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Synthesis of brasilibactin A and confirmation of absolute configuration of β-hydroxy acid fragment

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Abstract—A synthesis of brasilibactin A, a cytotoxic siderophore from the actinomycete of *Nocardia brasiliensis*, and three unnatural diastereomers of the natural product is described. Four possible diastereomers of the β-hydroxy acid fragment were prepared via asymmetric aldol reactions and used to synthesize brasilibactin A and its diastereomers. Careful analysis of ^{1}H NMR data confirmed that brasilibactin A possesses the 17*S*,18*R* absolute stereochemistry.

Brasilibactin A (**1**, Fig. 1) is a potent cytotoxic siderophore isolated from the actinomycete of *Nocardia brasiliensis* IFM 0995 by Tsuda et al.[1] The structure and stereochemistry of brasilibactin A was determined by extensive spectroscopic and chemical analysis.[1] Brasilibactin A (**1**) possesses a nearly identical molecular nucleus to many mycobactin-type siderophores, which includes a hydroxamic acid, an *N*-hydroxyformamide, and a 2-(2-hydroxyphenyl)-Δ^2-1,3-oxazoline which all serve as iron-chelating components.[2] Mitchell et al. recently reported the first total synthesis of **1** and revised the originally assigned *anti*-configuration[1] of the β-hydroxy acid fragment in **1** as *syn*-configuration by synthesizing two of the four possible diastereomers of the β-hydroxy acid fragment.[3] Brasilibactin A (**1**) was reported to exhibit potent cytotoxicity against murine leukemia L1210 and human epidermoid carcinoma KB cells (IC_{50}, 0.02 and 0.04 μg/mL, respectively) and to cause a concentration-dependent increase in the caspase-3 activity in HL60 cells.[1] However, the molecular mechanism of brasilibactin A (**1**) has yet to be established. We undertook the total synthesis of brasilibactin A (**1**) and its diastereomers to study the effect of configuration of the β-hydroxy acid fragment on biological activity (e.g., Fe^{3+}-chelation capability, cytotoxicity). Herein, we report a synthesis of brasilibactin A (**1**) and three unnatural diastereomers as well as an unambiguous confirmation of the absolute configuration of the β-hydroxy acid fragment of the natural product.

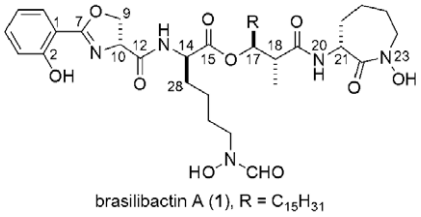

Figure 1. Structure of brasilibactin A.

Scheme 1 describes our approach to the synthesis of brasilibactin A and its diastereomers (**1**–**4**) via coupling of four fragments (**5**–**11**). Disconnection of an ester and two amide bonds of brasilibactin A yields four fragments, an oxazoline **5**, an *N*-hydroxyformamide **6**, a β-hydroxy acid (**7**–**10**), and a cyclic hydroxamic acid **11**. The oxazoline **5** could be prepared from a direct condensation of a derivative of salicylic acid **12** and D-serine benzyl ester (**13**). The *N*-hydroxyformamide **6** and the cyclic hydroxamic acid **11** could be derived from N^{α}-Cbz-D-lysine (**14**) via methodologies previously established by Miller et al. We anticipated that highly stereoselective aldol reactions of hexadecanal (**19**) with *N*-propanoyl-oxazolidinone and *O*-propanoyl-norephedrine could be used to prepare the four possible *syn*- and *anti*-diastereomers of the β-hydroxy acid (**7**–**10**).

As outlined in Scheme 2, oxazoline **5** was synthesized from commercially available methyl salicylate **20**.[2a] Protection of **20** with BnBr[4] and subsequent hydrolysis

