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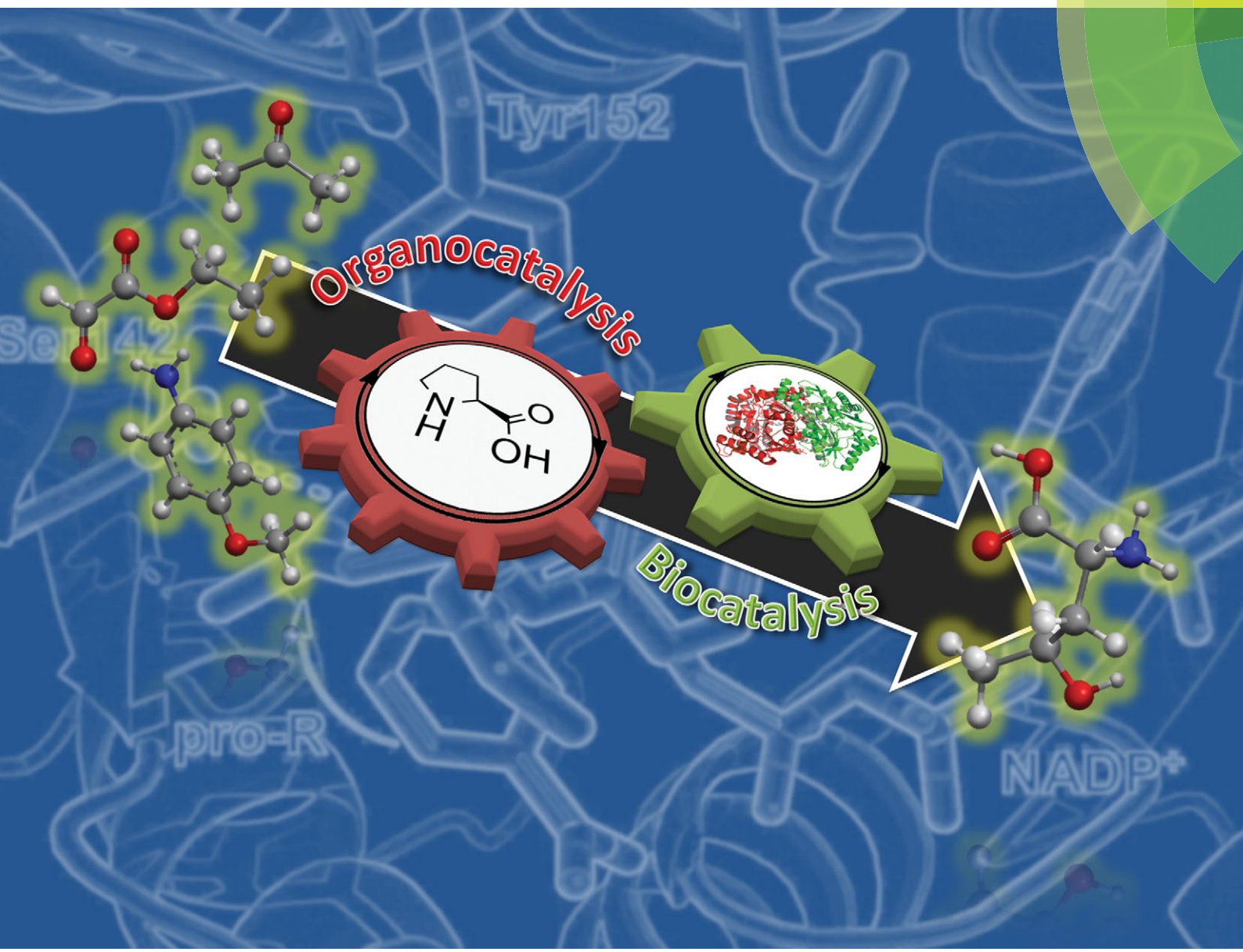

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Stereoselective synthesis of γ-hydroxynorvaline through combination of organo- and biocatalysis†

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An efficient route for the synthesis of all four diastereomers of PMP-protected α-amino-γ-butyrolacton to access γ-hydroxynorvaline was established. The asymmetric key steps comprise an organo-catalytic Mannich reaction and an enzymatic ketone reduction. Three reaction steps could be integrated in a one-pot process, using 2-PrOH both as solvent and as reducing agent. The sequential construction of stereogenic centres gave access to each of the four stereoisomers in high yield and with excellent stereocontrol.

