



# Reconstitution of Biosynthetic Machinery for the Synthesis of the Highly Elaborated Indole Diterpene Penitrem\*\*

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**Abstract:** Penitrem A is one of the most elaborated members of the fungal indole diterpenes. Two separate penitrem gene clusters were identified using genomic and RNA sequencing data, and 13 out of 17 transformations in the penitrem biosynthesis were elucidated by heterologous reconstitution of the relevant genes. These reactions involve 1) a prenylation-initiated cationic cyclization to install the bicyclo[3.2.0]heptane skeleton (PtmE), 2) a two-step P450-catalyzed oxidative processes forming the unique tricyclic penitrem skeleton (PtmK and PtmU), and 3) five sequential oxidative transformations (PtmKULNJ). Importantly, without conventional gene disruption, reconstitution of the biosynthetic machinery provided sufficient data to determine the pathway. It was thus demonstrated that the *Aspergillus oryzae* reconstitution system is a powerful method for studying the biosynthesis of complex natural products.

The increasing number of cryptic gene clusters prompted us to explore a general method that applies the genome mining approach to the functional analysis of individual biosynthetic steps and the production of unidentified metabolites.<sup>[1]</sup> Recently, we successfully employed the heterologous expression of entire biosynthetic genes for the diterpenes aphidico-

lin and paxilline (**1**) using five vectors with auxotrophic markers in *Aspergillus oryzae* NSAR1.<sup>[2,3]</sup> To increase the number of genes introduced into a single host, we developed new vectors with two cloning sites and tandem transformations, which allow us to introduce four genes in a single transformation. The use of this method enabled us to rapidly achieve full reconstitution of seven aflatrem biosynthetic genes in two rounds of transformations.<sup>[4]</sup>

To apply this reconstitution method to the elucidation of the biosynthetic pathway of a complex natural product, we chose tremogenic indole diterpene penitrem (**2**). Among the indole diterpenes, such as **1**, lolitrem, janthitrem, and nodulisporic acid, **2** is one of the most highly elaborated congeners and has a unique tricyclic system adjacent to the paxilline core structure (Figure 1). Herein, we describe the

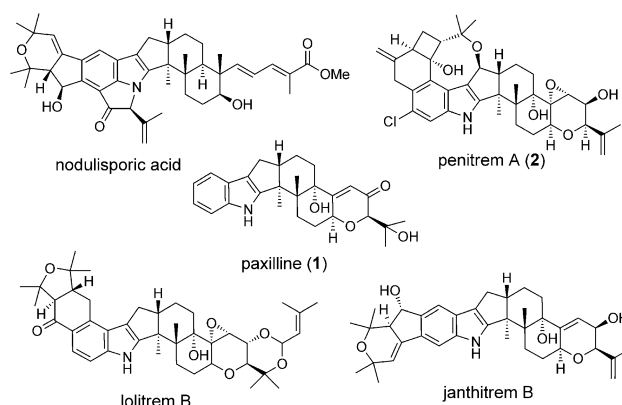


Figure 1. Representative indole diterpenes from various fungi.

reconstitution of 13 out of the 17 penitrem biosynthetic genes in the heterologous host *A. oryzae* to produce **2**. This process revealed a long biosynthetic pathway and a four-step reaction sequence for the construction of the characteristic tricyclic core scaffold, which features a cyclobutane and an eight-membered cyclic ether.

The tremogenic indole diterpene family consists of various members.<sup>[5,6]</sup> These metabolites have a common hexacyclic core structure, which is frequently modified by prenylation, aside from further modifications that give carbocycles and cyclic ethers (Figure 1).<sup>[5,6]</sup> Penitrem A (**2**) has been isolated from several *Penicillium* species, such as *P. crustosum*,<sup>[7]</sup> *P. janczewskii*,<sup>[8]</sup> and *P. simplicissimum*.<sup>[9]</sup> Among these strains, *P. crustosum* produces the penitrem homologues A–F<sup>[7]</sup> and paxilline-related metabolites, such as paspaline (**3**),<sup>[10]</sup> PC-M5 (**4**), and PC-M4<sup>[11]</sup> (**5**; Scheme 1).

