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Scalable Synthesis of L-*allo*-Enduracididine: The Unusual Amino Acid Present in Teixobactin

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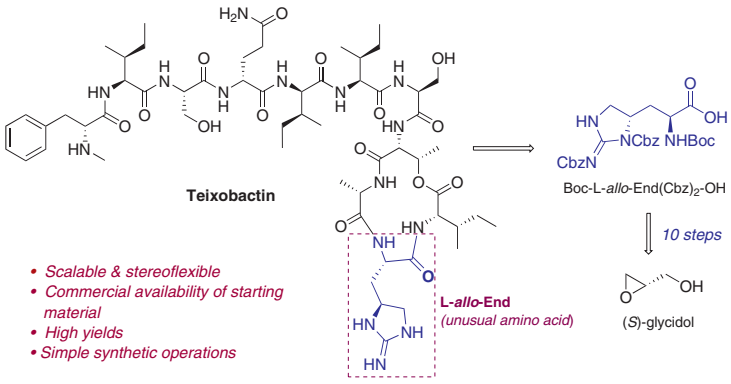

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Abstract A scalable synthesis of L-*allo*-enduracididine is achieved from commercially available (*S*)-glycidol in ten linear steps involving well-established synthetic transformations. The synthetic route is flexible and can be used to synthesize all four diastereomers by changing the stereochemistry of glycidol and Sharpless asymmetric dihydroxylation reagent.

Key words antibiotics, teixobactin, depsipeptide, unusual amino acid, L-*allo*-enduracididine, Staudinger reaction, Sharpless asymmetric dihydroxylation

According to WHO, ESKAPE pathogens appear as a major public health concern in hospital acquired infections in critically ill or immunocompromised patients.[1] In early 2015, a novel cyclic depsipeptide teixobactin (**1**) was isolated from screening of an unculturable β-proteobacteria (*Eleftheria terrae*) by iChip technique.[2] Teixobactin exhibits excellent activities against Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA, MIC 0.25 μg/mL), vancomycin-resistant *Enterococcus* species (VRE, MIC 0.5 μg/mL) and *Mycobacterium tuberculosis* (*Mtb*, MIC 0.125 μg/mL).[2] Teixobactin works as a lipase II inhibitor, like vancomycin, by not allowing pentapeptide incorporation into glycopeptidic cell wall of bacteria, thus rendering it susceptible to rupture.[3] In addition, **1** is also found to inhibit lipase III, another important component of bacterial cell-wall synthesis. Teixobactin is an undecapeptide and encompasses an unusual amino acid, L-*allo*-enduracididine[4] (L-*allo*-End) and four D-amino acids (Figure 1). The structure of teixobactin contains a depsipeptide macrolide and peptide side chain.

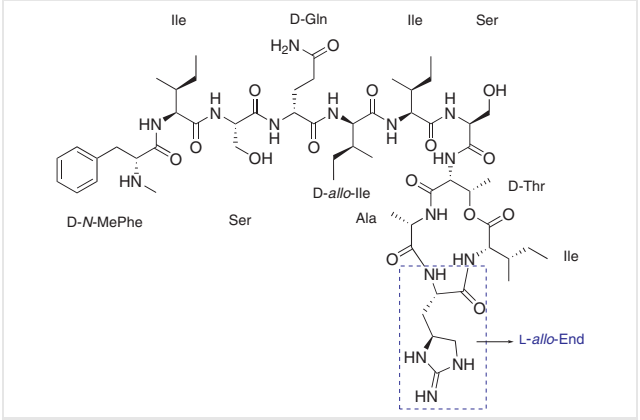

Figure 1 Structure of teixobactin (**1**)

The phenomenal biological activity of **1** prompted research groups to take up the total synthesis[5–9] of teixobactin and analogues[10] to elucidate its pharmacophore[11] towards discovering new antibiotics. So far, five total syntheses of **1** are reported, four solid-phase[5–7],[9] and one solution-phase.[8] The bottleneck in the synthesis of teixobactin is the availability of the unnatural amino acid, L-*allo*-enduracididine. A careful literature survey revealed easy access to L-*allo*-enduracididine will help in developing faster and affordable steps in synthesis of **1** and analogues on gram scale. The groups which achieved total[5–9] and partial[12] synthesis have relied on the synthesis of enduracididine either from (2*S*,3*R*)-4-hydroxy ornithine (which is obtained from L-aspartic acid)[13] developed by Rudolph et al. and Peoples et al. or from protected *trans*-hydroxyproline[14] developed by Yuan et al. Recently, Rao and co-workers reported L-*allo*-End precursor on gram scale via intramolecular guanidinylation followed by alcoholysis.[9]

