArS	10	>99	27:73	>99 (1*S*,2*R*)	94 (1*S*,2*S*)
26	**2a**	ArRmut11	10	>99	48:52	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
27	**2a**	ArR	10	85	98:2	>99 (1*R*,2*S*)	-

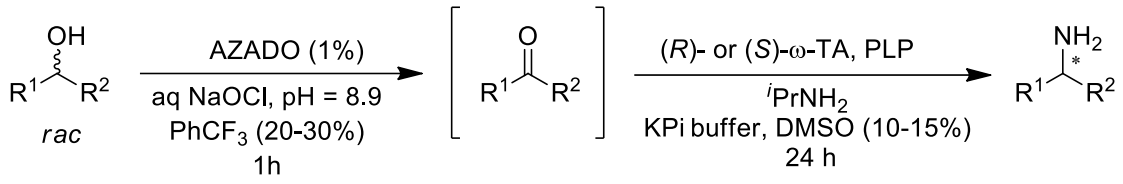
[a] Reaction conditions: Substrate (10 mM) in KPi buffer 100 mM pH 7.5-11.5 (900 μL) with PLP (1 mM) and *i*PrNH$_2$ (1 M), ω-TA (2 mg for the ω-TAs of Codexis and 5 mg for ArS, ArRmut11 and ArR), DMSO (5% v/v), for 24 h at 250 rpm and 30 ºC. [b] Determined by HPLC analysis. [c] The abbreviation ATA (amino transferase) was introduced by Codexis for its commercial ω-TAs.

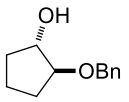
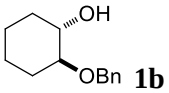
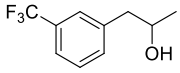
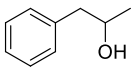
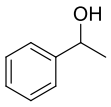
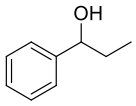
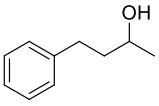
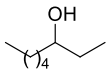
Once both catalytic steps were reliably established, we took the challenge of performing a one-pot fully convergent approach furnishing optically active amines from the analogue racemic alcohols. At this point, a number of concerns should be addressed to implement such a coupled process. A major challenge is the mutual compatibility of the involved catalysts as well as of their respective reaction conditions.[29] In order to carry out some compatibility tests, we took **1f** as a model substrate and investigated the impact of the biocatalyst (ω-TA) and its cofactor (PLP, pyridoxal-5'-phosphate) on the AZADO/NaOCl system. Whereas ω-TA was innocuous, the presence of PLP caused a decrease of the conversion (78%). However, the most important observation was the deactivation of PLP in this medium.[30] In addition, it had been already found that AZADO is totally inhibited by DMSO (see Table 1, entry 9), the co-solvent from the bioamination. On the other hand, another issue to consider was the substrate concentration, actually one of the most recalcitrant pitfalls when combining conventional and enzymatic catalysts. Indeed, despite both steps are conducted under basic aqueous medium and mild temperature, the alcohol oxidation was optimized at >200 mM, contrasting with the low concentration typically required by ω-TAs (10-20 mM). Thus, when the oxidation of **1b** was tested under identical conditions as above but at lower concentration (10 mM), no ketone was produced even after addition of an excess of NaOCl (up to 8.4 equiv) and the use of a higher amount of AZADO (10%).[31]

At this point, we have found several reasons precluding a concurrent process. Nevertheless, the inhibitor effect of DMSO and the absence of oxidation at a low substrate concentration opened up the possibility of a one-pot stepwise process preserving the integrity of PLP. Accordingly, we carried out the oxidation of **1b** at 250 mM upon the optimized conditions, namely 0.01 equiv of AZADO and 1.2 equiv of NaOCl. Once the oxidation was completed, the reaction mixture was diluted to 10 mM with a buffer solution (100 mM KPi, pH 11.5) containing *i*PrNH$_2$ (1.0 M) and PLP (1.0 mM), and DMSO (15% v/v) and the ω-transaminase ATA-303 were added. Then, the reaction mixture was incubated during 24 h at 30 ºC and 250 rpm. HPLC analysis showed complete conversion for the overall process, the corresponding *trans*-(1*R*,2*R*)-2-(benzyloxy)cyclohexanamine (**3b**) isolated as the sole product with

