



Synthesis and SAR of the antistaphylococcal natural product nematophin from *Xenorhabdus nematophila*

Frank Wesche^{1,2}, H el ene Adihou^{1,3,4}, Thomas A. Wichelhaus⁵ and Helge B. Bode^{*1,6,§}

Full Research Paper

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Address:

¹Molekulare Biotechnologie, Goethe University Frankfurt, Max-von-Laue-Str. 9, D-60438 Frankfurt am Main, Germany, ²present address: Respiratory, Inflammation and Autoimmunity, Innovative Medicines and Early Development, Biotech Unit, AstraZeneca, Pepparedsleden 1, M olndal 43183, Sweden, ³present address: Cardiovascular and Metabolic Diseases, Innovative Medicines and Early Development, Biotech Unit, AstraZeneca, Pepparedsleden 1, M olndal 43183, Sweden, ⁴present address: AstraZeneca MPI Satellite Unit, Abteilung Chemische Biologie, Max Planck Institut f ur Molekulare Physiologie, Otto-Hahn-Str. 11, 44227 Dortmund, Germany, ⁵Institut f ur Medizinische Mikrobiologie und Krankenhaushygiene, Universit atsklinikum Frankfurt, Paul-Ehrlich-Str. 40, D-60596 Frankfurt am Main, Germany and ⁶Buchmann Institute for Molecular Life Sciences (BMLS), Goethe University Frankfurt, Max-von-Laue-Str. 15, D-60438 Frankfurt am Main, Germany

Email:

Helge B. Bode^{*} - h.bode@bio.uni-frankfurt.de

* Corresponding author

§ Phone: 0049-69-798-29557

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Abstract

The repeated and improper use of antibiotics had led to an increased number of multiresistant bacteria. Therefore, new lead structures are needed. Here, the synthesis and an expanded structure–activity relationship of the simple and antistaphylococcal amide nematophin from *Xenorhabdus nematophila* and synthetic derivatives are described. Moreover, the synthesis of intrinsic fluorescent derivatives, incorporating azaindole moieties was achieved for the first time.

Introduction

Microorganisms present a rich source of bioactive natural products of pharmacological importance against an emerging number of multiresistant bacteria [1]. Such examples are *Xenorhabdus* sp., Gram-negative entomopathogenic bacteria

which live in symbiosis with soil-living nematodes of the genera *Steinernema* [2,3]. During a complex life cycle the nematode–bacteria pair infects and kills insect larvae, whereby *Xenorhabdus* produce a broad range of natural products with

antimicrobial properties [4–8]. As the *Steinernema–Xenorhabdus* complex is not pathogenic against humans, they are widely used as biocontrol agents in agriculture [9].

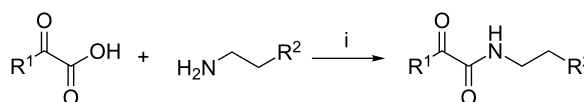
Natural products produced by bacteria play an important role in the bacteria/nematode/insect life cycle and most natural products are non-ribosomal peptides (NRP), e.g., rhabdopeptides [10,11] and polyketide–NRP hybrids, like xenocoumacins [12,13]. The quite simple amide nematophin (**1**) is another well-known member of natural products common in all *Xenorhabdus nematophila* strains and was first described by Li et al. in 1997 for their antimicrobial properties [14]. Simply, **1** is the condensation product of 3-methyl-2-oxopentanoic acid and tryptamine and showed good activities against different Gram-positive pathogens like *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA) comparable with activities of vancomycin. Recently, the biosynthesis of **1** was elucidated by our group as well as the 2-phenylethylamine derivative **2** with an α -keto amide moiety, which could be identified upon heterologous expression of the appropriate gene cluster in *E. coli* [15]. Moreover, elongated nematophin derivatives, namely nevaltophins from *Xenorhabdus* PB62.4, were described incorporating an additional valine. As **1** and nevaltophins act as prophenoloxidase activators, it is suggested that they have a specific role in the bacteria/nematode/insect symbiosis.

