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Zwittermicin A: Synthesis of analogs and structure–activity studies

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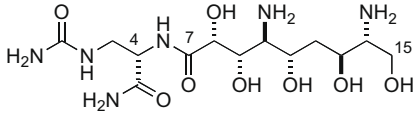
ABSTRACT

Analogs 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.

(+)-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 thuringensis* 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 Swern[12] 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-L-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 protection[15] 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 catalyst[16]) followed by N-benzylation[17] 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 conditions[19] afforded carboxylic acid **24** in (41% over two steps).

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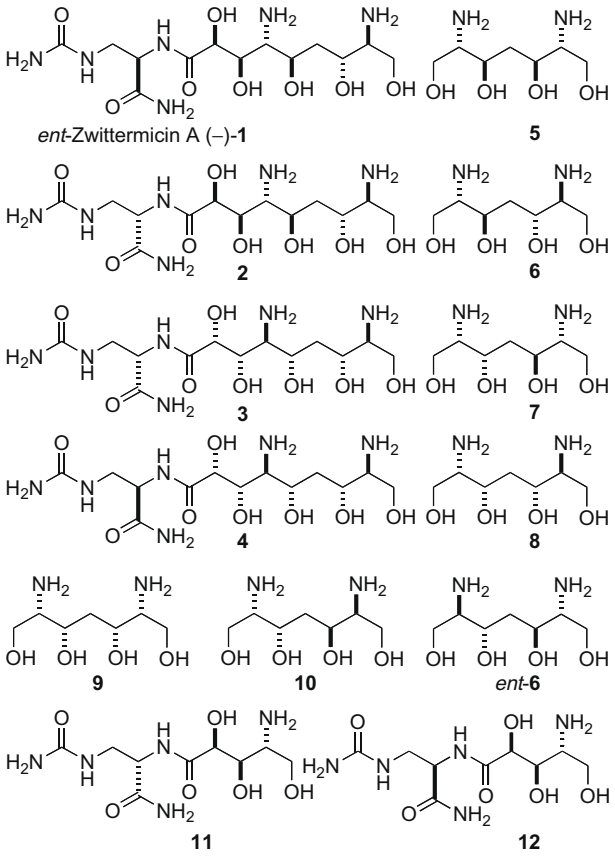
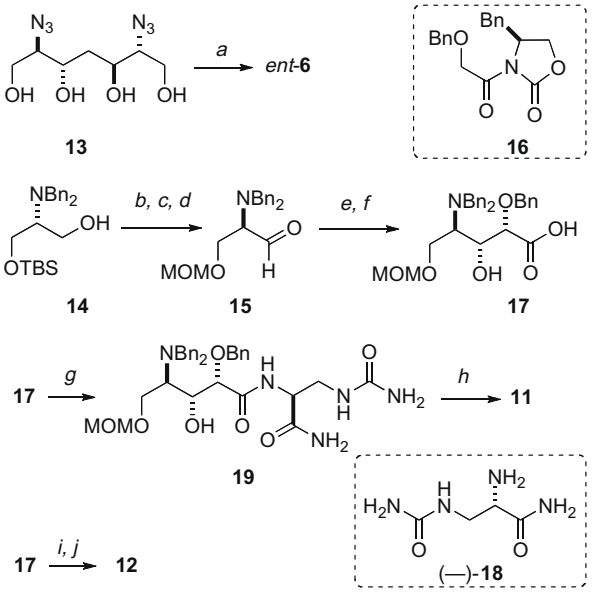
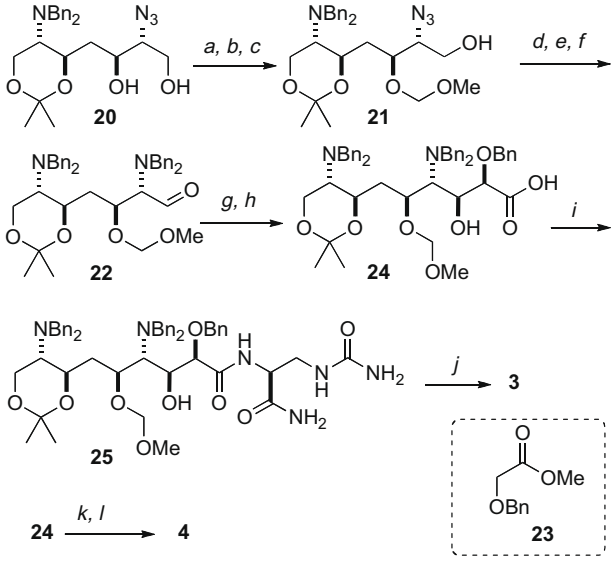


Figure 1. ZwA diastereomers and analogs for SAR studies.



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) $MeOCH_2Cl$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, 0 °C to rt, 14 h, 91%; (c) TBAF, THF, −10 °C, 16 h, 93%; (d) (i) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, (ii) Et_3N, 94%; (e) (i) **16**, *n*-Bu_2BOTf, 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) TBDPSCl, 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) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, (ii) Et_3N, 90%; (g) (i) **23**, *n*-Bu_2BOTf, 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%. TBDPSCl = *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**, *ent*-**6**, **11** and **12** MIC[a,b] (μg/mL)
Candida albicans 96–489	55.7	>128
C. glabrata	59.5	>128
C. albicans UCDFR1	>128	>128
C. albicans ATCC 14503	>128	>128
C. krusei	>128	>128
Erwinia carotovora	22.2	>128[c]
E. amylovora	18.8	>128[c]
Phytophthora infestans[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 (C_6), 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]

Acknowledgments

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