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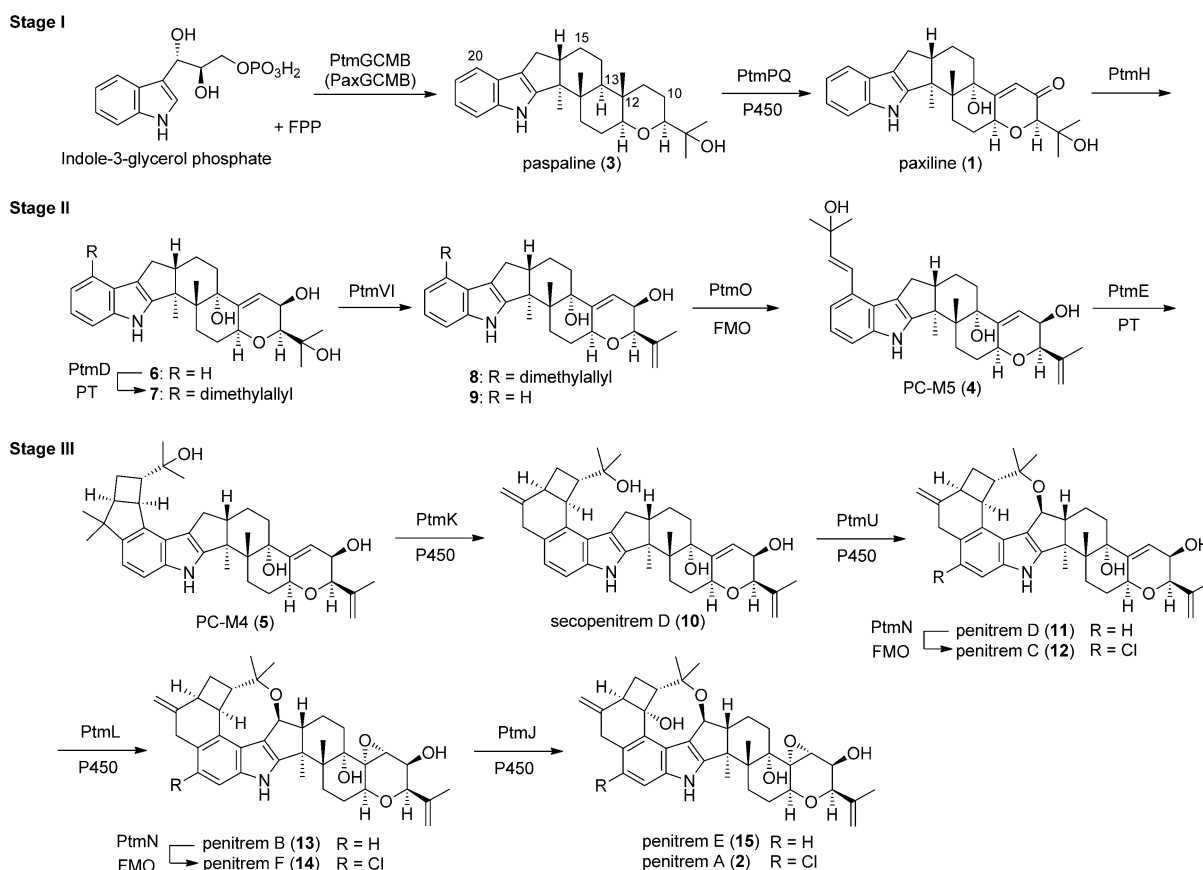
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**Scheme 1.** Elucidated biosynthetic pathway of the penitrems.

Biosynthetic studies with isotopically labeled precursors, including paxilline,<sup>[8,12]</sup> suggested that the characteristic penitrem core scaffold is derived from a diprenylated paxilline derivative. Isolation of the structurally related metabolites **4** and **5** allowed us to propose that the characteristic bicyclo[4.2.0]octane system is constructed by cyclization of diprenylated paxilline to bicyclo[3.2.0]heptane, subsequent oxidative ring expansion, and formation of the cyclic ether. Considering these findings, we divided the penitrem biosynthetic pathway into three stages: conversion of indole-3-glycerol phosphate and farnesyl diphosphate (FPP) into **1** (stage I), transformation of **1** into **5** (stage II), and conversion of **5** into **2**, which entails epoxidation, hydroxylation, and chlorination reactions after construction of the penitrem skeleton (stage III; Scheme 1).

