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Synthesis of Novel Cyclopeptide Candidates: I-Cyclo-[N^{α}-isophthaloyl-*bis*-(Glycine-Amino Acid)-L-Lysine] Derivatives with Expected Anticancer Activity

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THE SEARCH for potent cytotoxic agents, namely anticancers, presents and updated area of the organo-biochemical literature.

Herein, N^{α}-isophthaloyl bridged cyclo-pentapeptides, having the structure: Cyclo-[N^{α}- isophthaloyl-bis - (Glycine - Amino Acid)-L-Lys] -Y, 11 - 19**,** whereas, "Amino Acid" stands for "Glycine" or "L-Phenylalanine" or "Sarcosine" and Y represents: methyl ester or carboxylic or hydrazide group were, newly, synthesized.

Synthetically, hydrolysis of the starting linear tetra peptide bis-esters 5, 6 and 7 afforded the corresponding free acids, 8, 9 and 10, respectively, whilst upon their cyclization with L-lysine methyl ester, the cyclopeptide esters 11, 12 and 13 were, respectively, obtained. Hydrolysis or hdyrazinolysis of these cyclopeptide esters resulted in the cyclopeptide acids 14, 15 and 16 or hydrazides 17, 18 and 19.

Thus, Cyclo-[N^{α}-isophthaloyl-bis-(Gly-Gly)-L–Lys]-OMe, 11, Cyclo-[N^{α}-iso-phthaloyl-bis-(Gly-L-Phe)-L–Lys]-OMe, 12, Cyclo-(N^{α}-isophthaloyl- bis- (Gly-Sar)- L–Lys]-OMe, 13, Cyclo- [N^{α}-isophthaloyl- bis- (Gly-Gly)-L– Lys]- OH, 14, Cyclo-[N^{α}—iso-phthaloyl- bis- (Gly-L-Phe)-L–Lys]-OH, 15, Cyclo-[N^{α}-isophthaloyl-bis-(Gly-Sar)-L–Lys]-OH, 16, Cyclo- [N^{α}-isophthaloyl- bis (Gly-Gly)-L–Lys]-$NHNH_2$, 17, Cyclo-[N^{α}-isophthaloyl-bis-(Gly-L-Phe)-L–Lys]-$NHNH_2$, 18, Cyclo-[N^{α}-iso-phthaloyl-bis- (Gly-Sar)- L–Lys]-$NHNH_2$ 19, were rendered available, via conventional peptide synthesis, in solution.

A preliminary cytotoxicity evaluation (National Cancer Institute, Cairo, EGYPT), for a representative example, namely, candidate 12, against eight human cancer cell lines, seemed interesting. The detailed comparative results with those of five common anticancer drugs and their complementary biological and biochemical assays, for all the candidates, are envisioned, and will be published elsewhere.

Keywords: Anticancers, Cytotoxicity, Cyclopeptides and N^{α}-isophthaloyl-bis-peptides.

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Although peptides rarely, perfectly, function as drugs, due to their low bio-availability, they offer convenient preliminary synthetic drug candidates, due to their relative ease of assembly and versatile derivatization [1-5]. Thus, synthetic peptides, as initial biological leads, allow rapid identification of the molecular structural requirements of active drug modulators. A large number of natural, as well as, synthetic peptides having interesting biological activities are, progressively, reported [6-11].

However, synthetically, the conversion of the active linear peptides into their cyclic congeners, or the corresponding peptidomimetics, presents a successful approach for defining novel, more biologically active peptide candidates [12-19].

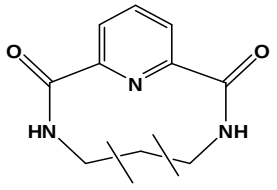
Physico-chemically, molecular cyclization imposes conventional conformational constraints, accompanied with lower entropy levels. Consequently, cyclopeptides are of general improved pharmacological characteristics and confer greater stability against the action of proteolytic enzymes, accordingly, having a prolonged bio-availability [20-23].

Drug design and discovery of anticancer cyclic peptides is, consequently, an updated research challenge [24-29].

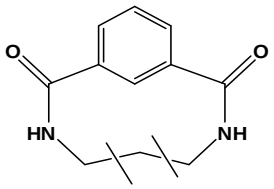
In such context, we have, previously, reported the synthesis of several cyclopeptides, searching for their non biological and, biological [30-37], namely, anticancer potency [38-40], A candidate example was, thus, previously, assayed against the 60 cell line panel test of the "American National Cancer Institute", (NCI, compound NSC: 719476 & September, 2012 Release)*.

Results and Discussion

In view of the, aforementioned, considerations and as a continuation of our previous works on anticancer cyclopeptides, we have reported (2004-2012) some novel Cyclo N^{α} - 2, 6-dipicolinoyl pentapeptides, as cytotoxic agents, with interesting findings [38-40]. Herein, as an extrapolation of the realized anticancer results, an isosteric molecular structural analogy was reviewed. Thus, the previously reported nitrogen heterocyclic dipicolinic acid bridged cyclopeptides: (I) were, consequently, planned to be replaced by the fully aromatic isophthalic acid bridged analogues: (II).



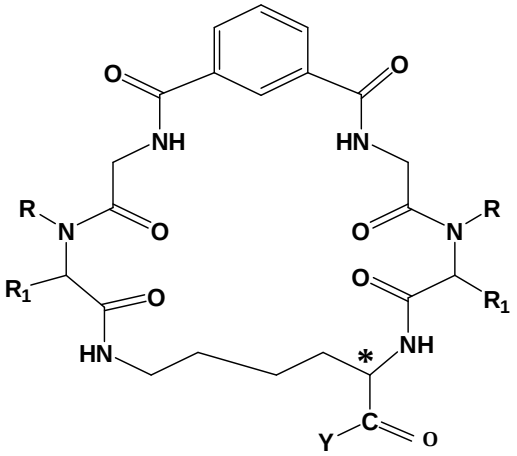
(I)



(II)

*http://dtp.nci.nih.gov/dtpstandard/servlet/MeanGraphSummary?searchtype=NSC&chemnameboolean=and&outputformat=html&searchlist=719476&Submit=Submit

Accordingly, a rational design, synthesis, purification and structural characterization of nine novel cyclic peptides congeners, namely, N^{α}-isophthaloyl bridged cyclopentapeptides, having the general structure: Cyclo-[N^{α}-isophthaloyl-bis-(Glycine-Amino Acid)-L-Lys]-Y, whilst the "Amino Acid" stands for, either Glycine, L-phenylalanine or Sarcosine and Y presents methyl ester, carboxylic or hydrazide group, were optimally synthesized, via conventional synthetic peptide coupling methods, in solution [1-5].



