Green Chemistry

Cutting-edge research for a greener sustainable future

www.rsc.org/greenchem

Volume 15 | Number 12 | December 2013 | Pages 3279–3490



ISSN 1463-9262

RSCPublishing

PAPER

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View Article Online View Journal | View Issue

Cite this: Green Chem., 2013, 15, 3318

Received 15th August 2013, Accepted 11th September 2013 DOI: 10.1039/c3gc41666f

www.rsc.org/greenchem

Introduction

The integration of several chemical transformations into onepot processes (often referred to as 'domino', 'tandem', or 'cascade' systems) offers advantages with respect to operational simplicity, operating time and costs, safety, and the consumption of energy and materials.¹ From an ecological perspective, the one-pot combination of catalytic methods² is particularly appealing, and recent years have brought about remarkable developments in the use of heterogeneous, homogeneous, organo- and biocatalysts in cascade systems.³ However, these approaches typically remain within one individual 'subfield' of catalysis, while the one-pot combination of

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One-pot combination of enzyme and Pd nanoparticle catalysis for the synthesis of enantiomerically pure 1,2-amino alcohols†

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One-pot combinations of sequential catalytic reactions can offer practical and ecological advantages over classical multi-step synthesis schemes. In this context, the integration of enzymatic and chemo-catalytic transformations holds particular potential for efficient and selective reaction sequences that would not be possible using either method alone. Here, we report the one-pot combination of alcohol dehydrogen-ase-catalysed asymmetric reduction of 2-azido ketones and Pd nanoparticle-catalysed hydrogenation of the resulting azido alcohols, which gives access to both enantiomers of aromatic 1,2-amino alcohols in high yields and excellent optical purity (ee >99%). Furthermore, we demonstrate the incorporation of an upstream azidolysis and a downstream acylation step into the one-pot system, thus establishing a highly integrated synthesis of the antiviral natural product (*S*)-tembamide in 73% yield (ee >99%) over 4 steps. Avoiding the purification and isolation of intermediates in this synthetic sequence leads to an unprecedentedly low ecological footprint, as quantified by the *E*-factor and solvent demand.

different types of catalysts is less explored. This is particularly true for combinations of chemical catalysts and enzymes, which are often complicated by divergent reaction conditions and detrimental interactions of the bio- and chemo-catalysts.⁴ Nevertheless, several excellent recent studies aimed at bringing chemo- and biocatalysis closer together clearly demonstrate the potential of chemo-enzymatic one-pot systems.⁵ In this context, true cascades – in which the biocatalytic and chemical reactions proceed concurrently – definitely represent the most elegant examples, but they are also most prone to the abovementioned difficulties.⁶ Sequential chemo-enzymatic one-pot reactions are more easily realised and offer essentially the same environmental advantages (*e.g.* reduction of solvent use due to elimination of work-up and purification steps).^{4a,c,7}

The 2-amino-1-aryl alcohol moiety is a common structural motif in biologically active compounds,⁸ as it forms the basic scaffold of adrenergics (*e.g.* the hormones adrenaline and nor-adrenaline, β -adrenergic blockers, and anti-asthma drugs), amphenicol antibiotics, and several bioactive natural products (Fig. 1). In addition, 1,2-amino alcohols have found broad use as chiral ligands and auxiliaries in asymmetric synthesis.⁹ For both pharmaceutical and synthetic applications, a high optical purity of chiral amino alcohols is desired, causing the need for highly selective asymmetric syntheses of these compounds. Chemical methods – such as asymmetric hydrogenation¹⁰ or transfer hydrogenation¹¹ of 2-amino ketones or their synthetic equivalents – have been intensively studied in recent years, yet

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[†]Electronic supplementary information (ESI) available: Complete results of ADH screening, additional optimisation studies, characterisation data of Pd-NPs, additional details on the environmental assessment of tembamide syntheses, additional experimental procedures, full characterisation data of compounds isolated from the chemo-enzymatic transformations, EATOS and Excel files used in the environmental impact assessment. See DOI: 10.1039/c3gc41666f



Fig. 1 Examples of biologically active 1,2-amino alcohols (2-amino-1-aryl alcohol motif highlighted in bold).

they often fail to afford enantiomerically pure products. As a result, classical resolution is still a widely used technique in the preparation of chiral 1,2-amino alcohols, either for upgrading the ee of an optically enriched product or for resolving the racemate.^{10a}

Biocatalysis, on the other hand, offers several options for the preparation of optically pure 1,2-amino alcohols in general, such as kinetic resolution or dynamic kinetic resolution of the racemate using lipases,¹² oxidative kinetic resolution of *N*-protected amino ketones by Baeyer–Villiger monooxygenases,¹³ the combination of aldolase-catalysed C–C bond formation and enzymatic decarboxylation,¹⁴ or the combination of lyase-catalysed C–C bond formation with reductive amination catalysed by ω -transaminases.¹⁵ However, biocatalytic asymmetric methods for the preparation of 2-amino-1-aryl alcohols in particular are comparably scarce.^{12e,14,15a,16a}

Herein, we describe a chemo-enzymatic approach for the asymmetric synthesis of this important substance class: a sequential one-pot combination of stereoselective azido ketone reduction by alcohol dehydrogenases and subsequent azide hydrogenation by palladium nanoparticles (Scheme 1), which provides access to both enantiomers of 2-amino-1-aryl alcohols in high yields and with excellent optical purities. The integration of an upstream azidolysis step into the one-pot process and *in situ* benzoylation of the crude amino alcohol have also been achieved, enabling the one-pot asymmetric synthesis of the natural product tembamide (Fig. 1) in four steps from the

corresponding commercially available bromo ketone **1e**. The ecological benefits of this one-pot concept are demonstrated by a basic environmental impact assessment.

Results and discussion

Azido alcohol hydrogenation catalysed by metal nanoparticles

In our one-pot approach, we envisioned combining the asymmetric reduction of 2-azido ketones catalysed by alcohol dehydrogenases (ADHs) with azide hydrogenation catalysed by recently reported¹⁷ lignin-stabilised metal nanoparticles (NPs). Since the latter reaction had not been investigated before, we began our studies by screening six different metal nanoparticle preparations (Pd or Pt, stabilised by three different lignin varieties) in the hydrogenation of 2-azido-1-phenylethanol 3a. Phosphate buffer containing 5% (v/v) of 2-propanol as organic co-solvent was chosen as a reaction medium to ensure compatibility with the conditions for the enzyme-catalysed step. As shown in Table 1, Pd-LC and Pd-LK nanoparticles (for explanation of nanoparticle types, see Table 1 footnotes) gave the best results, leading to full conversion within 4 h (entries 1 and 3). Minor amounts of unidentified side products were formed in all reactions, but subsequent experiments showed

	OH N ₃	H ₂ (10 bar), M-NPs (0.5 mol%)	OH NH2
		phosphate buffer 30 °C, 4 h	4a
Entry	NP type ^b	Conversion ^c [%]	Selectivity ^c [%]
1	Pd-LC	>99	86
2	Pd-LA	95	89
3	Pd-LK	>99	85
4	Pt-LC	52	89
5	Pt-LA	97	85
6	Pt-LK	97	87
7	$Pd-LK^d$	>99	>99

^{*a*} Conditions: 100 mM **3a**, 0.5 mM NPs, 10 bar H₂, 30 °C, 4 h. For details, see the Experimental section. ^{*b*} Lignin varieties: LC = sulfonated lignin with Ca^{2+} counterions, LA = sulfonated lignin with NH⁴⁺ counterions, LK = low-sulfonate Kraft lignin. ^{*c*} Determined by GC-FID analysis. ^{*d*} Reaction under basic conditions (pH 9).



Scheme 1 Chemo-enzymatic approach towards optically pure 1,2-amino alcohols via azidolysis, alcohol dehydrogenase (ADH) catalysed asymmetric reduction, and Pd nanoparticle (Pd-NP) catalysed azide hydrogenation.

that by carrying out the reduction under basic conditions (pH 9) the chemoselectivity can be raised further, such that 2-amino-1-phenyl-ethanol **4a** was the only product detectable by GC–FID and NMR analysis (Table 1, entry 7). The hydrogenation of **3b–f** under identical conditions proceeded with the same high level of selectivity, affording the corresponding 1,2-amino alcohols **4b–f** in essentially pure form.¹⁸

Asymmetric azido ketone reduction catalysed by ADHs

Next, we turned our attention to the ADH-catalysed asymmetric reduction of aromatic 2-azido ketones. Although there is literature precedence for this transformation,¹⁹ a broad survey of suitable enzymes has not yet been reported. Therefore, we carried out an extensive screening of 79 commercial ADHs of unspecified origin and four bacterial ADHs (ADH-A from Rhodococcus ruber DSM 44541, TbADH from Thermoanaerobium brockii, LkADH from Lactobacillus kefir, LbADH from Lactobacil*lus brevis*)²⁰ in the reduction of 2a to 3a. The screening was performed at 50 mM concentration of 2a and in the presence of 5% (v/v) 2-propanol, the latter serving both as co-solvent and co-substrate. Table 2 shows selected results of the ADH screening (for complete results, see ESI⁺). As a general trend, we identified more anti-Prelog-selective^{21,22} ADHs (18) with activity on 2a than Prelog-selective ones (9), and on average, the former showed about 5-10 times higher activity than the latter. Only eleven enzymes afforded optically pure 3a, whereby four gave the (R)-enantiomer and seven the (S)-enantiomer.²³ Based on the observed activities and stereoselectivities, we considered the commercial ADHs listed in Table 2 (entries 5-8) as well as ADH-A (entry 1), the most promising biocatalysts for the reaction under study.

