International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.1, pp 194-200, Jan-Mar 2012

Synthesis and Biological screening of Proline rich Cyclic Heptapeptide

Sachin Chaudhary[1,2*], Hitesh Kumar[2], Harishchandra Verma[2], Ashok Rajpoot[2]

[1]College of Pharmacy, IFTM, Lodhipur Rajput, Moradabad, U.P.244001.

[2]Moradabad Educational Trust, Faculty of Pharmacy, MIT Campus, Ram Ganga Vihar, Phase-II, Moradabad, U.P.-244001,India.

Corres.author: sachin.nehra84@gmail.com
Tel.: +919412825487

Abstract: In continuation of search of potent cyclic peptide, a new potent bioactive, proline-rich cyclic heptapeptide (**13**) was synthesized using the solution phase technique by cyclization of the linear peptide Boc-Pro-Phl-Tyr-Tryp-Phl-Pro-Tryp-OMe (**12**) after proper deprotection at carboxyl and amino terminals. Linear peptide segment will be prepared by coupling the tripeptide unit Boc-Pro-Phl-Tyr-OH (**10a**) with tetrapeptide unit Tryp-Phl-Pro-Tryp-OMe (**11a**) using dicyclohexyl carbodimide as the coupling agent and N-methyl morpholine as the base. Structures of all the new compounds were characterized by IR, ^{1}HNMR spectral data as well as elemental analysis using chemsketch software. The newly synthesized cyclopeptide was screened for its anthelmintic activity against pathogenic earthworm species. Compound **13** showed anthelmintic activity against earthworms *Megascoplex konkanesis* and *Eudrilus* species in comparison to Albendazole.

Keywords: Cyclic heptapeptide, antibacterial, antifungal, anthelmintic.

Introduction

Recently, cyclopolypeptides and related congeners have emerged as potent organic compounds due to their unique structures and wide pharmacological profile [1-3] which may prove better candidates to overcome the problem of wide spread increase of resistance towards conventional drugs. Cyclic peptides possess a wide range of pharmacological activities including cytotoxic activity, antimalarial activity, immunosuppressive activity, vasorelaxant activity and angiotension converting enzyme and tyrosinase inhibitory activity [4]. As part of ongoing efforts on synthetic aspects of bioactive cyclic peptides [5], the present investigation was aimed at the synthesis of novel proline rich cyclic heptapeptide, keeping in view significant bioactivities possessed by various cyclopeptides, the above synthetic peptide was further subjected to anthelmintic studies.

Experimental

Materials and equipment: All reactions requiring anhydrous conditions were conducted in a flame dried apparatus. Melting points were determined by the open capillary method and were uncorrected. L-Amino acids, di-*tert*-butylpyrocarbonate (Boc_2O), dicyclohexylcarbodiimide (DCC), trifluoroacetic acid (TFA), *p*-nitrophenol (pnp) and N-metylmorpholine (NMM) were obtained from Central drug house, New Delhi, India. IR spectra were recorded on a Shimadzu

8700 FTIR spectrophotometer(Shimadzu, Japan) using a thin film supported on KBr pellets for the synthesized cyclic heptapeptide and $CHCl_3$ as solvent for intermediate semisolids.^{1}H NMR were recorded on a Bruker AC NMR spectrometer (300MHz), (Brucker, USA) using tetramethylsilane (TMS) as internal standard. Purity of all compounds was checked by TLC on precoated silica gel G plates (Kieselgel 0.25 mm, 60G F254, Merck, Germany). Chloroform/methanol (9:1, *V/V*) was used as the developing solvent system and dark brown spots were detected on exposure to iodine vapours in a tightly closed chamber.

Synthesis of Boc-amino acids (1–3): L-Proline (2.31gm, 0.02mol) was dissolved in 1mol L^{-1} NaOH (20 ml) and *i*-propanol (20 ml). Boc_2O (6 ml) in *i*-propanol (10 ml) was added followed by 1mol L^{-1} NaOH (20 ml) to the resulting solution. The solution was stirred at room temperature (r.t.) for 2h and washed with light petroleum ether (b.p. 40–60^{0}C) (20 ml), acidified to pH 3.0 with 1 mol L^{-1} H_2SO_4 and finally extracted with chloroform (3x20 ml). The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure to give the crude product, which was crystallized from chloroform and petroleum ether (b.p.40–60^{0}C) to get pure Boc-Proline (**1**). Similarly Boc-Tryptophan (**2**) and Boc-Proline (**3**) were prepared by stirring Boc_2O (6 mL, 0.026 mol) with L-Tryptopahn (2.38 g, 0.02mol) and L-Proline (2.31 g, 0.02 mol), respectively.

