



Zwittermicin A: Synthesis of analogs and structure–activity studies

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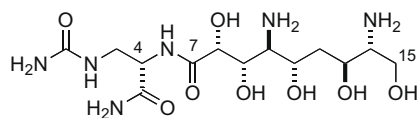
Synthesis

ABSTRACT

Analogues and diastereomers of the natural product zwittermicin A were prepared. SAR studies of these compounds reveal the antifungal activity to be dependent singularly upon the natural constitution and configuration.

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(+)-Zwittermicin A (**1**) (ZwA) is a highly polar aminopolyol antibiotic isolated from the soil-borne bacterium *Bacillus cereus*.¹ Compound **1** was first reported in 1994 and shows significant activity against human pathogenic yeast and phytopathogenic fungi.^{1,2} More importantly **1** has been shown to work synergistically with endo-toxin produced by *Bacillus thuringiensis* for control of gypsy moth.³ Research has shown strains of *B. cereus* producing **1** to be ubiquitous in the soil^{1c} and may be more benign to the environment than some synthetic pesticides.⁴ Biosynthesis of **1** arises from a non-ribosomal peptide synthetase/polyketide synthase pathway (NRPS/PKS) involving two new type 1 PKS extender units: hydroxymalonyl-acyl carrier protein (ACP) and aminomalonyl-ACP.⁵



(+)-Zwittermicin A (**1**)

Preliminary studies show that **1** appears to exhibit a unique mechanism of action.⁶ Investigations of ZwA-resistant mutants found that the resistance mapped to *rpoB* and *rpoC*, genes that encode subunits of RNA polymerase.⁶ However, **1** showed no effect on total RNA or DNA synthesis implying a mechanism of action that differs from that of other antibiotics that target RNA polymerase.⁶

Previously, we synthesized (–)-**1**, the enantiomer of ZwA, its diastereomer **2**, and analogs **5** through **10** (Fig. 1).⁷ This Letter describes the synthesis of the additional diastereomers **3** and **4** as well as analogs *ent*-**6**, **11** and **12** together with a comprehensive SAR study of all compounds.

During the course of our synthesis of (–)-**1** a number of models were made to evaluate synthetic strategies and some of these were later used to make analogs **11** and **12** as shown in Scheme 1. Analog *ent*-**6** was synthesized by reduction of diazide **13** (Scheme 1).⁸ The known alcohol **14**⁹ was converted to aldehyde **15** in three high-yielding steps; MOM protection of the primary OH group,¹⁰ desilylation of the primary OTBS group,¹¹ followed by Swern¹² oxidation. Evan's aldol addition of the boron enolate of chiral glycolate equivalent **16**¹³ to **15** (dr 47:1) followed by separation and removal of the chiral auxiliary under standard conditions afforded carboxylic acid **17** (57% over two steps).¹⁴ Coupling of **17** to known *N*-ureido-1,3-diaminopropionamide (–)-**18**⁷ gave **19** in 83% yield; global deprotection of the latter provided the truncated ZwA analog **11** in 76% yield. Similarly, analog **12** was prepared from (+)-**18**.

Azidodiol **20**, prepared from *L*-serine as described earlier,⁷ was refunctionalized by TBDPS protection¹⁵ of the terminal alcohol, MOM protection of the secondary alcohol and removal of the TBDPS group to give **21** in high yield (86% three steps, Scheme 2). Transformation of the azido group in **21** to an *N,N*-dibenzyl group by hydrogenolysis (Lindlar's catalyst¹⁶) followed by *N*-benzylation¹⁷ gave a primary alcohol that was easily oxidized to the stable aldehyde **22** (74% three steps).

Aldol addition of the glycolate equivalent **23**¹⁸ to aldehyde **22** (9:1 dr) followed by hydrolysis with lithium hydroxide under standard conditions¹⁹ afforded carboxylic acid **24** in (41% over two steps).

