How to cite: *Angew. Chem. Int. Ed.* **2023**, *62*, e202301601
doi.org/10.1002/anie.202301601

VIP **Biocatalysis** *Very Important Paper*

Methylation of Unactivated Alkenes with Engineered Methyltransferases To Generate Non-natural Terpenoids

*Benjamin Aberle, Daniel Kowalczyk, Simon Massini, Alexander-N. Egler-Kemmerer, Sebastian Gergel, Stephan C. Hammer, and Bernhard Hauer**

Abstract: Terpenoids are built from isoprene building blocks and have numerous biological functions. Selective late-stage modification of their carbon scaffold has the potential to optimize or transform their biological activities. However, the synthesis of terpenoids with a non-natural carbon scaffold is often a challenging endeavor because of the complexity of these molecules. Herein we report the identification and engineering of (*S*)-adenosyl-L-methionine-dependent sterol methyltransferases for selective *C*-methylation of linear terpenoids. The engineered enzyme catalyzes selective methylation of unactivated alkenes in mono-, sesqui- and diterpenoids to produce C_{11}, C_{16} and C_{21} derivatives. Preparative conversion and product isolation reveals that this biocatalyst performs *C–C* bond formation with high chemo- and regioselectivity. The alkene methylation most likely proceeds via a carbocation intermediate and regioselective deprotonation. This method opens new avenues for modifying the carbon scaffold of alkenes in general and terpenoids in particular.

Terpenoids form the largest group of natural organic compounds with a wealth of biological functions.[1,2] Application of terpenoids as flavor and fragrance compounds is well established, with examples such as farnesol,[3] rose oxide,[4] or the ionone family.[5] In addition, terpenoids have bioactive features that are relevant for use as pharmaceuticals, plant protection and more.[6–8] The biosynthesis of terpenes and terpenoids is based on coupling of C_5 diphosphates, which is why the carbon scaffolds of natural terpenoids usually contain a multiple of five carbon atoms. This observation is known as the isoprene rule and leads to the classification into monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}) and triterpenes (C_{30}).[9,10] The outstanding diversity of terpenoids in nature is mainly due to the numerous cyclization possibilities of isoprene precursors and subsequent oxo-functionalization reactions.[11,12]

Beyond natural terpenoids, the synthesis of non-natural derivatives is of great interest as it provides access to new bioactive compounds.[13–16] A powerful strategy to synthesize complex non-natural terpenoids is the selective functionalization of readily available natural products.[17] However, selective late-stage diversification of terpenoids remains a major challenge in catalyst development. This is particularly true for changes in the carbon scaffold that involve *C–C* bond formation at abundant *C–H* bonds or alkenes in terpenoids (Figure 1A). While alkene cyclopropanation, such as the Simmons–Smith methylation, is accessible and very reliable,[18] the simple methylation of unactivated alkenes in terpenoids remains elusive.[19–22] The seemingly small difference of a single methyl group in the carbon scaffold can have a large effect on the bioactivity of molecules. For example, the fragrance compounds ionones and irones differ only by one methyl group attached to the carbon scaffold, yet offer distinctive scents.[5] In medicinal chemistry, the screening of methylated compounds has a wide application outside of terpenoids. These derivatives

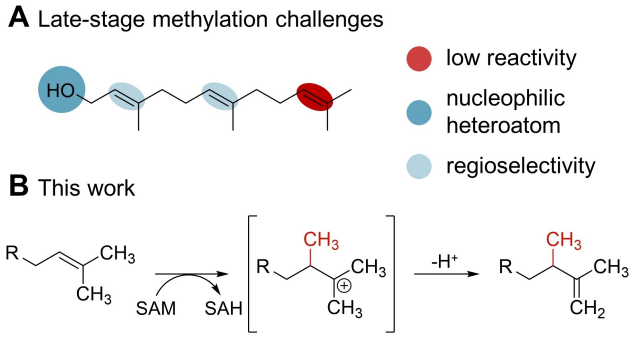

Figure 1. A) Major challenges of late-stage alkene methylation in terpenoids are the low activity of unactivated alkenes as nucleophiles as well as competing nucleophiles such as additional alkenes in repeating isoprene groups or free alcohol moieties. B) In this work we demonstrate the selective methylation of linear terpenoids with (*S*)-adenosyl-L-methionine (SAM)-dependent methyltransferases. A carbocation is proposed as an intermediate and the enzyme provides selective deprotonation.[33]

[*] B. Aberle, D. Kowalczyk, S. Massini, A.-N. Egler-Kemmerer, Dr. S. Gergel, Prof. Dr. S. C. Hammer, Prof. Dr. B. Hauer
Department of Technical Biochemistry, Institute of Biochemistry and Technical Biochemistry, University of Stuttgart
Allmandring 31, 70569 Stuttgart (Germany)
E-mail: bernhard.hauer@itb.uni-stuttgart.de
Dr. S. Gergel, Prof. Dr. S. C. Hammer
Faculty of Chemistry, Organic Chemistry and Biocatalysis, Bielefeld University
Universitätsstraße 25, 33615 Bielefeld (Germany)

often show significantly improved bioactive properties such as selectivity, solubility, half-life and binding affinity of small molecule drugs, known as the "magic methyl effect".[23–26]

