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Precursor-directed Diversification of Cyclic Tetrapeptidic Pseudoxylallemycins

Huijuan Guo,[a] Alexander Schmidt,[a] Philipp Stephan,[a] Luka Raguž,[a] Daniel Braga,[a] Marcel Kaiser,[c,d] Hans-Martin Dahse,[a] Christiane Weigel,[a] Gerald Lackner,[a,b] and Christine Beemelmanns*[a]

Abstract: Cyclic peptides containing non-proteinogenic amino acids often exhibit a broad bioactivity spectrum and many have entered clinical trials with good prospects for drug development. We recently reported the discovery of six cyclic tetrapeptides, the pseudoxylallemycins A–F (**1**–**6**), from a termite-associated *Pseudoxylaria* sp. X802. These compounds contain a rare *O*-homoallenyl-L-tyrosine moiety and showed promising antimicrobial activity against the Gram-negative pathogenic bacterium *Pseudomonas aeruginosa*. To perform more detailed structure-activity studies, we pursued a precursor-directed diversification strategy. Here, we report the purification, identification and testing of 21 new pseudoxylallemycin derivatives.

Introduction

Nonribosomally synthesized peptides are amongst the most widespread secondary metabolites in nature, with many possessing bioactivities that can be exploited for therapeutic applications.[1] They are biosynthesized by large, modular enzymes known as nonribosomal peptide synthetases (NRPS),[2] which incorporate proteinogenic as well as many non-proteinogenic amino acids.[3] Many NRPS-derived natural products are further enzymatically transformed by methylation, acylation or glycosylation.[1-4] The NRPS-based biosynthetic machinery frequently reveals a certain degree of promiscuity with respect to the accepted substrate scope resulting in the formation of several derivatives of the same compound family. The biosynthetic flexibility is proposed to provide a certain degree of evolutionary advantage to the producing organism,[1-4] as exemplified in the nonribosomal peptide synthetases (NRPS) based bacterial xenematide diversification strategy (*Xenorhabdus nematophila*).[5]

Fungi are known producers of a broad array of structurally diverse NRPS-derived natural products,[6] which include immunosuppressant cyclosporine from *Tolypocladium inflatum*,[7] insecticidal destruxin from *Metarhizium anisopliae*,[8] xyloallenolide A containing an aromatic allenic ether moiety from a mangrove-associated *Xylaria* sp. 2508,[9] as well as insecticidal and antitumor bassianolide and beauvericin from *Beauveria bassiana*.[10,11] We recently reported the isolation of the antibacterial tetracyclic peptides, named pseudoxylallemycins A–F (**1**–**6**), from the stowaway fungus *Pseudoxylaria* sp. X802 of fungus-growing termites (Figure **1**).[12,13]

Although many of those NRPS-derived natural products exhibit pharmacologically important activities, most compounds do not possess desirable selectivity and pharmacokinetic properties. To generate a broad substrate library for further biological testing, different approaches including labour-intensive (semi)synthesis or the re-engineering of NRPS variants have been applied. An alternative approach is to make use of the intrinsic substrate promiscuity of the underlying biosynthetic machinery, i.e. precursor-directed biosynthesis.[14-16]

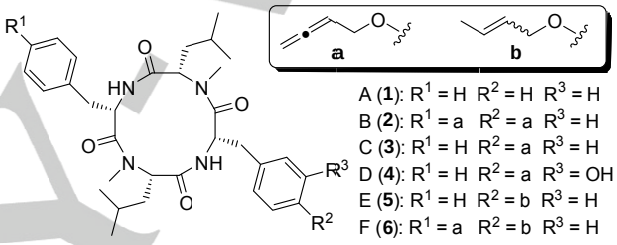

Figure 1. Structures of pseudoxylallemycins A–F (**1**–**6**).

