CHEMISTRY
A European Journal

A Journal of
ChemPubSoc Europe

Accepted Article

Title: Modular enzymatic cascade synthesis of vitamin B5 and its derivatives

Authors: Mohammad Zainal Abidin, Thangavelu Saravanan, Jielin Zhang, Pieter Tepper, Erick Strauss, and Gerrit Jan Poelarends

To be cited as: *Chem. Eur. J.* 10.1002/chem.201804151

Link to VoR: http://dx.doi.org/10.1002/chem.201804151

Supported by
ACES

WILEY-VCH

10.1002/chem.201804151

COMMUNICATION

WILEY-VCH

Modular enzymatic cascade synthesis of vitamin B_5 and its derivatives

Mohammad Z. Abidin,[a] Thangavelu Saravanan,[a] Jielin Zhang,[a] Pieter G. Tepper,[a] Erick Strauss,[b] Gerrit J. Poelarends*[a]

Abstract: Access to vitamin B_5 [(*R*)-pantothenic acid] and both diastereoisomers of α-methyl-substituted vitamin B_5 [(*R*)- and (*S*)-3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)-2-methylpropanoic acid] has been achieved using a modular three-step biocatalytic cascade involving 3-methylaspartate ammonia lyase (MAL), aspartate-α-decarboxylase (ADC), β-methylaspartate-α-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_5 and both diastereoisomers of α-methyl-substituted vitamin B_5, 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 might be tailored and used for the biocatalytic production of various vitamin B_5 derivatives by modifying the pantoyl or β-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.[1,2] An interesting class of new antimicrobials that target CoA biosynthesis is the pantothenamides (PanAms), which are secondary or tertiary amides of pantothenic acid (vitamin B_5, **5a**, Figure 1), the biosynthetic precursor of CoA. Various PanAms have been shown to possess potent antimicrobial activity against several organisms, including the pathogenic bacterium *Staphylococcus aureus*[3] as well as the malaria parasite *Plasmodium falciparum*.[4] However, pantetheinase enzymes that normally hydrolyse pantetheine in human serum also act on the PanAms, thereby reducing their efficacy.[5,6] Interestingly, pantetheinase-mediated hydrolysis of PanAms could be prevented by modifying the β-alanine moiety of the compounds.[7,8] Indeed, a PanAm with an added α-methyl group was shown to have superior antiplasmodial activity compared to its parent molecule.[9] However, such modifications introduces stereochemical complexity to the molecules that recent results have indicated is highly relevant to the antimalarial activity of

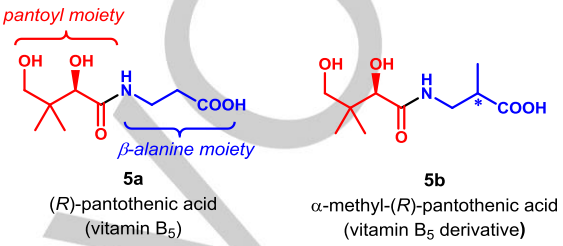
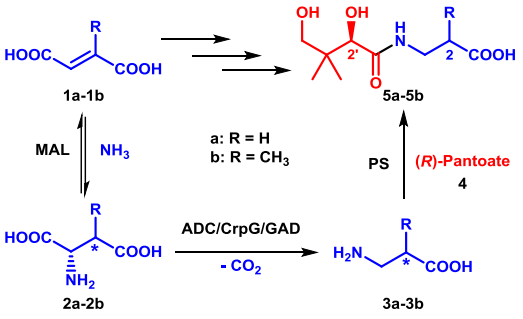


Figure 1. Structures of vitamin B_5 and its α-methyl-substituted derivative.