A further selection was made according to the enzymes' tolerance for higher substrate concentrations and their operational stability. We identified ADH-A and KRED-NADH-110 as the most robust of the investigated Prelog- and anti-Prelogselective enzymes, respectively, showing reasonable activity at 100 mM concentration of **2a** (Suppl. Fig. 1 and 2, ESI[†]), and a good performance over 24 h of reaction time (Suppl. Fig. 3 and 4, ESI[†]). The substrate scope of ADH-A and KRED-NADH-110 was investigated using the representative 2-azido-1-arylethanones **2a–f** as substrates. Both biocatalysts converted all six ketones tested with excellent stereoselectivity and with similar relative rates; only the reduction of **2f** by KRED-NADH-110 was surprisingly slow in comparison to the other 2-azido ketones (Fig. 2). Test transformations with 100 mM substrate concentration and optimised enzyme loadings (ADH-A: 1.5–2.5 mg mL⁻¹, KRED-NADH-110: 0.2–1.0 mg mL⁻¹; for details see ESI[†]) proceeded to complete conversion within 20 h in all cases.

Chemo-enzymatic one-pot transformations

After suitable ADHs had been identified, we proceeded with combining the enzymatic azido ketone reduction and Pd-NPcatalysed azide hydrogenation in a one-pot sequence. The biocatalytic reduction was run to completion (20 h) before adjusting the pH of the reaction mixture to 9 and adding a Pd-NP stock solution. First experiments showed that the activity of the nanoparticles was not impaired by the presence of the enzymes, and complete reduction of the intermediate azido alcohols 3a-f was achieved in 4 h. However, in addition to the expected products 4, the corresponding 2,2-dimethyloxazolidines 5 were also formed in minor amounts (4-10%), apparently by the reaction of 4 with acetone generated as by-product in the ADH-catalysed reduction (Scheme 2).²⁴ When the bioreduction was carried out under reduced pressure, so as to remove the acetone from the solution, the one-pot sequence afforded essentially pure 1,2-amino alcohols 4a-f. In semipreparative-scale experiments (5 mL volume, 0.5 mmol substrate converted), the desired products could be isolated in good yield and excellent enantiomeric excess (Table 3). Only the isolated yields of 4f fall behind compared to the other amino alcohols, which we attribute to the high aqueous solubility and the resulting difficult extraction of 4f.

Table 2 Activity and stereoselectivity of selected ADHs in the reduction of 2a to 3a^a

		0 N ₃ 2a	2-PrOH, ADH cat. phosphate buffer (pH 7) 30 °C, 2 h	OH N ₃ 3a		
Entry	Enzyme	Source/supplier	Cofactor ^b	Conv. ^{<i>c</i>} [%]	Act. ^{d} [U mg ⁻¹]	ee ^e [%]
1	ADH-A	R. ruber	NADH	12	0.5	>99 (R)
2	TbADH	T. brockii	NADPH	<1	<0.1	ndf
3	<i>Lk</i> ADH	L. kefir	NADPH	2	0.1	98 (S)
4	LbADH	L. brevis	NADPH	2	0.1	>99 (S)
5	KRED-NADH-110	Codexis	NADH	58	2.4	>99 (S)
6	KRED-P3-B03	Codexis	NADPH	5	0.2	>99 (R)
7	evo-1.1.030	evocatal	NADH	7	0.3	>99 (R)
8	evo-1.1.200	evocatal	NADH	73	3.1	>99 (S)

^{*a*} Conditions: 50 mM 2a, 0.1 mM NAD⁺, 0.1 mg mL⁻¹ ADH preparation, 5% (v/v) 2-PrOH, 30 °C, 2 h. For details, see the Experimental section. ^{*b*} Preferred cofactor according to literature or supplier's information. ^{*c*} Determined by GC–FID analysis. ^{*d*} Apparent activity [μ mol product/ (min mg enzyme preparation)] calculated from conversion. ^{*e*} Enantiomeric excess of 3a determined by chiral-phase GC–FID analysis. ^{*f*} *nd* = not determined.



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ADH-A (Prelog-selective, ee >99% in all cases)

KRED-NADH-110 (anti-Prelog-selective, ee >99% in all cases)



Fig. 2 Activity of ADH-A and KRED-NADH-110 in the reduction of azido ketones **2a–f**. Apparent activity [µmol product/(min mg enzyme preparation)] determined from conversion after 2 h; conditions: 50 mM **2**, 0.1 mM NAD⁺, 0.1 mg mL⁻¹ ADH preparation, 5% (v/v) 2-PrOH, 30 °C. For details, see the Experimental section.



Scheme 2 Rationalisation of the formation of 2,2-dimethyloxazolidines **5** in the ADH/Pd-NP one-pot sequence.

To further prove the preparative value and the scalability of the one-pot two-step reaction system, we performed the conversion of azidoketone **2b** into 1,2-amino alcohol (*R*)-**4b** on gram scale (75 mL, 7.5 mmol substrate). ADH-A (1.5 mg mL⁻¹) was used as a biocatalyst, and the target compound was isolated in 84% yield (1.08 g) and >99% ee.

Encouraged by the positive results of the ADH/Pd-NP combination, we sought to integrate the *in situ* formation of azido

 Table 3
 Chemo-enzymatic two-step one-pot transformation of azido ketones

 2a-f into amino alcohols 4a-f^a

0 L	2-PrOH, ADH cat.	H ₂ , Pd-NPs cat.	OH NH	2		
R 2a–f	buffer (pH 7) 30 °C, 20 h	buffer (pH 9) 30 °C, 4 h	R	2		
Subst.	ADH	GC yield $(4)^b$ [%]	Isol. yield (4) ^c [%]	ee (4) ^d [%]		
2a	ADH-A	97	87 (60 mg)	>99 (R)		
2b	ADH-A	98	89 (76 mg)	>99 (R)		
2c	ADH-A	99	86 (67 mg)	>99 (R)		
2d	ADH-A	99	85 (64 mg)	>99 (R)		
2e	ADH-A	98	85 (71 mg)	>99 (R)		
2f	ADH-A	>99	55 (35 mg)	$>99(S)^{e}$		
2a	KRED-NADH-110	98	86 (59 mg)	>99 (S)		
2 b	KRED-NADH-110	98	78 (67 mg)	>99 (S)		
2c	KRED-NADH-110	98	88 (68 mg)	>99 (S)		
2d	KRED-NADH-110	98	86 (65 mg)	>99 (S)		
2e	KRED-NADH-110	99	87 (73 mg)	>99 (S)		
2 f	KRED-NADH-110	>99	38 (24 mg)	$>99(R)^{e}$		
	R 2a-f Subst. 2a 2b 2c 2d 2e 2f 2a 2b 2c 2d 2e 2f 2d 2e 2f 2f	O RN32-PrOH, ADH cat. buffer (pH 7) 30 °C, 20 hSubst.ADH2aADH-A2b ADH-A2d ADH-A2d ADH-A2f ADH-A2b ADH-A2c ADH-A2d ADH-A2d ADH-A2d ADH-A2d ADH-A2d C ADH-A2d C ADH-A2d C C KRED-NADH-1102d C C KRED-NADH-1102d C KRED-NADH-1102d C KRED-NADH-1102f C KRED-NADH-1102f2fADH-Na	$\begin{array}{c c} O \\ R \\ \hline \\ 2a-f \\ \hline \\ 2a-f \\ \hline \\ 2a-f \\ \hline \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ $	$\begin{array}{c c} O \\ R \\ \hline \\ 2a-f \\ \hline \\ 2a-f \\ \hline \\ 2a-f \\ \hline \\ \\ 2a-f \\ \hline \\ \\ 2a-f \\ \hline \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ $		

^{*a*} Conditions: 100 mM 2, 0.1 mM NAD⁺, 0.2–2.5 mg mL⁻¹ ADH preparation, 0.5 mM Pd-NPs, 5% (v/v) 2-PrOH, 30 °C, 24 h. For details, see the Experimental section. ^{*b*} Relative amount of 4 in the crude product determined by GC-FID analysis. Rest to 100% is the corresponding 2,2-dimethyloxazolidine 5. ^{*c*} Isolated yield of pure 4 after column chromatography; semi-preparative scale (5 mL; 0.5 mmol substrate). ^{*d*} Enantiomeric excess of 4 as determined by chiral GC-FID analysis after conversion into the corresponding 2,2-dimethyloxazolidine 5. ^{*e*} Switch in substituent priorities according to Cahn–Ingold–Prelog rules.

ketones 2 from the corresponding halo ketones 1 into the onepot system. As a first test, 1a was reacted at 60 °C with 1.2 equiv. of NaN3 in buffer containing 5% (v/v) 2-propanol and varied amounts of potassium iodide as a nucleophilic substitution catalyst. With 5 and 10 mol% of iodide, the reaction proceeded almost equally fast, and 90% conversion was achieved within 4 h (Suppl. Fig. 5, ESI⁺). Transferring the same conditions (10 mol% KI) to the autoclave setup used for the one-pot sequence, the azidolysis reaction of 1a-e proceeded to completion within 5 h, while the furan derivative 1f only required 2 h for full conversion. In addition, we found that the ADH-catalysed reduction could be performed on the crude reaction mixture of the azidolysis step, as a minor decrease in enzyme activity was easily compensated by slightly raising the enzyme loading. Hence, the sequential combination of all three reactions (azidolysis, ADH reduction, and hydrogenation) proved feasible. All steps of the one-pot process could be run to complete conversion, and no accumulation of any side products was observed. Consequently, the 1,2-amino alcohols 4a-f were obtained with the same level of chemical and enantiomeric purity as in the two-step process (see Table 4).