In light of our recent discovery, we became particularly interested in the simultaneous formation of NRPS-derived pseudoxylallemycin A (**1**), B (**2**) and C (**3**) (Figure 1), all three exhibiting promising activity against Gram-negative pathogenic bacteria. Pseudoxylallemycin A (**1**) is a symmetric tetrapeptide containing two L-Phe-*N*Me-L-Leu units and is the most dominant tetrapeptide produced by X802. In contrast, derivative B (**2**) consists of two *O*-homoallenyl-L-Tyr-*N*Me-L-Leu units and is only produced in minor amounts, depending on the applied culture conditions. Currently, we hypothesize that pseudoxylallemycins are biosynthesized by an iterative NRPS containing one A-domain specific for an aliphatic amino acid (Leu) and one A-domain for aromatic amino acids (*O*-alkyl Tyr, Phe) respectively.[17] To understand the underlying amino acid specificity in detail, we applied a precursor-directed biosynthesis approach and tested the incorporation of differently substituted - synthetic and commercial - amino acids into the tetrapeptidic core structure.

The results of our precursor-directed biosynthesis experiments indicate promiscuous NRPS machinery that is able to tolerate more than ten different *para*-substituted aromatic amino acid derivatives. Here, we report the isolation of 21 new pseudoxylallemycin derivatives and the evaluation of their antimicrobial, antiparasitic, and cytotoxicity activity.

[a] Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Beutenbergstraße 11a, D-07745 Jena, Germany, Christine.beemelmanns@hki-jena.de
[b] Friedrich-Schiller University, Jena, Germany
[c] Dr. Marcel Kaiser, Parasite Chemotherapy Unit, Swiss Tropical and Public Health Institute, Socinstraße 57, 4002 Basel, Switzerland.
[d] University of Basel, Petersplatz 1, 4003 Basel, Switzerland.

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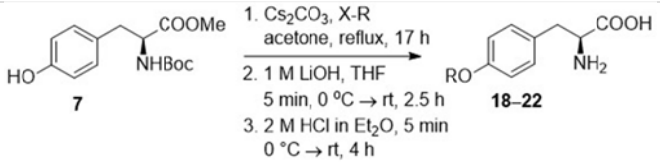
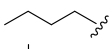
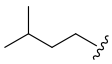
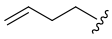
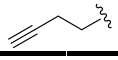
Results and Discussion

Precursor-directed Diversification

For subsequent cultivation experiments we first synthesized *O*-alkylated tyrosine derivatives **18**–**22** using a Williamson ether protocol (Table 1).[18] In short, Boc-L-Tyr(OH)-OMe (**7**) was deprotonated using Cs_2CO_3 and reacted with the respective alkyl halides under reflux (Method A) or subjected to Mitsunobu reaction conditions (Method B: 1-butinol, DIAD, PPh_3, see Supporting Information).[19] Subsequent saponification followed by acidic Boc-deprotection afforded the desired modified amino acids (**18**–**22**) in overall moderate to good yields (30%–80%).

Table 1. General synthetic procedure for preparation of L-tyrosinyl ethers (**18**–**22**).



entry	R	method[a]	product	Yield[b]
1		A	**18**	80%
2		A	**19**	83%
3		A	**20**	29%
4		A	**21**	69%
5		B	**22**	30%

[a] Method A: 1. Cs_2CO_3, R-X, reflux, 17 h; 2. 1 M LiOH, 0 °C to rt, 2.5 h; 3. 2 M HCl, 0 °C to rt, 4.0 h. Method B: 1. DIAD, PPh_3, 1-butinol, 0 °C, 30 min, then rt, o/n; 2. 10% LiOH, THF, rt 30 min; 3. HCl in Et_2O (4 M), 5 min, then HCl-gas, rt, o/n; [b] isolated yield over 3 steps. Isolated intermediates **8**–**17** not shown.

In a next step, we subjected the producing organism, *Pseudoxylaria* sp. X802 (from now on named X802) to cultivation conditions with and without the respective amino acids. In short, strain X802 was grown for 3 weeks on potato-dextrose-agar (PDA,6-well plates, 8 mL/well) containing 0.5 mM, 2.0 mM and 6.0 mM of the respective amino acid. Mycelium-covered agar was extracted using MeOH and the methanolic extracts were used to generate 10.0 mg/mL stock solutions for comparative UHPLC-MS and MS^2 analysis. Subsequently, MS^2 data was analysed using molecular networking GNPS[20] as pseudoxylallemycins were clearly detectable in a characteristic cluster arrangement (Figure S3).

