An Efficient Synthesis of Alterobactin A; A Super Siderophore of Marine Origin

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Abstract: Alterobactin A (**1**), a cyclic depsipeptide and a super siderophore isolated from an open-ocean bacterium, was efficiently synthesized for the first time by a convergent manner with the maximum protection of various functional groups.

Key words: alterobactin A, depsipeptidic siderophores, macrolactamization, selective OH protection and deprotection

Alterobactin A (**1**) was isolated together with alterobactin B (**2**) by Butler and co-workers from an open-ocean bacterium *Alteromonas luteoviolacea* collected off Chub Cay, Bahamas and identified as depsipeptidic siderophores[1] (Figure 1). Alterobactin A (**1**) has extraordinary affinity for ferric ion ($K_{Fe} = 10^{49–53}$), while alterobactin B (**2**) has a comparatively lower affinity for iron and may not be a true siderophore because it is formed by the base hydrolysis of alterobactin A (**1**) in the absence of bound iron(III). With the possible exception of enterobactin,[2] the ferric ion affinity of alterobactin A (**1**) is not exceeded by any other known siderophore and it is likely that the siderophores produced by open-ocean marine bacteria that live in an extremely low-iron environment may well have evolved unique structures with exceptional ferric ion affinities. Alterobactin A (**1**) is a macrocyclic depsipeptide, which contains two types of unusual amino acids (Figure 2), two L-threo-β-hydroxyaspartic acid (**3**, β-OH-Asp) and one (3*R*,4*S*)-4,8-diamino-3-hydroxyoctanoic acid (**4**, lysinestatine, LysSta) attached to a catechol carboxylate at the N^{ω}-site, through which ferric ion is coordinated to form a six-coordinate complex.[1b] Our interest in the exploitation of new methodology for the synthesis of aquatic natural products containing non-ribosomal amino acids[3] and siderophores[4] led us to investigate the synthesis of alterobactin A (**1**), a super siderophore of marine bacteria origin. Herein, we report the details of our synthetic work on alterobactin A (**1**).[5]

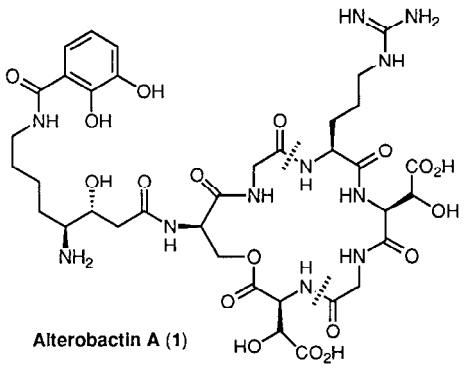


Figure 1. Structures of Alterobactin A **(1)** and B **(2)** and the Disconnection Crucial for the Convergent Synthesis of Alterobactin A **(1)**

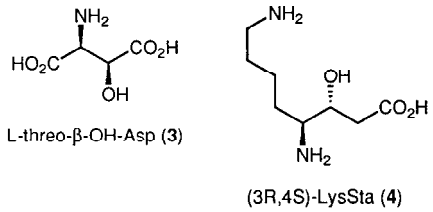


Figure 2. Structure of the Unusual Amino Acids **3** and **4**

Strategy

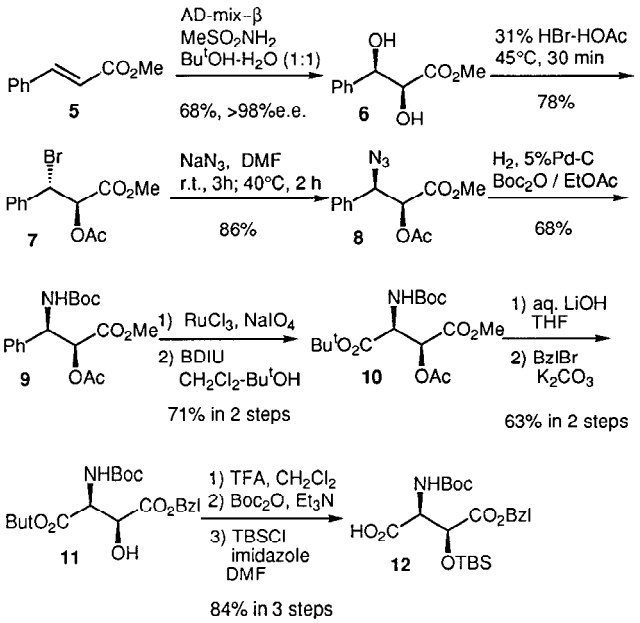
A [4+3] convergent strategy was adopted in our synthetic approach to alterobactin A (**1**). Fragment coupling of the two segments **28** and **31** was accomplished between Gly and Arg while macrolactamization was carried out between Gly and β-OH-Asp,[6] because the construction of the amide bonds at glycine as C-terminus would proceed without both racemization and steric constraint. Furthermore, a β-turn structure in alterobactin A (**1**) along Gly-Arg-β-OH-Asp-Gly sequence[1b] probably facilitates the macrolactamization of the linear precursor.[7] Alterobactin A (**1**) contains various reactive functional groups: one amino group, one guanidino group, two carboxyl groups, two phenol groups, and three hydroxyl groups. Thus, the choice of protecting groups, especially amino and carboxyl groups, was viewed as very critical to our ultimate success. It is necessary that the γ-amine of LysSta and the β-carboxylates of two β-OH-Asp should be kept inert in the amide-coupling process, and the phenol groups and hydroxyl groups should also be kept inert in the ester-forming step. The carbobenzoxy (Cbz) group was chosen to protect the γ-amino group of LysSta because it would per-

mit the selective deprotection of a *tert*-butyloxycarbonyl (Boc) group at the N-terminus of the peptide segments under acidic conditions. The benzyl (Bzl) group was chosen to block the carboxylates of two β-OH-Asp, whereas the 2,2,2-trichloroethyl (Tce) group was chosen to block the C-terminal carboxylates of the peptide segments, because the Bzl group would be kept intact under the conditions which cleave the Tce group with zinc. The *tert*-butyldimethylsilyl (TBS) or *tert*-butyldiphenylsilyl (TBDPS) group and the Bzl group were chosen to protect the other three hydroxyl groups and two phenol groups, respectively. The protection of the two phenol groups and the two carboxylates by Bzl, protection of the γ-amine by Cbz, and the three hydroxyl groups by TBS, set the stage for removing these protecting groups simultaneously through hydrogenation in aqueous HOAc/THF at the penultimate step of the synthesis. Although most sulfonyl groups are suitable to protect the guanidino function of arginine in our approach, the 2,3,6-trimethyl-4-methoxybenzenesulfonyl (Mtr) group is considered to be favorable, because removal of the Mtr group could be readily performed under relatively mild conditions,[8] and also there is no problem in selective deprotection of the Boc group.[9] The organophosphorus reagents, diethyl phosphorocyanidate [DEPC, $(EtO)_2$- P(O)CN][10] and pentafluorophenyl diphenylphosphinate [FDPP, $Ph_2P(O)OC_6F_5$],[11] could be applied here in the fragment-coupling and macrolactamization processes, respectively.

Synthesis

L-Threo-β-hydroxyaspartic Acid:

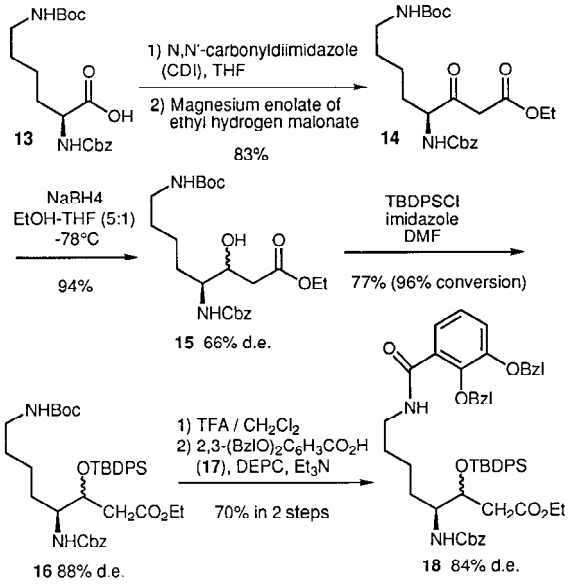
First, we synthesized the unusual amino acid β-OH-Asp derivative **12** from commercially available methyl cinnamate (**5**) through the stereocontrolled Sharpless dihydroxylation[12] and the transformation of the phenyl group, a carboxyl synthon,[13] to the carboxylate group (Scheme 1). The diol derivative **6** was obtained by the Sharpless asymmetric dihydroxylation of **5** by use of AD-mix-β in 68% yield with >98% ee. The enatiomeric excess (ee) was determined by ^{1}H NMR analysis of the bis-MTPA esters.[14] The bromo ester **7** was prepared from **6** by a stereo- and regioselective transformation with 31% HBr/HOAc[15] and then stereoselectively converted into the azido ester **8** with excess sodium azide. Hydrogenation and simultaneous protection of the resulting amino group in a one-pot process afforded the Boc-amino ester **9** in good yield and no *N*-acetyl product was produced.[11b] Ruthenium-catalyzed oxidation[13] of **9** and subsequent protection by using *O-tert*-butyl-*N*,*N*′-diisopropylisourea (BDIU) smoothly gave the fully protected β-OH-Asp derivative **10** in 71% yield, which was treated with aqueous lithium hydroxide in THF, followed by treatment with benzyl bromide and potassium carbonate to give the β-OH-Asp β-benzyl ester **11**. The desired building block, β-OH-Asp derivative **12** was produced in 84% yield by the simultaneous deprotection of the Boc group and *tert*-butyl ester, followed by the selective protection of the amino group as Boc and the hydroxyl group as the TBS group, respectively.



Scheme 1

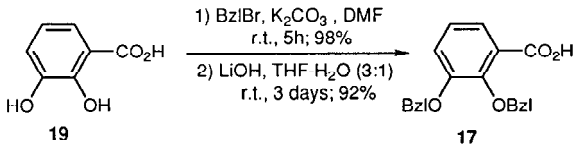
(3*R*,4*S*)-Lysinestatine:

Another building block (3*R*,4*S*)-LysSta derivative **21** was synthesized by the stereoselective reduction of the β-keto ester **14** and subsequent coupling with the catecholate **17**. The β-keto ester **14** was readily prepared from commercially available N^{α}-Cbz-N^{ω}-Boc-lysine (**13**) according to Rich.[16a] Reduction of **14** with sodium borohydride in EtOH/THF at low temperature afforded a mixture of diastereomers **15**,[16] whose selectivity was not influenced by the ratio of the solvents: ethanol/tetrahydrofuran (1:3, 64% de; 1:5, 66% de; 1:6, 69% de and 1:10, 66% de). Initially, the hydroxyl group of **15** was protected as the TBDPS group, and interestingly, the product **16** enriched in the (3*R*,4*S*)-diastereomer (88% de) was obtained. However, the mixture of diastereomers **16** was inseparable,[17] even if **16** was converted to its catecholate derivative **18** with 84% de (Scheme 2) by coupling with 2,3-



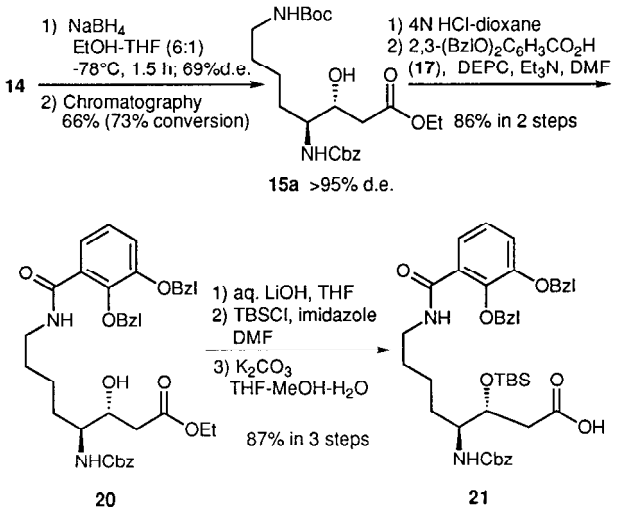
Scheme 2

bis(benzyloxy)benzoic acid (**17**), which was readily prepared from 2,3-catecholic acid (**19**) via protection of both the hydroxyl and carboxyl groups and then selective deprotection of the carboxylate group (Scheme 3).

