Natural Product Synthesis

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(+)-Zwittermicin A: Assignment of its Complete Configuration by Total Synthesis of the Enantiomer and Implication of D-Serine in its Biosynthesis**

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(+)-Zwittermicin A (**1**; Figure 1),[1] a water-soluble natural antibiotic that was reported in 1994 and isolated from the fermentation of the soil-borne bacterium *Bacillus cereus*,

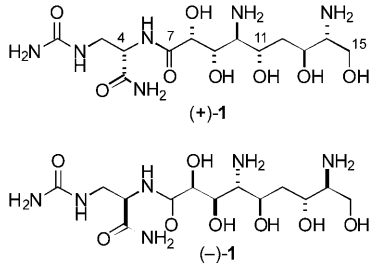

Figure 1. Natural (+)-zwittermicin A (**1**) and its enantiomer.

shows significant activity against phytopathogenic fungi.[2] Most importantly, **1** synergizes the bioactivity of the endotoxin produced by *Bacillus thuringensis* (BT), a "green" insecticide which is used globally for the protection of vegetable crops and for the eradication of gypsy moth from forest trees. [2,3] BT toxin and related biocontrol agents are important commodities used in the fight against declining agricultural production and rising third world food shortages.[3b] The biosynthesis of the sugarlike natural product **1** is very unusual; the molecule does not derive from carbohydrate metabolism, as the structure may suggest, but instead arises from a non-ribosomal peptide synthetase/polyketide synthase (NRPS/PKS) pathway that starts with an activated serine unit (Ser; C13–C15, zwittermicin A numbering). Zwittermicin A is the first polyketide described in which the two-carbon chain extensions occur by condensations of three-carbon units (and subsequent loss of CO_2), derived from hydroxymalonate (HM; C7–C8), aminomalonate (AM; C9–C10), and the more common extender, malonate (C11–C12).[4]

Combinatorial biosynthetic engineering of AM PKS modules has great potential for the production of exotic "non-natural" aminopolyketides[4c] and possible remodeling of PKS structures to alkaloids by exploiting the innate nucleophilicity of the NH_2 group. Despite high interest in **1**, the structure of zwittermicin A has eluded attempts to define its configuration for 14 years.[1c]

Herein, we report the complete absolute stereostructure of (+)-**1** by way of deductive reasoning and the first total synthesis of its enantiomer (−)-**1**. Our surprise finding—that C13–C15 formally derives from D-Ser, rather than L-Ser[5]—has implications for the structure–activity relationship of the loading domain in the NRPS/PKS complex which initiates the biosynthesis of (+)-**1**.

Azidodiol **2**, prepared from L-Ser as described earlier,[5] was refunctionalized by protection of the terminal alcohol with TBDPS,[6] protection of the secondary alcohol with MOM,[7] and removal of the TBDPS group[6] to give **3** in high yield (85% over 3 steps; Scheme 1).[8] Transformation of the azido group of **3** into an *N*,*N*-dibenzyl group by hydrogenolysis (Lindlar's catalyst[9]) and subsequent benzylation at the nitrogen center[6] gave a primary alcohol that was easily oxidized to the stable aldehyde **4** (84% over 3 steps).

Evan's aldol addition of the chiral glycolate equivalent **5**[10] to **4** and subsequent removal of the chiral auxiliary under standard reaction conditions afforded carboxylic acid **6** in 74% yield and 92% *de* (over 2 steps),[11] which is ready for coupling to *N*-ureido-L-1,3-diaminopropionamide ((−)-**8**), easily derived from the known amide **7**.[12]

Coupling of **6** and (−)-**8**[13] gave the amide **9** (81%) which was then globally deprotected[14] to afford (−)-**10** with the configuration proposed for (+)-**1**.[5] Although the ^1H and ^{13}C NMR spectra (400 MHz, D_2O) of (+)-**1**[15] and (−)-**10** were almost identical at C10–C15 (see the Supporting Information), chemical shift differences at H8 [(−)-**10**, $\delta = 4.53$ ppm, d, $J = 2.0$ Hz; (+)-**1**, $\delta = 4.56$ ppm, d, $J = 2.0$ Hz] were readily revealed upon analyzing the spectrum of a mixture of the two compounds (Figure 2 a). Additionally, the specific rotation of (−)-**10** ($[\alpha]_D = -23.0°$, H_2O) was opposite in sign and of larger magnitude than values measured for natural (+)-**1** ($[\alpha]_D = +8.1°$, H_2O; lit.[1a] $+8.9°$, H_2O) under the same conditions.