Progressive legislative regulations with respect to sustainability, safety and quality improvements have changed the focus of contemporary chemical research to designing more economic processes. In this context, (asymmetric) catalysis has become the method of choice to replace stoichiometric amounts of reagents and auxiliaries, thereby facilitating an efficient and energy saving process.[1] While each of the catalysis sub-fields (metal-, organo- and biocatalysis) has reached an impressive level of sophistication, efficiency can be further increased by combining methods from these different sub-fields. This allows exploiting and combining specific features of each type of catalyst in a highly cooperative fashion. For example, numerous reports have been published based on the combination of transition metals and organocatalysts;[2] furthermore, biocatalytic transformations have been coupled with diverse metals.[3] However, the combination of bio- and organocatalysts is still scarce.[4,5]

α-Amino-γ-butyrolactones are common structural elements in a series of natural products and pharmaceutical drugs that display diverse biological activities (*cf.* Fig. 1).[6] Moreover, the corresponding amino acid (γ-hydroxynorvaline) has been isolated from natural sources[7] and shows anti-diabetic properties.[8] The widespread occurrence of the γ-butyrolactone unit has led to the development of a number of stereoselective syntheses mainly based on chemo-catalysis,[9] while only few biocatalytic strategies have been developed.[10] Most of the procedures either require multiple steps, are auxiliary-assisted, or based on ex-chiral pool precursors.[11] Hence, access to only one or two out of four stereoisomers is provided. However, as the biological activity of bioactive compounds is related often to one specific unknown stereoisomer,[12] a convenient access to all four diastereomers, *e.g.* for SAR-studies, is desired.

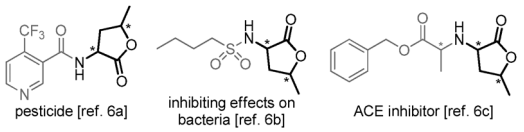

Fig. 1 Selected bioactive compounds containing α-amino-γ-butyro-lactones.

In this communication, a novel combination of organo- and biocatalysis is reported, which provides access to optically pure α-amino-γ-butyrolactones either in a step-wise fashion or in a cascade reaction.

In our study we focused initially on the bioreduction of racemic amino-ketoester *rac*-**1**, which is readily available by a proline catalysed Mannich reaction.[13,14] A set of homo- and heterologously expressed alcohol dehydrogenases (ADHs)[15] were assayed on analytical scale (5.6 mg, 20 mM) employing 2-propanol for $NAD(P)^+$ regeneration. Asymmetric reduction of the carbonyl group of amino-ketoester **1** afforded the corresponding diastereomers of alcohol **2**, which cyclised spontaneously to the desired 2-amino-lactones **3** (Scheme 1).

A number of ADHs transformed *rac*-**1** into the corresponding α-amino-lactones **3** with high conversion (selected results in Table 1; more details in the ESI†). For example, the (5*S*)-configured diastereomers (3*S*,5*S*)-**3** and (3*R*,5*S*)-**3** were accessible by the enzymes ADH-A[15a] (entry 1) and evo-1.1.030[15b] (entry 2), whereas the (5*R*)-diastereomers (3*S*,5*R*)-**3** and (3*R*,5*R*)-**3** were formed utilising evo-1.1.200[15b] (entry 5). The enzymes ADH-T[15c] (entry 3) and ADH-LS[15d] (entry 4) gave excellent results, too, with conversions

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† Electronic supplementary information (ESI) available: Detailed description of each experiment, full characterisation of all compounds and copy of HPLC traces and NMR data. See DOI: 10.1039/c4cc06230b

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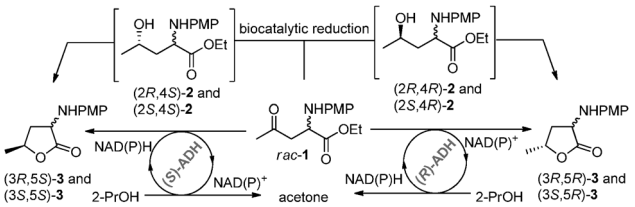

Scheme 1 Alcohol dehydrogenase catalysed reduction of racemic ketoester *rac*-**1** on analytical scale (20 mM, 5.6 mg). PMP = *para*-methoxyphenyl.