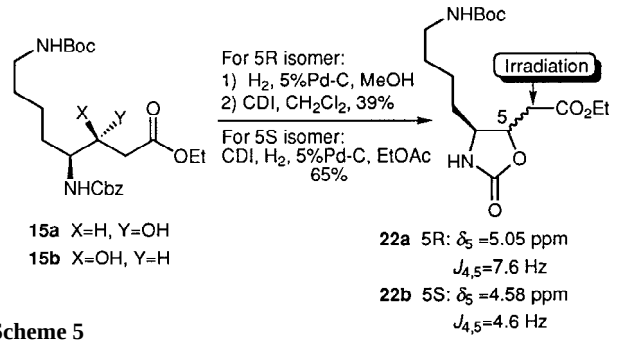


Scheme 3

Fortunately, the separation of the diastereomers **15** was effectively accomplished by flash chromatography on silica gel to provide the unusual amino acid subunit, (3*R*,4*S*)-LysSta derivative **15a** as the predominant isomer with >95% de (Scheme 4). The catecholate derivative **20** was produced by the deprotection of the Boc group of **15a** and subsequent coupling with **17**. Conversion of **20** to the required LysSta building block **21** was effected by hydrolysis of the ethyl ester and subsequent blocking of the hydroxyl group as the TBS moiety (Scheme 4). To determine the stereochemistry, **15a** and its diastereomer **15b** were converted into oxazolidinones **22a** and **22b**, respectively, and the configurations at C_5-methine of **22a** and **22b** were assigned by coupling-constant $J_{4,5}$ measurements (Scheme 5).[16b]



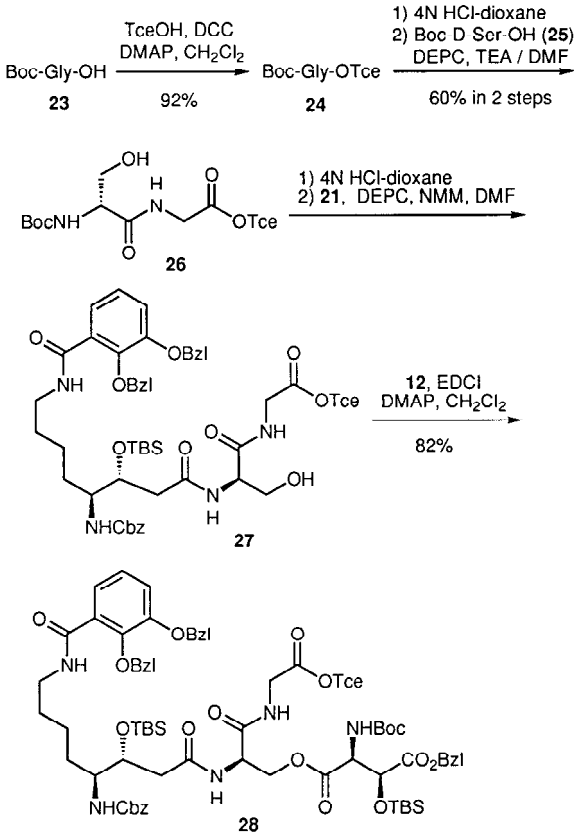
Scheme 4



Scheme 5

The Western Hemisphere (Tetradepsipeptide Segment):

Boc-Gly-OTce (**24**) was prepared from Boc-Gly-OH (**23**) by condensation of 2,2,2-trichloroethanol (TceOH) with *N*,*N*'-dicyclohexylcarbodiimide (DCC) in the presence of 4-(*N*,*N*-dimethylamino)pyridine (DMAP). Deprotection of **24** at the N-terminus with hydrogen chloride and subsequent coupling with Boc-D-Ser-OH (**25**) with DEPC in the presence of triethylamine (TEA) gave the dipeptide **26** in a moderate yield of 60%. Because of the non-protection of the hydroxyl group of **25**, phosphonate formation seems unavoidable even if the ratio of substrates and reagent (**24**/**25**/DEPC) was changed from 1:1.1:1.3 to 1:1.2:1.2. Although the isolated yield was improved to 70% by using FDPP, phosphinate formation was still not repressed. The dipeptide **26** was deprotected with hydrogen chloride and then coupled with the LysSta derivative **21** with DEPC to give the tripeptide **27**. The western hemisphere (the tetradepsipeptide segment) **28** was smoothly obtained by condensing **27** with the β-OH-Asp derivative **12** by the use of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDCI) and DMAP (Scheme 6).

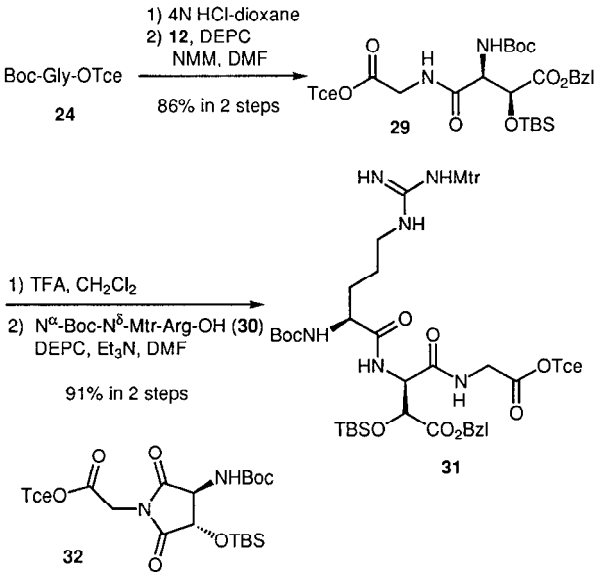


Scheme 6

The Eastern Hemisphere (Tripeptide Segment):

Construction of the eastern hemisphere **31** was also initiated from Boc-Gly-OTce (**24**), which underwent the Boc-deprotection followed by coupling with the β-OH-Asp derivative **12** using DEPC in the presence of TEA. However,

the products were an inseparable mixture of the dipeptide **29** and aspartimide derivative **32** in a ratio of 7:1. Fortunately, the formation of the undesired aspartimide **32** could be entirely suppressed when TEA was replaced with *N*-methylmorpholine (NMM) and the desired dipeptide **29** was formed with almost complete selectivity (>120:1 ratio). After selective deprotection of the Boc group of **29** with trifluoroacetic acid, coupling with N^{α}-Boc-Arg(Mtr)-OH (**30**) afforded **31** in a high yield of 91% (Scheme 7).

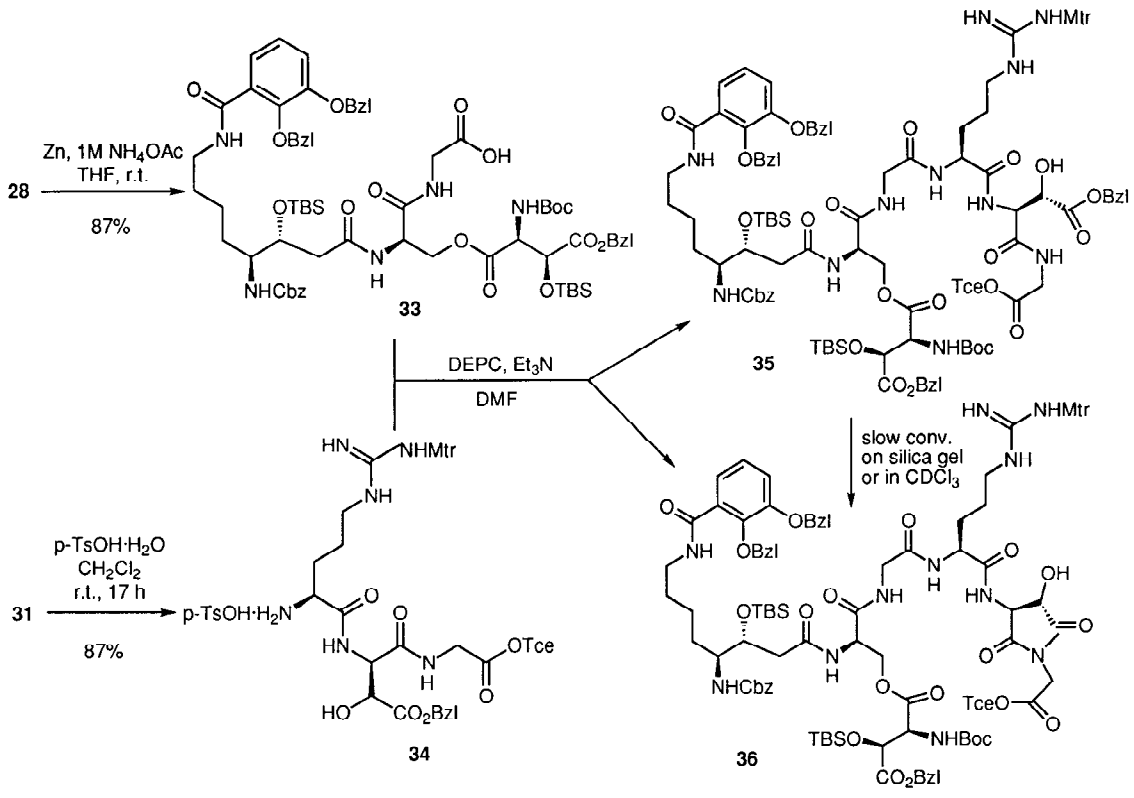


Scheme 7

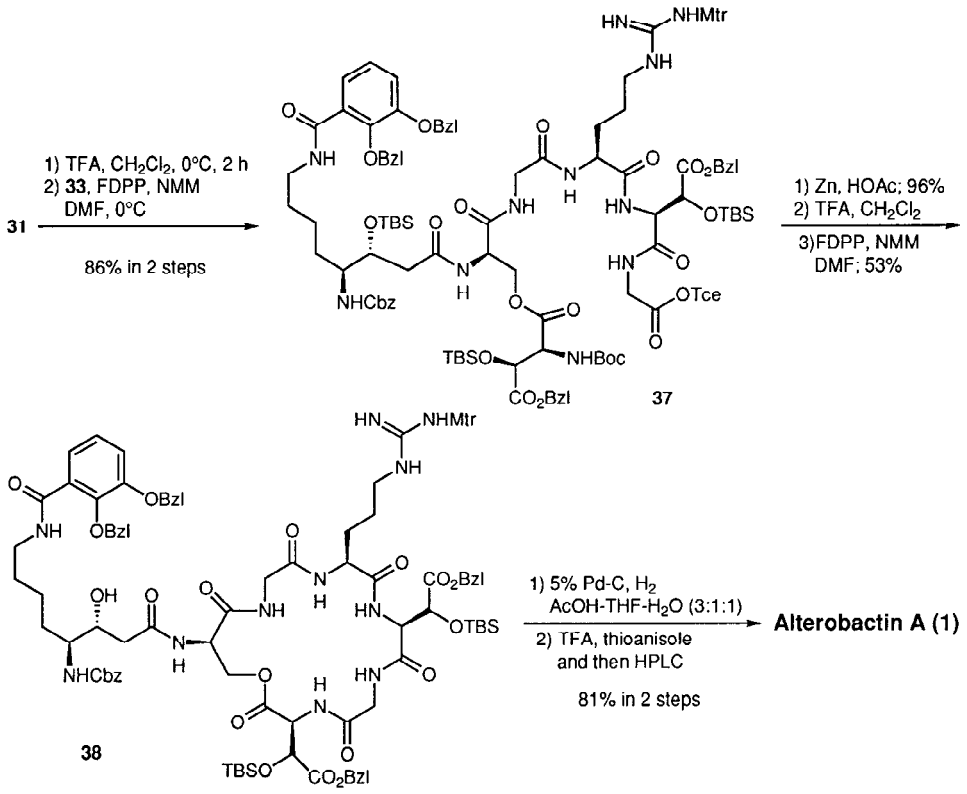
Alterobactin A:

Deprotection of the Tce group of the western hemisphere **28** was achieved by using zinc and 1M aqueous ammonium acetate, while treatment of the eastern hemisphere **31** with p-toluenesulfonic acid monohydrate resulted in the removal of the both Boc and TBS groups.[9c] Subsequently, coupling of **33** with **34** provided the expected depsipeptide **35** accompanied by the unexpected aspartimide derivative **36**. Furthermore, it was found that **35** slowly transformed into **36** on a silica gel column and in $CDCl_3$, an NMR solvent (Scheme 8).

