A Journal of the Gesellschaft Deutscher Chemiker

Angewandte International Edition Chemie

GDCh

www.angewandte.org

Accepted Article

Title: Total Synthesis and Structural Revision of the Antibiotic Tetrapeptide GE81112A

Authors: Gerrit Jürjens, Sören Manuel Michael Schuler, Michael Kurz, Sylvain Petit, Cédric Couturier, Frédéric Jeannot, Fabian Nguyen, Raffael Christoph Wende, Peter Eugen Hammann, Daniel N. Wilson, Eric Bacqué, Christoph Pöverlein, and Armin Bauer

To be cited as: *Angew. Chem. Int. Ed.* 10.1002/anie.201805901
Angew. Chem. 10.1002/ange.201805901

Link to VoR: http://dx.doi.org/10.1002/anie.201805901
http://dx.doi.org/10.1002/ange.201805901

WILEY-VCH

10.1002/anie.201805901

WILEY-VCH

Total Synthesis and Structural Revision of the Antibiotic Tetrapeptide GE81112A

Gerrit Jürjens,[b,c] Sören M. M. Schuler,[b] Michael Kurz,[d] Sylvain Petit,[e] Cédric Couturier,[e] Frédéric Jeannot,[e] Fabian Nguyen,[f] Raffael C. Wende,[b] Peter E. Hammann,[a] Daniel N. Wilson,[f] Eric Bacqué,[e] Christoph Pöverlein,*[d] and Armin Bauer*[a]

Abstract: The total synthesis of the naturally occurring antibiotic GE81112A, a densely functionalized tetrapeptide is reported. Comparison of spectral data with the natural product and lack of biological activity of the synthesized compound led us to revise the published configuration of the 3-hydroxypipecolic acid moiety. This hypothesis was fully validated by the synthesis of the corresponding epimer.

The world-wide problem of emergence of multi-drug resistant bacteria is compounded by the decline in new classes of antimicrobials entering into clinical practice. This has led to a renewed interest in novel scaffolds for the design of anti-microbial agents.[1] Such a novel chemical structure is represented by the GE81112 compounds which are produced by a *Streptomyces* sp.[2] GE81112 comprises three unusual tetrapeptide congeners (**1**-**3**, Figure 1) that display activity against a panel of both Gram-negative and Gram-positive pathogenic bacteria.[2,3] GE81112 inhibits protein synthesis by interacting with the small 30S ribosomal subunit using a binding site distinct from that of other known ribosome-targeting antibiotics.[2,4] The GE81112 antibiotics are virtually inactive on *E. coli* in standard antibacterial assays and display their activity against *E. coli* only in minimal media due to the mechanism of uptake of the GE81112 congeners into the bacteria by illicit transport through the oligopeptide permease (Opp) system.[3]

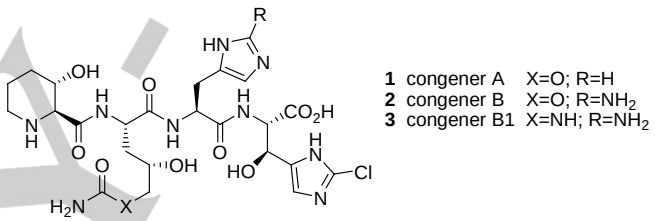


Figure 1. Reported structures of the GE81112 tetrapeptide congeners.

The isolation and characterization of GE81112 was published by Brandi *et al*. in 2006.[2] Subsequently, the biosynthesis of GE81112 was elucidated and the absolute configuration was postulated based on a combination of biosynthetic and chemical studies.[5] The tetrapeptide congeners consist of at least three unnatural amino acids: a *trans*-configured 3-hydroxypipecolic acid, a 2-amino-4-hydroxypentanoic acid bearing either a carbamate or urea moiety and a chloro-hydroxyhistidine. Furthermore, in two of the three congeners the histidine is replaced by an amino-histidine.

