

## Enzymatic Synthesis | Hot Paper |

Modular Enzymatic Cascade Synthesis of Vitamin B<sub>5</sub> and Its DerivativesMohammad Z. Abidin,<sup>[a]</sup> Thangavelu Saravanan,<sup>[a]</sup> Jieli Zhang,<sup>[a]</sup> Pieter G. Tepper,<sup>[a]</sup> Erick Strauss,<sup>[b]</sup> and Gerrit J. Poelarends<sup>\*[a]</sup>

**Abstract:** Access to vitamin B<sub>5</sub> [(*R*)-pantothenic acid] and both diastereoisomers of  $\alpha$ -methyl-substituted vitamin B<sub>5</sub> [(*R*)- and (*S*)-3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)-2-methylpropanoic acid] was achieved using a modular three-step biocatalytic cascade involving 3-methylaspartate ammonia lyase (MAL), aspartate- $\alpha$ -decarboxylase (ADC),  $\beta$ -methylaspartate- $\alpha$ -decarboxylase (CrpG) or glutamate decarboxylase (GAD), and pantothenate synthetase (PS) enzymes. Starting from simple non-chiral dicarboxylic acids (either fumaric acid or mesaconic acid), vitamin B<sub>5</sub> and both diastereoisomers of  $\alpha$ -methyl-substituted vitamin B<sub>5</sub>, which are valuable precursors for promising antimicrobials against *Plasmodium falciparum* and multidrug-resistant *Staphylococcus aureus*, can be generated in good yields (up to 70%) and excellent enantiopurity (>99% ee). This newly developed cascade process may be tailored and used for the biocatalytic production of various vitamin B<sub>5</sub> derivatives by modifying the pantooyl or  $\beta$ -alanine moiety.

Coenzyme A (CoA) is an essential enzyme cofactor in all organisms, and its biosynthetic pathway enzymes have been identified as attractive targets for new antimicrobial drugs.<sup>[1,2]</sup> An interesting class of new antimicrobials that target CoA biosynthesis is constituted by pantothenamides (PanAms), which are secondary or tertiary amides of pantothenic acid (vitamin B<sub>5</sub>, **5a**, Figure 1), the biosynthetic precursor of CoA. Various PanAms have been shown to possess potent antimicrobial activity against several organisms, including the pathogenic bac-

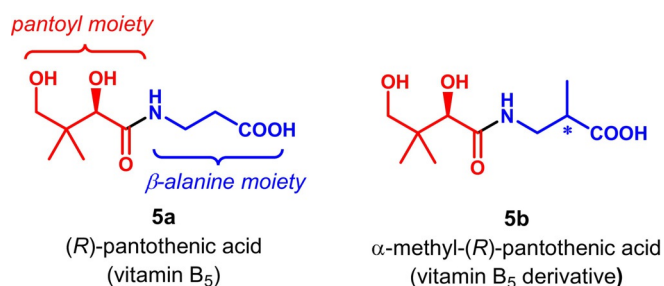


Figure 1. Structures of vitamin B<sub>5</sub> and its  $\alpha$ -methyl-substituted derivative.

terium *Staphylococcus aureus*<sup>[3]</sup> as well as the malaria parasite *Plasmodium falciparum*.<sup>[4]</sup> However, pantetheinase enzymes that normally hydrolyze pantetheine in human serum also act on the PanAms, thereby reducing their efficacy.<sup>[5,6]</sup> Interestingly, pantetheinase-mediated hydrolysis of PanAms could be prevented by modifying the  $\beta$ -alanine moiety of the compounds.<sup>[7,8]</sup> Indeed, a PanAm with an added  $\alpha$ -methyl group was shown to have superior antiplasmodial activity compared to its parent molecule.<sup>[9]</sup> However, such modifications introduce stereochemical complexity to the molecules, recent results of which have indicated the strong relevance to the anti-malarial activity of PanAm analogues.<sup>[10]</sup> However, the particularly challenging chemical synthesis of these compounds poses a significant barrier to the discovery of their clinical potential. Therefore, it is of high interest to develop an asymmetric biocatalytic synthetic strategy that provides efficient and step-economical access to pantothenic acid (**5a**) and both diastereoisomers of its  $\alpha$ -methyl substituted derivative (**5b**, Figure 1), avoiding (de-)protecting steps and intermediate purifications. The desired PanAms can be easily prepared from the corresponding pantothenic acids by transforming the carboxylic acid group to an amide.<sup>[11]</sup>

