N-(*L*-2-Aminopentanoyl)-*L*-phenylalanine dihydrate, a hydrophobic dipeptide with a non-proteinogenic residue

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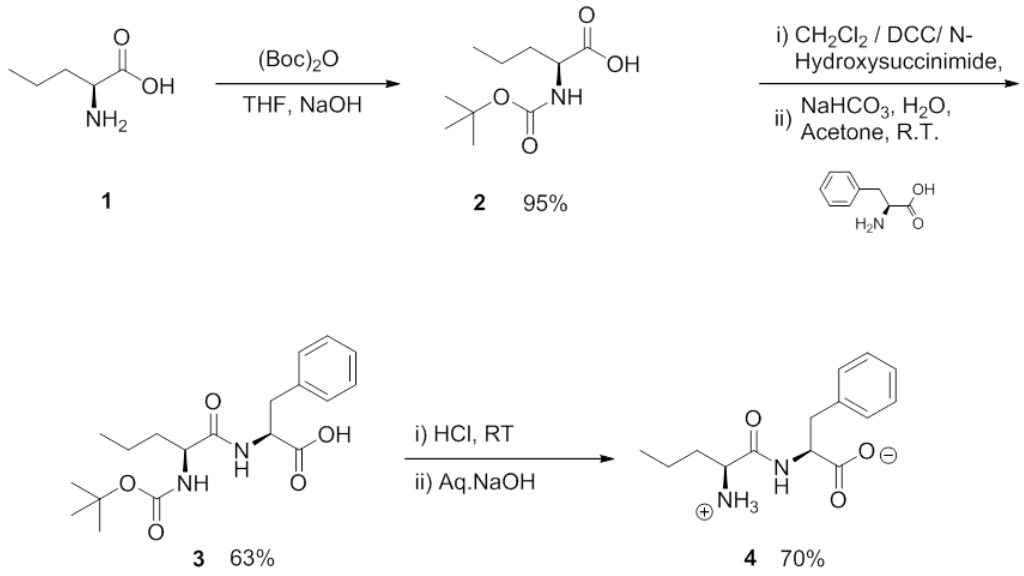
Supporting Information : Synthesis of *N*-(*L*-2-Aminopentanoyl)-*L*-phenylalanine dihydrate (Nva-Phe)



1) Synthesis of (*S*)-2-(*tert*-butoxycarbonylamino)pentanoic acid (2, Boc-Nva)

In a 100 mL round bottom flask (RBF) L-norvaline (**1**) (3 g, 2.56 mmol) was added to 50 mL of a chilled (0 - 5°C) tetrahydrofuran (THF) and water mixture (1:1). To this solution 5 mL of an aqueous solution of NaOH (1.025 g , 2.56 mmol) was gradually added (5 – 10 min) with continued stirring for an additional 10 min. Di-*tert*-butyl dicarbonate (7.0 mL, 30.8 mmol) was charged to the container, resulting in a turbid reaction mixture that was stirred at room temperature (RT) for 24 h to yield a clear solution. THF was then evaporated under vacuum at 30 °C on a rotary evaporator. The aqueous residue left behind was washed with diethyl ether (20ml x 2), the ether extract being discarded. The aqueous residue was cooled to 0 - 5°C, acidified with saturated $KHSO_4$ solution till the pH was 2-3 and extracted with fresh diethyl ether (30 mL x 3). The combined ether extract was washed by water

and brine solutions (20 mL each) separately and dried over anhydrous $MgSO_4$. Evaporation of the ether under vacuum afforded **2** (5.3 g, 95 %).

Optical rotation: $[\alpha]_{20589}$ = –13.6 (*c* = 1 g/100 ml, MeOH)

^{1}H-NMR (300MHz, DMSO-d_6), δ = 12.39 (s, 1H), 7.02-7.00 (d, *J* = 8.1 Hz, 1H), 3.90-3.82 (m, 1H), 1.62-1.50 (m, 2H), 1.38 (m, 11H), 0.88-0.83(t, *J* = 7.3 Hz, 3H).

^{13}C-NMR (300MHz, DMSO-d_6), δ 174.31 (*C*OOH), 155.60 (*C*O), 77.91 (>*C*<), 53.14 (*C*H), 32.88 (*C*H_2), 27.99 (3*C*H_3), 18.82 (*C*H_2), 13.48 (*C*H_3).

