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Towards the total synthesis of MKN-349A

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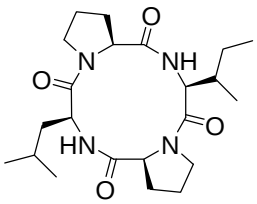
Attempts towards the total synthesis of MKN-349A and synthesis and characterization of a new and symmetric by-product, cyclooctapeptide is described. The characterization of the cyclooctapeptide is established using advanced NMR and MALDI studies.

Keywords: MKN-349A**,** cyclotetrapeptide, cyclooctapeptide, macrocyclisation, solution phase peptide synthesis

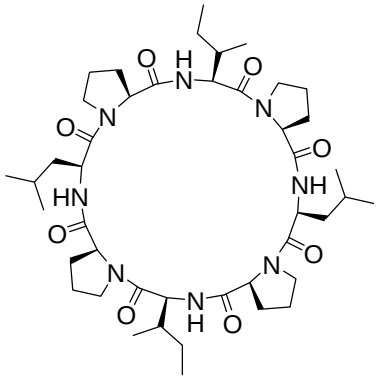
Marine microorganisms, present in diverse marine environment, have produced a variety of structurally unique and biologically active compounds. They are also recognized as emerging sources of secondary metabolites and the structural diversity has attracted considerable attention for their synthetic studies. One of the structurally interesting cyclic peptide MKN-349A **1**, with two L-proline moieties separated by hindered amino acids like L-leucine and L-isoleucine has attracted our attention, **Chart I**. The natural product, MKN-349A **1**, is isolated from an actinomycete of the genus *Nocardiopsis* from the Pacific deep-sea sediment and the dimer of MKN-349A **1** is an obvious by-product during the synthesis[1] **Chart I**.

Synthesis of cyclotetrapeptide **1**, consisting of all natural amino acids, is relatively difficult compared to cyclotetrapeptide with atleast one unnatural amino acid[2]. The synthesis of **1** involves cyclization of the tetrapeptide hydrochloride salt of Ile-Pro-Leu-Pro-Opfp **3**, as key transformation[3]. The tetrapeptide **3**, is obtained as per **Scheme I**.

The tetrapeptide **3**, thus obtained, is taken up for intramolecular/intermolecular cyclization to give the cyclic peptide shown in the **Chart I**. Treatment of the tetrapeptide hydrochloride salt in the presence of biphasic chloroform and 1.0 *N* sodium bicarbonate (ratio of 2:1) system furnished the crude product, **Scheme II**. The crude product indicated the formation of the desired tetrapeptide **1** with a molecular ion peak