It is well-known that aspartimide formation occurs slowly or not at all in trifluoroacetic acid,[18,19] and thus the selective deprotection of the Boc group of **31** was accomplished by using TFA in dichloromethane without liberation of the Mtr and TBS groups.[8] Condensation of this deprotected western hemisphere **31** with the eastern hemisphere **33** proceeded smoothly with FDPP to give the linear depsipeptide **37** in 86% yield (Scheme 9) and this implied that the steric protection of the hydroxyl group on the β-OH-Asp subunit could prevent aspartimide formation.[20] In this particular coupling process, DEPC was less effective and the yield was ~44–68%. Deprotection of **37** with zinc/1M aqueous ammonium acetate under the same conditions used for the removal of the Tce group of **28** afforded a complicated mixture. As a model experiment, treatment of the tripeptide **31** under the same condition gave only the debenzylation products, which were inferred to be the aspartimide derivative and its hydrolyzate based on ^{1}H NMR spectroscopy and TLC analysis. Surprisingly, however, treatment of **31** with zinc in HOAc

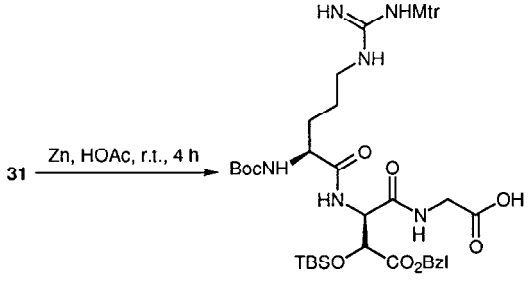


Scheme 8



Scheme 9

produced the Tce-cleaved compound as a single product[18] (**39**, Scheme 10). Therefore, after removal of the Tce group at the C-terminus of **37** with zinc in HOAc and then removal of the Boc group at the N-terminus with TFA, which simultaneously cleaved the TBS group on the side chain,[21] the macrolactamization was efficiently achieved by use of FDPP to give the macrocyclic depsipeptide **38** in a good yield of 53%.[9c,11] Cleavage of the protective groups, except the Mtr group, was effected by a one-pot catalytic hydrogenation over Pd-carbon in HOAc/THF/H_2O, and then deprotection of the Mtr group was readily performed with excess trifluoroacetic acid in the presence of thioanisole.[22] HPLC purification and lyophilization gave alterobactin A (**1**, Scheme 9), which was identical to the natural product by 500 MHz ^{1}H NMR, 125 MHz ^{13}C NMR, high-resolution FAB mass spectra, HPLC and TLC.[23] Interestingly, we found that the pH value of the solution strikingly affects the chemical shifts of the protons on the two β-OH-Asp moieties and the NMR experiments of alterobactin A **(1)** were performed in D_2O with pH 1.6.



Scheme 10

Thus, we have succeeded in synthesizing alterobactin A **(1)**, a super siderophore, which means that alterobactin B **(2)** has been also formally synthesized because alterobactin B is formed by base hydrolysis of *1*.[1]

Discussion

Two independent reports[24] have described the stereoselective preparation of the protected L-threo-β-hydroxyaspartic acids from D- and L-serine, respectively. Noteworthy features of our synthesis are as follows: (a) prochiral and commercially available methyl cinnamate (**5**) was used as starting material, (b) two stereogenic centers of L-threo-β-OH-Asp were built at the same time through Sharpless asymmetric dihydroxylation, (c) the α-carboxylate of threo-β-OH-Asp was efficiently generated by ruthenium oxidation of the phenyl group in good yield, and (d) all reactions proceeded under relatively mild conditions.

Alterobactin A (**1**) contains a β-OH-Asp-Gly sequence, which is similar to the Asp-Gly sequence. The tendency of the β-carboxylate in the aspartyl residue to acylate the amido group of the next amino acid residue in a sequence, and thereby the production of the aspartimide peptides, has been often reported.[18,19] Aspartimide formation is enhanced when the next residue is glycine.[18,19a] Indeed, aspartimide formation was frequently encountered in our approach. The coupling of **12** with the deprotected **24** with TEA gave a mixture of the dipeptide **29** and the aspartimide **32**. However, the subsequent removal of the Boc group

of **29** with TFA and coupling with N^{α}-Boc-Arg(Mtr)-OH (**30**) in the presence of TEA smoothly afforded the tripeptide **31** without any detectable formation of aspartimide. When the hydroxyl group of the aspartyl residue remains free, the aspartimide **36** was readily produced in the amide coupling-process of the deprotected tripeptide **33** and **34**. Furthermore, aspartimide formation predominates in the deprotection process of the tripeptide **31** at the C-terminus with zinc in 1M NH_4OAc-THF. Our results have shown that these aspartimide formations can be entirely suppressed under carefully selected conditions, which use a less basic amine, NMM, and protection of the hydroxyl group on the β-OH-Asp for the amide coupling, and use of a relatively weak acid, TFA and HOAc, for the deprotection at the N- and C-terminus, respectively.

Two glycyl residues and two β-OH-Asp residues are included in alterobactin A (**1**), and we have applied the same building units, **24** and **12**, for the construction of the peptide skeleton. In addition, the selected protective groups for the various reactive functional groups in alterobactin A (**1**), kept intact in the deprotection and the coupling of the peptide segments, were successfully removed in two steps under relatively mild conditions. Surprisingly, a selective deprotection of the Boc group in the tripeptide **31** which includes an N^{ω}-Mtr blocked arginine was achieved by using TFA in CH_2Cl_2 at 0 °C. To our knowledge, the selective deprotection is little known.[8]

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

Melting points were determined on a YAMATO MP-21 and are uncorrected. IR spectra were recorded on a SHIMADZU FTIR-8100 spectrometer. ^{1}H NMR spectra were recorded on a JEOL EX-270 (270 MHz), JEOL GSX-400 (400 MHz), or JEOL α-500 (500 MHz) spectrometer with TMS, or sodium 3-(trimethylsilyl)propanoate-d_4, or solvent as an internal standard, unless otherwise indicated. Abbreviations for NMR: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet, and br = broad. ^{13}C NMR spectra were obtained at 125 MHz on a JEOL α-500 (500 MHz) spectrometer with sodium 3-(trimethylsilyl)propanoate-d_4 as an internal standard. Fast atom bombardment mass spectra (FAB-MS) were recorded on a JEOL JMS-HX110A or JEOL JMS AX505HA high resolution spectrometer by using an argon beam in a glycerol or nitrobenzyl alcohol (NBA) matrix. Accurate mass measurement was obtained by using a JEOL JMS-HX110A high resolution spectrometer in the FAB mode (glycerol and NBA matrices). Optical rotations were measured on a JASCO DIP-140 digital polarimeter with a sodium lamp (λ = 589 nm, D line). Microanalysis was carried out on a YANACO CHN CORDER NT-5. TLC separations were conducted on 250 μm silica plates (Merck Art. 5715, Kieselgel 60 F_{254}) with visualization by UV fluorescence, and ninhydrin, or anisaldehyde, or phosphomolybdic acid staining by heating. Chromatography was carried out on a silica gel column with Fuji Silysia BW-820 or 200, and flash chromatography was accomplished on silica gel, Fuji Silysia BW-300. HPLC was carried out on YMC-Pack C_4 (preparative column: 250 × 20 mm I. D., S-5 μm, 120Å; analytical column: 150 × 4.6 mm I. D., S-5 μm, 120Å) connected in series, 280 nm or 320 nm on a SHIMADZU SPD-10A UV-vis detector.

THF was distilled from sodium/benzophenone ketyl. Et_2O was distilled from $LiAlH_4$. CH_2Cl_2 was distilled from CaH_2. *N*-Methylmorpholine (NMM) was distilled and stored over KOH pellets. Et_3N was dried over sodium wire. DMF was dried over 4Å molecular sieves. All other commercially available reagents were used as received.

Methyl (2*S*,3*R*)-2,3-Dihydroxy-3-phenylpropionate (6):
A 500-mL three-necked flask, equipped with a mechanical stirrer, was charged with *t*-BuOH (100 mL), H_2O (100 mL), and AD-mix-β [28.0 g, 60.0 mmol of $K_3Fe(CN)_6$, 60.0 mmol of K_2CO_3, 0.2 mmol of $(DHQD)_2$-PHAL, and 0.04 mmol of $K_2OsO_2(OH)_4$]. Stirring at r.t. produced two clear phases with the lower aqueous phase appearing as bright yellow. $MeSO_2NH_2$ (1.90 g, 20.0 mmol) was added at this point. The mixture was cooled in an ice-bath whereupon some of the dissolved salts precipitated. Methyl cinnamate (**5**; 3.24 g, 20.0 mmol) was added at once in one portion, and the heterogeneous slurry was stirred vigorously at this temperature for 12 h and then warmed to r.t. for 12 h. After the mixture was recooled in an ice-bath, Na_2SO_3 (30 g, 238 mmol) was added and the mixture was allowed to warm to r.t. and stirred for 1 h. EtOAc was added to the reaction mixture, and after separation of the organic layer, the aqueous phase was further extracted three times with EtOAc. The combined organic extracts were washed with H_2O, dried (Na_2SO_4), and concentrated in vacuo. The crude residue was purified by chromatography (1:1 EtOAc/hexane) to afford the 1,2-diol **6** as white crystals (2.67 g, 68%; >98% ee, by ^{1}H NMR analysis of the (+)-MTPA-diester); mp 80–81 °C (EtOAc/hexane); $[\alpha]_D^{26}$ –9.7 (*c* = 1.07, $CHCl_3$).
IR (KBr): ν = 3492, 3384, 1717, 1458, 1323, 1308, 1275, 1223, 1111, 1049, 722, 700 cm^{-1}.
^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.31–7.42 (m, 5 H, arom), 5.02 (dd, *J* = 2.8, 6.8 Hz, 1 H), 4.38 (dd, *J* = 3.0, 5.9 Hz, 1 H), 3.82 (s, 3 H, OCH_3), 3.10 (d, *J* = 5.9 Hz, 1 H), 2.72 (d, *J* = 6.9 Hz, 1 H).
Anal. $C_{10}H_{12}O_4$ (196.2): calcd C, 61.22; H, 6.16; found: C, 61.14; H, 6.19.

Methyl (2*R*,3*S*)-2-Acetoxy-3-bromo-3-phenylpropionate (7):
To the diol **6** (2.67 g, 13.6 mmol) was added 31% HBr/HOAc (21 mL), and the mixture was heated at 45 °C for 30 min. After cooling to r.t., the mixture was quenched by slowly pouring into an ice-saturated aq $NaHCO_3$ solution, and the aqueous layer was extracted three times with Et_2O. The combined organic extracts were washed once with H_2O and twice with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by chromatography (1:12.5 EtOAc/hexane) to give **7** as white crystals (3.19 g, 78%); mp 78–79 °C (EtOAc/hexane); $[\alpha]_D^{26}$ +100.3 (*c* = 1.36, $CHCl_3$).
IR (KBr): ν = 1763, 1748, 1493, 1451, 1433, 1377, 1260, 1219, 1198, 1090, 702 cm^{-1}.
^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.43–7.45 (m, 2 H), 7.31–7.35 (m, 3 H), 5.64 (d, *J* = 6.3 Hz, 1 H), 5.34 (d, *J* = 6.6 Hz, 1 H), 3.71 (s, 3 H, OCH_3), 2.11 (s, 3 H, $OCCH_3$).
Anal. $C_{12}H_{13}O_4Br$ (301.2): calcd C, 47.86; H, 4.35; found: C, 47.73; H, 4.33.