Little or nothing is known about the mode of action of this simple amide against *S. aureus*. Structure–bioactivity studies revealed that the α -keto moiety and the amide moiety itself are required for bioactivity [16]. These findings suggest a specific interaction of this electrophilic moiety with a nucleophile. Such an interaction was previously reported for the macrocyclic peptide cyclotheonamide A, isolated from marine sponge *Theonella* sp. Cyclotheonamide A is described as a potent inhibitor of various proteases, in particular trypsin and thrombin [17–19]. Hereby, the α -keto amide covalently binds to the serine oxygen in the active site under formation of a stable tetrahedral hemiketal. Furthermore, substitution of the indole hydrogen by alkyl, aryl or benzyl improves the in vitro antistaphylococcal activity. In contrast, the incorporation of smaller heterocycles like pyridine and imidazole as well as isosteric benzimidazole instead of the indole moieties lead to a loss of antibacterial activity. Kennedy et al. could synthesize a 2-phenyl derivative that showed nanomolar activity against *S. aureus* [20]. To the detriment of this compound class, all derivatives lose their antibacterial activity in the presence of serum in vitro in serial broth and agar dilution method [16,20]. Even with the use of charged groups as modifiers, serum-protein binding could not be avoided. However, we were interested in expanding the aforementioned structure–activity studies regarding the substitution of the indole moiety by different aromatic systems as well as

substitution of side chains in the α -keto carboxylic acids to generate more derivatives of this fascinating small and bioactive amide.

Results and Discussion

We first synthesized **1** and 1-methylnematophin (**3**) as standards to confirm preliminary results. We then initiated the synthesis of derivatives **2**, and **4–12**. Briefly, the appropriate α -keto carboxylic acid was coupled to the respective amine. Amide bond formation was achieved using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), 1-hydroxybenzotriazole (HOBt) and *N,N*-diisopropylethylamine (DIPEA) in DMF under microwave irradiation (Scheme 1). Racemic **1** was synthesized using tryptamine and (*rac*)-3-methyl-2-oxopentanoic acid. Similarly, compounds **2–7** were synthesized using different amines including 2-phenylethylamine (for **2**), 1-methyltryptamine (for **3**), 2-(2-naphthyl)ethylamine hydrochloride (for **4**), 2-(1-naphthyl)ethylamine hydrochloride (for **5**), 2-(1*H*-inden-3-yl)ethylamine hydrobromide (for **6**) [21], and 2-(1-benzofuran-3-yl)ethylamine (for **7**), respectively. Compounds **8–12** were synthesized by coupling tryptamine with different α -keto carboxylic acids, including 3-furylglyoxylic acid (for **8**), 3-indoleglyoxylic acid (for **9**), phenylglyoxylic acid (for **10**), and isomeric 3-methylpent-2-enoic acid (for **11** and **12**) [22], respectively. Compounds **11** and **12** were separated during the isolation process and initially constructed to enable target identification. Compounds **11** and **12** might act as Michael acceptor (α,β -unsaturated carbonyl) and attach irreversibly and covalently to a potential target [23]. After purification and characterization, the above mentioned compounds were tested against different pathogenic strains, i.e., methicillin-susceptible *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), *Enterococcus faecalis*, and *Micrococcus luteus*. The yields and bioactivities are summarized in Table 1 (and Table S1, Supporting Information File 1).



Scheme 1: General procedure for the synthesis of nematophin and related derivatives. i) 1.5 equiv α -keto carboxylic acid, 1.0 equiv amine, 1.5 equiv EDC·HCl, 1.5 equiv HOBt, 2.0 equiv DIPEA in DMF (c 0.1 M), 25 W, 75 °C, 20 min.

As previously described, the minimum inhibitory concentration (MIC) of **1** improves by alkylation of the indole moiety as seen for **3** [16] even **1** and **3** show a good activity against MRSA. Although for all other tested compounds no better activity than that of the original natural product **1** was observed, **6** showed an

Table 1: Summary of synthesized nematophin derivatives (1–12) and their bioactivity (MIC in $\mu\text{g/mL}$) against *S. aureus* (MSSA and MRSA).

