



Research Article

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## Total synthesis of Hirsutellide A

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### ABSTRACT

The total synthesis of Hirsutellide A was described. Macro cyclisation was successfully performed on the fully deprotected amino acid by Yamaguchi esterification protocol was carried out for the intramolecular cyclisation of the linear hexadepsipeptide to give the coupling product (Hirsutellide A) with the reagents TCBC, Et<sub>3</sub>N and DMAP in 22% yield. The linear hexapeptide precursor was synthesized in 25% from TBS-phe-ile-N-Me-gly-Cbz by undergoing three reactions with LiOH, CSA, DCC-DMAP respectively. Tripeptide and Dipeptide are synthesized by common coupling reactions, the coupling reagents used are HOBt, EDCI, HOAt, HATU and DIPEA. In addition to two standard amino acids, N-methylated glycine and L-isoleucine, I used an unusual hydroxyl D-phenylalanine amino acid.

**Keywords:** Hirsutellide A; cyclodepsipeptide; coupling reagents, yamaguchi esterification; tripeptide; dipeptide.

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### INTRODUCTION

Hirsutellide A, an 18 membered cyclic depsipeptide isolated from the cell extracts of *Hirsutella kobayasii*, BCC 1660, exhibits antimycobacterial activity with a MIC (Minimum Inhibitory Concentration) of 6-12 µg/ml with no cytotoxic effects towards Vero cells at 50 µg/ml and weak in vitro antimalarial activity with IC<sub>50</sub> value of 2.812 µg/ml. The Retrosynthetic analysis of Hirsutellide A is shown in the figure 1.

Ring closure can be done by either amide bond or ester bond formation. In my work, the macro cyclisation of linear precursor 2 through the formation of ester bond from the hydroxyl function of the D-phenylalanine residue and the carboxylic acid function of the N-methyl glycine residue. Now the sub target molecule 2 was divided into two tridepsipeptide units 3 and 4. Further divisions leads to four sub-units, 2-hydroxy-3-phenylpropanoic acid 5, 2-amino-3-methyl-pentanoic acid methyl ester 6, (N-methyl amino) acetic acid methyl ester 7, (N-Cbz-N-methyl amino) acetic acid tertiary butyl ester 8.