Methyl (2*S*,2*R*)-2-Acetoxy-3-azido-3-phenylpropionate (8):
To a solution of **7** (2.93 g, 9.72 mmol) in DMF (35 mL) was added NaN_3 (2.53 g, 38.9 mmol), and the mixture was stirred at r.t. for 3 h and then heated at 40 °C for 2 h. After cooling to r.t., the mixture was diluted with EtOAc, and the organic layer was washed once with H_2O and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude product was purified by chromatography (1:10 EtOAc/hexane) to afford the azide **8** as a colorless oil (2.20 g, 86 %); $[\alpha]_D^{26}$ –104.2 (*c* = 2.33, $CHCl_3$).
IR (neat): ν = 2109, 1825, 1755 (br), 1455, 1439, 1375, 1221 (br), 702 cm^{-1}.
^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.35–7.40 (m, 5 H, arom), 5.23 (d, *J* = 5.0 Hz, 1 H, α-H), 5.06 (d, *J* = 5.0 Hz, 1 H, β-H), 3.69 (s, 3 H, OCH_3), 2.14 (s, 3 H, $OCCH_3$).

Anal. $C_{12}H_{13}N_3O_4$ (263.2): calcd C, 54.75; H, 4.97; N, 15.96; found: C, 54.89; H, 5.14; N, 16.04.

Methyl (2*S*,3*R*)-2-Acetoxy-3-(*tert*-butoxycarbonylamino)-3-phenylpropionate (9):

A suspension of 5% Pd/C (532 mg) in EtOAc (7 mL) was stirred at r.t. under an H_2 atmosphere for 15 min, whereupon a solution of di-*tert*-butyl dicarbonate (4.36 g, 20 mmol) and the azide **8** (1.32 g, 5.0 mmol) in EtOAc (3 mL) was added. The mixture was stirred under an H_2 atmosphere for 6 h, and then filtered through a pad of Celite. The organic phase was washed with ice-cold 1M aq $KHSO_4$, sat. $NaHCO_3$, and brine, dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by chromatography (gradient from 1:5 to 1:4 EtOAc/hexane) to give **9** as a colorless oil (1.14 g, 68%); $[\alpha]_D^{26}$ +27.6 (c = 1.25, $CHCl_3$).

IR (neat): ν = 3375, 1755, 1751, 1717, 1514, 1499, 1370, 1229 (br), 1169, 1086, 758, 702 cm^{-1}.

^1H NMR (270 MHz, $CDCl_3$): δ = 7.25–7.37 (m, 5 H, arom), 5.40 (br s, 2 H, NH, α-H), 5.30 (s, 1 H, β-H), 3.77 (s, 3 H, OCH_3), 2.08 (s, 3 H, $OCCH_3$), 1.43 [s, 9 H, $OC(CH_3)_3$].

Anal. $C_{17}H_{23}NO_6$ (337.4): calcd C, 60.53; H, 6.87; N, 4.15; found: C, 60.54; H, 7.16; N, 4.10.

α-*tert*-Butyl β-Methyl (2*S*,3*S*)-*N*-Boc-β-Acetoxyaspartic Diester (10):

To a stirred solution of **9** (1.14 g, 3.38 mmol) in EtOAc (10 mL) and MeCN (10 mL) was added a suspension of $NaIO_4$ (18.07 g, 84.5 mmol) in H_2O (70 mL) followed by $RuCl_3 \cdot H_2O$ (46 mg, 0.20 mmol), and the heterogeneous slurry was vigorously stirred at r.t. for 11 h. The mixture was diluted with EtOAc, and after separation of the layers, the aqueous layer was further extracted twice with EtOAc. The combined extracts were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was dissolved in Et_2O, and the solution was filtered through a pad of Celite. The solvent was evaporated in vacuo to give a brown foam (981 mg). The crude acid was dissolved in CH_2Cl_2 (3.5 mL) and *t*-BuOH (14 mL) was added followed by BDIU (3.2 mL, 13.5 mmol). The mixture was heated at 50 °C for 11 h under an argon atmosphere, and after cooling to r.t., it was filtered through a pad of Celite and concentrated in vacuo. The residue was dissolved in EtOAc, and the solution was filtered through a pad of Celite and then washed with ice-cold 1 M aq $KHSO_4$, sat. $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by chromatography (gradient from 1:7 to 1:6 EtOAc/hexane) to afford the acetoxy diester **10** as a colorless wax (854 mg, 70%); mp 51–52 °C (EtOAc/hexane); $[\alpha]_D^{24}$ +47.2 (c = 0.57, $CHCl_3$).

IR (KBr): ν = 3337, 2980, 1761, 1750, 1742, 1700, 1528, 1512, 1370, 1283, 1233, 1225, 1154, 1092, 1065 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 5.53 (d, J = 2.4 Hz, 1 H, β-H), 5.22 (d, J = 9.8 Hz, 1 H, NH), 4.86 (d, J = 9.8 Hz, 1 H, α-H), 3.76 (s, 3 H, OCH_3), 2.13 (s, 3 H, $OCCH_3$), 1.45 (s, 9 H), 1.43 (s, 9 H).

Anal. $C_{16}H_{27}NO_8$ (361.4): calcd C, 53.18; H, 7.53; N, 3.88; found: C, 53.02; H, 7.61; N, 3.85.

α-*tert*-Butyl β-Benzyl (2*S*,3*S*)-*N*-Boc-β-Hydroxyaspartic Diester (11):

To a solution of **10** (722 mg, 2.0 mmol) in THF (45 mL) cooled in an ice-bath was added a 0.31 N aq LiOH solution (15 mL). The mixture was stirred at this temperature for 3 h, and then quenched by adding 1.0 N aq HCl (4.6 mL). The aqueous layer was extracted three times with EtOAc and the combined extracts were dried (Na_2SO_4) and filtered. To remove the residual HOAc, the combined filtrate was concentrated to about 15 mL, followed by azeotropic removal of the HOAc in vacuo with heptane (15 mL). The resulting acid was dissolved in DMF (7 mL) and cooled in an ice-bath, whereupon benzyl bromide (0.48 mL, 4.0 mmol) was added. Then, ground K_2CO_3 (41.5 mg, 3.0 mmol) was added to the mixture, and the suspension was stirred at this temperature for 5 h and partitioned between ice-cold 0.2 N aq HCl and EtOAc. The organic layer was separated and washed once with H_2O and brine, and dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by chromatography (gradient from 1:9 to 1:7 EtOAc/hexane) to afford the alcohol **11** as a white solid (500 mg, 63%); mp 100–101 °C (EtOAc/hexane); $[\alpha]_D^{22}$ +19.6 (c = 1.08, $CHCl_3$).

IR (KBr): ν = 3407, 2984, 1744, 1698, 1509, 1370, 1350, 1298, 1258, 1206, 1152, 1096, 1064, 747, 698 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 7.27–7.43 (m, 5 H, arom), 5.24 (overlapping, 1 H, NH), 5.19 (ABq, J = 12.0 Hz, 2 H, CH_2Ph), 4.69 (overlapping, 1 H, α-H), 4.67 (dd, J = 2.2, 6.6 Hz, 1 H, β-H), 3.12 (d, J = 6.2 Hz, 1 H, β-OH), 1.49 (s, 9 H), 1.43 (s, 9 H).

Anal. $C_{20}H_{29}NO_7$ (395.5): calcd C, 60.75; H, 7.39; N, 3.54; found: C, 60.76; H, 7.43; N, 3.45.

(2*S*,3*S*)-*N*-Boc-β-(*tert*-Butyldimethylsilyloxy)aspartic Acid β-Benzyl Ester (12):

To a stirred solution of the alcohol **11** (466 mg, 1.18 mmol) in CH_2Cl_2 (3.5 mL) cooled in an ice-bath was added dropwise trifluoroacetic acid (TFA, 3.5 mL). The resulting mixture was stirred at this temperature for 1 h and allowed to warm to r.t. over 4 h. The mixture was concentrated in vacuo, and the residual TFA was removed twice by diluting with CH_2Cl_2 (1 mL) and benzene (3 mL) and concentrating in vacuo. The resulting amine salt was dissolved in H_2O (6 mL) and dioxane (6 mL) and cooled in an ice-bath, whereupon Et_3N (0.41 mL, 3.0 mmol) was added dropwise with stirring, followed by Boc_2O in one portion. The mixture was stirred at this temperature for 20 min and allowed to warm to r.t. for 4 h, and then concentrated in vacuo. Ice–saturated $NaHCO_3$ solution (v/v, 1:1, 20 mL) was added to the residue, and then the aqueous layer was washed twice with Et_2O and acidified with 6 N aq HCl to pH 4 in an ice-bath and subsequently extracted twice with CH_2Cl_2. The combined extracts were dried (Na_2SO_4), filtered, and concentrated in vacuo to give a white foam (468 mg). The resulting acid and imidazole (803 mg, 11.8 mmol) were dissolved in DMF (4 mL) and cooled in an ice-bath, whereupon TBSCl (889 mg, 5.9 mmol) was added in one portion. The mixture was stirred at this temperature for 30 min and allowed to warm to r.t. over 40 h under an argon atmosphere. Subsequently, the mixture was poured into ice/1 M aq $KHSO_4$ (v/v, 1:1) and the aqueous layer was extracted three times with EtOAc. The combined organic extracts were washed once with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude product was purified by chromatography (gradient from 1:8 to 1:2 EtOAc/hexane) to give **12** as an oily white solid (448 mg, 84%); $[\alpha]_D^{24}$ –6.8 (c = 1.88, $CHCl_3$).

IR (neat): ν = 1761, 1723, 1667, 1501, 1256, 1165, 1132, 839, 781 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.31–7.38 (m, 5 H, arom), 5.26 (d, J = 8.6 Hz, 1 H, NH), 5.15 (ABq, J = 11.9 Hz, 2 H, CH_2Ph), 4.84 (br s, 2 H, α-H, β-H), 1.43 [s, 9 H, $OC(CH_3)_3$], 0.86 [s, 9 H, $SiC(CH_3)_3$], 0.08 (s, 3 H, $SiCH_3$), 0.01 (s, 3 H, $SiCH_3$).

Anal. $C_{22}H_{35}NO_7Si$ (453.6): calcd C, 58.26; H, 7.77; N, 3.09; found: C, 57.73; H, 7.76; N, 3.06.