Cyclo-[N^{α}-isophthaloyl-bis-(Glycine-Amino Acid)-L-Lys]-Y, 11-19

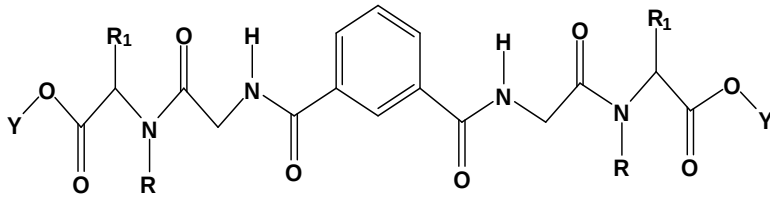
No.	Structure	Y	R	R_1
11	Cyclo-[N^{α}-isophthaloyl-bis-(Gly-Gly)-L-Lys]-Y	OMe	H	H
12	Cyclo-(N^{α}-isophthaloyl)-bis-[Gly-L-Phe]-L-Lys-Y	OMe	H	CH_2-C_6H_5
13	Cyclo-(N^{α}-isophthaloyl)-bis-[Gly-Sar]-L–Lys-Y	OMe	CH_3	H
14	Cyclo-(N^{α}-isophthaloyl)-bis-[Gly-Gly]-L–Lys-Y	OH	H	H
15	Cyclo-(N^{α}-isophthaloyl)-bis-[Gly-L-Phe]-L–Lys-Y	OH	H	CH_2-C_6H_5
16	Cyclo-(N^{α}-isophthaloyl)-bis-[Gly-Sar]-L–Lys-Y	OH	CH_3	H
17	Cyclo-(N^{α}-isophthaloyl)-bis-[Gly-Gly]-L–Lys-Y	$NHNH_2$	H	H
18	Cyclo-(N^{α}-isophthaloyl)-bis-[Gly-L-Phe]-L–Lys-Y	$NHNH_2$	H	CH_2-C_6H_5
19	Cyclo-(N^{α}-isophthaloyl)-bis-[Gly-Sar]-L–Lys-Y	$NHNH_2$	CH_3	H

The selection of the amino acids, constituting the lateral sides of the N^{α}-isophthaloyl bridged peptide cycle, to be: "Glycine", "Sarcosine" or "L-

Phenylalanine", was based upon the variations in the steric hindrance of their side chains, which, possibly, interact, variably, with the hypothetical biological receptors. Thus, while Glycine having a minimally hindered side chain, with only one hydrogen atom, Sarcosine seems moderate with a (N-CH_3) group, while L-phenylalanine has the most bulky group (-CH_2-phenyl). Such variation, as well as, their alternating sequence in the pentapeptide cycle, could permit a significant structure /cytotoxic activity relationships for the novel candidates.

Thus, nine novel cyclo-pentapeptides, namely*:* Cyclo-[N^{α}-isophthaloyl-bis-(Gly-Gly)-L-Lys]-OMe, 11, Cyclo-[N^{α}-isophthaloyl-bis-(Gly-L-Phe)-L-Lys]-OMe 12, Cyclo-(N^{α}-isophthaloyl-bis-(Gly-Sar)-L–Lys]-OMe, 13, Cyclo-[N^{α}-isophthaloyl-bis-(Gly-Gly) -L-Lys]-OH, 14, Cyclo-[N^{α}-isophthaloyl-bis-(Gly-L-Phe)-L–Lys]-OH, 15, Cyclo-[N^{α}-iso-phthaloyl-bis-(Gly-Sar)-L–Lys]-OH, 16, Cyclo-[N^{α}-isophthaloyl-bis(Gly-Gly)-L-Lys]-$NHNH_2$,17,Cyclo-[N^{α}isophthaloyl-bis-(Gly-L-Phe)-L-Lys]-$NHNH_2$,18, Cyclo-[N^{α}-iso-phthaloyl-bis-(Gly-Sar)-L–Lys]-$NHNH_2$, 19, were, newly, synthesized.

Synthetically, cyclization of corresponding three linear tetra peptide acid precursors 8, 9 and 10 with L-lysine methyl ester (*e.g. via,* mixed anhydride, DCC, azide, Diisopropylcarbodiimide and N-hyroxysuccinimide active ester coupling methods), afforded the cyclic peptide esters 11, 12 and 13, respectively, in an acceptable yield, as well as, chemical purity. Their hydrolysis or hyrazinolysis gave the corresponding cyclopeptide acids, 14, 15 and 16 or hydrazides 17, 18 and 19, respectively.



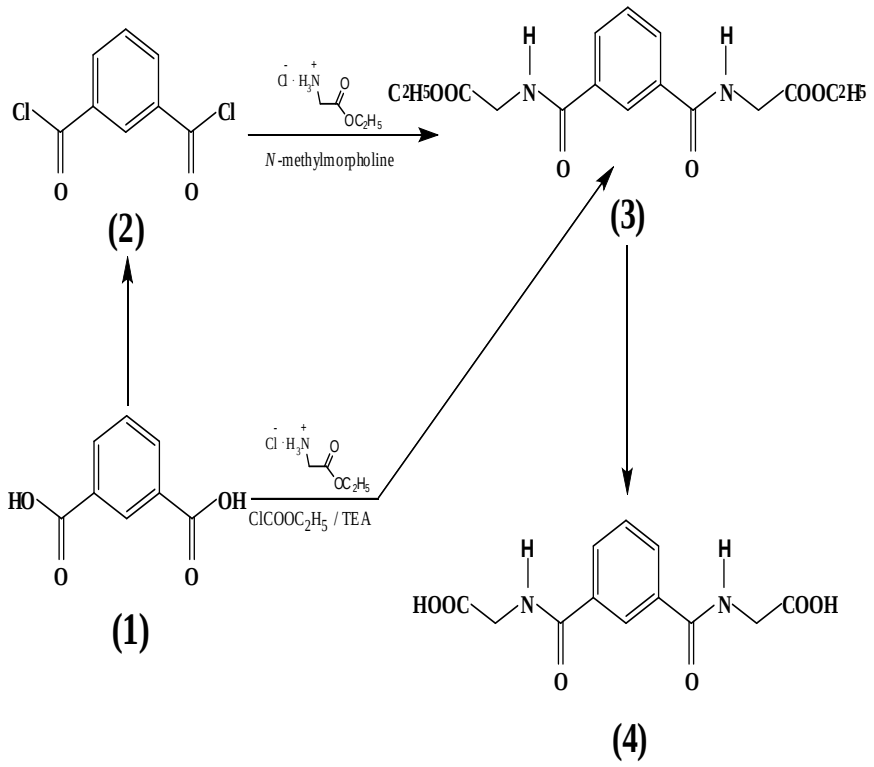
N^{α}-isophthaloyl)-bis-[Glycine - Amino Acid]-Y, 5-10

No.	Structure	Y	R	R_1
5	N^{α} -isophthaloyl-bis-[Gly - Gly -Y]	OMe	H	H
6	N^{α} -isophthaloyl-bis-[Gly - L - Phe -Y]	OMe	H	CH_2-C_6H_5
7	N^{α}-isophthaloyl-bis-[Gly - Sar -Y]	OMe	CH_3	H
8	N^{α}-isophthaloyl-bis-[Gly - Gly -Y]	OH	H	H
9	N^{α}-isophthaloyl-bis-[Gly - L - Phe-Y]	OH	H	CH_2-C_6H_5
10	N^{α}-isophthaloyl-bis-[Gly - Sar -Y]	OH	CH_3	H

Synthesis of the start N^{α}-isophthaloyl-*bis*-(Gylcine) ethyl ester 3 was based on N^{α}-isophthaloyl dicarbonyl dichloride 2, obtained by conversion of N^{α}-isophthalic dicarboxylic acid 1 *via* the reaction with thionyl chloride. The acid

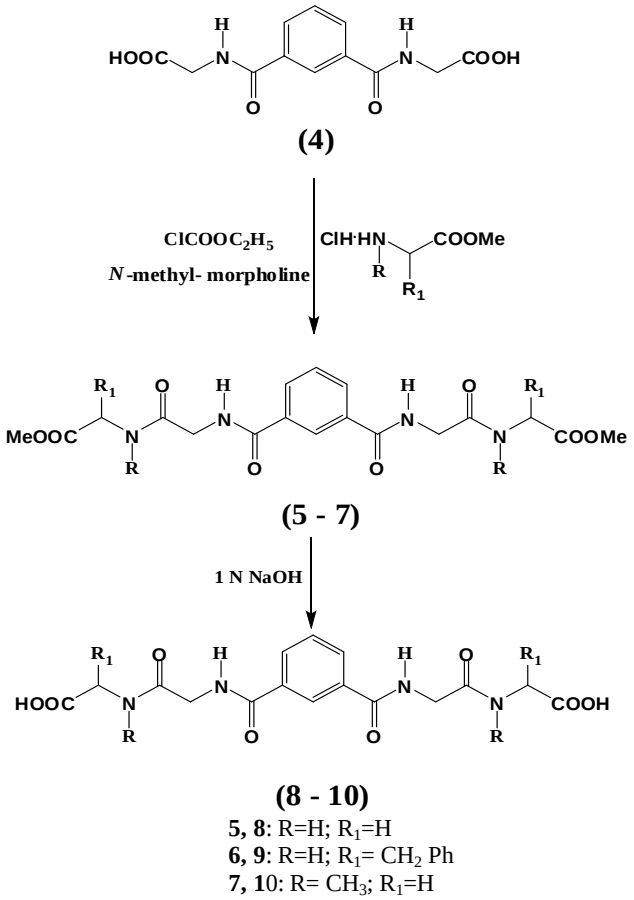
chloride was then coupled, at low temperature, with *Glycine ethyl ester*, in the presence of triethylamine, as organic base.

