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First Total Synthesis and Biological Potential of a Heptacyclopeptide of Plant Origin

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Synthesis of a natural glycine-rich heptacyclopeptide − mahafacyclin A (**7**) was accomplished by solution-phase technique of peptide synthesis *via* coupling of tetrapeptide unit Boc-*L*-Thr-*L*-Ile-*L*-Leu-Gly-OH with tripeptide unit *L*-Val-*L*-Phe-Gly-OMe followed by cyclization of linear heptapeptide fragment. Structure of the newly synthesized cyclopolypeptide was confirmed by means of chemical, spectroscopic analyses and subjected to antibacterial, antifungal and anthelmintic activity studies. Bioactivity results showed potent antifungal and anthelmintic activities of the synthesized peptide against dermatophytes *T. mentagrophytes*, *M. audouinii* and earthworm species *M. konkanensis*, *P. corethruses* and *E. eugeniea*.

Keywords *Jatropha mahafalensis*, peptide synthesis, cyclopolypeptide, natural product, pharmacological activity

Introduction

Medicinal plants have been used to treat health disorders and to prevent diseases since time immemorial.[1] Recently, the World Health Organization has estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care. Natural products from medicinal plants provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity.[2] Plant-derived cyclopeptides have complex structures with modified and/or unusual amino acid moieties and are concerned with a number of bioactivities including antifungal activity,[3] insecticidal activity,[4] tyrosinase inhibitory activity,[5] anthelmintic activity,[6] anti-inflammatory activity,[7,8] vasorelaxant activity,[9,10] estrogen-like activity,[11] antimalarial activity,[12] and anticancer activity.[13,14] A natural cyclic heptapeptide-mahafacyclin A with β-bulge characteristics, has been isolated from *Jatropha mahafalensis* latex and its structure was elucidated by a combination of chemical degradation, LSIMS data and 2D NMR experiments.[15] Minute quantities of this bioactive cyclopeptide obtained from natural resources (328 mg from 250 g of dry latex of *J. mahafalensis*) restricted scientists to investigate its biological profile in detail.

Keeping in view broad spectrum of bioactivities exhibited by plant-derived cyclopolypeptides[16-21] and an effort to obtain a potent bioactive compound in good yield, present investigation was directed toward solution-phase synthesis, structure elucidation and screening of glycine-rich heptacyclopeptide − mahafacyclin A (**7**) for antimicrobial and anthelmintic potential.

Experimental

Material and methods

All the reactions requiring anhydrous conditions were conducted in flame dried apparatus. Melting point was determined by open capillary method and was uncorrected. *L*-Amino acids, dicyclohexylcarbodiimide (DCC), *N*,*N*′-diisopropylcarbodiimide (DIPC), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC • HCl), trifluoroacetic acid (TFA), pentafluorophenol (*pfp*), *N*-methylmorpholine (NMM), triethylamine (TEA), di-*tert*-butylpyrocarbonate (Boc_2O) and pyridine (C_5H_5N) were obtained from Spectrochem Limited/Sigma-Aldrich (Mumbai, India). IR spectra were recorded on a Shimadzu 8700 FTIR spectrophotometer and $^1H/^{13}C$ NMR spectra were taken on a Bruker AC NMR spectrometer (300 MHz) using deuterated methanol as solvent and TMS as internal standard. A JMS-DX 303 Mass spectrometer operating at 70 eV by FABMS was utilized to record mass spectrum. Optical rotation was measured on a automatic polarimeter using sodium lamp. Elemental analyses of all compounds were performed on Vario EL III elemental analyzer and purity of all synthesized peptide deriva-

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tives was checked by TLC on precoated silica gel G plates.

Preparation of linear di/tri/tetrapeptide units (1—5)

To a solution of *L*-amino acid methyl ester hydrochloride/dipeptide methyl ester (0.01 mol) in chloroform (20 mL), TEA (2.8 mL, 0.021 mol) was added at 0 ℃ and the reaction mixture was stirred for 10 min. Boc-*L*-amino acid/Boc-dipeptide (0.01 mol) was dissolved in $CHCl_3$ (20 mL) followed by addition of DCC/DIPC/EDC•HCl (2.1 g/1.26 g/1.92 g, 0.01 mol) and HOBt (1.34 g, 0.01 mol). The resulting mixture was added to the above solution with constant shaking and stirring was continued for 24 h. The reaction mixture was filtered and the residue was washed with $CHCl_3$ (20 mL) and added to the filtrate. The filtrate was washed with 5% $NaHCO_3$ and saturated NaCl solutions. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum. The crude product was recrystallized from a mixture of chloroform and petroleum ether (b.p. 40—60 ℃) followed by cooling at 0 ℃ to get the title compounds **1—5**.