Table 1 Selected results of the ADH catalysed reduction of ketoester *rac*-**1** yielding lactone **3** (analytical scale, double determination)[a]

Entry	Enzyme	Conv.[b] [%]	d.r. [*syn*/*anti*][b]	ee_{syn} **3**[c] [%]	ee_{anti} **3**[c] [%]
1	ADH-A	99	52/48	>99 (3*R*,5*S*)	>99 (3*S*,5*S*)
2	evo-1.1.030	99	51/49	>99 (3*R*,5*S*)	>99 (3*S*,5*S*)
3	ADH-T	83	12/88	>99 (3*R*,5*S*)	>99 (3*S*,5*S*)
4	ADH-LS	86	71/29	>99 (3*S*,5*R*)	>99 (3*R*,5*R*)
5	evo-1.1.200	99	52/48	>99 (3*S*,5*R*)	>99 (3*R*,5*R*)
6	ADH-LB	49	99/1	>99 (3*S*,5*R*)	—
7	evo-1.1.270	49	99/1	>99 (3*S*,5*R*)	—

[a] Reaction conditions: ketoester *rac*-**1** (5.6 mg, 20 mM), KPi buffer (100 mM, pH 6.8), $NAD(P)^+$ (1.0 mM), 10 vol% 2-PrOH, ADH (for units see ESI), 30 °C, 700 rpm on a thermoshaker (horizontal position), 24 h. [b] Determined *via* GC on an achiral stationary phase. [c] Determined *via* HPLC on a chiral stationary phase.

of 83% and 86%, respectively. In all cases, the enantiomeric excess (ee) of all diastereomers of lactones **3** was >99%.

Among the enzymes tested, ADH-LB[15e] and evo-1.1.270[15b] gave unexpected results as in both cases mainly one diastereomer (3*S*,5*R*)-**3** was detected (entries 6 and 7). Since the conversions remained below 50%, we assumed a selective kinetic resolution with respect to the existing stereogenic centre in β-position to the carbonyl group. In order to gain a better understanding of such a rare case of distant stereorecognition by ADHs,[16] further investigations were performed: racemic and enantiopure Mannich-bases *rac*-/(*R*)-/(*S*)-**1** were incubated with ADH-LB and the reaction progress was monitored over time (Fig. 2). Thereby it was found that *rac*-**1** gave the (3*S*,5*R*)-**3** diastereomer as sole product with 40% conversion after eight hours, while (*R*)-**1** was not transformed. The pure enantiomer (*S*)-**1** reacted fastest, reaching 70% conversion within the same time.

The experimental observations were additionally supported by *in silico* docking studies: only substrate (*S*)-**1** was found to fit in the active site enabling a productive binding mode; the substrate occupies therein a pro-*R* conformation in which the carbonyl group can be activated by two hydrogen bonds through amino acids Tyr152 and Ser142 (Fig. 3). Attempts to dock the opposite enantiomer (*R*)-**1** into the active site failed to provide a reasonable productive binding mode (see ESI†). Hence, the structure of ADH-LB allows a chiral recognition of a remote stereocentre enabling a kinetic resolution.[16] Attempts to couple the diastereoselective reduction of **1** with a racemisation of **1** in a dynamic kinetic resolution failed so far due spontaneous degradation of **1** *via* elimination of anisidine over time.

The next step was the synthesis of all four stereoisomers of amino-lactone **3**; for this purpose, the biocatalysed reduction of ketoester **1** was optimised using alcohol dehydrogenases

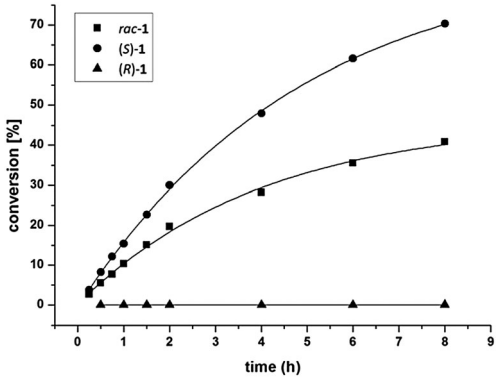

Fig. 2 Time course of the biocatalytic reduction of racemic and enantiopure Mannich-base **1** with the enzyme ADH-LB (double determination).

