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Synthesis of N-Methyl L-Phenylalanine for Total Synthesis of Pepticinnamin E

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The target compounds **3**, **10** and **11** were synthesized through different N-methylation strategies. The concise and efficient preparation of them in large scale was developed and **3**, **10** and **11** were obtained in suitable form used for both nitrogen-end and oxygen-end extension in next coupling reaction in peptides synthesis, specifically in total synthesis of natural pepticinnamin E.

Key Words: Synthesis, N-Methyl-L-phenylalanine, N-Methylation, Pepticinnamin E.

INTRODUCTION

N-Methyl amino acids are important modified amino acids and have been widely used in medicinal chemistry and biochemistry to change the conformation, restrict the flexibility and enhance the potency of the molecule. Incorporation of N-methyl amino acids into peptides often increase pharmacokinetically useful parameters such as membrane permeability[1,2], photolytic stability[1,3] and conformational rigidity[1,4,5].

The synthesis of N-methyl amino acids in suitable form for further solution or solid-phase transformation attracts attention of chemists due to the demonstrable biological activity associated with these subunits as part of larger peptides natural products[6-8] or lead compounds[9]. Various protocols have been developed for synthesis of N-methyl amino acids[10-20]. Such as, synthesis of N-methyl amino acids and N-alkyl amino esters from O'Donnelrs Schiff Bases[10]; synthesis of N-methyl amino acids containing peptides by reductive methylation of amino groups on the solid phase[11]; application of the Fukuyama amine synthesis to prepare the Fmoc N-methyl amino acids on solid phase[12-15]; reductive cleavage of different precursor oxazolidinones with Et_3SiH/TFA to give N-protected N-methyl amino acids[16-20]; synthesis of N-methyl amino acids containing dipeptides with dimethyl sulfate in the presence of sodium hydride and a catalytic amount of water[21]. The strategy *via* intermediate oxazolidinones is one of the most widely used to afford N-methyl amino acid used for oxygen-end extension in peptides synthesis.

In our total synthesis study[22,23] of pepticinnamin E (Fig. 1), the N-methyl L-phenylalanine might be incorporated into peptides chain under different coupling conditions both from

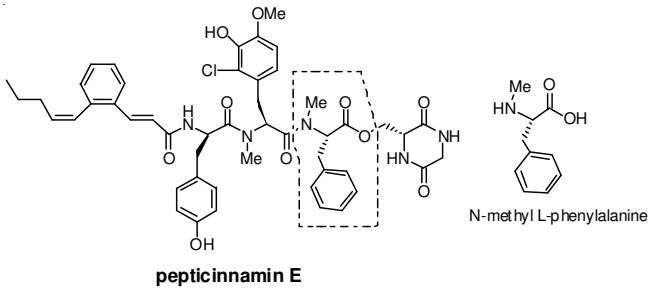

Fig. 1. Structures of pepticinnamin E and the N-methyl-L-phenylalanine

nitrogen-end or oxygen-end. Therefore, large scale of the N-protected-N-methyl-L-phenylalanine or N-methyl-L-phenylalanine methyl ester must be obtained. After comparing different strategies, we reported the best methodology to reach our target compounds **3**, **10** and **11** for further peptides synthesis, specifically in the total synthesis of pepticinnamin E.

EXPERIMENTAL

Melting points were determined with an electrothermal digital melting point apparatus and were uncorrected. Optical rotations were recorded on a Perkin-Elmer Model 341 polarimeter, at the sodium D line. Elemental analyses were recorded on Carlo-1106 model automatic instrument. Infrared spectra (IR) were run on Nicolet MX-1 and Nicolet-560 MAGNA. ^1H NMR and spectra were run either on Bruker-200 and Bruker-300 or on Varian-400 at 25 ºC; ^{13}C NMR was given by Bruker-200 at 25 ºC. MS-EI and MS-FAB mass spectra were obtained on V.G.7070E and ZAB-HS, respectively. All solvents were handled with standard ways before use.

