Total Synthesis of Alterobactin A, a Super Siderophore from an Open-Ocean Bacterium†

Jingen Deng, Yasumasa Hamada,* and Takayuki Shioiri*

Department of Synthetic Organic Chemistry, Faculty of Pharmaceutical Sciences, Nagoya City University Tanabe-dori, Mizuho-ku, Nagoya 467, Japan

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Alterobactin A was recently isolated by Butler and co-workers from an open-ocean bacterium, *Alteromonas luteoviolacea*, together with alterobactin B.[1a] The structure of alterobactin A was determined to be **1** by spectroscopic methods,[1b] and it was identified as a siderophore[2] possessing an extraordinary affinity for ferric ion.[1b] Alterobactin A (**1**) contains two unusual amino acids: L-threo-β-hydroxyaspartic acid (L-threo-β-OH-Asp) and (3*R*,4*S*)-4,8-diamino-3-hydroxyoctanoic acid (lysinestatine, Lys-Sta), attaching a catechol carboxylate at the ω-amino site. We now report the first total synthesis of **1**.

A [4 + 3] convergent strategy was adopted in our synthesis of **1** (see Figure 1 and Scheme 5). We thought that coupling of two fragments and macrocyclization should be accomplished between Gly-Arg and Gly-β-OH-Asp,[3] respectively, since the construction of the amide bonds at Gly as a C-terminal would proceed without both racemization and steric constraint. In addition, a β-turn structure existing in **1** along the Gly-Arg-β-OH-Asp-Gly sequence[1b] probably facilitates the macrocyclization of the linear precursor.[4] Furthermore, the synthetic challenge to alterobactin A (**1**) may be the appropriate protection of the various reactive groups involving one amino group, one guanidino group, two phenolic groups, three hydroxyl groups, and two carboxyl groups.

First, we synthesized the β-OH-Asp derivative **6**, as shown in Scheme 1. A key intermediate, **3**, was obtained from methyl cinnamate (**2**) using the enantiocontrolled Sharpless dihydroxylation (>98% ee) with AD-mix-β,[5a] followed by stereo- and regioselective transformations.[5b] Treatment of the bromo ester **3** with sodium azide, followed by hydrogenation and simultaneous protection, afforded the Boc-amino ester **4**. Ruthenium-catalyzed oxidation of **4** and subsequent protection by use of *O-tert*-butyl-*N*,*N*′-diisopropylisourea (BDIU)[2,6] gave the fully protected β-OH-Asp derivative **5**, which underwent deprotection and reprotection in five steps to yield the desired building block **6** (>98% de).

Synthesis of the Lys-Sta unit **9** (Scheme 2) started from the known[7] β-keto ester **7**, which was reduced with $NaBH_4$ at low temperature to give a mixture of diastereomers with 69% diastereomeric excess.[8] Separation by flash chromatography on silica gel was effectively accomplished to give the (3*R*,4*S*)-Lys-Sta derivative **8** as a predominant isomer (>95% de).[9] The

