Towards the total synthesis of halolitoralin-C

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Efforts towards the synthesis of halolitoralin-C is described using solution phase peptide synthesis through a highly convergent approach. The peptide coupling reactions have been carried out with EDC as a coupling agent with very high yields.

Marine microorganisms are the biggest reservoir providing a wide variety of structurally unique, biologically significant non-ribosomal peptides, especially cyclopeptides derivatives[1]. Small cyclopeptides are more promising lead structures in drug discovery due to their reduced conformational flexibility and high *in vivo* stability compared to their linear counterparts[2]. Antimicrobial peptides are particularly useful lead compounds and played a pivotal role in the pharmaceutical research as biomedically useful agents[3]. Halolitoralin C **1**, halolitoralin A **2**, and halolitoralin B **3** (ref. 4), were isolated from the ferment broth of a marine sediment-derived Halobacillus litoralis[5] YS3106, and exhibited moderate antifungal activity both in human fungi as well as crop-threatening fungi[3] (**Chart I**). The above three cyclopeptides also exhibited moderate anti-human gastric tumor activities *in vitro*. The stereo chemistries of the amino acids in all these cyclopeptides **1-3**, were determined as L-configuration through hydrolysis of the cyclopeptide followed by Marfey's analysis[6].
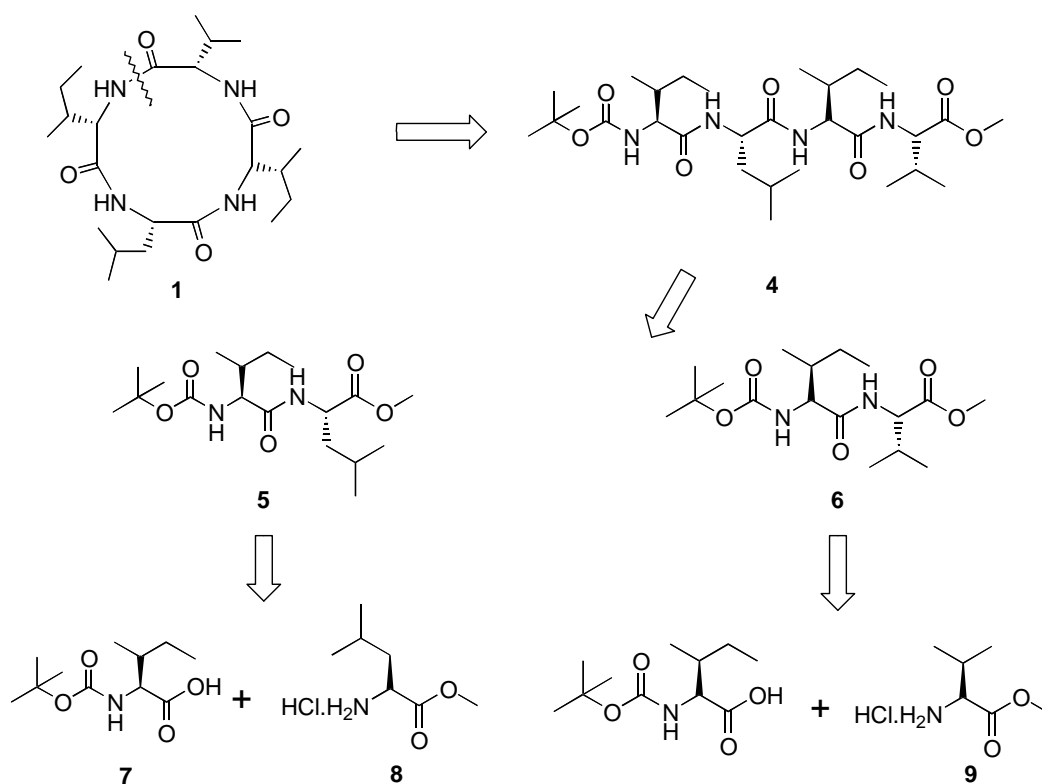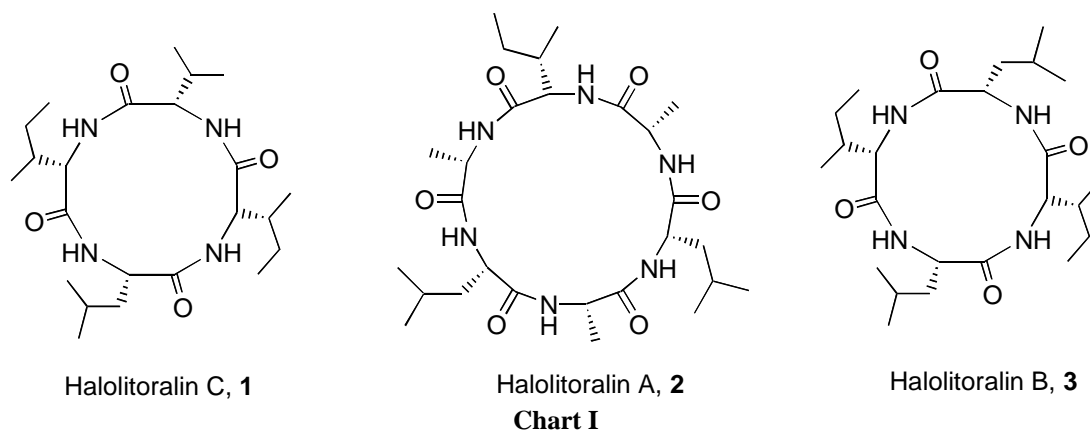
In continuation to an on going program on the solution phase synthesis of cyclopeptides, we got interested in the total synthesis of one of the above cyclo tetrapeptide Halolitoralin C **1**. The protected tetrapeptide could be envisaged as an advanced intermediate through the retro synthesis as shown in the **Chart II**. Deprotection of the amino protecting group in **4**, followed by hydrolysis of the ester functionality and intramolecular cyclization is expected to give the desired cyclotetrapeptide **1**. The tetrapeptide **4**, in turn, could be obtained from the corresponding dipeptides **5** and **6** through a convergent approach. The dipeptides **5** and **6** could easily be prepared from the suitably protected amino acids L-leucine methyl ester hydrochloride **8**, Boc-L-isoleucine **7** and L-valine methyl ester hydrochloride **9** as shown in the **Chart II**.