Keywords: Brasilibactin A; Siderophore; Absolute configuration; β-Hydroxy acid.
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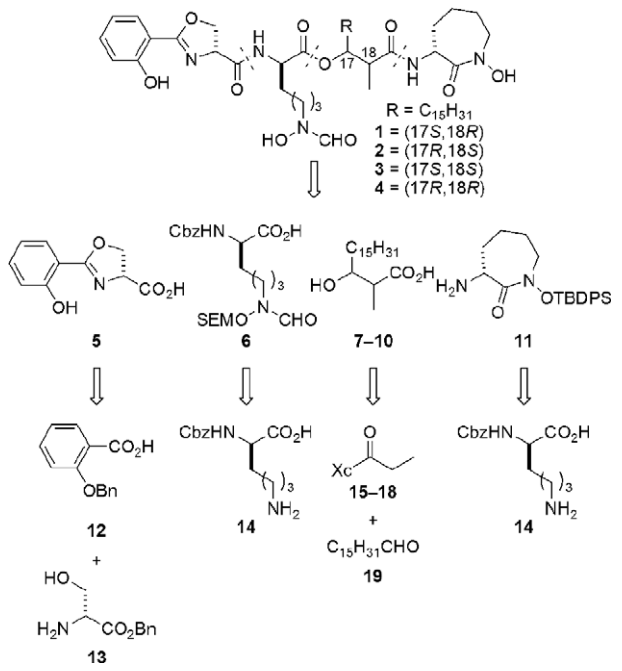
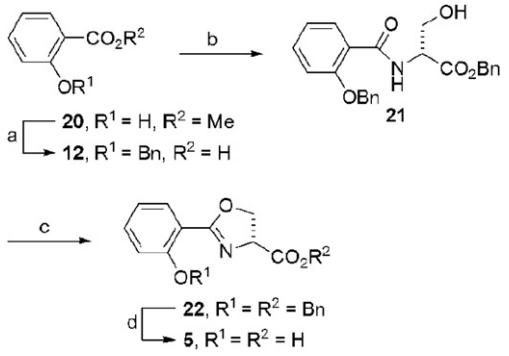


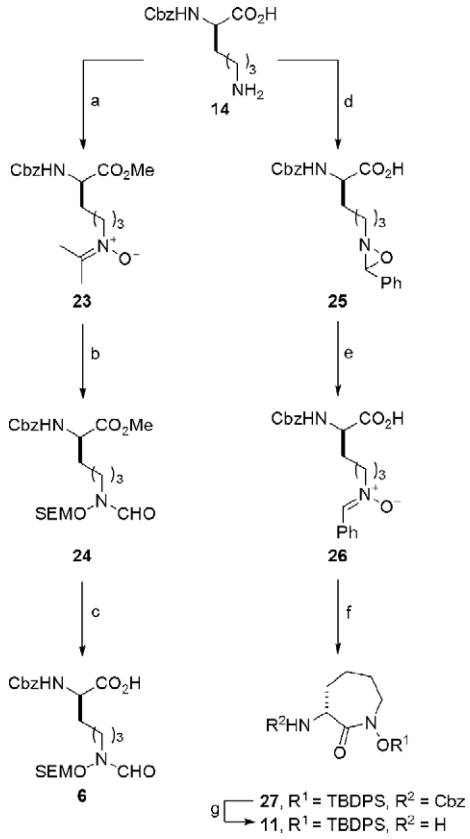
Scheme 1. Retrosynthetic analysis of brasilibactin A.



Scheme 2. Synthesis of oxazoline **5**. Reagents and conditions: (a) (i) BnBr, K_2CO_3, KI, THF, rt, 24 h; (ii) KOH, MeOH, H_2O, 64% for two steps; (b) **13**, EDC, Et_3N, CH_2Cl_2, rt, 24 h, 64%; (c) Burgess reagent, THF, reflux, 30 min, 70%; (d) 10% Pd–C, H_2, MeOH, rt, 2 h, >90%.

(KOH, MeOH)[5] provided **12** (64% for two steps). A coupling of **12** to D-serine benzyl ester (**13**)[6] followed by treatment of **21** with Burgess reagent provided **22**.[2a] Final deprotection of the Bn protecting groups in **22** under conventional conditions (H_2/Pd–C) completed the synthesis of **5** (>90%).

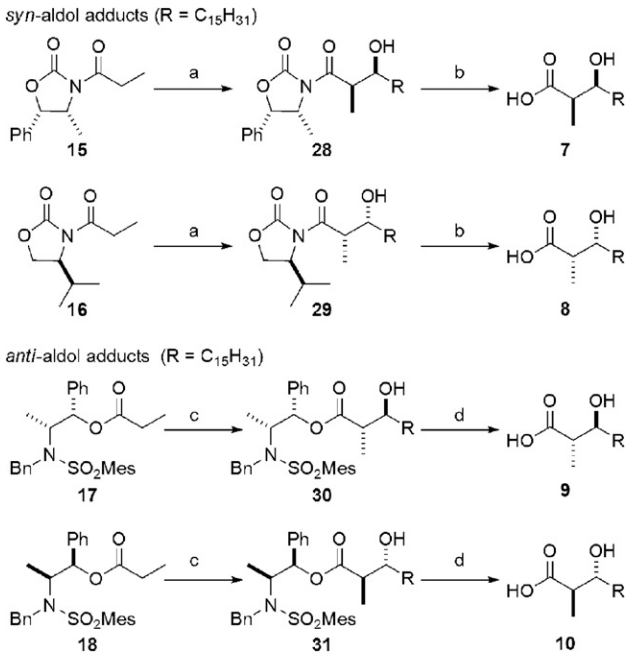
The *N*-hydroxyformamide **6** and the cyclic hydroxamic acid **11** were prepared following the procedures established by Miller et al.[7] As shown in Scheme 3, N^{α}-Cbz-D-lysine (**14**) was converted to the corresponding methyl ester followed by oxidation with dimethyldioxirane in acetone to give nitrone **23**.[7a] Treatment of **23** with $NH_2OH{\cdot}HCl$, coupling with formic acid, protection with SEMCl, and hydrolysis under basic conditions completed the synthesis of *N*-hydroxyformamide **6**.[7b] N^{α}-Cbz-D-lysine (**14**) was treated with benzaldehyde under basic conditions followed by oxidation with *m*-CPBA and TFA-promoted isomerization to provide