To determine the biosynthetic gene cluster of **2**, a genomic analysis of *P. simplicissimum* was employed. Using gene cluster searching methods (a local Blast search and the 2ndFind program),<sup>[13]</sup> we identified the contiguous sequence that contained genes homologous to those of the paxilline gene cluster. Using our proposed biosynthetic pathway, we speculated that this cluster (44 kb) consisting of the 15 putative genes lacked several genes for the oxidative modifications of the indole ring (Figure 2; see also the Supporting Information, Table S1). Therefore, another penitrem producer, namely *P. crustosum*, was also subjected to an RNA sequencing analysis, which identified 15 orthologous genes



**Figure 2.** Biosynthetic gene clusters of penitrem in *Penicillium simplicissimum*. The genes *ptmGAQMBCP* are highly homologous to those of the paxilline gene cluster.

and 5 further genes that showed close correlation to the production of penitrems in the expression profile (Table S2). Using these sequences, we identified a second 11 kb cluster consisting of five additional genes in the *P. simplicissimum* genome. The penitrem gene cluster designated as *ptm* consisted of seven orthologous paxilline biosynthetic genes, *ptmGCMBPQA*, the transcriptional regulator *ptmS* and the transporter *ptmT*, two prenyltransferase genes *ptmDE*, and six genes *ptmJKLNOU* that encode the oxidative transformation enzymes (four cytochrome P450 monooxygenases and two flavin adenine dinucleotide (FAD) dependent monooxygenases). The cluster also included three additional genes, namely *ptmH* (oxidoreductase), *ptmV* (acetyltransferase), and *ptmI*, which showed very low homology (<20% identity) to the aromatase/cyclase genes in the biosynthesis of bacterial aromatic polyketides.

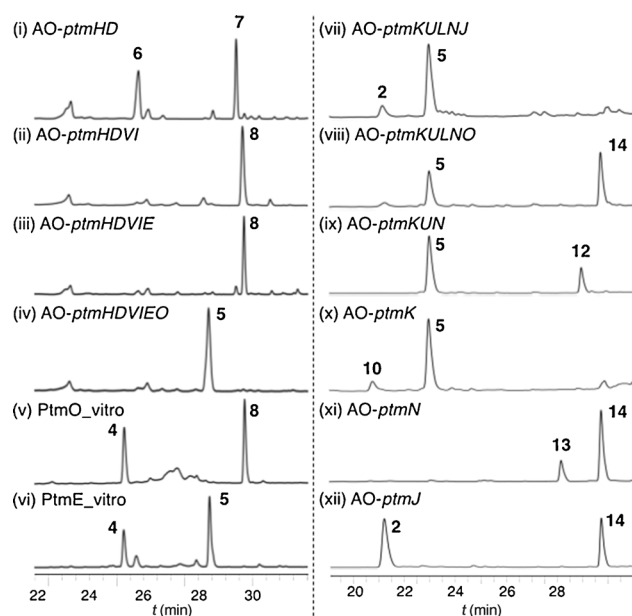
Initially, we examined the stage I transformation. Scott and co-workers had shown that **1**, aflatrem, and lolitrem are biosynthesized via a common intermediate, namely paspaline (**3**), based on a series of gene disruption experiments with four orthologous genes *paxGCMB*.<sup>[14]</sup> On the other hand, oxidative modifications of **3** with orthologous P450 gene sets (*paxPQ*, *atmPQ*, and *ltmPQ*) gave slightly different results.<sup>[14]</sup> As **2** shares a common hexacyclic core structure with these metabolites, we speculated that **3** is a precursor of **2** as in the case of aflatrem.<sup>[4]</sup> To confirm the putative functions of the *ptmPQ* genes, we introduced these genes to the paspaline-producing transformant AO-*paxGCMB*.<sup>[4]</sup> The incubation of the resultant strain AO-*paxGCMBptmPQ* produced **1** (Figure S1), confirming its intermediacy (Scheme 1; stage I: titer of **1**: 8.8 mg kg<sup>-1</sup>). This result was in good agreement with previous biosynthetic studies<sup>[8,12]</sup> and excluded the possibility that  $\beta$ -paxitriol (**6**) is directly formed by PtmP/PtmQ-dependent oxidative modifications.<sup>[15]</sup>