Alternatively, *bis*-ester 3, was comparably prepared from N^{α}-isophthalic dicarboxylic acid 1 and Glycine ethyl ester, via the mixed anhydride with ethyl chloroformate. Hydrolysis of 3 with 1N metabolic NaOH afforded the corresponding N^{α}-isophthaloyl-*bis*-Glycine 4 (Scheme 1).



Scheme 1. Synthetic routes for compound 3 and 4.

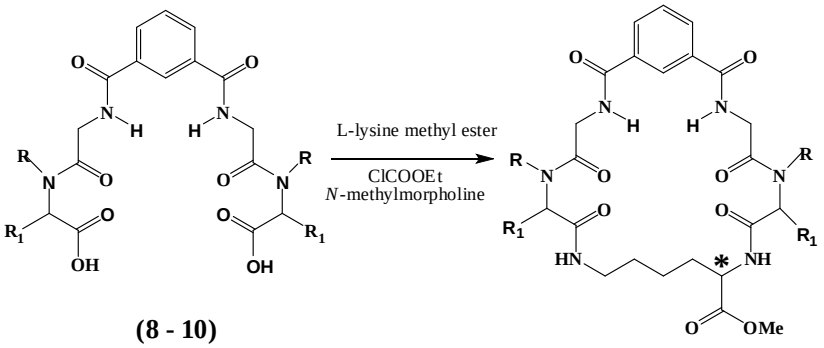
Synthesis of N^{α}-isophthaloyl-*bis*-[Dipeptide] - methyl esters, 5-7, was based on the treatment of the N^{α}-isophthaloyl-*bis*-Glycine, 4, with amino acid methyl ester hydrochloride in the presence of ethyl chloroformate, thus affording the corresponding esters. Hydrolysis with sodium hydroxide afforded the corresponding N^{α}-isophthaloyl-bis-[dipeptide]-OH, 8-10, respectively (Scheme 2).



Scheme 2. Synthetic routes for compounds 5 -10.

Cyclization of the *bis* dipeptides 8-10, respectively with L-Lysine methyl ester, was realized via different coupling methods, namely, mixed anhydride, DCC, azide, diisopropylcarbodiimide and active ester (Scheme 3).

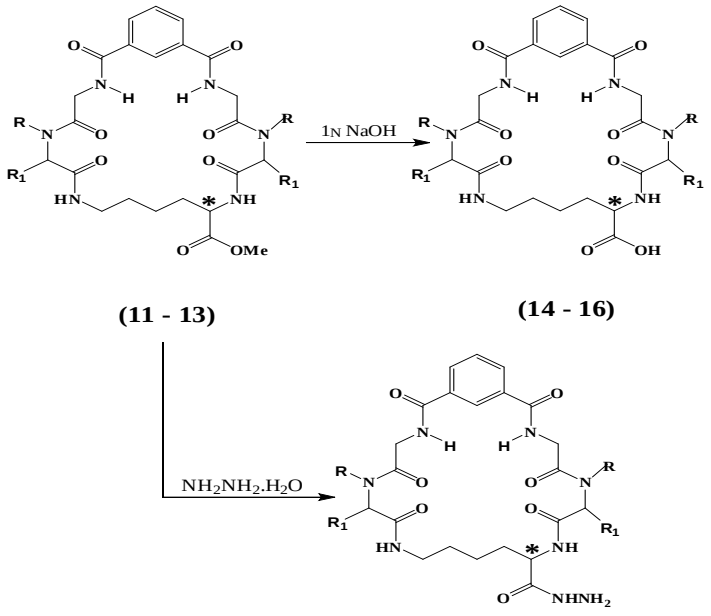
Finally, the cyclic pentapeptide esters 11–13, were converted to the corresponding acids, via the alkaline hydrolysis (1N metabolic sodium hydroxide), affording the acids 14-16, respectively. Parallelly, hydrazinolysis of 11–13, with hydrazine hydrate, afforded the cyclic pentapeptide hydrazides 17-19, respectively (Scheme 4).



(8 - 10) **(11 - 13)**

8, 11: R=H; R_1=H
9, 12: R=H; R_1= CH_2 Ph
10, 13: R= CH_3; R_1=H

Scheme 3. Synthetic routes for compounds 11–13.



(11 - 13) **(14 - 16)**

(17 - 19)

11, 14, 17: R=H; R_1=H
12, 15, 18: R=H; R_1= CH_2 Ph
13, 16, 19: R= CH_3; R_1=H

Scheme 4. Synthetic routes for compounds 14-19.

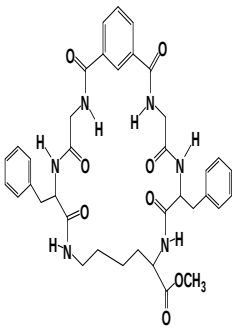
Preliminary cytotoxic activity of a representative compound, 12

Table 1 presents the realized cytotoxic activity data (IC_{50}) for a representative compound: Cyclo-*[N^{α}-iso-phthaloyl-bis-(Gly-L-Phe)-L–Lys]-OMe, 12* (The Egyptian National Cancer institute, Cairo,). Eight human cancer lines were targeted by the candidate and comparative results with those of five common anticancer drugs, actually manipulated in the current clinical practice, are presented.

The candidate seems promising as cytotoxic agent, with comparable or superior activity with the anticancer drugs.

The complementary conventional biological assays for the, candidate, as well as, the other candidates, are under investigation and will be published elsewhere.

TABLE 1. IC_{50} and comparative cytotoxic activity of Cyclo-[N^{α}-iso-phthaloyl-bis-(Gly-L-Phe)-L–Lys]-OMe, 12.

Structure	Human Cancer Cell Line	Breast (MCF-7)	Liver (HEPG2)	Colon (HCT 116)	Cervical (HELA)	Larynx (HEP2)	Prostate (PC3)	Breast (T47D)	Intestinal (Caco)
	IC_{50} (µg/ml)	7.4	7.55	9.83	14.8	10.0	8.60	4.28	10.7
	Ref. drug	% Compound Activity Relative to the Reference Drug							
	T	101	103.8	-----	---	----	---	----	-----
	Carbo	--	96.3	93.7	---	----	---	----	-----
	5F	--	98	113.4	--	101.1	---	----	-----
	Doxo	95.4	96	93.7	88.9	93.5	96	103.8	92.6
	Cis	95.1	95.6	101.2	88.1	93.1	103.6	103.6	92.6

**Reference drugs (T: Tamoxifen®, Carbo: Carboplatin®, 5F: 5- Fluorouracil®, Doxo: Doxorubicin hydrochloride®, Cis: Cisplatin®).