The unique binding mode, together with the activity of GE81112 on *E. coli*, attracted our attention to this tetrapeptide family as a promising starting point for a total synthesis-based approach towards the generation of new lead structures with the potential to treat drug-resistant Gram-negative bacteria.

Herein we report the first total synthesis of GE81112A. Moreover, based on analytical and biological activity data the *cis*-configuration of the hydroxypipecolic acid was proven.

[a] Dr. A. Bauer, Prof. Dr. P. E. Hammann
R&D Therapeutic Area Infectious Diseases
Sanofi-Aventis Deutschland GmbH
Industriepark Höchst, 65926 Frankfurt am Main, Germany
E-mail: Armin.Bauer@sanofi.com

[b] Dr. G. Jürjens, Dr. S. M. M. Schuler, Dr. R. C. Wende
Fraunhofer IME, Projektgruppe Bioressourcen
Winchesterstraße 2, 35394 Gießen, Germany

[c] Helmholtz Zentrum München, Institut für Medizinalchemie
Schneiderberg 1b, 30167 Hannover, Germany

[d] Dr. M. Kurz, Dr. C. Pöverlein
R&D Integrated Drug Discovery
Sanofi-Aventis Deutschland GmbH
Industriepark Höchst, 65926 Frankfurt am Main, Germany
E-mail: Christoph.Poeverlein@sanofi.com

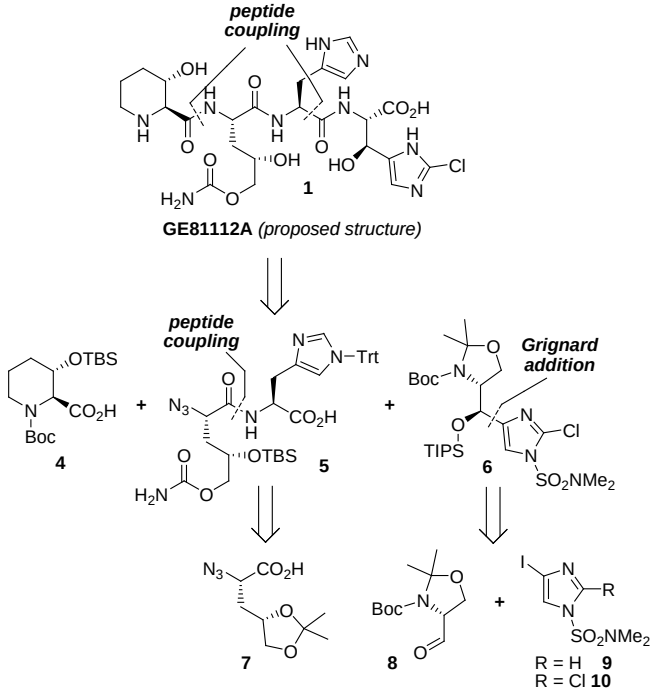
[e] Dr. S. Petit,[#] Dr. F. Jeannot, Dr. C. Couturier, Dr. E. Bacqué
Therapeutic Area Infectious Diseases
Sanofi R&D
1541, Avenue Marcel Mérieux, 69280 Marcy L'Etoile, France

[f] F. Nguyen,[+] Prof. Dr. D. N. Wilson[+]
Gene Center, Department for Biochemistry and Center for Protein Science Munich (CiPSM)
Ludwig-Maximilians-Universität
Feodor-Lynen-Str. 25, 81375 München, Germany

[+] present address:
Institut für Biochemie und Molekularbiologie
Universität Hamburg
Martin-Luther-King-Platz 6, 20146 Hamburg, Germany