As depicted Figure 2 and Figure 3, we detected the incorporation of almost all tested *O*-alkyl, *O*-alkenyl and *O*-alkyne-L-tyrosine derivatives into the pseudoxylallemycin backbone (**23**–**40**), except sterically demanding *O*-*tert*-butyl L-tyrosine and tyrosine itself (Supporting Information). In general, pseudoxylallemycin A (**1**) ((L-Phe-*N*Me-L-Leu)$_2$) was the most abundant tetracyclic peptide, followed by the non-symmetric pseudoxylallemycin C derivatives, in which one Phe residue is replaced by the supplemented modified amino acid.

Tyrosine derivatives **18**, **20** and **22** (Table 1) are suspected to be potential biosynthetic precursors of *O*-homoallenyl-L-Tyr (Figure 3), the aromatic amino acid building block of pseudoxylallemycins B–D (**2**–**4**). However, supplementation of **18** or **20** did not increase the production rates of allene-containing pseudoxylallemycins B–D (**2**–**4**) (Figure 2). Instead, the respective *O*-alkyne (**23**–**28**) and *O*-alkenyl-L-tyrosine-containing derivatives (**29**–**31**) were isolated (Figure 3, and Supporting Information). In contrast, supplementation of *O*-homopropargyl tyrosine derivative **22** to the fermentation broth of X802 significantly shifted the production rates towards the unsymmetric allene-containing pseudoxylallemycin C (**3**) (Figure 2). We then analysed if commercial *para*-substituted L-phenylalanine derivatives were incorporated into the tetrapeptide backbone. Again, LC-MS analysis of culture extracts clearly indicated the formation of 4-methyl-, 4-fluoro-, 4-chloro- and 4-bromo-substituted pseudoxylallemycins (**41**–**52**). However, no incorporation of 4-nitro and 4-amino-phenylalanine into the pseudoxylallemycin core structure was detectable by HRMS and MS^2, presumably as result of their electronic and cytotoxic properties (lower growth rates, decreased biomass formation) at higher millimolar concentrations.