An exemplary gram-scale conversion (75 mL, 7.5 mmol substrate) of **1c** into (*R*)-**4c** using ADH-A (2.0 mg mL⁻¹) as a biocatalyst provided the 1,2-amino alcohol in 84% isolated yield (0.97 g) and optically pure form (ee >99%).

Finally, we wanted to apply our newly developed chemoenzymatic one-pot reaction system to the asymmetric synthesis of a biologically active molecule. As a target compound, we

Table 4Chemo-enzymatic three-step one-pot transformation of halo ketones1a-f into amino alcohols $4a-f^a$

o ∐	x	NaN ₃ , KI cat.	2-PrOH, ADH cat.	H ₂ , Pd-NPs cat	, OF	I NHa
R´` 1a	∽⁄∧ ` ı–f	buffer (pH 7) 60 °C, 2–5 h	buffer (pH 7) 30 °C, 20 h	buffer (pH 9 30 °C, 4 h) R 4a	-f
Entry	Subst	. ADH	GC (4)	yield Isol ${}^{b}[\%]$ (4) ^c	. yield [%]	ee (4) ^d [%]
1	1a	ADH-A	>9	9 83 (57 mg)	>99 (R)
2	1b	ADH-A	>9	9 75 (63 mg)	>99 (R)
3	1c	ADH-A	>9	9 89 (69 mg)	>99 (R)
4	1d	ADH-A	>9	9 81 (61 mg)	>99 (R)
5	1e	ADH-A	>9	9 75 (63 mg)	>99 (R)
6	1f	ADH-A	>9	9 39 (25 mg)	$>99(S)^{e}$
7	1a	KRED-NA	DH-110 9	9 76 (52 mg)	>99 (S)
8	1b	KRED-NA	DH-110 >9	9 80 (69 mg)	>99 (S)
9	1c	KRED-NA	DH-110 >9	9 79 (61 mg)	>99 (S)
10	1d	KRED-NA	DH-110 >9	9 82 (62 mg)	>99 (S)
11	1e	KRED-NA	DH-110 >9	9 86 (72 mg)	>99 (S)
12	1f	KRED-NA	DH-110 >9	9 36 (23 mg)	$>99 (R)^{e}$

^{*a*} Conditions: 100 mM 1, 120 mM NaN₃, 10 mM KI, 0.1 mM NAD⁺, 0.3–4.0 mg mL⁻¹ ADH preparation, 0.5 mM Pd-NPs, 5% (v/v) 2-PrOH, 60–30 °C, 26–29 h. For details, see the Experimental section. ^{*b*} Relative amount of 4 in the crude product determined by GC-FID analysis. Rest to 100% is the corresponding 2,2-dimethyloxazolidine 5. ^{*c*} Isolated yield of pure 4 after column chromatography; semi-preparative scale (5 mL; 0.5 mmol substrate). ^{*d*} Enantiomeric excess of 4 as determined by chiral GC-FID analysis after conversion into the corresponding 2,2-dimethyloxazolidine 5. ^{*e*} Switch in substituent priorities according to Cahn–Ingold–Prelog rules.

chose (*S*)-tembamide, a naturally occurring benzamide derivative, for which antiviral (HIV) activity has been reported.²⁵ The synthesis required benzoylation of amino alcohol (*S*)-**4e** as the final step, which was achieved by simply adding a solution of benzoyl chloride (1.2 eq.) in MTBE to the alkaline, aqueous reaction mixture obtained after the Pd-NP-catalysed hydrogenation step (Scheme 3). A gram-scale reaction (50 mL, 5.0 mmol substrate, 0.5 mg mL⁻¹ KRED-NADH-110 as biocatalyst) yielded 0.98 g (73% from **1e**) of (+)-(*S*)-tembamide, thus providing access to this natural product in a four-step one-pot operation.

Environmental impact assessment

Because of its highly 'integrative' nature we considered the tembamide synthesis a suitable test case for assessing the ecological benefits of the multi-step one-pot concept. Therefore, we performed a basic environmental impact analysis, in which we compared our four-step one-pot preparation of tembamide to previously reported asymmetric syntheses of this compound. We chose Sheldon's *E*-factor²⁶ (mass of waste produced per mass of desired product) as a simple metric. We also decided to exclude solvents from the *E*-factor analysis and calculate the solvent demand as a second, independent indicator, since solvent waste and non-volatile waste (particularly salts) require very different processing. Both metrics can only provide a rough estimation of environmental impact, as they do not take into account the chemical composition (and hence the toxicity) of the waste, the energy demand of the involved processes, or the waste generated in the preparation of starting materials and catalysts. On the other hand, such a basic analysis is easily performed, and can thus serve as a quick ecoassessment of several synthetic options.

Table 5 provides an overview of the environmental performance of the six synthetic sequences under investigation. The chemo-enzymatic four-step one-pot system presented herein achieves the second-highest yield and has clear environmental advantages over the previously published procedures. Only the synthesis developed by Brown *et al.* reaches comparable values for yield, *E*-factor and solvent demand; however, it requires highly toxic hydrogen cyanide as a reagent in the asymmetric key step.

Differentiation of the *E*-factor into the contributions of the reaction itself (excess of reagents, coupled products, byproducts, catalysts) and the down-stream processing reveals the main advantage of the one-pot concept: the elimination of the isolation and purification steps leads to significant reductions in waste generation, which for all syntheses except the one reported by Yadav *et al.* (which uses large quantities of carrot root as a catalyst), is mainly linked to work-up and purification rather than loss of material in the reaction itself (Fig. 3). Nevertheless, our synthesis also features the lowest reaction-linked *E*-factor contribution (3.2) of the six procedures analysed.

Differentiation by the type of waste (see Suppl. Fig. 10, ESI[†]) shows that silica gel contributes substantially to the *E*-factor of the syntheses that make use of it. Those sequences that do not require chromatographic purifications (Baeza *et al.*, Brown *et al.*, and the present work), mainly produce inorganic salts (NaBr, NaCl, NaOH, Na₂SO₄, MgSO₄, and others) as waste, which are arguably less problematic.

Finally, a more detailed analysis of the different types of solvents used (see Suppl. Table 7, ESI[†]) shows that our chemoenzymatic sequence generally employs more environmentally acceptable solvents³¹ (mostly water, ethyl acetate, and ethanol) than the other processes, especially because it avoids the use of chlorinated solvents and does not require eluents for chromatography, which often contain large amounts of hexane.



Scheme 3 Asymmetric synthesis of (S)-tembamide in a chemo-enzymatic four-step one-pot sequence

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Table 5 Environmental impact comparison of catalytic asymmetric syntheses of tembamide

Article	Steps ^a	Asymmetric key step	Yield ^b [%]	<i>E</i> -factor ^{<i>c</i>}	Solvent ^d $[mL g^{-1}]$	Ref.
Present work	4 (1)	Asymmetric ketone reduction (ADH)	73	11.1	309	_
Lee et al. 2007	5 (5)	Asymmetric ketone reduction (Rh catalyst)	62	57.8	1600	27
Baeza et al. 2005	3 (2)	Asymmetric cyano- <i>O</i> -phosphorylation (Lewis acid/Brønsted base catalyst)	65	23.3	1031	28
Kamal <i>et al.</i> 2004	5(4)	Enantioselective transesterification (lipase)	42	114.9	1801	29
Yadav et al. 2001	$3(2)^{e}$	Asymmetric ketone reduction (carrot root)	85	97.5	826	16 <i>b</i>
Brown et al. 1993, 1994	3 (3)	Asymmetric hydrocyanation (peptide catalyst)	72	14.6	483	30

^{*a*} Total number of chemical transformations. The number of steps carried out individually (with product isolation) is given in parentheses. ^{*b*} Overall yield. ^{*c*} Overall *E*-factor (excluding solvents). ^{*d*} Overall solvent demand. ^{*e*} Please note that this synthesis starts from 2-azido ketone 2e, which is not commercially available, and therefore also needs to be synthesised.