Preparation of linear heptapeptide unit and its cyclized form (6, 7)

Boc-tetrapeptide, Boc-*L*-Thr-*L*-Ile-*L*-Leu-Gly-OH (5.02 g, 0.01 mol) was dissolved in 30 mL of THF and 2.23 mL (0.021 mol) of NMM was added at 0 ℃ and the resulting mixture was stirred for 15 min. Tripeptide methyl ester, *L*-Val-*L*-Phe-Gly-OMe (3.35 g, 0.01 mol) was dissolved in 30 mL of THF and DCC/DIPC/EDC•HCl (2.1 g/1.26 g/1.92 g, 0.01 mol) and HOBt (1.34 g, 0.01 mol) were added to the above mixture with stirring. Stirring was continued for 36 h, after which the reaction mixture was filtered and the filtrate was washed with 30 mL each of 5% $NaHCO_3$ and saturated NaCl solutions. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum. The crude product was recrystallized from a mixture of chloroform and petroleum ether (b.p. 40—60 ℃) followed by cooling at 0 ℃ to get Boc-*L*-Thr-*L*-Ile-*L*-Leu-Gly-*L*-Val-*L*-Phe-Gly-OMe **6** as yellowish semisolid mass. Linear heptapeptide unit **6** (4.1 g, 0.005 mol) was deprotected at carboxyl terminal using lithium hydroxide (LiOH, 0.18 g, 0.0075 mol) to obtain Boc-*L*-Thr-*L*-Ile-*L*-Leu-Gly-*L*-Val-*L*-Phe-Gly-OH. To a solution of the deprotected heptapeptide (4.03 g, 0.005 mol) in $CHCl_3$ (50 mL), pentafluorophenol (1.23 g, 0.0067 mol) and DCC (1.06 g, 0.005 mol) were added followed by stirring at r.t. for 12 h. Filtrate of the above reaction mixture was washed with 10% $NaHCO_3$ (20 mL×3) and 5% HCl (20 mL×2) solutions to obtain corresponding pentafluorophenyl ester Boc-*L*-Thr-*L*-Ile-*L*-Leu-Gly-*L*-Val-*L*-Phe-Gly-O*pfp*. Boc-group of resulting unit (3.89 g, 0.004 mol) was removed using TFA (0.91 g, 0.008 mol) to get deprotected product *L*-Thr-*L*-Ile-*L*-Leu-Gly-*L*-Val-*L*-Phe-Gly-O*pfp* which was dissolved in $CHCl_3$ (25 mL) and TEA or NMM or pyridine (2.8 mL/2.21 mL/1.61 mL, 0.021 mol) was added. Then, whole contents were kept at 0 ℃ for 7 d. The reaction mixture was washed with 10% $NaHCO_3$ (25 mL×3) and 5% HCl (25 mL×2) solutions. The organic layer was dried over anhydrous Na_2SO_4 and crude cyclized compound was recrystallized from CH_2Cl_2/*n*-hexane to obtain pure product cyclo (*L*-threonyl-*L*-isoleucyl-*L*-leucyl-glycyl-*L*-valyl-*L*-phenylalanyl-glycyl) (**7**). Physical characterization data and elemental analysis data for all the newly synthesized di/tri/tetra/heptapeptide intermediates and cyclic products **1—7** are given in Table 1 and Table 2.

1: ^{1}H NMR ($CDCl_3$) δ: 6.59 (br s, 1H, NH, Thr), 6.45 (br s, 1H, NH, Ile), 4.54 (t, J=3.95 Hz, 1H, H-α, Thr), 4.42 (br s, 1H, OH, Thr), 4.17 (t, J=8.65 Hz, 1H, H-α, Ile), 3.69—3.64 (m, 1H, H-β, Thr), 3.49 (s, 3H, OCH_3), 2.04—1.98 (m, 1H, H-β, Ile), 1.69—1.64 (m, 2H, H-γ, Ile), 1.52 (s, 9H, *tert*-Butyl), 1.26 (d, J=4.9 Hz, 3H, H-γ, Thr), 0.95 (t, J=7.75 Hz, 3H, H-δ, Ile), 0.87 (d, J=5.9 Hz, 3H, H-γ', Ile); IR ($CHCl_3$) ν: 3129, 3121 (N—H str, amide), 2969—2962, 2919 (C—H str, CH_3 and CH_2), 1744 (C=O str, ester), 1640, 1632 (C=O str, amide), 1539, 1532 (N—H def, amide), 1422, 1329 (O—H def), 1386, 1368 (C—H def, *tert*-Butyl), 1272 (C—O str, ester) cm^{-1}.

2: ^{1}H NMR ($CDCl_3$) δ: 6.78 (br s, 1H, NH, Gly), 6.05 (br s, 1H, NH, Leu), 4.25 (q, 1H, H-α, Leu), 4.09 (d, J=5.15 Hz, 2H, H-α, Gly), 3.65 (s, 3H, OCH_3), 1.93 (t, J=7.95 Hz, 2H, H-β, Leu), 1.56 (s, 9H, *tert*-Butyl), 1.52—1.47 (m, 1H, H-γ, Leu), 1.02 (d, J=6.25 Hz, 6H, H-δ, Leu); IR (KBr) ν: 3126 (N—H str, amide), 2965—2961, 2925—2519 (C—H str, CH_3 and CH_2), 1742 (C=O str, ester), 1642, 1635 (C=O str, amide), 1536, 1531 (N—H def, amide), 1389, 1368 (C—H def, *tert*-Butyl), 1269 (C—O str, ester) cm^{-1}.