>99% *ee* (Table 3, entry 4) and the same excellent *dr* (24:1) as that obtained starting from ketone (see Table 2, entry 12). Interestingly, after a simple extraction-based work up, amine **3b** was recovered pure without need of further purification with very high yield (94%). Then, once demonstrated the viability of this one-pot fully convergent approach, we extended the study to other alcohols. As shown in Table 3, all of them were quantitatively oxidized in a concentration ranging from 230-320 mM, and the subsequent bioaminations, performed at 10 mM, worked exceptionally well with all the biocatalysts leading to results (conversions, *ee*, and *dr* values) nearly identical to those measured in the single step.[32] As a result, fine tuning the oxidation conditions and adequate biocatalyst selection[33] provided a set of synthetically and pharmacologically interesting amines[34] with high overall yields (up to 97%) without need of chromatographic purification. Particularly remarkable is the simplicity of this chemoenzymatic setup, since the only required experimental setting, once finished the oxidation step in 1 h, was a dilution of the reaction medium before the addition of the biocatalyst. It should be noted that ω-TAs have been previously engineered via directed evolution for practical application in a manufacturing setting, leading to improved biocatalysts working at concentrations up to 500 mM.[21d,e] Although beyond the scope of this study, the implementation of such evolved enzymes in the chemoenzymatic process disclosed herein would enable to circumvent the dilution issue, upgrading its potential for translation into a truly cascade process applicable on scale.

Table 3. Catalytic amination of racemic alcohols into optically active amines using AZADO/NaOCl and a ω-transaminase.[a,b]