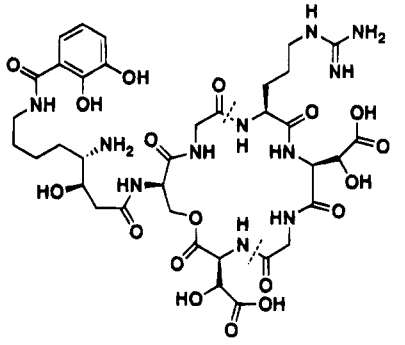
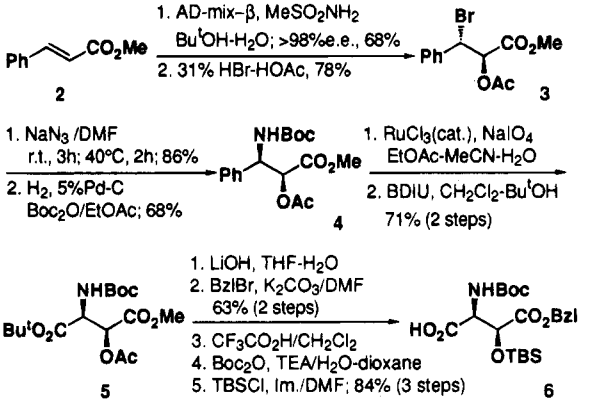
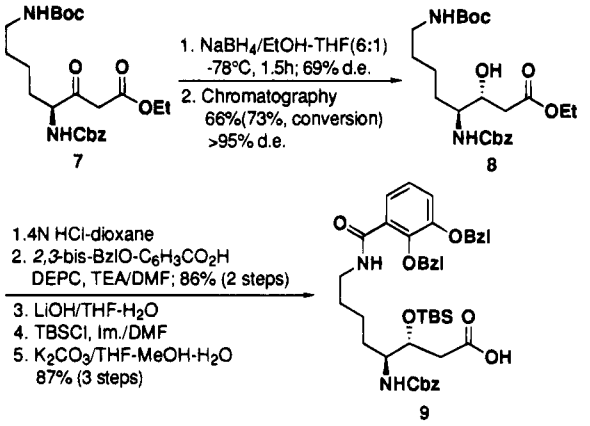


Figure 1. Alterobactin A (**1**).

Scheme 1



Scheme 2



required Lys-Sta building block **9** was obtained from **8** through (1) acidic removal of the Boc group, (2) coupling with 2,3-bis-(benzyloxy)benzoic acid with diethyl phosphorocyanidate (DEPC, $(C_2H_5O)_2P(O)CN$),[10] (3) alkaline hydrolysis, (4) treatment with *tert*-butyldimethylsilyl (TBS) chloride, and (5) alkaline deprotection of the TBS ester.

The tripeptide **11** was prepared from *N*-Boc-glycine trichloroethyl (Tce) ester (Boc-Gly-OTce, **10**) by attachment of Boc-D-serine and then the Lys-Sta derivative **9** through acidic deprotection of the N-terminal Boc group and coupling by use of DEPC. Condensation of **11** with the β-OH-Asp derivative **6** was effectively carried out by use of *N*-ethyl-*N*′-(3-(dimethylamino)propyl)carbodiimide (EDCI) to give the left-side depsitetrapeptide **12**, shown in Scheme 3.

The construction of the right-side tripeptide **15** was also started from Boc-Gly-OTce (**10**), which underwent the Boc

† Dedicated to Sir Derek Barton on the occasion of his 77th birthday (Kiju in Japanese).

(1) (a) For isolation and purification of alterobactins, see: Reid, R. T.; Butler, A. *Limnol. Oceanogr.* **1991**, *36*, 1783−1792. (b) For determination of structure and iron affinity, see: Reid, R.T.; Live, D. H.; Faulkner, D. J.; Butler, A. *Nature* **1993**, *366*, 455−458. (See also *Chem. Br.* **1994**, *30*, 174.)

(2) For reviews on the synthesis of phytosiderophores, see: (a) Shioiri, T.; Matsuura, F.; Hamada, Y. *Pure Appl. Chem.* **1994**, *66*, 2151−2154. (b) Shioiri, T.; Hamada, Y.; Matsuura, F. *Tetrahedron* **1995**, *51*, 3939−3958.

(3) Symbols and abbreviations are in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature. *Eur. J. Biochem.* **1984**, *138*, 9−37.

(4) Cf. Kopple, K. D. *J. Pharm. Sci.* **1972**, *61*, 1345−1356.

(5) (a) Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.; Kwong, H.; Morikawa, K.; Wang, Z.; Xu, D.; Zhang, X. *J. Org. Chem.* **1992**, *57*, 2768−2771. (b) Fleming, P. R.; Sharpless, K. B. *J. Org. Chem.* **1991**, *56*, 2869−2875.

(6) Mathias, L. J. *Synthesis* **1979**, 561.

(7) Maibaum, J.; Rich, D. H. *J. Org. Chem.* **1988**, *53*, 869−873.