PanAm analogues.[10] However, the more challenging chemical syntheses of these compounds poses a significant barrier to the discovery of their clinical potential. Therefore, the development of an asymmetric biocatalytic synthesis strategy that provides efficient and step-economic access to pantothenic acid (**5a**) and both diastereoisomers of its α-methyl substituted derivative (**5b**, Figure 1), avoiding (de-)protecting steps and intermediate purifications, is of high interest. The desired PanAms can be easily prepared from the corresponding pantothenic acids by transforming the carboxylic acid group to an amide.[11]

We envisioned that pantothenic acid (**5a**) and its α-methyl substituted derivative (**5b**) could be prepared from fumaric acid (**1a**) and mesaconic acid (**1b**), respectively, via a modular three-step enzymatic cascade involving 3-methylaspartate ammonia lyase (MAL), an appropriate decarboxylase such as aspartate-α-decarboxylase (ADC), β-methylaspartate-α-decarboxylase (CrpG) or glutamate decarboxylase (GAD), and pantothenate synthetase (PS) (Scheme 1). 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 amination (as catalyzed by MAL), or diastereospecific decarboxylation by one of the decarboxylase enzymes.



Scheme 1. Proposed enzymatic cascade synthesis of vitamin B_5 and its derivatives.

[a] M. Z. Abidin, Dr. T. Saravanan, J. Zhang, P. G. Tepper, Prof. Dr. G. J. Poelarends
Department of Chemical and Pharmaceutical Biology
Groningen Research Institute of Pharmacy, University of Groningen
Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands
E-mail: g.j.poelarends@rug.nl
[b] Prof. Dr. E. Strauss
Department of Biochemistry
Stellenbosch University
Private Bag X1, Matieland 7602 South Africa

Supporting information for this article is given via a link at the end of the document.

MAL of *Clostridium tetanomorphum* is part of a catabolic pathway for L-glutamate, where it catalyzes the conversion of L-*threo*-3-methylaspartate to ammonia and mesaconate.[12] 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** is formed at a rate much faster than 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.[13] In addition, MAL accepts fumarate (**1a**) as 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 reported previously.[13] 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 L-aspartic acid derivatives.[14]

ADC of *Escherichia coli* is part of the biosynthesis pathway for pantothenate, where it catalyzes the decarboxylation of **2a** to give β-alanine (**3a**).[15] This enzyme has a limited substrate scope and showed no decarboxylation activity towards **2b**.[16] The enzyme β-methylaspartate-α-decarboxylase (CrpG) of *Nostoc* sp. ATCC53789 is involved in a biosynthetic pathway for cryptophycin, where it catalyzes the decarboxylation of L-*erythro*-**2b** to yield (*R*)-3-amino-2-methylpropanoic acid (**3b**).[17] CrpG is the only enzyme known that can 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.[18] In this study, we demonstrate that this enzyme exhibits decarboxylase activity towards **2b**, but with highest activity towards the L-*threo* isomer. Note that ADC and CrpG have to 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 ATP-dependent condensation of (*R*)-pantoate (**4**) and β-alanine (**3a**) to form (*R*)-pantothenate (vitamin B_5, **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.[15,19]

Initially, we set out to combine MAL and ADC in one-pot to prepare product **3a**. Accordingly, substrate **1a** and NH_4Cl were incubated with MAL and ADC, and the reaction was monitored by TLC (Figure S3). After 24 h, **1a** was completely converted into product **3a**, as confirmed by ^{1}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_4Cl (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).

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 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). This indicates that CrpG is highly diastereoselective, with detectable activity only towards the L-*erythro* isomer.

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

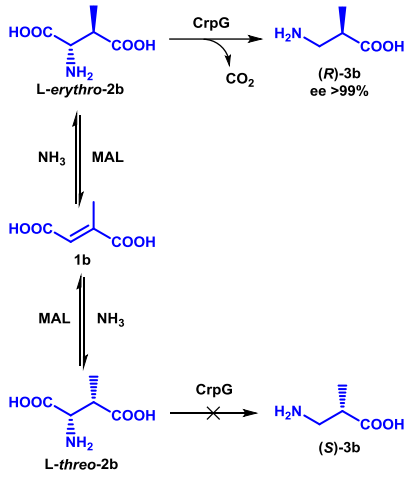
Product	Enzymes	Conversion (%)[b]	Isolated yield (%)[c]	ee (%)[d]
3a	MAL and ADC	> 99	85	-
(*R*)-**3b**	MAL and CrpG	> 99	78	> 99
(*S*)-**3b**	MAL-H194A and GAD	75	63	>99

