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Gram-Scale Solution-Phase Synthesis of Heptapeptide Side Chain of Teixobactin[1]

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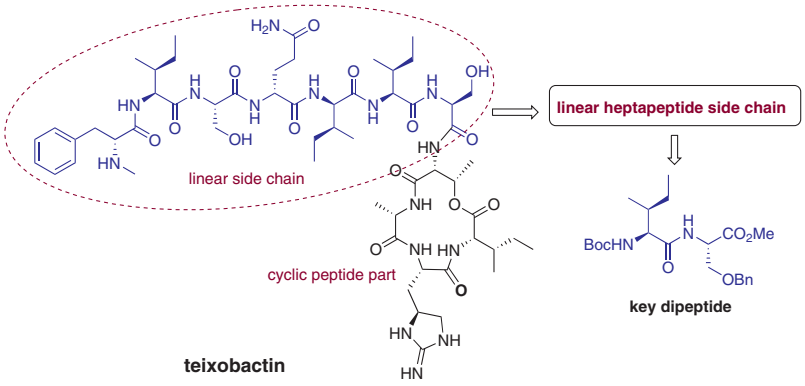

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Abstract We report herein a scalable synthesis of linear heptapeptide side chain of the depsipeptide natural product teixobactin through solution phase. The synthesis of heptapeptide was achieved through an efficient coupling of suitably protected tripeptide and tetrapeptide comprising of three D-amino acids and four usual L-amino acid subunits.

Keywords peptides, amino acids, antibiotics, solution-phase synthesis, heptapeptide, depsipeptide

The World Health Organization (WHO) recently released a list of 12 drug-resistant bacteria which are a threat to the human population.[2] These bacteria have developed resistance to most of the known antibiotics available in the market. This includes strains resistant to vancomycin, which is considered last line of treatment due to its activity of inhibiting lipid II. In the light of this grim situation, discovery of teixobactin (**1**) from β-proteobacteria, *Eleftheria terrae*, is considered a groundbreaking achievement.[3] Teixobactin has inhibited growth of Gram-positive bacteria including *Mycobacterium tuberculosis*, and resistance was not detected under the laboratory conditions. Teixobactin (**1**) has been shown to inhibit lipid II and lipid III resulting in disruption of formation of bacterial cell wall. The accumulation of cell-wall precursor UDP-MurNAc-pentapeptide in the teixobactin treated *Staphylococcus aureous* cells indicated that one of the steps in membrane formation were inhibited.[3,4] Teixobactin comprises of 11 amino acids out of which four are D-amino acids and is a cyclic depsipeptide with linear heptamer side chain (1–7) and macrocycle (8–11) (Figure 1). The presence of unusual amino acid, L-*allo*-enduracididine[5] (end), 13-membered macrocycle, and its very important biological activity has prompted several groups to take up total[6] and analogues,[6c,7–12] synthesis of teixobactin and to elucidate its pharmacophore.[10,12–14] Most of these use solid-phase synthesis to achieve the desired target.

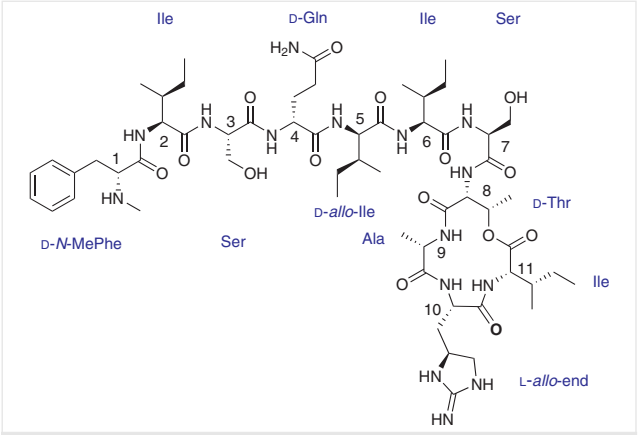

Figure 1 Structure of teixobactin (**1**)

Our group has been working in the area of peptides and peptidomimetics involving synthesis of unusual amino acids.[15,16] With the global focus shifting to antimicrobial resistance (AMR), we have initiated synthesis of some of the new antibacterials. Peptide-containing antibacterials are of special interest to us and in line with this, total synthesis of teixobactin was commenced in our group with an objective to find out new potent lead molecules.