Fig. 3 Contributions of reaction steps, product isolation steps, and product purification steps to the overall E-factor (excluding solvents) of catalytic asymmetric syntheses of tembamide

Conclusions

In summary, we have developed chemo-enzymatic one-pot reaction sequences that provide access to enantiomerically pure 1,2-amino alcohols either in two steps from the corresponding 2-azido ketones or in three steps from 2-halo ketones. The biocatalytic reduction of 2-azido ketones using alcohol dehydrogenases (ADHs) is the asymmetric key step in these processes, and by selecting suitable ADHs, both enantiomers of the target compounds can be obtained in excellent enantiomeric excess (ee >99%). The 2-amino-1-arylethanol derivatives 4a-f thus prepared are important building blocks in pharmaceutical research, for instance in the synthesis of anti-inflammatory, antiviral, or antitumour agents.³²

Furthermore, the one-pot concept has been applied to the asymmetric synthesis of the antiviral natural product (S)-tembamide, obtained in 73% yield over four steps and >99% ee from commercially available bromo ketone 1e. This synthesis reaches high catalyst turnover (TON = 200 for Pd, 1000 for NAD⁺, several 10 000 for the ADH),³³ uses only a small excess of reagents (1.2 eq. of NaN₃ and BzCl), and affords a chemically pure product after a final recrystallisation as the sole purification step. Due to these features, our method compares favourably with previous syntheses of tembamide not only in

terms of yield, but especially regarding its ecological impact, as quantified by E-factor and solvent demand. Hence, our study highlights the advantages of chemo-enzymatic one-pot processes in the multi-step synthesis of chiral compounds, and since it uses catalysts that are either commercially available or easily prepared, we believe that it will also be of practical value to synthetic chemists.

Experimental

General materials and methods

Unless otherwise noted, reagents and organic solvents were obtained from chemical suppliers in reagent grade quality and used without further purification. Petroleum ether (boiling range 40-60 °C) and ethyl acetate used for extraction and column chromatography were purchased in technical grade quality and were distilled prior to use. Pro analysi (p.a., >99% purity) grade solvents were used for handling the 1,2-amino alcohols 4a-f to avoid any undesired oxazolidine formation due to contaminating acetone. Sulfonated lignin with either calcium or ammonium counterions was obtained from Burgo Group S.p.A., Tolmezzo, Italy. Low-sulfonate Kraft lignin was obtained from Sigma-Aldrich. The halo ketones 1a-e as well as both enantiomers of amino alcohol 4a were obtained commercially; all other substrates and reference compounds were synthesised as described in the ESI.⁺

The proprietary enzymes used in this study are part of the Codexis Codex KRED screening kit, the Almac selectAZyme CRED screening kit, and the evocatal ADH screening kit. The ADHs from Lactobacillus kefir and Thermoanaerobium brockii were obtained from Sigma-Aldrich. ADH-A from Rhodococcus ruber DSM 44541 and LbADH from Lactobacillus brevis were heterologously expressed in E. coli as described in the ESI.⁺

Hydrogenation reactions, as well as analytical-scale (2 mL) and semi-preparative scale (5 mL) chemo-enzymatic one-pot transformations were carried out in magnetically stirred stainless steel autoclaves (16 mL total volume) that are part of a HEL PolyBlock8 parallel reactor system, and reactor temperature as well as stirring speed were controlled using the associated HEL WinISO software (v. 2.3.85.1). Gram-scale chemoenzymatic one-pot transformations were carried out in a

mechanically stirred *Parr* 4560 series stainless steel autoclave (452HC2 bomb cylinder, 160 mL total volume), and the reactor temperature was controlled using a *Parr* 4841 heater/controller. Non-enzymatic reactions were generally stirred at 500 rpm, while biotransformations were stirred at 300 rpm (to minimise mechanical stress).

Thin layer chromatography was carried out on silica gel 60 F₂₅₄ plates (Merck) and compounds were visualized either by UV or by dipping into cerium ammonium molybdate stain [50 g L⁻¹ (NH₄)₆Mo₇O₂₄·4H₂O, 2 g L⁻¹ Ce(SO₄)₂·4H₂O in 10% (v/v) sulfuric acid] or basic permanganate stain (50 g L^{-1} Na_2CO_3 , 10 g L⁻¹ KMnO₄, 0.85 g L⁻¹ NaOH in demineralised water). Melting points were determined in open capillary tubes on a Büchi B-540 apparatus and are uncorrected. ¹H-NMR and ¹³C-NMR spectra were recorded in CDCl₃ solution on a Bruker Avance 400 instrument at 400 and 100 MHz, respectively. Chemical shifts are given in parts per million (ppm) relative to the residual CHCl₃ peak (¹H: δ = 7.26 ppm, ¹³C: δ = 77.2 ppm) and coupling constants (J) are reported in Hertz (Hz). Specific optical rotation values $\left[\alpha\right]_{D}^{20}$ were determined on a *Perkin-Elmer* Model 343 Polarimeter at 20 °C and a wavelength of 589 nm (sodium D-line) using a cuvette of 1 dm path length.

Azido alcohol hydrogenation catalysed by metal nanoparticles

Screening of metal nanoparticles for activity and selectivity in the hydrogenation of azido alcohol 3a. In a small-scale autoclave reactor (16 mL), 2-azido-1-phenylethanol (3a; 32 mg, 200 µmol; final conc. 100 mM) was dissolved in 2-propanol (100 µL; final conc. 5% v/v). Potassium phosphate buffer (1.72 or 1.82 mL, depending on nanoparticle type; 100 mM, pH 7.0, 1 mM MgSO₄) and a stock solution of metal nanoparticles in water (Pd: 180 µL of a 5.6 mM stock, Pt: 83 µL of a 12 mM stock; final conc. 0.5 mM) were added, and the mixture was stirred at 30 °C and 500 rpm under hydrogen atmosphere (10 bar) for 4 h. The reaction mixture was extracted with EtOAc (800 µL), the extract was dried over MgSO₄ and conversion was determined by GC–FID analysis.

Investigation of the tolerance of Pd nanoparticles towards the presence of ADHs and NAD(P)⁺. Reactions were set up as described above, but contained 0.1–1.5 mg mL⁻¹ of different ADHs and 100 μ M NAD⁺ or NADP⁺.

Hydrogenation of azido alcohols 3a–f catalysed by Pd nanoparticles. Reactions were set up as described above, using 200 µmol (final conc. 100 mM) of azido alcohols 3a–f as substrates. After 4 h, the reaction mixture was transferred to microcentrifuge tubes, the product was extracted into EtOAc (2 × 1 mL), and the extract was dried over MgSO₄. Evaporation of the solvent under reduced pressure afforded the crude amino alcohols 4a–f. The conversion as well as the purity of the crude products were determined by GC–FID and NMR analysis.

Biotransformations

Screening of ADHs for activity and stereoselectivity in the reduction of azido ketone 2a. In a microcentrifuge tube (2 mL), 2-azidoacetophenone (2a; 4 mg, 25 μ mol; final conc. 50 mM) was dissolved in 2-propanol (25 μ L; final conc. 5% v/v,

approx. 650 mM). Potassium phosphate buffer (425 μ L; 100 mM, pH 7.0, 1 mM MgSO₄) and a stock solution of ADH (1 mg mL⁻¹; final conc. 0.1 mg mL⁻¹) and NAD(P)⁺ (0.7 mg mL⁻¹, 1 mM; final conc. 100 μ M) in potassium phosphate buffer (50 μ L) were added, and the mixture was shaken at 30 °C and 1000 rpm on a thermoshaker for 2 h. The reaction mixture was extracted with EtOAc (800 μ L), the extract was dried over MgSO₄ and conversion as well as product ee were determined by GC–FID analysis.

Investigation of the substrate scope of ADH-A and KRED-NADH-110. In a microcentrifuge tube (2 mL), azido ketone 2a–f (25 µmol; final conc. 50 mM) was dissolved in 2-propanol (25 µL; final conc. 5% v/v, approx. 650 mM). Potassium phosphate buffer (425 µL; 100 mM, pH 7.0, 1 mM MgSO₄) and a stock solution of ADH (1 mg mL⁻¹; final conc. 0.1 mg mL⁻¹) and NAD(P)⁺ (0.7 mg mL⁻¹, 1 mM; final conc. 100 µM) in potassium phosphate buffer (50 µL) were added, and the mixture was shaken at 30 °C and 1000 rpm on a thermoshaker for 2 h. The reaction mixture was extracted with EtOAc (800 µL), and the extract was dried over MgSO₄. Conversion was determined by GC–FID analysis, while product ee was determined either by GC–FID analysis (**3a**, **3e**, **3f**) or by HPLC analysis (**3b–d**).

Test transformations to ensure complete conversion under the conditions of the one-pot sequence were carried out in small-scale autoclave reactors (16 mL): azido ketone **2a–f** (200 µmol; final conc. 100 mM) was dissolved/dispersed in 2-propanol (100 µL; final conc. 5% v/v, approx. 650 mM). Potassium phosphate buffer (1.7 mL; 100 mM, pH 7.0, 1 mM MgSO₄) and a stock solution of ADH (2–25 mg mL⁻¹; final conc. 0.2–2.5 mg mL⁻¹, see Table 6) and NAD(P)⁺ (0.7 mg mL⁻¹, 1 mM; final conc. 100 µM) in potassium phosphate buffer (200 µL) were added, and the mixture was stirred at 30 °C and 300 rpm for 20 h. The reaction mixture was transferred to microcentrifuge tubes, the product was extracted into EtOAc (2 × 1 mL), and the extract was dried over MgSO₄. Conversion was determined by GC–FID analysis (**3a**, **3f**) or HPLC analysis (**3b–e**).