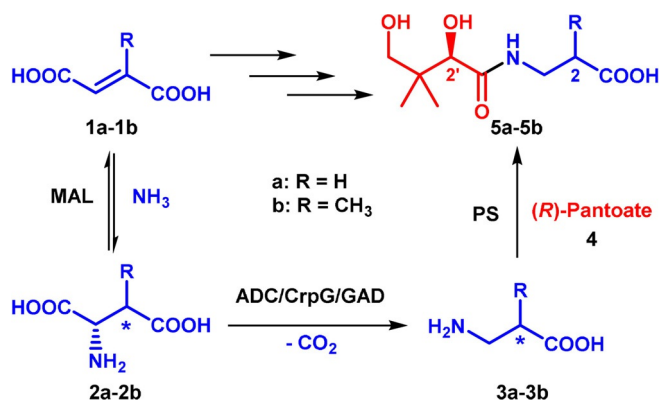
We envisioned that pantothenic acid (**5a**) and its  $\alpha$ -methyl substituted derivative (**5b**) could be prepared from fumaric acid (**1a**) and mesaconic acid (**1b**), respectively, through a modular three-step enzymatic cascade (Scheme 1) involving 3-methylaspartate ammonia lyase (MAL), an appropriate decarboxylase such as aspartate- $\alpha$ -decarboxylase (ADC),  $\beta$ -methylaspartate- $\alpha$ -decarboxylase (CrpG) or glutamate decarboxylase (GAD), and pantothenate synthetase (PS). The expected chemoselectivity of each biocatalyst could allow for a one-pot reaction sequence due to the orthogonal reactivity of each enzyme. In this process, the new stereogenic center in product **5b** can be established by either regio- and diastereoselective

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**Scheme 1.** Proposed enzymatic cascade synthesis of vitamin B<sub>5</sub> and its derivatives.

amination (as catalyzed by MAL), or diastereospecific decarboxylation by one of the decarboxylase enzymes.

MAL of *Clostridium tetanomorphum* is part of a catabolic pathway for L-glutamate, in which it catalyzes the conversion of L-threo-3-methylaspartate to ammonia and mesaconate.<sup>[12]</sup> Using a large molar excess of ammonia, the enzyme also efficiently catalyzes the amination of mesaconate (**1b**) to give both (2*S*,3*S*)-3-methylaspartate (L-threo-**2b**) and (2*S*,3*R*)-3-methylaspartate (L-erythro-**2b**) as products (Scheme 1). It was found that L-threo-**2b** forms at a much faster rate than that of L-erythro-**2b**, but at equilibrium (using a 65-fold molar excess of ammonia over **1b** at pH 9) the molar ratio of these diastereoisomers is approximately 1.<sup>[13]</sup> In addition, MAL accepts fumarate (**1a**) as a substrate, which is converted to L-aspartate (**2a**). The mechanism-inspired engineering of a MAL mutant (H194A) with strongly enhanced diastereoselectivity in the amination of **1b**, giving exclusively L-threo-**2b**, has been previously reported.<sup>[13]</sup> Moreover, the substrate scope of MAL has been expanded by structure-guided site-saturation mutagenesis, allowing for the biocatalytic production of a broad range of valuable 3-substituted derivatives of L-aspartic acid.<sup>[14]</sup>

ADC of *E. coli* is part of the biosynthetic pathway for pantothenate, in which it catalyzes the decarboxylation of **2a** to give β-alanine (**3a**).<sup>[15]</sup> This enzyme has a limited substrate scope and showed no decarboxylation activity towards **2b**.<sup>[16]</sup> The enzyme β-methylaspartate-α-decarboxylase (CrpG) of *Nostoc* sp. ATCC 53789 is involved in a biosynthetic pathway for cryptophycin, in which it catalyzes the decarboxylation of