To obtain information on the biosynthetic reaction sequence in stage II, and especially on the timing of the two prenylation reactions, two genes *ptmHD* that encode NAD(P<sup>+</sup>)-dependent oxidoreductase and prenyltransferase (31%/55% identity/similarity to *paxD*)<sup>[14a,16]</sup> were introduced into *A. oryzae* NSAR1 with pUSA2-*ptmHD*. The resultant transformant AO-*ptmHD* converted **1** into the new metabolites **6** and **7** (Figure 3). The molecular formulae (**6**: C<sub>27</sub>H<sub>35</sub>NO<sub>4</sub>; **7**: C<sub>32</sub>H<sub>43</sub>NO<sub>4</sub>) were determined by HR-MS, and the structures of **6** and **7** were determined to be  $\beta$ -paxitriol<sup>[15]</sup> and its monoprenylated analogue by extensive spectroscopic analysis (Scheme 1). We hypothesized that **7** would be further modified by a second prenylation (reverse mode) and dehydration. Therefore, we introduced possible candidate

genes, namely *ptmE* (prenyltransferase), *ptmI* (unknown), and *ptmV* (acetyltransferase), into AO-*ptmHD* to yield AO-*ptmHDV*, AO-*ptmHDVI*, AO-*ptmHDIE*, and AO-*ptmHDVIE*. Whereas incubation of AO-*ptmHDV* and AO-*ptmHDIE* with **1** did not give any predominant products, incubation of AO-*ptmHDVI* gave new product **8** (Figure 3; Figure S4). Based on HR-MS data (**8**: *m/z* 487.2811 [*M*+H]<sup>+</sup>), the molecular formula of **8** was determined to be C<sub>32</sub>H<sub>41</sub>NO<sub>3</sub>, which corresponds to the dehydration product of **7**. The structure was determined by spectroscopic analysis to be the prenylated form of penijanthe (**9**),<sup>[17]</sup> which was isolated from *P. janthinellum* (Scheme 1). Both PtmV and PtmI are likely required for the elimination. Similar two-step elimination (acetylation and elimination) processes have been reported for the biosynthesis of tetronate antibiotics,<sup>[18]</sup> although different types of enzymes are involved in the penitrem and tetronate biosyntheses. To determine the exact reaction sequence of stage II, we employed an in vitro functional analysis of PtmD. The enzymatic reactions of the three substrates **1**, **6**, and **9**, which were synthesized from paxilline, with PtmD in the presence of dimethylallyl diphosphate (DMAPP) showed a clear preference in the order of **6**, **9**, and **1** (Figures S2 and S3). This result suggests that the three-step process consists of C10 ketoreduction (PtmH) followed by C20 prenylation (PtmD) and then dehydration (PtmV, PtmI). For the AO-*ptmHDVIE* reaction, the HPLC profile was essentially identical to that of AO-*ptmHDVI*, indicating that the timing of the second prenylation is critical for the construction of the bicyclo[4.2.0]octane skeleton. Thus, we turned our attention to the functional analysis of the oxidation enzyme genes to reduce the number of possible reaction pathways.

To address the critical issue of whether **4** and **5** are shunt metabolites or actual intermediates, we prepared the transformants AO-*ptmKULNJ* and AO-*ptmKULNO*. The microbial transformations of **5** with AO-*ptmKULNJ* and AO-*ptmKULNO* afforded **2** and penitrem F (**14**), respectively [Stage III; conversions: 20% (**5**→**2**), 40% (**5**→**14**)]. These results showed that **5** is an intermediate in penitrem biosynthesis and also that five genes *ptmKULNJ* are essential for the transformations in stage III, and that PtmJ catalyzes the last benzylic hydroxylation (Scheme 1). To elucidate the detailed biosynthetic pathway in stage III, we prepared the transformants AO-*ptmKUN*, AO-*ptmK*, AO-*ptmN*, and AO-*ptmJ*. The reaction of **5** with AO-*ptmKUN* yielded penitrem C (**12**; Figure 3, Figure S4), indicating that these genes are involved in the formation of the key bicyclic ring and that PtmL catalyzes the epoxidation. Incubation of AO-*ptmK* with **5** gave new product **10** (Figure 3, Figure S4). Its molecular formula C<sub>37</sub>H<sub>47</sub>NO<sub>4</sub> was determined by HR-MS (*m/z* 569.3577 [*M*+H]<sup>+</sup>), which gave an *m/z* value that was 2 units lower than that of **5**, and the <sup>1</sup>H NMR spectrum showed additional *exo* olefin signals at 4.73 and 4.84 ppm. Together with other spectral data, the structure of **10**, designated as secopenitrem D, was determined to be the one shown in Scheme 1. Each biosynthetic reaction was further confirmed by the transformant carrying a single gene (Figure 3, Figure S4).