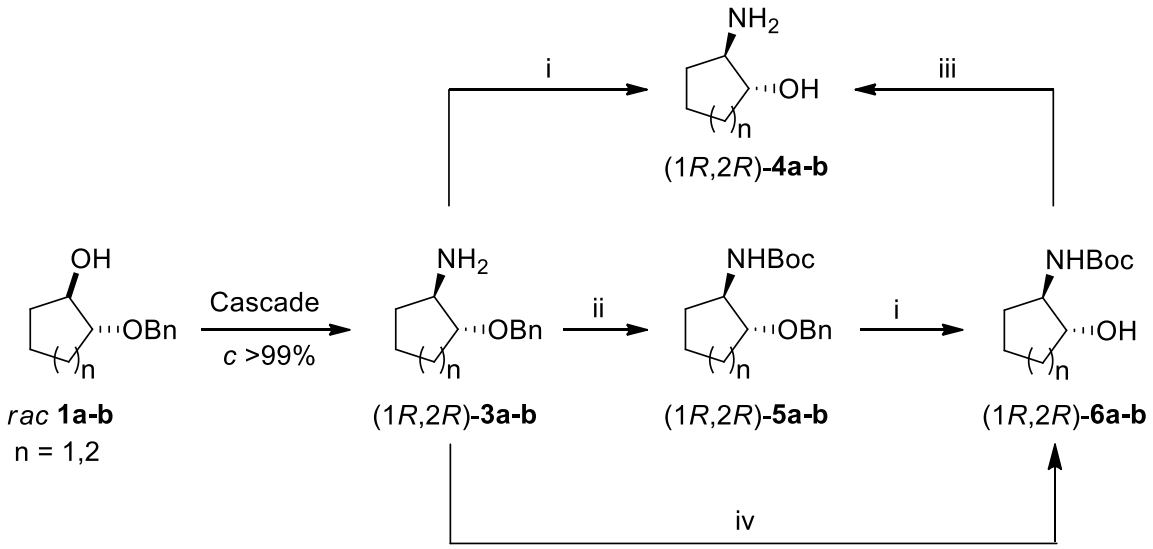


Entry	Substrate	ω-TA	Ratio (%)[b]			*trans:cis*	*ee* (%)[b]
			Alcohol	Ketone	Amine		
1	 **1a**	ATA-303	<1	<1	>99	86:14	>99 (1*R*,2*R*)[c]
2		ArS	<1	<1	>99	73:27	94 (1*S*,2*S*)[c]
3		ATA-P2-A01	<1	<1	>99	5:95	>99 (1*R*,2*S*)[c]
4	 **1b**	ATA-303	<1	<1	>99	96:4	>99 (1*R*,2*R*)[c]
5		ArR	<1	42	58	5:95	>99 (1*R*,2*S*)[c]
6	 **1c**	ATA-P2-B01	<1	<1	>99	-	>99 (*R*)
7		ArS	<1	<1	>99	-	92 (*S*)
8	 **1d**	ATA-251	<1	4	96	-	>99 (*S*)
9		ArRmut11	<1	<1	>99	-	>99 (*R*)
10	 **1e**	ATA-P1-A06	<1	20	80	-	>99 (*S*)
11	 **1f**	ATA-033	<1	13	87	-	>99 (*R*)
12	 **1g**	ATA-P1-A06	<1	<1	>99	-	>99 (*S*)
13		ArR	<1	<1	>99	-	>99 (*R*)
14	 **1h**	ATA-033	<1	15	85	-	97 (*R*)

[a] Oxidation step following the optimized conditions described in Table S3 in the SI for each substrate. Once completed, addition of ω-TA, DMSO [10% v/v (for **1c**-**h**) or 15% v/v (for **1a**,**b**)] and KPi buffer 100 mM pH 7.5 (for **1c**-**h**), pH 10 (for **1a**,**b**, entries 2, 3, and 5) or pH 11.5 (for **1a**,**b**, entries 1 and 4) with ^{i}Pr-NH$_2$ (1.0 M) PLP (1.0 mM) until a concentration of 10 mM and stirring at 250 rpm and 30 ºC. [b] Determined by GC or HPLC. [c] *ee* and configuration of the major diastereomer.

Finally, the synthetic utility of the organo-enzymatic platform developed herein was showcased by submitting the resulting optically active amino alcohol derivatives (1*R*,2*R*)-**3a** and (1*R*,2*R*)-**3b** (Table 3, entries 1 and 4) to a panel of chemical transformations to provide a set of valuable chiral synthons. First, a mild hydrogenolysis of **3a**,**b** furnished the free amino alcohols (1*R*,2*R*)-**4a**,**b** in quantitative

yield. On the other hand, treatment of **3a**,**b** with di-*tert*-butyl dicarbonate led to the orthogonally protected derivatives (1*R*,2*R*)-**5a**,**b**. Actually, orthogonally protected amino alcohols are highly valuable molecules in both asymmetric catalysis and medicinal chemistry.[17] Debenzylation of **5a**,**b** gives entry to *N*-protected amino alcohols (1*R*,2*R*)-**6a**,**b**, thus completing the practical syntheses of an interesting set of derivatives by means of quantitative and simple processes.



Scheme 2. Organo-enzymatic platform towards valuable optically active β-aminocycloalkanol derivatives. i) H_2, Pd-C 10%, MeOH; ii) $(Boc)_2O$, CH_2Cl_2, iii) CF_3CO_2H, CH_2Cl_2; iv) H_2, Pd-C 10%, $(Boc)_2O$, MeOH.