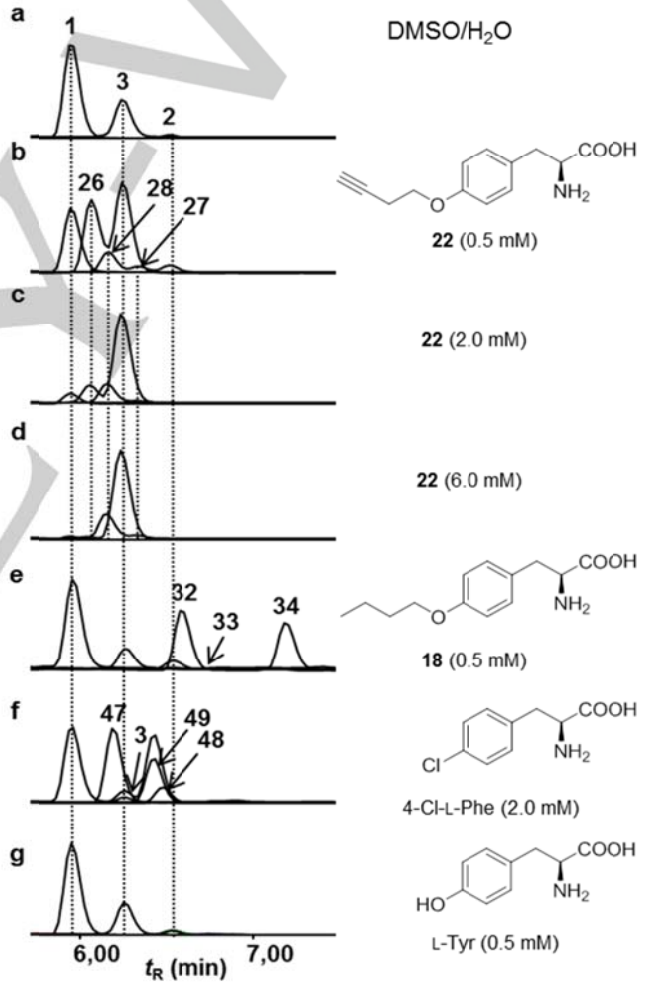


Figure 2. Comparative LC-MS analysis of culture extracts of strain X802 grown on solid media supplemented with: a) solvent control DMSO/H_2O; b) *O*-homopropargyl-L-Tyr **22** (0.5 mM); c) *O*-homopropargyl-L-Tyr **22** (2.0 mM); d) *O*-homopropargyl-L-Tyr **22** (6.0 mM); e) *O*-butyl-L-Tyr **18** (0.5 mM); f) 4-Cl-L-Phe (2.0 mM); g) L-Tyr (0.5 mM) (Y-axis represents the relative intensity of selected ion).

To test the promiscuity of the adenylation domain recognizing aliphatic amino acids (Leu), we also supplemented growth

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medium with L-allylglycine, L-propargylglycine, *N*-methyl-L-valine and *N*-methyl-L-alanine (Table S2 and Figure S2). Subsequent LC-MS analysis revealed only trace amounts of three possible *N*Me-L-valine derivatives (**53**–**55**), but no incorporation of allyl or propargylglycine was observed (Figure S2).

Isolation of pseudoxylallemycins G1–M3

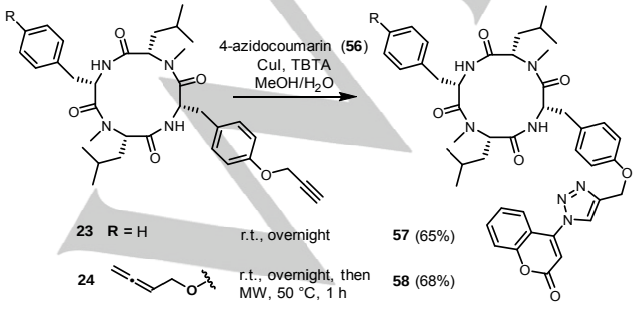
To pursue the isolation and characterization of the most prominently formed pseudoxylallemycin derivatives, we performed a large-scale cultivation of X802 on PDA (5 L) supplemented with 2 mM (or 0.5 mM) of the respective amino acid precursors (*O*-methyl-, *O*-homoallyl- (**20**), *O*-propargyl- (**21**), *O*-homopropargyl-L-tyrosine (**22**), 4-methyl-, 4-chloro- and 4-bromo-phenylalanine). Again, X802 was cultivated for four weeks until the agar was fully covered with mycelium. Agar pieces were extracted twice using MeOH and concentrated extracts were purified using pre-activated SPE-C18 cartridges.[12] Subsequent Sephadex LH20 and semipreparative HPLC purification resulted in the isolation of the corresponding pseudoxylallemycins. Overall, 21 new derivatives obtained in 0.2–2.0 mg were fully characterized using comparative NMR and ESI-HRMS/MS analysis (Figure 3, Supporting Information).

Bioactivities

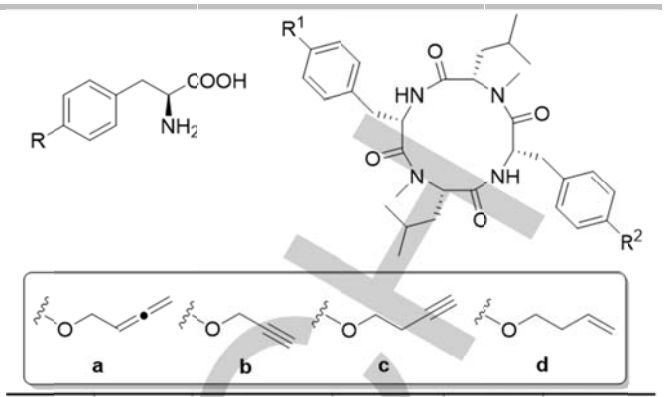
Subsequently, we evaluated the cytotoxicity, antibacterial and antiparasitic activity of our extended compound library. Similar to previous reports,[12] pseudoxylallemycins G1–M3 (**23**–**31**, **38**–**43**, and **47**–**52**) showed moderate inhibitory activity against *Pseudomonas aeruginosa* (MIC ~ 12.5 µg/mL) and towards human umbilical vein endothelial cells HUVEC independently of the introduced side chain modifications (GI_{50} 9–15 µg/mL; Table S33). As part of our bioactivity screening efforts pseudoxylallemycins A–C (**1**–**3**) were also subjected to antiparasitic activity assays. Overall, pseudoxylallemycin B (**2**) and C (**3**) showed antiparasitic, but due to their cytotoxic properties, unselective activity against *Trypanosoma brucei rhodesiense* (Table S34).

