ORGANIC LETTERS

2007
Vol. 9, No. 3
437−440

Asymmetric Synthesis of Diastereomeric Diaminoheptanetetraols. A Proposal for the Configuration of (+)-Zwittermicin A

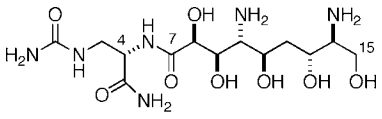
Evan W. Rogers[†] and Tadeusz F. Molinski*,[†]

Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093, and Department of Chemistry, University of California, Davis, One Shields Avenue, Davis, California 95616

tmolinski@ucsd.edu

Received November 17, 2006

ABSTRACT



Zwittermicin A (1)

A proposed absolute configuration for the 7 stereocenters in (+)-zwittermicin A is described based on asymmetric synthesis of six diastereomeric 2,6-diamino-1,3,5,7-heptanetetraols corresponding to the C9–C15 segment, pairwise ^{13}C NMR chemical shift difference analysis of the models with the natural product, interpretation of enantiospecificity of the serine loading domain of the zwittermicin A biosynthetic gene cluster, and degradation of the natural product.

(+)-Zwittermicin A (**1**), a water-soluble natural antibiotic, was isolated from fermentation of the soil-borne bacterium *Bacillus cereus*.[1] Compound **1** is of significant interest for control of crop diseases both as an antifungal agent and an adjuvant with BT toxin for biocontrol.[2]

Despite the appearance of its structure, **1** is not a sugar, but a polyketide derived from a serine starter unit followed by consecutive additions of aminomalonate, malonate, and two hydroxymalonates, each with a concomitant loss of CO_2.[3] Although the original isolation and structure elucidation—with partial relative configuration at C8−C10—were reported 12 years ago, the complete relative and absolute configuration remained unsolved. Neither the natural product nor any of the derivatives prepared to date exhibit crystallinity suitable for X-ray analysis. Applications of "*J*-based" NMR methods for assignment of relative configuration in **1** failed due to lack of stereorelayed scalar couplings across the C12 methylene group: a problem related to the stereotopicity of the corresponding ^1H NMR signals (vide infra).[4] Thus, this rare *diamino*-polyol represents a significant challenge for stereochemical elucidation. Herein, we assign the configuration of **1** using a combination of model synthesis, paired ^{13}C NMR chemical shift comparisons, Marfey's analysis,[5] and a bioinformatic interpretation of the gene sequence for zwittermicin A synthase.[3] A flexible preparation of the C9−C15 core of **1** is revealed that exploits Miyashita conditions

[†] Present address: University of California, San Diego.

(1) (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.

(2) (a) Silo-Suh, L. A.; Stabb, E. V.; Raffel, S. J.; Handelsman, J. *Curr. Microbiol.* **1998**, *37*, 6. (b) Broderick, N. A.; Goodman, R. M.; Raffa, K. F.; Handelsman, J. *Environ. Entomol.* **2000**, *29*, 101. (c) Stohl, E. A.; Brady, S. F.; Clardy, J.; Handelsman, J. *J. Bacteriol.* **1999**, *181*, 5455.

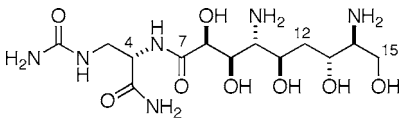
(3) (a) Emmert, E. A.; Kilmowicz, A. K.; Thomas, M. G.; Handelsman, J. *Appl. Environ. Microbiol.* **2004**, *70*, 104. (b) Stohl, E. A.; Milner, J. L.; Handelsman, J. *Gene* **1999**, *237*, 403.

(4) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866. HETLOC or HMBC experiments (600 MHz, D_2O) did not extract useful $^{2,3}J_{CH}$ couplings across the C11−C12−C13 sequence, while $^1H-^1H$ couplings to the C12 methylene group showed second-order effects as discusssed later in the text.

(5) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591.

10.1021/ol062804a CCC: $37.00
Published on Web 01/11/2007

for regioselective 2,3-epoxy-1-alkanol ring opening by azide and is amenable for the total synthesis of the natural product.



Zwittermicin A (**1**)

The *pseudo*-symmetry present in the C9−C15 portion of **1** suggested a unified strategy for construction of six model compounds that embody all possible relative configurations for the pair of diads: C10,11 and C13,14.