3: ^{1}H NMR ($CDCl_3$) δ: 7.12 (t, J=5.65 Hz, 1H, H-*p*, Phe), 7.02—6.97 (m, 2H, H-*m*, Phe), 6.85 (dd, J=7.2, 4.1 Hz, 2H, H-*o*, Phe), 6.75 (br s, 1H, NH, Phe), 6.43 (br s, 1H, NH, Val), 4.35 (q, J=5.6 Hz, 1H, H-α, Phe), 4.28 (t, J=5.85 Hz, 1H, H-α, Val), 3.54 (s, 3H, OCH_3), 2.95 (d, J=4.8 Hz, 2H, H-β, Phe), 1.87—1.82 (m, 1H, H-β, Val), 1.54 (s, 9H, *tert*-Butyl), 1.07 (d, J=4.6 Hz, 6H, H-γ, Val); IR ($CHCl_3$) ν: 3124 (N—H str, amide), 3069—3062 (C—H str, aromatic ring), 2923 (C—H str, CH_2), 1744 (C=O str, ester), 1640, 1632 (C=O str, amide), 1562, 1435 (skeletal bands), 1536, 1532 (N—H def, amide), 1385, 1362 (C—H def, *iso*-Propyl), 1388, 1369 (C—H def, *tert*-Butyl), 1271 (C—O str, ester), 716, 689 (C—H def, oop, aromatic ring) cm^{-1}.

4: ^{1}H NMR ($CDCl_3$) δ: 8.89 (br s, 1H, NH, Leu), 7.35 (br s, 1H, NH, Ile), 6.58 (br s, 1H, NH, Thr), 6.51 (br s, 1H, NH, Gly), 4.55 (t, J=8.6 Hz, 1H, H-α, Ile), 4.43 (br s, 1H, OH, Thr), 4.37 (t, J=3.9 Hz, 1H, H-α, Thr), 4.05 (d, J=5.2 Hz, 2H, H-α, Gly), 3.78 (q, 1H, H-α, Leu), 3.71—3.65 (m, 1H, H-β, Thr), 3.61 (s, 3H, OCH_3), 2.07—2.02 (m, 1H, H-β, Ile), 1.75 (t, J=7.9 Hz, 2H, H-β, Leu), 1.67—1.62 (m, 2H, H-γ, Ile), 1.55 (s, 9H, *tert*-Butyl), 1.51—1.45 (m, 1H, H-γ, Leu), 1.27 (d, J=4.85 Hz, 3H, H-γ, Thr), 1.04 (d, J=5.85 Hz, 3H, H-γ',

Ile), 1.01 (d, J=6.2 Hz, 6H, H-δ, Leu), 0.94 (t, J=7.8 Hz, 3H, H-δ, Ile); IR ($CHCl_3$) ν: 3128, 3125−3122 (N−H str, amide), 2969−2962, 2925, 2518 (C−H str, CH_3 and CH_2), 1743 (C=O str, ester), 1643−1637 (C=O str, amide), 1539−1535, 1531 (N−H def, amide), 1424, 1325 (O−H def), 1388, 1366 (C−H def, *tert*-Butyl), 1270 (C−O str, ester) cm^{-1}.

5: ^1H NMR ($CDCl_3$) δ: 7.60 (br s, 1H, NH, Phe), 7.24−7.19 (m, 2H, H-*m*, Phe), 6.99 (t, J=5.7 Hz, 1H, H-*p*, Phe), 6.83 (dd, J=7.15, 4.1 Hz, 2H, H-*o*, Phe), 6.52 (br s, 1H, NH, Gly), 6.40 (br s, 1H, NH, Val), 4.54 (q, J=5.55 Hz, 1H, H-α, Phe), 4.20 (t, J=5.9 Hz, 1H, H-α, Val), 4.05 (d, J=5.15 Hz, 2H, H-α, Gly), 3.67 (s, 3H, OCH_3), 2.77 (d, J=4.8 Hz, 2H, H-β, Phe), 1.89−1.84 (m, 1H, H-β, Val), 1.56 (s, 9H, *tert*-Butyl), 1.05 (d, J=4.55 Hz, 6H, H-γ, Val); IR ($CHCl_3$) ν: 3126, 3122 (N−H str, amide), 3065, 3059 (C−H str, aromatic ring), 2926−2921 (C−H str, CH_2), 1742 (C=O str, ester), 1643−1639, 1633 (C=O str, amide), 1565, 1432 (skeletal bands), 1535, 1529 (N−H def, amide), 1384, 1363 (C−H def, *iso*-Propyl), 1389, 1367 (C−H def, *tert*-Butyl), 1268 (C−O str, ester), 714, 687 (C−H def, oop, aromatic ring) cm^{-1}.