Methylation is also a common reaction in nature, usually controlled by (*S*)-adenosyl-L-methionine (SAM)-dependent methyltransferases (MTs). These enzymes use SAM as cosubstrate to transfer a methyl group to a nucleophilic substrate (Figure S1). Several MTs have been reported to methylate the alkene of isoprene moieties, which is remarkable because alkenes are not particularly good nucleophiles. Currently, however, natural MTs are limited to specific terpenoid substrates, namely complex sterols (C_{30}) and terpenoids carrying diphosphate moieties, which limits their application for late-stage derivatization.[27–32] We aimed to utilize MTs for selective methylation in a one-step reaction with readily available mono-, sesqui-, and diterpenoids (Figure 1B).

Here we report the engineering of a SAM-dependent methyltransferase from *Chlamydomonas reinhardtii* for the selective *C*-methylation of readily available mono-, sesqui- and diterpenoids (Figure 1). The engineered enzyme generates C_{11}, C_{16} and C_{21} derivatives by chemo-, regio- and stereoselective methylation of unactivated alkenes, a catalytic reaction that is currently out of reach for small molecule catalysts.

The enzyme family of sterol C24-methyltransferases (SMTs) is known to accept various sterols as substrate for methylation of the terminal prenyl moiety.[27] The reaction is believed to proceed via a carbocation intermediate that can generate different products (Figure 2A).[33] First, the natural substrate promiscuity of SMTs was explored. Based on a sequence similarity network that was generated from well-characterized SMT amino acid sequences, we have chosen a panel of 16 enzymes to cover the natural diversity (Figure 2B). Eleven of these SMTs were previously studied regarding the *C*-methylation of sterols *in vitro* and five of the sequences have not yet been characterized (Table S4, entry 12–16). To confirm activity of our enzyme panel, lanosterol (**1**) was chosen as model substrate, a steroid substrate accepted by many SMTs.[34–43] In line with literature, ten out of the 16 SMTs accepted **1** as substrate in vitro (Figure 2C), which includes the previously uncharacterized sequences from *Phycomyces blakesleeanus* (PhybSMT) and *Acidomyces richmondensis* (ArSMT).

Since SMTs are membrane-associated enzymes that typically show low activity and incomplete conversion with substrate concentrations in the low μM range,[34] it was important to identify reaction conditions that enable sufficient activity for in vitro substrate promiscuity screening. This was achieved by either adding 1% (*w*/*V*) of CHAPS detergent to the biotransformation with homogenized *E. coli* cells (Supporting Information II.e) or by detergent-mediated solubilization of the SMTs from the cell membrane prior to the biotransformation. Under optimized conditions (Supporting Information II.f), the reaction reached full conversion of **1** at 1 mM substrate concentration, which is 14 times higher than previously reported in vitro activities of such enzymes.[34]

After establishing a protocol for SMT enzyme preparation, we screened the enzyme panel for promiscuous activity towards 15 readily available terpenoids (Figure S3). As the generated non-natural terpenoids are not commercially available and synthesis of potential products required challenging multistep synthesis, promiscuous activity was identified using mass spectrometry. Analysis of the biotransformations was based on the specific mass shift for methylation (GC/MS-SIM, Table S3) and compared to negative controls. Promiscuous activity was found for five linear terpenoid substrates, including farnesol (**3**), pseudoionone (**4**) and linalool (**6**; Figure 2D). The promiscuous activities were generally very low, resulting in <1% product formation. Remarkably, SMTs from different clusters of the sequence similarity network revealed promiscuous activity (Figure 2B), with the enzyme from *Chlamydomonas reinhardtii* (CrSMT) and *Trypanosoma brucei* (TbSMT) accepting more than one mono-, sesqui- or diterpenoid as substrate.

We aimed to engineer one of these enzymes, to show that activity and selectivity can be optimized by directed evolution and to determine the product selectivity in these reactions. We decided to evolve an enzyme for the conversion of (*E*,*E*)-farnesol (**3**), as this molecule is applied as fragrance ingredient and has interesting pharmacological properties (e.g. antitumor and antibiotic activity).[6,7] It is also found to be a quorum sensing molecule in fungi and was investigated to build antibiofilm surfaces.[46–48] Selective modification of the carbon scaffold of readily available farnesol might thus give access to non-natural farnesol derivatives with altered or enhanced biological properties. CrSMT has been chosen as starting point for enzyme evolution as this enzyme showed the highest promiscuous activity with **3** as a substrate. Biotransformations with **3** yielded two different methylated products in a 60:40 ratio with ca. 0.5% conversion (Figure S4). Based on steroid binding studies in SMTs and on a homology model created with Robetta,[42,44,45,49] 44 amino acids were identified as potential active site residues (Supporting Information II.c). Please note that there is currently no crystal structure available for this entire family of enzymes.