The amino protected amino acid Boc-L-isoleucine **7** is found to be common in both the dipeptide intermediates **5** and **6**.

The amino group in isoleucine **10** is reacts with ditertiarybutydicarbonate in the presence of a base to give the corresponding N-protected isoleucine, Boc-Ile-OH **7** (ref. 7) in excellent yield. Similarly, the carboxyl group of L-Leucine **11** is treated with thionylchloride in methanol to furnish the corresponding L-leucine methyl ester hydrochloride **8** (ref. 8) in good yield, which on further coupling with Boc-L-Ile-OH **7**, in the presence of EDC and HOBt delivers dipeptide methyl ester **5** (ref. 9), in excellent yield, **Scheme I**.

Similarly, the other key intermediate dipeptide **6**, could be prepared from suitably protected amino acids

Halolitoralin C, **1**   Halolitoralin A, **2**   Halolitoralin B, **3**

Chart I

Chart II

7 and **9**. The carboxylic group of ʟ-valine **12** is reacted with thionyl chloride in the presence of methanol to give the corresponding methyl ester hydrochloride **9** (ref. 10) in excellent yield as a good white solid. The valine methyl ester hydrochloride, thus obtained, is coupled with Boc-Ile-OH **7** in the presence of EDC and HOBt to give the corresponding dipeptide **6** (ref. 11) in 85% yield, **Scheme II**.