L-erythro-**2b** to yield (R)-3-amino-2-methylpropanoic acid (**3b**).<sup>[17]</sup> CrpG is the only enzyme known to catalyze the decarboxylation of **2b**, with significant activity towards the L-erythro isomer only. GAD of the hyperthermophilic archaeon *Thermococcus kodakarensis* has been reported to function as an ADC and is most likely responsible for the production of β-alanine necessary for pantothenate biosynthesis.<sup>[18]</sup> In this study, we demonstrate that this enzyme exhibits decarboxylase activity towards **2b**, but with the highest activity towards the L-threo isomer. Notably, ADC and CrpG must undergo self-processing leading to formation of the catalytic pyruvoyl group, whereas GAD is a PLP-dependent decarboxylase that does not require autocatalytic self-processing.

Pantothenate synthetase (PS) of *E. coli* is involved in the last step of pantothenate biosynthesis and catalyzes the adenosine triphosphate (ATP)-dependent condensation of (R)-pantoate (**4**) and β-alanine (**3a**) to form (R)-pantothenic acid (vitamin B<sub>5</sub>, **5a**). PS enzymes typically accept a variety of β-alanine analogues in the condensation reaction, albeit with reduced catalytic efficiency compared to that with the natural substrate.<sup>[15,19]</sup>

Initially, we set out to combine MAL and ADC in one pot to prepare product **3a**. Accordingly, substrate **1a** and NH<sub>4</sub>Cl were incubated with MAL and ADC, and the reaction was monitored by TLC (Figure S3, Supporting Information). After 24 h, **1a** was completely converted to product **3a**, as confirmed by <sup>1</sup>H NMR spectroscopy. These initial results showed that the two enzymes MAL and ADC are compatible for cascade synthesis in one pot. To further demonstrate the preparative usefulness of this two-step cascade system, a 100 mg-scale synthesis was performed. Accordingly, substrate **1a** (25 mM) and NH<sub>4</sub>Cl (500 mM) were incubated with MAL (0.02 mol%) and ADC (0.6 mol%) in one pot (25 mL of buffer, pH 8). Under these conditions, excellent conversion (>99% after 24 h) and good isolated yield of product **3a** (85%) were achieved (Table 1, Figure S16 in the Supporting Information).

Unfortunately, ADC showed no decarboxylase activity towards either L-threo-**2b** or L-erythro-**2b**. Therefore, we cloned, expressed, and purified the decarboxylase CrpG, and then incubated it with a 1:1 mixture of L-threo-**2b** and L-erythro-**2b**. Under these conditions, the L-erythro isomer was fully decarboxylated, whereas the L-threo isomer was not converted, not even after prolonged incubation for 7 d (Figure S11, Supporting Information). This indicates that CrpG is highly diastereose-

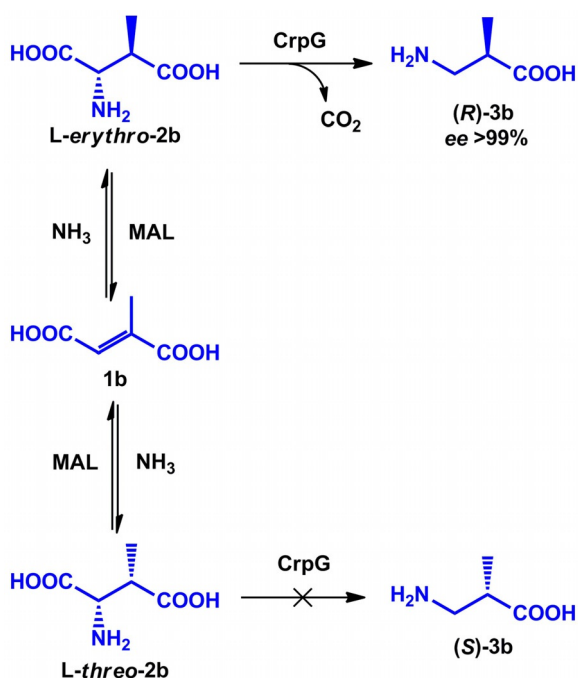
**Table 1.** Two-step enzymatic cascade synthesis of β-alanine (**3a**) and both enantiomers of 3-amino-2-methylpropanoic acid (**3b**).<sup>[a]</sup>