Ethyl (4*S*)-4-(Benzyloxycarbonylamino)-8-(*tert*-butyloxycarbonylamino)-3-oxooctanoate (14):

To a stirred solution of N^{α}-Cbz-N^{ω}-Boc-Lys-OH (**13**; 1.90 g, 5.0 mmol) in THF (15 mL) at 0 °C was added carbonyldiimidazole (CDI; 973 mg, 6.0 mmol). The mixture was stirred at this temperature for 1 h and at r.t. for 3 h, and then cooled to –18 °C before the magnesium enolate solution in THF (15 mL) [prepared from ethyl hydrogen malonate (1.32 g, 10.0 mmol) and 2 M *i*-PrMgCl (30 mmol) in THF (12.5 mL) at –18 °C for 45 min and at r.t. for 2.5 h] was added dropwise. After the mixture was stirred at –18 °C for 1 h and allowed to warm to r.t., stirring was continued for 4.5 h, and then the mixture was poured into ice/1 M aq $KHSO_4$. The aqueous layer was extracted three times with EtOAc and the combined organic phases were washed with sat. $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated

in vacuo. The crude product was purified by chromatography (gradient from 1:2.5 to 1:2 EtOAc/hexane) to give the β-keto ester **14** as a white solid (1.86 g, 83%); mp 70–71 °C (EtOAc/hexane); $[\alpha]_D^{26}$ –19.9 (*c* = 1.97, MeOH).
IR (KBr): ν = 3357, 1736, 1717, 1686, 1678, 1526, 1277, 1252, 1169, 1032 cm^{-1}.
^1H NMR (270 MHz, $CDCl_3$): δ = 7.36 (br s, 5 H, arom), 5.54 (d, *J* = 6.3 Hz, 1 H, 4-NH), 5.11 (s, 2 H, CH_2Ph), 4.58 (br s, 1 H, 8-NH), 4.40–4.47 (m, 1 H, 4-H), 4.19 (q, *J* = 6.9 Hz, 2 H, OCH_2Me), 3.55 (ABq, *J* = 15.8 Hz, 2 H, 2-CH_2), 3.07–3.11 (m, 2 H, 8-CH_2), 1.88–1.95 (m, 1 H, 5-H), 1.32–1.67 (m, 5 H, 5-H, 6- and 7-CH_2), 1.42 [s, 9 H, OC(CH_3)$_3$], 1.27 (t, *J* = 6.9 Hz, 3 H, CH_3).
Anal. $C_{23}H_{34}N_2O_7$ (450.5): calcd C, 61.32; H, 7.60; N, 6.22; found: C, 61.25; H, 7.49; N, 6.24.

Ethyl (3*R*,4*S*)-4-(Benzyloxycarbonylamino)-8-(*tert*-butyloxycarbonylamino)-3-hydroxyoctanoate (15a):
To a stirred solution of the oxo ester **14** (2.70 g, 6.0 mmol) in EtOH/THF (v/v, 6:1; 105 mL) at –78 °C was added portionwise $NaBH_4$ (248 mg, 7.5 mmol). The mixture was stirred at this temperature for 1.5 h, and the reaction was quenched by pouring the mixture into ice-cold 10 % aq citric acid (50 mL). The mixture was concentrated in vacuo, and then diluted with H_2O (50 mL). The aqueous layer was extracted three times with EtOAc and the combined organic phase was washed once with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo to give a mixture of the starting material **14** and diastereomers of the alcohol **15** [(3*R*,4*S*)/(3*S*,4*S*) 85:15 by ^1H NMR analysis] as a white solid. The crude product was purified by flash chromatography (2:1 Et_2O/hexane) to recover **14** (245 mg, 9%) and provide the desired diastereomer (3*R*,4*S*)-**15a** as a white solid (1.80 g, 66%, 73 % conversion; >95% de by ^1H NMR analysis); mp 91–92 °C (Et_2O/hexane). The analytical sample was recrystallized twice from Et_2O/hexane (>98% de); $[\alpha]_D^{26}$ –9.4 (*c* = 1.10, $CHCl_3$).
IR (KBr): ν = 3355, 3326, 1732, 1690, 1541, 1281, 1244, 1175, 1061, 1019 cm^{-1}.
^1H NMR (500 MHz, $CDCl_3$): δ = 7.29–7.39 (m, 5 H, arom), 5.10 (s, 2 H, CH_2Ph), 4.97 (d, *J* = 7.7 Hz, 1 H, NH), 4.56 (br s, 1 H, NH), 4.16 (q, *J* = 7.1 Hz, 2 H, OC$H_2$$CH_3$), 4.14–4.19 (m, 1 H, 3-H), 3.63–3.65 (m, 1 H, 4-H), 3.35 (br s, 1 H, 3-OH), 3.09 (br s, 2 H, 8-CH_2), 2.45–2.54 (m, 2 H, 2-CH_2), 1.59–1.62 (m, 3 H), 1.42 [s, 9 H, OC(CH_3)$_3$], 1.30–1.49 (m, 3 H), 1.26 (t, *J* = 7.1 Hz, 3 H, OCH$_2$CH_3).
Anal. $C_{23}H_{36}N_2O_7$ (452.6): calcd C, 61.05; H, 8.01; N, 6.19; found: C, 60.82; H, 7.84; N, 6.13.

2,3-Bis(benzyloxy)benzoic Acid (17):
To a solution of catecholic acid (**19**; 1.54 g, 10.0 mmol) and benzyl bromide (5.4 mL, 45.0 mmol) in DMF (20 mL) was added pulverized K_2CO_3 (8.29 g, 60.0 mmol). The mixture was stirred at r.t. for 5 h and then poured into ice-water. The aqueous layer was extracted three times with EtOAc and the combined organic phases were washed with ice-cold 1 M aq $KHSO_4$, H_2O, and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by chromatography (gradient from 1:15 to 1:10 EtOAc/hexane) to give a tribenzylated product as a white wax (4.15 g, 98%); mp 40 °C (EtOAc/hexane).
IR (KBr): ν = 1709 (br), 1578, 1497, 1469, 1456, 1375, 1366, 1319, 1283, 1258, 1132, 1035, 750, 746, 696 cm^{-1}.
^1H NMR (270 MHz, $CDCl_3$): δ = 7.24–7.45 (m, 16 H, arom), 7.14 (dd, *J* = 1.8, 7.9 Hz, 1 H, catechol 4-H), 7.08 (dd, *J* = 8.3, 15.9 Hz, 1 H, catechol 5-H), 5.31 (s, 2 H, CH_2Ph), 5.13 (s, 2 H, CH_2Ph), 5.06 (s, 2 H, CH_2Ph).
Anal. $C_{28}H_{24}O_4$ (424.5): calcd C, 79.23; H, 5.70; found: C, 79.12; H, 5.78.
The resulting tribenzylated product (2.53 g, 5.96 mmol) was dissolved in THF (75 mL) and cooled in an ice-bath, whereupon a solution of LiOH•H_2O (1.00 g, 23.8 mmol) in H_2O (25 mL) was added. The mixture was allowed to warm to r.t. and stirred for 3 days, and then diluted with H_2O (75 mL). The aqueous layer was washed twice with Et_2O and then acidified with 1 N aq HCl (24 mL) to pH ca. 4, and then extracted three times with CH_2Cl_2. The combined organic phases were dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude product was recrystallized from EtOAc/hexane to provide the acid **17** as white crystals (1.84 g, 92%); mp 113–114 °C (EtOAc/hexane).
IR (KBr): ν = 3032, 1694 (br), 1578, 1474, 1456, 1416, 1377, 1314 (br), 1262, 1086, 752, 698 cm^{-1}.
^1H NMR (270 MHz, $CDCl_3$): δ = 11.34 (br s, 1 H, CO_2H), 7.73–7.76 (dd, *J* = 1.8, 9.8 Hz, 1 H, catechol 4-H), 7.16–7.50 (m, 12 H, arom), 5.27 (s, 2 H, CH_2Ph), 5.20 (s, 2 H, CH_2Ph).
Anal. $C_{21}H_{18}O_4$ (334.4): calcd C, 75.44; H, 5.42; found: C, 75.40; H, 5.43.

Ethyl (3*R*,4*S*)-4-(Benzyloxycarbonylamino)-8-[2,3-bis(benzyloxy)-benzoylamino]-3-hydroxyoctanoate (20):
The lysinestatine derivative **15a** (1.58 g, 3.5 mmol) was treated with 4 N HCl/dioxane (15 mL) at 0 °C for 1 h and then at r.t. for 45 min. The mixture was concentrated in vacuo, and the residual HCl was removed by diluting with dioxane (3 mL) and toluene (9 mL) and evaporating in vacuo. The resulting amine salt (a white solid) and 2,3-bis(benzyloxy)benzoic acid (**17**; 1.46 g, 4.4 mmol) were dissolved in DMF (15 mL) and cooled in an ice-bath, whereupon DEPC (0.67 mL, 4.4 mmol) was added dropwise with stirring. After 15 min, Et_3N (1.12 mL, 8.1 mmol) was added, and the mixture was stirred at 0 °C for 4 h and subsequently allowed to warm to r.t. over 13 h. The mixture was diluted with EtOAc/benzene (v/v, 3:1; 60 mL), and the organic layer was washed twice with ice-cold 1 M aq $KHSO_4$, once with sat. $NaHCO_3$, and once with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by chromatography (gradient from 1:1 to 1.5:1 EtOAc/hexane) to give **20** as a white solid (2.02 g, 86%); mp 114–115 °C (EtOAc/hexane); $[\alpha]_D^{26}$ –9.3 (*c* = 1.22, $CHCl_3$).
IR (KBr): ν = 3306, 1728, 1684, 1639, 1576, 1541, 1246, 1057, 747, 696 cm^{-1}.
^1H NMR (400 MHz, $CDCl_3$): δ = 7.94 (br s, 1 H, LysSta 8-NH), 7.72 (dd, *J* = 1.6, 7.8 Hz, 1 H, catechol 6-H), 7.28–7.48 (m, 15 H, 3 × C_6H_5), 7.12 (d, *J* = 6.9 Hz, 1 H, catechol 4-H), 7.06 (t, *J* = 7.9 Hz, 1 H, catechol 5-H), 5.15 (s, 2 H, CH_2Ph), 5.09 (br s, 1 H), 5.06 (s, 2 H, CH_2Ph), 5.05 (ABq, *J* = 12.0 Hz, 2 H, CH_2Ph), 4.16 (q, *J* = 7.1 Hz, 2 H, OC$H_2$$CH_3$), 3.98–4.01 (m, 1 H, 3-H), 3.57–3.60 (m, 1 H, 4-H), 3.41 (d, *J* = 4.0 Hz, 1 H, 3-OH), 3.32–3.39 (m, 1 H, 8-H), 3.11–3.17 (m, 1 H, 8-H), 2.46–2.51 (m, 2 H, 3-CH_2), 1.54–1.57 (m, 1 H), 1.24–1.44 (m, 5 H), 1.27 (t, *J* = 7.1 Hz, 3 H, OCH$_2$CH_3).
Anal. $C_{39}H_{44}N_2O_8$ (668.9): calcd C, 70.04; H, 6.63; N, 4.19; found: C, 69.86; H, 6.62; N, 4.01.