[a] For synthesis of **3a**, the reaction mixture contained MAL (0.02 mol%), ADC (0.6 mol%), **1a** (25 mM), NH_4Cl (500 mM), and $MgCl_2$ (25 mM) in 25 mL Tris-HCl buffer (pH 8, 100 mM). For synthesis of (*R*)-**3b**, the reaction mixture contained MAL (0.02 mol%), CrpG (0.47 mol%), **1b** (30 mM), NH_4Cl (500 mM), and $MgCl_2$ (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%), *Tk*GAD (0.6 mol%), **1b** (10 mM), NH_4Cl (500 mM), PLP (1 mM), and $MgCl_2$ (25 mM) in 25 mL potassium phosphate buffer (pH 8, 100 mM); [b] Conversion was analysed by ^{1}H NMR spectroscopy; [c] Products were purified by cation exchange chromatography; [d] Enantiomeric excess (ee) was analysed by chiral HPLC.

Having established the preference of CrpG for L-*erythro*-**2b**, a two-step enzymatic cascade reaction was performed at analytical scale by incubation of **1b** and NH_4Cl with MAL and CrpG in one pot. Interestingly, full conversion of starting substrate **1b** was observed (Figure S4), yielding solely the (*R*)-enantiomer of product **3b**, as confirmed by ^{1}H NMR spectroscopy and chiral HPLC analysis. This is explained by a dynamic kinetic asymmetric transformation[20,21] of the diastereoisomeric mixture of **2b** (Scheme 2). In the first cascade step, MAL produces both L-*erythro*- and L-*threo*-**2b** as intermediate products. Subsequently, in the second step CrpG only decarboxylates L-*erythro*-**2b** to give exclusively (*R*)-**3b**. The remaining L-*threo*-**2b** is also converted into L-*erythro*-**2b** by MAL, leading to the full conversion of the starting material (**1b**) into the desired product (*R*)-**3b**.

Several control experiments were also performed. First, when **1b** and NH_4Cl were incubated with MAL only, a ~1:1 mixture of L-*threo*-**2b** and L-*erythro*-**2b** was obtained (Figure S12A). 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). Second, incubation of **1b** and NH_4Cl 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**, consistent with the inability of CrpG to decarboxylate L-*threo*-**2b** (Figure S9). These results confirm that MAL is responsible for both the synthesis and epimerisation 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**.

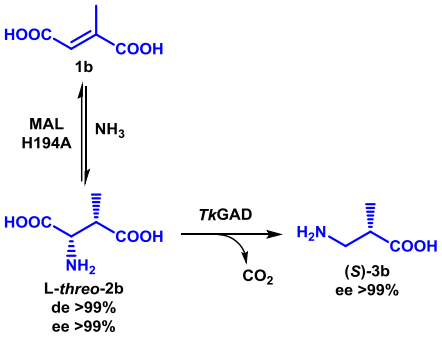


Scheme 2. One pot two-step enzymatic cascade reaction involving MAL and CrpG that fully converts mesaconate (**1b**) to only (*R*)-**3b**. This is due to CrpG only acting on L-*erythro*-**2b**, and the MAL-mediated dynamic kinetic asymmetric transformation of the L-*threo*-**2b** into the desired diastereomer.

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_4Cl (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).

CrpG displays activity towards L-*erythro*-**2b**, enabling the enzymatic synthesis of (*R*)-**3b**. In order 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**. We therefore cloned and produced two PLP-dependent GAD enzymes and tested their ability to decarboxylate L-*threo*-**2b**. Initially, we worked on the GAD from *Pyrococcus furiosus* (*Pf*GAD), which was reported to accept L-aspartate, L-glutamate and L-tyrosine as substrates.[22] Although many different expression conditions were tested, we were not able to produce *Pf*GAD in a soluble form in an *Escherichia coli* host. In an attempt to produce 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 *Pf*GAD limited the effectiveness of this approach. Therefore, we selected the GAD from the hyperthermophilic archaeon *Thermococcus kodakarensis* (*Tk*GAD), which has 71% sequence similarity with *Pf*GAD (Figure S2). *Tk*GAD reportedly catalyzes the decarboxylation of L-glutamate and L-aspartate.[23] The gene encoding *Tk*GAD was cloned and expressed, and the corresponding enzyme purified, yielding soluble and active protein (Figure S1). Initially, *Tk*GAD activity was tested towards L-aspartate (**2a**) and L-*erythro*- and L-*threo*-**2b** (Figure S10). 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 *Tk*GAD, yielding the desired (*S*)-**3b** with more than 70% conversion.