Chemical Modifications

The observed anti-microbial activity prompted us to synthesize conjugates **57** and **58** containing a fluorophore tag to be able to pursue potential mode of action studies. First, pseudoxylallemycin G1–G2 (**23**–**24**) was subjected to click chemistry conditions[21] and coumarin fluorophore (**57**) to yield derivatives **57** and **58** (Scheme 1). It is interesting to note that compounds, such as **24** and **58**, are bifunctional molecules that are suitable for further in vivo modifications and target identification.[22]



Scheme 1. Chemical modification of pseudoxylallemycins G1–G2 (**23**–**24**).



Entry	R	Additive	R^1	R^2	Nr
1	**a**	control	-H	-H	A (**1**)
			a	**a**	B (**2**)
			-H	**a**	C (**3**)
2	**b**	21	-H	**b**	G1 (**23**)
			a	**b**	G2 (**24**)
			b	**b**	G3 (**25**)
3	**c**	22	-H	**c**	H1 (**26**)
			a	**c**	H2 (**27**)
			c	**c**	H3 (**28**)
4	**d**	20	-H	**d**	I1 (**29**)
			a	**d**	I2 (**30**)
			d	**d**	I3 (**31**)
5	$-OCH_3$	OMe-L-Tyr	-H	$-OCH_3$	J1 (**38**)
			a	$-OCH_3$	J2 (**39**)
			$-OCH_3$	$-OCH_3$	J3 (**40**)
6	$-CH_3$	4-Me-L-Phe	-H	$-CH_3$	K1 (**41**)
			a	$-CH_3$	K2 (**42**)
			$-CH_3$	$-CH_3$	K3 (**43**)
7	-Cl	4-Cl-L-Phe	-H	-Cl	L1 (**47**)
			a	-Cl	L2 (**48**)
			-Cl	-Cl	L3 (**49**)
8	-Br	4-Br-L-Phe	-H	-Br	M1 (**50**)
			a	-Br	M2 (**51**)
			-Br	-Br	M3 (**52**)

Figure 3. Newly isolated and characterized pseudoxylallemycins produced by X802 using a precursor-directed biosynthesis strategy.

Conclusions

Pseudoxylallemycins are presumably of NRPS origin that contains two A-domains of different promiscuity.[17] Supplementation of either *O*-modified tyrosine or *para*-substituted phenylalanine derivatives to the fermentation broth of X802 resulted in the incorporation of the unnatural amino acid into the pseudoxylallemycin core structure. In all cases, the simultaneous presence of new pseudoxylallemycin derivatives

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and previously reported derivatives **1**–**4** in culture extracts suggest a certain degree of amino acid concentration dependency on pseudoxylallemycin formation. In total, 21 new derivatives were isolated and characterized. Subsequent bioactivity studies of isolated derivatives indicated structure-independent inhibitory activities against the bacterial Gram-negative pathogen *Pseudomonas aeruginosa*. Furthermore, pseudoxylallemycins can be chemically functionalized using click chemistry, modifications that can be used for future target identification. Future work is now dedicated towards understanding the biosynthetic pathway of both, the allenic moiety and pseudoxylallemycins, in greater detail to enable future engineering of this intriguing pathway.

Experimental Section

Supplementary Information (SI) available: fermentation procedures; isolation procedures, ESI-HRMS, ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra as well as chemical modifications.