Most of the reports involving synthesis of teixobactin and analogues are based on solid-phase peptide synthesis.[6–12] However, the solution-phase synthesis has its advantages over solid phase in large-scale feasibility and economically viable production of small-to-medium-length peptides for industrial purposes.[17] Two groups have reported the synthesis of unusual amino acid, the L-*allo*-enduracididine part

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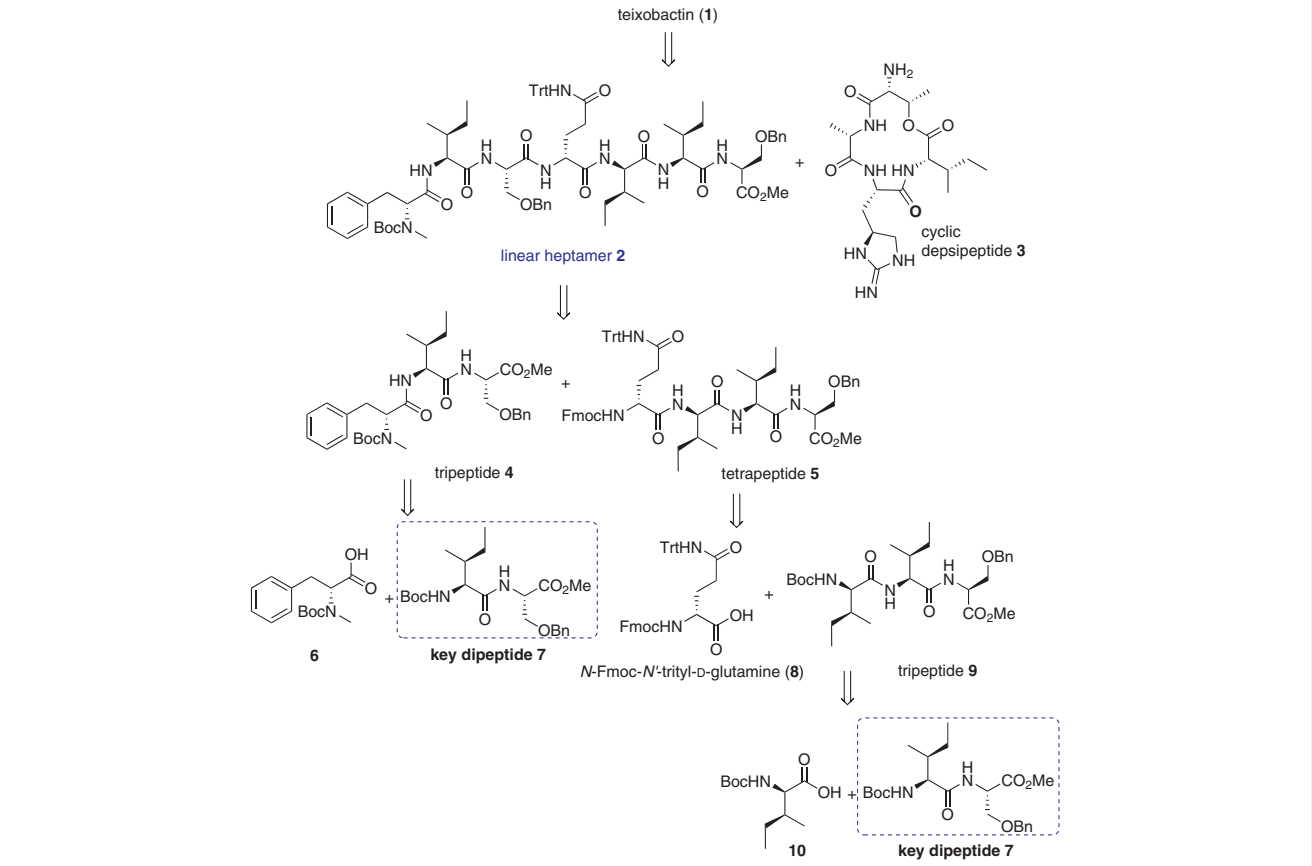
of the macrocyle of **1**.[6b,18] Furthermore, several groups have concentrated on the synthesis of teixobactin analogues by changing the side-chain residues[8a,9,10,13,19] and enduracididine residue of the macrocycle,[7,8,12,19] whose biological properties were compared with the natural product. The studies reveal that changes in the side chain result in decreasing the activity, and replacement of enduracididine residue (end) in the macrocycle does not affect potency of the antibacterial activity. In addition, the X-ray crystallographic structure of a derivative of teixobactin reveals that the unique pattern of hydrophobicity and stereochemistry of side chain residues (1–7) results in formation of amyloid-like fibrils, which is responsible for the activity against Gram-positive bacteria.[20] Hence, the conjugates of this heptapeptide side chain with different active/antibiotic motifs may result in the identification and development of new potent analogues against antibiotic-resistant bacterial strains. In a recent publication, Xu et al. completed the synthesis[21] of **1** and Reddy et al. achieved analogue synthesis[22] using solution phase. Both these groups based their synthetic strategy on hexapeptide (1–6) and macrolide (7–11) as key intermediates. The approach used by Xu et al. focuses on coupling of two tripeptides to obtain hexapeptide (1–6), whereas Reddy et al. have added all six amino acids in a linear fashion. We observed that the dipeptide part (2–3) and dipeptide part (5–6) of the heptapeptide side chain are identical (–NH–Ile–Ser–CO–). Thus, we planned to prepare this dipeptide in large quantity and couple with the required amino acids so that a concise (short) and scalable synthesis could be achieved that will enable total as well as analogue synthesis of teixobactin. We present herein a solution-phase synthesis of the heptamer side chain of **1**.