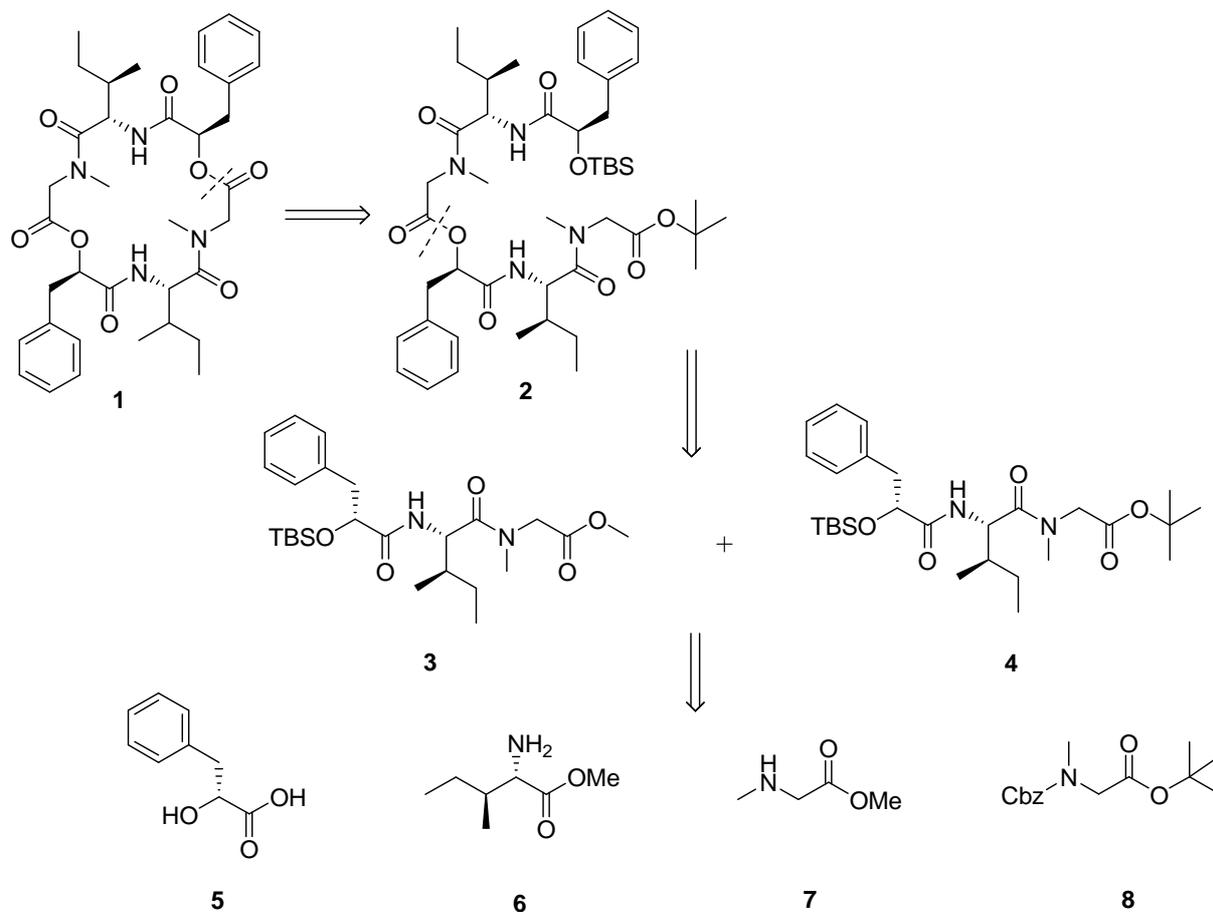
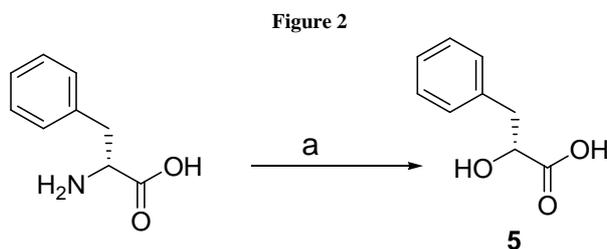


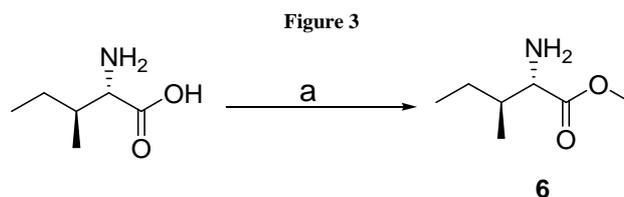
Figure 1: Retrosynthetic analysis of Hirsutellide A.

### EXPERIMENTAL SECTION

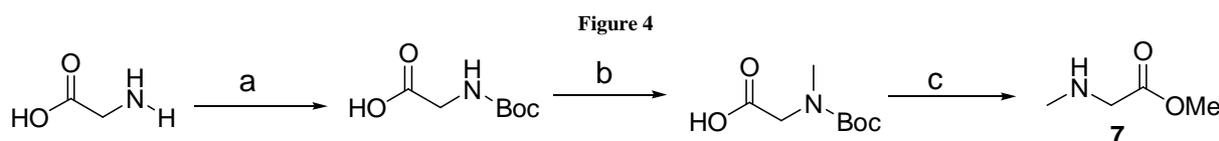
Compound **5** was obtained from D-phenylalanine through the diazotization hydrolysis (Scheme 1). In the first step the compound obtained as a white solid.