Product	Enzymes	Conversion [%] <sup>[b]</sup>	Isolated yield [%] <sup>[c]</sup>	ee [%] <sup>[d]</sup>
<b>3a</b>	MAL and ADC	> 99	85	–
( <i>R</i> )- <b>3b</b>	MAL and CrpG	> 99	78	> 99
( <i>S</i> )- <b>3b</b>	MAL-H194A and GAD	75	63	> 99

[a] For the synthesis of **3a**, the reaction mixture contained MAL (0.02 mol%), ADC (0.6 mol%), **1a** (25 mM), NH<sub>4</sub>Cl (500 mM), and MgCl<sub>2</sub> (25 mM) in 25 mL Tris-HCl buffer (pH 8, 100 mM). For the synthesis of (*R*)-**3b**, the reaction mixture contained MAL (0.02 mol%), CrpG (0.47 mol%), **1b** (30 mM), NH<sub>4</sub>Cl (500 mM), and MgCl<sub>2</sub> (25 mM) in 25 mL potassium phosphate buffer (pH 8, 100 mM). For the synthesis of (*S*)-**3b**, the reaction mixture contained MAL-H194A (0.04 mol%), TkGAD (0.6 mol%), **1b** (10 mM), NH<sub>4</sub>Cl (500 mM), PLP (1 mM), and MgCl<sub>2</sub> (25 mM) in 25 mL potassium phosphate buffer (pH 8, 100 mM). [b] Conversion was analyzed by <sup>1</sup>H NMR spectroscopy. [c] Products were purified by cation exchange chromatography. [d] ee values were determined by chiral HPLC.

lective, with detectable activity only towards the *L*-erythro isomer.

Having established the preference of CrpG for *L*-erythro-**2b**, a two-step enzymatic cascade reaction was performed at the analytical scale by incubation of **1b** and NH<sub>4</sub>Cl with MAL and CrpG in one pot. Interestingly, full conversion of starting substrate **1b** was observed (Figure S4, Supporting Information), yielding solely the (*R*)-enantiomer of product **3b**, as confirmed by <sup>1</sup>H NMR spectroscopy and chiral HPLC analysis. This is explained by a dynamic kinetic asymmetric transformation<sup>[20,21]</sup> of the diastereoisomeric mixture of **2b** (Scheme 2). In the first cascade step, MAL produced both *L*-erythro- and *L*-threo-**2b** as intermediate products. Subsequently, in the second step CrpG only decarboxylated *L*-erythro-**2b** to give exclusively (*R*)-**3b**. The remaining *L*-threo-**2b** was also converted into *L*-erythro-**2b** by MAL, leading to full conversion of the starting material (**1b**) to the desired product (*R*)-**3b**.



**Scheme 2.** One-pot, two-step, enzymatic cascade reaction, involving MAL and CrpG, that fully converts mesaconate (**1b**) to only (*R*)-**3b**. This is due to CrpG only acting on *L*-erythro-**2b** and to the MAL-mediated dynamic kinetic asymmetric transformation of the *L*-threo-**2b** to the desired diastereomer.

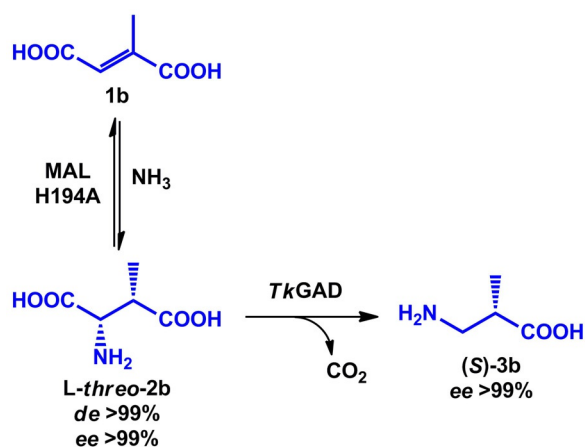
Several control experiments were also performed. Firstly, when **1b** and NH<sub>4</sub>Cl were incubated with MAL alone, an approximately 1:1 mixture of *L*-threo-**2b** and *L*-erythro-**2b** was obtained (Figure S12A, Supporting Information). After removal of MAL from the reaction mixture by heat inactivation and filtration, CrpG was added. After prolonged incubation (7 d), a mixture of unreacted *L*-threo-**2b** and product **3b** was obtained (Figure S12B, Supporting Information). Secondly, incubation of **1b** and NH<sub>4</sub>Cl with the diastereospecific mutant of MAL (MAL-H194A) and CrpG in one pot resulted in the formation of *L*-threo-**2b** but did not yield product **3b**, which is consistent