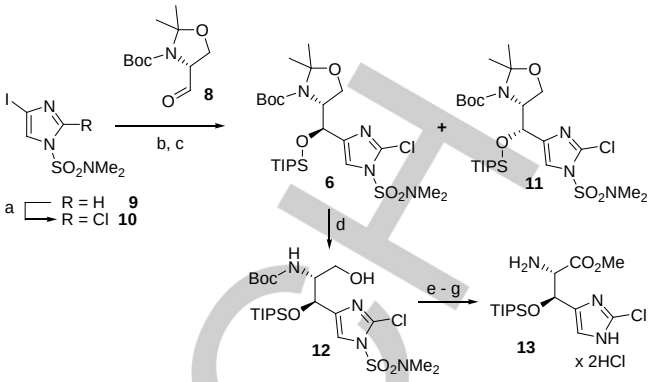
[#] present address:
UCB Pharma SA,
Chemin du Foriest, 1420 Braine-L'Alleud, Belgium

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



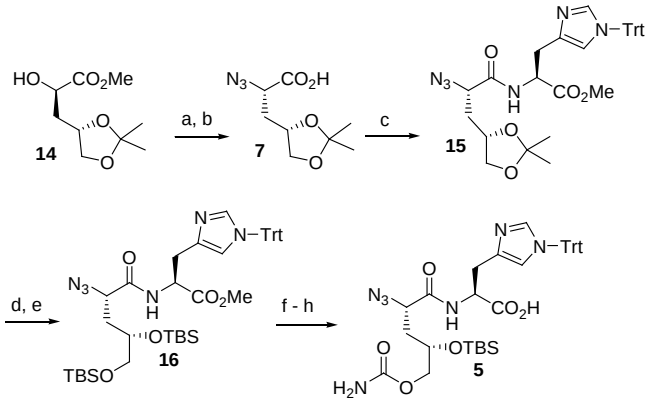
Scheme 1. Retrosynthetic analysis of GE81112A.

Our retrosynthetic analysis regarding GE81112A **1** disconnected tetrapeptide **1** between the first and the third of the three amide bonds to obtain protected 3-hydroxypipecolic acid **4**, central dipeptide **5**, and precursor **6** for the chlorinated 3-hydroxyhistidine (Scheme 1). 3-Hydroxypipecolic acid derivatives like **4** are accessible through an olefin ring-closing metathesis approach starting from protected *N*-allyl glycine.[6] Dipeptide **5** could be assembled from a chiral α-azido acid **7** derived from D-xylose and the commercially available trityl protected L-histidine methyl ester. Using dipeptide **5** with a masked amino function was designed to circumvent two possible obstacles in a putative linear sequence: firstly, formation of a 2,5-diketopiperazine, that could be formed during carbamate deprotection of the dipeptide consisting of chlorinated 3-hydroxyhistidine methyl ester and a carbamate protected histidine[7] and secondly, tendency of γ-hydroxy carboxylic acid or esters to form the corresponding γ-lactone, which is prevented by the presence of the amide.[8] The only price to pay is the risk of α-epimerisation during activation of dipeptide **5** – a known phenomenon for τ-protected and π-unprotected histidines.[9] Chlorinated 3-hydroxyhistidine methyl ester **13** was envisioned to be available by the addition of an anion derived from a 4-iodo imidazole like **9** or **10** to Garner's aldehyde (**8**). Both 3-hydroxy epimers should be accessible, which might be useful since the absolute configuration at that position was uncertain.



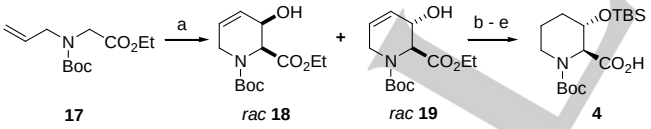
Scheme 2. Synthesis of the histidine derivative **13**. Reagents and conditions: a) LDA, THF, −78 °C, 90 min, then C_2Cl_6, −78 °C, 45 min, then rt, 4 h, 90%; b) **10**, EtMgBr, CH_2Cl_2, 0 °C, 20 min, then **8**, −60°C to −30°C, 4 h, 73%; c) TIPSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 2 h, 48% of **6** and 29% of **11**; d) **6**, $BiBr_3$, H_2O, MeCN, 0 °C, 20 min, 66%; e) NaOCl (10% in H_2O), TEMPO, $NaClO_2$, MeCN, rt, 3 h; f) $TMSCHN_2$, CH_2Cl_2/MeOH, rt, 24 h, 63% over 2 steps; g) HCl in 1,4-dioxane, THF, rt, crude product directly used in the next step (cf. Scheme 3).