Synthesis of L-amino acid methyl ester hydrochlorides (4–6): Thionyl chloride (1.4 ml, 0.02mol) was slowly added to methanol (100 ml) at 0 ^{0}C and L-phenylalanine (2.34gm, 0.02mol) was added to the above solution. The resulting mixture was refluxed for 8h at ambient temperature. Methanol was evaporated and the residue was triturated with ether at 0 ^{0}C until excess dimethyl sulphite was removed. The crude solid was crystallized from methanol and ether at 0 ^{0}C to get L-Phenylalanine methyl ester hydrochloride (**4**). Similarly, L-tryptophan methyl ester hydrochloride (**5**) and L-tyrosine methyl ester hydrochloride (**6**) were prepared by refluxing L-tryptophan (3.3 g, 0.02 mol)/L-tyrosine (2.38 g, 0.02 mol) with methanol (100 mL) in the presence of thionyl chloride (1.4 mL, 0.02 mol).

Synthesis of Boc-dipeptide methyl esters (7–9): A mixture of compound **4** (L-Phenylalanine methyl ester hydrochloride 1.67gm, 0.01mol) in $CHCl_3$ (20 ml), NMM (2.3 ml, 0.021mol) was added at 0 ^{0}C. The reaction mixture was stirred for 15 min. Compound **1** (Boc-Proline 3.1gm, 0.01mol) in $CHCl_3$ (20 ml) and DCC (2.1gm, 0.01mol) was added under stirring to the above mixture. After 36h the reaction mixture was filtered and the residue was washed with $CHCI_3$ (30 ml) and added to the filtrate. The filtrate was washed with 5% $NaHCO_3$ and saturated NaCl solution (25 ml each). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum. The crude product was crystallized from a mixture of chloroform and petroleum ether (b.p. 40–60 ^{0}C), followed by cooling at 0 ^{0}C to get Boc-Pro-Phl-OMe (**7**). Similarly, Boc-Tryp-Phl-OMe (**8**) and Boc-Pro-Tryp-OMe (**9**) were prepared by stirring compounds **2** and **3** with amino acid methyl ester hydrochlorides **4** and **5** respectively, in the presence of DCC and NMM.

Deprotection of dipeptides at carboxyl end (7a, 8a): To a solution of compound **7** (3.76 g, 0.01 mol) in THF/H_2O (1:1, 36 mL), LiOH (0.36 g, 0.015 mol) was added at 0 ^{0}C. The mixture was stirred at r.t. for 1 h and then acidified to pH 3.5 with 0.5 mol L^{-1} H_2SO_4. The aqueous layer was extracted with Et_2O (3 x 25 mL).Combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was crystallized from methanol and ether to get Boc-Pro-Phl-OH (**7a**). Similarly, compound **8** was hydrolyzed under alkaline conditions to obtain Boc-Pro-Phl-OH (**8a**).

Deprotection of dipeptide at amino end (9a): Compound **9** (4.42 g, 0.01 mol) was dissolved in $CHCl_3$ (15 mL) and treated with trifluoroacetic acid (2.28 g, 0.02 mol). The resulting solution was stirred at r.t. for 1 h and washed with saturated $NaHCO_3$ solution (25 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by crystallization from $CHCl_3$ and petroleum ether (b.p. 40–60 ^{0}C) to give pure Pro-Tryp-OMe (**9a**).

Synthesis of Boc-tri/tetrapeptide methyl esters (10, 11): To synthesize Boc-Pro-Phl-Tyr-OMe (10), dipeptide unit 7a (3.62 g, 0.01 mol) was coupled with amino acid methyl ester hydrochloride 6 (1.7 g, 0.01 mol) in the presenceof DCC and NMM the following the same procedure as adopted for the synthesis of Boc-dipeptide methyl esters 7–9. Similarly, Boc-Tryp-Phl-Pro-Tryp-OMe (11) was prepared by coupling deprotected dipeptide units 8a (3.16 g, 0.01 mol) and 9a (3.42 g, 0.01 mol) using DCC as the coupling agent and NMM as the base.