Chemo-enzymatic one-pot transformations

One-pot, two-step transformation of azido ketones 2a-f into amino alcohols 4a-f (analytical scale, 2 mL). A small-scale autoclave reactor (16 mL) was charged with the azido ketone 2

Table 6	ADH concentration	s used for the two-ste	ep one-pot transfor	rmation of
azido ket	ones 2a–f into amin	o alcohols 4a–f		

Substrate	$c(\text{ADH-A})^a$ [mg mL ⁻¹]	$c(\text{KRED-NADH-110})^a$ [mg mL ⁻¹]
2a	1.5	0.2
2b	1.5	0.2
2c	1.5	0.2
2d	1.5	0.2
2e	2.5	0.4
2f	1.5	1.0

 $^{\it a}$ Final concentration of crude ADH preparation in the reaction mixture.

(200 µmol; final conc. 100 mM), 2-propanol (100 µL; final conc. 5% v/v, approx. 650 mM), potassium phosphate buffer (1.7 mL; 100 mM, pH 7.0, 1 mM MgSO₄) and a stock solution of ADH (ADH-A: $15-25 \text{ mg mL}^{-1}$, final conc. $1.5-2.5 \text{ mg mL}^{-1}$; Codexis KRED-NADH-110: $2.0-10 \text{ mg mL}^{-1}$, final conc. $0.2-1.0 \text{ mg mL}^{-1}$; see Table 6) and NAD⁺ (0.7 mg mL⁻¹, 1 mM; final conc. 100 µM) in potassium phosphate buffer (200 µL), and the mixture was stirred at 30 °C and 300 rpm. The vent valve of the autoclave reactor was connected to a vacuum pump via a pressure control valve, and the reactor was evacuated to 200 mbar. After a reaction time of 18 h, the temperature was raised to 45 °C (to increase acetone evaporation) and stirring was continued for 2 h. A sample (50 µL) was taken, extracted with EtOAc (800 µL), the extract was dried over MgSO₄ and conversion was determined by GC analysis. The reaction mixture was then made alkaline (pH 9) by the addition of 4 M aq. NaOH solution (25 µL), a stock solution of Pd nanoparticles (180 µL of a 5.6 mM stock; final conc. 0.5 mM) was added, and the mixture was stirred for another 4 h at 30 °C and 500 rpm under hydrogen atmosphere (10 bar). The reaction mixture was extracted with EtOAc (800 μ L), the extract was dried over MgSO₄ and conversion as well as product ee were determined by GC analysis.

One-pot, two-step transformation of azido ketones 2a-f into amino alcohols 4a-f (semi-preparative scale, 5 mL). A smallscale autoclave reactor (16 mL) was charged with the azidoketone 2 (500 µmol; final conc. 100 mM), 2-propanol (250 µL; final conc. 5% v/v), potassium phosphate buffer (4.25 mL; 100 mM, pH 7.0, 1 mM MgSO₄) and a stock solution of ADH (ADH-A: 15–25 mg mL⁻¹, final conc. 1.5–2.5 mg mL⁻¹; Codexis KRED-NADH-110: 2.0-10 mg mL⁻¹, final conc. 0.2-1.0 mg mL^{-1} ; see Table 6) and NAD⁺ (0.7 mg mL⁻¹, 1 mM; final conc. 100 µM) in potassium phosphate buffer (500 µL), and the mixture was stirred at 30 °C and 300 rpm. The vent valve of the autoclave reactor was connected to a vacuum pump via a pressure control valve, and the reactor was evacuated to 200 mbar. After a reaction time of 18 h, the temperature was raised to 45 °C (to increase acetone evaporation) and stirring was continued for 2 h. The reaction mixture was then made alkaline (pH 9) by the addition of 4 M aq. NaOH solution (65 μ L), a stock solution of Pd nanoparticles (450 μ L of a 5.6 mM stock; final conc. 0.5 mM) was added, and the mixture was stirred for an additional 4 h at 30 °C and 500 rpm under hydrogen atmosphere (10 bar). The reactor was depressurised, 4 M aq. NaOH solution (500 µL) was added, and the reaction mixture was saturated with NaCl. The product was extracted into EtOAc $(4 \times 5 \text{ mL})$, the combined extracts were dried over MgSO₄ and evaporated under reduced pressure to give the crude amino alcohols as orange oils or solids. Column chromatography (~0.6 g of silica gel 60 in a Pasteur pipette; MTBE \rightarrow MTBE-MeOH-NH₄OH = 90/9/1) afforded the pure amino alcohols 4a-f.

(*R*)-2-Amino-1-phenylethanol [(*R*)-4a]. 60 mg (87%) off-white solid. mp: 63–64 °C (lit.³⁴ 57–59 °C). TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f} = 0.10. \ [\alpha]_{\rm D}^{20} = -58.8$ (CHCl₃, c = 1.31); lit.³⁵ (*S*) +60.6 (CHCl₃, c = 0.5). ¹H-NMR (400 MHz,

CDCl₃): δ [ppm] = 2.25 (3H, br s, OH, NH₂), 2.80 (1H, dd, J = 12.8 Hz, 7.7 Hz, CH₂), 2.93 (1H, dd, J = 12.9 Hz, 3.9 Hz, CH₂), 4.62 (1H, dd, J = 7.8 Hz, 4.0 Hz, CH-OH), 7.26–7.37 (5H, m, Ar). ¹³C-NMR (100 MHz, CDCl₃): δ [ppm] = 49.3, 74.4, 125.9, 127.5, 128.4, 142.6. GC–MS (EI, 70 eV): m/z = 137 (M⁺, <1), 118 (3), 107 (31), 91 (11), 79 (100), 77 (79), 65 (6), 51 (37). The NMR data are in accordance with literature values.³⁶

(*S*)-2-Amino-1-phenylethanol [(*S*)-4a]. 59 mg (86%) off-white solid. mp: 61–62 °C (lit.³⁴ 57–59 °C). TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f} = 0.10$. $[\alpha]_{\rm D}^{20} = +58.5$ (CHCl₃, c = 1.53); lit.³⁵ +60.6 (CHCl₃, c = 0.5). NMR and MS data were in accordance with those of the opposite enantiomer.

(*R*)-2-Amino-1-(4-chlorophenyl)ethanol [(*R*)-4b]. 76 mg (89%) off-white solid. mp: 94–95 °C (lit.³⁷ 92–94 °C). TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f} = 0.11. \ [\alpha]_{\rm D}^{20} = -64.5$ (CHCl₃, c = 1.08); lit.³⁸ (*S*) +67.4 (CHCl₃, c = 0.35). ¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 2.30 (3H, br s, OH, NH₂), 2.73 (1H, dd, J = 12.8 Hz, 7.8 Hz, CH₂), 2.92 (1H, d, J = 11.3 Hz, CH₂), 4.57 (1H, dd, J = 8.0 Hz, 3.9 Hz, CH-OH), 7.26 (2H, d, J = 8.3 Hz, Ar-o), 7.31 (2H, d, J = 8.1 Hz, Ar-m). ¹³C-NMR (100 MHz, CDCl₃): δ [ppm] = 49.2, 73.5, 127.2, 128.5, 133.1, 141.1. GC–MS (EI, 70 eV): m/z = 171 (M⁺, 1), 143 (5), 141 (16), 115 (4), 113 (13), 77 (100), 51 (28), 50 (14). The NMR data are in accordance with literature values.³⁶

(S)-2-Amino-1-(4-chlorophenyl)ethanol [(S)-4b]. 67 mg (78%) off-white solid. mp: 94–95 °C (lit.³⁷ 92–94 °C). TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f}$ = 0.11. [α]_D²⁰ = +64.7 (CHCl₃, c = 0.71); lit.³⁸ +67.4 (CHCl₃, c = 0.35). NMR and MS data were in accordance with those of the opposite enantiomer.

(*R*)-2-Amino-1-(4-fluorophenyl)ethanol [(*R*)-4c]. 67 mg (86%) off-white solid. mp: 83–84 °C (lit.³⁸ 63–65 °C). TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f}$ = 0.10. $[\alpha]_{\rm D}^{20}$ = -66.8 (CHCl₃, *c* = 1.08); lit.³⁸ (*S*) +40.8 (EtOH, *c* = 1.68). ¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 2.24 (3H, br s, OH, NH₂), 2.75 (1H, dd, *J* = 12.8 Hz, 7.9 Hz, CH₂), 2.94 (1H, dd, *J* = 12.6 Hz, 3.9 Hz, CH₂), 4.59 (1H, dd, *J* = 8.0 Hz, 3.9 Hz, CH-OH), 7.03 (2H, t, *J* = 8.5 Hz, Ar-*m*), 7.31 (2H, dd, *J* = 8.3 Hz, 5.4 Hz, Ar-*o*). ¹³C-NMR (100 MHz, CDCl₃): δ [ppm] = 49.3, 73.6, 115.2 (d, *J*_{CF} = 21.3 Hz), 127.5 (d, *J*_{CF} = 8.0 Hz), 138.3 (d, *J*_{CF} = 3.1 Hz), 162.2 (d, *J*_{CF} = 245 Hz). GC–MS (EI, 70 eV): *m*/*z* = 155 (M⁺, 1), 125 (39), 123 (16), 109 (14), 97 (100), 95 (41), 77 (56), 75 (22), 70 (8), 57 (11), 51 (25), 50 (14). The NMR data are in accordance with literature values.³⁸

(*S*)-2-Amino-1-(4-fluorophenyl)ethanol [(*S*)-4c]. 68 mg (88%) off-white solid. mp: 83–84 °C (lit.³⁸ 63–65 °C). TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f}$ = 0.10. $[\alpha]_{\rm D}^{20}$ = +66.7 (CHCl₃, *c* = 0.90); lit.³⁸ +40.8 (EtOH, *c* = 1.68). NMR and MS data were in accordance with those of the opposite enantiomer.