A key step of the synthesis of hydroxyhistidine **13** was the diastereoselective addition of a Grignard reagent prepared by halogen-metal exchange of a 4-iodo imidazole derivative to *R*-Boc-Garner's aldehyde **8** (Scheme 2). Our first approach was performed with **9**. Since anions generated at the imidazole 4(5)-position are known to be prone to equilibrate to the 2-position[10] we were aware that a careful selection of solvent and reaction conditions would be required in order to control the regioselectivity of the subsequent addition to the electrophile. We chose to apply Lindell's protocol[10] and performed the halogen-metal exchange in dichloromethane as a non-complexing solvent. Although in some cases we were able to obtain the desired addition products with *R*-Boc-Garner's aldehyde **8**[11] at the 4-position (which could be further elaborated to the key intermediate **6** after TIPS protection and chlorination at the 2-position) the reaction suffered from poor reproducibility and addition at the 2-position was observed frequently (see Supporting Information). Therefore, the hitherto undisclosed imidazole **10** with a blocked 2-position was prepared from **9** by regioselective chlorination and subsequently used. Halogen-metal exchange of **10** with EtMgBr in dichloromethane proceeded smoothly without any equilibration to the 2-position but addition to *R*-Boc-Garner's aldehyde **8**[11] under Cram-chelation control gave an inseparable ~2:1 mixture of addition products in favor of the required *syn* diastereomer. Both epimers **6** and **11** could be separated after silylation of the hydroxyl group. The acetonide was then cleaved using bismuth bromide.[12] This deprotection step required careful reaction monitoring since partial removal of the TIPS group was also observed. The resulting primary alcohol **12** was oxidized in a two-step sequence and the so formed acid was protected as the methyl ester. Simultaneous deprotection of both nitrogen atoms with hydrogen chloride in 1,4-dioxane resulted in hydrochloride **13** that was used directly in the coupling reaction with dipeptide **5**.



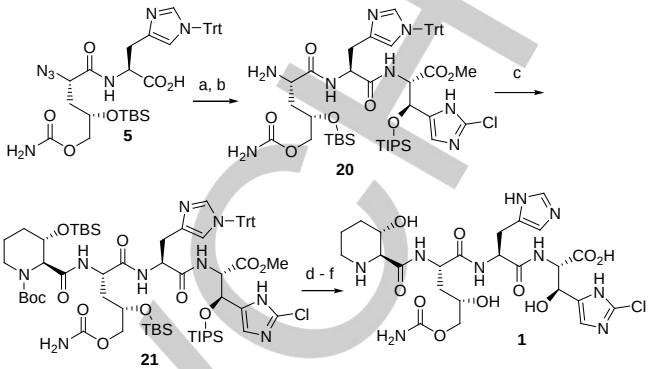
Scheme 3. Synthesis of dipeptide **5**. Reagents and conditions: a) PPh_3, DPPA, DEAD, THF, −78 °C to rt, 3 h; b) LiOH, THF/H_2O, rt, 4 h; c) H-His(1-Trt)-OMe·HCl, Oxyma, EDCI·HCl, $NaHCO_3$, DCM/DMF (1:1), −15 °C to rt, 18 h, 87% over 3 steps; d) HOAc/HCl (9:1), 0 °C, 3.5 h; e) TBSOTf, 2,6-lutidine, DCM, −78 °C to rt, on, 95% over 2 steps; f) HF·pyridine (70%), pyridine, THF, 0 °C to rt, 40 h, 45%; g) CDI, THF, rt, 3 h, then conc. NH_3, rt, 2 h, 82%; h) LiOH, THF/H_2O, rt, 1 h, 97%.