6: ^1H NMR ($CDCl_3$) δ: 8.88 (br s, 1H, NH, Leu), 8.29 (br s, 1H, NH, Gly-1), 7.99 (br s, 1H, NH, Val), 7.92 (br s, 1H, NH, Phe), 7.37 (br s, 1H, NH, Ile), 7.20−7.15 (m, 2H, H-*m*, Phe), 6.98 (t, J=5.65 Hz, 1H, H-*p*, Phe), 6.84 (dd, J=7.2 Hz, 4.15 Hz, 2H, H-*o*, Phe), 6.56 (br s, 1H, NH, Thr), 6.49 (br s, 1H, NH, Gly-2), 4.53 (t, J=8.55 Hz, 1H, H-α, Ile), 4.45 (br s, 1H, OH, Thr), 4.38 (t, J=3.85 Hz, 1H, H-α, Thr), 4.08 (d, J=5.15 Hz, 2H, H-α, Gly-1), 4.04 (d, J=5.2 Hz, 2H, H-α, Gly-2), 3.92 (q, 1H, H-α, Leu), 3.85 (q, J=5.55 Hz, 1H, H-α, Phe), 3.78 (t, J=5.85 Hz, 1H, H-α, Val), 3.72−3.67 (m, 1H, H-β, Thr), 3.63 (s, 3H, OCH_3), 2.98 (d, J=4.75 Hz, 2H, H-β, Phe), 2.09−2.04 (m, 1H, H-β, Ile), 1.82 (t, J=7.85 Hz, 2H, H-β, Leu), 1.66−1.61 (m, 2H, H-γ, Ile), 1.59 (s, 9H, *tert*-Butyl), 1.56−1.51 (m, 1H, H-β, Val), 1.50−1.44 (m, 1H, H-γ, Leu), 1.29 (d, J=4.9 Hz, 3H, H-γ, Thr), 1.06 (d, J=5.9 Hz, 3H, H-γ', Ile), 0.99 (d, J=6.15 Hz, 6H, H-δ, Leu), 0.96 (d, J=4.6 Hz, 6H, H-γ, Val), 0.93 (t, J=7.75 Hz, 3H, H-δ, Ile); ^{13}C NMR ($CDCl_3$) δ: 174.5, 172.8 (2C, C=O, Ile and Thr), 170.6, 169.1 (2C, C=O, Phe and Leu), 168.2, 166.9 (2C, C=O, Gly-2 and Val), 166.5, 149.2 (2C, C=O, Gly-1, Boc), 138.4 (C-γ, Phe), 130.2 (2C, C-*o*, Phe), 127.7 (2C, C-*m*, Phe), 127.1 (C-*p*, Phe), 79.7 (C-α, Boc), 69.4 (C-β, Thr), 62.1, 58.5 (2C, C-α, Thr and Val), 53.8 (C-α, Phe), 51.9 (OCH_3), 49.8, 48.1 (2C, C-α, Ile and Leu), 41.3, 39.4 (2C, C-α, Gly-2 and Gly-1), 38.8, 36.7 (2C, C-β, Leu and Phe), 35.5, 33.2 (2C, C-β, Ile and Val), 29.4 (3C, C-β, Boc), 26.6, 23.2 (2C, C-γ, Ile and Leu), 22.6 (2C, C-δ, Leu), 20.5 (C-γ, Thr), 18.8 (2C, C-γ, Val), 16.9 (C-γ', Ile), 10.3 (C-δ, Ile); IR ($CHCl_3$) ν: 3129−3126, 3123, 3119 (N−H str, amide), 3069, 3056 (C−H str, aromatic ring), 2969, 2965−2959, 2924−2916 (C−H str, CH_3 and CH_2), 1741 (C=O str, ester), 1643−1638, 1634−1629 (C=O str, amide), 1568, 1435 (skeletal bands), 1538−1533, 1529 (N−H def, amide), 1426, 1322 (O−H def), 1389, 1366 (C−H def, *tert*-Butyl), 1383, 1362 (C−H def, *iso*-Propyl), 1269 (C−O str, ester), 717, 689 (C−H def, oop, aromatic ring) cm^{-1}.