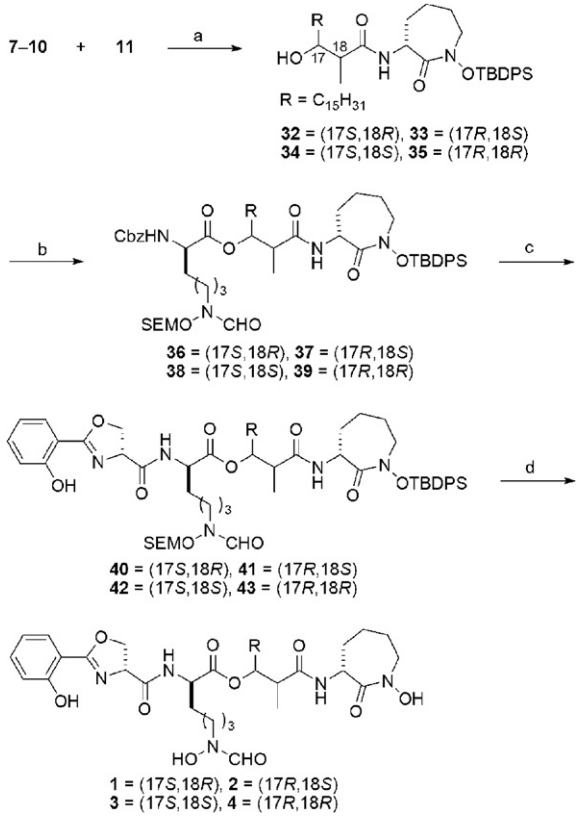
Scheme 3. Synthesis of *N*-hydroxyformamide **6** and cyclic hydroxamic acid **11**. Reagents and conditions: (a) $SOCl_2$, MeOH, rt, 24 h; then dimethyldioxirane, acetone, −78 °C, 15 min, 58% for two steps; (b) (i) $NH_2OH{\cdot}HCl$, MeOH, 40 °C, 12 min; (ii) HCO_2H, EDC, CH_2Cl_2, 0 °C, 2 h; then *i*-Pr_2NEt, MeOH, rt, 48 h, 70% for two steps; (iii) SEMCl, *i*-Pr_2NEt, DMAP, CH_2Cl_2, rt, 24 h, 80%; (c) aq. LiOH, THF, 0 °C, 30 min, rt, 1 h, 90%; (d) PhCHO, KOH, MeOH, 3 Å MS, rt, 24 h, then *m*-CPBA, MeOH, 0 °C, 2 h; (e) TFA, CH_2Cl_2, rt, 1 h; then PhCHO, rt, 24 h, 50% for two steps; (f) (i) $NH_2OH{\cdot}HCl$, MeOH, 60 °C, 20 min; (ii) EDC, HOAt, $NaHCO_3$, CH_3CN/DMF (7:2), rt, 48 h; (iii) TBDPSCl, imidazole, DMF, 35 °C, 24 h, 40% for three steps; (g) 10% Pd–C, H_2, MeOH, rt, 2 h, >90%.

the corresponding nitrone **26**.[7c,d] Since hydrolysis of **26** resulted in the formation of a hydroxylamine, addition of benzaldehyde to the reaction mixture improved the yield of the reaction (50% for two steps).[7c] Treatment of **26** with $NH_2OH{\cdot}HCl$, cyclization of the corresponding hydroxylamine under standard conditions (EDC, HOAt, $NaHCO_3$), and protection of the cyclic hydroxamic acid with TBDPSCl afforded **27**.[2a,7c] Final deprotection of the Cbz protecting group in **27** under conventional conditions (H_2/Pd–C) provided **11** (>90%).