The synthesis of dipeptide **5** (Scheme 3) started with alcohol **14**, an intermediate obtained starting with D-xylose according to a known procedure.[13] Alcohol **14** was transformed to the corresponding azide under Mitsunobu conditions. Saponification yielded acid **7**, that was then coupled with trityl-protected L-histidine methyl ester to furnish dipeptide **15**. Selective acetonide deprotection while retaining the trityl protecting group using 1N hydrogen chloride in acetic acid and careful work-up followed by double TBS-protection and selective deprotection of the primary TBS group with HF-pyridine complex in THF or NH_4F in methanol resulted in a mono-protected diol. The corresponding primary alcohol was transformed into a carbamate by sequential treatment with CDI and aqueous ammonia. Finally, saponification of the methyl ester furnished the central intermediate **5**.



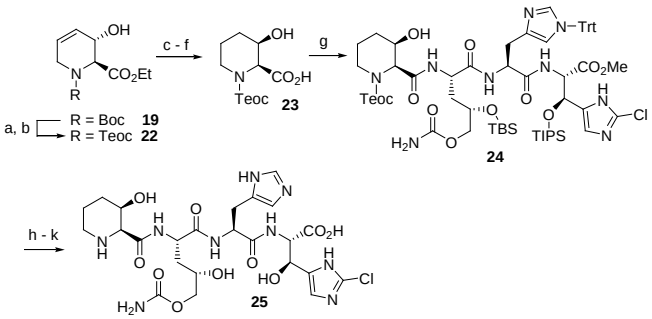
Scheme 4. Synthesis of *trans*-3-hydroxypipecolic acid **4**. Reagents and conditions: a) 2 steps, see ref [6]; b) chromatographic separation on Chiralpak™ AD-H/148; c) H_2, Pd/C, EtOAc, rt, on; d) TBSCl, imidazole, DMF, 40 °C, 2 h; e) NaOH, MeOH/H_2O, rt, on, then 40 °C, 91% over 3 steps.

Since we planned to develop the structure-activity relationships in the GE81112 series a synthetic route was considered that would deliver all four stereoisomers of the 3-hydroxypipecolic acid (Scheme 4). Such an approach was disclosed by the Takahata group.[6] Thus, racemic tetrahydropyridine derivatives **18** and **19** (ratio *trans*:*cis*: 4:1) were prepared from protected glycine **17**,[14] as reported. The epimers were separated by chromatography utilizing a chiral column (in contrast to the reported lipase-catalyzed kinetic resolution[6]) and the corresponding 2*S* isomer of **19** was further converted to the required intermediate **4**.



Scheme 5. Synthesis of GE81112A (**1**, proposed structure). Reagents and conditions: a) **13**, Oxyma, EDCI·HCl, $NaHCO_3$, CH_2Cl_2/DMF (1:1), −40 °C to rt, 48 h, 73%; b) PMe_3, THF/H_2O (5:1), rt, 12 h; c) **4**, Oxyma, EDCI·HCl, $NaHCO_3$, CH_2Cl_2/DMF (1:1), 0 °C to rt, 12 h, 36% over 3 steps; d) LiOH, MeOH/H_2O, rt, 7 d; e) TASF, H_2O/DMF, rt, 7 d, 62% over 2 steps; f) TFA, CH_2Cl_2, 0 °C, 1 h; g) HCOOH, 2,2,2-trifluoroethanol, rt, 13 h, 28% over 2 steps.