% Relative cytotoxicity: [100-(IC_{50} Compound)] x 100 / [100 – (IC_{50} Reference drug)]

Experimental

Melting points were determined in opened glass capillary tubes with an "Electro Thermal" Digital melting point apparatus, (model: IA9100) and are uncorrected. Elemental micro-analysis for carbon, hydrogen and nitrogen (Microanalytical Unit, NRC) was found within the acceptable limits of the calculated values. Infrared spectra (KBr) were recorded on a Nexus 670 FTIR Nicolet, Fourier Transform infrared spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra and carbon nuclear magnetic resonance (^{13}C-NMR) were run in (DMSO-d_6) on JöEL 270 MHz or 500 MHz instruments. Mass spectra (EI, 70 eV), were run on a MAT Finnigan SSQ 7000 spectrometer, using the electron impact technique (EI). Analytical thin layer chromatography (TLC) was performed on silica gel aluminum sheets, 60 F_{254} (E. Merck). The following solvent systems (by volume) were used as eluents for the development of the plates: S: chloroform/methanol/acetic acid (85/10/5); S_1: S/ petroleum ether (B.P. 40-60 ^{o}C) (3/2) and S_2: n-butanol/water/acetic acid/pyridine (120/48/12/40, by volume). Specific optical rotations were measured with a A. Krawss, Optronic, P8000 polarimeter, in a 1 dm length observation tube, at the indicated conditions, and according to the equation: $[\alpha]_{D}^{T} = 100 * \alpha / (c * l)$, where: α**,** observed rotation angle, D, sodium line (λ 589 nm), C, concentration (g/100 ml), l = path length, in dm and T = experimental temperature (^{o}C).

Synthesis of N^{α}-isophthaloyl-bis-[Glycine- ethyl ester]: 3

Method A: acid chloride method

A cold dichloromethane solution of free Glycine ethyl ester, was dropwisely added to a cold dichloromethane solution of isophthaloyl dicarbonyl dichloride 2 (-20 ^{o}C, 3 g, 14.78 mmol). The reaction mixture was stirred for additional 3 hr at the same temperature, then for 24 hr at room temperature, washed with water, 1N sodium bicarbonate, 1N potassium hydrogen sulphate, followed by water, then dried for 24 hr (0 ^{o}C) over anhydrous sodium sulphate. The solvent was evaporated to dryness and the obtained residue was solidified by trituration with pt.ether (B.P. 40-60 ^{o}C). The solid was then, filtered off, dissolved in methanol and precipitated by pet. ether, to give the ester 3.

Method B: mixed anhydride method

Ethyl chloroformate (2.9 ml, 30.1 mmol) was added to a stirred and cold (-20 ^{o}C) dichloromethane solution (50 ml) of isophthalic acid 1 (5 g, 30.1 mmol) and N-methyl-morpholine (6.6 ml, 60.2 mmol). The reaction mixture was stirred for additional 30 min and then free Glycine ethyl ester (8.4 g, 60.2 mmol), in cold dichloromethane (50 ml, -20 ^{o}C) was added. Stirring was maintained for additional 3 hr at -20 ^{o}C, followed by keeping for 24 hr at room temperature.

The reaction mixture was washed with water, 1N sodium bicarbonate, 1N potassium hydrogen sulphate, followed by water, then dried over anhydrous sodium sulphate. The solvent was evaporated to dryness and the obtained oily

residue was solidified by trituration with pt.ether (B.P. 40-60 ^{o}C). The obtained solid was collected by filtration and precipitated from MeOH/pet. ether, to give compound 3, as identified by its melting point and TLC, in comparison with authentic samples, prepared according to method A.

3: Yield, %: 86 [A], 72 [B]; m.p. 99-102 ^{o}C, *IR (cm^{-1}):* 3311 (NH, str.), 3069 (CH-arom.), 2979 (CH-aliph.), 1734 (C=O, ester), 1643 and 1524 (C=O, amide I and II, respectively) cm^{-1}, *MS (m/ z , %):* 336 (M^+, 5.63), 337 (M^++1, 1.12), 291 (3.37), 263 (18.92%), 234 (100), 160 (12.71), 132 (10.69) 104 (29.43), 76 (20.36), 63 (3.29), 50 (4.48), *Molecular formula (M.wt.):* $C_{16}H_{20}N_2O_6$ (336.34), C*alculated analysis (%):* C, 57.14, H, 5.99, N, 8.33; *Found (%):* C, 57.20, H, 6.02, N, 8.45.

Synthesis of N$^{\alpha}$-isophthaloyl-bis-[Glycine]: 4

To a stirred and cold ethanolic solution (-5 ^{o}C, 20 ml) of the corresponding dipeptide ester 3 (1 mmol), 1 N sodium hydroxide (25 ml) was gradually added. The reaction mixture was stirred for 2 hr at the same temperature, then for 3 hr at room temperature. The solvent was concentrated under reduced pressure and the remaining aqueous solution was cooled and acidified with 1 N hydrochloric acid to pH ~ 3. The obtained solid was, then, filtered off, washed with water, dried and crystallized from ethanol/water to give the corresponding dipeptide 4.

4: Yield, 74 %; m.p. 224-226 ^{o}C, *IR (cm^{-1}):* 3336 (NH, str.), 3267 (CH-Arom.), 2983 (CH-aliph.), 1693 (C=O, acid), 1607 and 1545 (C=O, amide I and II, respectively) cm^{-1}, *MS(m/z ,%):* 280 (M^+, 0.08), 281 (M^+ +1, 0.05), 228 (0.22), 178 (3.71), 166 (57.49), 149 (100), 121 (34.34), 105 (6.98), 76 (12.87), 65 (54.68), 50 (19.27). *Molecular formula (M.wt.),* $C_{12}H_{12}N_2O_6$ (280.07): C*alculated analysis (%):* C 46.16, H, 3.87, N, 8.97; *Found (%):* C, 46.24, H, 3.90, N, 8.90.

Synthesis of N$^{\alpha}$-isophthaloyl-bis-[dipeptide]-methyl ester, 5-7,(mixed anhydride method)

Ethyl chloroformate (2.9 ml, 30.1 mmol) was added to a stirred and cold dichloromethane solution (-20 ^{o}C, 50 ml) solution of N$^{\alpha}$-isophthaloyl-*bis*-[Glycine]: 4, (5 gm, 30.1 mmol) and N-methylmorpholine (6.6 ml, 60.2 mmol). The reaction mixture was stirred for additional 30 min and then the free amino acid ester (60.2 mmol, -20 ^{o}C) in dichloromethane (100 ml) was added. Stirring was maintained for additional 3 hr at -20 ^{o}C, then for 24 hr at room temperature.

The reaction mixture was then washed with water, 1N sodium bicarbonate, 1N potassium hydrogen sulphate and water, and then dried over anhydrous sodium sulphate (24 hr at 0 ^{o}C). The solvent was evaporated to dryness and the obtained oily residue was solidified by trituration with pt.ether (B.P. 40-60 ^{o}C). The residual solid was collected by filtration and precipitated from MeOH/ pet. ether to give the corresponding esters 5, 6 and 7, respectively .