(*R*)-2-Amino-1-(4-tolyl)ethanol [(*R*)-4d]. 64 mg (85%) offwhite solid. mp: 59–60 °C (lit.³⁷ 67–69 °C). TLC (silica, MTBE– MeOH–NH₄OH = 90/9/1): $R_{\rm f}$ = 0.11. [α]_D²⁰ = -65.5 (CHCl₃, *c* = 1.00); lit.³⁹ –32 (CHCl₃, *c* = 5.0). ¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 2.19 (3H, br s, OH, NH₂), 2.35 (3H, s CH₃), 2.79 (1H, dd, *J* = 12.8 Hz, 7.8 Hz, CH₂), 2.93 (1H, d, *J* = 11.1 Hz, CH₂), 4.58 (1H, dd, J = 7.8 Hz, 4.0 Hz, CH-OH), 7.16 (2H, d, J = 7.7 Hz, Ar-*m*), 7.23 (2H, d, J = 7.6 Hz, Ar-*o*). ¹³C-NMR (100 MHz, CDCl₃): δ [ppm] = 21.1, 49.3, 74.3, 125.8, 129.1, 137.1, 139.7. GC-MS (EI, 70 eV): m/z = 151 (M⁺, 3), 122 (15), 121 (100), 119 (12), 93 (93), 91 (96), 77 (75), 65 (28), 51 (18). The NMR data are in accordance with literature values.^{10d}

(*S*)-2-Amino-1-(4-tolyl)ethanol [(*S*)-4d; CAS 149403-05-4]. 65 mg (86%) off-white solid. mp: 59–60 °C (lit.³⁷ 67–69 °C). TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f} = 0.11. \ [\alpha]_{\rm D}^{20} = +65$ (CHCl₃, c = 1.00); lit. (R)³⁹ –32 (CHCl₃, c = 5.0). NMR and MS data were in accordance with those of the opposite enantiomer.

(*R*)-2-Amino-1-(4-methoxyphenyl)ethanol [(*R*)-4e]. 71 mg (85%) off-white solid. mp: 102–103 °C. TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f}$ = 0.09. $[\alpha]_{\rm D}^{20}$ = -61.4 (CHCl₃, *c* = 0.93); lit.³⁸ –39.9 (CHCl₃, *c* = 1.03). ¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 1.94 (3H, br s, OH, NH₂), 2.79 (1H, dd, *J* = 12.8 Hz, 7.8 Hz, CH₂), 2.97 (1H, dd, *J* = 12.5 Hz, 4.0 Hz, CH₂), 3.81 (3H, s, OCH₃), 4.59 (1H, dd, *J* = 7.9 Hz, 4.1 Hz, CH-OH), 6.90 (2H, d, *J* = 8.7 Hz, Ar-*m*), 7.29 (2H, d, *J* = 8.8 Hz, Ar-*o*). ¹³C-NMR (100 MHz, CDCl₃): δ [ppm] = 49.3, 55.3, 74.1, 113.8, 127.1, 134.6, 159.1. GC–MS (EI, 70 eV): *m/z* = 167 (M⁺, 3), 138 (10), 137 (100), 109 (42), 94 (43), 77 (40), 66 (14), 65 (10), 51 (9). The NMR data are in accordance with literature values.^{10d}

(*S*)-2-Amino-1-(4-methoxyphenyl)ethanol [(*S*)-4e; CAS 46084-19-9]. 73 mg (87%) off-white solid. mp: 102–103 °C. TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f} = 0.09$. $[\alpha]_{\rm D}^{20} = +61.6$ (CHCl₃, c = 1.01); lit. (R)³⁸ –39.9 (CHCl₃, c = 1.03). NMR and MS data were in accordance with those of the opposite enantiomer.

(*S*)-2-Amino-1-(2-furyl)ethanol [(*S*)-4f]. 35 mg (55%) yellowish solid. mp: 80–81 °C. TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f} = 0.14$. $[\alpha]_{\rm D}^{20} = -33.5$ (CHCl₃, c = 0.93); lit.⁴⁰ –38.6 (CHCl₃, c = 1.80). ¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 2.53 (3H, br s, OH, NH₂), 2.98 (2H, d, J = 5.8 Hz, CH₂), 4.61 (1H, t, J = 5.8 Hz, CH-OH), 6.24 (1H, d, J = 3.3 Hz, Ar-3), 6.32 (1H, dd, J = 3.2 Hz, 1.8 Hz, Ar-4) 7.35 (1H, d, J = 1.8 Hz, Ar-5). ¹³C-NMR (100 MHz, CDCl₃): δ [ppm] = 46.0, 68.2, 106.3, 110.2, 142.0, 155.5. GC–MS (EI, 70 eV): m/z = 127 (M⁺, 12), 98 (82), 97 (86), 81 (8), 69 (33), 53 (17), 51 (15), 42 (24), 41 (100). The NMR data are in accordance with literature values.⁴¹

(*R*)-2-Amino-1-(2-furyl)ethanol [(*R*)-4f]. 24 mg (38%) yellowish solid. mp: 81–82 °C. TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f} = 0.14$. $[\alpha]_{\rm D}^{20} = +33.6$ (CHCl₃, c = 0.85); lit. (*S*)⁴⁰ –38.6 (CHCl₃, c = 1.80). NMR and MS data were in accordance with those of the opposite enantiomer.

The ADH concentrations used for the one-pot, two-step transformation of azido ketones **2a–f** into amino alcohols **4a–f** are summarised in Table 6.

One-pot, two-step transformation of azido ketone 2b into amino alcohol (*R*)-4b (gram scale, 75 mL). An autoclave reactor (160 mL) was charged with 2-azido-4'-chloroacetophenone (2b; 1.47 g, 7.5 mmol; final conc. 100 mM), 2-propanol (3.75 mL; final conc. 5% v/v), potassium phosphate buffer (66.25 mL; 100 mM, pH 7.0, 1 mM MgSO₄) and a stock solution of ADH-A (113 mg; final conc. 1.5 mg mL⁻¹) and NAD⁺ (6 mg; final conc. 100 μ M) in potassium phosphate buffer

(5 mL), and the mixture was stirred at 30 °C and 300 rpm. The vent valve of the autoclave reactor was connected to a vacuum pump via a pressure control valve, and the reactor was evacuated to 200 mbar. After a reaction time of 18 h, the temperature was raised to 45 °C and stirring continued for 2 h. The reaction mixture was cooled to room temperature, and complete consumption of the 2b was verified by TLC analysis. The reaction mixture was then made alkaline (pH 9) by the addition of 4 M aq. NaOH solution (1 mL), a stock solution of Pd nanoparticles (6.7 mL of a 5.6 mM stock; final conc. 0.5 mM), was added, and the mixture was stirred for an additional 16 h (overnight) at 30 °C and 500 rpm under hydrogen atmosphere (10 bar). Complete consumption of 3b was verified by TLC analysis, 4 M aq. NaOH solution (10 mL) was added, and the reaction mixture was saturated with NaCl. The product was extracted into EtOAc (5×40 mL; phase separation accelerated by centrifugation), and the combined extracts were dried over MgSO₄ and evaporated under reduced pressure to give 1.30 g of an orange solid. Column chromatography (silica gel 60, MTBE \rightarrow MTBE-MeOH-NH₄OH = 90/9/1) afforded 1.08 g (84%) of (R)-4b as an off-white solid. mp: 94-95 °C (lit.³⁷ 92-94 °C). TLC (silica, MTBE-MeOH-NH₄OH = 90/9/1): $R_{\rm f}$ = 0.11. $\left[\alpha\right]_{D}^{20} = -64.4$ (CHCl₃, c = 0.94); lit.³⁸ (S) +67.4 (CHCl₃, c =0.35). NMR and MS data were in accordance with those obtained for the product of the semi-preparative scale experiment.

One-pot, three-step transformation of halo ketones 1a-f into amino alcohols 4a-f (analytical scale, 2 mL). A small-scale autoclave reactor (16 mL) was charged with the halo ketone 1 (200 µmol; final conc. 100 mM), 2-propanol (100 µL; final conc. 5% v/v, approx. 650 mM) and potassium phosphate buffer (1.9 mL; 100 mM, pH 7.0, 1 mM MgSO₄) containing NaN_3 (7.8 mg mL⁻¹, 120 mM) and KI (1.7 mg mL⁻¹, 10 mM), and the mixture was stirred at 60 °C and 500 rpm for 2–5 h. A sample (50 µL) was taken, extracted with EtOAc (800 µL), the extract was dried over MgSO4 and conversion was determined by GC analysis. Additional 2-propanol (100 µL; to supplement the material lost through evaporation) and a stock solution of ADH (ADH-A: 20–30 mg mL⁻¹, final conc. 2.0–3.0 mg mL⁻¹; Codexis KRED-NADH-110: $3.0-15 \text{ mg mL}^{-1}$, final conc. 0.3–1.5 mg mL⁻¹; see Table 7) and NAD⁺ (0.7 mg mL⁻¹, 1 mM; final conc. 100 µM) in potassium phosphate buffer (200 µL) were added, and the mixture was stirred at 30 °C and 300 rpm.