These 44 residues were initially mutated to amino acids with substantially different size to find hot spots for mutagenesis that influence conversion or selectivity (Figure S35).

Positions with beneficial effects on activity or selectivity were chosen for iterative site saturation mutagenesis.[50] Three rounds of evolution yielded the triple mutant CrSMT-125 (E224A, I109L, T216H) that showed a 55-fold increase in conversion of (*E*,*E*)-farnesol compared to the wild type CrSMT (Figure 3B). Further optimization of reaction parameters led to a conversion of 45 ± 1%. Besides a significant increase in activity, the engineered enzyme completely controlled the termination of the reaction, leading to a single methylated product **3-Me** with >99% selectivity. The homology model of CrSMT suggests that the selectivity determining mutation E224A is at the interface of the substrate and SAM/SAH binding sites (Figure 3C). The subtle mutation I109L might optimize the position of the neighboring residue Y110, which is proposed to stabilize the

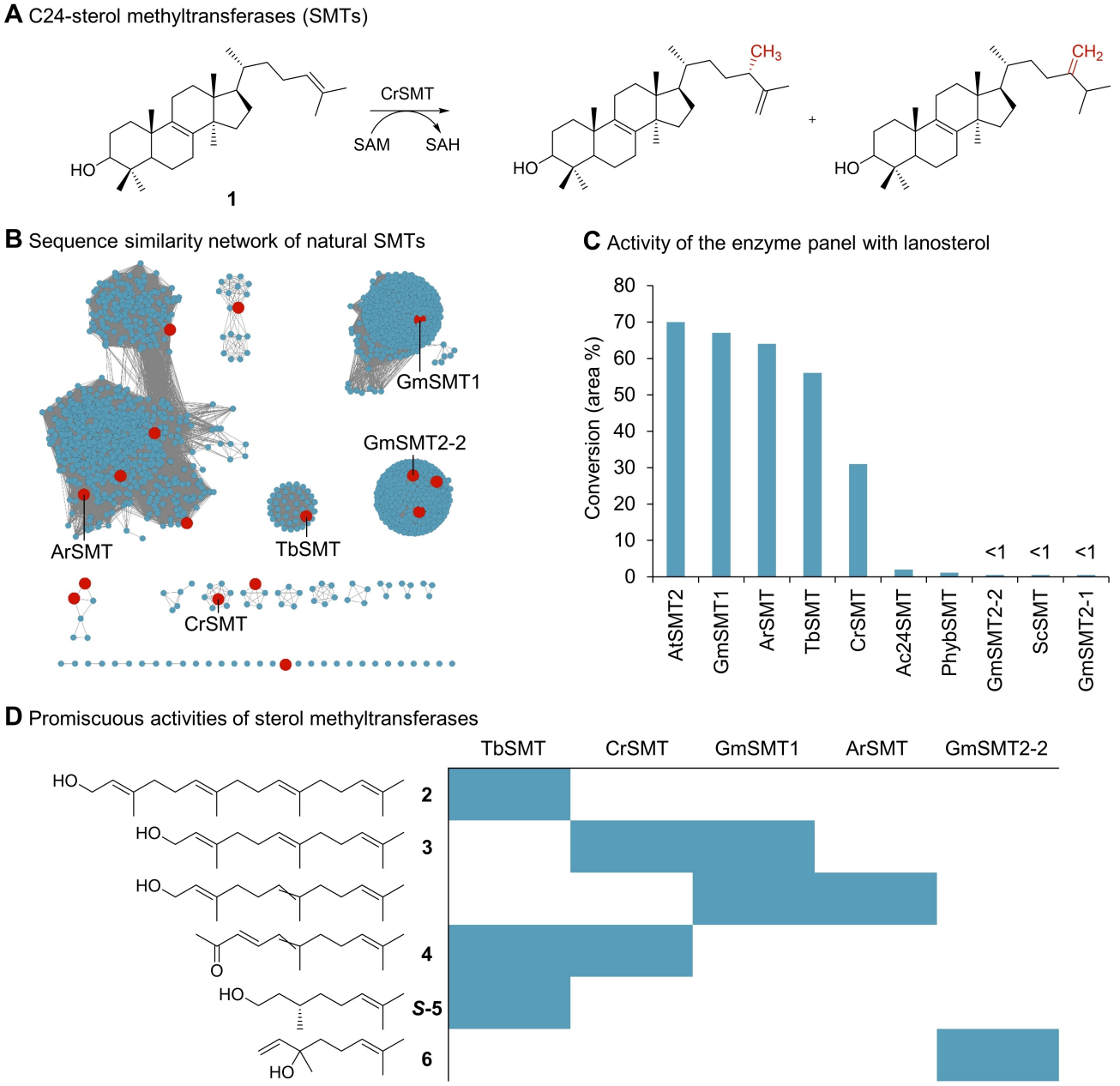

Figure 2. A) Methylation of lanosterol by the sterol methyltransferase from *Chlamydomonas reinhardtii* (CrSMT) is stereoselective and yields two products, which in one case includes a 1,2-hydride migration-deprotonation sequence.[34] B) Sequence similarity network of known and putative SMT-sequences found with BLAST. Sequences selected for in vitro screening shown as red dots. Detailed annotation in Figure S2 and Table S4. C) In vitro activity of the panel of SMTs with 1 mM lanosterol (**1**) at 37 °C for 4 h (Supporting Information II.e). D) Promiscuous activities of SMT wild type enzymes for geranylgeraniol (**2**), farnesol (**3**), pseudoionone (**4**), (*S*)-β-citronellol (***S*-5**) and linalool (**6**). The screening covered 15 terpenoids (Figure S3) and 16 SMTs (Table S4). Blue color indicates detectable promiscuous activity in the screening.

cation intermediate.[34] The sequence region around T216 is described to be involved in SAM binding,[42] yet, in the homology model the position T216 and thus the T216H mutation is located at the protein surface (Figure 3C). Aside from this, the functional role of the beneficial mutations remains ambiguous and needs further investigation.

To characterize the product **3-Me**, a preparative biotransformation of **3** was performed with the engineered variant CrSMT-125. The reaction product was partially isolated by preparative HPLC yielding 8.5 mg (Supporting Information II.g). The product structure was elucidated by 2D NMR as well as high resolution MS (Supporting Information I.b.). The structure reveals a methylation of the terminal prenyl unit of **3**, followed by selective deprotonation of the carbocation intermediate to generate 10-methyl-11-ene-(*E*,*E*)-farnesol (**3-Me**), a currently unknown farnesol derivative (Figure 3A). Analysis of the optical rotation confirmed chirality of the compound with $[\alpha]D22 = -8.0$