For the final assembly of GE81112A (Scheme 5) the central building block **5** was coupled with the chloro-histidine ester **13** in good yields (73%) albeit some racemization (16%) was observed. Staudinger reduction furnished the tripeptide **20** that was subsequently coupled with 3-hydroxypipecolic acid **4** to give the GE81112A precursor **21**. Long reaction times were needed and unsatisfactory yields were observed, presumably due to the high steric hindrance caused by the protecting groups of **4** resulting in attenuated reactivity in the active ester formation from **4** during the coupling step. Although the deprotection to **1** followed a carefully concerted sequence it was met with difficulties and we experienced considerable loss of material, i. a. methyl ester saponification and subsequent treatment with TASF to remove the silyl group required several days and partial decomposition was observed upon TFA-mediated Boc cleavage. Due to the high polarity of **1**, a pure batch could finally only be obtained by subsequent Hydrophilic Interaction Chromatography (HILIC) and RP-HPLC steps (see Supporting Information). However, compared to the reported data[2,3] of the isolated natural product **NP**, our synthesized product **1** showed significant differences in the NMR spectra shifts of the pipecolinic acid moiety signals (Table 1) and furthermore did not display any activity in antibacterial assays (Table 2) nor in an *E. coli* lysate-based transcription-translation assay[15] (Figure 2). With these data in hand, we reasoned that the stereochemistry of the pipecolinic acid was assigned wrongly. Since no epimerization domain in the biosynthetic cluster of GE81112A had been detected[5] it seemed likely that the absolute configuration of the secondary hydroxyl group was assigned incorrectly. This hypothesis was also underlined by results from additional biochemical studies with the putative pipecolic acid hydroxylase GeF.[16]



Scheme 6. Synthesis of GE81112A (**25**, revised structure). Reagents and conditions: a) HCl in 1,4-dioxane, rt, 45 min; b) Teoc-OSu, NEt_3, 1,4-dioxane/H_2O (1:1), rt, on, 81% over 2 steps; c) AcOH, DEAD (in PhMe), PPh_3, THF, 0 °C to rt, 6 h, 56%; d) LiOH, MeOH/H_2O, −30 °C, 15 min, 59%; e) LiOH, THF/H_2O, −30 °C to rt, 24 h, 84%; f) H_2, Pd/C, EtOAc, rt, on; 99%; g) **20**, Oxyma™, EDCI·HCl, $NaHCO_3$, CH_2Cl_2/DMF (1:1), 0 °C to rt, 16 h, 41%; h) LiOH, THF/H_2O, rt, 40%; i) TASF (in DMF), H_2O/DMF, rt, 42 h; k) HCOOH, 2,2,2-trifluoroethanol, rt, 24 h, 78% over 2 steps.

Therefore, we set out to synthesize the corresponding tetrapeptide with the *cis*-hydroxypipecolic acid incorporated (Scheme 6). Due to the problems encountered with the final cleavage of the protecting groups in the synthesis of **1**, we switched from Boc to Teoc, a protecting group that was expected to be removable together with the other silyl protecting groups. Since silyl ethers of *cis*-3-hydroxy pipecolinic acids are known to be unstable[17] we utilized the unprotected compound **23** in the coupling step. Although the *cis* isomer of **4** would be accessible via **18**, we also designed an alternative route from the major diastereomer **19** employing a Mitsunobu inversion. The final steps to the revised GE81112A structure **25** were performed in analogy to the synthesis of **1** under optimized conditions and furnished **25** in decent yields.

Not only the NMR data of **25** (Table 1), but also the biological activity pattern (Table 2 and Figure 2) matched completely with those of the natural product **NP**[2] (see Supporting Information for full NMR spectral data).

Table 1, NMR shifts (ppm) of the pipecolic acid moieties in the natural product isolate (NP; literature data)[2], **1** and, **25**.

	^{1}H **1**	^{1}H **NP**	^{1}H **25**	^{13}C **1**	^{13}C **NP**	^{13}C **25**
NH_2^+	9.30	9.07	9.02	-	-	-
NH_2^+	8.85	8.30	8.30	-	-	-
α	3.55	3.94	3.95	61.2	59.7	59.7
β	3.70	4.40	4.40	66.7	64.1	64.1
γ1	2.00	1.81	1.82	31.0	28.4	28.4
γ2	1.46	1.62	1.64	-	-	-
δ1	1.81	1.88	1.89	19.9	15.9	15.9
δ2	1.69	1.52	1.53	-	-	-
ε1	3.18	3.15	3.16	42.6	42.9	42.9
ε2	2.87	2.90	2.91	-	-	-

Table 2. Comparison of the biological profiles of the synthetic GE81112As **1** and **25** with literature data of the natural product isolate **NP**[2].