Synthesis of Boc-heptapeptide methyl ester (12): To synthesize Boc-Pro-Phl-Tyr-Tryp-Phl-Pro-Tryp-OMe (**12**), tripeptide unit 10 (4.77 g, 0.01 mol)

was deprotected at carboxyl end to get Boc-Pro-Phl-Tyr-OH (**10a**) following the same procedure as adopted for the synthesis of compounds **7a** and **8a** from compounds**7** and **8**, respectively. Tetrapeptide unit **11** (6.4 g, 0.01 mol) was deprotected at amino end to get Tryp-Phl-Pro-Tryp-OMe (**11a**) following the same procedure as adopted for the synthesis of compound **9a** from compound **9**. The deprotected tripeptide unit **10a**(4.63 g, 0.01 mol) and tetrapeptide unit **11a** (5.4 g, 0.01 mol) were coupled in the presenceof DCC and NMM to get linear heptapeptide unit **12** under same the experimental conditionsas adopted for the synthesis of Boc-dipeptide methyl esters **7**–**9**.

Synthesis of cyclic heptapeptide (13): To synthesize Cyclo(Pro-Phl-Tyr-Tryp-Phl-Pro-Tryp) (**13**), linear heptapeptide unit **12** (4.93 g, 0.005 mol) was deprotected at carboxyl end using LiOH (0.18 g) toget Boc-Pro-Phl-Tyr-Tryp-Phl-Pro-Tryp-OH (**12a**) following the same procedure as adopted for the synthesis of compounds **7a** and **8a** from compounds **7** and **8** respectively. The deprotected heptapeptide unit **12a** (4.86 g, 0.005 mol) was dissolved in $CHCl_3$ (50 mL) at 0 ^{0}C. To the above solution, pnp (0.94 g, 0.0067 mol) was added and stirred at r.t. for 12h. The reaction mixture was filtered and the filtrate washed with 10% $NaHCO_3$ solution until excess of pnp was removed and finally washed with 5% HCI to get the corresponding p-nitrophenyl ester Boc-Pro-Phl-Tyr-Tryp-Phl-Pro-Tryp-O-pnp (**12b**).

To compound **12b (**4.37 g, 0.004 mol)dissolved in $CHCl_3$ (35 ml), TFA (0.91 g) was added, stirred at r.t. for 1h and washed with 10% $NaHCO_3$ solution (2 x 25ml). The organic layer was dried over anhydrous Na_2SO_4 to get Pro-Phl-Tyr-Tryp-Phl-Pro-Tryp-O-pnp (**12c**), which was dissolved in $CHCl_3$ (25 mL) and NMM (2.3 ml) was added. Then, all contents were kept at 0 ^{0}C for 7 days. The reaction mixture was washed with 10% $NaHCO_3$ solution until the by-product *p*-nitrophenol was removed completely and finally washed with 5% HCl (15ml). The organic layer was dried over anhydrous Na_2SO_4. Finally, chloroform was distilled off and the crude cyclized product was crystallized from $CHCI_3$ and hexane to get the pure Cyclo(Pro-Phl-Tyr-Tryp-Phl-Pro-Tryp) (**13**).The spectral data and physicochemical data for compound **1-13** are given in Tables 1 and 2.