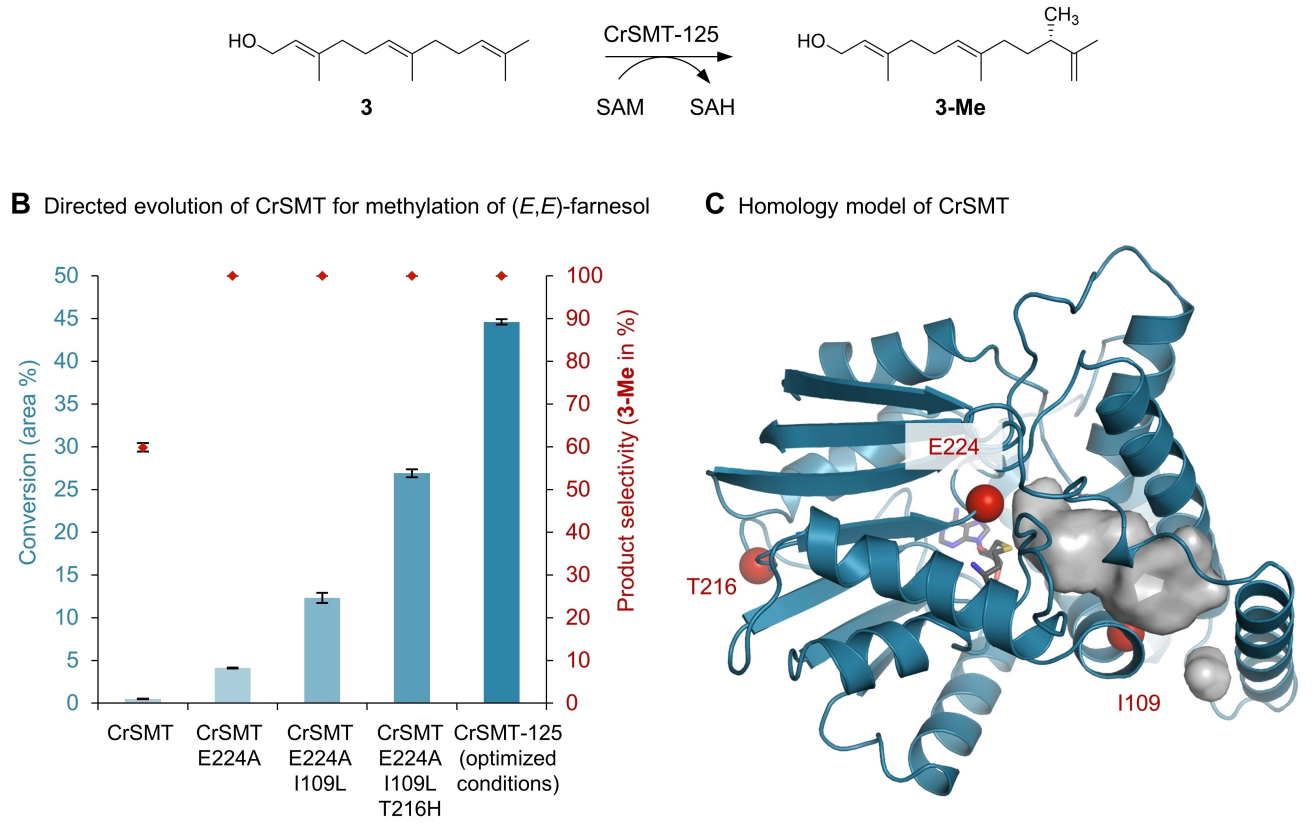

Figure 3. A) Methylation of (*E*,*E*)-farnesol (**3**) by engineered CrSMT-125 yields a single product after catalyst-controlled deprotonation. The structure of 10-methyl-11-ene-(*E*,*E*)-farnesol (**3-Me**) was determined with high-resolution mass spectrometry and NMR. Optical rotation of the isolated product indicates that the catalyst is stereoselective. The absolute configuration of the chiral center is assumed analogue to the lanosterol product.[33,34] B) Iterative site saturation of three positions (E224, I109, T216) increased conversion of **3** to **3-Me** with high selectivity (method: Supporting Information II.f; optimized conditions: 2 mM SAM, 300 µL solubilized enzyme solution). Shown data is the average of triplicates, error bars indicate standard deviation. C) Homolgy model of CrSMT designed with Robetta with highlighted amino acids E224, I109 and T216 that have been targeted in iterative site saturation.[44,45] The position of coproduct SAH is derived from an alignment with a crystal structure of the MT RebM (pdb: 3bus).

($c = 0.1$ in $CHCl_3$), supporting that CrSMT-125 catalyzes a stereoselective *C*-methylation by enantiofacial discrimination of the alkene moiety as described for the natural reaction.[34] Since **3-Me** is not available as racemic mixture, the exact enantioselectivity is currently unknown. In analogy to the product of sterol methylation (Figure 2A), it is likely that **3-Me** is produced as the (*S*)-enantiomer with high selectivity by the enzyme. Altogether, this late-stage methylation produced a non-natural terpenoid with stereo-, regio- and a remarkable chemoselectivity, as the nucleophilic methylation targets an alkene in the presence of an alcohol