CONCLUSIONS

In conclusion, we disclosed an expedient, stereoselective and operationally simple protocol for the synthesis of a variety of optically active amines, through the unprecedented one-pot combination of: *i*) organocatalyzed oxidation of secondary alcohols; *ii*) subsequent enantioselective bioamination of the transiently formed ketones. In all cases, the desired final amines were isolated in high yields and with excellent diastereo- and enantiomeric excesses. Likewise, it is worth noting the use of a one-pot methodology, in which the aqueous reaction medium from the organocatalyzed reaction feeds the

enzymatic amination. This methodology, which exploits the advantages of merging *two catalytic worlds* such as organo- and enzymatic catalysis, represents one of the few contributions of this kind of combination in water. Moreover, it is a robust alternative to the existing methodologies, with proven efficacy for a broad variety of secondary alcohols.

ACKNOWLEDGEMENTS

E. Liardo acknownledges funding from the European Union's Horizon 2020 MSCA ITN-EID program under grant agreement No 634200 (Project BIOCASCADES). N.R-L acknowledges MINECO for funding under Torres-Quevedo program (PTQ-12-05 407). Authors from University of Oviedo thank to Principado de Asturias for financial support (FC-15-GRUPIN-14-002). Authors also thank Wolfgang Kroutil the generous gift of the ω-TAs Cv, ArS, ArR and ArRmut11.

SUPPORTING INFORMATION

Supporting Information Available: Experimental procedures, screenings for the oxidation and enzymatic transamination steps, characterization data, copies of HPLC chromatograms and NMR spectra. This material is available free of charge on the ACS Publications website at http://pubs.acs.org.

REFERENCES

1 (a) Bruggink, A.; Schoevaart, R.; Kieboom, T. *Org. Process Res. Dev.* **2003**, ***7***, 622-640; (b) Lee, J. M.; Na, Y.; Han, H.; Chang, S. *Chem. Soc. Rev*. **2004**, *33*, 302-312; (c) Wender, P. A.; Miller, B. L. *Nature* **2009**, *460*, 197-201.

2 (a) Ricca, E.; Brucher, B.; Schrittwieser, J. H. *Adv. Synth. Catal.* **2011**, *353*, 2239-2262; (b) Simon, R. C.; Richter, N.; Busto, E.; Kroutil, W. *ACS Catal*. **2014**, *4*, 129-143; (c) Muschiol, J.; Peters, C.; Oberleitner, N.; Mihovilovic, M. D.; Bornscheuer, U. T.; Rudroff, F. *Chem. Commun*. **2015**, *51*,

5798-5811; (d) France, S. P.; Hepworth, L. J.; Tuner, N. J.; Flitsch, S. L. *ACS Catal.* **2017**, *7*, 710-724; (e) Schrittwieser, J. H.; Velikogne, S.; Hall, M.; Kroutil, W. *Chem. Rev*. **2017**, DOI**:** 10.1021/acs.chemrev.7b00033.

3 Yamada, H.; Shimizu, S. *Angew. Chem., Int. Ed*. **1988**, *27*, 622-642.

4 Gröger, H.; Hummel, W. *Curr. Opin. Chem. Biol.* **2015**, *19*, 171-179.

5 For examples of Cu-catalyzed 'click' reactions, Ru-catalyzed allylic isomerizations, the Wacker oxidation or Suzuki and Heck cross-coupling reactions combined with bioreduction and bioamination processes, see: (a) Cuetos, A.; Bisogno, F. R.; Lavandera, I.; Gotor, V. *Chem. Commun.* **2013**, *49*, 2625-2627; (b) Ríos-Lombardía, N.; Vidal, C.; Cocina, M.; Morís, F.; García-Álvarez, J.; González-Sabín, J. *Chem. Commun*. **2015**, *51*, 10937-10940; (c) Ríos-Lombardía, N.; Vidal, C.; Liardo, E.; Morís, F.; García-Álvarez, J.; González-Sabín, J. *Angew. Chem., Int. Ed.* **2016**, *55*, 8691-8695; (d) Sato, H.; Hummel, W.; Gröger, H. *Angew. Chem., Int. Ed.* **2015**, *54*, 4488-4492; (e) Burda, E.; Hummel, W.; Gröger, H. *Angew. Chem., Int. Ed.* **2008**, *47*, 9551-9554; (f) Boffi, A.; Cacchi, S.; Ceci, P.; Cirilli, R.; Fabrizi, G.; Prastaro, A.; Niembro, S.; Shafir, A.; Vallribera, A. *ChemCatChem* **2011**, *3*, 347-353.