1

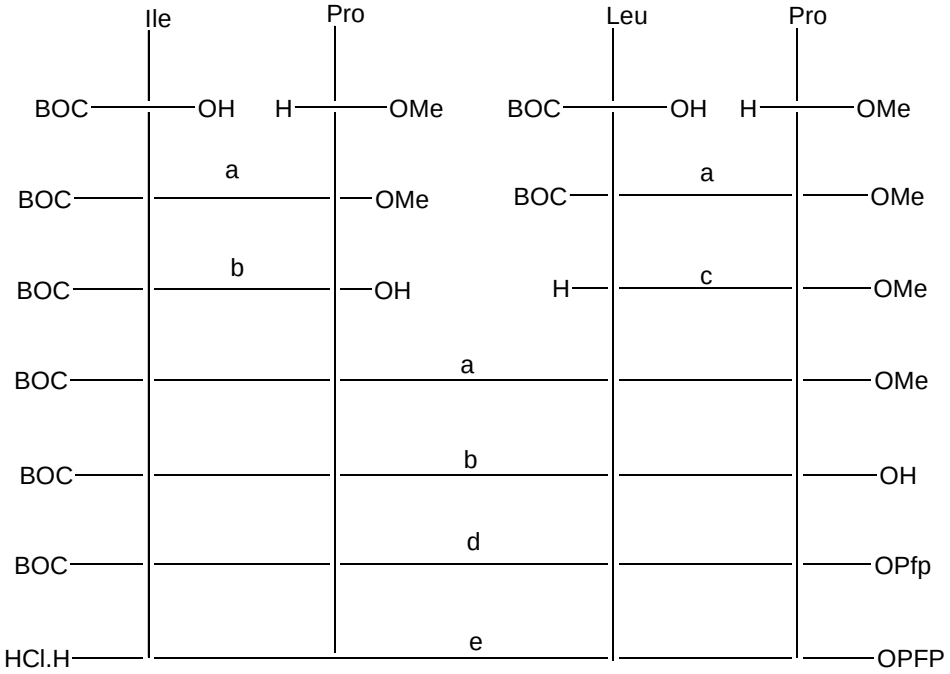


2

Chart I

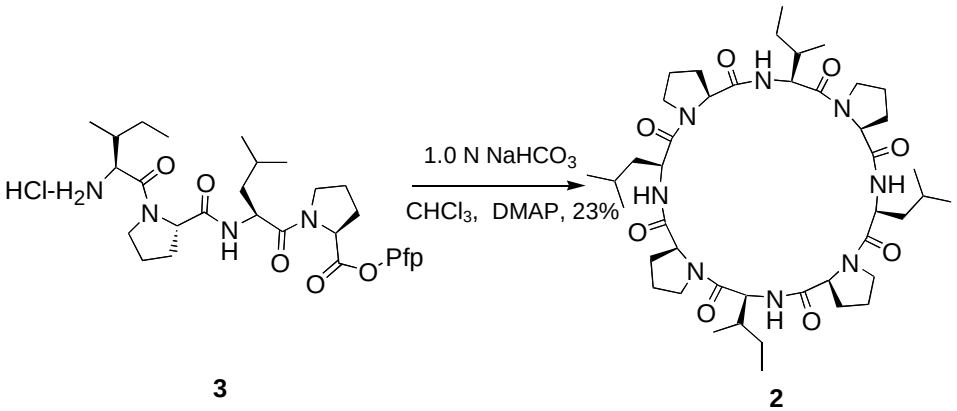
in mass at 421.3 along with the corresponding cyclooctapeptide **2**. The reaction was repeated under different dilutions and reaction conditions to get the desired tetrapeptide **1**, in good concentration. However, efforts to isolate and purify the cyclic tetrapeptide **1**, from the crude reaction mass were unsuccessful, possibly due to its presence in very low concentrations.

The by-product cyclooctapeptide **2**, could be isolated in pure form using column chromatography. The positive ES-MS spectrum displayed a protonated molecular ion at *m/z* 841.8 and MALDI studies[4] substantiated the formation of **2**. The proton NMR spectrum showed characteristic peptidic α protons in the region of 4.2-5.0 ppm integrating to eight protons. This observation is in agreement with the eight cross-peaks seen in the gHSQC experiment. Further, there are eight characteristic amide carbon signals observed between 169-173 ppm in the ^{13}C NMR spectrum. Hence it is reasonable to conclude the presence of eight amino acid moieties in **2**. It is interesting to know that four methylene protons were observed between 3.4-3.8 ppm, which were characteristic of the four proline δ methylene and the spin system of each proline were confirmed by COSY and TOCSY experiments. The presence of two leucine and



Reagents and conditions: a. DCC, HOBt. b. 1 *N* NaOH, MeOH. c. TFA, DCM. d. EDC, NMM, PFP, DCM e. 1 *M* 1, 4-dioxane.HCl.

Scheme I



Scheme II

isoleucine each, were inferred from the methyl signals between 0.8-1.1 ppm and their corresponding exchangeable protons (four) were observed at 6.1-6.3 and 8.6-9.0 ppm. Hence the presence of eight amino acids namely four prolines, two leucines and two isoleucines were confirmed by 1D and 2D NMR experiments. Based on the synthetic methodology employed, and the molecular ion information obtained from MS data, the structure of the cyclic peptide **2** has been deduced.

In summary, synthesis of a new dimeric cyclic peptide is described using solution phase synthesis starting from commercially available basic amino acids. The difficult purification of the same could be achieved from structurally closely related moieties and the structure of the cyclic peptide is assigned based on advanced NMR and mass spectral data.

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 Mercury plus model 400 MHz instrument, (chemical shift in δ, ppm) with TMS as internal standard; and mass spectra run on PESEIEXAPI-3000 model.