With the exception of C11, the ^{13}C NMR chemical shifts of these remote centers are expected to be relatively independent of the remainder of the molecule. Consequently, pairwise comparison of chemical shifts in the models with the corresponding values in **1** should converge upon a unique configurational assignment.

The six models **2**−**7** were synthesized starting with serine (Scheme 1). *O*-TBS-*N*,*N*-dibenzylserinal (**8**), prepared from L-serine, was converted to epoxide **9** by using the method of Concellón.[6,7] Carbon chain extension of **9** by a BF_3·Et_2O-mediated epoxide opening with the anion derived from *O*-TBS propargyl ether afforded **10**. Protecting group adjustment followed by Red-Al reduction of the triple bond gave *E*-olefin **11**, which was treated with *m*-CPBA to give diastereomeric epoxides **12** and **13** in a ratio of 1:1.8.

Separation of the diastereomers required protection of the primary alcohol and HPLC separation followed by deprotection to give the pure epoxides. Regioselective elaboration of the contiguous 2-amino-1,3-diol motif was projected based on Miyashita's boron-directed azide opening of 1,2-epoxyalkanols.[8] In the event, separate azide opening of epoxides **12** and **13** with Miyashita's method provided 1,3-diols **14** (regioselectivity 9:1) and **15** (regioselectivity 2.3:1), respectively, in good yields.[9] Acid-catalyzed deprotection of **14** and **15**, with concomitant hydrogenolysis of the benzyl and azido groups, afforded models **2** and **3**, respectively, as their hydrochloride salts. The configurations of the two diastereomers were readily apparent by ^{1}H and ^{13}C NMR spectroscopy, which revealed C_{2v} symmetry in **2**.

The remaining four models were synthesized from the L-serine methyl ester derivative **16** (Scheme 2) by using the complementary *syn*-selective epoxide formation[7] to provide **17**, the C2 epimer of **9**. Chain extension of **17** was achieved as before to give propargyl alcohol **18**, which was separately converted to *E*- and *Z*-allylic alcohols **19** and **20** by Red-Al reduction or hydrogenation over Lindlar catalyst, respectively. Epoxidation of olefin **19** with *m*-CPBA was less successful due to the lability of the diastereomeric products; however, oxidation of **19** with methyltrioxorhenium gave a mixture of distereomeric epoxides that was carried forward with Miyashita's method followed by acetonide protection to give azides **21** and **22**. Since neither of the two diaminotetraols anticipated from conversion of **21** and **22** were expected to show symmetry (C_1 space group), the configu-