As the penitrem gene cluster encodes six oxidation enzymes, the functional analysis of the five genes *ptmKULNJ*



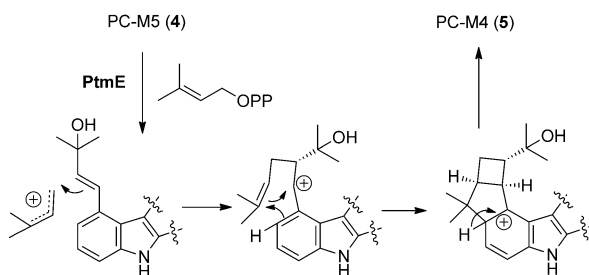
**Figure 3.** HPLC profiles of extracts from biotransformations with *A. oryzae* transformants and of in vitro reactions: i) AO-*ptmHD* with **1**; ii) AO-*ptmHDVI* with **1**; iii) AO-*ptmHDVIE* with **1**; iv) AO-*ptmHDVIEO* with **1**; v) PtmO reaction with **8**; vi) PtmE reaction with **4**; vii) AO-*ptmKULNJ* with **5**; viii) AO-*ptmKULNO* with **5**; ix) AO-*ptmKUN* with **5**; x) AO-*ptmK* with **5**; xi) AO-*ptmN* with **13**; xii) AO-*ptmJ* with **14**.

left a putative flavin-dependent monooxygenase gene *ptmO*. The in vitro transformation of **8** with recombinant PtmO gave a single, acid-labile product, which was identical to **4**<sup>[11a]</sup> (Figure 3, Scheme 1). Based on this result, we introduced two genes *ptmOE* into AO-*ptmHDVI*, and the resultant AO-*ptmHDVIOE* was incubated with **1**. HPLC analysis of the extract showed a prominent product that was identical to **5** (Figure 3). To confirm this highly unusual reaction, we employed in vitro analysis of PtmE. Incubation of **4** with recombinant PtmE in the presence of Mg<sup>2+</sup> and DMAPP gave **5** (Figure 3; for the proposed reaction mechanism, see Figure 4). PtmE did not accept any other precursors, includ-

options will greatly facilitate the study of biosynthetic pathways of fungal natural products. This powerful method enabled us to not only characterize each individual enzyme reaction step, but also to synthesize intermediates and the final natural product. We would like to emphasize that the functional analysis of 13 out of the 17 genes involved in the penitrem biosynthesis was achieved without using the conventional gene disruption method. In turn, this indicates that the *A. oryzae* heterologous expression system constitutes a highly reliable method for studying fungal natural product biosynthesis.<sup>[26]</sup>

**Keywords:** biosynthesis · heterologous expression · indole diterpenes · natural products · penitrem

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**Figure 4.** Proposed mechanism of the PtmE-catalyzed reaction.

ing **8**. These data established that the stage II biosynthetic pathway proceeds from **1** to **5** via the key intermediates **8** and **4** (Scheme 1; stage II: 87% conversion (**1**→**5**)).

Considering the overall enzymatic transformation, the mechanism of the construction of the bicyclo[4.2.0]octane skeleton is especially intriguing. Prenylation-initiated cationic cyclizations (addition of a prenyl group to an olefin) are rather rare transformations in natural product biosynthesis. It has been reported that chrysanthemyl diphosphate synthase (cyclopropane) and cyclolavandulyl diphosphate synthase (cyclohexene) catalyze head-to-head couplings of DMAPP and the subsequent cyclization (Figure S5).<sup>[19]</sup> In the biosynthesis of the antidepressant agent hyperforin, prenylation of the geranyl group of the phloroglucinol moiety was proposed to trigger nucleophilic attack of the internal enol (Figure S5).<sup>[20]</sup> In the second step, the oxidative ring expansion is a unique process in the P450-catalyzed reaction although the P450 monooxygenase TenA catalyzes ring expansion of tetramic acid to 2-pyridone in tenellin biosynthesis.<sup>[21]</sup> A similar ring expansion catalyzed by a non-heme Fe/α-ketoglutarate-dependent dioxygenase has been reported in the biosynthesis of cephalosporin.<sup>[22]</sup> In the last step, the formation of the eight-membered oxocane cyclic ether occurred in the presence of cytochrome P450 monooxygenase PtmU. In organic synthesis, the formation of such medium-sized rings is rather difficult to achieve owing to non-bonding transannular repulsion. The P450-catalyzed tetrahydrofuran formation in the biosynthesis of aureothin is a preceding example of oxidative cyclic ether formations.<sup>[23]</sup>