After successfully preparing the desired advanced intermediate dipeptides **5** and **6**, the next step was to prepare the key intermediate, the tetrapeptide **4**, **Scheme III**. Thus, deprotection of the amino

protecting group in **6** using trifluroacetic acid in DCM furnished the corresponding TFA salt of the corresponding amino ester **14**. Hydrolysis of the ester functionality in **5**, under mild alkaline conditions, delivered the amino protected carboxylic acid **13**, in excellent yield. Coupling of the two advanced intermediates **13** and **14** in presence of EDC and HOBt gave the corresponding tetrapeptide ester **4**, in 87% yield, **Scheme III**.

With the key intermediate tetrapeptide ester **4**, in hand, our next objective was to deprotect the protecting groups on both the ester and amino

functionality to facilitate the intramolecular cyclization. Thus, hydrolysis of the tetrapeptide ester **4**, under mild alkaline conditions in methanol furnished carboxylic acid **15** in very good yield. The acid **15**, thus obtained, is treated with TFA in dichloromethane as solvent furnished crude TFA salt **16**. However, intramolecular cyclization of the amino acid **16** under a variety of conditions was proved to be unsuccessful resulting in complex reaction mixtures. As the direct coupling reaction was not promising, recourse was thus taken to have a stepwise protocol by activating the acid functionality followed by deprotection of the amino protection and intramolecular cyclization. The acid functionality in **15**, is activated with pentafluorophenol followed by deprotection of the Boc group and neutralization with a base also didn't result in the formation of the product **1**, **Scheme IV**.

In summary, efforts towards the synthesis of the cyclotetrapeptide **1** using a convergent solution phase synthesis is described. The advanced intermediates could be prepared in very high yields using EDC and HOBt as the peptide coupling protocol.
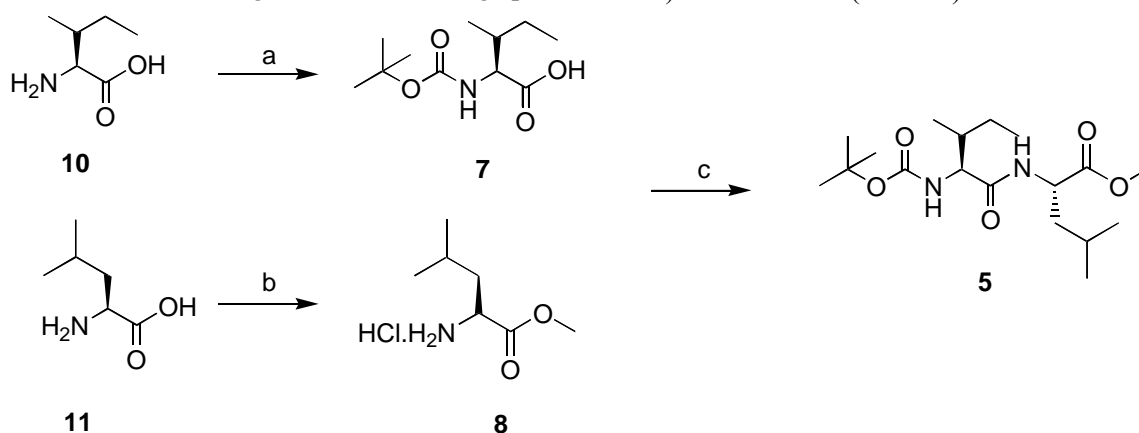
Experimental Section