moiety. Regarding chemical methods, such as the Simmons–Smith methylation, which produces a cyclopropane from a prenyl group,[22] this catalyst offers distinct control over the product selectivity without the need for a directing group and without methylation of multiple nucleophilic targets in the substrate.

Engineering of CrSMT also substantially increased activity towards other linear terpenoids in the C_{10}-C_{20} range. CrSMT-125 converted all tested terpenoids providing access to many non-natural carbon scaffolds. In total, five reactions could be scaled up to isolate and identify the products (Figure 4B, Supporting Information II.b). This includes the conversion of (*E*,*E*,*E*)-geranylgeraniol (**2**, 75 %), (*E*,*E*)-farnesol (**3**, 47 %), (*E*,*E*)-farnesylacetone (**7**, 59 %), (*E*,*E*)-homofarnesol (**8**, 94 %) and (*E*)-geranylacetone (**9**, 44 %). In all cases, the isoprene unit opposite to the oxygen was methylated, followed by deprotonation at one of the terminal methyl groups. This indicates a broad substrate scope while maintaining high catalyst control and selectivity for the methylation and deprotonation position. Furthermore, pseudoionone (**4**, 21 %) as well as (*S*)- and (*R*)-β-citronellol (***S*-5** and ***R*-5**, 3.6 % and 1.2 %) were converted but product isolation was hampered by low activity (Figure 4C).

In conclusion, we have developed a new biocatalyst that allows *C*-methylation of alkenes in readily available mono-, sesqui- and diterpenes. This enzymatic reaction generates non-natural terpenoids with carbon scaffolds that do not follow the isoprene rule. The reaction is highly chemo-, regio- as well as stereoselective and generates terpenoid derivatives that are very difficult to access otherwise.[51] We