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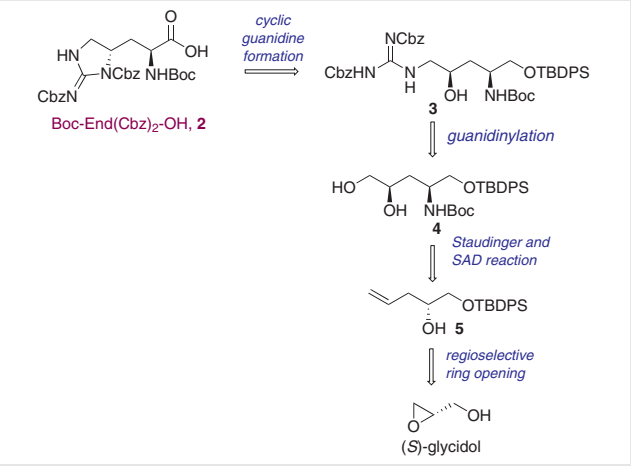
Our own efforts to complete the total synthesis of teixobactin are hinged on the commercial nonavailability of enduracididine. We have already achieved teixobactin peptide side-chain synthesis in solution phase as well as in solid phase.[15] Thus, we desired to develop an alternate synthesis of L-*allo*-enduracididine which will be scalable and stereoflexible. Herein, we report the synthesis of this unusual amino acid from (*S*)-glycidol which is commercially available.

Accordingly, the retrosynthetic analysis envisioned the construction of suitably protected L-*allo*-enduracididine (Boc-End(Cbz)$_2$-OH, **2**) through an intramolecular nucleophilic substitution of guanidine compound **3**, which in turn could be achieved from diol **4** through guanidinylation. The diol **4** could be obtained from homoallylic alcohol **5** by Staudinger reaction followed by Sharpless asymmetric dihydroxylation (SAD). The homoallylic alcohol **5** could be synthesized by regioselective ring opening of (*S*)-glycidol (Scheme 1).



Scheme 1 Retrosynthesis of L-*allo*-enduracididine (Boc-End(Cbz)$_2$-OH, **2**)

Based on the retrosynthetic analysis, (*S*)-glycidol was converted into **2** (Scheme 2). The primary hydroxyl group of commercially available (*S*)-glycidol was protected as *tert*-butyldiphenylsilyl ether (in 95% yield)[16] and regioselective ring opening of epoxide was carried out using a reported procedure which gave homoallylic alcohol **5** in 100 g scale.[16,17] The regioselective opening of epoxide was achieved with CuI catalyst and vinylmagnesium bromide to get alcohol **5** in 96% yield. Mesylation of alcohol **5** followed by azide displacement using NaN_3 gave azido pentenol **6** with inversion of configuration at C-2 and 90% yield over two steps. The azide **6** was reduced under Staudinger reaction conditions using TPP in THF–H_2O (3:1) in the presence of $(Boc)_2O$ to provide *N*-Boc-protected amine **7** in 92% yield. The second chirality was introduced via Sharpless asymmetric dihydroxylation[18] using AD mix-β and methanesulfonamide in *t*-BuOH–H_2O (1:1) at 0 °C for 20 h to realize the diol **4** in 92% yield as a separable diastereomeric mixture (by silica gel column chromatography) in 7:3 ratio with the required diastereomer being the major isomer. Our plan was to convert this diol into amino alcohol to couple with *N*,*N*′-Di-Cbz-1*H*-pyrazole-1-carboxamidine to introduce guanidine moiety. Initially, the diol **4** was monotosylated in situ with Ts_2O/2,4,6-collidine/pyridine in CH_2Cl_2 at ≤ –10 °C, treated with ammonium hydroxide in EtOH at 60 °C to give amino alcohol via epoxide[19] which on further treatment with *N*,*N*′-di-Cbz-1*H*-pyrazole-1-carboxamidine[5,12] gave guanidine derivative **3** in 52% overall yield for four sequential transformations without purification of intermediates. To improve the yield of guanidine derivative **3** further, we thought of an alternative synthetic sequence. Selective mesylation of primary alcohol in compound **4** with MsCl/Et_3N in CH_2Cl_2 at ≤ –30 °C, followed by treatment with NaN_3 in DMF at 70 °C gave azido alcohol **8** in 87% yield. Then, the azide **8** was reduced under Staudinger reaction conditions (TPP, THF–H_2O) to provide amino alcohol which on further treatment with *N*,*N*′-di-Cbz-1*H*-pyrazole-1-carboxamidine[5,12] gave the guanidine derivative **3** in 85% yield (Scheme 2).[20]