6 For examples of metal-catalyzed olefin metatheses coupled to enzymatic decarboxylation, hydrolysis or aromatization processes, see: (a) Baraibar, A. G.; Reichert, D.; Mügge, C.; Seger, S.; Gröger, H., Kourist, R. *Angew. Chem., Int. Ed.* **2016**, *55*, 1-6; (b) Tenbrink, K.; Sebler, M.; Schatz, J. Gröger, H. *Adv. Synth. Catal.* **2011**, *353*, 2363-2367; (c) Scalacci, N.; Black, G. W.; Mattedi, G.; Brown, N. L.; Turner, N. J.; Castagnolo, D. *ACS Catal*. **2017**, *7*, 1295-1300. For other successful combinations of metals and enzymes, see: (d) Wang, Z. J.; Clary, K. N.; Bergman, R. G.; Raymond K. N.; Toste, F. D. *Nat. Chem.* **2013**, *5*, 100-103; (e) Denard, C. A.; Huang, H.; Bartlett, M. J.; Lu, L.; Tan, Y.; Zhao H.; Hartwig, J. F. *Angew. Chem., Int. Ed.* **2014**, *53*, 465-469; (f) Latham, J.; Henry, J.-M.; Sharif, H.

H.; Menon, B. R. K.; Shepherd, S. A.; Greaney M. F.; Micklefield, J. *Nat. Commun.* **2016**, *7*, 11873-11880.

7 (a) Rulli, G.; Duangdee, N.; Baer, K.; Hummel, W.; Berkessel, A.; Gröger, H. *Angew. Chem., Int. Ed.*, **2011**, *50*, 7944-7947; (b) Hickey, D. P.; McCammant, M. S.; Giroud, F.; Sigman, M. S.; Minteer, S. D. *J. Am. Chem. Soc*. **2014**, *136*, 15917-15920.

8 (a) Newman, D. J.; Cragg, G. M. *J. Nat. Prod*. **2016**, *79*, 629−661. (b) Talapatra, S. K.; Talapatra, B. *Chemistry of Plant Natural Products*; Springer-Verlag Berlin Heidelberg, **2015**. (c) *Pharmaceuticals: Classes, Therapeutic Agents, Areas of Application*; McGuire, J. L., Ed.; Wiley-VCH: Weinheim, Germany, **2000**; Vol. 1-4.

9 Kohls, H.; Steffen-Munsberg, F.; Höhne, M. *Curr. Opin. Chem. Biol*. **2014**, 19, 180-192 and references therein.

10 Barta, K.; Ford, P. C. *Acc. Chem. Res*. **2014**, *47*, 1503–1512

11 (a) Tauber, K.; Fuchs, M.; Sattler, J.; Pitzer, J.; Pressnitz, D.; Koszelewski, D.; Faber, K.; Pfeffer, J.; Haas, T.; Kroutil, W. *Chem. Eur. J.* **2013**, *19*, 4030-4035; (b) Mutti, F. G.; Knaus, T.; Scrutton, N. S.; Breuer, M.; Turner, N. J. *Science* **2015**, *349*, 1525-1529.

12 (a) Fuchs, M.; Tauber, K.; Sattler, J.; Lechner, H.; Pfeffer, J.; Kroutil, W.; Faber, K. *RSC Adv.* **2012**, *2*, 6262-6265; (b) Pickl, M.; Fuchs, M.; Glueck, S. M.; Faber, K. *ChemCatChem.* **2015**, *7*, 3121-3124.

