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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**), [1] a water-soluble natural antibiotic reported in 1994 from the fermentation of the soil-borne bacterium *Bacillus cereus*, shows significant activity against phytopathogenic fungi.[2] Most importantly, **1** synergizes the bioactivity of the endotoxin produced by *Bacillus thuringensis* (BT), a 'green' insecticide used globally protection of vegetable crops and 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 sugar-like **1** is very unusual; the molecule does not derive from carbohydrate metabolism, as the structure may suggest, but arises from a non-ribosomal peptidyl-polyketide synthase (NRPS-PKS) pathway, starting with an activated serine (Ser, C13–C15, zwittermicin A numbering). Zwittermicin A is the first described polyketide in which C_2 chain extensions occur by condensations of C_2 units (followed by loss of CO_2) derived from hydroxymalonate (HM, C7–C8) and aminomalonate (AM, C9–C10) in addition to the more common extender, malonate. [4]

Combinatorial biosynthetic engineering of AM PKS modules has great potential for production of exotic 'non-natural' amino-polyketides[4c] and possible remodeling of PKS structures alkaloids by exploitation of 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 until now.[1c]

We report here 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 structure-function of the loading domain in the NRPS-PKS complex that initiates biosynthesis of (+)-**1**.

Azidodiol **2**, prepared from L-serine as described earlier, [5] was refunctionalized by TBDPS protection[6] of the terminal alcohol, MOM protection[7] of the secondary alcohol and removal of the TBDPS group[6] to give **3** in high yield (85% three steps, Scheme 1).[8] Transformation of the azido group in **3** to an *N*,*N*-dibenzyl group by hydrogenolysis

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(Lindlar's catalyst[9]) followed by *N*-benzylation[6] gave a primary alcohol that was easily oxidized to the stable aldehyde **4** (84% three steps).

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

Coupling of **6** and **8**[13] gave an amide **9** (81%) that was globally deprotected[14] to afford compound (−)-**10** with the configuration proposed for (+)-**1**.[5] Although the ^{1}H and ^{13}C NMR spectra (400 MHz, D_2O) of (+)-**1**[15,16] and (−)-**10** were almost identical at C10–C15 (see Supporting information), chemical shift differences at H8 [(−)-**10**, δ 4.53, d, J = 2.0 Hz: (+)-**1**, δ 4.56, d, J = 2.0 Hz] were readily revealed upon measurements of a mixture of the two compounds (Figure 2). 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°) under the same conditions.

The relative configuration of the C8–C15 segment of (+)-**1** was certain from analysis of ^{1}H NMR spin system topicities and ^{13}C NMR chemical shift differences from a C_2 symmetric diamino tetraol derived from **2**.[5] Considering that the amino acid configuration in (+)-**1** was unequivocally L-, [5] the ^{1}H and ^{13}C NMR signals at C10–C15 showed negligible differences, and the largest ^{1}H NMR difference occurred at H8, we hypothesized that the mismatch was due to inversion of *all* configurations in the diaminopolyol-carboxylate moiety of (−)-**10**: C8–C11, C13, and C14. The latter 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 require involvement of D-Ser. In order to test this hypothesis, **12**, a diastereomer of **9**, was prepared (Scheme 2) by coupling carboxylic acid **6** with D-α-aminoamide (+)-**8** (88%) (the latter compound was derived in two steps from the known acyl azide **11**[17] by Curtius rearrangement followed by ammoniolysis). Deprotection of **12** under the conditions used previously (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** and NMR measurements gave a single discrete set of ^{1}H (Figure 1) 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 of 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. The configurational assignment described here has implications for the biosynthesis of (+)-**1**. Three scenarios can be considered to explain the unexpected 14*R* configuration of zwittermicin A; direct incorporation of D-Ser at C13–C15, similar to that observed for the starter D-Ala residue of cyclosporine, [18a] α-epimerization of a carrier protein-bound L-Ser by an embedded epimerization domain, or the involvement of a dual function condensation/epimerization domain, such as those operating in the biosynthesis of arthrofactin[18b] and enduracidin.[18c] In the latter case, a single catalytic domain may be responsible for inversion of the α-configuration and coupling of the resultant thio-acyl D-Ser residue with a down-stream acceptor residue, however, this mechanism has yet to be associated with a mixed NRPS-PKS system. Although details have been reported for gene products ZmAG-ZmAI responsible for the AM extender unit, [4c] the identification of the genes and a mechanism responsible for the Ser loading domain and incorporation into C13–C15 of **1** are still unclear. Resolution of this mystery awaits more detailed annotation of the gene cluster for biosynthesis of (+)-**1**.