Table 1. Spectral data of compounds 1–13

Compd No.	IR ($CHCl_3$, cm^{-1}), ^{1}HNMR ($CDCl_3$, δ ppm).
1	3580 **(**OH str), 1217 (C-O str, COOH), 1739 (C=O str), 2970 (CH str), 1163(C-C str), 1209 (C-N str).
	4.01 (s, C**H**-COOH), 1.80-1.82 (d, C**H$_2$**), 1.74-1.76 (d, C**H$_2$**), 3.43-3.33 (d, C**H$_2$**); 10.80 (s, O**H**); 1.37 (3H, C**H$_3$**).
2	2960 (CH ring str) (2960); 1650 (C=C ring str), 870(C=C ring bend), 1160(C-C str), 2950 (CH str, CH_3) 3350 (NH str), 1750 (C=O str), 3300(OH str), 1250(C-O str, COOH).
	4.15 (s, C**H**-COOH); 3.01-2.98 (C**H$_2$**); 10.80 (N**H**); 6.88 (=C**H**-NH); 11.05 (O**H**); 1.32 (C**H$_3$**); 7.13 (C**H**).
3	3580 **(**OH str), 1217 (C-O str, COOH), 1739 (C=O str), 2970 (CH str), 1163(C-C str), 1209 (C-N str).
	4.01 (s, C**H**-COOH), 1.80-1.82 (d, C**H$_2$**), 1.74-1.76 (d, C**H$_2$**), 3.43-3.33 (d, C**H$_2$**); 10.80 (s, O**H**); 1.37 (3H, C**H$_3$**).
4	2820, (NH str), 3100 (Ar-H str), 1470 (C=C Ring str), 2970 (CH str, methyl), 1747 (C=O str), 1250, (C-O str), 1180 (CN str).
	7.09 (s, m, Ar-**H**); 7.11 (s, p, Ar-**H**); 7.16 (s, o, Ar-**H**); 4.05 (s, N**H**); 3.49 (s, OC**H$_3$**)**.**
5	3050 (C-H Ring str), 1670(C=C Ring str), 3300(NH str), 1640(C=O str), 1240, (C-O str), 2960 (C-H methyl str).
	2.40 (s, N**H$_2$**); 3.62 (s, C**H**); 3.03-3.30 (d, C**H$_2$**); 6.96 (s, C**H**); 11.09 (s, N**H**); 7.23 (s, C**H**).
6	2950 (N-H str), 3050 (C-H Ring str), 1650 (C=C Ring str), 2970(C-H str methyl, 1780 (C=O str), 1240 (C-O str), 3200 (O-H str).
	2.48 (s, N**H$_2$**); 4.11 (s, O**H**)**;** 2.48 (s, C**H$_3$**); 6.72 (s, C**H**).
7	8.11 (s, N-**H**); 6.68-6.88 (m, Ar-**H**); 2.24- 4.23 (m, C-**H**); 1.10-1.78 (d, C**H$_2$**)**.**

7a	1650, (C=O str), 3480, (OH str), 1475 (C=C Aromatic str), 3000 (C-H Aromatic str), 2850 (C-H str methyl), 1250 (C-O stretching), 3600(N-H str).
	10.55 (s, O**H**); 6.68-6.88 (m, Ar-**H**); 2.24- 4.23 (m, C-**H**); 1.10-1.78 (d, C**H$_2$**).
8	8.59 (s, N-**H**); 11.12 (s, O-**H**); 5.39-5.41(d, C**H$_2$**); 7.81 (s, N-**H**); 6.52- 7.36 (m, C-**H**); 10.59 (s, N-**H**).
8a	1650 (C=O str), 3480 (O-H str), 1475 (C=C Aromatic str), 3000 (C-H Aromatic str), 2850 (CH str methyl), 1250 (C-O str), 3600 (NH str).
	8.59 (s, N-**H**); 11.12 (s, O-**H**); 5.39-5.41(d, C**H$_2$**); 7.81 (s, N-**H**); 6.52- 7.36 (m, C-**H**); 10.59 (s, N-**H**).
10	8.29 (s, N-**H**); 10.76 (s, O-**H**); 1.36 (s, C**H$_3$**); 9.19 (s, N-**H**), 5.60 (s, O-**H**); 6.58-7.72 (m, C-**H**); 1.70-4.55 (m, C**H$_2$**).
10a	1630 (C=C Aromatic str), 3050 (C-H Aromatic str), 1240 (C-O stre), 3300 (OH str), 2900 (NH str), 2850 (C-H str methyl), 1350 (C-N str).
	8.29 (s, N-**H**); 10.76 (s, O-**H**); 1.36 (s, C**H$_3$**); 9.19 (s, N-**H**), 5.60 (s, O-**H**); 6.58-7.72 (m, C-**H**); 1.70-4.55 (m, C**H$_2$**).
11a	10.85 (s, N-**H**); 6.63-7.51 (m, C-**H**); 1.05-2.85(m, C**H$_2$**); 8.29 (s, N-**H**); 3.42(s, OC**H$_3$**); 5.71(s, N-**H**).
12a	1625 (C=C aromatic str), 3050 (CH Aromatic str), 3300 (NH str), 1750 (C=O str), 3450 (OH str), 2850 (C-H methyl str), 1180 (C-N str), 1260 (C-O str).
	5.71(s, N-**H**), 6.60-7.58 (m, C-**H**); 1.34-3.67 (m, C-**H$_2$**).
13	1625 (C=C aromatic str), 3050 (CH Aromatic str), 3300 (NH str), 1750 (C=O str), 3450 (OH str), 2850 (C-H methyl str), 1180 (C-N str), 1260 (C-O str).
	10.64(s, N-**H**); 6.89 (s, O-**H**); 3.11(s, N-**H**); 7.46-7.49(m, C-**H**); 0.76-2.24(m, C**H$_2$**).