Synthesis of N-methyl-L-phenylalanine methyl ester (3) *via* the Fukuyama amine synthesis

N-(2-Nitrobenzenesulfonamide)L-phenylalanine methyl ester (4): To a mixture of L-phenylalanine methyl ester hydrochloride **1** (1.079 g, 5 mmol) and Et_3N (1.5 mL, 1.1 mmol) in dry dichloromethane (40 mL) was added dropwise the solution of 2-nitrobenzenesulfonyl chloride (1.767 g, 7.8 mmol) in CH_2Cl_2 (18 mL) at -5 ºC. After stirring 5 min at the same temperature, the reaction was stirred at room temperature for 6 h. Then the reaction was diluted with dichloromethane (50 mL) and washed with cold 1N $KHSO_4$ and brine (Fig. 2). The organic layer was dried over anhydrous Na_2SO_4 and concen-trated in vacuum to give crude product, which was purified by flash chromatography to give **4** (1.78 g, 97.8 %) as light yellow solid. m.p. 89-90 ºC; $[\alpha]_D^{28}$ = -5.9 (c = 0.80, CH_2Cl_2); FT-IR (Neat, ν_{max}, cm^{-1}): 3330, 2985, 1740, 1542, 1420, 1352, 1303, 1255, 1168, 1102, 856, 738, 593; ^1H NMR (200 MHz, $CDCl_3$) δ ppm: 7.99-7.65 (m, 4H, ArH), 7.21 (m, 5H, ArH), 6.01 (d, *J* = 8.7 Hz, 1H, NH), 4.46 (d, *J* = 6.5 Hz, 1H, α-CH), 3.52 (s, 3H, OCH_3), 3.12 (t, *J* = 6.5 Hz, 2H, β-CH_2); MS-EI (m/z): 365(M^+ + 1).

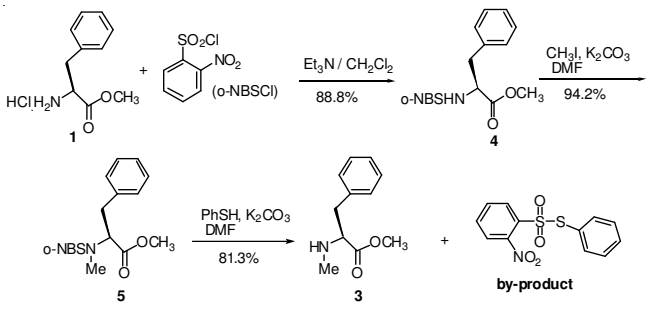

Fig. 2. Preparation of compound **3** *via* fukuyama amine synthesis

N-Methyl-N-(2-nitrobenzenesulfonamide) L-phenylalanine methyl ester (5): To a mixture of compound **4** (2.019 g, 5.55 mmol), powdered anhydrous K_2CO_3 (3.827 g, 2.77 mmol) in anhydrous DMF (20 mL)was added dropwise the MeI (1.11 mL, 1.72 mmol). After the yellow colour became light, the reaction was stirred at 52 ºC for 5 h. Then the residual was diluted with ice water (50 mL), extracted with chloroform three times (3 × 30 mL). The combined organic layer was washed with brine and dried over anhydrous $MgSO_4$, concentrated *in vacuo* to give yellow slurry, which was recrystallized from petroleum ether/ethyl acetate to afford compound **5** (1.85 g, 88.2 %) as colourless solid. m.p. 92-93 ºC $[\alpha]_D^{28}$ = -4.5 (c = 0.5, CH_2Cl_2); anal. calcd. (%) for $C_{17}H_{18}N_2O_6S$: C 53.77, H 4.66, N 7.41. Found (%): C 53.86, H 4.91, N 7.25; FT-IR (Neat, ν_{max}, cm^{-1}): 3091, 2958, 1750, 1541, 1456, 1349, 1253, 1199, 1160, 1000, 750, 594; ^1H NMR (200 MHz, $CDCl_3$) δ ppm: 7.78-7.53 (m, 4H, Ar-H), 7.21 (s, 5H, Ar-H), 4.93 (dd, *J* = 7.8 Hz, 3.4 Hz, 1H, α-CH), 3.64 (s, 3H, OCH_3), 3.35, 2.95 (dd, *J* = 7.8 Hz, 6.1 Hz, 2H, β-CH_2), 3.03 (s, 3H, NMe); EI-MS (m/z): 379 (M^+ + 1).