with the inability of CrpG to decarboxylate *L*-threo-**2b** (Figure S9, Supporting Information). These results confirm that MAL is responsible for both the synthesis and epimerization of *L*-threo- and *L*-erythro-**2b**, and that CrpG displays activity towards *L*-erythro-**2b** only, allowing for the selective synthesis of (*R*)-**3b** starting from the simple non-chiral dicarboxylic acid **1b**.

To demonstrate the synthetic usefulness of this two-step enzymatic cascade, a 100 mg-scale synthesis was performed. Accordingly, substrate **1b** (30.8 mM) and NH<sub>4</sub>Cl (500 mM) were incubated with MAL (0.02 mol%) and CrpG (0.47 mol%) in one pot (25 mL of buffer, pH 8). High conversion (>99% after 7 d), good isolated yield (78%), and excellent enantiopurity of product (*R*)-**3b** (>99% ee) were achieved (Table 1, Figures S17 and S25, Supporting Information).

CrpG displays activity towards *L*-erythro-**2b**, enabling the enzymatic synthesis of (*R*)-**3b**. To synthesize the opposite enantiomer of **3b**, a decarboxylase with activity towards *L*-threo-**2b** was required. Our attempts to obtain CrpG variants by directed evolution through screening of single-site saturation mutagenesis libraries did not yield any mutants with detectable activity towards *L*-threo-**2b**. Therefore, we cloned and produced two pyridoxal phosphate (PLP)-dependent GAD enzymes, and tested their ability to decarboxylate *L*-threo-**2b**. Initially, we worked on the GAD from *Pyrococcus furiosus* (*PfGAD*), which was reported to accept *L*-aspartate, *L*-glutamate and *L*-tyrosine as substrates.<sup>[22]</sup> Although many different expression conditions were tested, we were not able to produce *PfGAD* in a soluble form in an *E. coli* host. In an attempt to produce the soluble protein, different constructs were made as fusions with three solubility enhancers: maltose-binding protein (MBP), small ubiquitin-like modifier protein (SUMO) and Fh8, a small protein secreted by the parasite *Fasciola hepatica*. However, inefficient solubilization of *PfGAD* limited the effectiveness of this approach. Therefore, we selected the GAD from the hyperthermophilic archaeon *Thermococcus kodakarensis* (*TkGAD*), which has 71% sequence similarity with *PfGAD* (Figure S2, Supporting Information). *TkGAD* reportedly catalyzes the decarboxylation of *L*-glutamate and *L*-aspartate.<sup>[23]</sup> The gene encoding *TkGAD* was cloned and expressed, and the corresponding enzyme purified, yielding the soluble and active protein (Figure S1, Supporting Information). Initially, *TkGAD* activity was tested towards *L*-aspartate (**2a**) and *L*-erythro- and *L*-threo-**2b** (Figure S10, Supporting Information). The enzyme completely converted **2a** to **3a**, whereas the reaction with *L*-erythro-**2b** showed <10% conversion. To our delight, *L*-threo-**2b** was also accepted as substrate by *TkGAD*, yielding the desired (*S*)-**3b** with more than 70% conversion.

Considering that *TkGAD* displays activity towards both diastereoisomers of **2b**, a dynamic kinetic asymmetric transformation approach, in which MAL and *TkGAD* are combined in one pot, would not yield the enantiopure (*S*)-**3b** product. Hence, for the one-pot, two-step, enzymatic cascade synthesis of (*S*)-**3b**, *TkGAD* was used in combination with the diastereospecific MAL-H194A mutant, which produces exclusively *L*-threo-**2b** upon amination of **1b** (Scheme 3 and Figure S5 in the Supporting Information). Accordingly, substrate **1b** (10 mM) and NH<sub>4</sub>Cl (500 mM) were incubated with MAL-H194A (0.02 mol%)