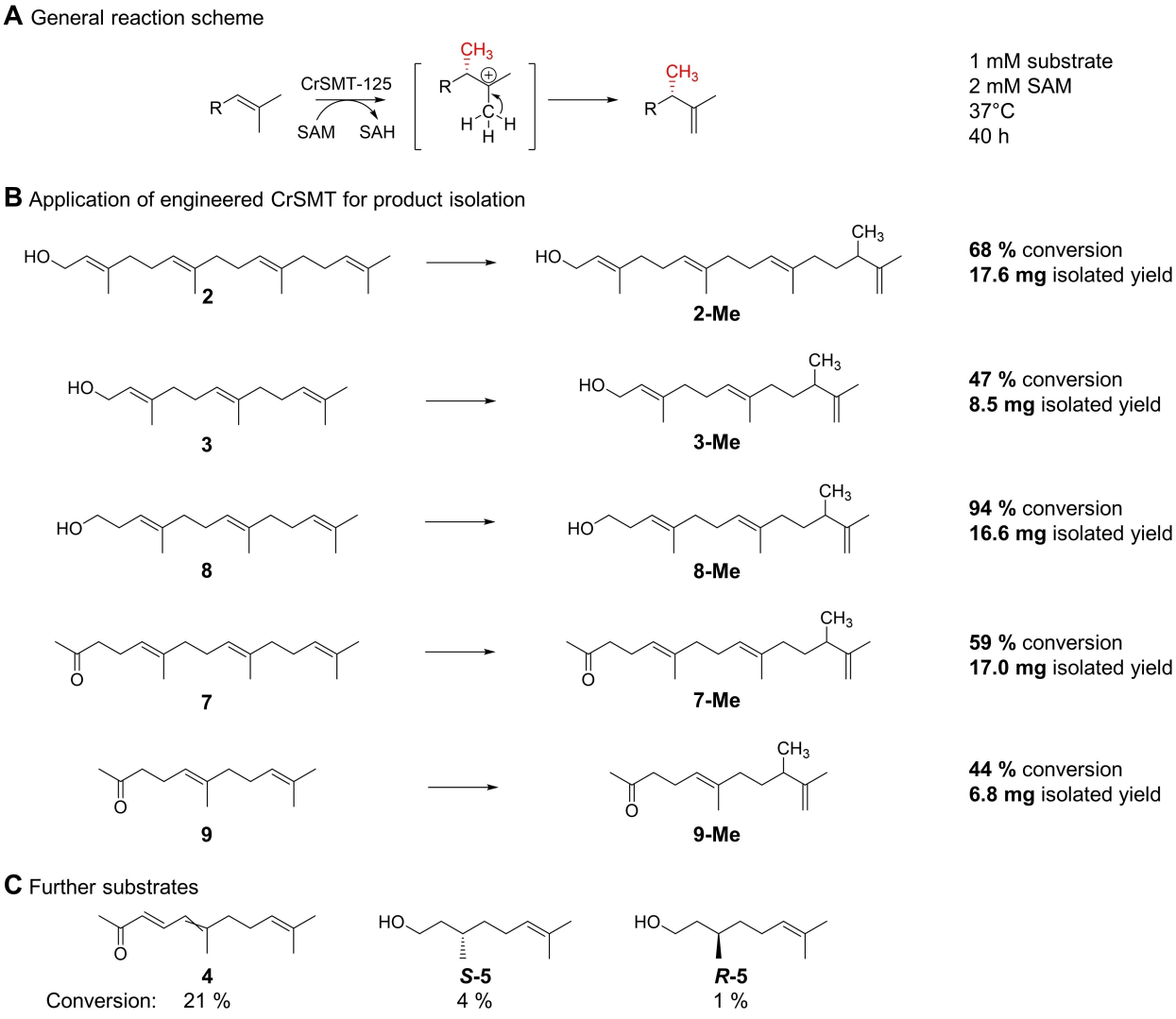

Figure 4. A) General reaction scheme proposed for the engineered CrSMT variant. B) Substrate scope of the engineered enzyme CrSMT-125 (E224A, I109L, T216H). Conversion is determined as GC peak area ratio of product and substrate (Supporting Information II.f). Preparative biotransformations were used to isolate the products and elucidate the structure by 2D NMR and high-resolution mass spectra (Supporting Information II.g, 40 h, 37 °C). C) Substrates with lower conversions, where the products could not be isolated, yet a methylation product was identified by GC/MS.

envision that this enzymatic late-stage modification has the potential to generate many new carbon scaffolds in one simple synthetic operation. We have shown that products can be isolated on a preparative scale, which can be useful to screen for improved or altered bioactivity. For larger scale applications, the stoichiometric use of SAM is a major drawback. This was addressed in the past by developing regeneration systems that use cheaper methyl sources like methionine and polyphosphate or even methyl iodide.[52,53] Furthermore, it is likely that this concept for late-stage terpene modification is not limited to methylation. SAM-dependent enzyme chemistry is currently developing towards a platform for selective biocatalytic alkylations using simple haloalkanes as alkyl source.[54–59] It will be interesting to see what kind of challenging *C–C* bonds can be formed with readily available terpenoids by further exploring the catalytic properties of SMTs and related enzymes.

Acknowledgements

We thank Prof. Dr. W. D. Nes and his group for kindly providing plasmids for expression of AtSMT2, GmSMT1 and GmSMT2-1. We thank Dr. A. Schneider, Dr. L. Bengel, Dr. J. Wissner and Dr. P. Heinemann for fruitful discussions. This work was funded by the "Bundesministerium für Bildung und Forschung (031B0359B)". Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: Alkene Methylation · Biocatalysis · Late-Stage Modification · Methyltransferase · Terpenoids

