Natural Product Synthesis

(+)-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 twocarbon chain extensions occur by condensations of three-

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9500 Gilman Drive, La Jolla, CA, 92093-0358 (USA) Fax: (+1) 858-822-0386 E-mail: tmolinski@ucsd.edu 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 NH₂ 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 $\mathbf{5}^{[10]}$ to $\mathbf{4}$ and subsequent removal of the chiral auxiliary under standard reaction conditions afforded carboxylic acid $\mathbf{6}$ in 74% yield and 92% *de* (over 2 steps),^[11] which is ready for coupling to *N*-ureido-L-1,3-diaminopropionamide ((–)- $\mathbf{8}$), easily derived from the known amide $\mathbf{7}$.^[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 ¹³C NMR spectra (400 MHz, D₂O) 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 ($[\alpha]_D = -23.0^\circ$, H₂O) was opposite in sign and of larger magnitude than values measured for natural (+)-1 ($[\alpha]_D = +8.1^\circ$, H₂O; lit.^[1a] + 8.9°, H₂O) 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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^[**] Financial support for this work was provided by the National Institutes of Health (RO1 AI039987). We are grateful to Prof. T. M. Zabriskie (Oregon State University) and Prof. M. Burkart (UC San Diego) for helpful discussions. HRMS measurements were carried out by R. New (UC Riverside) and Y. X. Su (UC San Diego). We thank D. Manker (Novo Nordisk, Entotech Inc, Davis, CA) for a gift of authentic (+)-1.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200801561.



Scheme 1. Synthesis of (-)-10. Reagents and conditions: a) TBDPSCl, imidazole, DMF, 0°C \rightarrow RT, 4 h, 91%; b) MeOCH₂Cl, Hünig's base, CH₂Cl₂, 0°C \rightarrow RT, 56 h, 98%; c) TBAF, THF, -10°C, 4 h, 95%; d) Lindlar cat., H₂, (1 atm), EtOH, 14 h, 98%; e) BnBr, K₂CO₃, CH₃CN, 31 h, 91%; f) 1. (COCl)₂, DMSO, CH₂Cl₂, -78°C; 2. Et₃N, 94%; g) 1. **5**, *n*Bu₂BOTf, Et₃N, CH₂Cl₂, -78 \rightarrow 0°C, 3 h; 2. **4**, -78 \rightarrow 0°C, 2.5 h, 77%, d.r. 24:1; h) H₂O₂, LiOH, 0°C, 30 min, 96%; i) TFA, 0°C, 1 h, 98%; j) 1. **6**, EDCl, HOBt, DMF, 0°C, 10 min; 2. (-)-**8**, Et₃N, 0°C \rightarrow RT, 1 h, 81%; k) 1. HCl, MeOH, H₂ (5 atm), Pd/C, 1 h; 2. HCl, H₂O, H₂ (5 atm), Pd/C, 1 h, 76%. Bn = benzyl, Boc = *tert*-butoxycarbonyl, DMF = *N*,*N*-dimethylaminopropyl)carbodiimide hydrochloride, HOBt = 1-hydroxybenzotriazole, MOM = methoxymethyl, TBAF = tetra*n*-butylammonium fluoride, TBDPS = *tert*-butyldiphenylsilyl, TFA = trifluoroacetic acid.

topicities and ¹³C 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 ¹³C 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



Figure 2. ¹H NMR spectra (400 MHz, D₂O): 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, NH₃, 30 min; 3. NH₃ (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, Et₃N, 0°C \rightarrow RT, 1 h, 88%; d) 1. HCl, MeOH, H₂ (5 atm), Pd/C, 1 h; 2. HCl, H₂O, H₂ (5 atm), Pd/C, 1 h, 75%.

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 ¹³C signals corresponding to those of natural (+)-zwittermicin A.^[1a]

Finally, the specific rotation of synthetic (-)-1 $([a]_D = -7.9^\circ, H_2O)$ was opposite in sign and equal in magnitude to natural zwittermicin A. Therefore the configuration of zwittermicin A ((+)-1) is $4S_{,8}R_{,9}S_{,1}10S_{,1}13S_{,1}4R$ 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 14R 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]

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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) a 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* ATCC14503 (MIC = 55.7 μ g mL⁻¹) and the fluconazole-resistant strain *C. albicans* 96-489 (MIC = 59.5 μ 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 μ g mL⁻¹) under the same conditions. This interesting result implies that the activity of (+)-1 is not

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

Fungal strains	(+)-1 MIC $[\mu g m L^{-1}]^{[a]}$	(–)- 1 MIC [µg 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* ATCC14053 by passage through subinhibitory fluconazole. See the Supporting Information for details of culture conditions.

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.

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.

Received: April 3, 2008 Published online: September 16, 2008

Keywords: amino alcohols · configuration determination · natural products · polyketides · total synthesis

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