**Scheme 3.** The one-pot, two-step, enzymatic cascade reaction that converts mesaconate (**1b**) to only (*S*)-**3b** and relies on a diastereospecific mutant MAL (MAL-H194A) and the newly discovered stereoselectivity of *TkGAD* towards *L*-threo-**2b**.

and *TkGAD* (0.3 mol%) in one pot (25 mL of buffer, pH 8). Due to a partial precipitation of the protein (caused by the instability of *TkGAD*), the same amount of each enzyme was added again after 24 h of incubation. Using these conditions, good conversion (75 % after 48 h), good isolated yield (63 %), and excellent enantiopurity of product (*S*)-**3b** (> 99% *ee*) were achieved (Table 1, Figures S18 and S25 in the Supporting Information).

Having developed one-pot, two-step, enzymatic cascade reactions for the production of **3a**, (*R*)-**3b** and (*S*)-**3b**, we next verified whether the PS enzyme is able to accept these compounds as substrates in condensation with (*R*)-pantoate (**4**) using small-scale (1 mL) reactions. We were pleased to find that PS accepted **3a**, (*R*)-**3b** and (*S*)-**3b** as substrates in the condensation reaction, yielding the corresponding pantothenic acid products **5a** and **5b**, as confirmed by <sup>1</sup>H NMR spectroscopy (Figure S13, Supporting Information).

Having established that PS can be used to set up a three-step enzymatic cascade reaction, the enzymes MAL, ADC, and PS were combined in one pot. Initially, the reaction was tested by adding the three enzymes simultaneously and using an equimolar ratio of **1a**, **4** and ATP. Although full conversion of

the starting substrate **1a** was observed after 24 h, the desired product (*R*)-**5a** was obtained in a crude yield of only 50% along with accumulation of **3a**. This indicated that further optimization of the reaction conditions was necessary to improve the yield of product (*R*)-**5a**. After testing different ratios of **4** and ATP, a molar ratio of 2:3 was found to be best, which resulted in excellent conversion of the starting substrate into the desired product **5a** (crude yield > 99%; Figures S6 and S14, Supporting Information). Hence, under these conditions, no significant accumulation of intermediate product **3a** was observed.

To assess the performance of this one-pot multi-enzymatic cascade, a 32 mg-scale synthesis was performed. The three-step cascade reaction was performed by addition of all components simultaneously including the three enzymes. After 24 h, the starting material **1a** was completely consumed (conversion > 99%, Figure S15 A, Supporting Information) and the desired product (*R*)-**5a** was obtained in good isolated yield (70%) and with excellent *ee* (> 99%) (Table 2, Figures S19 and S20, Supporting Information). Importantly, the modularity of this enzymatic cascade approach also allows for the facile synthesis of both diastereoisomers of  $\alpha$ -methyl-substituted vitamin B<sub>5</sub>, that is (*2R,2'R*)-**5b** and (*2S,2'R*)-**5b** (Figures S7 and S8, Supporting Information), with one stereogenic center being set by the selected combination of enzymes and the other by the substrate (*R*)-pantoate (**4**). Under suitable reaction conditions (for details, see section 8.2 in the Supporting Information), and using the appropriate combination of enzymes in one-pot, the desired products (*2R,2'R*)-**5b** and (*2S,2'R*)-**5b** were obtained with excellent *de* and *ee* values (> 99%) and in 46–49% isolated yield (Table 2; Figures S15, S21–S24, and S26 in the Supporting Information).

In conclusion, we have successfully developed a one-pot cascade process for the synthesis of enantiomerically pure vitamin B<sub>5</sub> starting from fumarate and utilizing MAL, ADC, and PS enzymes. Starting from mesaconic acid, the stereoselective synthesis of both diastereoisomers of  $\alpha$ -methyl-substituted vitamin B<sub>5</sub>, an important antibiotic precursor, was achieved by using either the CrpG or GAD enzyme instead of ADC, with one stereogenic center being set by the selected combination of MAL/CrpG or MAL-H194A/GAD and the other derived from one of the starting substrates. Given the availability of engi-

**Table 2.** Three-step enzymatic cascade synthesis of pantothenic acid (**5a**) and both diastereoisomers of its  $\alpha$ -methyl-substituted derivative **5b**.<sup>[a]</sup>