Boc-Ile-Pro-Leu-Pro-OMe 4. Boc-Leu-Pro-OMe (51 g, 0.15 mole) was dissolved in dichloromethane (102 mL) and cooled to 10-15°C. To this trifluoro-acetic acid (102 mL) was added at the same temperature for 30 min and reaction temperature brought to RT. The reaction was stirred at the same temperature for 1 hr and concentrated completely under reduced pressure at below 40°C afforded compound trifluoroacetate salt of Leu-Pro-OMe. The compound Boc-Ile-Pro-OH (44.0 g, 0.13 mole) in dichloromethane (140 mL) was cooled to 0-5°C and HOBT (20.0 g, 0.149 mole) was added and stirred for 10 min at same temperature. DCC (20.0 g, 0.15 mole) was added stirred for 1 hr at 0-5°C. The crude trifluoroacetate salt of Leu-Pro-OMe in dichloro-methane (200 mL) was neutralized with NMM, added to the reaction. After completion of addition the reaction temperature brought to RT and stirred for 12 hr. The reaction mixture cooled to 0-5°C, filtered, and filtrate was concentrated completely under reduced pressure. The crude was dissolved in ethyl acetate 600 mL and washed successively with 0.5 *N* HCl (2 × 100 mL), water (100 mL), 10% aq. $NaHCO_3$ solution (2 × 100 mL) and water (3 × 100 mL). Finally the organic part was dried over Na_2SO_4, concentrated afforded **4** (60 g, 83%) as pale yellow syrup. ES-MS: 553.7(M+1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.01 (d, *J*=12.5Hz, 1H), 5.17 (d, *J*=9.4Hz, 1H), 4.74-4.68 (m, 1H), 4.54-4.48 (m, 2H), 4.31-3.80 (m, 1H), 3.80-3.71 (m, 2H), 3.7 (s, 3H), 3.62-3.57 (m, 2H), 2.26-2.16 (m, 2H), 2.20-1.96 (m, 7H), 1.76-1.63 (m, 2H), 1.55-1.51 (m, 3H), 1.42 (s, 9H), 0.95-0.87 (m, 12H). Anal. Calcd for $C_{28}H_{48}N_4O_7$: C, 60.85; H, 8.75; N, 10.14. Found: C, 60.75; H, 8.63; N, 10.09%.

Boc-Ile-Pro-Leu-Pro-OH 5. Boc-Ile-Pro-Leu-Pro-OMe **4** (55.0 g, 0.10 mole) was dissolved in methanol (275 mL, 5.0 vol) and cooled to 0-10°C. To this 2.0 *N* sodium hydroxide solution (76.5 mL) was added drop-wise at the same temperature for 1 hr. The reaction was stirred at RT for 8 hr. The crude was diluted with water (300 mL) and washed with ethyl acetate (3 × 100 mL) to remove impurities. The aqueous part acidified with 15% aq. $KHSO_4$ and extracted with ethyl acetate (3 × 150 mL). The combined organic part was washed with water (3 × 100 mL) and the organic part was dried over Na_2SO_4 filtered and concentrated under reduced pressure at below 40°C afforded **5** (50.0 g, 93%) as pale yellow fluffy solid. ES-MS: 539.3(M+1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29-7.25 (m, 1H), 5.27 (d, *J*=9.1Hz, 1H), 4.75-4.70 (m, 1H), 4.58-4.55 (m, 2H), 4.30 (t, *J*=7.25Hz, 1H), 3.81-3.77 (m, 2H), 3.63-3.58 (m, 2H), 2.26-2.16 (m, 1H), 2.20-1.96 (m, 8H), 1.76-1.63 (m, 2H), 1.55-1.51 (m, 2H), 1.43-1.42 (s, 9H), 1.20-1.10 (m, 1H), 0.95-0.87 (m, 12H). Anal. Calcd for $C_{27}H_{46}N_4O_7$: C, 60.20; H, 8.61; N, 10.40. Found: C, 59.99; H, 8.59; N, 10.10%.

Boc-Ile-Pro-Leu-Pro-Opfp 6. Boc-Ile-Pro-Leu-Pro-OH **5** (20.0 g, 0.037 mole) and pentafluorophenol (pfp) (8.2 g, 0.04 mole) was dissolved in dichloromethane (120 mL) and cooled to –5 to 0°C. To this EDC.HCl (8.5 g, 0.044 mole) was added and neutralized the reaction with *N*-methyl morpholine at –5 to 0°C. The reaction stirred at the same temperature for 1 hr and at RT for 16 hr. The reaction mixture concentrated at 30°C and diluted with ethyl acetate (200 mL), washed with 1.0 *N* sulphuric acid (3 × 80 mL), water (4 × 100 mL). The organic part was dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography using ethyl acetate/pet. ether (1:1) afforded Boc-Ile-Pro-Leu-Pro-Opfp **6** as pale red syrup (20.9 g, 80%). ES-MS: 705.3 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$):