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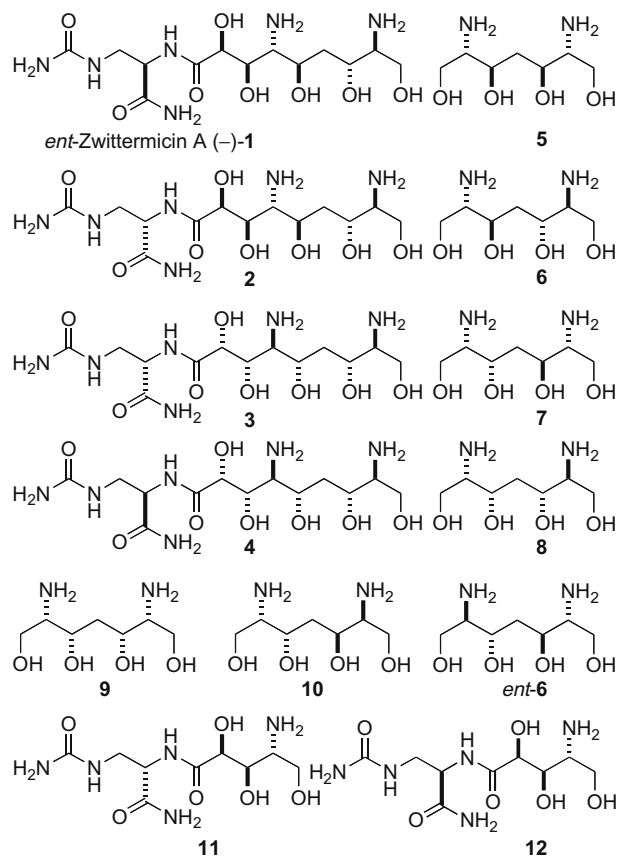
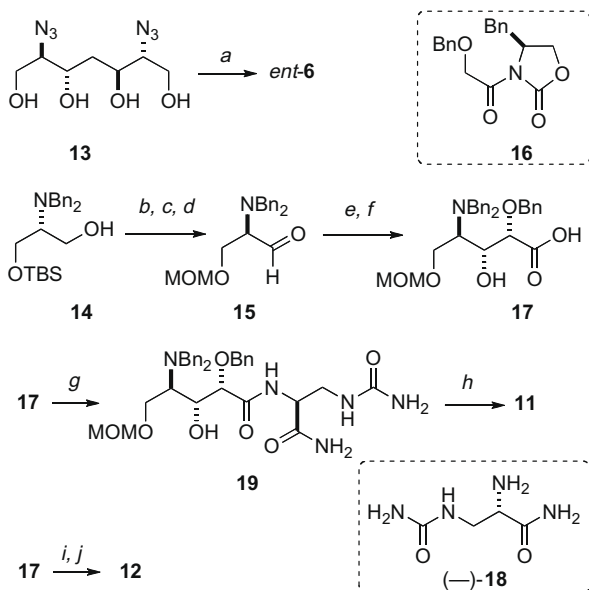
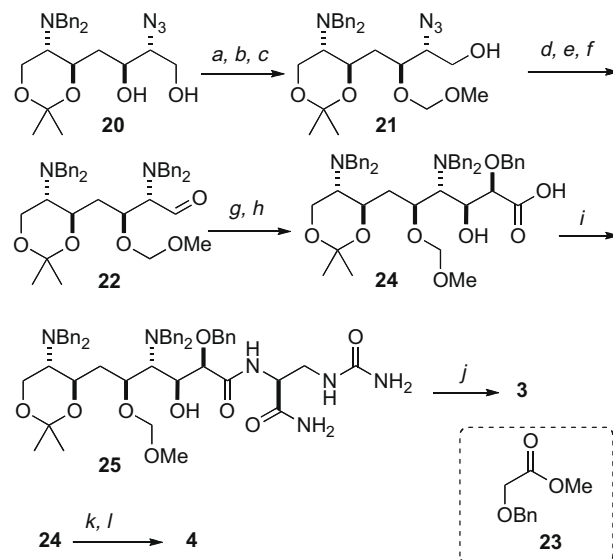


Figure 1. ZWA diastereomers and analogs for SAR studies.