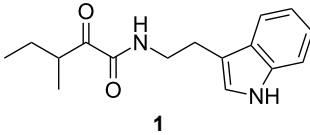
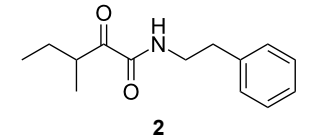
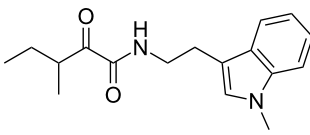
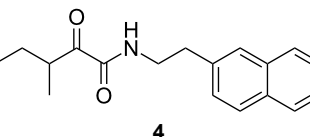
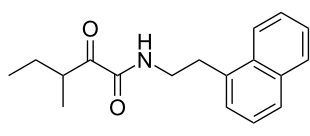
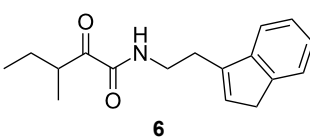
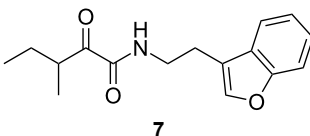
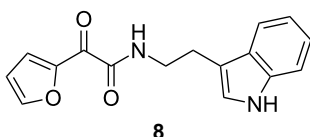
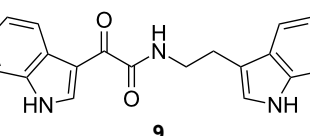
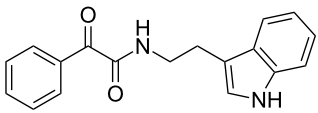
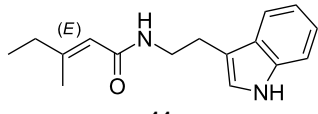
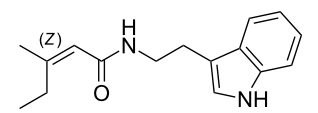
structure	yield ^a	MIC ($\mu\text{g/mL}$) ^b	
		<i>S. aureus</i> (MSSA) ATCC 29213	MRSA (MRSA) ATCC 43300
 1	46%	0.5	0.5
 2	56%	16	32
 3	74%	0.125	0.031
 4	48%	2	4
 5	70%	1	4
 6	26%	1	1
 7	54%	>128	>128
 8	59%	64	>64
 9	84%	>64	>64

Table 1: Summary of synthesized nematophin derivatives (1–12) and their bioactivity (MIC in $\mu\text{g/mL}$) against *S. aureus* (MSSA and MRSA). (continued)

	47%	8	8
10			
	59% ^c	>128	>128
11			
	59% ^c	>128	>128
12			

^aPurification using column chromatography on silica gel. ^bMedian of three experiments. ^cDerivatives were synthesized using an isomeric mixture of 3-methylpent-2-enoic acid.