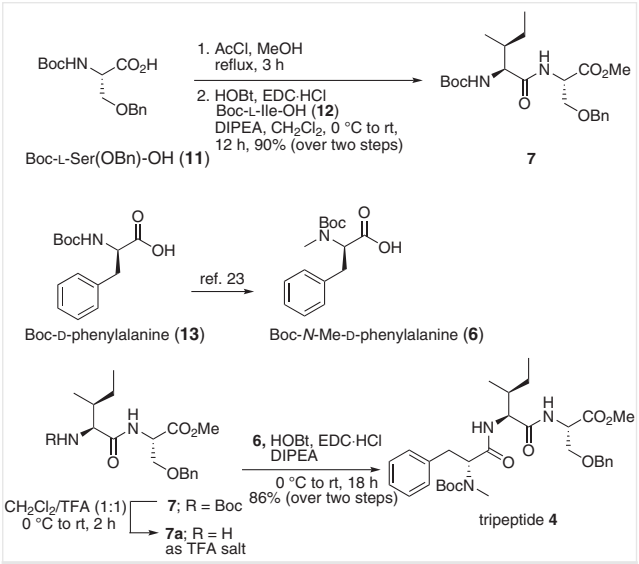
Retrosynthesis of teixobactin revealed the presence of a linear heptamer **2** and cyclic depsipeptide **3**. The linear heptamer fragment **2** would be obtained via coupling of a tripeptide **4** and tetrapeptide **5** which in turn could be obtained from a key dipeptide **7** (Scheme 1).