Synthesis of four possible diastereomers of the β-hydroxy acid fragment (**7**–**10**) was achieved by employing the highly stereoselective *syn*-aldol reactions of the *N*-propanoyl-oxazolidinones (**15** and **16**)[8] and *anti*-aldol reactions of the *O*-propanoyl-norephedrines (**17** and **18**)[9] (Scheme 4). The aldol reactions of hexadecanal (**19**)[10] with **15** and **16** in the presence of *n*-Bu_2-BOTf and *i*-Pr_2NEt provided the desired *syn*-aldol adducts **28** (85%) and **29** (85%), respectively, as a single



Scheme 4. Synthesis of β-hydroxy acid (**7**–**10**). Reagents and conditions: (a) (i) $(n\text{-Bu})_2$BOTf, $i\text{-Pr}_2$NEt, CH_2Cl_2, 0 °C, 30 min; (ii) **19**, CH_2Cl_2, −78 °C, 2 h, 85%; (b) 30% H_2O_2, LiOH, THF/H_2O (4:1), 0 °C, 1 h, 90%; (c) (i) $(c\text{-hex})_2$BOTf, Et_3N, CH_2Cl_2, 0 °C, 30 min; (ii) **19**, CH_2Cl_2, −78 °C, 2 h, 80%; (d) LiOH, THF/MeOH/H_2O (2:3:2), rt, 4 days, 85%.



Scheme 5. Synthesis of **1**–**4**. Reagents and conditions: (a) EDC, CH_2Cl_2, rt, 24 h, 64–68%; (b) **6**, DCC, DMAP, toluene, rt, 48 h, 76–89%; (c) 10% Pd–C, H_2, MeOH, rt, 2 h; then **5**, EDC, CH_2Cl_2, rt, 24 h, 48–57%; (d) TFA, CH_2Cl_2, rt, 1.5 h, 68–73%.

diastereomer. Hydrolysis of the β-hydroxy amides (**28** and **29**) under conventional conditions (30% H_2O_2, LiOH) provided the corresponding β-hydroxy acids (**7** and **8**) in 90% yield, respectively. In a similar manner, the aldol reactions of hexadecanal (**19**) with the chiral derivatives of norephedrine (**17** and **18**) in the presence of $c\text{-Hex}_2$BOTf and Et_3N followed by the hydrolysis of the corresponding esters (**30** and **31**) were utilized for the preparation of the *anti*-β-hydroxy acids (**9** and **10**).

The synthesis of brasilibactin A (**1**) and its diastereomers (**2**–**4**) began with coupling **7**–**10** and **11** as shown in Scheme 5. EDC-coupling[2a] of **7** to **11** followed by subsequent coupling[3,7b] of **32** to **6** provided **36**. Alternative methods for the formation of the ester bond (DEAD or DIAD, PPh_3; 2,4,6-trichlorobenzoyl chloride, NEt_3, then DMAP; EDC, DMAP) failed to react with this sterically hindered substrate. Deprotection of the Cbz protecting group in **36** and coupling of the corresponding amine to **5** completed the synthesis of the protected depsipeptide (**40**).[3] Final deprotection of the SEM and TBDPS protecting groups in **40** by treatment with TFA afforded **1** in 68% yield.[3,7b] The diastereomers of brasilibactin A (**2**–**4**) were also prepared in the same manner. ^{1}H NMR data for **1**–**4** were carefully compared with the authentic material (Table 1). The chemical shifts and coupling patterns of **1**, in particular those of H-14 (δ 4.25, m), H-17 (δ 4.90, dt, $J = 2.8$, 8.8 Hz), H-18 (δ 2.62, m), and H-20 (δ 8.13, d, $J = 7.2$ Hz), were identical to those of the natural product.[1,3] The comparison unambiguously showed that brasilibactin A possesses the 17*S*,18*R* absolute stereochemistry, which is consistent with the assignment by Mitchell et al.[3]

In summary, we completed a synthesis of brasilibactin A (**1**), a structurally and biologically interesting linear depsipeptide, and its unnatural diastereomers (**2**–**4**). The convergent synthetic strategy should be broadly applicable to the synthesis of a diverse set of analogs

Table 1. Comparison of ^{1}H NMR data for **1**–**4** with natural brasilibactin A[a]

H no.	Natural **1**[1]	**1** (17*S*,18*R*)	**2** (17*R*,18*S*)	**3** (17*S*,18*S*)	**4** (17*R*,18*R*)	(17*S*,18*R*) in Ref. 3	(17*R*,18*S*) in Ref. 3
18	2.62	2.62	2.66	2.70	2.74	2.61	2.66
14	4.25	4.25	4.21	4.23	4.15	4.24	4.22
21	4.44	4.44	4.46	4.40	4.42	4.42	4.47
9	4.47	4.47	4.48	4.48	4.51	4.47	4.46
17	4.90	4.90	4.95	4.93	4.95	4.90	4.95
20	8.11	8.13	8.15	7.88	7.93	8.15	8.16

[a] Chemical shifts (ppm) in DMSO-d_6.

of **1**. Further studies to assess their biological activity and identify molecular targets of **1** are in progress.

Acknowledgment

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Supplementary data

Copies of ^{1}H NMR data for compounds **1**–**4**, **7**–**10**, **22**, **24**, and **27**–**43** and comparison of ^{1}H NMR data for **1**–**4**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.09.112.

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