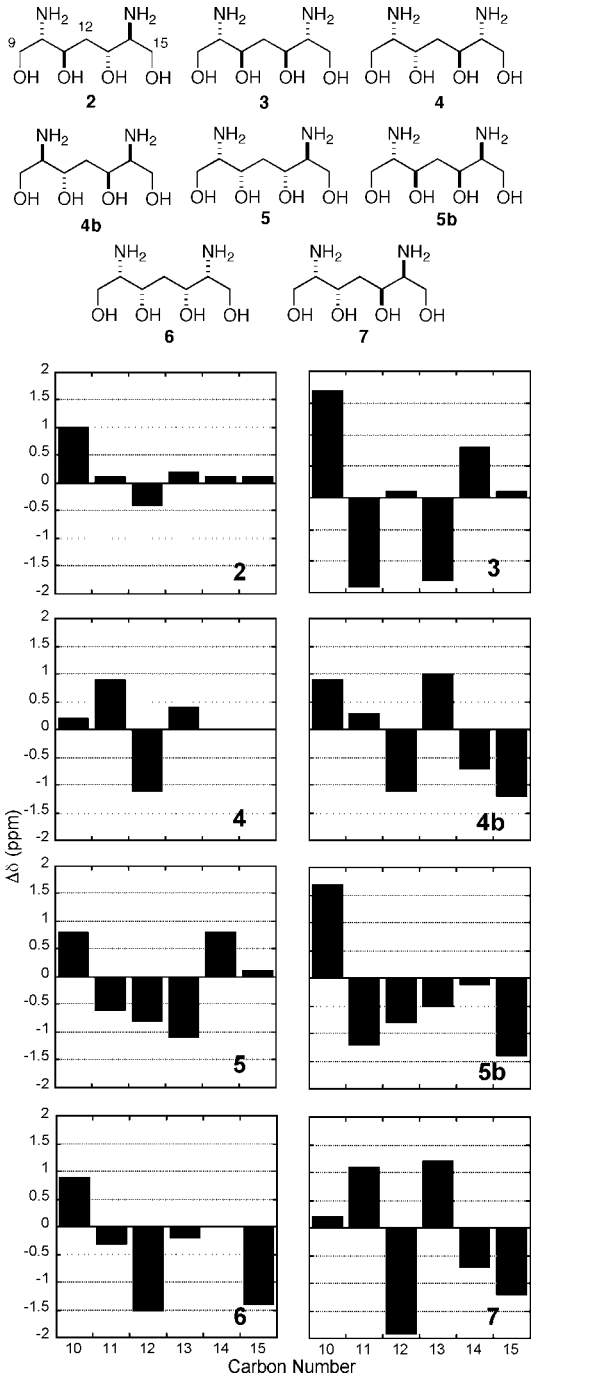


Figure 1. ^{13}C NMR (100 MHz, D_2O, reference internal CH_3CN, δ 1.47 ppm) $\Delta\delta$ values (δ_C model − δ_C **1**) of model compounds **2**−**7**. "**4b**" and "**5b**" are "virtual isomers" of **4** and **5**, respectively, by reversing the order of ^{13}C δ assignments for the purpose of comparison with **1**.

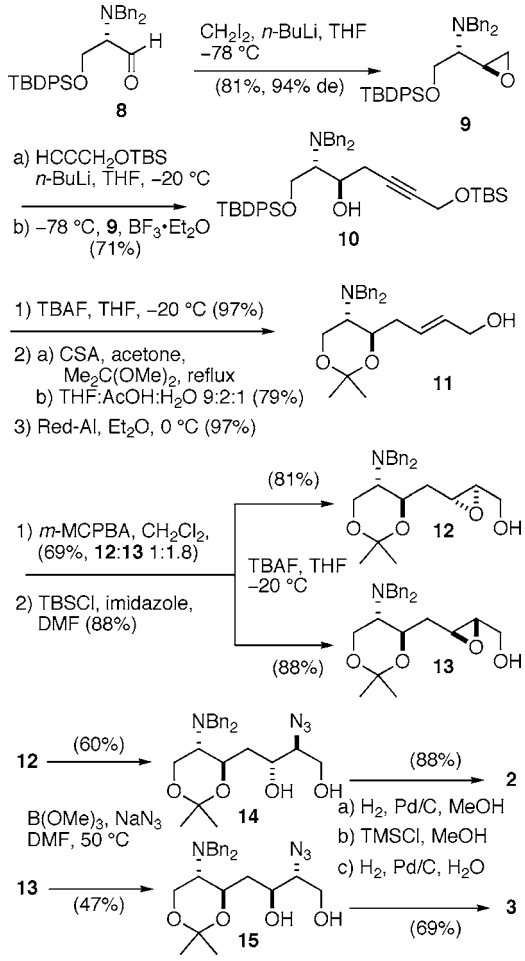
(6) (a) Hulme, A. N.; Montgomery, C. H.; Henderson, D. K. *J. Chem. Soc., Perkin. Trans. 1* **2000**, 1837. (b) Laïb, T.; Chastanet, J.; Zhu, J. *J. Org. Chem.* **1998**, *63*, 1709.

(7) Concellón, J. M.; Riego, E.; Rodríguez-Solla, H.; Plutín, A. M. *J. Org. Chem.* **2001**, *66*, 8661.

(8) Sasaki, M.; Tanino, K.; Hirai, A.; Miyashita, M. *Org. Lett.* **2003**, *5*, 1789.

(9) Under standard conditions, in the absence of $B(OMe)_3$, yields and regioselectivity were poor (e.g., **12** → **14**; NH_4Cl, NaN_3, DMF, 44%, regioselectivity 1:1.4).

Scheme 1



rational assignments of these molecules from NMR were in doubt. Fortunately, azide **22** crystallized as colorless needles (mp 138 °C) and X-ray analysis (Figure 2) provided the configuration of the 4-substituted (4*R*,5*S*)-2,2-dimethyl-5-azidodioxane ring [(13*R*,14*S*), zwittermicin A numbering]. It follows that **21** is the (4*S*,5*R*)-diastereomer.