The synthesis of both tri- and tetrapeptides began with commercially available amino acids. The synthesis of tripeptide **4** was started with a key dipeptide **7** intermediate. It was obtained from the coupling of commercially available Boc-L-Ser(OBn)-OH (**11**) and Boc-L-Ile-OH (**12**). The compound **11** was treated with acetyl chloride in MeOH under reflux for simultaneous conversion of acid into methyl ester and Boc deprotection to get H-Ser(OBn)-OMe as a hydrochloride salt, which was coupled with the acid **12** using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl) and hydroxybenzotriazole



Scheme 1 Retrosynthesis of teixobactin (**1**)

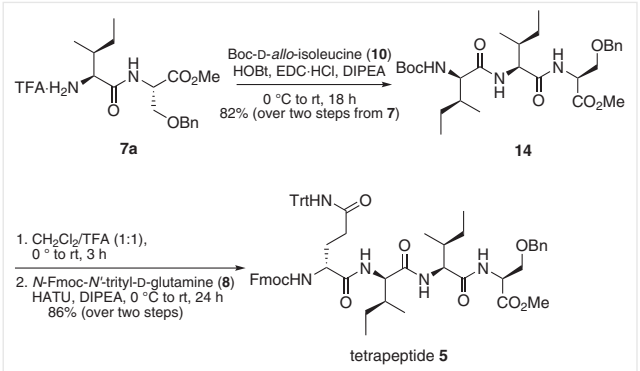
(HOBt) as coupling reagents and DIPEA as base in dichloromethane to give dipeptide **7** in 90% yield over two steps. *N*-Methylation[23] on Boc-protected D-phenylalnine (**13**) using NaH and MeI in THF gave Boc-*N*-Me-D-phenylalanine (**6**) in 95% yield. The dipeptide **7** upon Boc deprotection,[24] with TFA/CH_2Cl_2 (1:1) to get free amine as a TFA salt **7a**, followed by coupling with the acid **6** using HOBt, EDC·HCl and DIPEA in CH_2Cl_2 gave tripeptide **4** in 86% yield over two steps (Scheme 2).



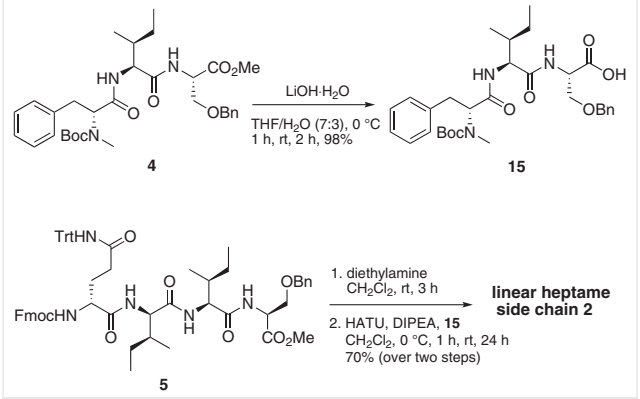
Scheme 2 Synthesis of tripeptide **4**

The dipeptide **7** was also used for the synthesis of tetrapetide **5**. The amine **7a** (as TFA salt), obtained from Boc deprotectction of dipeptide **7** using TFA in CH_2Cl_2, was coupled with Boc-D-*allo*-isoleucine (**10**) using HOBt, EDC·HCl and DIPEA in CH_2Cl_2 to give tripeptide **14** in 82% yield over two steps. The Boc deprotection of tripeptide **14** gave the corresponding amine which was further coupled with *N*-Fmoc-*N*′-trityl-D-glutamine (**8**) using 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxide hexafluorophosphate (HATU) and DIPEA to give tetrapeptide **5** (Scheme 3).

Ester hydrolysis of tripeptide **4** using LiOH·H_2O in THF/H_2O gave acid **15**. The Fmoc group in tetrapeptide **5** was deprotected with diethylamine[25] in CH_2Cl_2, and the obtained free amine was coupled with acid **15** using HATU and DIPEA in CH_2Cl_2 to accomplish heptamer side chain of teixobactin **2** in 70% yield over two steps (Scheme 4). The epimerization of amino acids is a major concern in peptide synthesis. Here, the amount of epimerization during heptamer synthesis was quantified by HPLC. The analysis showed less than 2% epimerization (see Supporting Information).