Table 2: Physical and analytical data of compound 1-13

Compound No.	Physical state	M.p. (^{0}C)	Yield (%)	Molecular formula (Mf)	Elemental analysis			
					C	H	N	O
1	White crystals	133-134^{0}C	83%	$C_{10}H_{17}NO_4$	55.8	7.96	6.51	29.73
2	White crystals	130-132 ^{0}C	72%	$C_{16}H_{20}N_2O_4$	63.14	6.62	9.2	21.03
3	White crystals	133-134^{0}C	83%	$C_{10}H_{17}NO_4$	55.8	7.96	6.51	29.73
4	White crystals	160-162 ^{0}C	65%	$C_{10}H_{14}ClNO_2$	55.69	6.54	6.49	14.84
5	White crystals	180-184 ^{0}C	85%	$C_{12}H_{15}ClNO_2$	56.58	5.94	11	12.56
6	White crystals	190-192 ^{0}C	84%	$C_{11}H_{16}NO_3$	51.84	6.09	6.05	20.72
7	Semisolid mass	-	65%	$C_{20}H_{28}N_2O_5$	62.05	6.94	8.04	22.96
8	Semisolid mass	-	75%	$C_{27}H_{31}N_3O_5$	67.48	7.13	8.74	16.65
9	Semisolid mass	-	78%	$C_{22}H_{29}N_3O_5$	64.02	7.71	9.74	18.54
7a	White solid	145-147^{0}C	71%	$C_{19}H_{26}N_2O_5$	62.97	7.23	7.73	22.07
8a	White solid	189-190^{0}C	72%	$C_{25}H_{30}N_3O_5$	66.93	6.91	9.01	17.15
9a	Semisolid mass	-	68%	$C_{17}H_{21}N_3O_3$	65.23	7.6	12.68	14.48
10	Semisolid mass	-	72%	$C_{29}H_{37}N_3O_7$	64.96	7.27	7.58	20.19
11	Semisolid mass	-	72%	$C_{42}H_{50}N_6O_7$	67.76	7.11	10.78	14.36
10a	White crystals	111-112^0 C	70%	$C_{28}H_{35}N_3O_7$	64.43	7.08	7.77	20.72
11a	Semisolid mass	-	65%	$C_{37}H_{39}O_5N_6$	68.9	6.97	12.36	11.77
12	Semisolid mass	-	70%	$C_{65}H_{73}N_9O_{11}$	67.92	6.96	10.48	14.64
13	Light brown solid	165-166^{0}C	70%	$C_{59}H_{60}N_9O_8$	69.07	6.67	12.07	12.26

Earthworms: The newly synthesized cyclic heptapeptide **13** was for anthelmintic activity against earthworm, *Eudrilus sp.* (ICARBC 042) and *Megascoplex konkanensis* (ICARBC 211). The earthworms were obtained from pharma garden of institute.