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Keywords: natural products • non-ribosomal peptide synthase • allenes • bioactivities • amino acids

[1] a) R. Finking, M. A. Marahiel, *Annu. Rev. Microbiol*. **2004**, *58*, 453; b) E. A. Felnagle, E. E. Jackson, Y. A. Chan, A. M. Podevels, A. D. Berti, M. D. McMahon, M. G. Thomas, *Mol. Pharmaceutics* **2008**, 5, 191; c) C. T. Walsh. *Nat Prod Rep*. **2016**, *33*, 127; d) R. D. Süssmuth, A. Mainz, *Angew. Chem. Int. Ed.* **2017**, *56*, 3770.

[2] a) M. A. Fischbach, C. T. Walsh, *Chem. Rev.* **2006**, *106*, 3468; b) K. J. Weissman, *Nat. Chem. Biol*. **2015**, *11*, 660; c) K. Bloudoff, T. M. Schmeing, *Biochim Biophys Acta*. **2017**, *1865*, 1587.

[3] C. T. Walsh, R. V. O'Brien, C. Khosla, *Angew. Chem. Int. Ed.* **2013**, *52*, 7098.

[4] a) M. A. Fischbach, J. Clardy, *Nat. Chem. Biol.* **2007**, *3*, 353; b) H. Wang, D. P. Fewer, L. Holm, L. Rouhiainen, K. Sivonen, *Proc. Natl. Acad. Sci.* **2014**, *111*, 9259–9264.

[5] J. M. Crawford, C. Portmann, R. Kontnik, C. T. Walsh, J. Clardy, *Org. Lett.* **2011**, *13*, 5144.

[6] X. Wang, M. Lin, D. Yu, D. Lai, L. Zhou, *Molecules* **2017**, *22*, 2069.

[7] D. M. Canafax, N. L. Ascher, *Clin. Pharm.* **1983**, *2*, 515.

[8] K. Sowjanya Sree, V. Padmaja, Y. L. Murthy, *Pest Manag. Sci.* **2008**, *64*, 119.

[9] S. Wang, Z. Xu, W. Mao, Z. She, N. Tan, C. Li, Y. Lin. *Nat. Prod. Res.* **2008**, *22*, 612.

[10] Y. Fan, X. Liu, N. O. Keyhani, G. Tang, Y. Pei, W. Zhang, S. Tong, *Proc. Natl. Acad. Sci. U S A*. **2017**, *114*, E1578.

[11] S. E. Helaly, B. Thongbai, M. Stadler, *Nat. Prod. Rep.* **2018**, DOI: 10.1039/c8np00010g

[12] H. Guo, N. B. Kreuzenbeck, S. Otani, M. Garcia-Altares, H. M. Dahse, C. Weigel, D. K. Aanen, C. Hertweck, M. Poulsen, C. Beemelmanns, *Org. Lett.* **2016**, *18*, 3338.

[13] a) S. Yan, S. Li, W. Wu, F. Zhao, L. Bao, R. Ding, H. Gao, H. A. Wen, F. Song, H. W. Liu, *Chem. Biodivers.* **2011**, *8*, 1689; b) F. Song, S. Wu, Y. Zhai, Q. Xuan, T. Wang, *Chem. Biodivers.* **2014**, *11*, 673.

[14] R. Traber, H. Hofmann, H. Kobel, *J. Antibiot.* **1989**, *42*, 591.

[15] H. Kries, R. Wachtel, A. Pabst, B. Wanner, D. Niquille, D. Hilvert, *Angew. Chem. Int. Ed.* **2014**, *53*, 10105.

[16] X. Wang, M. Lin, D. Yu, D. Lai, L. Zhou, *Molecules* **2017**, *22*, 2069.

[17] a) C. Steiniger, S. Hoffmann, A. Mainz, M. Kaiser, K. Voigt, V. Meyer, R. D. Süssmuth, *Chem. Sci.* **2017**, *8*, 7834; b) D. Yu, F. Xu, S. Zhang, J. Zhan, *Nat. Commun.* **2017**, *8*, 15349.

[18] S. L. Solar, R. R. Schumaker, *J. Org. Chem.* **1966**, *31*, 1996.

[19] O. Mitsunobu, M. Yamada, *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2380.