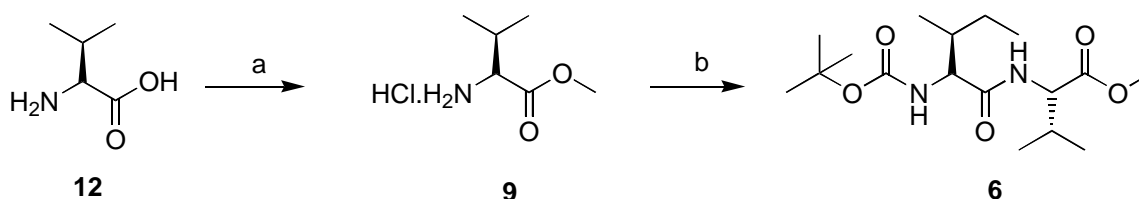Melting points were recorded on a Buchi Melting point apparatus in open capillary tubes and are uncorrected. TLC checking was done using pre-coated silica gel sheets obtained from Merck, Germany. ^{1}H NMR spectra were obtained on a Varian Gemini 2000 model 400 MHz instrument. (Chemical shift in δ, ppm) with TMS as internal standard and mass spectrum run on HP5989 spectrometer.

Boc-Isoleucine 7. To a cooled solution of L-Isoleucine **10** (50 g, 0.38 mole) in 1,4-dioxane (200 mL) was added 1.0 *N* sodium hydroxide solution (450 mL) followed by ditertiarybutyldicarbonate (99.4 g, 0.45 mole) under stirring. The reaction mixture was warmed to R T with further stirring for 15 h. After completion of reaction, the reaction mixture was cooled to 0 to 10°C and acidified with 15% aq. potassium hydrogensulphate. The reaction mixture was extracted with ethyl acetate (3 × 200 mL) and the combined organic part was washed with brine solution (200 mL). The organic part was dried over sodium sulphate, filtered and concentrated under reduced pressure to give Boc-Ileu-OH **7** (80 g, 90%). MS (ES): 232 (M[+]+1); ^{1}H NMR (400 MHz, CDCl$_3$): δ 10.9 (br s, 1H), 5.09 (d, *J* = 6.52 Hz, 1H), 4.31-4.27 (dd, 1H), 1.98-1.94 (m, 1H), 1.45 (s, 9H), 1.27-1.15 (m, 2H), 0.98 (d, *J* = 6.87 Hz, 3H), 0.93 (t, *J* = 7.52 Hz, 3H); m. p. 65-68°C (Reported: 66-69°C); [α]$_D^{26}$ +3 (*c* 2.0, ACOH).

L-**leu-OMe. HCl 8**. Thionyl chloride (93.5 mL) was added to a mixture of L-leucine **11** (50 g, 0.38 mole) and methanol (200 mL) for 1 h below 45°C and
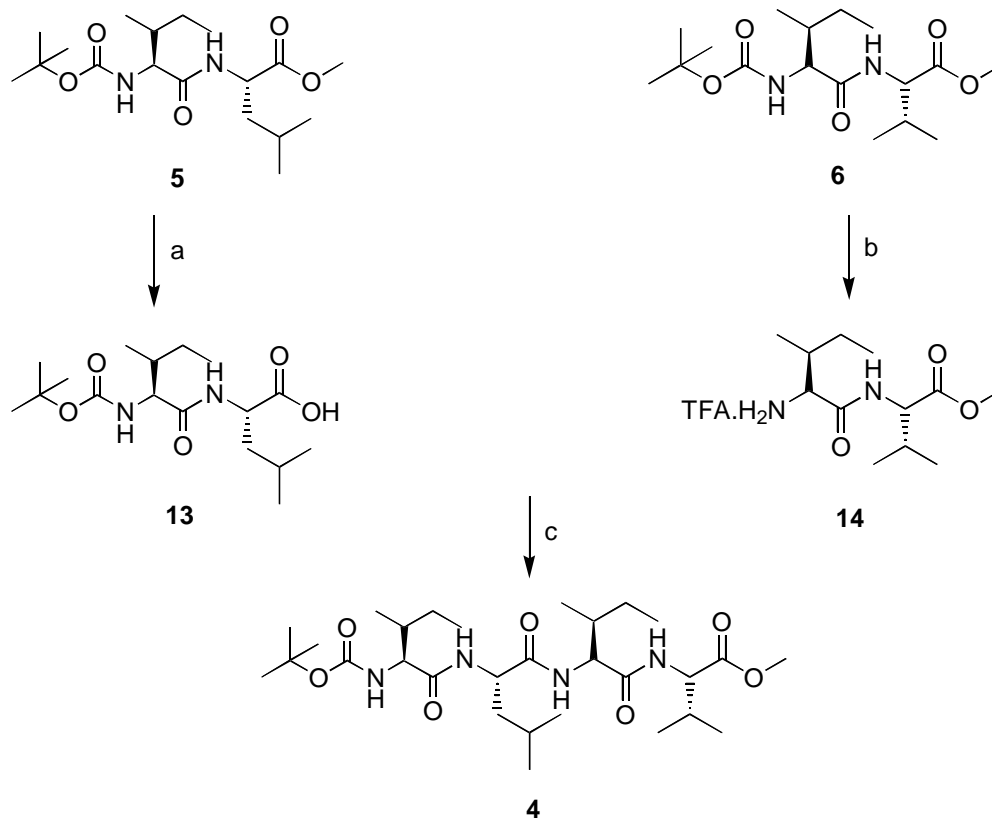