 Table 7
 ADH concentrations used for the three-step one-pot transformation of halo ketones 1a-f into amino alcohols 4a-f

Substrate	$c(\text{ADH-A})^a$ [mg mL ⁻¹]	$c(\text{KRED-NADH-110})^a$ [mg mL ⁻¹]
2a	2.0	0.3
2b	2.0	0.3
2c	2.0	0.3
2d	2.0	0.3
2e	3.0	0.5
2f	2.0	1.5

 $^{\it a}$ Final concentration of crude ADH preparation in the reaction mixture.

The vent valve of the autoclave reactor was connected to a vacuum pump *via* a pressure control valve, and the reactor was evacuated to 200 mbar. After a reaction time of 18 h, the temperature was raised to 45 °C (to increase acetone evaporation) and stirring was continued for 2 h. A sample (50 μ L) was taken, extracted with EtOAc (800 μ L), the extract was dried over MgSO₄ and conversion was determined by GC analysis. The reaction mixture was then made alkaline (pH 9) by the addition of 4 M aq. NaOH solution (25 μ L), a stock solution of Pd nanoparticles (180 μ L of a 5.6 mM stock; final conc. 0.5 mM) was added, and the mixture was stirred for another 4 h at 30 °C and 500 rpm under hydrogen atmosphere (10 bar). The reaction mixture was dried over MgSO₄ and conversion as well as product ee were determined by GC analysis.

One-pot, three-step transformation of halo ketones 1a-f into amino alcohols 4a-f (semi-preparative scale, 5 mL). A smallscale autoclave reactor (16 mL) was charged with the halo ketone 1 (500 µmol; final conc. 100 mM), 2-propanol (250 µL; final conc. 5% v/v, approx. 650 mM) and potassium phosphate buffer (4.9 mL; 100 mM, pH 7.0, 1 mM MgSO₄) containing NaN_3 (7.8 mg mL⁻¹, 120 mM) and KI (1.7 mg mL⁻¹, 10 mM), and the mixture was stirred at 60 °C and 500 rpm for 2-5 h. After cooling to room temperature, any larger agglomerates of solid 2 were broken into smaller pieces using a stirring rod, additional 2-propanol (250 µL; to supplement the material lost through evaporation) and a stock solution of ADH (ADH-A: 20-35 mg mL⁻¹, final conc. 2.0-3.5 mg mL⁻¹; Codexis KRED-NADH-110: 3.0–15 mg mL⁻¹, final conc. 0.3–1.5 mg mL⁻¹; see Table 7) and NAD⁺ (0.7 mg mL⁻¹, 1 mM; final conc. 100 μ M) in potassium phosphate buffer (500 µL) were added, and the mixture was stirred at 30 °C and 300 rpm. The vent valve of the autoclave reactor was connected to a vacuum pump via a pressure control valve, and the reactor was evacuated to 200 mbar. After a reaction time of 18 h, the temperature was raised to 45 °C (to increase acetone evaporation) and stirring was continued for 2 h. The reaction mixture was then made alkaline (pH 9) by the addition of 4 M aq. NaOH solution (65 µL), a stock solution of Pd nanoparticles (450 µL of a 5.6 mM stock; final conc. 0.5 mM) was added, and the mixture was stirred for an additional 4 h at 30 °C and 500 rpm under hydrogen atmosphere (10 bar). The reactor was depressurised, 4 M aq. NaOH solution (500 μ L) was added, and the reaction mixture was saturated with NaCl. The product was extracted into EtOAc $(4 \times 5 \text{ mL})$, the combined extracts were dried over MgSO₄ and evaporated under reduced pressure to give the crude amino alcohols as orange oils or solids. Column chromatography (~0.6 g of silica gel 60 in a Pasteur pipette; MTBE \rightarrow MTBE-MeOH-NH₄OH = 90/9/1) afforded the pure amino alcohols 4a-f.

(*R*)-2-Amino-1-phenylethanol [(*R*)-4a]. 57 mg (83%) off-white solid. mp: 62–63 °C (lit.³⁴ 57–59 °C). TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f}$ = 0.10. $[\alpha]_{\rm D}^{20}$ = -58.8 (CHCl₃, *c* = 1.31); lit. (*S*)³⁵ +60.6 (CHCl₃, *c* = 0.5). NMR and MS data were in accordance with those obtained for the product of the two-step one-pot sequence.

(S)-2-Amino-1-phenylethanol [(S)-4a]. 52 mg (76%) off-white solid. mp: 61–62 °C (lit.³⁴ 57–59 °C). TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f}$ = 0.10. $[\alpha]_{\rm D}^{20}$ = +59.0 (CHCl₃, *c* = 1.02); lit.³⁵ +60.6 (CHCl₃, *c* = 0.5). NMR and MS data were in accordance with those obtained for the product of the two-step one-pot sequence.

(*R*)-2-Amino-1-(4-chlorophenyl)ethanol [(*R*)-4b]. 63 mg (75%) off-white solid. mp: 93–95 °C (lit.³⁷ 92–94 °C). TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f} = 0.11$. $[\alpha]_{\rm D}^{20} = -64.2$ (CHCl₃, c = 1.08); lit. (*S*)³⁸ +67.4 (CHCl₃, c = 0.35). NMR and MS data were in accordance with those obtained for the product of the two-step one-pot sequence.

(S)-2-Amino-1-(4-chlorophenyl)ethanol [(S)-4b]. 69 mg (80%) off-white solid. mp: 94–95 °C (lit.³⁷ 92–94 °C). TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f}$ = 0.11. [α]_D²⁰ = +64.4 (CHCl₃, *c* = 1.10); lit.³⁸ +67.4 (CHCl₃, *c* = 0.35). NMR and MS data were in accordance with those obtained for the product of the two-step one-pot sequence.

(*R*)-2-Amino-1-(4-fluorophenyl)ethanol [(*R*)-4c]. 69 mg (89%) off-white solid. mp: 82–83 °C (lit.³⁸ 63–65 °C). TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f}$ = 0.10. $[\alpha]_{\rm D}^{20}$ = -66.9 (CHCl₃, *c* = 1.04); lit. (*S*)³⁸ +40.8 (EtOH, *c* = 1.68). NMR and MS data were in accordance with those obtained for the product of the two-step one-pot sequence.

(S)-2-Amino-1-(4-fluorophenyl)ethanol [(S)-4c]. 61 mg (79%) off-white solid. mp: 83–84 °C (lit.³⁸ 63–65 °C). TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f}$ = 0.10. $[\alpha]_{\rm D}^{20}$ = +66.7 (CHCl₃, c = 1.04); lit.³⁸ +40.8 (EtOH, c = 1.68). NMR and MS data were in accordance with those obtained for the product of the two-step one-pot sequence.

(*R*)-2-Amino-1-(4-tolyl)ethanol [(*R*)-4d]. 64 mg (85%) offwhite solid. mp: 56–58 °C (lit.³⁷ 67–69 °C). TLC (silica, MTBE– MeOH–NH₄OH = 90/9/1): $R_{\rm f}$ = 0.11. [α]_D²⁰ = -64.5 (CHCl₃, *c* = 1.02); lit.³⁹ –32 (CHCl₃, *c* = 5.0). NMR and MS data were in accordance with those obtained for the product of the two-step one-pot sequence.

(*S*)-2-Amino-1-(4-tolyl)ethanol [(*S*)-4d]. 62 mg (82%) offwhite solid. mp: 57–58 °C (lit.³⁷ 67–69 °C). TLC (silica, MTBE– MeOH–NH₄OH = 90/9/1): $R_{\rm f}$ = 0.11. $[\alpha]_{\rm D}^{20}$ = +64.8 (CHCl₃, *c* = 1.00); lit. (*R*)³⁹ –32 (CHCl₃, *c* = 5.0). NMR and MS data were in accordance with those obtained for the product of the two-step one-pot sequence.

(*R*)-2-Amino-1-(4-methoxyphenyl)ethanol [(*R*)-4e]. 71 mg (85%) off-white solid. mp: 102–103 °C. TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f}$ = 0.09. $[\alpha]_{\rm D}^{20}$ = -61.4 (CHCl₃, *c* = 0.93); lit.³⁸ –39.9 (CHCl₃, *c* = 1.03). NMR and MS data were in accordance with those obtained for the product of the two-step one-pot sequence.

(*S*)-2-Amino-1-(4-methoxyphenyl)ethanol [(*S*)-4e]. 72 mg (86%) off-white solid. mp: 103–104 °C. TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f}$ = 0.09. $[\alpha]_{\rm D}^{20}$ = +61.4 (CHCl₃, *c* = 1.24); lit. (*R*)³⁸ –39.9 (CHCl₃, *c* = 1.03). NMR and MS data were in accordance with those obtained for the product of the two-step one-pot sequence.

(S)-2-Amino-1-(2-furyl)ethanol [(S)-4f]. 25 mg (39%) yellowish solid. mp: 79–81 °C. TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f} = 0.14$. $[\alpha]_{\rm D}^{20} = -33.9$ (CHCl₃, c = 0.77); lit.⁴⁰ -38.6 (CHCl₃, c = 1.80). NMR and MS data were in accordance with those obtained for the product of the two-step one-pot sequence.