[1] S. D. Tetali, *Planta* **2019**, *249*, 1–8.
[2] J. Gershenzon, N. Dudareva, *Nat. Chem. Biol.* **2007**, *3*, 408–414.
[3] A. Lapczynski, S. P. Bhatia, C. S. Letizia, A. M. Api, *Food Chem. Toxicol.* **2008**, *46*, S149–S156.
[4] T. Yamamoto, H. Matsuda, Y. Utsumi, T. Hagiwara, T. Kanisawa, *Tetrahedron Lett.* **2002**, *43*, 9077–9080.
[5] M. Gautschi, J. A. Bajgrowicz, P. Kraft, *Chimia* **2001**, *55*, 379–387.
[6] J. H. Joo, A. M. Jetten, *Cancer Lett.* **2010**, *287*, 123–135.
[7] G. de Araújo Delmondes, D. S. Bezerra, D. de Queiroz Dias, A. de Souza Borges, I. M. Araújo, G. Lins da Cunha, P. F. R. Bandeira, R. Barbosa, H. D. Melo Coutinho, C. F. B. Felipe, J. M. Barbosa-Filho, I. R. Alencar de Menezes, M. R. Kerntopf, *Food Chem. Toxicol.* **2019**, *129*, 169–200.
[8] A. C. Huang, A. Osbourn, *Pest Manage. Sci.* **2019**, *75*, 2368–2377.
[9] L. Ruzicka, *Experientia* **1953**, *9*, 357–396.
[10] H. V. Thulasiram, H. K. Erickson, C. D. Poulter, *Science* **2007**, *316*, 73–76.
[11] E. Pichersky, J. P. Noel, N. Dudareva, *Science* **2006**, *311*, 808–811.
[12] Y. Gao, R. B. Honzatko, R. J. Peters, *Nat. Prod. Rep.* **2012**, *29*, 1153–1175.
[13] A. Stepanyuk, A. Kirschning, *Beilstein J. Org. Chem.* **2019**, *15*, 2590–2602.
[14] C. Oberhauser, V. Harms, K. Seidel, B. Schröder, K. Ekramzadeh, S. Beutel, S. Winkler, L. Lauterbach, J. S. Dickschat, A. Kirschning, *Angew. Chem. Int. Ed.* **2018**, *57*, 11802–11806.
[15] T. Nishimura, J. Kawai, Y. Oshima, H. Kikuchi, *Org. Lett.* **2018**, *20*, 7317–7320.
[16] P. Bashyal, R. P. Pandey, S. B. Thapa, M. K. Kang, C. J. Kim, J. K. Sohng, *ACS Omega* **2019**, *4*, 9367–9375.
[17] D. J. Jansen, R. A. Shenvi, *Future Med. Chem.* **2014**, *6*, 1127–1148.
[18] H. Cang, R. A. Moss, K. Krogh-Jespersen, *J. Am. Chem. Soc.* **2015**, *137*, 2730–2737.
[19] G. Yan, A. J. Borah, L. Wang, M. Yang, *Adv. Synth. Catal.* **2015**, *357*, 1333–1350.
[20] Y. Chen, *Chem. Eur. J.* **2019**, *25*, 3405–3439.
[21] S. D. Friis, M. J. Johansson, L. Ackermann, *Nat. Chem.* **2020**, *12*, 511–519.
[22] J. Liu, D. Liu, W. Nie, H. Yu, J. Shi, *Org. Chem. Front.* **2022**, *9*, 163–172.
[23] E. J. Barreiro, A. E. Kümmerle, C. A. M. Fraga, *Chem. Rev.* **2011**, *111*, 5215–5246.
[24] H. Schönherr, T. Cernak, *Angew. Chem. Int. Ed.* **2013**, *52*, 12256–12267.
[25] J. Boström, D. G. Brown, R. J. Young, G. M. Keserü, *Nat. Rev. Drug Discovery* **2018**, *17*, 709–727.
[26] C. S. Leung, S. S. F. Leung, J. Tirado-Rives, W. L. Jorgensen, *J. Med. Chem.* **2012**, *55*, 4489–4500.
[27] W. D. Nes, *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **2000**, *1529*, 63–88.
[28] C. Ignea, M. Pontini, M. S. Motawia, M. E. Maffei, A. M. Makris, S. C. Kampranis, *Nat. Chem. Biol.* **2018**, *14*, 1090–1098.
[29] L. Drummond, M. J. Kschowak, J. Breitenbach, H. Wolff, Y.-M. Shi, J. Schrader, H. B. Bode, G. Sandmann, M. Buchhaupt, *ACS Synth. Biol.* **2019**, *8*, 1303–1313.
[30] M. J. Kschowak, F. Maier, H. Wortmann, M. Buchhaupt, *ACS Synth. Biol.* **2020**, *9*, 981–986.
[31] S. von Reuss, D. Domik, M. C. Lemfack, N. Magnus, M. Kai, T. Weise, B. Piechulla, *J. Am. Chem. Soc.* **2018**, *140*, 11855–11862.
[32] A. Hou, L. Lauterbach, J. S. Dickschat, *Chem. Eur. J.* **2020**, *26*, 2178–2182.
[33] W. D. Nes, *Phytochemistry* **2003**, *64*, 75–95.