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

The minimum inhibitory activities of authentic natural (+)-**1** against *Candida albicans* ATCC 14503 (MIC 55.7 μg/ml) and the Fluconazole-resistant strain *C. albicans* 96–489 (MIC 59.5 μg/ml) were found to comparable to the antifungal activities found by Handelsman et al. for (+)-**1** against a range of plant pathogenic fungi of agricultural importance. *ent*-Zwittermicin A (−)-**1**, on the other hand was inactive (MIC > 128 μg/ml) under the same conditions. This interesting result implies that the activity of (+)-**1** is due not to non-specific interactions with the diaminopolyol, but 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 intra-cellular target.

In summary, the absolute stereostructure of (+)-zwittermicin A (**1**) has been assigned unambiguously 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 consistent with an antipodal configuration of the propagated Ser starter unit. Zwittermicin A and its enantiomer exhibit a differential activity against fungal pathogens that underscores the importance of chirality to the biological activity of these acyclic diaminopolyol natural products.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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15. We thank D. Manker (Novo Nordisk, Entotech, Inc, Davis, CA) for a gift of authentic (+)-**1**.
16. All NMR and optical rotations of **1** and **10** were recorded on the corresponding purified (HPLC) dihydrochloride salts (See Supporting Information). NMR spectra (D_2O) were referenced to internal CH_3CN set to δ2.06 and δ1.47 ppm for ^1H and ^{13}C, respectively (Gottlieb HE, Kotlyar V, Nudelman A. J Org Chem. 1997; 62:7512–7515. [PubMed: 11671879]
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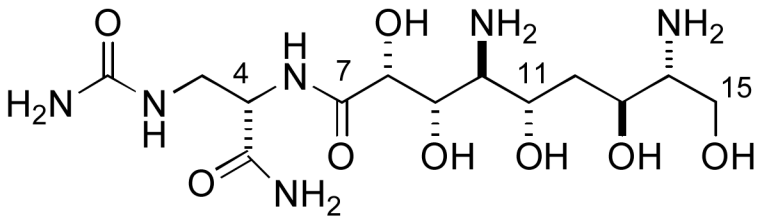


Figure 1.
Natural (+)-zwittermicin A (**1**)

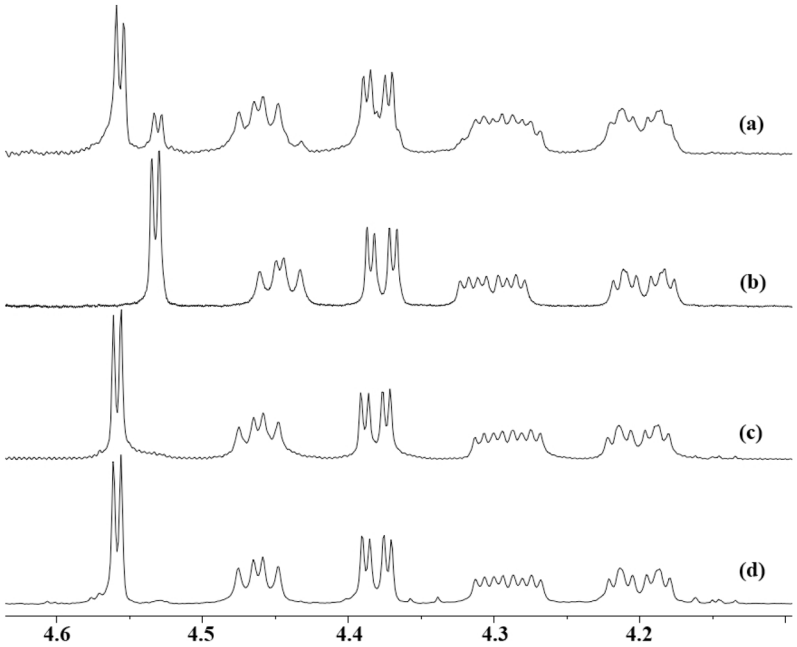
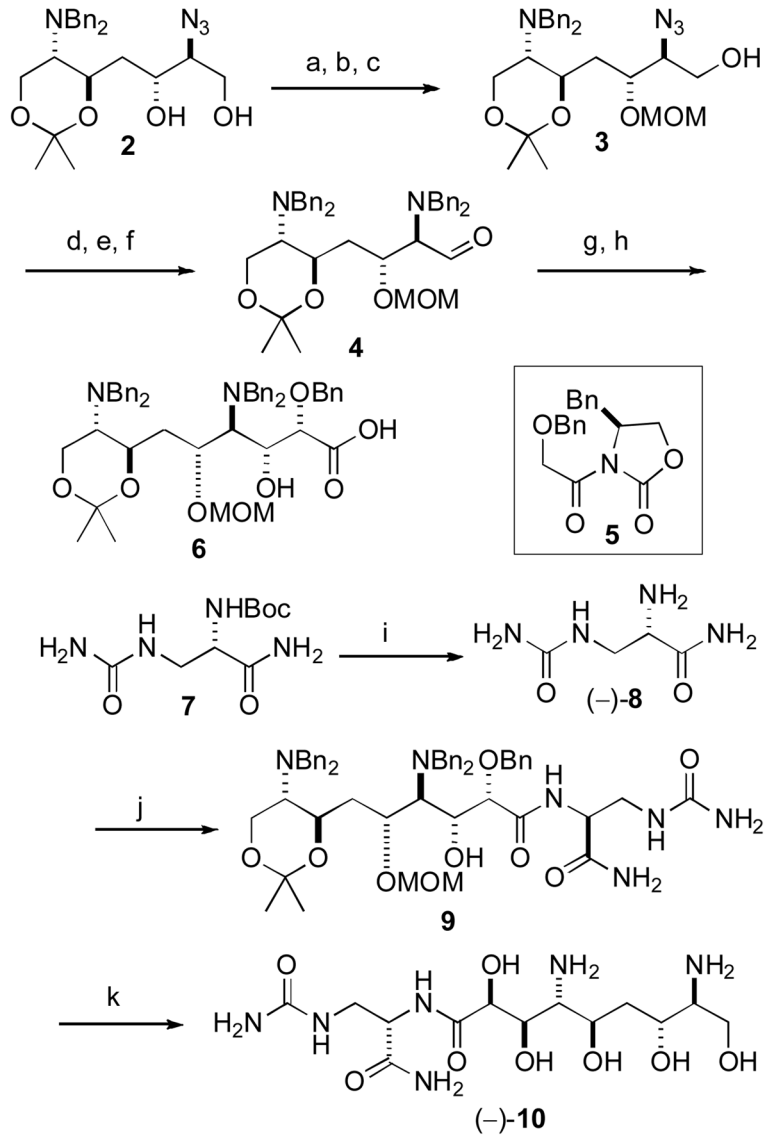