	MIC [µg mL^{-1}]		
	E. coli	***E. coli*** **(minimal media)**	***M. catarrhalis***
NP	> 512	0.06	2
1	> 64	> 64	> 64
25	> 64	< 0.03	16

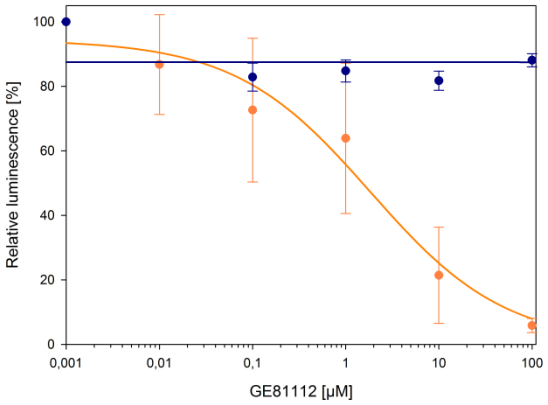


Figure 2. Translation inhibition by **1** (blue) or **25** (orange) of firefly luciferase production using an *E. coli in vitro*-coupled transcription-translation system.

In conclusion we have accomplished the first total synthesis of the proposed structure (**1**)[5] and the revised structure (**25**) of the densely functionalized tetrapeptide GE81112A with three unique amino acids. Our synthesis provides access to sufficient amounts of the natural product in order to pursue further investigations concerning its mode of action and its pharmacodynamic and pharmacokinetic properties. Our work also sets the stage for the establishment of structure-activity relationships and further lead optimization.

Acknowledgements

We thank Yakub Gungor, Silke Herok-Schöpper, Chiara Presenti and Thorsten Schäfer for technical assistance and Rolf Hirsch for performing antibacterial profiling.

Keywords: natural products • antibiotics • total synthesis • structural revision • peptides • non-ribosomal peptide synthesis

[1] G. D. Wright, *Nat. Prod. Rep.* **2017**, *34*, 694-701.

[2] L. Brandi, A. Lazzarini, L. Cavaletti, M. Abbondi, E. Corti, I. Ciciliato, L. Gastaldo, A. Marazzi, M. Feroggio, A. Fabbretti, A. Maio, L. Colombo, S. Donadio, F. Marinelli, D. Losi, C. O. Gualerzi, E. Selva, *Biochemistry* **2006**, *45*, 3692-3702.

[3] A. Maio, L. Brandi, S. Donadio, C. O. Gualerzi, *Antibiotics* **2016**, 5, 5020017/1-5020017/14.

[4] a) A. Fabbretti, A. Schedlbauer, L. Brandi, T. Kaminishi, A. M. Giuliodori, R. Garofalo, B. Ochoa-Lizarralde, C. Takemoto, S. Yokoyama, S. R. Connell, C. O. Gualerzi, P. Fucini, *Proc. Natl. Acad. Sci. USA* **2016**, *113*, E2286-E2295; b) J. P. López-Alonso, A. Fabbretti, T. Kaminishi, I. Iturrioz, L. Brandi, D. Gil-Carton, C. O. Gualerzi, P. Fucini, S. R. Connell, *Nucleic Acids Res.* **2017**, *45*, 2179-2187; c) D. N. Wilson, *Crit. Rev. Biochem. Mol. Biol.* **2009**, *44*, 393-433.

[5] T. M. Binz, S. I. Maffioli, M. Sosio, S. Donadio, R. Müller, *J. Biol. Chem.* **2010**, *285*, 32710-32719.

[6] Y. Yoshimura, C. Ohara, T. Imahori, Y. Saito, S. Miyauchi, I. Adachi, H. Takahata, *Bioorg. Med. Chem.* **2008**, *16*, 8273-8286.