Reagents and conditions: a. (Boc)$_2$O, NaOH, 90%; b. SOCl$_2$, Methanol, 85%; c. EDC, HOBt, DMF, 91%.
Scheme I

Reagents and conditions: a. SOCl$_2$, Methanol, 96%; b. **7**, EDC, HOBt, 85%.
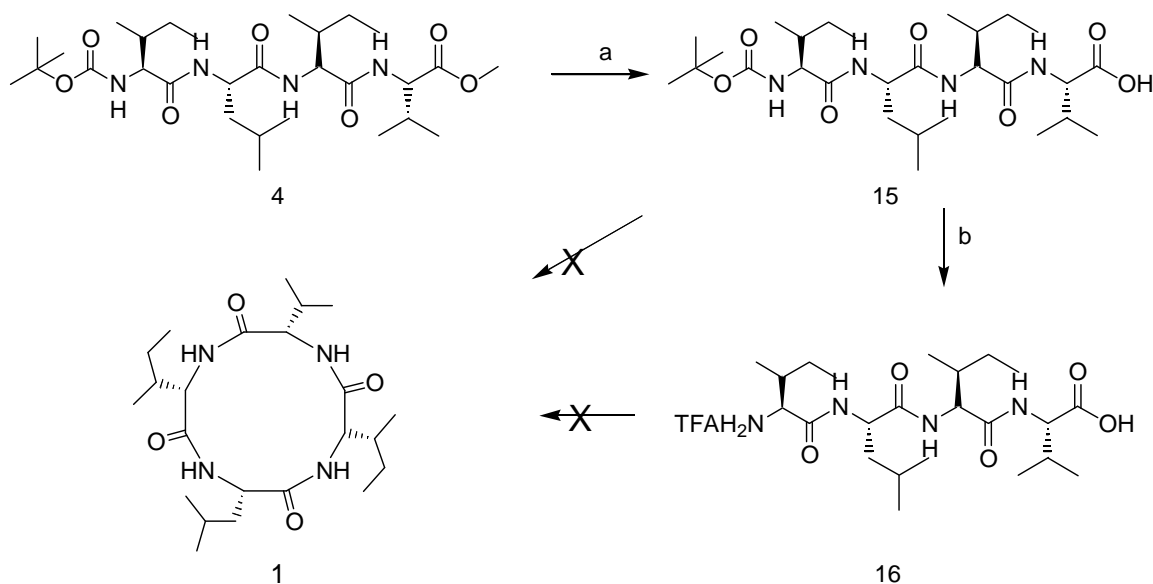**Scheme II**

refluxed for 4 h. The reaction mixture was concentrated and distilled with toluene to remove traces of thionyl chloride. The residue was dissolved in methanol and precipitated with diisopropyl ether (200

mL) and filtered to give **8** as a white solid (61.5 g, 85%). ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.57 (br s, 3H), 3.93 (t, J = 6.98 Hz, 1H), 3.74 (s, 3H), 1.79-1.72 (m, 1H), 1.67-1.63 (m, 2H), 0.89 (d, J = 6.45 Hz, 6H);

Reagents and conditions: a. TFA, DCM, 98%; b. 1.0 N NaOH, MeOH, 98%; c. EDC, HOBt, 87%.