The intramolecular cyclization of **3** via triflate[5,12] using triflic anhydride and *N*,*N*-diisopropylethylamine at –78 °C allowed us to construct the enduracididine skeleton **9** in 90% yield.[21] This upon deprotection of silyl group with TBAF in THF afforded alcohol **10** in 95% yield. Finally, the oxidation of the obtained primary alcohol **10** using DMP gave aldehyde which upon Pinnick–Lindgren oxidation using a combination of sodium chlorite and NaH_2PO_4 in *t*-BuOH–H_2O provided the target building block, L-*allo*-enduracididine (Boc-End(Cbz)$_2$-OH, **2**) in 74% yield over two steps, which is being used to complete the total synthesis of teixobactin. A small portion of the carboxylic acid **2** was converted into the corresponding methyl ester **11** using K_2CO_3/MeI in 76% yield. The present approach allows the synthesis of L-*allo*-enduracididine in gram scale due to commercial availability of starting material and simple synthetic operations.

In conclusion, a stereoflexible and scalable synthesis of Boc-End(Cbz)$_2$-OH, an unusual amino acid building block of potent depsipeptide antibiotic teixobactin, has been achieved in ten steps with an overall yield of 22.75%. By changing the stereochemistry of starting material, viz., glycidol and dihydroxylating agent, other diastereomers can be synthesized with equal ease.

Conflict of Interest

The authors declare no conflict of interest.

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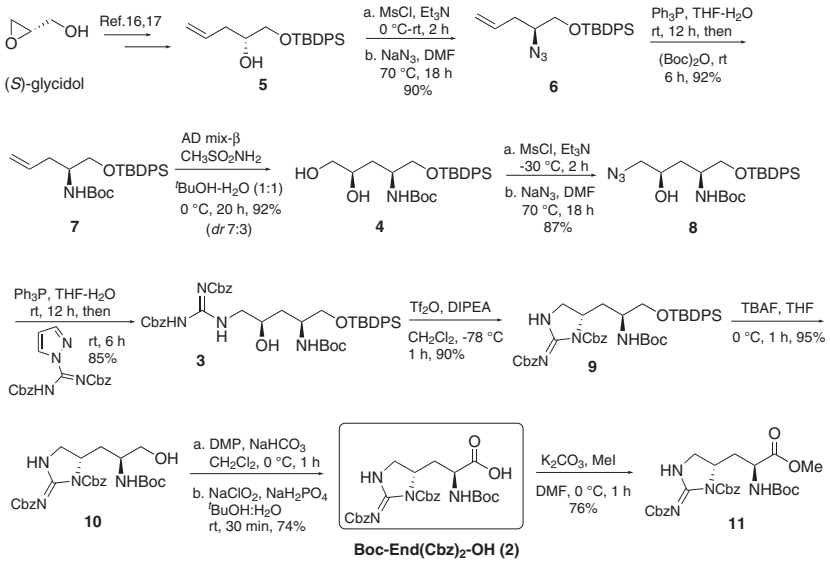

Scheme 2 Synthesis of L-*allo*-enduracididine **2**

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/a-1528-0625.