5: Yield: 52 %; m.p. 109-112 ^{o}C, *IR (cm^{-1}):* 3329 (NH stretching), 3282 (CH, arom.), 3073 (CH, aliphatic), 1743 (C=O, ester), 1647, 1543 and 1482 (C=O, amide I, II and III, respectively). *^{1}H-NMR (δ, ppm):* 9.10 (s, 4H, 4NH), 8.37 (m, 1H, aromatic H, C_2), 8.02 - 7.97 [m, 2H, aromatic H (C_4, C_6)], 759 (m, 1H, aromatic H, C_5), 4.02, 4.01 (s, 8H, 4CH_2, NHCH_2CO, α- Gly), 3.63, 3.60 (s, 6H, 2COOCH_3), *^{13}C-NMR: (δ, ppm):* 41.7-39.6 (4NHCH_2, α-Gly), 52.3 (2COOCH3), 127.1 (aromatic C_2), 129.2 (aromatic C_5), 130.7 (aromatic $C_{4,6}$), 134.4 (aromatic $C_{1,3}$), 166.7 (2COOCH_3, ,2NHCOPhe), 170.8 (2CONHCH_2COO). *MS(m/z %):* 422 (M^+, 24.72), 407 (24.99), 377 (78.08), 358 (44.03), 289 (40.79), 226 (42.57), 190 (45.16), 176 (90.14), 167 (69.06), 103 (50.17), 76 (64.96), 55 (100), 54 (26.09). *Molecular formula (M.wt.):* $C_{18}H_{22}N_4O_8$ (422.4): C*alculated analysis (%):* C, 51.18, H, 5.25, N, 13.26, *Found (%):* C, 51.26, H, 5.36, N, 13.40.

6: Yield: 63 %; $[\alpha]_D^{25}$ = -22.1 (C = 0.04, MeOH), *IR (cm^{-1}):* 3427 (NH, stretching), 2924 (CH, arom.), 2855 (CH, aliph.), 1638 (C=O, ester), 1436 and 1318 (C=O, amide I and II, respectively), *MS (m/z, %):* 603 (M^+, 21.85), 577 (35.06), 477 (25.31), 449 (30.80), 382 (26.19), 377 (65.80), 320 (41.80), 292 (61.74), 265 (69.71), 201 (59.68), 190 (88.22), 173 (45.73), 129 (82.76), 110 (84.19), 97 (41.35), 83(100), *Molecular formula (M.wt.):* $C_{32}H_{34}N_4O_8$ (602.6):C*alculated analysis(%):* C, 63.78, H, 5.69, N, 9.30; *Found (%):* C 63.89, H, 5.70, N 9.33.

7: Yield: 58 %; m.p. 116-119 ^{o}C , *IR (cm^{-1}):* 3438 (NH stretching), 2969 (CH, aromatic), 2361 (CH, aliphatic), 1631 (C=O, ester), 1539, 1458 and 1258 (C=O, amide I, II and III, respectively), *MS (m/z, %):* 450 (M^+, 77.14), 364 (77.14), 247 (77.14), 236 (78.57), 213 (73.57), 125 (78.57), 105 (100), 104 (92.14), 103 (78.57), 94 (79.29), 89 (78.57), 76 (87.14), 69 (44.29), 51 (4.29), *Molecular formula (M.wt.):* $C_{20}H_{26}N_4O_8$ (450.18): *Calculated analysis (%):* C, 53.33, H, 5.82, N, 12.44; *Found (%):* C 53.40, H, 5.71, N, 12.39.

Synthesis of N$^{\alpha}$-isophthaloyl-bis [Dipeptide], 8-10

To a stirred and cold methanolic solution (-5 ^{o}C, 20 ml) of the corresponding tetrapeptide ester 5-7, (1 mmol), sodium hydroxide (1N, 25 ml) was gradually added. The reaction mixture was stirred for 2 hr at the same temperature then for 3 hr at room temperature. The solvent was distilled off under reduced pressure, and the remaining aqueous solution was cooled and acidified with 1 N hydrochloric acid to pH ~ 3. The obtained solid was filtered off, washed with water, dried and crystallized from ethanol/water to give the corresponding tetrapeptides 8-10, respectively.

8: Yield: 60 %; m.p. 131-133 ^{o}C, IR *(cm^{-1}):* 3306 (NH stretching), 3066 (CH, aromatic), 2923 (CH, aliphatic), 1694 (C=O, acid), 1639, 1571 and 1542 (C=O amide I, II and III, respectively). *MS (m/z, %):* 395 (M^+ +1, 0.03), 383 (0.03), 308 (37.45), 277 (11.60), 249 (92.51), 220 (100), 188 (2.71), 160 (23.75), 104 (5.55), 76 (1.43), 51 (17.98), 50 (9.55). *Molecular formula (M.wt):* $C_{16}H_{18}N_4O_8$

(394.3): C*alculated analysis (%):* C 48.73, H 4.60, N 14.21; *Found(%):* C 48.80, H 4.66, N 14.25.

9: Yield: 65 %; m.p. 108-110 ^{o}C. $[\alpha]_D^{25}$ = -5.8 (C = 41.7, MeOH), *IR (cm^{-1}):* 3069 (NH stretching), 2557 (CH, aliphatic), 1691 (C=O, acid), 1578 and 1420 (C=O amide I and II, respectively). *MS (m/z ,%):* 575 (M^+, 0.25), 496 (0.26), 422 (0.37), 360 (0.71), 300 (0.48), 264 (0.57), 192 (11.06), 148 (65.09), 118 (11.97), 103 (17.39), 91 (100), 74 (50.46), 65 (15.58), 63 (4.02), 50 (1.35), *Molecular formula (M.wt.):* $C_{30}H_{30}N_4O_8$ (574.58): C*alculated analysis (%):* C 62.71, H 5.26, N 9.75, *Found (%) :* C 62.77, H 5.33, N 9.81.

10: Yield: 90 %; m.p. 136-139 ^{o}C, *IR, (cm^{-1}):* 3652 (NH stretching), 2964 (CH, arom.), 2667 (CH, aliphatic), 1690 (C=O, acid), 1611, 1577 and 1512 (C=O, amide I, II and III, respectively). *MS (m/z, %):* 422 (M^+, 0.50), 423 (M^++1, 4.60), 378 (22.03), 316 (10.90), 250 (10.20), 234 (14.57), 183 (10.61), 129 (19.49), 120 (33.01), 99 (19.17), 82 (12.97), 57 (100), 55 (26.28), 54 (11.57). *Molecular formula (M.wt.):* $C_{18}H_{22}N_4O_8$ (422.4): *Calculated analysis (%):* C 51.18, H 5.25, N 13.26; Found *(%):* C 51.30, H 5.33, N, 13.18.

Synthesis of cyclo - [N$^{\alpha}$ - isophthaloyl) – bis – Gly - amino acid) - L - Lys] -OMe (Cyclicpentapeptide methyl esters), 11-13

Method A: mixed anhydride method

Ethyl chloroformate (0.2 ml, 2 mmol) was added to a stirred and cold (-15^{o}C) dichloromethane solution (20 ml) of the corresponding *N*$^{\alpha}$-isophthoyl-*bis* [dipeptide], 8-10 (1 mmol), containing *N*-methylmorpholine (0.2 ml, 2 mmol). The reaction mixture was stirred for additional 20 min., then a cold (-15 ^{o}C) dichloromethane solution (20 ml) of free L-lysine methyl ester (1 mmol) was added. Stirring was maintained for 3 hr at -15 ^{o}C then for 12 hr at room temperature. The reaction mixture was washed with water, 1N sodium bicarbonate, 1N potassium hydrogen sulfate and water then dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to dryness and the obtained oily residue was solidified by trituration with dry ether/*n*-hexane mixture. The crude product was purified by preparative thin layer chromatography using S_3 as eluent to give the corresponding cyclic pentapeptide methyl esters 11, 12 and 13, respectively.

Method B: DCC method

A cold (-5 ^{o}C) tetrahydrofuran solution (20 ml) of free L-lysine methyl ester (1 mmol) was added to a stirred dry tetrahydrofuran solution (-5 ^{o}C, 20 ml) of the corresponding N$^{\alpha}$-isophthaloyl-*bis* [Dipeptide] 8, 9 and 10, respectively (1 mmol). dicyclohexylcarbodiimide (0.42 g, 2 mmol) was then added, in portions over 20 min at the same temperature to the reaction mixture. Stirring was maintained for 20 hr at room temperature. The reaction mixture was then diluted with acetonitrile (20 ml) and the formed dicyclohexylurea was filtered off and washed with acetonitrile (2 x 10 ml). The filtrate was kept in refrigerator overnight and the newly formed dicyclohexylurea was then filtered off.