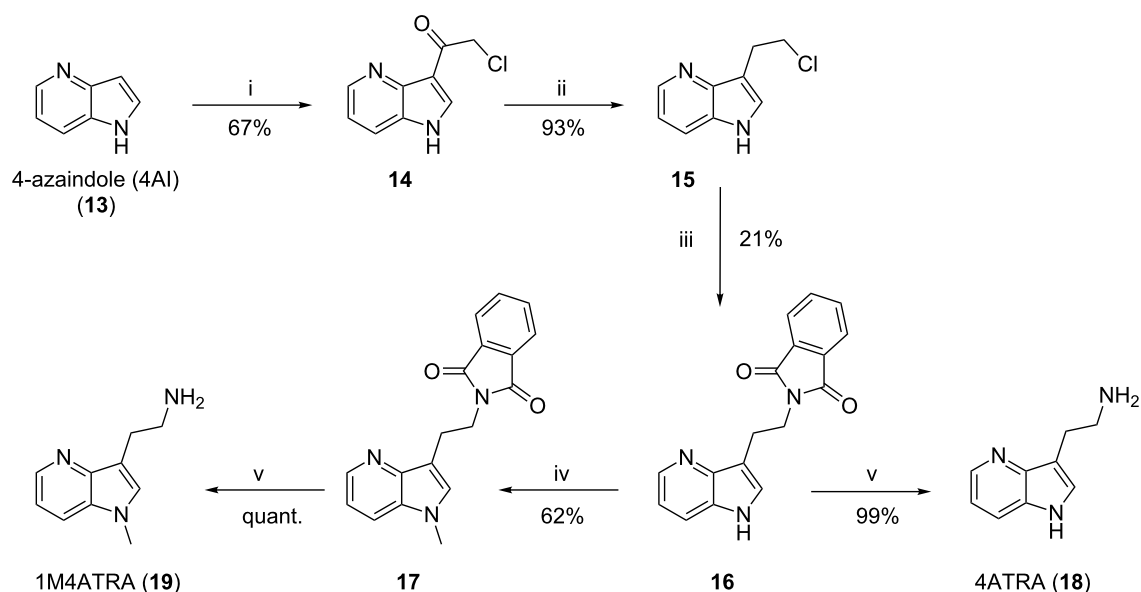
activity comparable to **1** against MRSA. Since only a slight activity could be observed for some compounds against additionally tested bacteria, i.e., *M. luteus* ATCC 9431 and *E. faecalis* ATCC 29212, a staphylococcal-specific target is suggested. All derivatives with α -keto- β -methylvaleric moiety (**1–7**) were active against *S. aureus*. Substitution of the side chain in the α -keto amide significantly affects the bioactivity, as seen for compounds **8**, **9** and **10**. Furthermore, attempts to identify a possible target by the incorporation of an α,β -unsaturated carbonyl moiety, as it should bind irreversible to a potential target, instead of the α -keto amide in **11** and **12** are stalled as it led to a complete loss of bioactivity.

To the best of our knowledge, no one has ever tried to incorporate azaindole moieties in small natural products and tried to use them for fluorescence imaging. This approach may circumvent the above-mentioned impasse and should allow at least localization studies in *S. aureus* cells. Azaindoles are isosteric to indole, whereas one of the endocyclic methines is substituted with nitrogen, and thus leads to an increased and red-shifted fluorescence [24–26]. Budisa and co-workers have already used azatryptophans to study proteins with intrinsic green and blue fluorescence [27,28]. The azaindole moiety allows a linker-less incorporation of a fluorescent label with minimal disturbance.

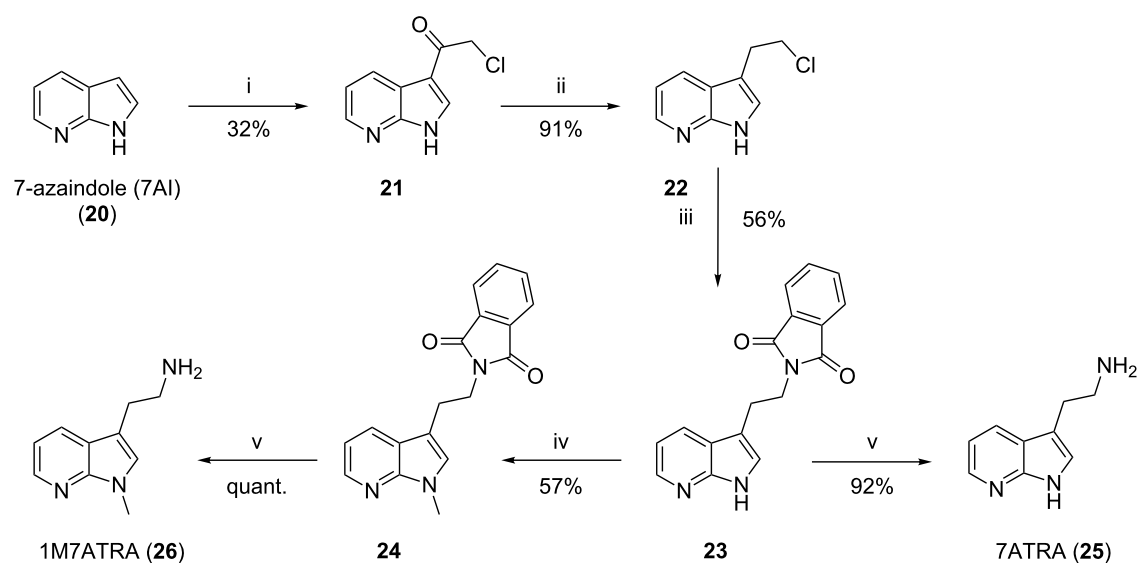
Therefore, four fluorescent derivatives of nematophin were designed and their synthesis initiated. The syntheses of the appropriate azatryptamine derivatives (**17**, **18**, **25**, and **26**) were achieved from the non-expensive and commercially available 4- and 7-azaindole (**13** and **20**), respectively. First, **13** and **20** were