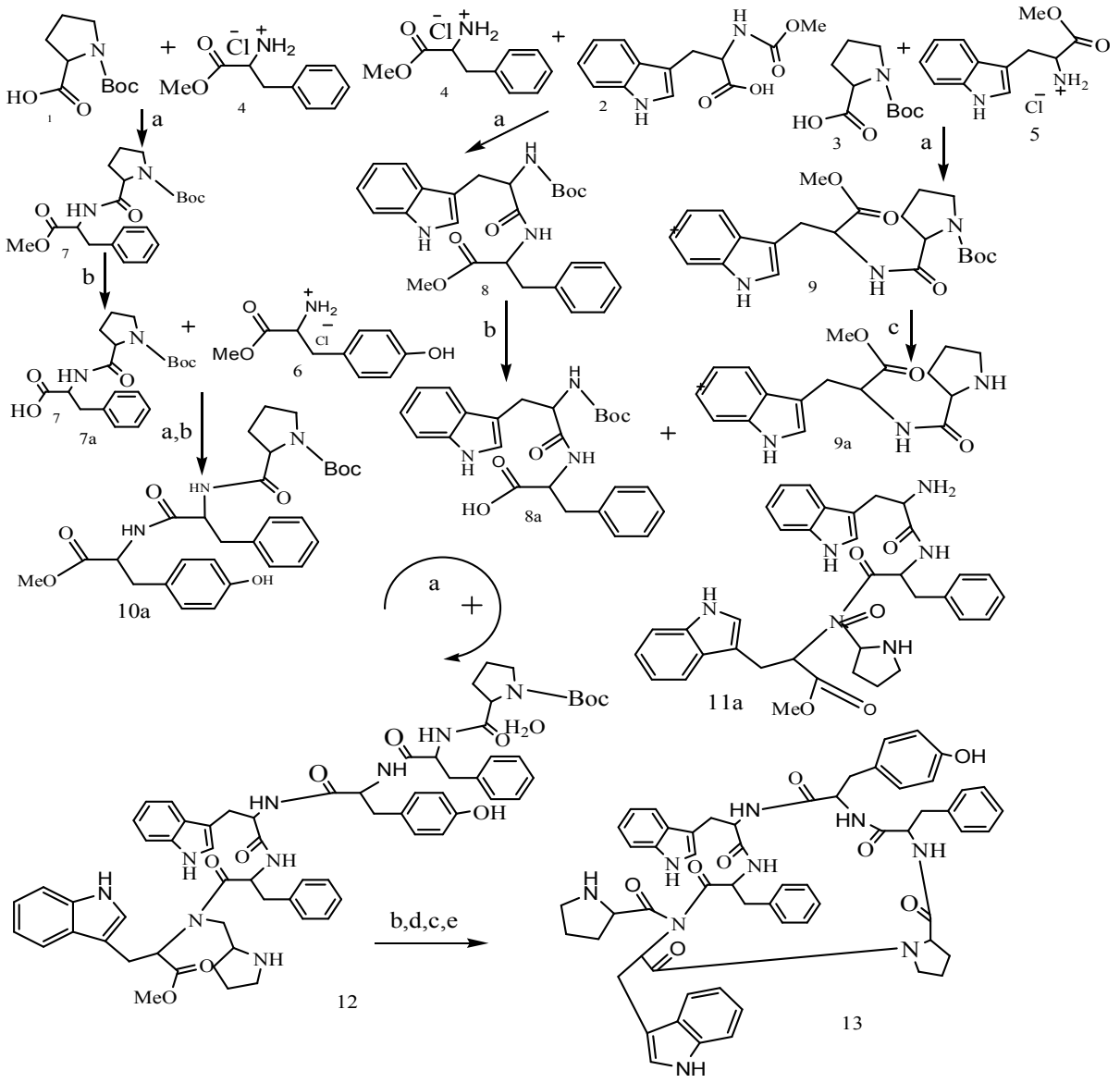
Anthelmintic activity: An anthelmintic activity study was perfomed for compound **13** and against earthworms according to Garg's method (6). Suspension of the samples were prepared by triturating 200 mg of synthesized compound **13** with 20 mL of Tween 80 (0.5%) and 20 mL of distilled water and the resulting mixture was stirred using a mechanical stirrer for 30 min. The suspension was diluted to contain 2 mg mL^{-1} of the test sample. Suspensions of reference drugs Albendazole was prepared in a similar way by triturating 100 mg of drug with 10 mL of Tween 80 (0.5%) and 10 mL of distilled water separately and finally diluted to contain 2 mg mL^{-1} of Albendazole. Two sets of five earthworms of similar sizes (5.1 cm in length) were placed in Petri plates of 10.2 cm diameter containing 50 mL of the suspension of the test sample and reference drugs (100 mg of the substance applied) at r.t. Another set of five earthworms was kept as control in a solution of 50 mL of Tween 80 (0.25%). The paralyzing and death times were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earth worms in warm water (50 °C), which stimulated movement if the worm was alive. Results of the anthelmintic activity of the test compound and reference drugs are listed in Table III.