Tetrahydrofuran was evaporated to dryness and the obtained residue was dissolved in dichloromethane, washed with 1N sodium bicarbonate, 1N potassium hydrogen sulfate and water, and then dried over anhydrous sodium sulfate. The solvent was evaporated to dryness, and the obtained oily residue was solidified by trituration with dry ether/n-hexane mixture. The obtained solid was collected by filtration and precipitated from EtOH/n-hexane mixture. The cyclic pentapeptide methyl esters 11, 12 and 13 were identified by melting point and TLC, in comparison with authentic samples prepared according to method A.

Method C: Azide method

To a stirred methanolic solution (20 ml) of N^{α} -isophthaloyl-*bis*-*[Gly-Gly]*-*OMe*, 5 (0.845 g, 2mmol), anhydrous hydrazine hydrate (0.7ml, 20mmol) was added. The reaction mixture was refluxed for 3 hr, after which the solvent was evaporated. The obtained residue was triturated with ether, filtered off and crystallized from methanol/ether to afford the corresponding dihydrazide (yield 75%, m.p. 66-68 ^{o}C).

A cold mixture (-15 ^{o}C) of the dihydrazide derivative (0.422 g, 1mmol) in hydrochloric acid (6N, 2 ml) and glacial acetic acid (1 ml) was stirred for 10 min, then an aqueous solution of sodium nitrite (5 M, 2 ml), was added. Stirring was maintained for 30 min at the same temperature, after which the reaction mixture was extracted with ether (60 ml), washed with cold water, 5% sodium bicarbonate and water then dried over anhydrous sodium sulphate. The cold ethereal azide solution (-15 ^{o}C) was added to free L-lysine methyl ester (1mmol). Stirring was maintained for 5 hr at the same temperature, then for 20 hr at room temperature. The reaction mixture was washed with water, 5% potassium hydrogen sulphate and water then dried over anhydrous sodium sulphate. Ether was evaporated to dryness and the obtained oily residue was solidified by trituration with dry ether/ n-hexane mixture to give the corresponding cyclic pentapeptide methyl ester 11, as identified by melting point and TLC, in comparison with authentic sample prepared according to method A.

Method D: Diisopropylcarbodiimide method

L-Lysine methyl ester (1 mmol) was added to a stirred solution of N^{α}-isophthaloyl-*bis*-[Gly-L-Phe], 9 (1mmol) in dichloromethane (20 ml, -5 ^{o}C). Diisopropyl carbodiimide (0.26 ml, 1mmol), was then added, and the reaction mixture was stirred for 20 hr at room temperature. The solution was washed with 1N sodium bicarbonate (2x3ml) 0.5 N HCl (2x3ml) and water (2x3ml), then dried over anhydrous sodium sulphate, and evaporated under reduced pressure. The obtained oily residue was solidified by trituration with dry ether/n-hexane mixture, filtered off and crystallized from ethanol/diethyl ether to give the corresponding cyclic pentapeptide methyl ester, 12 as identified by melting point and TLC, in comparison with an authentic sample prepared according to method A.

Method E: Active ester method

To a stirred cold (-5°C) dry tetrahydrofuran solution (20 ml) of N^{α}-isophthaloyl – *bis* - [Gly – L - Phe] , 9 (0.57g, 1mmol) containing N-hydroxysuccinimide (0.24gm, 2mmol), dicyclohexylcarbodiimide (0.42g, 2 mmol) was added in portions over 20 min to the reaction mixture. Free L-lysine methyl ester (1 mmol) was then added. Stirring was maintained for 20 hr at room temperature. The reaction mixture was then diluted with acetonitrile (20ml) and the formed dicyclohexylurea was filtered off and washed with acetonitrile (2x10 ml). The filtrate was kept in refrigerator overnight and the newly formed dicyclohexylurea was filtered off. Tetrahydrofuran was evaporated to dryness and the obtained residue was dissolved in dichloromethane, washed with 1N sodium bicarbonate, 1N potassium hydrogen sulphate and water then dried over anhydrous sodium sulphate. The solvent was evaporated to dryness and the obtained oily residue was solidified by trituration with dry ether/n-hexane mixture to give the corresponding cyclic pentapeptide methyl ester, 12, as identified by melting point and TLC, in comparison with authentic sample prepared according to method A.

11: Yield (%) 50 [A], 45 [B], 35 [C]; m.p. 70-72 °C. $[\alpha]_D^{25}$ = -3.6 (C = 0.05, MeOH), *IR, (cm^{-1}):* 3376 (NH stretching), 3070 (CH, aromatic), 2978 (CH, aliphatic), 1728 (C=O ester), 1665 and 1533(C=O amide I, and II, respectively), *^{1}H-NMR (δ. ppm)*: 9.09 (m, 1H, aromatic H, C_2), 8.88 - 8.37 (m, 2H, aromatic H (C_4, C_6)), 8.02, 8.01 (s, 8H, 8NH), 7.56 (m, 1H, aromatic H (C_5)), 4.31 (t, 1H, CH_2CHNH, α- CH, Lys), 4.13 – 3.93 (s , 8H, $4CH_2$, $4NHCH_2CO$, α Gly), 3.57 (s, 3H, $COOCH_3$), 3.17 (m, 2H, $NHCH_2CH_2$, εCH_2, Lys), 2.19 - 1.10 (m, 6H, 3CH2, $NHCH_2CH_2CH_2CH_2CHNH$, γ, δ, β-CH_2, Lys), *^{13}C-NMR (δ, ppm* : 32.1 - 23.2 ($CH_2CH_2CH_2CH_2CH$, γ, δ, β-CH_2, Lys), 40.1 - 39.8 ($4NHCOCH_2$, α -Gly), 51.9 ($NHCH_2CH_2CH_2$, ε-CH_2, Lys), 52.3 ($COOCH_3$), 62.3 (CH_2CH_2CHNH, α CH, Lys), 129.1 (aromatic C_2), 156.8, 156.5 (aromatic $C_{1, 3, 4, 5, 6}$), 173.6 ($COOCH_3$, 8 CONH), *MS (m/z, %): 518* (M^+, 5.57), 459 (6.04), 407 (9.60), 369 (13.28), 323 (4.317.298), 279 (18.99), 201 (31.37%), 177 (100), 101 (45.66), 77 (35.62), 58 (32.79), 50 (20.25). *Molecular formula (M.wt.):* $C_{23}H_{30}N_6O_8$ (518.5): *Calculated analysis (%):* C 53.28, H 5.83, N 16.21; *Found (%) :* C, 53.30, ,H 5.88, N, 16.32.