13 Martínez-Montero, L.; Gotor, V.; Gotor-Fernández, V.; Lavandera, I. *Green Chem.* **2017**, *19*, 474-480.

14 (a) Finnin, M. *Am. J. Health Syst. Pharm*. **2010**, *67*, 1157–1164; (b) Izawa, K.; Onishi, T. *Chem. Rev*. **2006**, *106*, 2811–2827.

15 Ager, D. J.; Prakash, I.; Schaad, D. R. *Chem. Rev*. **1996**, *96*, 835–876.

16 (a) Cao, Q.; Dornan, L. M.; Rogan, L.; Hughes, N. L.; Muldoon, M. J. *Chem. Commun*. **2014**, *50*, 4524-4543; (b) Tebben, L.; Studer, A. *Angew. Chem., Int. Ed.* **2011**, *50*, 5034-5068.

17 (a) Caron, S.; Dugger, R. W.; Ruggeri, S. G.; Ragan, J. A.; Brown Ripin, D. H. *Chem. Rev*. **2006**, *106*, 2943-2989; (b) Dugger, R. W.; Ragan, J. A.; Brown Ripin, D. H. *Org. Process Res. Dev*. **2005**, *9*, 253-258.

18 See the Supporting Information for details about the screening of nitroxyl radicals.

19 Shibuya, M.; Tomizawa, M. T.; Suzuki, I.; Iwabuchi, Y. *J. Am. Chem. Soc.* **2006**, *128*, 8412-8413.

20 Zhu, C.; Zhang, Z.; Ding, W.; Xie, J.; Chen, Y.; Wu, J.; Chen, X.; Ying, H. *Green Chem*. **2014**, *16*, 1131-1138.

21 For some reviews about ω-TAs, see: (a) Höhne, M.; Bornscheuer, U. in *Enzyme Catalysis in Organic Synthesis*, ed. K. Drauz, Gröger, H.; May, O., Wiley-VCH, 3rd edn, 2012, 779; (b) Mathew, S.; Yun, H. *ACS Catal.* **2012**, *2*, 993-1001; (c) Koszelewski, D.; Tauber, K.; Faber, K.; Kroutil, W. *Trends Biotechnol*. **2010**, *28*, 324-332; For some examples of bioamination applied to pharmaceutical relevant amines including industrial processes, see: (d) Savile, C. K.; Janey, J. M.; Mundorff, E. C.; Moore, J. C.; Tam, S.; Jarvis, W. R.; Colbeck, J. C.; Krebber, A.; Fleitz, F. J.; Brands, J.; Devine, P. N.; Huisman, G. W.; Hughes, G. J. *Science* **2010**, *329*, 305-309; (e) Girardin, M.; Ouellet, S. G.; Gauvreau, D.; Moore, J. C.; Hughes, G.; Devine, P. N.; O'Shea, P. D.; Campeau, L.-C. *Org. Process Res. Dev*. **2013**, *17*, 61-68; (f) Chung, C. K.; Bulger, P. G.; Kosjek, B.; Belyk, K. M.; Rivera, N.; Scott, M. E.; Humphrey, G. R.; Limanto, J.; Bachert, D. C.; Emerson, K. M. *Org. Process Res. Dev*. **2014**, *18*, 215-227; (g) Pavlidis, I. V.; Weis M. S.; Genz, M.; Spurr, P.; Hanlon S. P.; Wirz, B.; Iding, H.; Bornscheuer, U. T. *Nat, Chem*. **2016**, *8*, 1076-1082.

22 For an extensive overview about the DYRKR concept, see: (a) Applegate, G. A.; Berkowitz, D. B. *Adv. Synth. Catal*. **2015**, *357*, 1619-1632.