Results and Discussion

Chemistry: The solution-phase technique was selected for peptide synthesis because it is simple and economic compared to solid phase peptide synthesis, which involves complicated chemistry utilizing costly linker resins (7, 8). In the present work, pnp and a novel base, NMM, are used for esterification and cyclization during the synthesis of cyclopeptide **13** from the linear peptide unit **12**, affording compound **13** in 70 % yield. There are previous literature reports on the synthesis of cyclic peptides by Dahiya et al (5), who utilized pnp and pyridine for esterification and cyclization to get cyclopeptides in 72% yields. Pepide units were prepared by the Bodanszky method with certain modifications (11). Boc_2O was used to protect the amino group of L-amino acids. The carboxyl group of L-amino acids was protected by esterification with methanol utilizing $SOCl_2$. Furthermore, TFA was used to remove the Boc group and the ester group was removed by alkaline hydrolysis with lithium hydroxide.

Table 3. Anthelmintic activity of compound 13

Compd.	Eudrilus species		Megascoplex konkanensis	
	Mean paralyzing time ± SEM (min)	Mean death time ± SEM (min)	Mean paralyzing time ± SEM (min)	Mean death time ± SEM (min)
0.5 % Tween 80 In distiilled water	-	-	-	-
Albendazole	9.35 ± 0.12	21.15 ± 0.17	10.56±0.93	12.73±0.84
13	12.25 ± 0.23	21.96 ± 0.07	11.24 ± 0.71	12.87 ± 0.39



Scheme: 1

a = DCC, NMM, CHCl , r.t., 36 h; b = LiOH, THF/H O (1:1), r.t., 1 h
c = TFA, $CHCl_3$, r.t., 1 h; d = pnp, $CHCl_3$, r.t., 12 h
e = NMM, $CHCl_3$, 7 days, 0 ^{0} C

Biological activity: Comparison of anthelmintic data indicated that compound **13** exhibited higher anthelmintic activity against earthworms, *Eudrilus* species and *Megascoplex konkanensis*, in comparison to reference drug albendazole at 2 mg mL^{-1}. The results of anthelmintic activity are given in Table 3.

Conclusion

The solution phase technique employing catalytic amounts of the NMM base for cyclization and DCC as coupling agent provided yields effective for cyclopeptide synthesis. The antibacterial activity of the compound might be due to the presence of two proline moieties and anthelmintic activity may be attributed to the tryptophan and threonine moieties in the cyclic peptide.

Abbreviations and acronyms – Boc – *tert*-butyloxycarbonyl, Boc_2O – di-*tert* butylpyrocarbonate, DCC – dicyclohexylcarbodiimide, NMM – *N*-methylmorpholine, Phe – phenylalanine, pnp – *p*-nitrophenol, Pro – proline, TFA – trifluoroacetic acid, Thr – threonine, Tyrp – tryptophan.

Acknowledgements- The author is thankful to Prof. A.K. Wahi, College of pharmacy I.F.T.M, Moradabad for his kind support and guidance.

References

1. F. Sarabia, S. Chammaa, A. S. Ruiz, L. M. Ortiz and F. J. Herrera, Chemistry and biology of cyclic depsipeptides of medicinal and biological interest. Curr. Med. Chem., 2004, 11, 1309-1332.
2. M. M. Joullie, and D. J. Richard, Cyclopeptide alkaloids: chemistry and biology. Chem. Commun. (Camb), 2004, 21, 2011-2015.
3. Craik, D. J., Daly, N. L., Mulvenna, J., Plan, M. R., and Trabi M, Discovery, structure and biological activities of the cyclotides. Curr. Protein Pept. Sci., 2004, **5**, 297-315.
4. R. Dahiya., Synthesis and *in vitro* Cytotoxic Activity of a Natural Peptide of Plant Origin. J. Iran. Chem. Soc., 2008, 5(3), 445-452.
5. R. Dahiya., Synthesis of a Phenylalanine-Rich Peptide as Potential Anthelmintic and Cytotoxic Agent. Acta Poloniae Pharmaceutica, 2007, 64(6), 509-516.
6. W. Gu and R. B. Silverman, Solid-phase total synthesis of scytalidamide A, 2003, 68, 8774–8779.
7. L. Bourel-Bonnet, K. V. Rao, M. T. Hamann and A. Ganesan, Solid-phase total synthesis of kahalalide A and related analogues, 2005, 48, 1330–1335.
8. M. Bodanszky and A. Bodanszky, The Practice of Peptide Synthesis, Springer-Verlag, New York 1984, 78–143.

*****