Recently, the reconstitution method has become a practical method to study fungal metabolite biosynthesis.<sup>[24]</sup> Two different approaches are possible, namely integration with multiple plasmids, as described in this paper, and integration with a single plasmid carrying multiple genes.<sup>[25]</sup> These

- [1] a) S. Bergmann, J. Schumann, K. Scherlach, C. Lange, A. A. Brakhage, C. Hertweck, *Nat. Chem. Biol.* **2007**, *3*, 213–217; b) Y.-M. Chiang, E. Szewczyk, A. D. Davidson, N. P. Keller, B. R. Oakley, C. C. Wang, *J. Am. Chem. Soc.* **2009**, *131*, 2965–2970; c) R. B. Williams, J. C. Henrikson, A. R. Hoover, A. E. Lee, R. H. Cichewicz, *Org. Biomol. Chem.* **2008**, *6*, 1895–1897.
- [2] R. Fujii, A. Minami, T. Tsukagoshi, N. Sato, T. Sahara, S. Ohgiya, K. Gomi, H. Oikawa, *Biosci. Biotechnol. Biochem.* **2011**, *75*, 1813–1817.
- [3] K. Tagami, C. Liu, A. Minami, M. Noike, T. Isaka, S. Fueki, Y. Shichijo, H. Toshima, K. Gomi, T. Daiiri, H. Oikawa, *J. Am. Chem. Soc.* **2013**, *135*, 1260–1263.
- [4] K. Tagami, A. Minami, R. Fujii, C. Liu, M. Tanaka, K. Gomi, T. Daiiri, H. Oikawa, *ChemBioChem* **2014**, *15*, 2076–2080.
- [5] S. Saikia, M. J. Nicholson, C. Young, E. J. Parker, B. Scott, *Mycol. Res.* **2008**, *112*, 184–199.
- [6] a) *Handbook of Secondary Fungal Metabolites* (Eds.: R. J. Cole, B. B. Jarvis, M. A. Schweikert), Academic Press, New York, **2003**; b) W. B. Turner, D. C. Aldridge, *Fungal Metabolites*, Vol. 2, Academic Press, New York, **1982**.
- [7] a) A. E. De Jesus, P. S. Steyn, F. R. Van Heerden, R. Vleggaar, P. L. Wessels, W. E. Hull, *J. Chem. Soc. Perkin Trans. 1* **1983**, 1847–1856; b) A. E. De Jesus, P. S. Steyn, F. R. Van Heerden, R. Vleggaar, P. L. Wessels, W. E. Hull, *J. Chem. Soc. Perkin Trans. 1* **1983**, 1857–1861.
- [8] J. Penn, P. G. Mantle, *Phytochemistry* **1994**, *35*, 921–926.
- [9] H. Hayashi, Y. Asabu, S. Murao, M. Nakayama, M. Arai, *Chem. Express* **1993**, *8*, 177–180.
- [10] M. C. González, C. Lull, P. Moya, I. Ayala, J. Primo, E. P. Yúfera, *J. Agric. Food Chem.* **2003**, *51*, 2156–2160.
- [11] a) T. Hosoe, K. Nozawa, S. Udagawa, S. Nakajima, K. Kawai, *Chem. Pharm. Bull.* **1990**, *38*, 3473–3475; b) T. Yamaguchi, K. Nozawa, T. Hosoe, S. Nakajima, K. I. Kawai, *Phytochemistry* **1993**, *32*, 1177–1181.
- [12] A. E. De Jesus, C. P. Gorstallman, P. S. Steyn, F. R. Van Heerden, R. Vleggaar, P. L. Wessels, W. E. Hull, *J. Chem. Soc. Perkin Trans. 1* **1983**, 1863–1868.
- [13] 2ndFind: <http://biosyn.nih.gov/2ndfind/>.
- [14] a) C. Young, L. McMillan, E. Telfer, B. Scott, *Mol. Microbiol.* **2001**, *39*, 754–764; b) M. J. Nicholson, A. Koulman, B. J. Monahan, B. L. Pritchard, G. A. Payne, B. Scott, *Appl. Environ. Microbiol.* **2009**, *75*, 7469–7481; c) S. Saikia, D. Takemoto, B. A. Tapper, G. A. Lane, K. Fraser, B. Scott, *FEBS Lett.* **2012**, *586*, 2563–2569.
- [15] S. Saikia, E. J. Parker, A. Koulman, B. Scott, *J. Biol. Chem.* **2007**, *282*, 16829–16837.