N-Methyl-L-phenylalanine methyl ester (3): To a mixture of compound **5** (0.756 g, 2 mmol) and powdered anhydrous K_2CO_3 (0.83 g, 6 mmol) was added anhydrous DMF (20 mL) under N_2, then benzenethiol (0.26 mL 2.4 mmol) was added dropwise to mixture at room temperature. After being stirred for 2.5 h at the same temperature, the reaction was diluted with ice water (10 mL), extracted with ether three times (3 × 30 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under vacuum, the residue was chromatographed (SiO_2, 20:1 chloroform/methanol) to give **3** (0.314 g, 81.3 %) as slight yellow oil. $[\alpha]_D^{28}$ = -5.2 (c = 0.80, CH_2Cl_2); anal. calcd. (%) for $C_{11}H_{15}NO_2$: C 68.39, H 7.77, N 7.26. Found (%): C 68.94, H 7.69, N7.16; FT-IR (Neat, ν_{max}, cm^{-1}): 3336, 2950, 2798, 1735, 1495, 1454, 1199, 1172, 700; ^1H NMR (200 MHz, $CDCl_3$) δ ppm 7.30-7.15 (m, 5H, Ar-H), 3.67 (s, 3H, OCH_3), 3.47 (t, *J* = 6.8 Hz, 1H, α-CH), 2.97 (d, *J* = 6.8 Hz, 2H, β-CH_2), 2.37 (s, 3H, NCH_3), 1.82 (s, 1H, NH); EI-MS (m/z): 193 (M^+).

Synthesis of target compound 3, 10 and 11 *via* direct methylation of protected amino acids

N-Cbz-L-Phenylalanine methyl ester (6): To a solution of L-phenylalanine methyl ester hydrochloride **1** (1.079 g, 5 mmol) in H_2O (5 mL) and EtOAc (8 mL) was added the solution of Na_2CO_3 (0.583 g, 5.5 mmol) in H_2O (3 mL) and CbzCl ((0.81 mL, 5.5 mmol) at 0 ºC successively. The reaction was kept stirring at 16 ºC until no NH_2 group showed by ninhydrin stain (Fig. 3). Then ethyl acetate layer was separated and the water layer was extracted with ethyl acetate three times (3 × 10 mL). The combined organic layer was washed with solution of 20 % pyridine, 5 % HCl and brine. Then dried over anhydrous Na_2SO_4 and evaporated under vacuum to give **6** (1.56 g, 99.4 %) as colourless oil. $[\alpha]_D^{28}$ = -6.6 (c = 0.80, CH_2Cl_2); FT-IR (Neat, ν_{max}, cm^{-1}): 3334, 2953, 2793, 1735, 1705, 1491, 1453, 1189, 1168, 705; ^1H NMR (200 MHz, $CDCl_3$) δ ppm: 7.45-7.07 (m, 10H, Ar-H), 5.20 (bs, 1H, NH), 5.09 (s, 2H, OCH_2Ph), 4.69 (m, 1H, α-CH), 3.71 (s, 3H, OCH_3), 3.11 (m, 2H, β-CH_2).

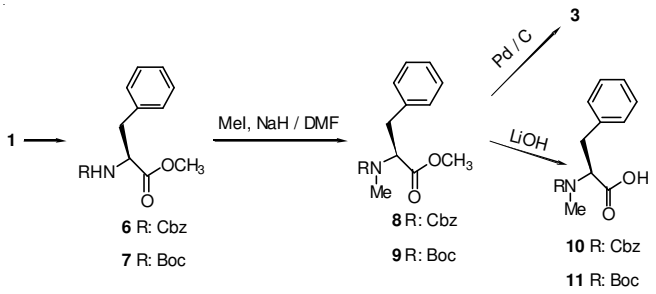

Fig. 3. Preparation of compounds **3**, **10** and **11** *via* direct methylation of protected amino acids