Scheme 1. Synthesis of *ent*-6, 11, and 12. Reagents and conditions: (a) H_2O , H_2 (1 atm), Pd/C, 2 h, 100%; (b) MeOCH_2Cl , $\text{EtN}(i\text{-Pr})_2$, CH_2Cl_2 , 0 °C to rt, 14 h, 91%; (c) TBAF, THF, –10 °C, 16 h, 93%; (d) (i) $(\text{COCl})_2$, DMSO, CH_2Cl_2 , –78 °C, (ii) Et_3N , 94%; (e) (i) 16, *n*-Bu₂BOTf, Et_3N , CH_2Cl_2 , –78 to 0 °C, 3 h, (ii) 15, –78 to 0 °C, 2.5 h, 85%, dr 47:1; (f) H_2O_2 , LiOH, 0 °C, 30 min, 67%; (g) (i) 17, EDCI, HOBT, DMF, 0 °C, 10 min, (ii) (–)-18, Et_3N , 0 °C to rt, 2.5 h, 83%; (h) (i) HCl, MeOH, H_2 (5 atm), Pd/C, 1 h, (ii) HCl, H_2O , H_2 (5 atm), Pd/C, 1 h, 76%; (i) (i) 17, EDCI, HOBT, DMF, 0 °C, 10 min, (ii) (+)-18, Et_3N , 0 °C to rt, 1.5 h, 67%; (j) (i) HCl, MeOH, H_2 (5 atm), Pd/C, 1 h, (ii) HCl, H_2O , H_2 (5 atm), Pd/C, 1 h, 73%. EDCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, HOBT = 1-hydroxybenzotriazole.



Scheme 2. Synthesis of ZWA diastereomers 3 and 4. Reagents and conditions: (a) TBDPSCI, imidazole, DMF, 0 °C to rt, 3.5 h, 91%; (b) MeOCH_2Cl , Hünig's base, CH_2Cl_2 , 0 °C to rt, 48 h, 96%; (c) TBAF, THF, –10 °C, 4 h, 98%; (d) Lindlar's cat., H_2 , (1 atm), EtOH, 15 h, 89%; (e) BnBr, K_2CO_3 , CH_3CN , 83 h, 92%; (f) (i) $(\text{COCl})_2$, DMSO, CH_2Cl_2 , –78 °C, (ii) Et_3N , 90%; (g) (i) 23, *n*-Bu₂BOTf, Hünig's base, Et_2O , –78 °C, 1.5 h, (ii) 22, –78 to 0 °C, 2 h, 49%; (h) LiOH, H_2O :MeOH:THF, 0 °C, 4 h, 84%; (i) (i) EDCI, HOBT, DMF, 0 °C, 10 min, (ii) (–)-18, Et_3N , 0 °C to rt, 2.5 h, 86%; (j) (i) HCl, MeOH, H_2 (5 atm), Pd/C, 1 h, (ii) HCl, H_2O , H_2 (5 atm), Pd/C, 1.3 h, 57%; (k) (i) 24, EDCI, HOBT, DMF, 0 °C, 10 min, (ii) (+)-18, Et_3N , 0 °C to rt, 1.5 h, 86%; (l) (i) HCl, MeOH, H_2 (5 atm), Pd/C, 1 h, (ii) HCl, H_2O , H_2 (5 atm), Pd/C, 1 h, 73%. TBDPSCI = *tert*-butyldiphenylsilyl chloride.

EDCI coupling of 24 to (–)-18 gave 25 (86% yield) and global deprotection provided zwittermicin A diastereomer 3 in 57% yield. In a similar manner, diastereomer 4 was synthesized from (+)-18 and 24.

Antifungal assay of natural (+)-1 and the 13 synthetic compounds was conducted against the fungal strains *Candida albicans* 96-489, *Candida glabrata*, *C. albicans* UCDFR1, *C. albicans* ATCC 144503, *Candida krusei*, and the phytopathogenic bacteria *Erwinia carotovora*, and *Erwinia amylovora* and oomycete *Phytophthora infestans* (Table 1). Natural zwittermicin A [(+)-1] showed activity against *C. albicans*, *C. glabrata*, *E. carotovora*, and *E. amylovora*. Despite structural similarities of the synthetic compounds to natural ZWA, particularly the enantiomer (–)-1 and stereoisomeric 3 and 4, only the natural product showed detectable activity.