(3*R*,4*S*)-4-(Benzyloxycarbonylamino)-8-[2,3-bis(benzyloxy)benzoylamino]-3-(*tert*-butyldimethylsilyloxy)octanoic Acid (21):
To a stirred solution of the ester **20** (1.20 g, 1.79 mmol) in THF (35 mL) was added dropwise an aq solution of 0.24 N LiOH at 0 °C, and then the mixture was stirred at this temperature for 3 h and diluted with H_2O (35 mL). Aq 1N HCl (0.31 mL) was added to the mixture to quench the reaction, and the aqueous layer was subsequently saturated with NaCl and extracted three times with EtOAc/benzene (v/v, 1:1). The combined extracts were dried (Na_2SO_4), filtered, and concentrated in vacuo to give a white solid (1.14 g). The resulting acid and imidazole (1.22 g, 17.9 mmol) were dissolved in DMF (6 mL) and cooled in an ice-bath, whereupon TBSCl (1.35 g, 8.97 mmol) was added in one portion. The mixture was stirred at 0 °C for 15 min and allowed to warm to r.t. over 17.5 h under an argon atmosphere. The reaction was quenched by partitioning between ice-cold 1 M aq $KHSO_4$ solution and EtOAc. The organic layer was separated and the aqueous layer was further extracted twice with EtOAc. The combined organic extracts were washed with ice-cold 1 M aq $KHSO_4$ and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was dissolved THF/MeOH (v/v, 2:1, 24 mL), and an aq solution of K_2CO_3

(273 mg, 1.97 mmol) in H_2O (8 mL) was added to this solution at 0 °C. The mixture was stirred at this temperature for 1.5 h, and then poured into ice-cold 0.2 N aq HCl to quench the reaction. The aqueous layers were extracted three times with EtOAc, and the combined extracts were washed once with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by chromatography (gradient from 1:4 to 2:1 EtOAc/hexane) to give the acid **21** as an oily white solid (1.17 g, 87%); $[\alpha]_D^{24}$ –10.1 (*c* = 1.11, $CHCl_3$).
IR ($CHCl_3$): ν = 3378, 1717 (br), 1653, 1647, 1576, 1539, 1456, 1262, 1217, 1084, 756, 698 cm^{-1}.
^1H NMR (500 MHz, $CDCl_3$): δ = 7.97 (br s, 1 H, 8-NH), 7.71 (dd, *J* = 1.8, 7.9 Hz, 1 H, catechol 6-H), 7.29–7.47 (m, 15 H, 3 × C_6H_5), 7.13 (d, *J* = 6.7 Hz, 1 H, catechol 4-H), 7.08 (t, *J* = 7.9 Hz, 1 H, catechol 5-H), 5.15 (s, 2 H, C*H*$_2$Ph), 5.06 (s, 2 H, C*H*$_2$Ph), 5.05 (ABq, *J* = 11.9 Hz, 2 H, C*H*$_2$Ph), 4.93 (d, *J* = 8.5 Hz, 1 H, 4-NH), 4.15 (br s, 1 H, 3-H), 3.60 (br s, 1 H, 4-H), 3.28 (br s, 1 H, 8-H), 3.20 (br s, 1 H, 8-H), 2.44–2.51 (m, 2 H, 3-CH_2), 1.56 (br s, 1 H), 1.22–1.33 (m, 5 H), 0.87 [s, 9 H, SiC$(CH_3)_3$], 0.06 (s, 3 H, SiCH_3), 0.05 (s, 3 H, SiCH_3).
Anal. $C_{43}H_{54}N_2O_8Si$ (755.0): calcd C, 68.41; H, 7.21; N, 3.71; found: C, 68.17; H, 7.30; N, 3.69.

Boc-Gly-OTce (24):
To a stirred solution of Boc-Gly-OH (**23**; 4.96 g, 28.3 mmol), 2,2,2-trichloroethanol (3.4 mL, 35.4 mmol) and DMAP (864 mg, 7.1 mmol) in CH_2Cl_2 (150 mL) at 0 °C was added DCC (6.72 g, 32.5 mmol) in one portion. The mixture was stirred at this temperature for 2 h, allowed to warm to r.t., stirred for 18 h, and then filtered through a pad of Celite. The filtrate was washed with ice-cold 1 M aq $KHSO_4$ solution, sat. $NaHCO_3$ solution and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by chromatography (gradient from 1:8 to 1:6 EtOAc/hexane) to give the ester **24** as a white solid (7.98 g, 92%); mp 70–71 °C (EtOAc/hexane).
IR (KBr): ν = 3416, 1782, 1771, 1690 (br), 1518 (br), 1371, 1281, 1262, 1154 (br), 819, 723 cm^{-1}.
^1H NMR (270 MHz, $CDCl_3$): δ = 5.01 (br s, 1 H, NH), 4.80 (s, 2 H, C*H*$_2$Tce), 4.07 (d, *J* = 5.6 Hz, 2 H, CH_2N), 1.46 [s, 9 H, OC$(CH_3)_3$].
Anal. $C_9H_{14}NO_4Cl_3$ (306.6): calcd C, 35.26; H, 4.60; N, 4.57; found: C, 35.11; H, 4.54; N, 4.57.

Boc-D-Ser-Gly-OTce (26):
Boc-Gly-OTce (**24**; 2.30 g, 7.5 mmol) was treated with 4 N HCl/dioxane (15 mL) at 0 °C. The mixture was stirred at this temperature for 1 h, then warmed to r.t. for 1.5 h, and concentrated in vacuo. The residue was dissolved in dioxane (3 mL) and toluene (9 mL), and the volatiles were removed in vacuo. The resulting amine salt and Boc-D-Ser-OH (**25**; 1.69 g, 8.25 mmol) were dissolved in DMF (20 mL), and then DEPC (1.5 mL, 9.75 mmol) at 0 °C was added to this solution with stirring. After 15 min, Et_3N (2.3 mL, 16.9 mmol) was added and the mixture was stirred at 0 °C for 5 h and then at r.t. for 2 h, and diluted with EtOAc/benzene (v/v, 3:1). The organic layer was washed twice with ice-cold 1 M aq $KHSO_4$ solution, once with sat. $NaHCO_3$ solution, and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by chromatography (gradient from 1:1 to 1.5:1 EtOAc/hexane) to afford the dipeptide **26** as a white solid (1.77 g, 60%); mp 92–93 °C (EtOAc/hexane); $[\alpha]_D^{26}$ +27.8 (*c* = 1.04, $CHCl_3$).
IR (KBr): ν = 3310 (br), 3092, 1767, 1680 (br), 1541, 1368, 1298, 1254, 1057, 927 cm^{-1}.
^1H NMR (500 MHz, $CDCl_3$): δ = 7.22 (br s, 1 H, Gly NH), 5.56 (br s, 1 H, Ser NH), 4.78 (ABq, *J* = 11.9 Hz, 2 H, CH_2CCl_3), 4.26 (dd, *J* = 6.1, 18.3 Hz, 1 H, Gly α-H), 4.24 (br s, 1 H, Ser α-H), 4.17 (dd, *J* = 5.5, 18.3 Hz, 1 H, Gly α-H), 4.12 (br s, 1 H, Ser β-H), 3.69 (br s, 1H, Ser β-H), 2.91 (br s, 1 H, OH), 1.46 [s, 9 H, OC$(CH_3)_3$].
Anal. $C_{12}H_{19}Cl_3N_2O_6$ (393.6): calcd C, 36.62; H, 4.86; N, 7.12; found: C, 36.51; H, 4.82; N, 7.08.

Tripeptide 27:
Boc-D-Ser-Gly-OTce (**26**; 673 mg, 1.71 mmol) was treated with 4 N HCl/dioxane (8 mL) at 0 °C. The mixture was stirred at this temperature for 30 min and then at r.t. for 1.5 h, and concentrated in vacuo. The residue was dissolved in dioxane (2 mL) and toluene (6 mL), and the volatiles were evaporated in vacuo. The resulting amine salt and the acid component **21** (1.17 g, 1.55 mmol) were dissolved in DMF (5 mL), and DEPC (0.26 mL, 1.71 mmol) was added to this solution at 0 °C. After the mixture was stirred for 15 min, NMM (0.36 mL, 3.26 mmol) was added, and the mixture was stirred at 0 °C for 40 h and then diluted with EtOAc/benzene (v/v, 3:1). The organic phases were washed twice with ice-cold 1 M aq $KHSO_4$ solution, once with sat. $NaHCO_3$ solution, and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by chromatography (gradient from 1.5:1 to 3.5:1 EtOAc/hexane) to recover the acid component **21** as a colorless oil (400 mg, 34% recovery) and to afford the tripeptide **27** as a white solid (813 mg, 51%, 77% conversion); mp 42–43 °C (EtOAc/hexane); $[\alpha]_D^{23}$ +9.2 (*c* = 0.96, MeOH).
IR (KBr): ν = 3380 (br), 2953, 2930, 1771, 1701, 1649 (br), 1576, 1536 (br), 1454, 1262, 1175, 1082 (br), 837, 777, 733, 698 cm^{-1}.
^1H NMR (270 MHz, $CDCl_3$): δ = 7.98 (t, *J* = 5.1 Hz, 1 H, LysSta 8-NH), 7.69 (dd, *J* = 2.0, 7.6 Hz, 1 H, catechol 6-H), 7.66 (t, *J* = 3.6 Hz, 1 H, Gly NH), 7.28–7.49 (m, 15 H, 3 × C_6H_5), 7.05–7.15 (m, 2 H, catechol 5-H, 4-H), 5.19 (s, 2 H, C*H*$_2$Ph), 5.08 (overlapping d, *J* = 5.3 Hz, 1 H, Ser NH), 5.05 (s, 2 H, C*H*$_2$Ph), 5.01 (s, 2 H, C*H*$_2$Ph), 4.70 (ABq, *J* = 11.9 Hz, 2 H, CH_2CCl_3), 4.54–4.55 (m, 1 H, Ser α-H), 4.20 (dd, *J* = 6.0, 17.9 Hz, 1 H, Gly α-H), 4.14 (overlapping dd, *J* = 4.3 Hz, 1 H, Ser β-H), 4.07 (overlapping m, 1 H, LysSta 3-H), 4.00 (dd, *J* = 5.1, 18.0 Hz, 1 H, Gly α-H), 3.67 (overlapping dd, *J* = 4.6 Hz, 1 H, Ser β-H), 3.59–3.66 (m, 1 H, LysSta 4-H), 3.17–3.34 (m, 2 H, LysSta 8-CH_2), 2.53 (br s, 2 H, LysSta 2-CH_2), 2.00 (br s, 1 H, Ser OH), 1.89 (br s, 1 H), 1.22–1.69 (m, 5 H), 0.89 [s, 9 H, SiC$(CH_3)_3$], 0.08 [s, 6 H, Si$(CH_3)_2$].
Anal. $C_{50}H_{63}Cl_3N_4O_{11}Si$ (1030.5): calcd C, 58.28; H, 6.16; N, 5.44; found: C, 58.08; H, 6.16; N, 5.48.

Depsitetrapeptide 28:
The tripeptide **27** (421 mg, 0.41 mmol), the acid component **12** (161 mg, 0.36 mmol), and DMAP (6 mg, 0.05 mmol) were dissolved in CH_2Cl_2 (2.5 mL) and cooled in an ice-MeOH bath, whereupon EDCI (102 mg, 0.53 mmol) was added. The mixture was stirred at this temperature for 30 min, and then in an ice-bath for 24 h. After dilution with EtOAc, the organic layer was washed with ice-cold 1 M aq $KHSO_4$ solution, sat. $NaHCO_3$ solution, and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by chromatography (3:5 then 1:1 EtOAc/hexane) to recover the tripeptide **27** (88 mg, 21% recovery) and give the depsipeptide **28** as a white solid (427 mg, 82%; 87% conversion); mp 53–54 °C (EtOAc/hexane); $[\alpha]_D^{23}$ +2.8 (*c* = 1.06, $CHCl_3$).
IR (KBr): ν = 3393, 2955, 2932, 1763, 1719, 1701, 1686, 1655, 1647, 1638, 1541, 1509, 1499, 1260, 1163, 1132, 839, 781, 735, 698 cm^{-1}.
^1H NMR (270 MHz, CDCl3): δ = 7.95 (br s, 1 H, LysSta 8-NH), 7.69 (dd, *J* = 2.3, 7.3 Hz, 1 H, catechol 6-H), 7.30–7.49 (m, 22 H, 4 × C_6H_5, Gly NH, β-OHAsp NH), 7.07–7.13 (m, 2 H, catechol 5-H, 4-H), 5.36 (d, *J* = 9.2 Hz, 1 H, Ser NH), 5.14 (s, 2 H, C*H*$_2$Ph), 5.13 (s, 2 H, C*H*$_2$Ph), 5.09 (overlapping br s, 1 H, LysSta 4-NH), 5.05 (d, *J* = 1.3 Hz, 2 H, C*H*$_2$Ph), 5.02 (s, 2 H, C*H*$_2$Ph), 4.81–4.84 (m, 3 H, Ser α-H, β-OHAsp α-H, β-H), 4.66 (s, 2 H, CH_2CCl_3), 4.51 (br s, 1 H, Ser α-H), 4.42 (dd, *J* = 5.3, 11.2 Hz, 1 H, Ser β-H), 4.04–4.15 (m, 3 H, LysSta 3-H, Gly α-CH_2), 3.59 (br s, 1 H, LysSta 4-H), 3.30 (br s, 1 H, LysSta 8-H), 3.19 (br s, 1 H, LysSta 8-H), 2.46 (br s, 2 H, LysSta 2-CH_2), 1.64 (br s 1 H), 1.41 [s, 9 H, OC$(CH_3)_3$], 1.27–1.42 (m, 5 H), 0.86 [s, 9 H, SiC$(CH_3)_3$], 0.84 [s, 9 H, SiC$(CH_3)_3$], 0.07 [s, 6 H, Si$(CH_3)_2$], 0.05 (s, 3 H, SiCH_3), –0.03 (s, 3 H, SiCH_3).
Anal. $C_{72}H_{96}Cl_3N_5O_{17}Si_2$ (1466.1): calcd C, 58.99; H, 6.60; N, 4.78; found: C, 58.79; H, 6.44; N, 4.87.