converted in a Friedel–Crafts acylation with chloroacetyl chloride (ClCH_2COCl) and aluminium chloride (AlCl_3) in DCM to give compounds **14** and **21**. Subsequent reduction was achieved with triethylsilane (Et_3SiH) in TFA to give **15** and **22**. For the synthesis of the primary amine, halides **15** and **22** were converted in a Gabriel synthesis with potassium phthalimide in DMF to the appropriate phthalimides **16** and **23** [29]. These intermediary compounds **16** and **23** also allowed an *N*-methylation of the azaindole moiety with sodium hydride (NaH) and methyl iodide (MeI) to yield **17** and **24**. By ethanolic hydrazinolysis and microwave irradiation the phthalimides (**16**, **17**, **23**, and **24**) were deprotected yielding the desired primary amines in good yields (**18**, **19**, **25**, and **26**) (Scheme 2 and Scheme 3). With all building blocks available, the synthesis of the appropriate fluorescent derivatives was performed as before, using (*rac*)-3-methyl-2-oxopentanoic acid. Yields after coupling and bioactivities are summarized in Table 2.

Whereas the biological data, for example against *S. aureus* or MRSA shows a decreased activity for all azaindole derivatives (compare **1** with **27** or **28**) as observed for isosteric benzimidazoles [20], an increased activity for all methylated derivatives (compare pairs of **1/3**, **27/28**, and **29/30**) could be observed for at least MSSA. This observation may correlate to the molecular polarity, and thus hydrophilicity of the cyclic moiety, whereas almost all hydrophobic moieties showed good antistaphylococcal activity as seen for naphthyl (**4** and **5**) and indene (**6**). However, the increase of hydrophobicity influences the passive membrane diffusion and therefore might influence how the compounds get to their actual target [30]. Moreover, a specific



Scheme 2: Synthesis of azatryptamines (4-azatryptamine (4ATRA) and 1-methyl-4-azatryptamine (1M4ATRA)). i) 5.0 equiv AlCl_3 , 5.0 equiv chloroacetyl chloride in DCM, overnight, rt 67%; ii) 7.0 equiv Et_3SiH in TFA, overnight, rt, 93%; iii) 1.1 equiv potassium phthalimide, in DMF, 100 °C, 21%; iv) 1.2 equiv NaH, 1.0 equiv MeI in DMF, overnight, rt, 62%; v) 5.0 equiv $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ in EtOH, 90 °C, 2 h, 25 W, 99% to quant.



Scheme 3: Synthesis of azatryptamines (7-azatryptamine (7ATRA) and 1-methyl-7-azatryptamine (1M7ATRA)). i) 5.0 equiv AlCl_3 , 5.0 equiv ClCH_2COCl in DCM, overnight, rt 32%; ii) 7.0 equiv Et_3SiH in TFA, overnight, rt, 93%; iii) 1.1 equiv potassium phthalimide, in DMF, 100 °C, 56%; iv) 1.2 equiv NaH, 1.0 equiv MeI in DMF, overnight, rt, 57%; v) 5.0 equiv $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ in EtOH, 2 h, 90 °C, 25 W, 92% to quant.