Scheme 3 Synthesis of tetrapeptide **5**



Scheme 4 Synthesis of linear heptamer **2**

In conclusion, we have completed a solution-phase convergent synthesis of heptapeptide side chain of the 'super antibiotic', teixobactin on a gram scale in 37% overall yield. The synthesis was achieved through an efficient coupling of suitably protected tripeptide and tetrapeptide which were prepared from a common dipeptide.[26,27] This synthetic scheme will allow us to synthesize analogues of the natural product. The synthesis of analogues of **1** and their biological evaluation will be presented in due course.

Funding Information

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0039-1690232.

References and Notes

(1) CSIR-IICT communication number: IICT/Pubs./2019/316.
(2) (a) Willyard, C. *Nature* **2017**, *543*, 7643. (b) Von Nussbaum, F.; Süssmuth, R. D. *Angew. Chem. Int. Ed.* **2015**, *54*, 6684; *Angew. Chem.* **2015**, *127*, 6784. (c) Arias, A.; Murray, C. B. E. *N. Engl. J. Med.* **2015**, *372*, 1168. (d) Tobin, K. C. *Nature Rev. Microbiol.* **2015**, *13*, 126. (e) Hunter, P. *EMBO Rep.* **2015**, *16*, 563. (f) Kostic, M. *Chem. Biol.* **2015**, *22*, 159. (g) Piddock, L. J. V. *J. Antimicrob. Chemother.* **2015**, *70*, 2679.
(3) Ling, L. L.; Schneider, T.; Peoples, A. J.; Spoering, A. L.; Engels, I.; Conlon, B. P.; Mueller, A.; Schaeberle, T. F.; Hughes, D. E.; Epstein, S.; Jones, M.; Lazarides, L.; Steadman, V. A.; Cohen, D. R.; Felix, C. R.; Fetterman, K. A.; Millett, W. P.; Nitti, A. G.; Zullo, A. M.; Chen, C.; Lewis, K. *Nature* **2015**, *517*, 455.
(4) (a) Ng, V.; Chan, W. C. *Chem. Eur. J.* **2016**, *22*, 12606. (b) Öster, C.; Walkowiak, G. P.; Hughes, D. E.; Spoering, A. L.; Peoples, A. J.; Catherwood, A. C.; Tod, J. A.; Lloyd, A. J.; Herrmann, T.; Lewis, K.; Dowson, C. G.; Lewandowski, J. R. *Chem. Sci.* **2018**, *9*, 8850.
(5) Atkinson, D. J.; Naysmith, B. J.; Furkert, D. P.; Brimble, M. A. *Beilstein J. Org. Chem.* **2016**, *12*, 2325.
(6) (a) Jin, K.; Sam, L. H.; Laam, Po. K. H.; Lin, D.; Ghazvini Zadeh, E. H.; Chen, S.; Yuan, Y.; Li, X. *Nat. Commun.* **2016**, *7*, 12394. (b) Giltrap, A. M.; Dowman, L. J.; Nagalingam, G.; Ochoa, J. L.; Linington, R. G.; Britton, W. J.; Payne, R. J. *Org. Lett.* **2016**, *18*, 2788. (c) Zong, Y.; Fang, F.; Meyer, K. J.; Wang, L.; Ni, Z.; Gao, H.; Lewis, K.; Zhang, J.; Rao, Y. *Nat. Commun.* **2019**, *10*, 3268.
(7) Jad, Y. E.; Acosta, G. A.; Naicker, T.; Ramtahal, M.; El-Faham, A.; Govender, T.; de la Torre, H. G.; Albericio, F. *Org. Lett.* **2015**, *17*, 6182.