δ 7.03 (d, *J*=8.2 Hz, 1H), 5.18 (d, *J*=9.2 Hz, 1H), 4.13 (dd, *J*=4.1,8.5 Hz, 1H), 4.75-4.69 (m, 1H), 4.55 (dd, *J*=3.5, 7.9 Hz, 1H), 4.31 (t, *J*=8.0 Hz, 1H), 3.93-3.81 (m, 1H), 3.81-3.71 (m, 1H), 3.71-3.56 (m, 2H), 2.43-2.38 (m, 1H), 2.27-2.12 (m, 6H), 2.04-1.91 (m, 2H), 1.76-1.63 (m, 2H), 1.55-1.50 (m, 2H), 1.42 (s, 9H), 1.25-1.11 (m, 1H), 1.00-0.88 (m, 12H), ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.36, 171.38, 171.12, 168.12, 155.77, 140.71, 139.57, 139.57, 137.80, 137.80, 124.85, 79.53, 59.83, 58.33, 56.22, 49.20, 47.68, 46.77, 41.43, 37.96, 29.08, 28.31(3C), 27.64, 25.11, 25.07, 24.38, 24.08, 23.26, 21.77, 15.40, 11.16. Anal. Calcd for $C_{33}H_{45}F_5N_4O_7$: C, 56.24; H, 6.44; N, 7.95. Found: C, 56.09; H, 6.40; N, 7.80%.

HCl-Ile-Pro-Leu-Pro-Opfp 3. To a solution of Boc-Ile-Pro-Leu-Pro-Opfp **6** (10.0 g, 0.014 mole), in 1,4-dioxane (47 mL) were added 1.0 *M* 1,4-dioxane. HCl. The reaction mixture was stirred for 2 hr at room temperature. The reaction mixture was concentrated and solid isolated from ethyl acetate afforded **3** (6.0 g, 66%) as a white coloured solid. ES-MS: 605 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55-8.25 (br s, 3H), 7.80 (d, *J*=8.5 Hz, 1H), 4.92-4.89 (m, 1H), 4.83-4.80 (m, 2H), 4.25-4.05 (br s, 1H), 4.00-3.75 (m, 2H), 3.75-3.45 (m, 2H), 2.55-2.30 (m, 1H), 2.25-1.85 (m, 8H), 1.80-1.20 (m, 5H), 1.12 (d, *J*=7.0 Hz, 3H), 1.05-0.85 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.81, 171.58, 168.18, 167.86, 141.06 (2C, *J*=251.4 Hz), 137.83 (2C, *J*=252.9), 139.53 (*J*=252 Hz) 125.02 (*J*=~25.0 Hz), 60.46, 58.39, 56.22, 49.43, 48.44, 46.89, 41.29, 36.42, 29.06, 28.88, 25.23, 24.98, 24.27, 24.04, 23.11, 21.92, 14.82, 11.25.

Cyclo (Ile-Pro-Leu-Pro-Ile-Pro-Leu-Pro) 2. The solution of **3**. (1.0 g, 1.56 mmol) in chloroform (150 mL) was added to well-stirred mixture of chloroform /1.0 *N* sodium bicarbonate (1350 mL, 2:1) containing 4-dimethylaminopyridine (38.0 mg) for 7 hr at RT. The organic layer was separated and washed with 1.0 *N* H_2SO_4 and water. The organic part was dried over Na_2SO_4, filtered and concentrated. Purification of the residue by preparative HPLC (eluent 0.1% trifluoro-acetic acid/acetonitrile = 40/60) afforded **2** (150 mg, 23%) as a white solid. ES-MS: 841.8(M+1); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.92 (br, s 1H), 8.80 (br s, 2H), 6.26 (br s, 1H), 4.89-4.80 (m, 2H), 4.80-4.70 (m, 2H), 4.50-4.40 (m, 2H), 4.4-4.3 (m, 2H), 3.70-3.42 (m, 8H), 2.65-2.45 (m, 2H), 2.30-1.50 (m, 22H), 1.50-1.14 (m, 4H), 1.07 (d, *J*=6.7Hz, 3H), 0.96 (d, *J*=7.0Hz, 3H), 0.94-0.88 (m, 15H), 0.86 (d, *J*=6.7Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.00, 172.25, 172.11, 171.95, 170.52, 170.31, 170.31, 169.76, 61.11, 60.23, 59.96, 59.84, 57.30, 55.59, 51.40, 49.98, 47.98, 47.41, 47.11, 46.75, 44.83, 39.00, 38.12, 37.00, 31.04, 29.02, 25.51, 25.50, 25.31, 25.31, 25.20, 25.20, 25.00, 24.98, 23.80, 23.73, 23.55, 21.95, 21.27, 20.71, 15.80, 15.27, 11.87, 11.37. Anal. Calcd for $C_{44}H_{72}N_8O_8$: C, 62.83; H, 8.63; N, 13.32. Found: C, 62.76; H, 8.63; N, 13.2%.

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4 The sample is analyzed by MALDI-TOF (Micro mass) mass spectrometer in reflectron mode and the spectra were processed by using the Mass Lynx 4.0 version software.