Because *Tk*GAD displays activity towards both diastereoisomers of **2b**, a dynamic kinetic asymmetric transformation approach in which MAL and *Tk*GAD are combined in one pot would not yield enantiopure (*S*)-**3b** product. Hence, for the one-pot, two-step enzymatic cascade synthesis of (*S*)-**3b**, *Tk*GAD 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). Accordingly, substrate **1b** (10 mM) and NH_4Cl (500 mM) were incubated with MAL-H194A (0.02 mol%) and *Tk*GAD (0.3 mol%) in one pot (25 mL of buffer, pH 8). Because some protein precipitation occurred (due to instability of *Tk*GAD), 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).



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

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 ^{1}H NMR spectroscopy (Figure S13).

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). 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 S15A) and the desired product (*R*)-**5a** was obtained in good isolated yield (70%) and with excellent ee (>99%) (Table 2, Figures S19 and S20). Importantly, the modularity of this enzymatic cascade approach also allows for the facile synthesis of both diastereoisomers of α-methyl-substituted vitamin B_5, that is (2*R*,2'*R*)-**5b** and (2*S*,2'*R*)-**5b** (Figures S7 and S8), 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 SI), and using the appropriate combination of enzymes in one-pot, the desired products (2*R*,2'*R*)-**5b** and (2*S*,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).

Table 2. Three-step enzymatic cascade synthesis of pantothenic acid (**5a**) and both diastereoisomers of its α-methyl-substituted derivative **5b**[a]

Product	Enzymes	Conv (%)[b]	Isolated yield (%)	de and ee (%)[e]
(*R*)-**5a**	MAL, ADC and PS	> 99	70[c]	> 99
(2*R*,2'*R*)-**5b**	MAL, CrpG and PS	> 99	49[c]	> 99
(2*S*,2'*R*)-**5b**	MAL-H194A, GAD and PS	75	46[d]	>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 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_4Cl (500 mM) and $MgCl_2$ (10 mM) in 20 mL Tris-HCl buffer (100 mM, pH 9). For synthesis of (2*R*,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_4Cl (500 mM) and $MgCl_2$ (10 mM) in 20 mL Tris-HCl buffer (100 mM, pH 9). For synthesis of (2*S*,2'*R*)-**5b**, the reaction mixture contained MAL-H194A (0.04 mol%), *Tk*GAD (0.6 mol%), PS (0.07 mol%), **1b** (10 mM), **4** (20 mM), ATP (30 mM), PLP (1 mM), NH_4Cl (500 mM) and $MgCl_2$ (10 mM) in 20 mL Tris-HCl buffer (100 mM, pH 9). [b] conversion was analysed by ^{1}H NMR spectroscopy; [c] product was purified by preparative HPLC; [d] product was purified by silica gel column chromatography; [e] de and ee were analysed by ^{1}H NMR spectroscopy and HPLC analysis.

In conclusion, we have successfully developed a one-pot cascade process for the synthesis of enantiomerically pure vitamin B_5 starting from fumarate and utilizing MAL, ADC, and PS enzymes. Starting from mesaconic acid, the stereoselective synthesis of both diastereoisomers of α-methyl-substituted vitamin B_5, 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 engineered 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.