(8) (a) Parmar, A.; Iyer, A.; Vincent, C. S.; Van Lysebetten, D.; Prior, S. H.; Madder, A.; Taylor, E. J.; Singh, I. *Chem. Commun.* **2016**, *52*, 6060. (b) Parmar, A.; Iyer, A.; Lloyd, D. G.; Vincent, C. S.; Prior, S. H.; Madder, A.; Taylor, E. J.; Singh, I. *Chem. Commun.* **2017**, *53*, 7788. (c) Parmar, A.; Lakshminarayanan, R.; Iyer, A.; Mayandi, V.; Goh, E. T. L.; Lloyd, D. G.; Chalasani, M. L. S.; Verma, N. K.; Prior, S. H.; Beuerman, R. W.; Madder, A.; Taylor, E. J.; Singh, I. *J. Med. Chem.* **2018**, *61*, 2009.
(9) Yang, H.; Chen, K. H.; Nowick, J. S. *ACS Chem. Biol.* **2016**, *11*, 1823.
(10) Abdel Monaim, S. A. H.; Jad, Y. E.; Acosta, G. A.; Naicker, T.; Ramchuran, E. J.; El-Faham, A.; Govender, T.; Kruger, H. G.; de la Torre, B. G.; Albericio, F. *RSC Adv.* **2016**, *6*, 73827.
(11) Abdel Monaim, S. A. H.; Jad, Y. E.; Ramchuran, E. J.; El-Faham, A.; Govender, T.; Kruger, H. G.; de la Torre, B. G.; Albericio, F. *ACS Omega* **2016**, *1*, 1262.
(12) Wu, C.; Pan, Z.; Yao, G.; Wang, W.; Fang, L.; Su, W. *RSC Adv.* **2017**, *7*, 1923.
(13) Parmar, A.; Prior, S. H.; Iyer, A.; Vincent, C.; Van Lysebetten, D.; Breukink, E.; Madder, A.; Taylor, E. J.; Singh, I. *Chem. Commun.* **2017**, *53*, 2016.
(14) Yang, H.; Du Bois, D. R.; Ziller, J. W.; Nowick, J. S. *Chem. Commun.* **2017**, *53*, 2772.
(15) For selected papers, see: (a) Chandrasekhar, S.; Babu, B. N.; Prabhakar, A.; Sudhakar, A.; Reddy, M. S.; Kiran, M. U.; Jagadeesh, B. *Chem. Commun.* **2006**, 1548. (b) Chandrasekhar, S.; Kiranmai, N.; Kiran, M. U.; Devi, A. S.; Reddy, G. P. K.; Idris, M.; Jagadeesh, B. *Chem. Commun.* **2010**, *46*, 6962; and the references cited therein.
(16) Chandrasekhar, S.; Pendke, M.; Muththe, C.; Akondi, S. M.; Mainkar, P. S. *Tetrahedron Lett.* **2012**, *53*, 1292.
(17) (a) Andersson, L.; Blomberg, L.; Flegel, M.; Lepsa, L.; Nilsson, B.; Verlander, M. *Biopolymers* **2000**, *55*, 227. (b) Chandrudu, S.; Simerska, P.; Toth, I. *Molecules* **2013**, *18*, 4373.
(18) Craig, W.; Chen, J.; Richardson, D.; Thorpe, R.; Yuan, Y. *Org. Lett.* **2015**, *17*, 4620.
(19) Parmar, A.; Iyer, A.; Prior, S. H.; Lloyd, D. G.; Goh, E. T. L.; Vincent, C. S.; Palmai-Pallag, T.; Bachrati, C. Z.; Breukink, E.; Madder, A.; Lakshminarayanan, R.; Tayler, E. J.; Singh, I. *Chem. Sci.* **2017**, *8*, 8183.
(20) Yang, H.; Wierzbicki, M.; Du Bois, D. R.; Nowick, J. S. *J. Am. Chem. Soc.* **2018**, *140*, 14028.