(8) The diastereoselectivity was dependent not on the solvent system (64, 66, and 66% de in THF/EtOH 1:3, 1:5, and 1:10, respectively) but on the protective groups.[7]

(9) The configuration was determined by ^1H NMR analysis of the corresponding oxazolidinone. Cf. Dufour, M.-N.; Jouin, P.; Poncet, J.; Pantaloni, A.; Castro, B. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1895−1899.

(10) Takuma, S.; Hamada, Y.; Shioiri, T. *Chem. Pharm. Bull.* **1982**, *30*, 3147−3153 and references therein.

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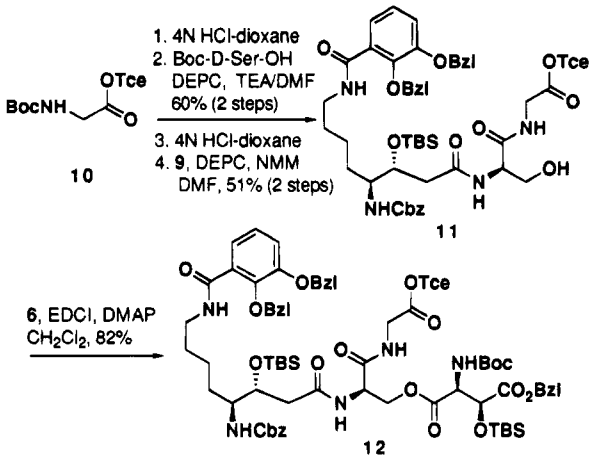
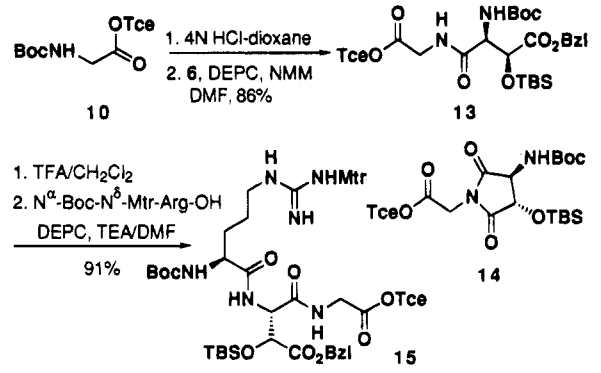
Scheme 3