7: ^1H NMR ($CDCl_3$) δ: 9.29 (br s, 1H, NH, Ile), 8.09 (br s, 1H, NH, Thr), 7.98 (br s, 1H, NH, Val), 7.82 (br s, 1H, NH, Leu), 7.62 (br s, 1H, OH, Thr), 7.58 (br s, 1H, NH, Phe), 7.19−7.15 (m, 2H, H-*m*, Phe), 7.09 (br s, 1H, NH, Gly-2), 7.02 (br s, 1H, NH, Gly-1), 6.99 (t, J=5.7 Hz, 1H, H-*p*, Phe), 6.87 (dd, J=7.15, 4.2 Hz, 2H, H-*o*, Phe), 6.54 (t, J=5.9 Hz, 1H, H-α, Val), 6.27 (q, 1H, H-α, Leu), 5.76 (q, J=5.6 Hz, 1H, H-α, Phe), 5.68 (t, J=3.9 Hz, 1H, H-α, Thr), 5.37 (d, J=5.15 Hz, 2H, H-α, Gly-2), 5.28 (t, J=8.6 Hz, 1H, H-α, Ile), 5.23 (d, J=5.2 Hz, 2H, H-α, Gly-1), 3.84−3.79 (m, 1H, H-β, Thr), 2.43 (d, J=4.8 Hz, 2H, H-β, Phe), 1.89−1.85 (m, 1H, H-β, Ile), 1.75−1.71 (m, 1H, H-β, Val), 1.69 (t, J=7.9 Hz, 2H, H-β, Leu), 1.67−1.63 (m, 1H, H-γ, Ile), 1.42 (d, J=4.85 Hz, 3H, H-γ, Thr), 1.18 (d, J=4.55 Hz, 6H, H-γ, Val), 1.02 (d, J=6.2 Hz, 6H, H-δ, Leu), 0.98 (d, J=5.85 Hz, 3H, H-γ', Ile), 0.94 (t, J=7.8 Hz, 3H, H-δ, Ile), 0.84−0.79 (m, 2H, H-γ, Leu); ^{13}C NMR ($CDCl_3$) δ: 174.9, 173.4 (2C, C=O, Ile and Val), 171.6, 169.9 (2C, C=O, Gly-2 and Gly-1), 169.2, 164.4 (2C, C=O, Thr and Leu), 162.8 (C=O, Phe), 138.0 (C-γ, Phe), 131.5 (2C, C-*o*, Phe), 129.8 (2C, C-*m*, Phe), 126.9 (C-*p*, Phe), 66.2 (C-β, Thr), 60.7, 59.4 (2C, C-α, Ile and Val), 58.0, 54.4 (2C, C-α, Thr and Phe), 49.1 (C-α, Leu), 44.0, 42.3 (2C, C-α, Gly-2 and Gly-1), 39.9 (C-β, Phe), 37.4, 35.0 (2C, C-α, Leu and Ile), 33.2 (C-β, Val), 25.8, 23.6 (2C, C-γ, Leu and Ile), 23.1 (2C, C-δ, Leu), 22.3 (C-γ, Thr), 18.5 (2C, C-γ, Val), 17.4 (C-γ', Ile), 9.9 (C-δ, Ile); IR (KBr) ν: 3128−3125, 3122, 3119 (N−H str, amide), 3066 (C−H str, aromatic ring), 2967, 2963−2958, 2925, 2917 (C−H str, CH_3 and CH_2), 1647, 1639, 1632−1627 (C=O str, amide), 1569, 1437 (skeletal bands), 1537, 1532, 1529−1525 (N−H def, amide), 1425, 1326 (O−H def), 1382, 1361 (C−H def, *iso*-propyl), 719, 686 (C−H def, oop, aromatic ring) cm^{-1}; FABMS *m/z* (%): 688.8 [$(M+H)^+$, 100], 660.8 [$(688.8–CO)^+$, 11], 631.7 [$(H\text{-}Thr\text{-}Ile\text{-}Leu\text{-}Gly\text{-}Val\text{-}Phe)^+$, 60], 603.7 [$(631.7–CO)^+$, 15], 589.7 [$(H\text{-}Phe\text{-}Gly\text{-}Thr\text{-}Ile\text{-}Leu\text{-}Gly)^+$, 44], 587.7 [$(H\text{-}Ile\text{-}Leu\text{-}Gly\text{-}Val\text{-}Phe\text{-}Gly)^+$, 58], 575.6 [$(H\text{-}Leu\text{-}Gly\text{-}Val\text{-}Phe\text{-}Gly\text{-}Thr)^+$, 41], 561.7 [$(589.7–CO)^+$, 12], 559.7 [$(587.7–CO)^+$, 10], 547.6 [$(575.6–CO)^+$, 11], 532.6 [$(H\text{-}Phe\text{-}Gly\text{-}Thr\text{-}Ile\text{-}Leu)^+$, 66], 530.6 [$(H\text{-}Ile\text{-}Leu\text{-}Gly\text{-}Val\text{-}Phe)^+$, 73], 504.6 [$(532.6–CO)^+$, 14], 502.6 [$(530.6–CO)^+$, 12], 484.6 [$(H\text{-}Thr\text{-}Ile\text{-}Leu\text{-}Gly\text{-}Val)^+$, 70], 474.5 [$(H\text{-}Leu\text{-}Gly\text{-}Val\text{-}Phe\text{-}Gly)^+$, 62], 456.6 [$(484.6–CO)^+$, 11], 446.5 [$(474.5–CO)^+$, 17], 419.5 [$(H\text{-}Phe\text{-}Gly\text{-}Thr\text{-}Ile)^+$, 54], 417.5 [$(H\text{-}Leu\text{-}Gly\text{-}Val\text{-}Phe)^+$, 36], 391.5 [$(419.5–CO)^+$, 11], 389.5 [$(417.5–CO)^+$, 10], 385.4 [$(H\text{-}Thr\text{-}Ile\text{-}Leu\text{-}Gly)^+$, 49], 383.5 [$(H\text{-}Ile\text{-}Leu\text{-}Gly\text{-}Val)^+$, 43], 357.4 [$(385.4–CO)^+$, 10], 355.4 [$(383.5–CO)^+$, 14], 306.3 [$(H\text{-}Phe\text{-}Gly\text{-}Thr)^+$, 61], 284.3 [$(H\text{-}Ile\text{-}Leu\text{-}Gly)^+$, 52], 278.3 [$(306.3–CO)^+$, 11], 270.3 [$(H\text{-}Leu\text{-}Gly\text{-}Val)^+$, 32],

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242.3 [(270.3–CO)$^+$, 10], 205.2 [(H-Phe-Gly)$^+$, 28], 177.2 [(205.2–CO)$^+$, 15], 171.2 [(H-Leu-Gly)$^+$, 22], 148.2 [(H-Phe)$^+$, 19], 143.2 [(171.2–CO)$^+$, 11], 120.2 [Phe immonium ion ($C_8H_{10}N$)$^+$, 24], 114.2 [(H-Leu)$^+$, 11], 91.1 [(C_7H_7)$^+$, 15], 86.1 [Leu/Ile immonium ion ($C_5H_{12}N$)$^+$, 25], 77.1 [(C_6H_5)$^+$, 11], 74.1 [(Thr immonium ion C_3H_8NO)$^+$, 15], 72.1 [Val immonium ion ($C_4H_{10}N$)$^+$, 17], 57.1 [(C_4H_9)$^+$, 8], 45.1 [(C_2H_5O)$^+$, 11], 43.1 [(C_3H_7)$^+$, 11], 30.0 [(Gly immonium ion CH_4N)$^+$, 16], 29.1 [(C_2H_5)$^+$, 9], 15.0 [(CH_3)$^+$, 11].