N-Cbz-N-Methyl-L-phenylalanine methyl ester (8): To a solution of compound **6** (1.56 g, 5 mmol) in dry DMF (16 mL) was added NaH (60 %, 0.6 g, 15 mmol) at 0 ºC. After the mixture was stirred for 2 min, the MeI (1.25 mL, 20 mmol) was added dropwise and the reaction was stirred at room temperature for another 2 h. Then quenched with ice water (10 mL) and extracted with ethyl acetate three times (3 × 20 mL). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give **8** (1.533 g, 93.7 %) as colourless oil. $[\alpha]_D^{28}$ = -5.7 (c = 0.86, CH_2Cl_2); anal. calcd. (%) for $C_{19}H_{21}NO_4$: C 69.92, H 6.42, N 4.28. Found (%) C 70.22, H 6.44, N 4.37. FT-IR (Neat, ν_{max}, cm^{-1}): 3029, 2952, 1743, 1700, 1496, 1454, 1398, 1316, 1264, 1216, 1157, 1105, 753, 699; ^1H NMR (300 MHz, $CDCl_3$) δ

ppm (main rotomer): 7.39-7.13 (m, 10H, ArH), 5.14-5.02 (m, 2H, OCH_2Ph), 4.85 (m, 1H, α-CH), 3.73 (s, 3H, OCH_3), 3.40-2.90 (m, 2H, β-CH_2), 2.79 (s, 3H, NCH_3); MS-FAB (m/z): 328 ($M^+ + 1$).

N-Cbz-N-Methyl-L-phenylalanine (10): To a solution of **8** (1.50 g, 4.58 mmol)in THF (20 mL) was added dropwise the solution of $LiOH·H_2O$ (1 g, 22.91 mmol) in water (10 mL) at 0 ºC. The reaction was stirred at room temperature for 2 h and then extracted with ether twice (1.5 mL*2). The water layer was acidified by 0.1N HCl until pH ≈ 3.5 and extracted with ethyl acetate three times (3 × 20 mL). The combined organic layer was washed with brine and dried over anhydrous $MgSO_4$, concentrated *in vacuo* to give **10** (1.148 g, 85.7 %) as bright yellow oil. $[\alpha]_D^{28}$ = -9.70 (c = 0.33, CH_2Cl_2); FT-IR (Neat, ν_{max}, cm^{-1}): 3031, 2944, 1730, 1705, 1455, 1403, 1317, 1213, 1142, 752, 698; ^1H NMR (300 MHz, $CDCl_3$) δ ppm (main rotomer): 7.32-7.14 (m, 10H, ArH), 5.11-5.03 (m, 2H, OCH_2Ph), 4.95 (m, 1H, α-CH), 3.39-3.02 (m, 2H, β-CH_2), 2.78 (s, 3H, NCH_3).

N-Boc-L-Phenylalanine methyl ester (7): The procedure for **7** is similar with that for **6**. The **7** was obtained as colourless oil, yield 99.8 %. $[\alpha]_D^{28}$ = -2.1 (c = 1.2, CH_2Cl_2); FT-IR (Neat, ν_{max}, cm^{-1}): 3369, 2978, 1746, 1717, 1498, 1366, 1251, 1214, 1168, 1062, 701; ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 7.32-7.11 (m, 5H, ArH), 4.89 (bs, 1H, NH), 4.58 (m, 1H, α-CH), 3.71 (s, 3H, $COOCH_3$), 3.09 (m, 2H, β-CH_2), 1.41 (s, 9H, Boc).