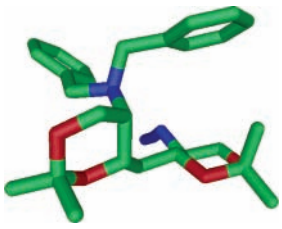
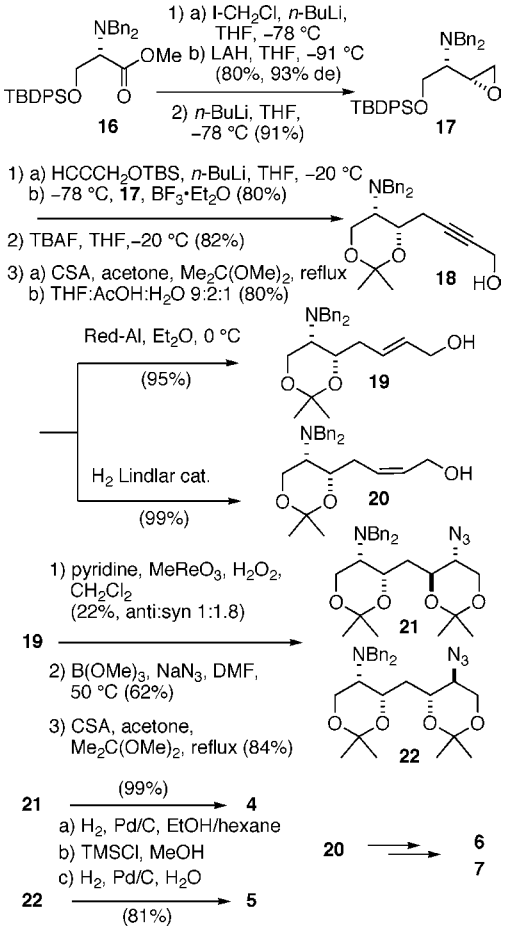


Figure 2. X-ray structure of **22**.

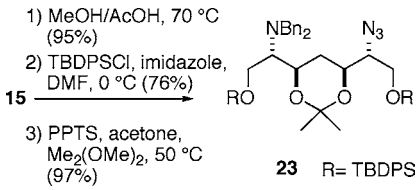
To further verify stereochemical assignments of the models, azide **15** was converted to the acetonide **23** (Scheme 3). The ^{1}H NMR spectrum of **23** showed the expected large diaxial vicinal couplings (δ 4.14, ddd, J = 10.4, 8.0, 2.4 Hz; δ 3.83, ddd, J = 11.6, 6.4, 2.4 Hz) for a *syn*-4,6-

Scheme 2



disubstituted 1,3-dioxane and large ^{13}C chemical shift differences for the *gem* CH_3 signals of the isopropylidene group (δ 29.9, q; 19.7, q).[10]

Scheme 3



Acid-catalyzed global deprotection and hydrogenolysis of **21** and **22** compounds provided models **4** and **5**, respectively, as their HCl salts. Models **6** and **7** were synthesized from olefin **20** with the same approach.

The diastereomeric family of model compounds comprise two *meso* compounds (**3** and **6**), two C_2 isomers (**2** and **7**), and two isomers lacking symmetry (C_1, **4** and **5**). As expected, the ^{1}H NMR signal of the C4 methylene protons

(10) Rychnovsky, S. D.; Rogers, B.; Yang, G. *J. Org. Chem.* **1993**, *58*, 3511−3515.

in each C_2 isomer (e.g., **2**, δ 1.66 m, 2H) appeared as a complex second-order multiplet owing to the fact that the H4 protons were chemical-shift equivalent but magnetically inequivalent. Conversely, the 4-CH_2 protons in the *meso* isomer **3** are both chemical shift inequivalent and magnetically inequivalent and appear as diastereotopic protons exhibiting a first-order ABX_2 pattern (δ 1.79 dt 1H, J = 14.4, 8.4 Hz; δ 1.84, dt, J = 14.4, 4.7 Hz, 1H). Analogous patterns were observed for C_2-symmetrical **7** and *meso*-**6**. Interestingly, the ^{1}H NMR signal of the corresponding C12 methylene group in **1** also exhibited a complex second-order pattern (400, 500, and 600 MHz) similar to those of **2** and **7**, but dissimilar to the 4-CH_2 signals of **3** and **6**, suggesting that the spin systems in **1**, **2**, and **7** reflected local C_2 or *pseudo*-C_2 symmetry, largely dictated by an *anti* relationship of the C11 and C13 OH groups.