Table 1 Physical characterization data for **1**—**7**

Compd.	Physical state	$[\alpha]_D^c$/(°)	R_f^f	Yield/%
1	Semisolid mass	+11.7	0.72	69
2	White solid[a]	–44.9[d]	0.86	77
3	Semisolid mass	–107.2	0.67	85
4	Semisolid mass	+82.9	0.58	80
5	Viscous mass	–51.6[d]	0.80	72
6	Semisolid mass	–56.7[d]	0.62	78
7	Pale yellow solid[b]	–78.4[e]	0.53[g]	84

[a] m.p. 87—88 ℃; [b] 93—95 ℃ (d); [c] *c*, 0.5 in MeOH; [d] *c*, 0.25 in MeOH; [e] *c*, 0.1 in MeOH; [f] ($CHCl_3$/MeOH, *V*∶*V*=9∶1); [g] ($CHCl_3$/MeOH , *V*∶*V*=7∶3).

Biological activity studies

Anthelmintic screening Newly synthesized linear heptapeptide and heptacyclopeptide **6** and **7** were subjected to anthelmintic activity studies against three earthworm species *Megascoplex konkanensis*, *Pontoscotex corethruses* and *Eudrilus eugeniea* at 2 mg/mL concentration using Garg's method.[22] Tween 80 (0.5%) in distilled water was used as control and mebendazole was used as standard drug. The results of anthelmintic screening are tabulated in Table 3.

Antibacterial and antifungal screening Antimicrobial activity studies for linear heptapeptide and heptacyclopeptide **6** and **7** were performed against Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, Gram-negative bacteria *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, dermatophytes *Microsporum audouinii*, *Trichophyton mentagrophytes*, diamorphic fungi *Candida albicans* and other fungal strains, including *Aspergillus niger* at 200–3.1 μg/mL concentration by using modified Kirby-Bauer disc diffusion method.[23] MIC values of test compounds were determined by tube dilution technique. The Petri plates inoculated with bacterial cultures were incubated at 37 ℃ for 18 h and those inoculated with fungal cultures were incubated at 37 ℃ for 48 h. Gatifloxacin and griseofulvin were used as reference drugs and DMF/DMSO were used as control. The results of antibacterial and antifungal studies are presented in Table 4 and Table 5. Experimental details of the biological activity studies are described in our previously published reports.[24,25]

Results and Discussion

An outline of the synthetic strategy for heptacyclopeptide **7** is illustrated in Scheme 1.

To prepare the target molecule, it was split into three dipeptide units *viz.* Boc-*L*-Thr-*L*-Ile-OMe (**1**), Boc-*L*-Leu-Gly-OMe (**2**), Boc-*L*-Val-*L*-Phe-OMe (**3**) and an amino acid unit Gly-OMe.HCl. The dipeptides were prepared by coupling Boc-amino acid *viz.* Boc-*L*-Thr-OH, Boc-*L*-Leu-OH, Boc-L-Val-OH with respective

Table 2 Elemental analysis data for **1**—**7**

Compd.	Mol. formula (M_r)	C (Calcd/found)/%	H (Calcd/found) /%	N (Calcd/found) /%
1	$C_{16}H_{30}N_2O_6$ (346)	55.47 (55.49)	8.73 (8.72)	8.09 (8.10)
2	$C_{14}H_{26}N_2O_5$ (302)	55.61 (55.58)	8.67 (8.65)	9.26 (9.25)
3	$C_{20}H_{30}N_2O_5$ (378)	63.47 (63.45)	7.99 (7.96)	7.40 (7.39)
4	$C_{24}H_{44}N_4O_8$ (516)	55.80 (55.79)	8.58 (8.60)	10.84 (10.85)
5	$C_{22}H_{33}N_3O_6$ (435)	60.67 (60.65)	7.64 (7.65)	9.65 (9.68)
6	$C_{40}H_{65}N_7O_{11}$ (819)	58.59 (58.57)	7.99 (8.01)	11.96 (11.95)
7	$C_{34}H_{53}N_7O_8$ (687)	59.37 (59.39)	7.77 (7.78)	14.25 (14.23)

Table 3 Anthelmintic activity data for **6** and **7**[a]

Compd.	Earthworm species		
	M. konk.	*P. core.*	*E. euge.*
	mpt (mdt)[b]	mpt (mdt)	mpt (mdt)
6	14.46±0.32[c] (23.43 ± 0.18)	19.26±0.22 (30.14±0.43)	14.33±0.40 (24.54±0.41)
7	09.18±0.13 (17.39±0.28)	13.44±0.40 (22.04±0.26)	11.28±0.19 (21.35±0.26)
Control[d]	—	—	—
Std[e]	13.65±0.42 (22.45±0.36)	17.57±0.47 (29.55±0.19)	13.51±0.43 (24.09±0.60)

[a] *M. konk.*: *Megascoplex konkanensis*, *P. core.*: *Pontoscotex corethruses*, *E. euge.*: *Eudrilus eugeniea*; [b] mean paralyzing time (mean death time) in minutes; [c] Data are given as mean±S.D. (*n*=3); [d] Tween 80 (0.5%) in distilled water; [e] Mebendazole.

Table 4 Antibacterial activity data for **6** and **7**[a]

Compd.	Diameter of zone of inhibition/mm Bacterial strains			
	B. sub.	*S. aur.*	*P. aeru.*	*K. pneu.*
6	—	13 (25)	11 (6.25)	15 (6.25)
7	—	17 (25)	16 (6.25)	18 (6.25)
Control[b]	—	—	—	—
Std[c]	18 (12.5)[d]	27 (6.25)	23 (6.25)	25 (6.25)

[a] *B. sub.*: *Bacillus subtilis*, *S. aur.*: *Staphylococcus aureus*, *P. aeru.*: *Pseudomonas aeruginosa*, *K. pneu.*: *Klebsiella pneumonia*; [b] DMF; [c] Gatifloxacin; [d] Values in bracket are MIC values (μg/mL).