While the decarboxylation step actually is stereochemically deconstructive, with the loss of one chiral centre, the cascade approach strongly benefits from the use of stereo-divergent decarboxylases. These enzymes not only allow the synthesis of both diastereoisomers of α-methyl-substituted vitamin B_5, 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.[24,25]

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.[26-28] However, as the starting materials (fumarate and mesaconate) can be efficiently produced in high yields by large-scale fermentation using metabolically engineered *E. coli* strains,[29,30] we envision to co-express the enzymes employed for the cascade in such a fermentation host in order to directly obtain vitamin B_5 and its α-methyl-substituted derivatives from cheap carbon and nitrogen sources. Indeed, such a cell-based approach would eliminate the need for ATP recycling.

Acknowledgements

The authors thank Sabry Younes, Hans Raj, Harshwardhan Poddar and Marianne de Villiers for insightful discussions. Robbert Cool is acknowledged for his expert assistance in construction and purification of the fusion proteins. We acknowledge financial support from the Netherlands Organization of Scientific Research (VICI grant 724.016.002) and from the European Research Council (PoC grant 713483). Mohammad Z. Abidin acknowledges funding from the Indonesia Endowment Fund for Education (LPDP) and Jielin Zhang acknowledges funding from the China Scholarship Council.

Keywords: biocatalysis, enzyme cascade, pantothenic acid

[1] C. Spry, K. Kirk, K. J. Saliba, *FEMS Microbiol. Rev.* **2008**, *32*, 56-106.

[2] W. J. A. Moolman, M. de Villiers, E. Strauss, *Biochem. Soc. Trans.* **2014**, *42*, 1080-1086.

[3] M. de Villiers, L. Barnard, L. Koekemoer, J. L. Snoep, E. Strauss, *FEBS J.* **2014**, *281*, 4731–4753.

[4] K. J. Saliba, C. Spry, *Biochem. Soc. Trans.* **2014**, *42*, 1087-1093.

[5] C. Spry, C. Macuamule, Z. Lin, K. G. Virga, R. E. Lee, E. Strauss, K. J. Saliba, *PLoS One* **2013**, *8*, e54974.

[6] S. Duprè, M. A. Rosei, L. Bellussi, E. Del Grosso, D. Cavallini, *Eur. J. Biochem.* **1973**, *40*, 103–107.

[7] M. de Villiers, C. Macuamule, C. Spry, Y.-M. Hyun, E. Strauss, K. J. Saliba, *ACS Med. Chem. Lett.* **2013**, *4*, 784–789.

[8] L. Barnard, K. J. Mostert, W. A. L. van Otterlo, E. Strauss, *ACS Infect. Dis.* **2018**, *4*, 736–743.

[9] C. J. Macuamule, E. T. Tjhin, C. E. Jana, L. Barnard, L. Koekemoer, M. de Villiers, K. J. Saliba, E. Strauss, *Antimicrob. Agents Chemother.* **2015**, *59*, 3666–3668.

[10] J. Schalkwijk, E. L. Allman, P. A. M. Jansen, L. E. de Vries, S. Jackowski, P. N. M. Botman, C. A. Beuckens-Schortinghuis, K. M. Koolen, J. M. Bolscher, M. W. Vos, K. Miller, S. Reeves, H. Pett, G. Trevitt, S. Wittlin, C. Scheurer, S. Sax, C. Fischli, G. Josling, T. W. A. Kooij, R. Bonnert, B. Campo, R. H. Blaauw, F. P. J. T. Rutjes, R. Sauerwein, M. Llinas, P. H. H. Hermkens, K. J. Dechering, *bioRxiv* **2018**, doi: http://dx.doi.org/10.1101/256669.

[11] J. L. Meier, A. C. Mercer, H. Rivera, M. D. Burkart, *J. Am. Chem. Soc.* **2006**, *128*, 12174–12184.

[12] H. A. Barker, R. D. Smyth, R. M. Wilson, H. Weissbach, *J. Biol. Chem.* **1959**, *234*, 320–328.

[13] H. Raj, W. Szymanski, J. De Villiers, V. Puthan Veetil, W. J. Quax, K. Shimamoto, D. B. Janssen, B. L. Feringa, G. J. Poelarends, *Chem. Eur. J.* **2013**, *19*, 11148–11152.