[34] B. A. Haubrich, E. K. Collins, A. L. Howard, Q. Wang, W. J. Snell, M. B. Miller, C. D. Thomas, S. K. Pleasant, W. D. Nes, *Phytochemistry* **2015**, *113*, 64–72.
[35] W. Zhou, W. D. Nes, *Arch. Biochem. Biophys.* **2003**, *420*, 18–34.
[36] W. D. Nes, Z. Song, A. L. Dennis, W. Zhou, J. Nam, M. B. Miller, *J. Biol. Chem.* **2003**, *278*, 34505–34516.
[37] A. K. Neelakandan, Z. Song, J. Wang, M. H. Richards, X. Wu, B. Valliyodan, H. T. Nguyen, W. D. Nes, *Phytochemistry* **2009**, *70*, 1982–1998.
[38] M. Venkatramesh, D.-A. A. Guo, Z. Jia, W. D. Nes, *Biochim. Biophys. Acta Lipids Lipid Metab.* **1996**, *1299*, 313–324.
[39] W. D. Nes, B. S. McCourt, W.-X. Zhou, J. Ma, J. A. Marshall, L.-A. Peek, M. Brennan, *Arch. Biochem. Biophys.* **1998**, *353*, 297–311.
[40] T. D. Niehaus, S. Kinison, S. Okada, Y. S. Yeo, S. A. Bell, P. Cui, T. P. Devarenne, J. Chappell, *J. Biol. Chem.* **2012**, *287*, 8163–8173.
[41] W. Zhou, G. I. Lepesheva, M. R. Waterman, W. D. Nes, *J. Biol. Chem.* **2006**, *281*, 6290–6296.
[42] E. S. Kaneshiro, J. A. Rosenfeld, M. Basselin-Eiweida, J. R. Stringer, S. P. Keely, A. G. Smulian, J. L. Giner, *Mol. Microbiol.* **2002**, *44*, 989–999.
[43] M. E. Kidane, B. H. Vanderloop, W. Zhou, C. D. Thomas, E. Ramos, U. Singha, M. Chaudhuri, W. D. Nes, *J. Lipid Res.* **2017**, *58*, 2310–2323.
[44] D. E. Kim, D. Chivian, D. Baker, *Nucleic Acids Res.* **2004**, *32*, W526–W531.
[45] S. Raman, R. Vernon, J. Thompson, M. Tyka, R. Sadreyev, J. Pei, D. Kim, E. Kellogg, F. Dimaio, O. Lange, L. Kinch, W. Sheffler, B. H. Kim, R. Das, N. V. Grishin, D. Baker, *Proteins Struct. Funct. Bioinf.* **2009**, *77*, 89–99.
[46] M. Nowacka, A. Kowalewska, D. Kręgiel, *Surfaces* **2020**, *3*, 197–210.
[47] R. Kovács, L. Majoros, *J. Fungi* **2020**, *6*, 99.
[48] G. Ramage, S. P. Saville, B. L. Wickes, J. L. López-Ribot, *Appl. Environ. Microbiol.* **2002**, *68*, 5459–5463.
[49] W. D. Nes, J. A. Marshall, Z. Jia, T. T. Jaradat, Z. Song, P. Jayasimha, *J. Biol. Chem.* **2002**, *277*, 42549–42556.
[50] M. T. Reetz, J. D. Carballeira, *Nat. Protoc.* **2007**, *2*, 891–903.
[51] E. Romero, B. S. Jones, B. N. Hogg, A. Rué Casamajo, M. A. Hayes, S. L. Flitsch, N. J. Turner, C. Schnepel, *Angew. Chem. Int. Ed.* **2021**, *60*, 16824–16855.
[52] S. Mordhorst, J. Siegrist, M. Müller, M. Richter, J. N. Andexer, *Angew. Chem. Int. Ed.* **2017**, *56*, 4037–4041.
[53] C. Liao, F. P. Seebeck, *Nat. Catal.* **2019**, *2*, 696–701.
[54] L. L. Bengel, B. Aberle, A. Egler-Kemmerer, S. Kienzle, B. Hauer, S. C. Hammer, *Angew. Chem. Int. Ed.* **2021**, *60*, 5554–5560.

[55] Q. Tang, C. W. Grathwol, A. S. Aslan-Üzel, S. Wu, A. Link, I. V. Pavlidis, C. P. S. Badenhorst, U. T. Bornscheuer, *Angew. Chem. Int. Ed.* **2021**, *60*, 1524–1527.
[56] F. Ospina, K. H. Schülke, S. C. Hammer, *ChemPlusChem* **2022**, *87*, e202100454.
[57] J. Peng, C. Liao, C. Bauer, F. P. Seebeck, *Angew. Chem. Int. Ed.* **2021**, *60*, 27178–27183.
[58] I. J. W. McKean, P. A. Hoskisson, G. A. Burley, *ChemBioChem* **2020**, *21*, 2890–2897.
[59] K. H. Schülke, F. Ospina, K. Hörnschemeyer, S. Gergel, S. C. Hammer, *ChemBioChem* **2022**, *23*, e202100632.

Manuscript received: February 2, 2023
Accepted manuscript online: March 30, 2023
Version of record online: May 5, 2023