Table 5 Antifungal activity data for **6** and **7**[a]

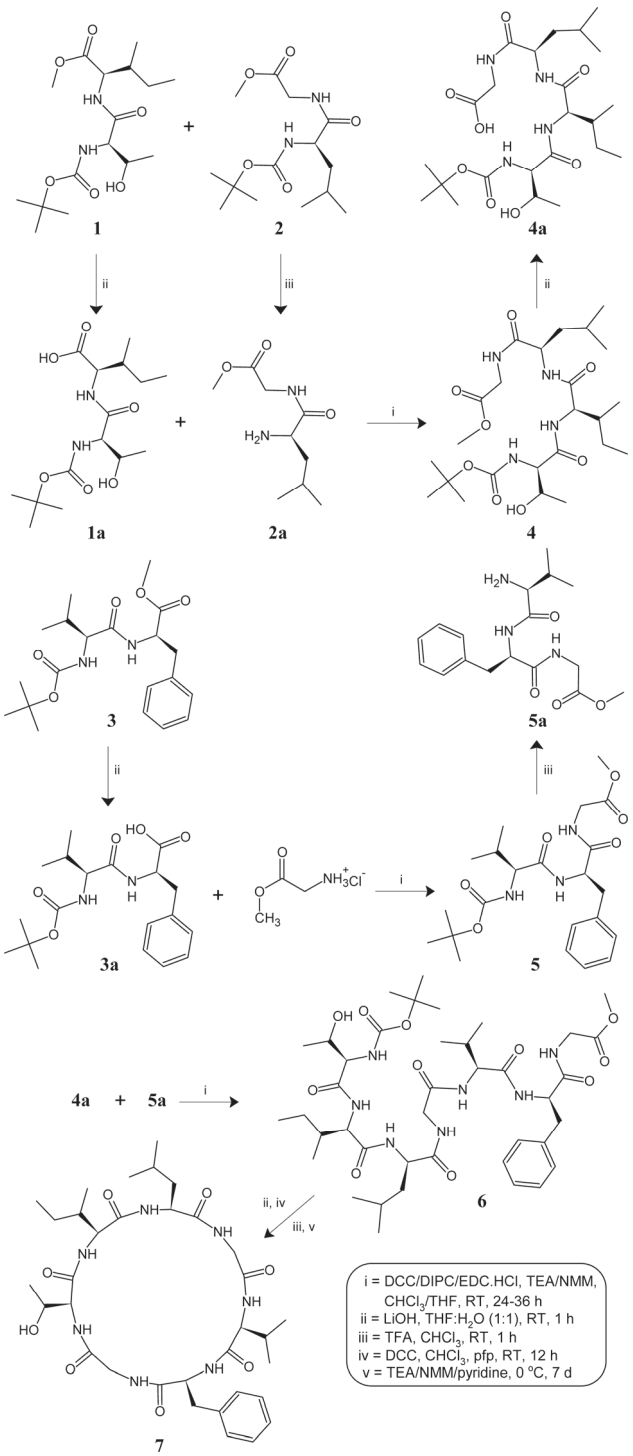
Compd.	Diameter of zone of inhibition/mm Fungal strains			
	C. alb.	*M. audo.*	*A. niger*	*T. menta.*
6	12 (6.25)	16 (6.25)	—	18 (6.25)
7	15 (6.25)	22 (6.25)	—	24 (6.25)
Control[b]	—	—	—	—
Std[c]	20 (6.25)[d]	18 (6.25)	20 (12.5)	19 (6.25)

[a] *C. alb.*: *Candida albicans*, *M. audo.*: *Microsporum audouinii*, *A. niger*: *Aspergillus niger*, *T. menta.*: *Trichophyon mentagrophytes*; [b] DMSO; [c] Griseofulvin; [d] Values in bracket are MIC values (μg/mL).

amino acid methyl ester hydrochlorides like *L*-Ile-OMe•HCl, Gly-OMe•HCl and *L*-Phe-OMe•HCl using dicyclohexylcarbodiimide (DCC)/*N,N'*-diisopropylcarbodiimide (DIPC)/1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC•HCl) as coupling agents and TEA/NMM as base.[26] The ester group of dipeptide unit **1** was removed using LiOH and deprotected peptide was coupled with another dipeptide unit **2** deprotected at amino terminal using trifluoroacetic acid (TFA), using DIPC as coupling agent and NMM as base, to get the linear tetrapeptide unit Boc-*L*-Thr-*L*-Ile-*L*-Leu-Gly-OMe (**4**).

Similarily, the ester group of dipeptide unit **3** was removed using LiOH and deprotected peptide was coupled with Gly-OMe•HCl using EDC•HCl as coupling agent and TEA as base, to get the tripeptide unit Boc-*L*-Val-*L*-Phe-Gly-OMe (**5**). Now, tetrapeptide unit **4** deprotected at carboxyl end was coupled with tripeptide unit **5** deprotected at amino terminal, using DIPC as coupling agent and NMM as base, to get linear heptapeptide unit Boc-*L*-Thr-*L*-Ile-*L*-Leu-Gly-*L*-Val-*L*-Phe-Gly-OMe (**6**). Finally, cyclization of the linear heptapeptide **6** was done by pentafluorophenyl ester method which involves replacement of methyl ester with pentafluorophenyl ester which is a better leaving group, through deprotection at carboxyl terminal using LiOH and coupling with pentafluorophenol using DCC, followed by removal of Boc-group using TFA and keeping the deprotected unit for 7 d at 0 ℃ in the presence of base (NMM/TEA/pyridine) to obtain the heptacyclopeptide *cyclo*-(*L*-threonyl-*L*-isoleucyl-*L*-leucyl-glycyl-*L*-valyl-*L*-phenylalanyl-glycyl) (**7**). Further, in order to describe the intermolecular forces of drug receptor interaction as well as transport and distribution of drugs in a quantitative manner, various steric and lipophilicity parameters are needed to be calculated for the synthesized linear and cycloheptapeptide.