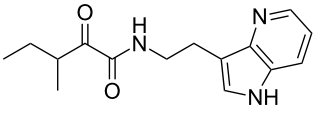
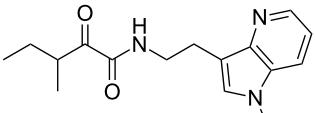
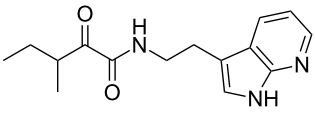
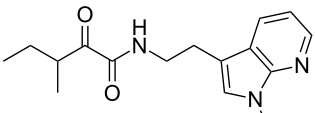
minimal size of the cyclic moiety must be fulfilled as, e.g., **2** with a phenyl moiety is less active in vitro.

Conclusion

Despite their lower bioactivities, the azaindole derivatives are currently under investigation upon their use for fluorescence

imaging. Furthermore, derivatives **3** and **6** will be further studied for identification of their actual target in *S. aureus*. This could be a serine protease that can also be addressed in the future by more stable compounds. Moreover, it might be possible to use nematophin and its derivatives for topical treatment of *S. aureus* infections.

Table 2: Summary of synthesized nematophin derivatives (27–30) and their bioactivity (MIC in µg/mL) against *S. aureus* (MSSA and MRSA).

structure	yield ^a	MIC (µg/mL) ^b	
		<i>S. aureus</i> (MSSA) ATCC 29213	MRSA (MRSA) ATCC 43300
 27	32%	32	64
 28	16%	16	>128
 29	72%	16	64
 30	28%	4	32

^aPurification using column chromatography on silica gel. ^bMedian of three experiments.

Supporting Information

Supporting Information File 1

General procedure, supplementary table and NMR spectra.

[<https://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-15-47-S1.pdf>]

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ORCID® IDs

Frank Wesche - <https://orcid.org/0000-0001-6340-3155>

Helge B. Bode - <https://orcid.org/0000-0001-6048-5909>

References

- Li, J. W.-H.; Vederas, J. C. *Science* **2009**, *325*, 161–165. doi:10.1126/science.1168243
- Herbert, E. E.; Goodrich-Blair, H. *Nat. Rev. Microbiol.* **2007**, *5*, 634–646. doi:10.1038/nrmicro1706
- Goodrich-Blair, H.; Clarke, D. J. *Mol. Microbiol.* **2007**, *64*, 260–268. doi:10.1111/j.1365-2958.2007.05671.x
- Shi, Y.-M.; Bode, H. B. *Nat. Prod. Rep.* **2018**, *35*, 309–335. doi:10.1039/c7np00054e
- Vizcaino, M. I.; Guo, X.; Crawford, J. M. *J. Ind. Microbiol. Biotechnol.* **2014**, *41*, 285–299. doi:10.1007/s10295-013-1356-5
- Klassen, J. L. *Curr. Opin. Insect Sci.* **2014**, *4*, 15–22. doi:10.1016/j.cois.2014.08.004
- Challinor, V. L.; Bode, H. B. *Ann. N. Y. Acad. Sci.* **2015**, *1354*, 82–97. doi:10.1111/nyas.12954
- Crawford, J. M.; Portmann, C.; Zhang, X.; Roeffaers, M. B. J.; Clardy, J. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 10821–10826. doi:10.1073/pnas.1201160109
- Shapiro-Ilan, D. I.; Gouge, D. H.; Koppenhöfer, A. M. Factors affecting commercial success: case studies in cotton, turf and citrus.. In *Entomopathogenic nematology*; Gaugler, R., Ed.; CABI: Wallingford, 2002; pp 333–355. doi:10.1079/9780851995670.0333
- Cai, X.; Nowak, S.; Wesche, F.; Bischoff, I.; Kaiser, M.; Fürst, R.; Bode, H. B. *Nat. Chem.* **2017**, *9*, 379–386. doi:10.1038/nchem.2671
- Reimer, D.; Cowles, K. N.; Proschak, A.; Nollmann, F. I.; Dowling, A. J.; Kaiser, M.; French-Constant, R.; Goodrich-Blair, H.; Bode, H. B. *ChemBioChem* **2013**, *14*, 1991–1997. doi:10.1002/cbic.201300205
- Reimer, D.; Luxenburger, E.; Brachmann, A. O.; Bode, H. B. *ChemBioChem* **2009**, *10*, 1997–2001. doi:10.1002/cbic.200900187