[20] a) J. Watrous, P. Roach, T. Alexandrov, B. S. Heath, J. Y. Yang, R. D. Kersten, M. van der Voort, K. Pogliano, H. Gross, J. M. Raaijmakers, B. S. Moore, J. Laskin, N. Bandeira, P. C. Dorrestein, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, E1743; b) M. Wang, J. J. Carver, V. V. Phelan, L. M. Sanchez, N. Garg, Y. Peng, D. D. Nguyen, J. Watrous, C. A. Kapono, T. Luzzatto-Knaan, C. Porto, A. Bouslimani, A. V. Melnik, M. J. Meehan, W.-T. Liu, M. Crüsemann, P. D. Boudreau, E. Esquenazi, M. Sandoval-Calderón, R. D. Kersten, L. A. Pace, R. A. Quinn, K. R. Duncan, C.-C. Hsu, D. J. Floros, R. G. Gavilan, K. Kleigrewe, T. Northen, R. J. Dutton, D. Parrot, E. E. Carlson, B. Aigle, C. F. Michelsen, L. Jelsbak, C. Sohlenkamp, P. Pevzner, A. Edlund, J. McLean, J. Piel, B. T. Murphy, L. Gerwick, C.-C. Liaw, Y.-L. Yang, H.-U. Humpf, M. Maansson, R. A. Keyzers, A. C. Sims, A. R. Johnson, A. M. Sidebottom, B. E. Sedio, A. Klitgaard, C. B. Larson, C. A. Boya P, D. Torres-Mendoza, D. J. Gonzalez, D. B. Silva, L. M. Marques, D. P. Demarque, E. Pociute, E. C. O'Neill, E. Briand, E. J. N. Helfrich, E. A. Granatosky, E. Glukhov, F. Ryffel, H. Houson, H. Mohimani, J. J. Kharbush, Y. Zeng, J. A. Vorholt, K. L. Kurita, P. Charusanti, K. L. McPhail, K. F. Nielsen, L. Vuong, M. Elfeki, M. F. Traxler, N. Engene, N. Koyama, O. B. Vining, R. Baric, R. R. Silva, S. J. Mascuch, S. Tomasi, S. Jenkins, V. Macherla, T. Hoffman, V. Agarwal, P. G. Williams, J. Dai, R. Neupane, J. Gurr, A. M. C. Rodríguez, A. Lamsa, C. Zhang, K. Dorrestein, B. M. Duggan, J. Almaliti, P.-M. Allard, P. Phapale, L.-F. Nothias, T. Alexandrov, M. Litaudon, J.-L. Wolfender, J. E. Kyle, T. O. Metz, T. Peryea, D.-T. Nguyen, D. VanLeer, P. Shinn, A. Jadhav, R. Müller, K. M. Waters, W. Shi, X. Liu, L. Zhang, R. Knight, P. R. Jensen, B. Ø Palsson, K. Pogliano, R. G. Linington, M. Gutiérrez, N. P. Lopes, W. H. Gerwick, B. S. Moore, P. C. Dorrestein, N. Bandeira, *Nat. Biotechnol.* **2016**, *34*, 828.

[21] T. R. Chan, R. Hilgraf, K. B. Sharpless, V. V. Fokin, *Org. Lett.* **2004**, *6*, 2853.

[22] M. Winn, J. K. Fyans, Y. Zhuo, J. Micklefield, *Nat. Prod. Rep.* **2016**, *33*, 317.

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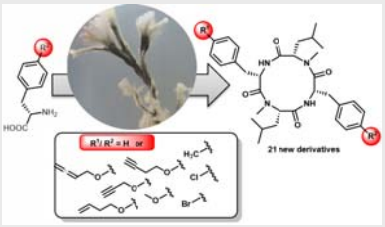
FULL PAPER

We report the identification of 21 new pseudoxylallemycin derivatives, the assessment of their antimicrobial activity and first studies towards their chemical derivatization.



*Huijuan Guo, Alexander Schmidt, Philipp Stephan, Luka Raguž, Daniel Braga, Marcel Kaiser, Hans-Martin Dahse, Christiane Weigel, Gerald Lackner, and Christine Beemelmanns**

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Precursor-directed Diversification of Cyclic Tetrapeptidic Pseudoxylallemycins

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