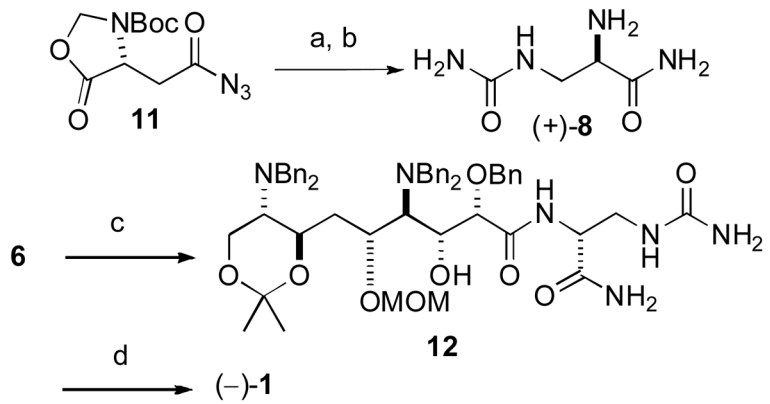
Figure 2.

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



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) (*i*) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, (*ii*) Et_3N, 94%; (g) (*i*) **5**, *n*-Bu_2BOTf, Et_3N, CH_2Cl_2, −78 to 0 °C, 3 h, (*ii*) **4**, −78 to 0 °C, 2.5 h, 77%, dr 24:1; (h) H_2O_2, LiOH, 0 °C, 30 min, 96%; (i) TFA, 0 °C, 1 h, 98%; (j) (*i*) **6**, EDCI, HOBt, DMF, 0 °C, 10 min, (*ii*) (−)-**8**, Et_3N, 0 °C-rt, 1 h, 81%; (k) (*i*) HCl, MeOH, H_2 (5 atm), Pd/C, 1 h, (*ii*) HCl, H_2O, H_2 (5 atm), Pd/C, 1 h, 76%. TBDPSCl= *tert*-butyldiphenylsilyl chloride, EDCI= 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloide, hOBt= 1-hydroxybenzotriazole.



Scheme 2.

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

Table 1

In vitro minimum inhibitory activities of natural (+)-**1** and (−)-**1**, against pathogenic *Candida* species.

Fungal Strains	(+)-1 MIC (μg/ml)[a]	(−)-1 MIC (μg/ml)[a]
Candida albicans 96–489[b]	55.7	>128
C. glabrata	59.5	>128
C. albicans UCDFR1[c]	>128	>128
C. albicans ATCC 14503	>128	>128
C. krusei	>128	>128

[a] Compounds tested as their free-bases. MIC defined as the lowest concentration eliciting 90% growth inhibition.

[b] clinical isolate.

[c] Fluconazole-resistant *Candida* strain raised from *C. albicans* ATCC 14503 by passage through sub-inhibitory Fluconazole. See Supporting Information for details of culture conditions.