The relative configuration of the C8–C15 segment of (+)-**1** was certain from the analysis of ^1H NMR spin system

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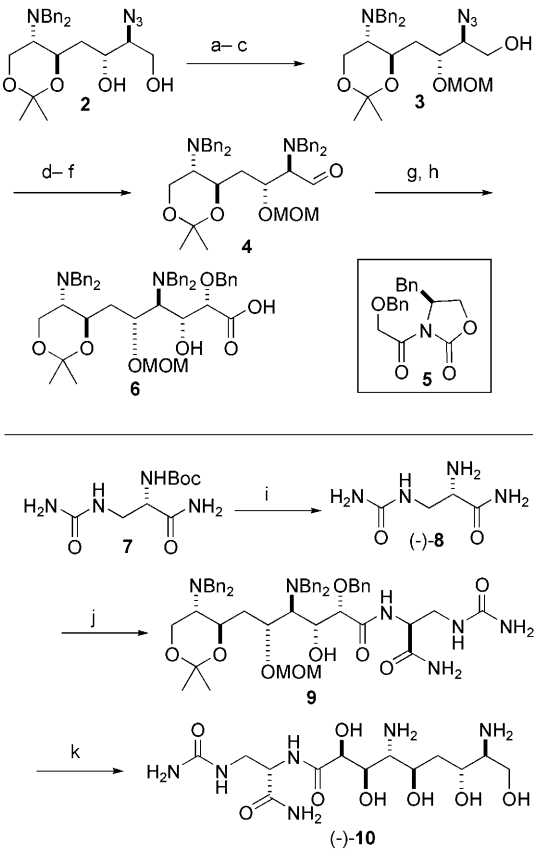


Scheme 1. Synthesis of (−)-**10**. Reagents and conditions: a) TBDPSCl, imidazole, DMF, 0 °C→RT, 4 h, 91 %; b) $MeOCH_2Cl$, Hünig's base, CH_2Cl_2, 0 °C→RT, 56 h, 98 %; c) TBAF, THF, −10 °C, 4 h, 95 %; d) Lindlar cat., H_2, (1 atm), EtOH, 14 h, 98 %; e) BnBr, K_2CO_3, CH_3CN, 31 h, 91 %; f) 1. $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; 2. Et_3N, 94 %; g) 1. **5**, nBu_2BOTf, Et_3N, CH_2Cl_2, −78→0 °C, 3 h; 2. **4**, −78→0 °C, 2.5 h, 77 %, d.r. 24:1; h) H_2O_2, LiOH, 0 °C, 30 min, 96 %; i) TFA, 0 °C, 1 h, 98 %; j) 1. **6**, EDCI, HOBt, DMF, 0 °C, 10 min; 2. (−)-**8**, Et_3N, 0 °C→RT, 1 h, 81 %; k) 1. HCl, MeOH, H_2 (5 atm), Pd/C, 1 h; 2. HCl, H_2O, H_2 (5 atm), Pd/C, 1 h, 76 %. Bn = benzyl, Boc = *tert*-butoxycarbonyl, DMF = *N*,*N*-dimethylformamide, DMSO = dimethyl sulfoxide, EDCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, HOBt = 1-hydroxybenzotriazole, MOM = methoxymethyl, TBAF = tetra-*n*-butylammonium fluoride, TBDPS = *tert*-butyldiphenylsilyl, TFA = trifluoroacetic acid.