3) Synthesis of (S)-2-((S)-2-(tert-butoxycarbonylamino)pentanamido)-3-phenylpropanoic acid (3, Boc-Nva-Phe)

Compound **2** (0.11 g, 0.50 mmol), dicyclohexyl dicarbodiimide (DCC) (0.125 g, 0.6 mmol) and *N*-hydroxysuccinimide (0.070 g, 0.6 mmol) were mixed in a dry RBF containing 10 mL of dry dichloromethane (DCM) under nitrogen atmosphere at 10 °C. The reaction mixture was heated to room temperature with continued stirring for 2 h. Dicyclohexyl urea, obtained as a byproduct, was filtered from the reaction suspension through a celite bed prepared in dichloromethane. The DCM filtrate was evaporated under vacuum to obtain a residue of the succinate ester of **2** (0.160 gm). In another RBF L-phenylalanine (0.085 g, 0.51 mmol) was added to 5 mL of water containing 0.129 g of $NaHCO_3$ at RT. To this, the succinate ester solution of **2** dissolved in acetone (5 mL) was added over 5 min with continued stirring for 30 min at RT. Acetone from the reaction was evaporated under vacuum, and the water residue extracted with 15 mL of diethyl ether. The water residue was diluted with 10 mL of additional water and cooled to 0 °C for subsequent acidification with 1M HCl solution till pH 2-3. The product was extracted from the aqueous solution with diethyl ether (20 mL x 3). The combined ether extract was washed with brine and water (10 mL each) followed by drying over anhydrous $MgSO_4$, and evaporation of ether under vacuum afforded **3** (0.117 g, 63 %).

MS ES^+ [M+H] = 365

^{1}H-NMR (300MHz, $CDCl_3$-*d*), δ = 7.77 (bs, 1H), 7.28-7.15 (m, 5H), 6.83-6.81 (d, *J* = 7.5 Hz, 1H), 5.15-5.12 (d, *J* = 8.4 Hz, 1H), 4.87-4.81 (m, 1H), 4.15 (m, 1H) 3.24-3.16 (m, 1H), 3.06-2.99 (m, 1H), 1.75-1.66 (m, 1H), 1.46 (s, 9H), 1.38-1.24 (m, 2H), 0.92-0.87 (t, *J* = 7.2 Hz, 3H)

^{13}C-NMR (75MHz, $CDCl_3$-*d*), δ = 174.39 (*C*OO^-), 172.66 (*C*ON), 156.26 (*C*O), 136.29 (Ph *C*), 129.83 (Ph *C*), 128.88 (Ph *C*), 127.45 (Ph *C*), 77.61 (>*C*<), 54.68 (*C*H), 53.63 (*C*H), 37.85 (*C*H_2), 34.82 (*C*H_2), 28.68 (3*C*H_3), 19.15 (*C*H_2), 14.06 (*C*H_3).

13) Synthesis of (S)-2-((S)-2-aminopentanamido)-3-phenylpropanoic acid (4, L-Nva-L-Phe)

In a 50 mL RBF compound **3** (0.117 g) was dissolved in 10 mL of dry THF and cooled to 0 - 5°C. 2 mL of 4M HCl solution in dioxane was then slowly added. The reaction mixture was stirred for 48 h at RT and the reaction progress monitored with thin layer chromatography analysis (20 % MeOH in DCM eluent system, followed by Ninhydrin staining). Evaporation of the THF and dioxane under vacuum gave the syrupy crude residue of **4**, which was then redissolved in 10 mL of water and extracted with diethyl ether (20 mL x 2) to remove the non-polar impurities. The aqueous solution was neutralized with 1N NaOH solution till the pH was 7-8. Slow evaporation of water from this solution at RT over three weeks afforded colorless crystals of **4,** L-Nva-L-Phe (0.060 g, 70%).

MS ES^+ [M+H] = 265

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.84-8.81 (d, J = 9 Hz, 1H), 8.25-8.10 (m, 3H), 7.34-7.14 (m, 5H), 4.48-4.40 (m, 1H), 3.76-3.70 (m, 1H), 3.09-3.03 (m, 1H), 2.96-2.88 (m, 1H), 1.69-1.65 (m, 2H), 1.33-1.27 (m, 2H), 0.86-0.81 (t, J = 7.3 Hz, 3H).

^{13}C-NMR (75MHz, DMSO-d_6) δ = 173.24 (*C*OO^-), 169.67 (*C*ON), 137.97 (Ph *C*), 129.88 (Ph *C*), 129.19 (Ph *C*), 127.49 (Ph *C*), 54.78 (*C*H), 52.80 (*C*H), 37.16 (*C*H_2), 34.04 (*C*H_2), 18.07 (*C*H_2), 14.45. (*C*H_3).