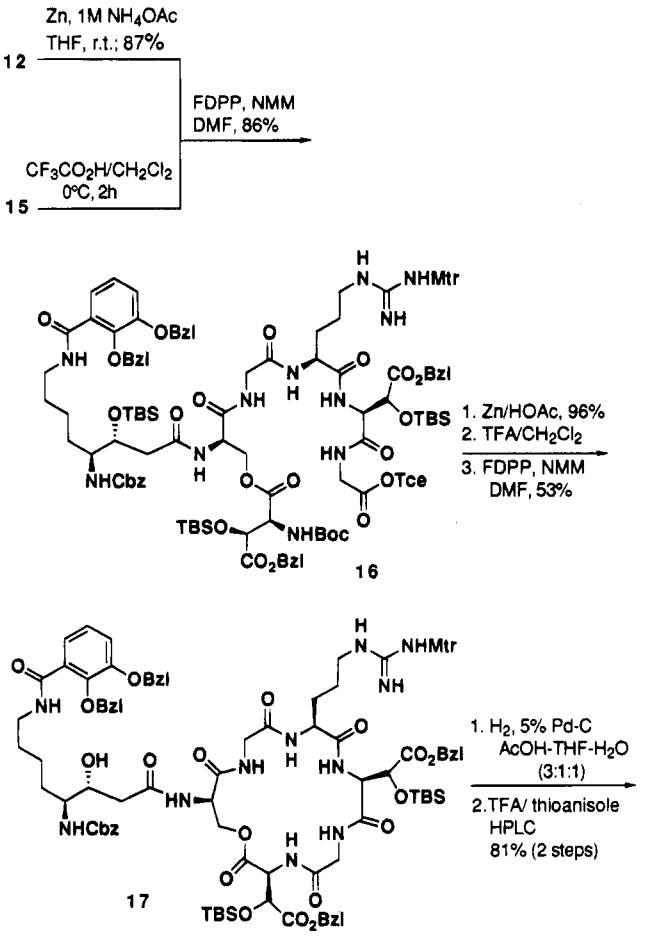
Scheme 4



deprotection followed by coupling with the β-OH-Asp derivative **6** by use of DEPC and triethylamine (TEA). The products were an inseparable mixture of the dipeptide **13** and aspartimide derivative **14** in a ratio of 7:1.[11] However, formation of the undesired aspartimide **14** could be entirely suppressed when TEA was replaced with *N*-methylmorpholine (NMM) (**13**:**14** > 120:1). After selective deprotection of the Boc group of **13** with trifluoroacetic acid (TFA), coupling with N^{α}-Boc-N^{δ}-2,3,6-trimethyl-4-methoxybenzenesulfonyl(Mtr)arginine afforded the right-side tripeptide **15** in high yield (Scheme 4).

Deprotection of the Tce group from **12** was achieved by use of zinc powder, while treatment of **15** with *p*-TsOH·H_2O removed the both Boc and TBS groups. Coupling of the deprotected **12** with the deprotected **15** resulted in the concomitant formation of the aspartimide derivative at the β-OH-Asp-Gly sequence. This implied that the steric protection of the hydroxyl group on the β-OH-Asp-residue could suppress the aspartimide formation.[12] Fortunately, selective deprotection of the Boc group from **15** was accomplished by use of TFA in CH_2Cl_2[13] without liberation of the Mtr and TBS groups, shown in Scheme 5. Condensation of the two deprotected building blocks smoothly proceeded with pentafluorophenyl diphenylphosphinate (FDPP)[14] to give the linear depsiheptapeptide **16**. After removal of the Tce group at the C-terminus with zinc and then removal of the Boc group at the N-terminus with TFA, which simultaneously cleaved the TBS group at the side chain, the macrolactamization was effectively achieved by use of FDPP,[14] to afford the macrocyclic depsipeptide **17** in good yield.

Scheme 5



Alterobactin A (1)

Finally, removal of all the protective groups in two steps and purification by preparative HPLC provided alterobactin A (**1**) in 81% yield, identical to the natural product by 500 MHz ^{1}H NMR, 125 MHz ^{13}C NMR, high-resolution FAB mass spectra, HPLC,[15] and TLC. Interestingly, we found that the pH value in the solution strikingly affects the chemical shifts of the protons on the two β-OH-Asp moieties.

Our synthesis of alterobactin A not only has proved the proposed structure **1**, but also promises availability of this super siderophore in quantities which will be useful for the elucidation of the siderophoric mechanism in bacteria.

Acknowledgment. We thank Professor Alison Butler of University of California, Santa Barbara, for kindly sending us the sample of natural alterobactins and their NMR spectra. The high-resolution FAB mass of alterobactin A was determined by Dr. Hideaki Murata of Meijo University, to whom the authors' thanks are due. J.D. is grateful to the Japan Society for the Promotion of Science for a postgraduate fellowship. This work was financially supported in part by the Ministry of Education, Science and Culture, Japan.

Supporting Information Available: Experimental procedures, characterization data ^{1}H NMR, ^{1}H−^{1}H COSY, ^{13}C NMR, ^{1}H−^{13}C COSY, and HMBC spectra for synthetic alterobactin A, ^{1}H NMR spectra of natural alterobactin A and compounds **6**, **9**, **12**, **15**, and **17**, and ^{1}H−^{1}H COSY spectrum of **17** (29 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be obtained from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(15) Column: YMC-pack C_4, 150 × 4.6 mm i.d.; mobile phase MeCN/H_2O 5:95 containing 0.1% TFA; flow rate, 1 mL/min; detection, UV 280 nm. Retention times: synthetic alterobactin A, 12.67 min; natural product, 12.69 min; mixture, 12.80 min.