23 (a) Cuetos, A.; Lavandera, I.; Gotor, V. *Chem. Commun*. **2013**, *49*, 10688-10690; (b) Limanto, J.; Ashley, E. R.; Yin, J.; Beutner, G. L.; Grau, B. T.; Kassim, A. M.; Kim, M. M.; Kaplars, A.; Liu, Z.; Strotman, H. R.; Truppo, M. D. *Org. Lett*. **2014**, *16*, 2716-2719; (c) Peng, Z.; Wong, J. W.; Hansen, E. C.; Puchlopek-Dermenci, A. L. A.; Clarke, H. J. *Org. Lett*. **2014**, *16*, 860-863.

24 Kaulmann, U.; Smithies, K.; Smith, M. E. B.; Hailes, H. C.; Ward, J. M. *Enzyme Microb. Technol*. **2007**, *41*, 628-637.

25 Pannuri, S.; Kamat, S. V.; García, A. R. M. (Cambrex North Brunswick Inc.) WO 2006/063336A2).

26 Mutti, F. G.; Fuchs, C. S.; Pressnitz, D.; Sattler, J. H.; Kroutil, W. *Adv.Synth. Catal*. **2011**, *353*, 3227-3233.

27 See Tables S4 and S6 in the Supporting Information.

28 For an efficient synthesis of *N*,*N*-disubstituted *cis*-β-aminocycloalkanols, see: Liu, S.; Xie, J.-H.; Wang, L.-X.; Zhou, Q.-L. *Angew. Chem., Int. Ed.* **2007**, *46*, 7506-7508.

29 (a) Bornscheuer, U. T. *Angew. Chem., Int. Ed.*, **2015**, *54*, 2-4; (b) Schmidt-Dannert, C.; López-Gallego, F. *Microb. Biotechnol*. **2016**, *9*, 601-609

30 To prove the deactivation of PLP, once the oxidation reaction was allowed to react during 1 h in the presence of PLP, all the ingredients for the subsequent transamination step, except PLP, were added (see conditions in Table 3). In this case, no amine was formed and only a mixture of ketone and alcohol was observed. In addition, when pyridine-4-carbaldehyde, an analogous of PLP, was treated with 0.4 M aq NaOCl (pH = 8.9), its oxidation to pyridine-4-carboxylic acid was totally produced after 12 h.

31 The stability of AZADO was a limiting factor and longer reaction times derived from diluted media led to inactivation of the organocatalyst. For a study of stability, see: Shibuya, M.; Nagasawa, S.; Osada, Y.; Iwabuchi, Y. *J. Org. Chem*. **2014**, *79*, 10256-10268.

32 For the full panel of enzymatic screenings, see Supporting Information. In the case of ketones **2a**,**b**, 10 mM substrate concentration was advisable to optimize diastereoselectivity. In the other cases, the bioaminations proceeded well at 25 mM.

33 For ketones **2f** and **2h**, the selection of the optimal ω-TA was based on previous reports (see ref. 5b).

34 Optically active amine **3c** and its acetamide derivative are precursors of fenfluramine, an anorectic agent: (a) Cerulli, J.; Lomaestro, B. M; Malone, M. *Ann Pharmacother* **1998**, *32*, 88-102; (b) Grignaschi, G.; Samanin, R. *Eur. J. Pharmacol.* **1992**, *212*, 287-289. Amphetamine **3d** is an stimulant and anorectic agent: (c) Taylor, K. M.; Snyder, S. H. *Science* **1970**, *168*, 1487–1489; (d) Hajòs, G. T.; Garattini, S. *J. Pharm. Pharmacol*. **1973**, *25*, 418–419. Optically active **3g** is precursor of the antihypertensive agent labetalol: (e) Gold, E. H.; Chang, W.; Cohen, M.; Baum, T.; Ehrreich, S.; Johnson, G.; Prioli, N.; Sybertz, E. J. *J. Med. Chem*. **1982**, *25*, 1363-1370.