- [16] C. W. Liu, M. Noike, A. Minami, H. Oikawa, T. Dairi, *Appl. Microbiol. Biotechnol.* **2014**, *98*, 199–206.
- [17] T. Itabashi, T. Hosoe, D. Wakana, K. Fukushima, K. Takizawa, T. Yaguchi, K. Okada, G. M. D. Takaki, K. Kawai, *J. Nat. Med.* **2009**, *63*, 96–99.
- [18] a) C. Kanchanabancha, W. Tao, H. Hong, Y. Liu, F. Hahn, M. Samborsky, Z. Deng, Y. Sun, P. F. Leadlay, *Angew. Chem. Int. Ed.* **2013**, *52*, 5785–5788; *Angew. Chem.* **2013**, *125*, 5897–5900; b) L. F. Wu, H. Y. He, H. X. Pan, L. Han, R. X. Wang, G. L. Tang, *Org. Lett.* **2014**, *16*, 1578–1581.
- [19] T. Ozaki, P. Zhao, T. Shinada, M. Nishiyama, T. Kuzuyama, *J. Am. Chem. Soc.* **2014**, *136*, 4837–4840.
- [20] a) P. Adam, D. Arigoni, A. Bacher, W. Eisenreich, *J. Med. Chem.* **2002**, *45*, 4786–4793; b) N. S. Bystrov, B. K. Chernov, V. N. Dobrynin, M. N. Kolosov, *Tetrahedron Lett.* **1975**, *16*, 2791–2794.
- [21] L. M. Halo, M. N. Heneghan, A. A. Yakasai, Z. Song, K. Williams, A. M. Bailey, R. J. Cox, C. M. Lazarus, T. J. Simpson, *J. Am. Chem. Soc.* **2008**, *130*, 17988–17996.
- [22] a) K. Valegård, A. C. T. van Scheltinga, A. Dubus, G. Ranghino, L. M. Öster, J. Hajdu, I. Andersson, *Nat. Struct. Mol. Biol.* **2004**, *11*, 95–101; b) K. Valegård, A. C. T. van Scheltinga, M. D. Lloyd, T. Hara, S. Ramaswamy, A. Perrakis, A. Thompson, H. J. Lee, J. E. Baldwin, C. J. Schofield, J. Hajdu, I. Andersson, *Nature* **1998**, *394*, 805–809.
- [23] M. E. A. Richter, N. Traitcheva, U. Knupfer, C. Hertweck, *Angew. Chem. Int. Ed.* **2008**, *47*, 8872–8875; *Angew. Chem.* **2008**, *120*, 9004–9007.
- [24] a) T. Itoh, K. Tokunaga, Y. Matsuda, I. Fujii, I. Abe, Y. Ebizuka, T. Kushiro, *Nat. Chem.* **2010**, *2*, 858–864; b) M. N. Heneghan, A. A. Yakasai, L. M. Halo, Z. S. Song, A. M. Bailey, T. J. Simpson, R. J. Cox, C. M. Lazarus, *ChemBioChem* **2010**, *11*, 1508–1512; c) Y. Matsuda, T. Awakawa, T. Wakimoto, I. Abe, *J. Am. Chem. Soc.* **2013**, *135*, 10962–10965; d) Y. Matsuda, T. Wakimoto, T. Awakawa, T. Mori, I. Abe, *J. Am. Chem. Soc.* **2014**, *136*, 15326–15336; e) Y. Matsuda, T. Iwabuchi, T. Wakimoto, T. Awakawa, I. Abe, *J. Am. Chem. Soc.* **2015**, *137*, 3393–3401.
- [25] Z. Wasil, K. A. K. Pahirulzaman, C. Butts, T. J. Simpson, C. M. Lazarus, R. J. Cox, *Chem. Sci.* **2013**, *4*, 3845–3856.
- [26] *Penicillium simplicissimum* and *P. janthinellum* were recently renamed as *P. ochrochloron* and *P. cremeogriseum*, respectively; see: C. M. Visagie, J. Houbraken, J. C. Frisvad, S. B. Hong, C. H. W. Klaassen, G. Perrone, K. A. Seifert, J. Varga, T. Yaguchi, R. A. Samson, *Stud. Mycol.* **2014**, *78*, 343–371.

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