Scheme 1[figure 2]: Reagents and conditions:  $\text{NaNO}_2$ , 1M  $\text{H}_2\text{SO}_4$ , 36h, 98%

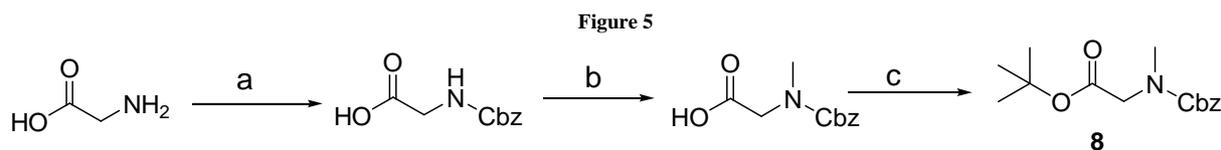
Subunit **6** was obtained from L-isoleucine by esterification with  $\text{CH}_3\text{COCl}$  (Scheme 2).  $\text{CH}_3\text{COCl}$  was drop wise added in 24h to push the reaction to completion.

Scheme 2[figure 3]: Reagents and conditions: CH<sub>3</sub>COCl, Dry MeOH, 24h, 0 °C to rt

Subunit **7** was N-methyl glycine methyl ester, was obtained from glycine through the N-Boc protection, N-methylation and esterification reaction (Scheme 3).

Scheme 3[Figure 4]: Reagents and conditions: a. (Boc)<sub>2</sub>O, 3N NaOH; Dioxane, 24h; b: NaH(60%), MeI, Dry THF, 24h, 0 °C to rt.; c: CH<sub>3</sub>COCl, Dry MeOH, 24h, 0 °C to rt

Subunit **8** was obtained from glycine through the N-Cbz protection, N-methylation and esterification reaction (Scheme 4).



Scheme 4[figure 5]: Reagents and conditions: a. Cbz chloride, 3N NaOH, 1,4-Dioxane, 24h, 0 °C to rt.; b. NaH(60%), MeI, Dry THF, 24h, 0 °C to rt.; c. DCC, DMAP, Dry DCM, 24h, 0 °C to rt

The synthetic sequence of compound **2** is shown in the Scheme 5. Coupling of **5** (1.0 equiv) and **6** (1.0 equiv) using HOBT (1.5 equiv) and EDCI (1.5 equiv) as coupling reagent, DIPEA (3 equiv) acts as a base yielded the dipeptide. The free hydroxyl group in phenylalanine moiety of above dipeptide is esterified with TBS-OTf (1.5 equiv) to yield TBS protected dipeptide (**9**). Removal of the methyl ester group (ester hydrolysis) of compound **9** by treating with LiOH (5 equiv) yields free carboxylic acid group compound (**9a**), and this compound is coupled with unit **7** (1.0 equiv) by using DIPEA (3 equiv) as base HOAt (1.3 equiv) – HATU (1.3 equiv) as coupling reagents yielded 60% of the tripeptide **3**. The compound **4** is synthesized from the dipeptide **9** which is used after treatment with LiOH to give dipeptide **9a** (1.0 equiv), then before coupling with the unit **8** (1.0 equiv), the unit **8** is Cbz protected and it is removed by H<sub>2</sub>-Pd/C as catalyst gives a compound **8a**, both **9a** and **8a** are coupled by using DIPEA (3 equiv) as base HOAt (1.3 equiv) – HATU (1.3 equiv) as coupling reagents. The compound **3** is ester hydrolysed by using LiOH gives compound **3a**, then the compound **4** is TBS deprotected by using CSA gives compound **4a**. Then compound **3a** and **4a** are coupled by using DCC-DMAP coupling reagent thus the formation of ester bond, yielded 50% of the linear hexadepsipeptide **2**.

**Scheme 5[figure 6]:** Reagents and conditions: a. Dry DCM, HOBT, EDCI, DIPEA, 24h, 0 °C to rt.; b. TBS-OTf, DRY DCM, 2,6-Lutidine, 0 °C to rt, 10 mins.; c. LiOH, THF:MeOH:H<sub>2</sub>O (3:1:1), 0 °C for 1hr.; d. Dry DCM, HOAt, HATU, DIPEA, 24h, 0 °C to rt.; e. H<sub>2</sub>-Pd/C, 1 atm, Dry MeOH, 1hr, rt.; f. Dry DCM, HOAt, HATU, DIPEA, 24h, 0 °C to rt.; g. LiOH, THF:MeOH:H<sub>2</sub>O (3:1:1), 0 °C for 1hr.; h. CSA, Dry MeOH, 0 °C for 1hr.; i. DCC, DMAP, Dry DCM, 24h, 0 °C to rt.