Scheme III

Reagents and conditions: a. 1.0 N aq. NaOH, MeOH, 83%; c. TFA, DCM; d. DPPA, NaHCO3, DMF.

Scheme IV

MS(CI): 146 (M$^+$+1); m. p. 150-153°C (Reported: 151-153°C); [α]$_D^{26}$ +20 (*c* 4.5, MeOH).

Boc-Ile-Leu-OMe 5. To a mixture of Boc-lleu-OH **7**, (2.0 g, 8.65 mmoles), DMF (10 mL) at room temperature was added HOBt (1.4 g, 10.38 mmoles). The reaction was cooled to 0-5°C and then EDC.HCl (1.99 g, 10.38 mmoles) in DMF was added and neutralized the reaction mixture with *n*-methylmorpholine. The reaction was stirred at the same temperature for 30 min. Leu-OMe. HCl **8**, (1.73 g, 9.52 mmoles) in DMF (10 mL) was neutralized with NMM and added to the reaction slowly at 0-5°C and continued for 1 h at same temperature. The reaction temperature brought to room temperature and stirred for 12 h. After the completion of the reaction, the reaction mass was diluted with water. The precipitated solid was filtered, washed with water and dried to give the **5**, as a white solid (2.81 g, 91%). MS (ES): 359.2(M+1); ^{1}H NMR (400 MHz, CDCl$_3$): δ 6.19 (d, *J* = 7.52 Hz, 1H), 5.01(br s, 1H), 4.65-4.59 (m, 1H), 3.91 (m, 1H), 3.72 (s, 3H), 1.86 (br s,1H), 1.67-1.62 (m, 2H), 1.59-1.50 (m, 2H), 1.44 (s, 9H), 1.19-1.12 (m, 1H), 0.94-0.89 (m, 12H).

Boc-Ile-Leu-OH 13. To a cooled solution of Boc-Ileu-OMe **5**, (1.0 g, 2.79 mmoles) in methanol (8.0 mL) was added 1.0 *N* NaOH (8.4 mL, 8.37 mmoles) slowly for 10 min. The reaction was stirred at room temperature for 1 h and acidified to *p*H 3 with 10% KHSO$_4$ solution, extracted with ethyl acetate, washed with water, dried over anhydrous sodium sulfate and concentrated over rotavapor to give **13** as a white solid (0.95 g, 98%). MS(ES): 345.2 (M$^+$+1); ^{1}H NMR (400 MHz, CDCl$_3$): δ 9.50 (br s, 1H), 6.50 (d, *J* = 7.2 Hz, 1H), 5.13 (d, *J* = 6.98 Hz, 1H), 4.63-4.57 (m, 1H), 3.94 (m, 1H), 1.85-1.74 (m, 1H), 1.74-1.62 (m, 2H), 1.59-1.52 (m, 2H), 1.43 (s, 9H), 1.19-1.09 (m, 1H), 0.95-0.88 (m, 12H).