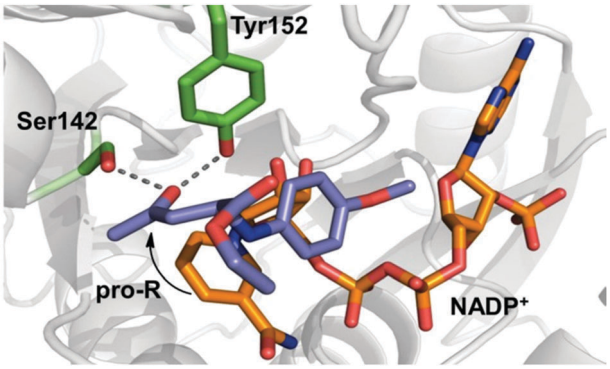

Fig. 3 Docking study: productive binding mode of Mannich-base (*S*)-**1** (purple) and the cofactor $NADP^+$ (orange) in the active site of ADH-LB.

ADH-A and evo-1.1.200. Investigating 2-PrOH as a cosolvent revealed that a substrate concentration of 30 mM (7.95 mg mL^{-1}, 10 vol% 2-PrOH) led to completion of the reaction in the case of the (*S*)-selective ADH-A within 24 h (for details, see ESI†). The (*R*)-selective enzyme evo-1.1.200 transformed even 50 mM (13.3 mg mL^{-1}, 30 vol% 2-PrOH) to completion within 24 h. The optimum pH was determined to be 6.3 in order to keep the partial racemisation of the optically pure Mannich adduct **1** at a minimum (see ESI†). In the subsequent semi-preparative scale transformations [56 mg (*S*)-**1**] >99% conversion was achieved; however, ring closure was incomplete, as mainly the optically pure amino-alcohol **2** rather than the lactone **3** was isolated (*ca.* 50–70%; see ESI†).[17] Assuming that the corresponding methyl ester of **1** would undergo faster lactonisation than the ethyl analogue, a transesterification step (HCl–MeOH) was integrated into the reaction sequence. The optimised conditions finally allowed conversion of the pure amino-ester (*R*)-/(*S*)-**1** (up to >300 mg), readily accessible by a proline-catalysed Mannich reaction of acetone and imine **4**,[14] into the corresponding lactones **3**. The *syn*- and *anti*-diastereomers were isolated in excellent yields (78–82%) and with remarkable stereocontrol (de >98 : 2 and ee >99% in all cases; see Scheme 2).

As an alternative to the conventional step-by-step synthesis (Scheme 2) two sequential approaches were established. In a first approach, aldimine **4** was formed in 2-PrOH, which also served as a solvent for the subsequent Mannich reaction and as

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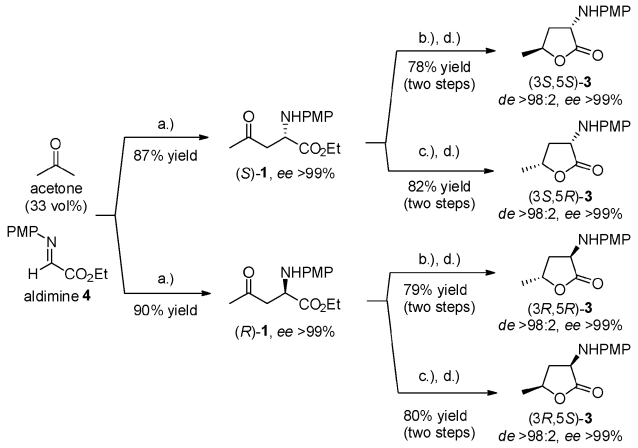
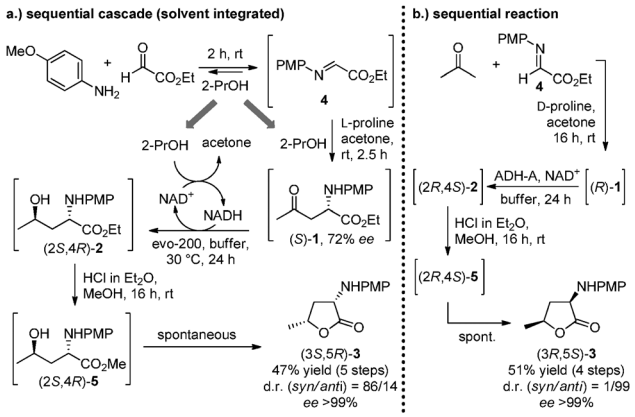
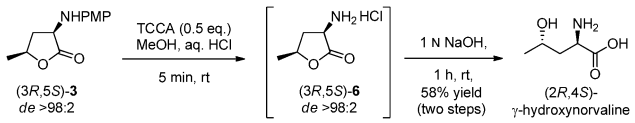