[14] H. Raj, W. Szymański, J. De Villiers, H. J. Rozeboom, V. Puthan Veetil, C. R. Reis, M. De Villiers, F. J. Dekker, S. De Wildeman, W. J. Quax, A. M. W. H. Thunnissen, B. L. Feringa, D. B. Janssen, G. J. Poelarends, *Nat. Chem.* **2012**, *4*, 478–484.

[15] M. E. Webb, A. G. Smith, C. Abell, *Nat. Prod. Rep.* **2004**, *21*, 695–721.

[16] M. E. Webb, E. Stephens, A. G. Smith, C. Abell, *Chem. Commun.* **2003**, 2416–2417.

[17] Z. Q. Beck, D. A. Burr, D. H. Sherman, *Chembiochem* **2007**, *8*, 1373–1375.

[18] H. Tomita, Y. Yokooji, T. Ishibashi, T. Imanaka, H. Atomia, *J. Bacteriol.* **2014**, *196*, 1222–1230.

[19] K. Miyatake, Y. Nakano, S. Kitaoka, *J. Nutr. Sci. Vitaminol.* **1978**, *24*, 243–253.

[20] J. Steinreiber, K. Faber, H. Griengl, *Chem. Eur. J.* **2008**, *14*, 8060-8072.

[21] B. M. Trost, R. C. Bunt, R. C. Lemoine, T. L. Calkins, *J. Am. Chem. Soc.* **2000**, *122*, 5968-5976.

[22] H. W. Kim, Y. Kashima, K. Ishikawa, N. Yamano, *Biosci. Biotechnol. Biochem.* **2009**, *73*, 224–227.

[23] S. J. Hong, I. Ullah, G. S. Park, C. Lee, J. H. Shin, *J. Korean Soc. Appl. Biol. Chem.* **2012**, *55*, 213–218.

[24] J. Steinreiber, M. Schürmann, M. Wolberg, F. van Assema, C. Reisinger, K. Fesko, D. Mink, H. Griengl, *Angew. Chem. Int. Ed.* **2007**, *46*, 1624-1626.

[25] E. Ricca, B. Brucher, J. H. Schrittwieser, *Adv. Synth. Catal.* **2011**, *353*, 2239 – 2262.

[26] J. N. Andexer, M. Richter, *ChemBioChem.* **2015**, *16*, 380 – 386.

[27] H. Zhao, W. A. van der Donk, *Curr. Opin. Biotechnol.* **2003**, *14*, 583–589.

[28] A. Alissandratos, K. Caron, T. D. Loan, J. E. Hennessy, C. J. Easton, *ACS Chem. Biol.* **2016**, *11*, 3289-3293.

[29] C. W. Song, S. Y. Lee, *Appl. Microbiol. Biotechnol.* **2015**, *99*, 8455–8464.

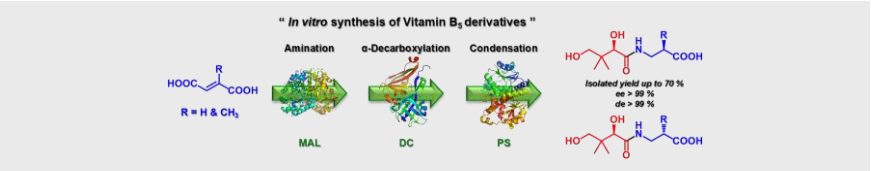
[30] J. Wang, K. Zhang, *Metab. Eng.* **2015**, *30*, 190–196.

10.1002/chem.201804151

WILEY-VCH

Entry for the Table of Contents

COMMUNICATION



Enzymes show "Unity In Diversity": Starting from simple non-chiral dicarboxylic acids enantiopure vitamin B_5 derivatives, which are valuable antimicrobial precursors, were prepared using a modular reaction cascade involving three different enzymes in one-pot.

*M. Z. Abidin,T. Saravanan, J. Zhang, P. G. Tepper, E. Strauss, G. J. Poelarends**

Page No. – Page No.

Modular enzymatic cascade synthesis of vitamin B_5 and its derivatives