Product	Enzymes	Conversion [%] <sup>[b]</sup>	Isolated yield [%]	<i>de</i> and <i>ee</i> [%] <sup>[e]</sup>
( <i>R</i> )- <b>5a</b>	MAL, ADC and PS	> 99	70 <sup>[c]</sup>	> 99
( <i>2R,2'R</i> )- <b>5b</b>	MAL, CrpG and PS	> 99	49 <sup>[c]</sup>	> 99
( <i>2S,2'R</i> )- <b>5b</b>	MAL-H194A, GAD and PS	75	46 <sup>[d]</sup>	> 99

[a] The enzymes were found to be compatible for cascade synthesis at pH 9 (MAL, MAL-H194A and PS, optimum pH: 9.0–10.0; ADC, CrpG and GAD, optimum pH: 7.5–8.0). The amounts of applied enzymes were adjusted such that high conversions were achieved. For the synthesis of **5a**, the reaction mixture contained MAL (0.01 mol%), ADC (0.3 mol%), PS (0.07 mol%), **1a** (10 mM), **4** (20 mM), ATP (30 mM), NH<sub>4</sub>Cl (500 mM) and MgCl<sub>2</sub> (10 mM) in 20 mL Tris-HCl buffer (100 mM, pH 9). For the synthesis of (*2R,2'R*)-**5b**, the reaction mixture contained MAL (0.01 mol%), CrpG (0.7 mol%), PS (0.07 mol%), **1b** (10 mM), **4** (20 mM), ATP (30 mM), NH<sub>4</sub>Cl (500 mM) and MgCl<sub>2</sub> (10 mM) in 20 mL Tris-HCl buffer (100 mM, pH 9). For the synthesis of (*2S,2'R*)-**5b**, the reaction mixture contained MAL-H194A (0.04 mol%), *TkGAD* (0.6 mol%), PS (0.07 mol%), **1b** (10 mM), **4** (20 mM), ATP (30 mM), PLP (1 mM), NH<sub>4</sub>Cl (500 mM) and MgCl<sub>2</sub> (10 mM) in 20 mL Tris-HCl buffer (100 mM, pH 9). [b] The conversion was analyzed by <sup>1</sup>H NMR spectroscopy. [c] The product was purified by preparative HPLC. [d] The product was purified by silica gel column chromatography. [e] *de* and *ee* values were determined by <sup>1</sup>H NMR spectroscopy and HPLC analysis.



neered MAL mutants and natural PS enzymes with a broad substrate scope, work is in progress to expand the substrate scope of CrpG and GAD by protein engineering.

Although the decarboxylation step is actually stereochemically deconstructive with the loss of one chiral center, the cascade approach strongly benefits from the use of stereo-divergent decarboxylases. These enzymes not only allow for the synthesis of both diastereoisomers of  $\alpha$ -methyl-substituted vitamin B<sub>5</sub>, but they also provide a strong driving force to pull the equilibrium of the MAL-catalyzed reaction towards product formation. The use of an irreversible decarboxylation step, with stereochemical kinetic distinction, is an important strategy in biocatalytic cascade synthesis to overcome thermodynamic limitations and maximize product yield.<sup>[24,25]</sup> A possible constraint on the use of the developed cascade for large-scale transformations would be the dependence of the PS enzyme on the expensive cofactor ATP. This could be addressed by the incorporation of an auxiliary enzyme-catalyzed step for efficient ATP recycling. Several ATP-recycling enzyme systems are available and a few have already been successfully implemented in preparative biocatalysis.<sup>[26–28]</sup> However, considering that the starting materials (fumarate and mesaconate) can be efficiently produced in high yields by large-scale fermentation using metabolically engineered *E. coli* strains,<sup>[29,30]</sup> we envision to co-express the enzymes employed for the cascade in such a fermentation host to directly obtain vitamin B<sub>5</sub> and its  $\alpha$ -methyl-substituted derivatives from cheap carbon and nitrogen sources. Indeed, such a cell-based approach would eliminate the need for ATP recycling.

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## Conflict of interest

The authors declare no conflict of interest.

**Keywords:** biocatalysis • cascade reaction • enzymes • pantothenic acid

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