Dipeptide 29:
A mixture of Boc-Gly-OTce (**24**; 115 mg, 0.38 mmol) and 4 N HCl/dioxane (2 mL) was stirred at 0 °C for 30 min and at r.t. for 1 h, and then concentrated in vacuo. The residue was dissolved in dioxane (1 mL) and toluene (3 mL), and the volatiles were removed in vacuo. The resulting amine salt and the acid component **12** (138 mg, 0.3 mmol) were dissolved in DMF (1 mL), whereupon DEPC (68 µL, 0.45 mmol) at 0 °C was added to this solution. After the mixture was stirred for 15 min, NMM (79 µL, 0.72 mmol) was added, and then the mixture was further stirred at 0 °C for 20 h. After dilution with benzene/EtOAc (v/v, 1:3, 20 mL), the organic layer was washed with ice-cold 1 M aq $KHSO_4$ solution, sat. $NaHCO_3$ solution, and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by chromatography (1:8 EtOAc/hexane) to give the dipeptide **29** as an oily white solid (165 mg, 86%; **29**, aspartimide **32** >120:1 from ^{1}H NMR).
IR (neat): ν = 3422, 3359, 2955, 2932, 1759, 1723, 1717, 1682, 1498, 1368, 1256, 1165, 1129, 839, 782, 727 cm^{-1}.
^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.31–7.35 (m, 5 H, arom), 7.01 (br s, 1 H, Gly NH), 5.36 (br s, β-OHAsp NH), 5.13 (ABq, J = 11.9 Hz, 2 H, CH_2Ph), 4.94 (d, J = 3.0 Hz, β-OHAsp β-H), 4.79 (ABq, J = 11.9 Hz, CH_2CCl_3), 4.66 (d, J = 8.3 Hz, β-OHAsp α-H), 4.18 (br s, 2 H, Gly α-CH_2), 1.44 [s, 9 H, $OC(CH_3)_3$], 0.86 [s, 9 H, $SiC(CH_3)_3$], 0.08 (s, 3 H, $SiCH_3$), 0.02 (s, 3 H, $SiCH_3$).
FAB-MS: m/z = 641, 643, 645 (MH^+).

Tripeptide 31:
To a solution of the dipeptide **29** (151 mg, 0.24 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C was added dropwise TFA (1.5 mL). The mixture was stirred at 0 °C for 2 h, and then concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (1 mL) and benzene (3 mL), and the volatiles were removed in vacuo. The resulting amine salt and Boc-Arg(Mtr)-OH (143 mg, 0.29 mmol) were dissolved in DMF (1 mL), whereupon DEPC (54 µL, 0.36 mmol) at 0 °C was added. After 15 min, TEA (79 µL, 0.57 mmol) was added and the mixture was stirred at 0 °C for 16 h, then diluted with EtOAc/benzene (v/v, 3:1, 20 mL). The organic layer was washed with ice-cold 1 M aq $KHSO_4$ solution, sat. $NaHCO_3$ solution, and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by chromatography (1:1 then 1.5:1 EtOAc/hexane) to give the tripeptide **31** as a white solid (217 mg, 91%); mp 73–74 °C (EtOAc/hexane); $[\alpha]_D^{24}$ –13.3 (c = 1.00, $CHCl_3$).
IR (KBr): ν = 3416, 3343, 2955, 2934, 1759, 1686, 1624, 1586, 1551, 1508, 1474, 1458, 1368, 1308, 1256, 1163, 1121, 839 cm^{-1}.
^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.53 (br s, 1 H, NH), 7.27–7.31 (m, 6 H, C_6H_5, NH), 6.94 (d, J = 8.9 Hz, 1 H, β-OHAsp NH), 6.53 (s, 1 H, Mtr 5-H), 6.19 (br s, guanidino), 5.11 (ABq, J = 12.0 Hz, 2 H, CH_2Ph), 4.97 (s, 1 H, β-OHAsp β-H), 4.90 (d, J = 8.6 Hz, β-OHAsp α-H), 4.75 (s, 2 H, CH_2CCl_3), 4.01–4.14 (m, 3 H, Arg α-H, Gly α-CH_2), 3.81 (s, 3 H, Mtr OCH_3), 3.12–3.14 (m, 2 H, Arg δ-CH_2), 2.70 (s, 3 H, Mtr 2-CH_3), 2.63 (s, 3 H, Mtr 6-CH_3), 2.12 (s, 3 H, Mtr 3-CH_3), 1.27–1.67 (m, 4 H, Arg β-CH_2, γ-CH_2), 1.42 [s, 9 H, $OC(CH_3)_3$], 0.88 [s, 9 H, $SiC(CH_3)_3$], 0.07 (s, 3 H, $SiCH_3$), 0.05 (s, 3 H, $SiCH_3$).
Anal. $C_{42}H_{63}Cl_3N_6O_{12}SSi$ (1010.9): calcd C, 49.92; H, 6.28; N, 8.32; found: C, 50.05; H, 6.33; N, 8.46.

Linear Depsiheptapeptide 37:
To a stirred solution of the depsitetrapeptide **28** (172 mg, 0.12 mmol) in THF (1.5 mL) was added zinc powder (460 mg, 7.04 mmol), followed by 1 M aq NH_4OAc (0.3 mL). The heterogeneous slurry was vigorously stirred at r.t. for 20 h, then diluted with EtOAc (20 mL), and filtered through a pad of Celite. The combined filtrate was washed with ice-cold 1 M aq $KHSO_4$ solution, and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by chromatography (1:1 EtOAc/hexane then 25:1 CH_2Cl_2/MeOH) to give the acid **33** as a white solid (136 mg, 87%).
IR (KBr): ν = 3450, 3350, 2953, 2932, 1759, 1721, 1655, 1649, 1576, 1541, 1499, 1456, 1260, 1213, 1163, 1130, 839, 781, 698 cm^{-1}.
^{1}H NMR (270 MHz, $CDCl_3$): δ = 8.20 (t, J = 5.4 Hz, 1 H, LysSta 8-NH), 7.66 (dd, J = 3.1, 6.7 Hz, 1 H, catechol 6-H), 7.24–7.48 (m, 20 H, 4 × C_6H_5), 7.12–7.20 (m, 2 H, catechol 5-H, 4-H), 7.02 (t, J = 4.6 Hz, 1 H, Gly NH), 5.62 (d, J = 9.9 Hz, 1 H, β-OHAsp NH), 5.29 (d, J = 9.2 Hz, 1 H, NH), 5.15 (s, 2 H, CH_2Ph), 5.08–5.12 (m, 4 H, CH_2Ph, CHPh, NH), 5.04 (s, 2 H, CH_2Ph), 4.90 (d, J = 12.5 Hz, 1 H, CHPh), 4.79 (d, J = 9.9 Hz, 1 H, β-OHAsp α-H), 4.74 (d, J = 2.3 Hz, 1 H, β-OHAsp β-H), 4.67–4.71 (m, 1 H, Ser α-H), 4.52–4.57 (m, 1 H, Ser β-H), 4.35 (dd, J = 6.3, 11.2 Hz, 1 H, Ser β-H), 4.19–4.20 (m, 1 H, LysSta 3-H), 3.99–4.07 (m, 1 H, Gly α-H), 3.72–3.79 (m, 1 H, Gly α-H), 3.49–3.54 (m, 1 H, LysSta 4-H), 3.20–3.31 (m, 2 H, LysSta 8-CH_2), 2.43–2.59 (m, 2 H, LysSta 2-CH_2), 1.62–1.65 (m, 1 H), 1.40 [s, 9 H, $OC(CH_3)_3$], 1.17–1.46 (m, 5 H), 0.87 [s, 9 H, $SiC(CH_3)_3$], 0.84 [s, 9 H, $SiC(CH_3)_3$], 0.10 (s, 3 H, $SiCH_3$), 0.08 (s, 3 H, $SiCH_3$), 0.05 (s, 3 H, $SiCH_3$), 0.02 (s, 3 H, $SiCH_3$).
To a stirred solution of the tripeptide **31** (38 mg, 0.038 mmol) in CH_2Cl_2 (1 mL) at 0 °C was added dropwise TFA (1 mL). The mixture was stirred at 0 °C for 2 h, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (1 mL) and benzene (3 mL), and the volatiles were removed in vacuo. The resulting amine salt and the above acid (**33**; 44 mg, 0.033 mmol) were dissolved in DMF (0.2 mL), and NMM (16 µL, 0.15 mmol) at 0 °C was added dropwise, followed by solid FDPP (38 mg, 0.098 mmol) in one portion. The mixture was stirred at 0 °C for 42 h, diluted with EtOAc. The organic layer was washed with ice-cold 1 M aq $KHSO_4$ solution, sat. $NaHCO_3$ solution, and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by chromatography (gradient from 1:1 to 2:1 EtOAc/hexane) to give the depsiheptapeptide **37** as a white solid (63 mg, 86%); mp 81–82 °C (EtOAc/hexane); $[\alpha]_D^{25}$ +2.5 (c = 0.86, $CHCl_3$).
IR (KBr): ν = 3351 (br), 2953, 2932, 1759, 1721, 1675 (br), 1541, 1501, 1260, 1163, 1123, 839, 781, 698 cm^{-1}.
^{1}H NMR (270 MHz, $CDCl_3$): δ = 8.10 (br s, 1 H, LysSta 8-NH), 7.78 (br s, 1 H, Gly NH), 7.62 (d, J = 7.3 Hz, 1 H, catechol 6-H), 6.96–7.45 (m, 31 H, arom, NH), 6.50 (s, 1 H, Mtr 5-H), 6.20 (br s, 3H, guanidino), 5.83 (br s, 1 H, NH), 5.34 (d, J = 8.9 Hz, 1 H, NH), 4.95–5.14 (m, 12 H, 5 × CH_2Ph, 2 × β-OHAsp α-H), 4.75 (s, 2 H, CH_2CCl_3), 4.60–4.72 (m, 3 H, 2 × β-OHAsp β-H, Ser α-H), 4.46 (d, J = 7.9 Hz, 1 H, Ser β-H), 3.96–4.24 (m, 5 H, Ser β-H, 3 × Gly α-H, LysSta 3-H), 3.79 (s, 3 H, Mtr OCH_3), 3.70 (d, J = 14.4 Hz, 1 H, Gly α-H), 3.43 (br s, 1 H, LysSta 4-H), 3.29 (br s, 1 H, LysSta 8-H), 3.12 (br s, 3 H, LysSta 8-H, Arg δ-CH_2), 2.70 (s, 3 H, Mtr 2-CH_3), 2.63 (s, 3 H, Mtr 6-CH_3), 2.43–2.53 (m, 2 H, LysSta 2-CH_2), 2.11 (s, 3 H, Mtr 3-CH_3), 1.75 (br s, 1 H), 1.41 [s, 9 H, $OC(CH_3)_3$], 1.25–1.47 (m, 9 H), 0.88 [s, 9 H, $SiC(CH_3)_3$], 0.83 [s, 18 H, 2 × $SiC(CH_3)_3$], 0.06 [s, 6 H, $Si(CH_3)_2$], 0.05 (s, 3 H, $SiCH_3$), 0.01 (s, 3 H, $SiCH_3$), –0.03 [s, 6 H, $Si(CH_3)_2$].
Anal. $C_{107}H_{148}Cl_3N_{11}O_{26}SSi_3$ (2227.8): calcd C, 57.71; H, 6.69; N, 6.92; found: C, 57.52; H, 6.66; N, 6.98.