Scheme 2 Synthesis of all four diastereomers of PMP-protected α-amino-γ-butyrolactone **3** through combination of organo- and biocatalysis. *Reagents and conditions*: (a) D-/L-proline (0.25 eq.), DMSO, 3 h, rt; (b) (*R*)-/(*S*)-**1** (30 mM), ADH-A, NAD^+, buffer (100 mM, pH 6.3), 2-PrOH (10 vol%), 30 °C, 24 h; (c) (*R*)-/(*S*)-**1** (50 mM), evo-1.1.200, NAD^+, buffer (100 mM, pH 6.3), 2-PrOH (30 vol%), 30 °C, 24 h; (d) HCl·Et_2O (2.0 eq.), MeOH, 16 h, rt. PMP = *para*-methoxyphenyl.



Scheme 3 Synthesis of α-amino-γ-butyrolactones by means of two different approaches: (a) sequential cascade reaction in which the solvent (2-PrOH) was integrated in the first three steps (5 steps, 2 pots); (b) conventional sequential reaction sequence with the preformed aldimine (4 steps, 2 pots).



Scheme 4 PMP deprotection to yield natural γ-hydroxynorvaline.

the reducing agent for the enzyme-mediated reduction (after dilution with buffer solution). All three steps were accomplished in one pot, after which the transesterification-lactonisation cascade was performed in a separate vessel. The whole sequence afforded the *syn*-configured lactone (3*S*,5*R*)-**3** in a remarkable 47% yield with a d.r. of 86/14 in favour of the *syn*-diastereomer (Scheme 3a: 5 steps in total, 2 pots, 90% average yield per step). The diminished d.r. in comparison to the previous results can be rationalised by the usage of 2-PrOH for the proline-catalysed reaction, which under these conditions afforded (*S*)-**1** with 72% ee.

In a second approach, the Mannich reaction was carried out with the preformed aldimine **4** in acetone as solvent, and aminoketoester (*R*)-**1** was obtained at prolonged reaction time (16 h) with >99% conversion and in optically pure form (ee >99%). After evaporation of the solvent, the crude product was subjected to reduction by ADH-A in buffer–2-PrOH. The final transesterification-lactonisation cascade furnished the diastereomerically pure amino lactone (3*R*,5*S*)-**3** in 51% yield with an excellent d.r. of 99:1 (Scheme 3b: 4 steps, 2 pots, 85% average yield per step).

Final PMP-deprotection of the diastereomerically pure α-amino-lactone **3** was achieved employing TCCA (trichloroisocyanuric acid) in MeOH,[18b] providing the hydrochloride salt of **6**. Unexpectedly, other oxidative deprotection methods [$PhI(OAc)_2$, CAN, HIO_6, laccase, *etc.*][18] were unsuccessful. Intensive optimisation of the purification of **6** with respect to the solvent, acid, reaction time and work-up procedure finally provided access to γ-hydroxynorvaline: the natural product was isolated diastereomerically pure after a base promoted ring opening (58% yield over two steps) as demonstrated for the (2*R*,4*S*)-diastereomer (Scheme 4).

In conclusion, a straightforward synthesis of all four diastereomers of PMP-protected α-amino-γ-butyrolactone to access γ-hydroxynorvaline has been established. The sequential construction of the stereogenic centres was accomplished by combination of organo- and biocatalysed key steps. In one approach, three sequential steps including the organo- as well as the biocatalysed transformations were performed in one pot, integrating 2-propanol first as solvent and then as reducing agent.

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