Synthesis of peptides 1-5

Peptides Piv-Pro-Gly-NHMe(**1**), Piv-Pro-βGly-NHMe(**2**), Piv-Pro-βGly-OMe(**3**), Piv-Pro-δAva-OMe(**4**) and Boc-Pro-γAbu-OH(**5**) were synthesized by conventional solution phase procedure. The peptides were purified by medium pressure liquid chromatography on a reverse-phase C_{18} column (40-60 micron pore size) using methanol-water gradients. The peptide fractions were pooled, concentrated and lyophilized. The homogeneity of the peptides was ascertained by HPLC (C_{18}, 5-10 micron). The purified peptides were characterized by electrospray ionization mass spectrometry (ESI-MS) using a HP LCMSD 1100 mass spectrometer. Complete structural characterization of these peptides was established by 500 MHz ^{1}H NMR spectroscopy.

A detailed synthetic procedure is described for Piv-Pro-Gly-NHMe (**1**), followed by brief descriptions for Piv-Pro-βGly-OMe (**3**), Piv-Pro-βGly-NHMe (**2**), Piv-Pro-δAva-OMe (**4**) and Boc-Pro-γAbu-OH (**5**)

Piv-Pro-Gly-NHMe (1)

2.0 g (10 mmol) of Piv-Pro-OH was dissolved in 10-15ml dry THF and cooled to -20°C, and to that 1.3ml (11 mmol) of isobutylchloroformate was added dropwise, followed by dropwise addition of dry triethylamine (~2ml). 2.5 g (20 mmol) of GlyOMe.HCl was dissolved in 10-15ml of dry tetrahydrofuran (THF) and pH was adjusted to ~8 by the addition of dry TEA. This was added to the pre-cooled reaction mixture and stirred for 30 min at -20°C. The reaction mixture was allowed to attain room temperature and was stirred for ~6 h. The completion of reaction was monitored by TLC. The solvent THF was removed under reduced pressure and the residue was dissolved in 20-30 ml of water and extracted with ethyl acetate (3 x 50 ml). The combined organic layer was washed

with 2N HCl (3 x 50 ml), brine (~ 50 ml), 1N sodium carbonate (3 x 50 ml) and brine (50 ml). The organic layer was dried over anhydrous sodium sulfate. The ethyl acetate was evaporated in *vacuo*. The peptide was obtained as a white solid (yield 2.1 g, 78%) and used directly for further reaction. 1.1 g of Piv-Pro-Gly-OMe was dissolved in 15-20 ml of dry methanol and saturated with methylamine gas and allowed to stand in a tightly stoppered flask at room temperature for 72 h. Methanol was evaporated and the residue was treated with petroleum-ether to give a white solid (yield 1.0g, 95%), ESI-MS: M_{calc} = 269.3; MNa^+ = 292.2.

Piv-Pro-βGly-OMe (3)

2.0 g (10 mmol) of Piv-Pro-OH, 2.8 g (20 mmol) of β-HGly-OMe.HCl, 1.3 ml (11 mmol) of isobutylchloroformate, 2 ml of dry triethylamine and dry THF were used to prepare Piv-Pro-β-HGly-OMe following the above procedure (yield 2.2 g, 76%). ESI-MS: M_{calc} = 302.4, MNa^+_{obs} = 325.1.

Piv-Pro-βGly-NHMe (2)

1.2 g of Piv-Pro-βGly-OMe in dry methanol was used to convert into Piv-Pro-βGly-NHMe following the procedure described for **1** (yield 1.1 g, ~92%). ESI-MS: M_{calc} = 301.4, MNa^+ = 324.6.

Piv-Pro-δAva-OMe (4)

2.0 g (10 mmol) of Piv-Pro-OH, 3.3 g (20 mmol) of δAva-OMe.HCl, 1.3 ml of isobutylchloroformate, 2 ml of dry triethylamine and dry THF were used to prepare Piv-Pro-δAva-OMe, following the procedure described for **1** (yield 2.1 g, ~67%), ESI-MS: M_{calc} = 328.4, MNa^+ = 351.1.

Boc-Pro-γAbu-OH (5)

The dipeptide ester Boc-Pro-γAbu-OMe was prepared from Boc-Pro and γAbuOMe.HCl. as described for **3**. Saponification to the dipeptide acid **5** was achieved by adding 2N NaOH and methanol. Standard workup procedures involve extraction of an acidified solution with ethyl acetate. The ethyl acetate layer was dried over grind Na_2SO_4 and evaporated to yield white solid. Single crystals were obtained from ethyl acetate solutions and used directly for diffraction studies.