Cyclic Depsiheptapeptide 38:
Linear depsiheptapeptide **37** (100 mg, 0.045 mmol) was dissolved in HOAc followed by zinc powder (294 mg, 4.5 mmol). The suspension was vigorously stirred at r.t. for 4.5 h, diluted with EtOAc, and filtered through a pad of Celite. The filtrate was washed with ice-cold 1 M aq $KHSO_4$ solution, and brine, dried (Na_2SO_4), and filtered. The organic solvent was concentrated in vacuo to 15 mL followed by azeotropic removal of HOAc in vacuo with heptane (15 mL) to afford the free acid as a white solid (90 mg, 96%).
^{1}H NMR (270 MHz, $CDCl_3$): δ = 8.10 (br s, 1 H, NH), 7.72 (br s, 1 H, NH), 7.62 (d, J = 7.6 Hz, 1 H, catechol 6-H), 7.05–7.47 (m, 31 H, arom, catechol, and NH), 6.50 (s, 1 H, Mtr 5-H), 6.35 (br s, guanidino), 5.60 (br s, 1 H, NH), 5.33 (d, J = 8.9 Hz, 1 H, NH), 4.92–5.18 (m, 12 H, 5 × CH_2Ph, 2 × β-OHAsp α-H), 4.74–4.78 (m, 3 H, 2 × β-OHAsp α-H, Ser α-H), 4.52–4.57 (m, 1 H), 4.37 (br s, 1 H), 4.01–4.20 (m, 3 H), 3.80 (br s, 1 H), 3.79 (s, 3 H, Mtr OCH_3), 3.48 (br

s, 1 H, LysSta 4-H), 3.10 (br s, 4 H, LysSta 8-CH_2), 2.67 (s, 3 H, Mtr 2-CH_3), 2.60 (s, 3 H, Mtr 6-CH_3), 2.51 (br s, 2 H, LysSta 2-CH_2), 2.10 (s, 3 H, Mtr 3-CH_3), 1.25–1.66 (m, 10 H), 1.39 [s, 9 H, $OC(CH_3)_3$], 0.85 [s, 9 H, $SiC(CH_3)_3$], 0.81 [s, 9 H, $SiC(CH_3)_3$], 0.80 [s, 9 H, $SiC(CH_3)_3$], 0.06 (s, 3 H, $SiCH_3$), 0.03 (s, 3 H, $SiCH_3$), 0.02 [s, 6 H, $Si(CH_3)_2$], –0.04 (s, 3 H, $SiCH_3$), –0.06 (s, 3 H, $SiCH_3$).

The resulting acid (90 mg, 0.043 mmol) was treated with TFA (2.5 mL) in CH_2Cl_2 (2.5 mL) at 0°C. The mixture was vigorously stirred at this temperature for 2.5 h, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (1 mL) and benzene (3 mL), and the volatiles were removed in vacuo. The deprotected product was dissolved in DMF (10 mL). NMM (24 μL, 0.22 mmol) at 0°C was added to this mixture, followed by a solution of FDPP (33 mg, 0.086 mmol) in DMF (1 mL). The mixture was stirred at 0°C for 30 min and then allowed to warm to r.t. for 38 h. After removal of DMF by distillation below 20°C, the residue was dissolved in EtOAc and the organic layer was washed with ice-cold 1 M aq $KHSO_4$ solution, sat. $NaHCO_3$ solution, and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by chromatography (25:1 CH_2Cl_2/MeOH) to give the cyclic depsipeptide **38** as white crystals (42 mg, 53%); mp 108–109°C (CH_2Cl_2/hexane); $[\alpha]_D^{25}$ –28.8 (c = 0.83, $CHCl_3$).

IR (KBr): ν = 3335 (br), 2951, 2932, 1744, 1620 (br), 1539 (br), 1456, 1379, 1308, 1260 (br), 1121, 889, 783, 698 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.81 (br s, 0.5 H, guanidino), 8.56 (d, J = 4.4 Hz, 1 H, Arg NH), 8.27 (d, J = 8.3 Hz, 1 H, β-OHAsp NH), 8.13 (t, J = 5.4 Hz, 1 H, LysSta 8-NH), 8.04 (br s, 1H, Gly NH), 7.75 (d, J = 7.8 Hz, 1 H, Ser NH), 7.49 (d, J = 7.1 Hz, 1 H, catechol 6-H), 7.10–7.45 (m, 29 H, arom, Gly NH, β-OHAsp NH), 6.97(d, J = 8.8 Hz, 1 H, LysSta 4-NH), 6.67 (s, 1 H, Mtr 5-H), 6.41 (br s, guanidino), 5.19 (s, 2 H, CH_2Ph), 5.10 (ABq, J = 12.5 Hz, 2 H, CH_2Ph), 5.05 (s, 2 H, CH_2Ph), 5.01 (s, 2 H, CH_2Ph), 4.97 (ABq, J = 10.6 Hz, 2 H, CH_2Ph), 4.85 (d, J = 5.0 Hz, 2 H, CH_2Ph), 4.85 (d, J = 5.0 Hz, 2 H, β-OHAsp α-H, LysSta 3-OH), 4.83 (d, J = 3.7 Hz, 1 H, β-OHAsp α-H), 4.76 (d, J = 4.6 Hz, 1 H, β-OHAsp β-H), 4.67 (br s, 2H, β-OHAsp β-H, Ser α-H), 4.24 (d, J = 10.5 Hz, 1 H, Ser β-H), 4.11–4.13 (m, 1 H, Ser β-H), 4.06 (dd, J = 7.8, 12.2 Hz, 1 H, Gly α-H), 3.82–3.85 (m, 2 H, Arg α-H, Gly α-H), 3.77 (s, 5 H, Mtr OCH_3, LysSta 3-H, Gly α-H), 3.67 (d, J = 13.4 Hz, 1 H, Gly α-H), 3.31–3.32 (overlapping m, 1 H, LysSta 4-H), 3.21–3.24 (overlapping m, 1 H, LysSta 8-H), 3.11–3.13 (m, 1 H, LysSta 8-H), 3.01 (d, J = 4.4 Hz, 2 H, Arg α-CH_2), 2.59 (s, 3 H, Mtr 2-CH_3), 2.52 (s, 3 H, Mtr 6-CH_3), 2.30–2.33 (d, J = 12.5 Hz, 1 H, LysSta 2-H), 2.21 (dd, J = 9.16, 15.0 Hz, 1 H, LysSta 2-H), 2.04 (s, 3 H, Mtr 3-CH_3), 1.15–1.55 (m, 10 H), 0.79 [s, 9 H, $SiC(CH_3)_3$], 0.78 [s, 9 H, $SiC(CH_3)_3$], –0.01 (s, 3H, $SiCH_3$), –0.02 (s, 3 H, $SiCH_3$), –0.05 [s, 6 H, $Si(CH_3)_2$].

Anal. $C_{94}H_{123}N_{11}O_{23}SSi_2$ (1864.0): calcd C, 60.60; H, 6.65; N, 8.27; found: C, 60.52; H, 6.79; N, 8.42.

Alterobactin A (1):

To a solution of the cyclic depsipeptide **38** (37 mg, 0.020 mmol) in HOAc/THF/H_2O (v/v/v, 3:1:1; 4.5 mL) was added 5% Pd/C. The suspension was stirred at r.t. for 12.5 h under a H_2 atmosphere, filtered through a pad of Celite, and the pad was washed twice with HOAc/THF/H_2O (3:1:1) and three times with THF/H_2O (v/v, 1:1). The combined filtrate was concentrated in vacuo and any residual HOAc and H_2O were removed by adding heptane and toluene, and concentrating in vacuo. The residue was triturated and washed three times with Et_2O to afford the product retaining the Mtr group as a white solid (24 mg). The above Mtr-protected product was treated with TFA (6 mL), followed by thioanisole (0.24 mL). The mixture was vigorously stirred at r.t. for 7 h and concentrated in vacuo. The residual thioanisole was removed under high vacuum. The crude product was triturated and washed three times with Et_2O and then further purified by reverse-phase HPLC (C_4; MeCN/H_2O/TFA, 10:90:0.1; 7.5 mL/min) and lyophilized to afford alterobactin A (**1**) as a grey-white solid (15 mg, 81%); R_f 0.32 (silica gel, BuOH/H_2O/AcOH, 3:3:1).

^{1}H NMR (500 MHz, D_2O): δ = 7.21 (d, J = 7.9 Hz, 1 H, catechol 6-H), 7.08 (d, J = 7.9 Hz, 1 H, catechol 4-H), 6.87 (t, J = 7.9 Hz, 1 H, catechol 5-H), 5.16 (d, J = 3.1 Hz, 1 H, β-OHAsp α-H), 5.03 (d, J = 3.1 Hz, 1 H, β-OHAsp α-H), 4.96 (d, J = 3.1 Hz, 1 H, β-OHAsp β-H), 4.86 (d, J = 3.1 Hz, 1 H, β-OHAsp β-H), 4.70 (overlapping m, 1 H, Ser α-H), 4.64 (dd, J = 5.2, 11.3 Hz, 1 H, Ser β-H), 4.40 (dd, J = 1.8, 10.9 Hz, 1 H, Ser β-H), 4.35 (m, 1 H, LysSta 3-H), 4.12 (overlapping m, 1 H, Arg α-H), 4.08 (ABq, J = 17.1 Hz, 2H, Gly α-CH_2), 4.03 (ABq, J = 17.1 Hz, 2 H, Gly α-CH_2), 3.39 (m, 3 H, LysSta 4-H and 8-H), 3.20 (t, J = 6.7 Hz, 2 H, Arg δ-CH_2), 2.58 (d, J = 5.5 Hz, 2 H, LysSta 2-CH_2), 1.77–1.86 (m, 2 H, Arg β-CH_2), 1.60–1.76 (m, 6 H, Arg γ-CH_2, LysSta 5-CH_2 and 7-CH_2), 1.54–1.58 (m, 1 H, LysSta 6-H), 1.41–1.48 (m, 1 H, LysSta 6-H).

^{13}C NMR (125 MHz, D_2O): δ = 177.5 (Arg C=O), 177.3 (OHAsp β-C=O), 176.9 (OHAsp β-C=O), 175.4 (LysSta C=O), 175.2 (Gly C=O), 173.9 (Gly C=O), 173.7 (Ser C=O), 173.3 (OHAsp α-C=O), 173.0 (DHB C=O), 172.3 (OHAsp α-C=O), 159.6 (Arg C=NH), 149.6 (DHB C-2), 147.4 (DHB C-3), 122.5 (DHB C-5), 122.2 (DHB C-4), 121.7 (DHB C-6), 119.9 (DHB C-1), 73.0 (OHAsp C_β), 72.8 (OHAsp C_β), 70.0 (LysSta C-3), 67.7 (Ser C_β), 58.9 (OHAsp C_α), 58.3 (Arg C_α), 58.2 (OHAsp C_α), 58.0 (LysSta C-4), 54.5 (Ser C_α), 45.3 (Gly C_α), 45.2 (Gly C_α), 43.2 (Arg C_δ), 41.6 (LysSta C-8), 40.7 (LysSta C-2), 30.9 (LysSta C-7), 30.3 (Arg C_β), 29.2 (LysSta C-5), 26.9 (Arg C_γ), 25.0 (LysSta C-6).

FAB-MS in H_2O: m/z = 928 (MH^+).

FAB-HRMS (NBA) in H_2O: $C_{36}H_{54}N_{11}O_{18}$ (MH^+): m/z calcd 928.3649, found 928.3723.

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