12: Yield (%) 65 [A], 50 [B], 40 [C], 25 [D]; $[\alpha]_D^{25}$ = -14.4 (C = 0.13, MeOH), *IR (cm^{-1}):* 3366 (NH stretching), 2945 (CH aromatic), 2834 (CH aliphatic), 1653 (C=O, ester), 1453 and 1414 (C=O amide I and II, respectively, *^{1}H-NMR (δ, ppm):* 8.57 (m, 1H, aromatic H (C_2)), 8.12 (m, 2H, aromatic H (C_4, C_6)), 8.1, 8 (s, 6H, 6NH, D_2O exchangeable, amide), 7.89 (m, 1H, aromatic H(C_5)), 7.19-7.10 (m, 10H, aromatic H, L-Phe), 4.96 (t, 2H, $NHCHCH_2$ Phe), 4.69 (t, 1H, $NHCH_2CH_2CH_2CH_2CHNH$, α-CH, Lys, methino), 4.16 (s, 4H, $2CH_2$, $NHCH_2CO$, α-Gly, methylene), 3.59 (s, 3H, $COOCH_3$), 3.12 (d, 4H, $2CH_2$, CH_2Phe), 2.06 (m, 2H, $NHCH_2CH_2CH_2CH_2CHNH$, ε-CH_2, Lys, methylene), 2.17 , 2.19 (m , 6H, 3CH2, $NHCH_2CH_2CH_2CH_2CHNH$, γ-, δ-, β-

CH_2, Lys), *^{13}C-NMR (δ, ppm)*: 22.6 ($NHCH_2CH_2CH_2CH_2CHNH$, δ-CH_2, Lys), 29.4($NHCH_2CH_2CH_2CH_2CHNH$,γ-CH_2, Lys), 30.3 ($NHCH_2CH_2CH_2CH_2CHNH$, β-CH_2,Lys), 31.7, 31.4 (2NH-CH_2CO, α Gly), 38.9 ($NHCH_2CH_2CH_2CH_2CHNH$, ε-CH_2,Lys),52.3($COOCH_3$),53.8($NHCHCH_2$Phe),60.6 ($NHCH_2CH_2CH_2CH_2CHNH$, α- CH, Lys), 126.8, 126.6 (2 aromatic C_2, L-Phe), 128.5, 128.9 (aromatic C_2, C_4, C_5, C_6), 129.3 (2aromatic C_2,C_6, L-Phe), 130.6 (2 aromatic C_3,C_5, L-Phe), 133.7 (2 aromatic C_1, L-Phe), 146.6, 151.3 (aromatic C_1, C_3), 157.1, 156.6 (2NHCOPhe), 165.2 (2$NHCOCH_2$), 172 (2NHCOCH), 173.3 ($COOCH_3$), *MS (m/z, %):* 698 (M^+, 19.91), 609 (35.75), 568 (13.69), 477 (30.18), 407 (29.61), 350 (30.85), 313 (43.16), 291 (40.84), 243 (55.38), 232 (58.24), 197 (70.58), 161 (86.16), 107 (68.32), 98 (100), 95 (68.21), 88 (24.90), 54 (49.14). *Molecular formula (M.wt.):* $C_{37}H_{42}N_6O_8$ (698.8): C*alculated analysis: (%)* C, 63.60, H, 6.06, N, 12.03, *Found (%):* C, 63.67, H, 6.05, N, 12.12.

13: Yield (%) 82 [A], 45 [B]; m.p. 144-147 °C. $[\alpha]_D^{25}$ = -5.22 (C = 0.17, MeOH). *IR (cm^{-1}):* 3366 (NH stretching), 2945 (CH, aromatic), 2833 (CH, aliphatic), 1651 (C=O, ester), 1537, 1453 and 1417 (C=O amide I, II and III, respectively). *^{1}H-NMR (δ* ppm): 9.00 (m, 1H, aromatic H (C_2)), 8.56 (m, 2H, aromatic H (C_4 , C_6)), 8.12 (s, 8H, 8NH, D_2O exchangeable, sec. amide), 7.50 [m, 1H, aromatic H (C_5)], 4.65 (t, 1H, $NHCH_2CH_2CH_2CH_2CHNH$, α-CH, Lys, methino), 3.91 (s, 8H, 4CH_2, $NHCH_2CO$, α- Gly, α-Sar, methylene), 3.41 (s, 9H, ($COOCH_3$, 2NCH_3), 2.95 (m, 2H, $NHCH_2CH_2CH_2CH_2CHNH$, ε-CH_2, Lys, methylene), 1.76, 1.10 (m , 6H, 3CH2, $NHCH_2CH_2CH_2CH_2CHNH$, γ-, δ-, β-CH_2, Lys), *^{13}C-NMR (δ, ppm)*: 21.8 ($CH_2CH_2CH_2CH_2CH$, δ- CH_2, Lys), 29.6 ($CH_2CH_2CH_2CH_2CH$, γ-CH_2, Lys), 31.4 ($CH_2CH_2CH_2CH_2CH$, β- CH_2, Lys), 39.9, 39.7 (2NCH_3), 40.2, 40 (2NCH_3COCH_2, α- Sar), 40.4 ($NHCH_2CH_2CH_2CH_2CHNH$), 41 ($COOCH_3$), 55.1, 55 (2$NHCOCH_2$) 60 ($NHCH_2CH_2CH_2CH_2CHNH$), 140.4 (aromatic $C_{1\text{-}6}$), 156.8 (2NCH_3COPhe), 157.2 (2 NHCO CH_2, α Gly), 173 (2 NCH_3CO CH_2), 195.7 ($COOCH_3$). *MS (m/z ,%):* 546 (M^+, 16.19), 521 (10.36), 468 (15.41), 390 (15.09), 368 (25.97), 285 (13.80), 236 (66.06), 165 (33.16), 129 (74.29), 85 (100), 73 (70.60), 60 (38.54), 57 (94.24), 55 (95.40), 51 (16.39), *Molecular formula (M.wt.)*, $C_{25}H_{34}N_6O_8$ (546.6): C*alculated analysis (%):* C, 54.94, H, 6.27, N,15.38; *Found (%):* C, 54.86, ,H 6.26, N,15.49.

Synthesis of cyclo-(N$^{\alpha}$-isophthaloyloyl)-bis-[Gly- Amino acid]-L-Lys,(Cyclic pentapeptides), 14-16

To a stirred and cold methanolic solution (-5 °C, 20 ml) of the corresponding cyclic pentapeptide methyl ester, 11-13, respectively, (1 mmol), sodium hydroxide (1N, 25 ml) was gradually added. The reaction mixture was stirred for 3 hr at the same temperature then for 24 hr at room temperature. The solvent was distilled off under reduced pressure, and the remaining aqueous solution was cooled and acidified with 1 N hydrochloric acid to pH ~ 3. The obtained solid was filtered off, washed with water, dried and crystallized from ethanol/water to give the corresponding cyclic pentapeptides, 14-16, respectively.

14: Yield: 70 %; m.p. 91-93 °C. $[\alpha]_D^{25}$ = -12.2 (C = 0.05, MeOH), *IR, (cm^{-1}):* 3347 (NH stretching), 3079 (CH, aromatic), 2929 (CH aliphatic), 1694 (C=O, acid), 1580, 1529 and 1422 (C=O, amide I, II and III, I respectively), *^{1}H-NMR (δ, ppm)*: 13.21 (s, 1 H, OH, D_2O exchangeable), 8.79 (m, 1H, aromatic H, C_2), 8.65 (m, 1H, aromatic H, C_2), 8.44, 8.13 (s, 6 H, 6NH, D_2O exchangeable,sec. amide), 7.63 (m, 1H, aromatic H, C_5), 3.90 (t, 1 H, $NHCH_2CH_2CH_2CH_2CHNH$, α-CH, Lys, methino), 3.33 (s, 8H, $4CH_2$ ($4NHCH_2CO$, α-Gly), 2.48 (m, 2 H,$NHCH_2CH_2CH_2CH_2CHNH$, ε-CH_2, Lys, methylene), 2.43, 1.19 (m , 6 H, 3 CH_2, $NHCH_2CH_2CH_2CH_2CHNH$, γ-, δ-, β-CH_2, Lys). *MS (m/z ,%) :* 504 (M^+, 0.08), 431 (0.10), 327 (0.58), 345 (0.28), 166 (83.97), 149 (100), 121 (34.10), 93 (6.37), 74 (30.51), 65 (53.55), 50 (7.41). *Molecular formula (M.wt.)*, $C_{22}H_{28}N_6O_8$ (504.5): *Calculated analysis (%):* C, 52.38, H, 5.59, N, 6.66; *Found (%):* C, 52.47, H, 5.60, N, 16.76.