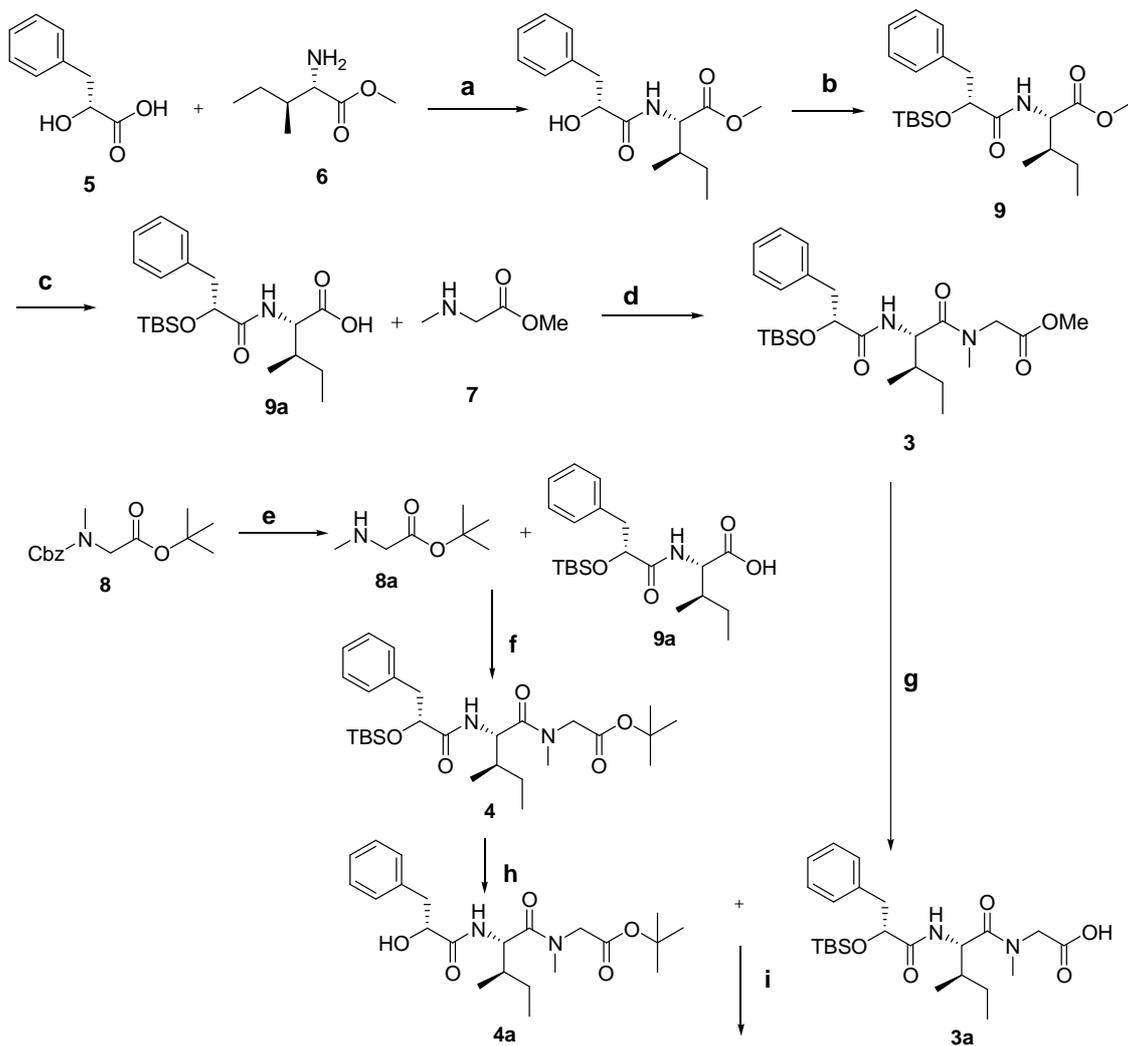
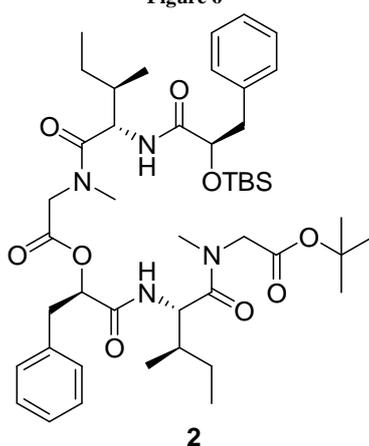