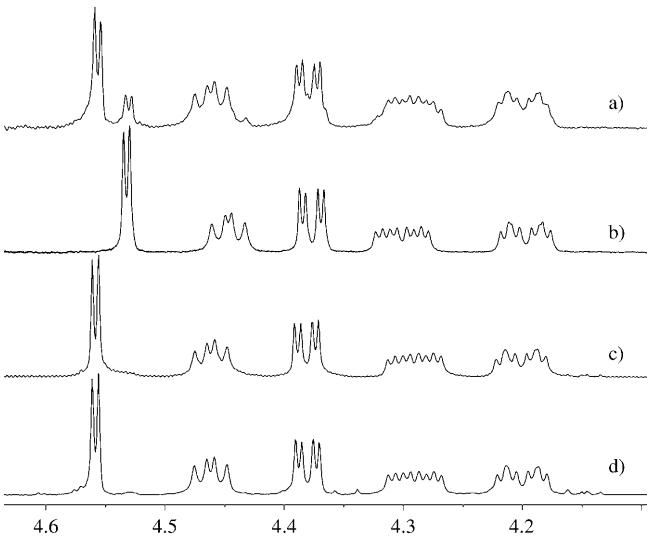
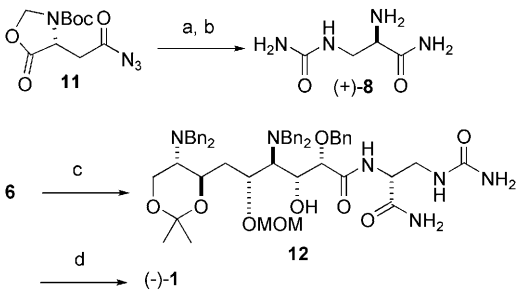


Figure 2. ^1H NMR spectra (400 MHz, D_2O): a) 1:3 mol ratio of synthetic (−)-**10** and natural (+)-**1**; b) synthetic (−)-**10**; c) synthetic (−)-**1**; d) 1:2 mol ratio of synthetic (−)-**1** and natural (+)-**1**. Concentrations of approximately 10 mM, no solvent suppression.

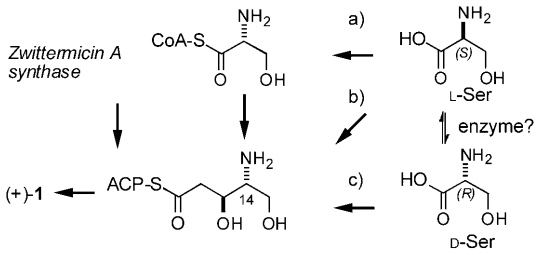


Scheme 2. Synthesis of model (−)-**1**. Reagents and conditions: a) 1. μW, toluene, 110 °C, 15 min; 2. THF, NH_3, 30 min; 3. NH_3 (2 M), MeOH, 5 h; 4. NaOH (1 N), MeOH, 4.5 h, 62 %; b) TFA, 0 °C, 1 h, 99 %; c) 1. EDCI, HOBt, DMF, 0 °C, 10 min; 2. (+)-**8**, Et_3N, 0 °C→RT, 1 h, 88 %; d) 1. HCl, MeOH, H_2 (5 atm), Pd/C, 1 h; 2. HCl, H_2O, H_2 (5 atm), Pd/C, 1 h, 75 %.

topicities and ^{13}C NMR chemical shift differences of a C_2-symmetric diamino tetraol derived from **2**.[5] Considering that the configuration of the amino acid in (+)-**1** was unequivocally L,[5] the ^1H and ^{13}C NMR signals at C10–C15 showed negligible differences, and the largest ^1H NMR difference in chemical shift occurred at H8, we hypothesized that the mismatch resulted from the inversion of all configurations in the diaminopolyol–carboxylate moiety of (−)-**10**: C8–C11, C13, and C14. The latter (inversion of configuration at C14) would negate the original assumption of a formal biosynthesis of **1** derived from an L-Ser starter unit[4a,5] in the NRPS loading domain and would therefore require the involvement of D-Ser. To test this hypothesis compound **12**, a diastereomer of **9**, was prepared by coupling carboxylic acid **6** with D-α-aminoamide (+)-**8** (88 %; Scheme 2, where (+)-**8** was derived from the known acyl azide **11**[16] in 2 steps by Curtius rearrangement and subsequent ammoniolysis). Removal of the protecting groups of **12** under the reaction conditions that were previously used in Scheme 1[14] gave (−)-**1** in 75 % yield.