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(20) **Synthetic Procedure for Guanidine Derivative 3** Triphenylphosphine (2.0 g, 7.2 mmol) was added to a stirred solution of azide **8** (1.27 g, 2.5 mmol) in THF–H_2O (15 mL, 3:1) at 0 °C. Then the reaction was allowed to warm to room temperature and stirred for 12 h. After this period, to the reaction Goodman's reagent (*N,N*'-di-Cbz-1*H*-pyrazole-1-carboxamidine, 968 mg, 2.5 mmol) was added, and the mixture was stirred for another 6 h. The reaction was extracted with EtOAc (2 × 100 mL), the organic layer was washed with brine (75 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum. The residue was purified by column chromatography on silica gel using 85:15 hexanes–EtOAc (v/v) as eluent to give **3** (1.7 g, 85%) as a white solid. TLC: R_f = 0.5 (hexanes–EtOAc, 7:3); mp 92 °C; $[\alpha]_D^{20}$ +6.2 (*c* 1.1, $CHCl_3$). IR (neat): ν_{max} = 3370, 3334, 2946, 1640, 1057 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 11.65 (s, 1 H), 8.71–8.68 (br s, 1 H), 7.57–7.50 (m, 4 H), 7.40–7.16 (m, 16 H), 5.10 (s, 2 H), 5.04 (s, 2 H), 4.82 (d, *J* = 8.8 Hz, 1 H), 4.55 (s, 1 H), 3.86–3.75 (m, 1 H), 3.75–3.59 (m, 3 H), 3.51 (dd, *J* = 10.3, 3.7 Hz, 1 H), 3.23–3.08 (m, 1 H), 1.65–1.53 (m, 1 H), 1.37 (s, 9 H), 1.35–1.25 (m, 1 H), 0.98 (s, 9 H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 163.6, 157.3, 156.1, 153.5, 136.7, 135.6, 135.5, 134.6, 132.9, 132.7, 129.9, 129.8, 128.6, 128.5, 128.4, 128.3, 128.1, 127.8, 127.8, 80.1, 68.0, 67.0, 66.3, 48.6, 46.3, 38.1, 28.3, 26.8, 19.2. HRMS (ESI): *m/z* calcd for $[M + H]^+$: $C_{43}H_{55}N_4O_8Si$: 783.3779; found: 783.3783.

(21) **Synthetic Procedure for Intramolecular Cyclization of Guanidine Derivative 3; (S)-Benzyl-2-{[(benzyloxy)carbonyl]imino}-5-{(S)-2-[(tert-butoxycarbonyl)amino]-3-[(tert-butyl diphenylsilyl)oxy]propyl}imidazolidine-1-carboxylate (9)**

To a solution of 3 (3.2 g, 4 mmol) in anhydrous CH_2Cl_2 (20 mL) was added DIPEA (3.6 mL, 20 mmol), followed by Tf_2O (0.76 mL, 4.5 mmol) dropwise at –78 °C under nitrogen atmosphere. After stirring for 1 h, the reaction was quenched by the addition of ammonium chloride (100 mL), the two layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (100 mL). The combined organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum to give a colorless oil. The residue was purified by column chromatography on silica gel using 70:30 hexanes–EtOAc (v/v) as eluent to give 9 (2.81 g, 90%) as a white solid. TLC: R_f = 0.5 (hexanes–EtOAc, 1:1); mp 84 °C; $[\alpha]_D^{20}$ –7.5 (*c* 1.0, $CHCl_3$). IR (neat): ν_{max} = 3758, 3709, 3481, 3367, 2940, 1710, 1259, 1159 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.82–8.41 (br s, 1 H), 7.63–7.52 (m, 4 H), 7.47–7.27 (m, 16 H), 5.27 (s, 2 H), 5.16 (q, *J* = 12.4 Hz, 2 H), 4.63 (d, *J* = 8.9 Hz, 1 H), 4.34 (t, *J* = 8.4 Hz, 1 H), 3.79 (t, *J* = 8.4 Hz, 1 H), 3.72–3.60 (m, 2 H), 3.52 (dd, *J* = 9.9, 3.4 Hz, 1 H), 3.43 (d, *J* = 9.5 Hz, 1 H), 2.07 (t, *J* = 12.0 Hz, 1 H), 1.63 (t, *J* = 11.8 Hz, 1 H), 1.43 (s, 9 H), 1.01 (s, 9 H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 156.0, 135.6, 135.6, 135.3, 133.1, 132.9, 130.1, 130.0, 128.8, 128.4, 128.2, 128.0, 127.9, 79.8, 68.4, 67.5, 66.5, 54.0, 48.7, 36.1, 28.5, 26.9, 19.3. HRMS (ESI): *m/z* calcd for $[M + H]^+$: $C_{43}H_{53}N_4O_7Si$: 765.3662; found: 765.3678.