Table 1
Biological testing of zwittermicin A and synthetic compounds

Pathogenic microbes	(+)-1 MIC ^{a,b} (μg/mL)	(–)-1, 2–10, <i>ent</i> -6, 11 and 12 MIC ^{a,b} (μg/mL)
<i>Candida albicans</i> 96-489	55.7	>128
<i>C. glabrata</i>	59.5	>128
<i>C. albicans</i> UCDFR1	>128	>128
<i>C. albicans</i> ATCC 14503	>128	>128
<i>C. krusei</i>	>128	>128
<i>Erwinia carotovora</i>	22.2	>128 ^c
<i>E. amylovora</i>	18.8	>128 ^c
<i>Phytophthora infestans</i> ^d	>32	>32 ^c

^a The MIC endpoint is defined as the lowest concentration (μg/mL) with 90% growth inhibition.

^b (+)-1, (–)-1, 2–4, 11 and 12 were converted to the free amine before testing. Compounds 5–10 were used as their HCl salts.

^c 5–10 not tested.

^d The maximum dose used was 32 μg/disk due to limitations of the nutrient agar well diffusion assay.

The biological data indicate that the mechanism of action is highly stereospecific. The enantiomer (–)-**1** and all of the diastereomers were inactive.⁷ Changing the configuration of **1** at only two stereocenters (C-13 and C-14 in **3**) resulted in complete loss of activity, even in the most susceptible strain, *E. amylovora*. None of the truncated analogs (Fig. 1) showed activity. In fact, the activity of (+)-**1** is quite singular; it not mimicked by simple synthetic stereoisomers or analogs.

Antifungal activity in simple vicinal aminoalkanols has been observed for other natural products, such as oceanapiside,²⁰ oceanalin,²¹ even sphingosine and its synthetic short-chain analogs (C₆), irrespective of the relative configurations.²² Consequently, the stringent stereochemical requirements observed for antifungal activity properties in **1** were unexpected and surprising. The mechanism of action of **1** is presently unknown, but clearly differs from that of the former compounds which appear to interdict sphingolipid metabolism.²³

In summary, two zwittermicin A diastereomers and three analogs were synthesized and compared with natural zwittermicin A and eight other previously synthesized analogs and stereoisomers in antifungal assays. The microbial susceptibility profile of **1–12** indicates that the mechanism of action is highly stereospecific, and the complete complement of functionality found in the natural enantiomer zwittermicin A [(+)-**1**] is required for efficacy. Further investigations to identify the mechanism of action may benefit from *selective* affinity tagging of **1** and deployment in cell-free pull-down experiments to identify the cellular target.²⁴