The NMR spectra of synthetic (−)-**1** and natural (+)-**1** were identical in all respects; co-addition of natural (+)-**1** to (−)-**1** gave a single discrete set of ^1H (Figure 2 d) and ^{13}C signals corresponding to those of natural (+)-zwittermicin A.[1a]

Finally, the specific rotation of synthetic (−)-**1** ($[\alpha]_D = -7.9°$, H_2O) was opposite in sign and equal in magnitude to natural zwittermicin A. Therefore the configuration of zwittermicin A ((+)-**1**) is 4*S*,8*R*,9*S*,10*S*,11*S*,13*S*,14*R* as depicted (Figure 1). The assignment of configuration described here has implications for the biosynthesis of (+)-**1**. Three scenarios can be considered to explain the unexpected 14*R* configuration of zwittermicin A; 1) direct incorporation of D-Ser at C13–C15 (path a); Scheme 3), which is similar to that observed for the D-Ala starter residue of cyclosporine,[17a]



Scheme 3. Possible pathways for the propogation of D-Ser in the biosynthesis of (+)-**1**). a) Loading of L-Ser and inversion at C14 of L-Ser thioester catalyzed by an epimerase domain. b) Loading of L-Ser and condensation with malonyl thioester with concomitant epimerization. c) Loading of D-Ser.

2) α epimerization of a carrier-protein-bound L-Ser by an embedded epimerization domain (path b); Scheme 3), or 3) the involvement of a dual-function condensation/epimerization domain (path c); Scheme 3), such as those operating in the biosynthesis of arthrofactin[17b] and enduracidin.[17c] In the latter case, a single catalytic domain may be responsible for inversion of the α configuration and coupling of the resultant thioacyl D-Ser residue with a downstream acceptor residue, however, this mechanism is yet to be associated with a mixed NRPS/PKS system. Although details have been reported for gene products ZmAG-ZmAI, which are responsible for the AM extender unit,[4c] the identification of the genes and a mechanism responsible for the Ser loading domain and its incorporation into C13–C15 of **1** are still unclear. Resolution of this mystery awaits more detailed annotation of the gene cluster involved in the biosynthesis of (+)-**1**.

We have briefly compared the biological activity of (+)-zwittermicin A with that of its synthetic enantiomer (−)-**1**, by measuring the susceptibility of pathogenic fungi and fluconazole-resistant pathogens of the genus *Candida* (Table 1).

The minimum inhibitory activities of authentic natural (+)-**1** against *Candida albicans* ATCC 14503 (MIC = 55.7 $\mu g\,mL^{-1}$) and the fluconazole-resistant strain *C. albicans* 96-489 (MIC = 59.5 $\mu g\,mL^{-1}$) were found to be comparable to the antifungal activities found by Handelsman et al.[2a] for (+)-**1** against a range of plant pathogenic fungi of agricultural importance. On the other hand, (−)-zwittermicin A was inactive (MIC > 128 $\mu g\,mL^{-1}$) under the same conditions. This interesting result implies that the activity of (+)-**1** is not related to non-specific interactions with the diaminopolyol unit, but is more closely allied to either transport across the cell wall or membrane, or a mechanism that implicates a more subtle chiral recognition motif at an as-yet unidentified intracellular target.

Table 1: In vitro minimum inhibitory activities against pathogenic *Candida* species for natural (+)-**1** and synthetic (−)-**1**.

Fungal strains	(+)-**1** MIC [$\mu g\,mL^{-1}$][a]	(−)-**1** MIC [$\mu g\,mL^{-1}$][a]
C. albicans 96-489[b]	55.7	>128
C. glabrata	59.5	>128
C. albicans UCDFR1[c]	>128	>128
C. albicans ATCC 14053	>128	>128
C. krusei	>128	>128

[a] Compounds tested as their free bases. The MIC is defined as the lowest concentration eliciting 90% growth inhibition. [b] A clinical isolate. [c] Fluconazole-resistant *Candida* strain was raised from *C. albicans* ATCC 14053 by passage through subinhibitory fluconazole. See the Supporting Information for details of culture conditions.

In summary, the absolute stereostructure of (+)-zwittermicin A ((+)-**1**) has been unambiguously assigned by total synthesis of (−)-**1** in an overall yield of 1.9% (20 steps from *N*,*N*-dibenzyl-L-serine methyl ester). Interpretation of the configuration of (+)-**1** implicates a "D-Ser" motif in the biosynthesis of C13–C15, which is consistent with an antipodal configuration of the propagated Ser starter unit. Zwittermicin A and its enantiomer exhibit a pattern of differential activity against fungal pathogens that underscores the importance of chirality to the biological activity of these acyclic diaminopolyol natural products.

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