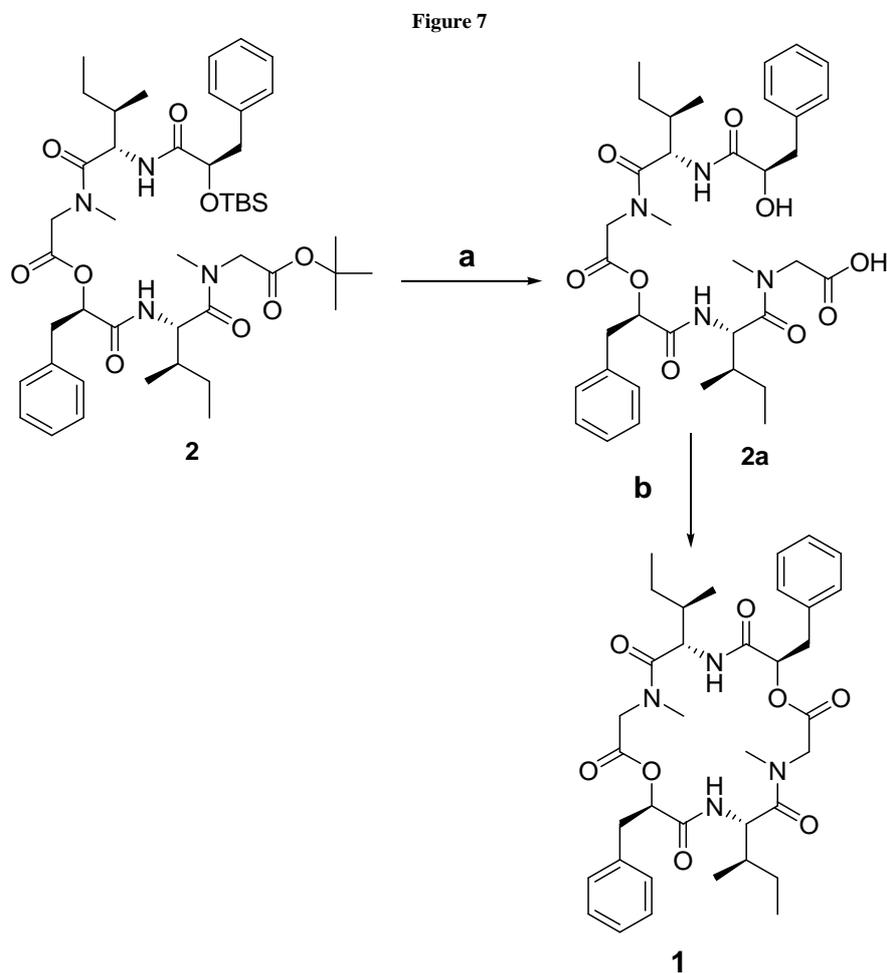