Scheme 1 Synthetic route for heptacyclopeptide **7**



Disappearance of absorption bands at 1741 and 1269 cm^{-1} and 1389, 1366 cm^{-1} ($C{=}O_{str}$ and $C{-}O$ str, me-

thyl ester group and C—H bend, *tert*-butyl group) in FT-IR spectrum of **7** clearly indicated cyclization of linear heptapeptide unit. This fact was further supported by disappearance of two singlets at δ 1.59 and 3.63, corresponding to protons of *tert*-butyl and methyl ester groups, in ^{1}H NMR spectrum and disappearance of singlets at δ 79.7, 29.4 and 51.9, corresponding to carbon atoms of *tert*-butyl and methyl ester groups, in ^{13}C NMR spectrum of **7**. Seven signals between δ 6.54—5.23 in the proton spectrum of **7** suggested a peptidic structure for the synthesized product, with these signals being attributable to the α-protons of all seven amino acid units and values being in a slightly higher range when compared to natural mahafacyclin A.[15] The ^{1}H NMR spectrum of cyclized product showed the presence of seven broad singlets between δ 9.29—7.58, 7.09—7.02 corresponding to the imino protons of the isoleucine, threonine, valine, leucine, phenylalanine and two glycine moieties and ^{13}C NMR spectrum of cyclopeptide 7 indicated the presence of seven signals between δ 174.9—162.8 due to carbonyl carbons of seven amino acids, which were in agreement with δ values for imino protons and carbonyl carbons of natural mahafacyclin A,[15] indicating similarity of the structure of the newly synthesized heptacyclopeptide with that of the natural molecule. Moreover, ^{1}H/^{13}C NMR spectra of the cyclized product **7** showed characteristic peaks confirming the presence of all the 53 protons and 34 carbon atoms. Presence of pseudomolecular ion peak at *m/z* 688.8 corresponding to the molecular formula $C_{34}H_{54}N_7O_8$ in mass spectra of **7**, along with other fragment ion peaks resulting from cleavage at 'Leu-Ile', 'Phe-Val', 'Thr-Gly' and 'Ile-Thr' amide bond levels, showed the exact sequence of attachment of all the seven amino acid units in a chain and was in agreement with protonated ion peak of natural mahafacyclin A.[15] In addition, elemental analysis data of **7** afforded values ($\pm$0.02) strictly in accordance to the molecular composition. Anthelmintic activity studies revealed that the synthesized heptacyclopeptide **7** possessed potent anthelmintic activity at 2 mg/mL concentration in tween 80 (0.5%) and distilled water. From the comparison of anthelmintic activity data, it is observed that cyclopeptide **7** displayed more activity than its corresponding linear precursor **6** against all three earthworm species *M. konkanensis*, *P. corethruses* and *E. eugeniea*, in comparison to standard drug mebendazole (Table 3). Moreover, heptacyclopeptide **7** displayed remarkable bioactivity against dermatophytes *M. audouinii* and *T. mentagrophytes* with MIC values of 6 μg/mL. Analysis of antimicrobial activity data indicated that cyclic heptapeptide **7** displayed moderate level of bioactivity against pathogenic *Candida albicans* and Gram-negative bacteria, in comparison to standard drugs griseofulvin and gatifloxacin. However, no significant antimicrobial activity was observed against Gram-positive bacteria and *Aspergillus niger* (Table 4).

Conclusions

The first total synthesis of naturally occurring heptacyclopeptide mahafacyclin A (**7**) was carried out successfully in a good yield from the linear precursor via coupling reactions utilizing carbodiimide chemistry. DIPC was found to be a good coupling agent in comparison to DCC, EDC•HCl and pentafluorophenyl ester proved to be better for the activation of acid functionality of linear heptapeptide unit. Moreover, NMM proved to be a good base for intramolecular cyclization of linear heptapeptide fragment in comparison to pyridine and TEA. The synthesized heptacyclopeptide **7** displayed potent anthelmintic activity against *M. konkanensis*, *P. corethruses* and *E. eugeniea*, alongwith remarkable antifungal activity against dermatophytes *M. audouinii* and *T. mentagrophytes*, in comparison to reference drugs mebendazole and griseofulvin. The newly synthesized cyclic peptide **7** displayed better activity than its linear counterpart **6** due to the conformational rigidity of cyclic form. The rigidity of cyclic form decreases the entropy term of the Gibbs free energy and therefore, allows the enhanced binding toward target molecules or receptor selectivity. Further studies are needed to develop SAR of natural/synthetic cycloheptapeptide by replacing phenylalanine unit of mahafacyclin A with other units like *N*-methylphenylalanine, tyrosine, histidine, tryptophan units and indicate effect of modification on biological activities. On passing toxicity tests, newly synthesized heptacyclopeptide **7** may prove a good candidate for clinical studies and can be a new anthelmintic and antidermatophyte drug of future.

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