(21) Gao, B.; Chen, S.; Hou, Y. N.; Zhao, Y. J.; Ye, T.; Xu, Z. *Org. Biomol. Chem.* **2019**, *17*, 1141.
(22) (a) Gunjal, V. B.; Reddy, D. S. *Tetrahedron Lett.* **2019**, *60*, 1909. (b) Dhara, S.; Gunjal, V. B.; Handore, K. L.; Reddy, D. S. *Eur. J. Org. Chem.* **2016**, 4289.
(23) (a) Reddy, G. V.; Kumar, R. S. C.; Shankaraiah, G.; Babu, K. S.; Rao, J. M. *Helv. Chim. Acta* **2013**, *96*, 1590. (b) Malkov, A.; Varnkova, K.; Cerny, M.; Kocovsky, P. *J. Org. Chem.* **2009**, *74*, 8425. (c) Ferrie, L.; Reymond, S.; Capdevielle, P.; Cossy, J. *Org. Lett.* **2006**, *8*, 3441.
(24) Hartwig, S.; Nguyen, M. M.; Hecht, S. *Polym. Chem.* **2010**, *1*, 69.
(25) Long, B.; Zhang, J.; Tang, X.; Wu, Z. *Org. Biomol. Chem.* **2016**, *14*, 9712.
(26) **Synthetic Procedure for Key Dipeptide Intermediate, Methyl *O*-Benzyl-*N*-[(*tert*-butoxycarbonyl)-L-isoleucyl]-L-serinate (7)**
To a mixture of commercially available Boc-L-Ser(OBn)-OH (**11**, 5 g, 16.9 mmol, 1.0 equiv) in MeOH (50 mL) under nitrogen atmosphere, was added MeCOCl (1.8 mL, 25.3 mmol, 1.5 equiv) slowly at 0 °C. The reaction mixture was stirred at reflux for 3 h, and the solvent was distilled off in *vacuo* to get L-Ser(OBn)-OMe as a hydrochloride (4.1 g, 16.9 mmol). In another round-bottomed flask, Boc-L-isoleucine (**12**, 3.9 g, 16.9 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (50 mL) under nitrogen atmosphere, and HOBt (2.5 g, 18.5 mmol, 1.1 equiv) and EDC·HCl (4.85 g, 25.3 mmol, 1.5 equiv) were added sequentially at 0 °C and stirred for 15 min. To this reaction mixture was added dropwise a solution of the above-obtained L-Ser(OBn)-OMe hydrochloride salt (4.1 g, 16.9 mmol, 1.0 equiv) and DIPEA (11.5 mL, 67.6 mmol, 4.0 equiv) in dry CH_2Cl_2 (20 mL) at 0 °C and stirred for 1 h. Then reaction mixture was maintained at room temperature for 12 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with saturated aqueous NH_4Cl solution (2 × 75 mL). The organic layer was separated and washed with saturated aqueous $NaHCO_3$ solution (2 × 75 mL) followed by brine solution (75 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography (15% EtOAc in hexanes) to give key dipeptide **7** (6.39 g, 90%) as white solid. R_f = 0.5 (20% EtOAc in hexanes); mp 160–162 °C; $[\alpha]_D^{20}$ +26.30 (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.36–7.31 (m, 2 H), 7.31–7.23 (m, 3 H), 6.67 (d, *J* = 6.9 Hz, 1 H), 5.10 (d, *J* = 7.4 Hz, 1 H), 4.73 (dt, *J* = 7.9, 3.1 Hz, 1 H), 4.50 (q, *J* = 12.2 Hz, 2 H), 4.08–3.98