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References and notes

- (a) He, H.; Silo-Suh, L. A.; Handelsman, J.; Clardy, J. *Tetrahedron Lett.* **1994**, 35, 2499; (b) Silo-Suh, L. A.; Lethbridge, B. J.; Raffel, S. J.; He, H.; Clardy, J.; Handelsman, J. *Appl. Environ. Microbiol.* **1994**, 60, 2023; (c) Athukorala, S. N. P.; Fernando, W. G. D.; Rashid, K. Y. *Can. J. Microbiol.* **2009**, 55, 1021.
- (a) Silo-Suh, L. A.; Stabb, E. V.; Raffel, S. J.; Handelsman, J. *Curr. Microbiol.* **1998**, 37, 6; (b) Milner, L.; Stohl, E. A.; Handelsman, J. *J. Bacteriol.* **1996**, 178, 4266; (c) Stohl, E. A.; Brady, S. F.; Clardy, J.; Handelsman, J. *J. Bacteriol.* **1999**, 181, 5455.
- (a) Broderick, N. A.; Goodman, R. M.; Handelsman, J.; Raffa, K. F. *Environ. Entomol.* **2003**, 32, 387; (b) Broderick, N. A.; Goodman, R. M.; Raffa, K. F.; Handelsman, J. *Environ. Entomol.* **2000**, 29, 101.
- (a) Stabb, E. V.; Jacobson, L. M.; Handelsman, J. *Appl. Environ. Microbiol.* **1994**, 60, 4404; (b) Raffel, S. J.; Stabb, E. V.; Milner, J. L.; Handelsman, J. *Microbiology* **1996**, 142, 3425.
- (a) Stohl, E. A.; Milner, J. L.; Handelsman, J. *Gene* **1999**, 237, 403; (b) Emmert, E. A.; Kilmowicz, A. K.; Thomas, M. G.; Handelsman, J. *Appl. Environ. Microbiol.* **2004**, 70, 104; (c) Chan, Y. A.; Boyne, M. T., II; Podels, A. M.; Klimowicz, A. K.; Handelsman, J.; Kelleher, N. L.; Thomas, M. G. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, 103, 14349; (d) Zhao, C.; Luo, Y.; Song, C.; Liu, Z.; Chen, S.; Yu, Z.; Sun, M. *Arch. Microbiol.* **2007**, 187, 313.
- Stabb, E. V.; Handelsman, J. *Mol. Microbiol.* **1998**, 27, 311.
- (a) Rogers, E. W.; Molinski, T. F. *Org. Lett.* **2007**, 9, 437; (b) Rogers, E. W.; Dalisay, D. S.; Molinski, T. F. *Angew. Chem., Int. Ed.* **2008**, 47, 8086.
- (a) Rogers, E. W.; Molinski, T. F. *J. Org. Chem.* **2009**, 74, 7660; (b) Rogers, E. W., PhD Thesis, UC San Diego, 2008.
- Laib, T.; Chastanet, J.; Zhu, J. *J. Org. Chem.* **1998**, 63, 1709.
- Stork, G.; Takahashi, T. *J. Am. Chem. Soc.* **1977**, 99, 1275.
- Novachek, K. A.; Meyers, A. I. *Tetrahedron Lett.* **1996**, 37, 1743.
- Yakelis, N. A.; Roush, W. R. *J. Org. Chem.* **2003**, 68, 3838.
- Evans, D. A.; Gage, J. R. *Org. Synth.* **1990**, 68, 83.
- Evans, D. A.; Britton, T. C.; Ellman, J. A. *Tetrahedron Lett.* **1987**, 28, 6141.
- Hulme, A. N.; Montgomery, C. H.; Henderson, D. K. *J. Chem. Soc., Perkin. Trans. 1* **2000**, 1837.
- Corey, E. J.; Nicolaou, K. C.; Balanson, R. D.; Machida, Y. *Synthesis* **1975**, 590.
- Reetz, M. T. *Chem. Rev.* **1999**, 99, 1121.
- Bertrand, M. B.; Wolfe, J. P. *Org. Lett.* **2006**, 8, 4661.
- Vicario, J. L.; Rodriguez, M.; Badia, D.; Carrillo, L.; Reyes, E. *Org. Lett.* **2004**, 6, 3171.
- (a) Nicholas, G. M.; Hong, T. W.; Molinski, T. F.; Lerch, M. L.; Cancilla, M. T.; Lebrilla, C. B. *J. Nat. Prod.* **1999**, 62, 1678; (b) Nicholas, G. M.; Molinski, T. F. *J. Am. Chem. Soc.* **2000**, 122, 4011.
- Makariev, T. N.; Denisenko, V. A.; Dmitrenko, P. S.; Guzii, A. G.; Santalova, E. A.; Stonik, V. A.; MacMillan, J. B.; Molinski, T. F. *Org. Lett.* **2005**, 7, 2897.
- Nicholas, G. N.; Li, R.; MacMillan, J. B.; Molinski, T. F. *Bioorg. Med. Chem. Lett.* **2002**, 12, 2159.
- Dalisay, D. S.; Molinski, T. F., unpublished.
- Hughes, C. C.; MacMillan, J. B.; Gaudencio, S. P.; Fenical, W.; La Clair, J. J. *Angew. Chem., Int. Ed.* **2009**, 48, 728.