Val-OMe. HCl 9. Thionyl chloride (93.5 mL) was added to a mixture of L-valine 12 (50 g) and methanol (200 mL) for 1 h below 45°C and refluxed for 4 h. The reaction mixture was concentrated and distilled with toluene to remove traces of thionyl chloride. The residue was dissolved in methanol and precipitated with diisopropyl ether (200 mL) and filtered to give **9**, as a white solid (54 g, 96%). MS(ES): 131 (M$^+$+1); ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.72 (br s, 3H), 3.80 (d, *J* = 4.83 Hz, 1H), 3.74 (s, 3H), 2.22-2.17 (m, 1H), 0.99 (d, *J* = 6.98 Hz, 3H), 0.94 (d, *J* =6.71, 3H); m. p. 166-170°C (Reported. 165-170°C); [α]$_D^{26}$ +23.7 (*c* 2.0, MeOH).

Boc-Ile-Val-OMe 6. HOBt (1.4 g, 10.38 mmoles) was added to a mixture of Boc-lleu-OH **7**, (2.0 g, 8.65 mmoles) in DMF (10 mL) at room temperature. The reaction mixture was cooled to 0-5°C and then EDC.HCl (1.99 g, 10.38mmoles) dissolved in DMF was added and neutralized the reaction mixture. The reaction was stirred at the same temperature for 30 min. Val-OMe HCl **9**, (1.6 g, 9.52 mmoles) in DMF (10 mL) was neutralized with NMM and then added to the above reaction slowly at 0-5°C and continued the stirring for 1 h at same temperature. The reaction temperature brought to room temperature and stirred for 12 h. After the completion of the reaction, the reaction mass is diluted with water and the precipitated solid was filtered, washed with water and dried to give **6** as a white solid (2.53 g, 85%).); MS(ES): 345.3 (M$^+$+1); ^{1}H NMR (400 MHz, CDCl3): δ 6.37 (d, *J* = 7.79 Hz, 1H), 5.01(br s, 1H), 4.53 (m, 1H), 3.93 (m,1H), 3.73 (s, 3H), 2.20-2.15 (m, 1H),2.20-2.15 (m, 1H), 1.90-1.85 (m, 1H), 1.59-1.50 (m, 1H), 1.44 (s, 9H), 1.19-1.12 (m, 1H), 0.94-0.90 (m, 12H); m. p. 165-166°C; [α]$_D^{26}$ +15.7 (*c* 1.0, MeOH).

Boc-Ileu-Leu-Ileu-Val-OMe 4. TFA-Ileu-Val-OMe **14**-TFA (2.0 mL) was added to a mixture of cold Boc-Ileu-Val-OMe **8** (1.07 g, 3.13 mmoles) in DCM (2.0 mL) for 10 min and stirred at RT for 1 h, concentrated to give **14**, (1.09 g, 98%). This was used directly for the reaction.

HOBt (422 mg, 3.13 mmoles) was added to a mixture of Boc-lleu-Leu-OH **13**, (900 mg, 2.61 mmoles), DMF (10 mL) at room temperature. The reaction was cooled to 0-5°C and then EDC.HCl (600 mg, 3.13 mmoles) in DMF was added and neutralized the reaction mixture. The reaction was stirred at the same temperature for 30 min at the same temperature. TFA-Ileu-Val-OMe **14**, (1.07 g, 303 mmoles) in DMF (10 mL) was neutralized with NMM and added to the reaction slowly at 0-5°C and continued for 1 h at same temperature. The reaction temperature brought to room temperature and stirred for 12 h. The reaction was diluted with water and precipitated solid was filtered and washed with water and dried to give **4** as white solid (1.3 g, 87%). MS(ES): 571.7 (M$^+$+1); ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.05 (d, *J* = 7.8 Hz, 1H), 7.87 (d, *J* = 8.05 Hz, 1H), 7.72 (d, *J* = 8.86 Hz, 1H), 6.73 (d, *J* = 8.86 Hz, 1H) 4.40-4.37 (m, 1H), 4.27 (t, *J* = 8.05 Hz, 1H), 4.14-4.10 (m, 1H), 3.72 (t, *J* = 8.86 Hz, 1H), 3.60 (s, 3H), 2.05-2.00 (m, 1H), 1.73-1.56 (m, 3H), 1.44-1.40 (m, 3H), 1.36 (s, 9H), 1.10-1.0 2 (m, 3H), 0.88-0.77 (m, 24H); CHN Analysis: C,

61.10; H, 9.44; N, 9.62; O, 19.23. (Calculated for $C_{29}H_{54}N_4O_7$: C, 61.03; H, 9.54; N, 9.82; O, 19.62).