An unequivocal assignment of relative configuration for the diaminotetraol segement in **1** was made by pairwise comparisons of the differences in the ^{13}C chemical shifts ($\Delta\delta$) for C10−C15 of **1** and model compounds (Figure 1).[11] There are only six diastereomers of the symmetrically substituted diaminoheptanetetraol models but eight diastereomers of the C10−C15 segment in **1**. To complete the comparison, the ^{13}C δ assignments of C_1 isomers **4** and **5** were reversed to give the remaining two isomers—virtual compounds "**4b**" and "**5b**". The C_{2v} symmetric **2** is the only model compound with a close match to **1** for every carbon (Figure 1) except C9, which is the point of difference between **1** and the models and expected to show an "outlying" $\Delta\delta$ in every case.

Importantly, the other C_{2v} isomer **7** had the largest mismatch, which secures confidence for assignment of *erythro* relationships in each of the C10,11 and C13,14 diads. Elimination of the mismatched *meso* isomers **3** and **6**, as suggested by ^{1}H NMR and stereotopicity analysis of the 12-CH_2 signal (above), is now corroborated by ^{13}C NMR.

Therefore, compounds **1** and **2** share the same relative configuration at the stereogenic centers corresponding to C10, C11, C13, and C14 of **1**. The data in Figure 1, in conjunction with the relative configurations at C8−C10,[1] now allow us to extend the assignment of relative configuration of **1** to C8−C15.

Although no direct evidence for the absolute configuration of C8−C15 is yet available, analysis of the published sequence of the gene cluster for biosynthesis of zwittermicin A highly suggests that the C14 shares the same configuration as L-serine.[3] Zwittermicin A is synthesized by a hybrid polyketide synthase−nonribosomal peptide synthase (PKS−NRPS) that comprises nine open reading frames including a loading domain for the starter unit that is homologous with serine adenylation domains found in gene clusters for biosynthesis of iturin A and mycosubtilin. Since the proposed gene sequence for production of **1** shows C13−C15 originating from L-serine and epimerase domains are absent, it is highly likely that C14 is L and the absolute stereochemistry for C8−C14 in **1** is as depicted.

The absolute configuration at the remaining C4 stereocenter in **1** was determined as 4*S* by Marfey's analysis.[5] Acid hydrolysis of authentic **1** (6 N HCl, 24 h, 110 °C) and derivatization of the products with 2,4-dinitrophenyl-5-fluoro-L-alaninamide (Marfey's reagent) under standard conditions, followed by analysis (C_{18} HPLC-MS) gave one peak that matched the peak (coinjection, MS spectrum) obtained by similar treatment of commercially available (−)-(*S*)-N^3-ureido-2,3-diaminopropionic acid (*S*-albizziin).

In conclusion, we have assigned the configuration of **1** as (4*S*,8*S*,9*R*,10*R*,11*R*,13*R*,14*S*). using an integrated approach based on synthesis and pairwise comparisons with model compounds, Marfey's analysis, and published data. This sets the stage for completion of **1** by chain extension of a suitably protected derivative of **2** and attachment of the N^3-ureido-2,3-diaminopropionamide side chain, which is the subject of current research in our laboratories.

Acknowledgment. We thank Mark Zabriskie (Oregon State University) for helpful discussions, and Entotech, Inc. (Davis, CA) for a sample of authentic zwittermicin A. X-ray analysis was carried out by A. Rheingold (UCSD). HRMS measurements were carried out by R. New (UC Riverside MS Facility), Y. Su (UC San Diego MS Facility), and the Scripps Center for Mass Spectrometry (La Jolla, CA). The UC Davis 400 MHz NMR and LCMS were purchased with funds provided by instrument grants NSF CHE-9808183 and RR14701-01, respectively. This work was supported by a grant from the NIH (to T.F.M., RO1 AI39987) and a fellowship from the Ecotoxicology Lead Campus Program (to E.W.R., UC Davis).

Supporting Information Available: Experimental procedures, X-ray data for **22**, and selected ^{1}H and ^{13}C NMR. This material is available free of charge via the Internet at http://pubs.acs.org.

OL062804A

(11) ^{13}C NMR measurements of the hydrochloride salts of **1** and models **2**−**7** were carried out under essentially identical conditions (D_2O, 25 °C) with internal CH_3CN as reference.