(*R*)-2-Amino-1-(2-furyl)ethanol [(*R*)-4f]. 23 mg (36%) yellowish solid. mp: 80–81 °C. TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f} = 0.14$. [α]²⁰_D = +33.8 (CHCl₃, c = 0.75); lit. (*S*)⁴⁰ –38.6 (CHCl₃, c = 1.80). NMR and MS data were in accordance with those obtained for the product of the two-step one-pot sequence.

The ADH concentrations used for the one-pot, three-step transformation of halo ketones **1a–f** into amino alcohols **4a–f** are summarised in Table 7.

One-pot, three-step transformation of halo ketone 1c into amino alcohols (R)-4c (gram scale, 75 mL). An autoclave reactor (160 mL) was charged with the 2-bromo-4'-fluoroacetophenone (1c; 1.63 g, 7.5 mmol; final conc. 100 mM), 2-propanol (3.75 mL; final conc. 5% v/v, approx. 650 mM) and potassium phosphate buffer (71.25 mL; 100 mM, pH 7.0, 1 mM MgSO₄) containing NaN₃ (0.59 g, 9 mmol; final conc. 120 mM) and KI (0.13 g, 0.75 mmol; final conc. 10 mM), and the mixture was stirred at 60 °C and 500 rpm for 5 h. The reaction mixture was cooled to room temperature, and the complete consumption of 1c was verified by TLC analysis. Any larger agglomerates of solid 2c were broken into smaller pieces using a stirring rod, additional 2-propanol (3.75 mL; to supplement the material lost through evaporation) and a stock solution of ADH-A (150 mg; final conc. 2.0 mg mL⁻¹) and NAD⁺ (5.3 mg; final conc. 100 µM) in potassium phosphate buffer (5 mL) were added, and the mixture was stirred at 30 °C and 300 rpm. The vent valve of the autoclave reactor was connected to a vacuum pump via a pressure control valve, and the reactor was evacuated to 200 mbar. After a reaction time of 18 h, the temperature was raised to 45 °C (to increase acetone evaporation) and stirring was continued for 2 h. The reaction mixture was cooled to room temperature, and complete consumption of 2c was verified by TLC analysis. The reaction mixture was then made alkaline (pH 9) by the addition of 4 M aq. NaOH solution (1 mL), a stock solution of Pd nanoparticles (6.7 mL of a 5.6 mM stock; final conc. 0.5 mM) was added, and the mixture was stirred for an additional 4 h at 30 °C and 500 rpm under hydrogen atmosphere (10 bar). Complete consumption of 3c was verified by TLC analysis, 4 M aq. NaOH solution (10 mL) was added, and the reaction mixture was saturated with NaCl. The product was extracted into EtOAc (5 \times 40 mL; phase separation accelerated by centrifugation), and the combined extracts were dried over MgSO4 and evaporated under reduced pressure to give 1.06 g of an orange solid. Column chromatography (silica gel 60, MTBE \rightarrow MTBE-MeOH-NH₄OH = 90/9/1) afforded 0.97 g (84%) of (R)-4c as an off-white solid. mp: 83-84 °C (lit.³⁸ 63–65 °C). TLC (silica, MTBE–MeOH–NH₄OH = 90/9/1): $R_{\rm f} = 0.10. \ [\alpha]_{\rm D}^{20} = -66.5 \ ({\rm CHCl}_3, \ c = 0.92); \ {\rm lit.} \ (S)^{38} + 40.8$ (EtOH, c = 1.68). NMR and MS data were in accordance with those obtained for the product of the semi-preparative scale experiment.

One-pot, four-step transformation of halo ketone 1e into (S)tembamide [CAS 15779-24-5] (gram scale, 50 mL). An autoclave reactor (160 mL) was charged with the 2-bromo-4'-methoxyacetophenone (1e; 1.15 g, 7.5 mmol; final conc. 100 mM), 2-propanol (2.5 mL; final conc. 5% v/v, approx. 650 mM) and potassium phosphate buffer (47.5 mL; 100 mM, pH 7.0, 1 mM $MgSO_4$) containing NaN₃ (0.39 g, 6 mmol; final conc. 120 mM) and KI (0.08 g, 0.5 mmol; final conc. 10 mM), and the mixture was stirred at 60 °C and 500 rpm for 5 h. The reaction mixture was cooled to room temperature, and complete consumption of 1e was verified by TLC analysis. Any larger agglomerates of solid 2e were broken into smaller pieces using a stirring rod, additional 2-propanol (2.5 mL; to supplement the material lost through evaporation) and a stock solution of Codexis KRED-NADH-110 (25 mg; final conc. 0.5 mg mL^{-1}) and NAD^+ (3.3 mg; final conc. 100 µM) in potassium phosphate buffer (5 mL) were added, and the mixture was stirred at 30 °C and 300 rpm. The vent valve of the autoclave reactor was connected to a vacuum pump via a pressure control valve, and the reactor was evacuated to 200 mbar. After a reaction time of 18 h, the temperature was raised to 45 °C (to increase acetone evaporation) and stirring was continued for 2 h. The reaction mixture was cooled to room temperature, and complete consumption of 2e was verified by TLC analysis. The reaction mixture was then made alkaline (pH 9) by the addition of 4 M aq. NaOH solution (1 mL), a stock solution of Pd nanoparticles (6.7 mL of a 5.6 mM stock; final conc. 0.5 mM) was added, and the mixture was stirred for an additional 4 h at 30 °C and 500 rpm under hydrogen atmosphere (10 bar). Complete consumption of 3e was verified by TLC analysis, 4 M aq. NaOH solution (1.5 mL) and a solution of benzoyl chloride (0.84 g, 6.0 mmol) in MTBE (15 mL) were added, and stirring was continued at room temperature for 2 h. Complete consumption of 4e was verified by TLC analysis, EtOAc (20 mL) was added, and the phases were separated (accelerated by centrifugation). The aqueous phase was extracted with EtOAc (3×30 mL; phase separation accelerated by centrifugation), the combined extracts were washed with 1 M aq. NaOH solution (40 mL) and brine (10 mL), and dried over MgSO₄. The solvent was evaporated under reduced pressure to give 1.40 g of an off-white solid. Recrystallisation (EtOH-water = 8/2) afforded 0.98 g (73%) of (S)-tembamide as a colourless, crystalline solid. mp: 148-149 °C (lit.²⁷ 145-147 °C). TLC (silica, MTBE-MeOH-NH₄OH = 90/9/1): $R_f = 0.10. [\alpha]_D^{20} = +54.9$ (CHCl₃, c = 0.52); lit.²⁹ +56.9 (CHCl₃, c = 0.54). ¹H-NMR (400 MHz, DMSO-d₆): δ [ppm] = 3.28-3.35 (1H, m, CH₂), 3.43-3.49 (1H, m, CH₂), 3.73 (3H, s, OCH₃), 4.74 (1H, dt, J = 7.7 Hz, 4.7 Hz, CH-OH), 5.41 (1H, d, J = 4.4 Hz, OH), 6.90 (2H, d, J = 8.6 Hz, Ar-m), 7.29 (2H, d, J = 8.6 Hz, Ar-o), 7.45 (2H, t, J = 7.3 Hz, Ar-m'), 7.51 (1H, t, J = 7.2 Hz, Ar-p'), 7.84 (2H, dd, J = 7.0 Hz, 1.6 Hz, Ar-o'), 8.46 (1H, t, J = 5.7 Hz, NH). ¹³C-NMR (100 MHz, DMSO-d₆): δ [ppm] = 47.7, 55.0, 70.7, 113.4, 127.1, 127.2, 128.2, 131.0, 134.6, 135.8, 158.3, 166.4. GC-MS (EI, 70 eV): m/z = 271 (M⁺, <1), 150 (39), 137 (34), 135 (76), 134 (100), 109 (20), 105 (57), 94 (19), 77 (57), 66 (7), 51 (15). The NMR data are in accordance with the literature values.42

Environmental impact assessment

E-factor calculations were performed using the EATOS (v. 1.1) software tool,⁴³ while solvent demand was calculated using Microsoft Excel (determining the solvent use of each step separately and carrying forward the solvent demand of all intermediates). All reactions were treated as proceeding to complete conversion, hence all losses in yield are accounted for as 'unknown by-products'. In cases where the exact quantities of reagents or auxiliary materials were not given in the literature, the following estimations were used: solvents for diluting solu*tions*: 3 times the initial volume, *Celite for filtration*: 0.1 g mL^{-1} of filtrate, solvents for extraction: 50 mL g^{-1} of crude product, aqueous solutions for washing extracts: 20% of extract volume, except brine: 10% of extract volume, anhydrous salts for drying extracts: 0.02 g mL⁻¹ of extract volume, solvents for recrystallisa*tion*: 10 mL g^{-1} of crude product, *silica gel for chromatography*: 20 g g⁻¹ of crude product, *eluents for chromatography*: 500 mL g⁻¹ of crude product (total eluent volume). The EATOS and Excel files used for the calculations are available as ESI.[†]

Acknowledgements

This study has been financed by the Austrian Science Fund (FWF project J3244-N17). The authors would like to thank Florian Mayer for assistance with the NMR measurements, Maarten Gorseling and Remco van Oosten for general technical assistance, and Caroline Paul for proof-reading the manuscript.

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