N-Boc-N-methyl-L-phenylalanine methyl ester (9): The procedure for compound **9** is similar with that for compound **8**. The compound **9** was obtained as slight yellow oil, yield 99 %. $[\alpha]_D^{28}$ = -1.73 (c = 1.24, CH_2Cl_2); anal. calcd. (%) for $C_{16}H_{23}NO_4$: C 65.53, H 7.85, N 4.78. Found (%): C 65.64, H 7.94, N 4.72. FT-IR (Neat, ν_{max}, cm^{-1}): 2975, 2951, 1745, 1696, 1454, 1367, 1256, 1151, 1103, 701; ^1H NMR (300 MHz, $CDCl_3$) δ ppm (main rotomers): 7.31-7.09 (m, 5H, ArH), 3.72 (s, 3H, $COOCH_3$), 3.69-3.21 (m, 1H, α-CH), 3.05-2.91 (m, 2H, β-CH_2), 2.71, 2.41 (s, 3H, NCH_3), 1.38, 1.26 (s, 9H, Boc-CH_3); MS-FAB (m/z): 294 ($M^+ + 1$).

N-Boc-N-methyl-L-phenylalanine (11): The procedure for compound **11** is similar with that for compound **10**. The compound **11** was obtained as slight yellow soft solid, yield 97.5 %. $[\alpha]_D^{28}$ = -14.8 (c = 0.55, CH_2Cl_2); FT-IR (KBr, ν_{max}, cm^{-1}): 2972, 1715, 1693, 1454, 1438, 1296, 1250, 1157, 1076, 698; ^1H NMR (300 MHz, $CDCl_3$) δ ppm (main rotomers): 7.85 (bs, 1H, COOH), 7.30-7.17 (m, 5H, ArH), 4.84-4.62 (m, 1H, α-CH), 3.28 (m, 1H, β-CH_2), 3.11 (m, 1H, β-CH_2), 2.75 (s, 3H, NCH_3), 1.39 (s, 9H, Boc-CH_3).

Preparation of 3 *via* hydrogenation of compound 8: Compound **8** (3.27 g, 0.1 mol) was dissolved in 90 mL of methanol and 10 % Pd/C (0.4 g, 10 % wt) was added into the reaction solution. Then reaction mixture was hydrogenated under H_2 at room temperature until TLC showed the starting material **8** was consumed completely. The solid in reaction was filtered off through a celite pad and the filtrate was concentrated *in vacuo* to give **3** (1.91 g, 99 %) as colourless oil.

RESULTS AND DISCUSSION

It was reported[11] that N-methyl amino acids could be prepared by reductive methylation of amino group using B_2H_6/Me_2S system. Even the yield in first step of this strategy was excellent, however, target compound **3** was obtained only in middle yield in our case (Fig. 4).

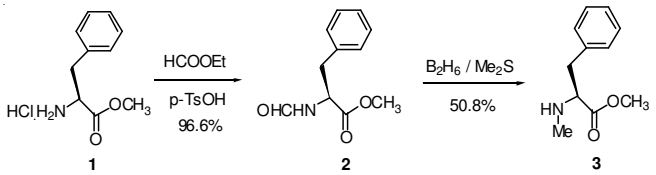

Fig. 4. Preparation of compound **3** *via* reductive methylation of amino acids

Following the way of fukuyama amine synthesis[12-15] (Fig. 3), sulfonylation of commercially available **1** by 1.58 eq of *o*-NBSCl gave N-NBS-protected intermediate **4** which was treated with MeI under basic condition to afford methylated sulfonamide **5** in high yield. However, the cleavage of *o*-NBS group resulted in producing a by-product as bright yellow colour solid with strong UV colour on TLC plate compared with that of target compound **3**. The colour of by-product was easy to cover that of compound **3** on TLC plate since R_f value of the by-product was very close to that of compound **3**. Therefore, this condition caused possibility to lose desired product **3** during purification. The compound **3** obtained in Figs. 3 and 4 can only be used for nitrogen-end extension in next coupling reaction.

Besides nitrogen-end extension in total synthesis of pepticinnamin E, oxygen-end extension would be needed. The directed methylation of Cbz-protected **6** and Boc-protected **7** gave important intermediates **8** and **9**, respectively in excellent yields (Fig. 3). Both **8** and **9** can be used for nitrogen-end extension in coupling reaction after cleavage of Cbz group under catalytically hydrogenation and removing Boc group by TFA, respectively. Both **8** and **9** could be hydrolyzed under mild basic condition to afford acids **10** and **11**, respectively without extensive purification, which could be employed in oxygen-end extension coupling reaction.

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