Boc-Ileu-Leu-Ileu-Val-OH 15. To a cooled solution of Boc-Ileu-Leu-Ileu-Val-OMe **4** (50 mg) in methanol (4 mL) was added 1.5 N aq. NaOH (6 eq) was added and stirred at RT for 12 h. After the completion of the reaction, the reaction mass is acidified with 10% aq. $KHSO_4$ solution, extracted into ethyl acetate and washed with water. The ethyl acetate layer is concentrated to give **15**, as a white solid (40 mg, 83%). MS(ES): 557.5 (M^++1); ^1H NMR (400 MHz, $CDCl_3$): δ 12.45 (bs, 1H) 7.88-7.82 (m, 2H), 7.75 (d, $J = 8.86$ HZ, 1H) 6.73 (d, $J = 9.1$ Hz, 1H), 4.41-4.00 (m, 1H), 4.27 (t, $J = 8.32$ Hz, 1H), 4.11-4.00 (m, 1H), 3.78 (t, $J = 8.32$ Hz,1H), 2.06-2.00 (m, 1H), 1.73-1.52 (m, 3H), 1.44-1.40 (m, 3H), 1.36 (s, 9H), 1.10-1.02 (m, 3H), 0.88-0.77 (m, 24H); CHN Analysis: C, 60.18; H, 9.23; N, 9.88; O, 20.23. (Calculated for $C_{28}H_{52}N_4O_7$: C, 60.41; H, 9.41; N, 10.06; O, 20.12).

Acknowledgements

References

1 Fusetani N & Matsunaga S, *Chem Rev*, 93, **1993**, 1793.
2 Wopf P, *Chem Rev*, 95, **1995**, 2115.
3 Zasloff M, *Nature*, 415, **2002**, 389.
4 Yang L, Tan R X, Wang Q, Huang W –Y & Yin Y –X, *Tetrahedron Lett*, 43, **2002**, 6545.
5 Holt J G, *Bergey's Manual of Determinative Bacteriology*, 9th Edn.; **1994**.
6 (a) Marfey P, *Carlsberg Res Commun*, 49, **1984**, 591.(b) Fujii K, Ikai Y, Oka H, Suzuki M & Harada K –I, *Anal Chem*, 69, **1997**, 5146. (c) Fujii K, Shimoya T, Ikai Y, Oka H & Harada K –I, *Tetrahedron Lett*, 39, **1998**, 2579.
7 (a) John G C, David N D, Eric S F, Annie M H, Robert B M, Aaron B M, Lisa M S, Derril H W Jr & Lois L. W, *Bioorganic & Medicinal chemistry letters*, 14, **2004**, 275.
8 (a) Eisenbarth S & Steffan B, *Tetrahedron*, 56, **2000**, 363. (b) Laschat S, Froelich R, Wobbling B, *J Org Chem*, 61 **1996**, 2829.
9 Hashimoto C & Muramastu I, *Bull Chem Soc Jpn*, 66, **1993**, 181.
10 (a) Easton Christopher J, Kociuba K & Radom L, *Tetrahedron asymmetry,* 18, **2003**, 2919. (b) Swarnalaxmi S, Balakumar R & Velmathi S, *Indian J Chem*, 41B, **2002**, 1666.
11 (a). Suresh Babu V V & Tantry S J, *Indian J Chem*, 43B, 2004, 2708. (b) Daniel C & Michel W, *Tetrahedron letters*, 35, **1994**, 9561.