13. McInerney, B. V.; Taylor, W. C.; Lacey, M. J.; Akhurst, R. J.; Gregson, R. P. *J. Nat. Prod.* **1991**, *54*, 785–795. doi:10.1021/np50075a006
14. Li, J.; Chen, G.; Webster, J. M. *Can. J. Microbiol.* **1997**, *43*, 770–773. doi:10.1139/m97-110
15. Cai, X.; Challinor, V. L.; Zhao, L.; Reimer, D.; Adihou, H.; Grün, P.; Kaiser, M.; Bode, H. B. *Org. Lett.* **2017**, *19*, 806–809. doi:10.1021/acs.orglett.6b03796
16. Himmler, T.; Pirro, F.; Schmeer, N. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2045–2050. doi:10.1016/s0960-894x(98)00358-8
17. Lee, A. Y.; Hagihara, M.; Karmacharya, R.; Albers, M. W.; Schreiber, S. L.; Clardy, J. *J. Am. Chem. Soc.* **1993**, *115*, 12619–12620. doi:10.1021/ja00079a065
18. Maryanoff, B. E.; Qiu, X.; Padmanabhan, K. P.; Tulinsky, A.; Almond, H. R.; Andrade-Gordon, P.; Greco, M. N.; Kauffman, J. A.; Nicolaou, K. C.; Liu, A. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 8048–8052. doi:10.1073/pnas.90.17.8048
19. Fusetani, N.; Matsunaga, S.; Matsumoto, H.; Takebayashi, Y. *J. Am. Chem. Soc.* **1990**, *112*, 7053–7054. doi:10.1021/ja00175a045
20. Kennedy, G.; Viziano, M.; Winders, J. A.; Cavallini, P.; Gevi, M.; Micheli, F.; Rodegher, P.; Seneci, P.; Zumerle, A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1751–1754. doi:10.1016/s0960-894x(00)00331-0
21. Liu, S.; Motta, A.; Delferro, M.; Marks, T. J. *J. Am. Chem. Soc.* **2013**, *135*, 8830–8833. doi:10.1021/ja4039505
22. Tiseni, P. S.; Peters, R. *Chem. – Eur. J.* **2010**, *16*, 2503–2517. doi:10.1002/chem.200902896
23. Gersch, M.; Kreuzer, J.; Sieber, S. A. *Nat. Prod. Rep.* **2012**, *29*, 659–682. doi:10.1039/c2np20012k
24. Chen, Y.; Rich, R. L.; Gai, F.; Petrich, J. W. *J. Phys. Chem.* **1993**, *97*, 1770–1780. doi:10.1021/j100111a011
25. Cash, M. T.; Schreiner, P. R.; Phillips, R. S. *Org. Biomol. Chem.* **2005**, *3*, 3701–3706. doi:10.1039/b506652b
26. Carnerero, J. M.; González-Benjumea, A.; Carmona, C.; Balón, M. *Spectrochim. Acta, Part A* **2012**, *97*, 1072–1078. doi:10.1016/j.saa.2012.07.099
27. Lepthien, S.; Hoesl, M. G.; Merkel, L.; Budisa, N. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 16095–16100. doi:10.1073/pnas.0802804105
28. Hoesl, M. G.; Larregola, M.; Cui, H.; Budisa, N. *J. Pept. Sci.* **2010**, *16*, 589–595. doi:10.1002/psc.1263
29. Osby, J. O.; Martin, M. G.; Ganem, B. *Tetrahedron Lett.* **1984**, *25*, 2093–2096. doi:10.1016/s0040-4039(01)81169-2
30. Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615–2623. doi:10.1021/jm020017n

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