(m, 1 H), 3.89 (dd, *J* = 9.5, 3.2 Hz, 1 H), 3.72 (s, 3 H), 3.66 (dd, *J* = 9.5, 3.2 Hz, 1 H), 1.95–1.79 (m, 1 H), 1.51–1.45 (m, 1 H), 1.44 (s, 9 H), 1.20–1.09 (m, 1 H), 0.95 (d, *J* = 6.8 Hz, 3 H), 0.90 (t, *J* = 7.4 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.4, 170.5, 155.7, 137.5, 128.6, 128.0, 127.8, 79.9, 73.4, 69.6, 59.2, 52.6, 37.8, 28.4, 24.8, 15.6, 11.7 ppm. IR (thin film): ν_{max} = 3285, 2964, 2928, 1707, 1642, 1512, 1367, 1165, 863, 749, 666 cm^{-1}. HRMS (ESI): *m/z* calcd for $[M + Na]^+$ $C_{22}H_{34}N_2O_6Na$: 445.2315; found: 445.2313.

(27) **Synthetic Procedure for Tripeptide, Methyl *O*-Benzyl-*N*-(*tert*-butoxycarbonyl)-D-*allo*isoleucyl-L-isoleucyl-L-serinate (14)**

To a solution of Boc-Ile-Ser(OBn)-OMe (**7**, 5.0 g, 11.83 mmol, 1.0 equiv) in dry CH_2Cl_2 (5.0 mL) under nitrogen atmosphere was added TFA (5.0 mL, 65.2 mmol, 5.53 equiv) at 0 °C and stirred for 30 min at this temperature. Then reaction was maintained at room temperature for 2 h. After completion, the volatiles were removed in *vacuo*. The residue was dried under high vacuum for 1 h to get the corresponding amine salt (5.2 g, crude) as a pale brown thick liquid which was used for the next step directly. Boc-D-*allo*-isoleucine (**10**, 2.73 g, 11.80 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (30 mL) under nitrogen atmosphere. HOBt (1.75 g, 12.98 mmol, 1.1 equiv) and EDC·HCl (3.4 g, 17.7 mmol, 1.5 equiv) were added sequentially at 0 °C. A mixture of amine salt (5.2 g, crude, ca. 11.9 mmol, 1.0 equiv) and DIPEA (8.08 mL, 47.32 mmol, 4.0 equiv) in dry CH_2Cl_2 (50 mL) was added dropwise to the above reaction mixture at 0 °C and stirred for 30 min. Then reaction mixture was maintained at room temperature for 18 h. After completion of reaction, the reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with saturated aqueous NH_4Cl solution (2 × 75 mL). The organic layer was separated and washed with saturated aqueous $NaHCO_3$ solution (2 × 75 mL) followed by brine solution (75 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (25% EtOAc in hexanes) to give tripeptide **14** (5.16 g, 82%, over 2 steps) as white solid. R_f = 0.5 (30% EtOAc in hexanes); mp 134–136 °C; $[\alpha]_D^{20}$ +13.80 (*c* 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.37–7.24 (m, 5 H), 6.75–6.64 (m, 2 H), 5.02 (d, *J* = 6.6 Hz, 1 H), 4.73 (dt, *J* = 8.0, 3.2 Hz, 1 H), 4.57–4.43 (m, 2 H), 4.41 (dd, *J* = 8.6, 6.3 Hz, 1 H), 4.24–4.10 (m, 1 H), 3.89 (dd, *J* = 9.5, 3.3 Hz, 1 H), 3.73 (s, 3 H), 3.64 (dd, *J* = 9.5, 3.0 Hz, 1 H), 2.07–1.95 (m, 1 H), 1.95–1.85 (m, 1 H), 1.58–1.48 (m, 1 H), 1.44 (s, 9 H), 1.42–1.34 (m, 1 H), 1.24–1.12 (m, 2 H), 0.98–0.87 (m, 9 H), 0.82 (d, *J* = 6.8 Hz, 3 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.8, 170.8, 170.4, 155.8, 137.5, 128.6, 128.1, 127.8, 80.1, 73.4, 69.5, 58.3, 57.5, 52.7, 37.7, 37.3, 28.4, 26.6, 24.9, 15.4, 14.2, 11.8, 11.5 ppm. IR (thin film): ν_{max} = 3323, 2966, 2930, 1747, 1658, 1501, 1365, 1211, 1163, 1020, 867, 749, 665 cm^{-1}. HRMS (ESI): *m/z* calcd for $[M + Na]^+$ $C_{28}H_{45}N_3O_7Na$: 558.3155; found: 558.3156.