[7] A. D. Borthwick, *Chem. Rev*. **2012**, 112, 3641-3716.

[8] W. Zhao, J. Sun, *Synlett* **2014**, *25*, 303-307.

[9] A. Isidro-Llobet, M. Álvarez, F. Albericio, *Chem. Rev*. **2009**, *109*, 2455-2504.

[10] R. M. Turner, S. D. Lindell, S. V. Ley, *J. Org. Chem.* **1991**, *56*, 5739-5740.

[11] a) P. Garner, *Tetrahedron Lett*. **1984**, *25*, 5855-5858; b) M. Passiniemi, A. M. P. Koskinen, *Beilstein J. Org. Chem*. **2013**, *9*, 2641-2659.

[12] X. Cong, F. Hu, K.-G. Liu, Q.-J. Liao, Z.-J. Yao, *J. Org. Chem.* **2005**, *70*, 4514-4516.

[13] M. Okabe, R. C. Sun, G. B. Zenchoff, *J. Org. Chem*. **1991**, *56*, 4392-4397.

[14] S. Schleich, G. Helmchen, *Eur. J. Org. Chem.* **1999**, 2515-2521.

[15] a) Y. S. Polikanov, A. L. Starosta, M. F. Juette, R. B. Altman, D. S. Terry, W. Lu, B. J. Burnett, G. Dinos, K. A. Reynolds, S. C. Blanchard, T. A. Steitz, D. N. Wilson, *Mol. Cell* **2015**, *58*, 823-844; b) A. C. Seefeldt, F. Nguyen, S. Antunes, N. Pérébaskine, M. Graf, S. Arenz. K. K. Inampudi, C. Douat, G. Guichard, D. N. Wilson, C. A. Innis, *Nat. Struct. Mol. Biol.* **2015**, *22*, 470-475; c) A. L. Starosta, V. V. Karpenko, A. V. Shishkina, A. Mikolajka, N. V. Sumbatyan, F. Schluenzen, G. A. Korshunova, A. A. Bogdanov, D. N. Wilson, Chem. Biol. **2010**, *17*, 504-514.

[16] S. Thies, PhD thesis, Saarland University, Germany, **2013**.

[17] N. Liang, A. Datta, *J. Org. Chem*. **2005**, *70*, 10182-10185.

10.1002/anie.201805901

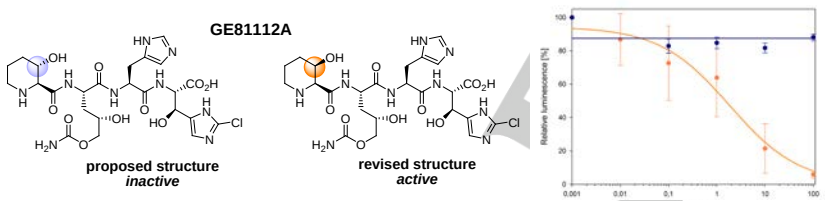
WILEY-VCH

Entry for the Table of Contents (Please choose one layout)

Layout 1:

Layout 2:

COMMUNICATION



One center makes the difference: The first total synthesis of the antibacterial tetrapeptide GE81112A is described. Its structure was revised and the correct absolute configuration was determined. Remarkably, the compound with the proposed structure differing only in one position did not show any bioactivity.

Dr. Gerrit Jürjens, Dr. Sören M. M. Schuler, Dr. Michael Kurz, Dr. Sylvain Petit, Dr. Cédric Couturier, Dr. Frédéric Jeannot, Fabian Nguyen, Dr. Raffael C. Wende, Prof. Dr. Peter E. Hammann, Prof. Dr. Daniel N. Wilson, Dr. Eric Bacqué, Dr. Christoph Pöverlein, and Dr. Armin Bauer**

Page No. – Page No.

Total Synthesis and Structural Revision of the Antibiotic Tetrapeptide GE81112A