Figure 6



Subsequent reductive removal of the TBS and the cleavage of the tertiary butyl ester group in compound **2** gave the free functional hydroxyl group and carboxylic acid group contained compound **2a** (scheme 6).



**Scheme 6[figure 7]:** Reagents and conditions: **a.** TFA, Dry DCM, 0 °C to rt, 1hr.; **b.**TCBC (2,4,6-trichlorobenzoyl chloride), Et<sub>3</sub>N, Dry THF, 0 °C to rt, 1hr, DMAP, Toluene, 80 °C.

The macro cyclisation proceeded with Yamaguchi reagent (1.3 equiv), Et<sub>3</sub>N (10 equiv) and DMAP (20.64 equiv) in THF in under low dilute conditions in 15% yield for hirsutellide A **1**. The structure was determined by mass spectrometry.

### CONCLUSION

In conclusion, the antimycobacterial cyclohexadepsipeptide hirsutellide A **1** has been prepared in ten steps starting from 2-TBS hydroxy-3-phenylpropanoic acid **5**, 2-amino-3-methyl-pentanoic acid methyl ester **6**, (N-methyl amino) acetic acid methyl ester **7**, (Cbz-N-methyl amino) acetic acid tertiary butyl ester **8**. The linear hexadepsipeptide precursor **2** was synthesised in 50% yield based on the compounds **3** and **4** using coupling reagents like HOAt, HATU, DCC and DMAP. The macro cyclisation was successfully performed on the fully deprotected amino acid **2a** with TCBC-DMAP in 15% yield.

**Acknowledgment**

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- [5] Analytical data of compound 8: Rf: 0.5 (SiO<sub>2</sub>, Ethyl acetate in petroleum ether). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.43-7.26 (m, 5H), 5.14 (d, J=7.55Hz, 2H), 3.90 (d, J=21.15Hz, 2H), 2.99 (d, J=4.5Hz, 3H), 1.53-1.36(m, 9H). ESI-MS: m/z 297 [M+H<sub>2</sub>O]<sup>+</sup>, 302[M+Na]<sup>+</sup>.
- [6] Analytical data of compound 7: Rf: 0.5 (SiO<sub>2</sub>, Ethyl acetate in petroleum ether).
- [7] Analytical data of compound 5: ESI-MS: m/z 184 [M+H<sub>2</sub>O]<sup>+</sup>, 189 [M+Na]<sup>+</sup>.
- [8] Analytical data of compound 6: ESI-MS: m/z 146 [M+H]<sup>+</sup>.
- [9] Analytical data of compound 9: Rf: 0.3 (SiO<sub>2</sub>, Ethyl acetate in petroleum ether). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.3-7.19 (m, 5H), 7.08 (d, J=8.85 Hz, 1H), 4.57-4.51(m, 1H), 4.32-4.28 ( m, 1H), 3.69 (s, 3H), 8.41 (bs, 1H), 3.23-3.16 ( m, 1H), 2.89-2.82 (m, 1H), 1.9-1.8 (m, 1H), 1.45-1.35 (m, 1H), 1.18-1.08 (m, 1H), 0.92-0.82 (m, 6H). ESI-MS: m/z 294 [M+H]<sup>+</sup>, 316 [M+Na]<sup>+</sup>.
- [10] Analytical data of compound 3: Rf: 0.5 (SiO<sub>2</sub>, Ethyl acetate in petroleum ether). ESI-MS: m/z 479 [M+H]<sup>+</sup>, 501 [M+Na]<sup>+</sup>, 517 [M+K]<sup>+</sup>.
- [11] Analytical data of compound 4: Rf: 0.5 (SiO<sub>2</sub>, Ethyl acetate in petroleum ether). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.69-7.5 (m, 5H), 7.54 (d, J=9.30 Hz, 1H), 5.23(t, J=7.6 Hz, 2H), 5.07-4.93 (m, 1H), 4.77-4.67 (m, 1H), 3.56 (s, 3H), 3.23-3.15 (m, 2H), 2.23-2.16(m, 1H), 2.01-1.19 (m, 2H), 1.90-1.85 (m, 6H), 1.38-1.24 (m, 18H), 0.41 (s, 3H), 0.32 (s, 3H). ESI-MS: m/z 522 [M+H]<sup>+</sup>, 544 [M+Na]<sup>+</sup>.
- [12] Analytical data of compound 2: Rf: 0.5 (SiO<sub>2</sub>, Ethyl acetate in petroleum ether). ESI-MS: m/z 876 [M+Na]<sup>+</sup>.
- [13] Analytical data of compound 1: ESI-MS: m/z 666 [M+H]<sup>+</sup>, 666 [M+Na]<sup>+</sup>, 704 [M+K]<sup>+</sup>.