15: Yield: 60 %; m.p. 180-182 °C. $[\alpha]_D^{25}$ = -17.8 (C = 0.03, MeOH), *IR (cm^{-1}):* 3413 (NH stretching), 2925 (CH aromatic), 2863 (CH aliphatic), 1724 (C=O, acid), 1660 and 1597 (C=O, amide I and II respectively), *^{1}H-NMR (δ, ppm)*: 12.85 (s, 1 H, OH, D_2O exchangeable), 8.61 (m, 4 H, aromatic H), 8, 7.96 (s, 6 H, 6 NH, D_2O exchangeable, sec. amide), 7.89, 7.87 (m, 4 H, 2 aromatic H (C_3 and C_5, L-Phe), 7.22, 7.18 (m, 4H, 2 aromatic H (C_2 and C_6, L-Phe), 7.15, 7.12 (m, 2 H, aromatic H, C_4, L-Phe), 4.60, 4.32 (t, 2 H, $NHCHCH_2Phe$), 4.31-3.92 (t, 1 H, $NHCH_2CH_2CH_2CH_2CHNH$, α-CH, Lys, methino), 3.6-3.53 (s, 4 H, 2 CH_2, $NHCH_2CO$, α Gly, methylene), 3.38-3.13 (dd, 6 H, 3 CH_2, CH_2, Phe), 3.05 (m, 2H, $NHCH_2CH_2CH_2CH_2CHNH$, ε-CH_2, Lys, methylene), 2.4-1.3 (m , 6 H, 3 CH2, $NHCH_2CH_2CH_2CH_2CHNH$, *γ-, δ-, β-* CH_2, Lys). *MS: m/z (%):*684 (M^+, 0.05), 598 (0.05), 509 (0.07), 410 (0.11), 373 (0.35), 345 (2.49), 300 (2.02), 273 (5.10), 192 (5.79), 149 (7.57), 120 (10.72), 91 (100), 74 (7.24), 65 (10.93), 56 (2.72), 50 (0.40). *Molecular formula (M.wt.):* $C_{36}H_{40}N_6O_8$ (684.7): C*alculated analysis (%):* C 63.15, H 5.89, N, 12.27; Found *(%):* C, 63.25, H 5.90, N, 12.22.

16: Yield: 92 %; m.p. 179-181 °C. $[\alpha]_D^{25}$ = -5.6 (C = 0.42, MeOH). *IR, (cm^{-1}):* 3394 (NH stretching), 2944 (CH, aromatic), 2836 (CH aliphatic), 1735 (C=O, acid), 1648, 1455 and 1241 (C=O, amide I, II and III, respectively), *^{1}H-NMR (δ, ppm):* 7.67 (m, 4H, aromatic H), 6.82- 6.60 (s, 6 H, 6 NH, D_2O exchangeable)(sec. amide), 4.58 (t, 1 H, $NHCH_2CH_2CH_2CH_2CHNH$, α CH, methino), 4.09 (s, 8H, $4CH_2$ ($2NHCH_2CO$, α- Gly, $2NCH3CH_2CO$, α- Sar, methylene), 3.39 (s, 6 H, 2 CH_3, NCH_3), 2.75 (m, 2 H,$NHCH_2CH_2CH_2CH_2CHNH$, εCH_2, Lys, methylene), 2.14, 1.88 (m, 6 H, 3 CH_2, $NHCH_2CH_2CH_2CH_2CHNH$, γ-, δ-, β- CH_2, Lys), *^{13}C-NMR (δ,ppm):* 22.9 ($CH_2CH_2CH_2CH_2CH$, δ- CH_2, Lys), 29.50 ($CH_2CH_2CH_2CH_2CH$, γ CH_2, Lys), 30.90 ($CH_2CH_2CH_2CH_2CH$, β- CH_2, Lys), 40, 39.90 ($2NCH_3$), 40.30, 40.20 ($2NCH_3COCH_2$, α- Sar), 41.50 ($NHCH_2CH_2CH_2CH_2CHNH$, ε-CH_2, Lys), 51.50 ($2NHCOCH_2$, α- Gly) 60.90 ($NHCH_2CH_2CH_2CH_2CHNH$, α-CH, Lys), 125.4 (aromatic $C_{2,5}$), 134, 132

(aromatic $C_{4, 6}$), 139.6, 139.4 (aromatic $C_{1, 3}$), 170.4 ($2NCH_3COPhe$), 171 (2 $NHCO\ CH_2$), 172 (2 $NCH_3CO\ CH_2$), 174 (COOH). *MS (m/z, %):* 532 (M^+, 0.19), 475 (4.58), 410 (4.55), 355 (4.36), 260 (4.43), 225 (5.67), 162 (4.43), 132 (5.63), 109 (3.04), 78 (53.12), 63 (100), 60 (4.58), 51 (1.39). *Molecular formula (M.wt.):* $C_{24}H_{32}N_6O_8$ (532.5): *Calculated analysis (%):* C 54.13, H 6.06, N 15.78; *Found (%):* C 54.26, H 6.00, N 15.71.

Synthesis of Cyclo-[N^{α}-isophthaloyl-bis (Gly-Amino acid)-L-Lys]-$NHNH_2$, (Cyclic pentapeptide hydrazides), 17-19

To a stirred methanolic solution (50 ml) of the corresponding cyclic pentapeptide methyl ester 11-13, respectively (1 mmol), anhydrous hydrazine hydrate (0.35 ml, 10 mmol) was added. The reaction mixture was refluxed for 3hr, after which the solvent was evaporated. The obtained residue was triturated with ether, filtered off and crystallized from methanol/ether to afford the corresponding cyclic pentapeptide hydrazides 17-19, respectively.

17: Yield: 66 %; m.p. 131-133 ^oC. $[\alpha]_D^{25}$ = -31.5 (C = 0.04, MeOH), *IR (cm^{-1}):* 3308 (NH stretching), 3068 (CH, aromatic), 2975 (CH, aliphatic), 1653, 1535 and 1381 (C=O amide I, II and III, respectively), *^{1}H-NMR (δ, ppm):* 9.85 (s, 1H, $CONHNH_2$), 9.42 (m, 1 H, aromatic H) (C_2), 9.16 (m, 2 H, aromatic H,C_4, C_6), 8.30- 7.96 (s, 6 H, 6 NH, D_2O exchangeable, sec. amide), 7.51 (m, 1H, arom. H) (C_5), 4.67 (t, 1H, $CH_2CH_2CH_2CH_2CHNH$, α CH, Lys), 3.92, 3.82 (s, 8H, $4CH_2$ ($4NHCH_2CO$, α- Gly), 3.58 (s, 2H, $CONHNH_2$), 2.87 (m, 2H, $NHCH_2CH_2CH_2CH_2CHNH$, ε- CH_2, Lys), 2.46- 1.45 (m , 6H, 3CH2, $NHCH_2CH_2CH_2CH_2CHNH$, γ-, δ-, β-CH_2, Lys). *Molecular formula (M.wt.),* $C_{22}H_{30}N_8O_7$ (518.5): *Calculated analysis (%):* C 50.96, H 5.83, N 21.61; *Found (%):* C, 50.86, H, 5.80, N, 21.70.

18: Yield: 60 %; m.p. 206-208 ^oC. $[\alpha]_D^{25}$ = -60 (C = 0.03, MeOH), *IR (cm^{-1}):* 3297 (NH stretching), 3061 (CH, aromatic), 2979 (CH, aliphatic), 1692, 1648 and 1534 (C=O, amide I, II and III, respectively),*^{1}H-NMR (δ, ppm):* 9.75 (s, 1H, $CONHNH_2$), 9.00 (m, 4 H, aromatic H), 7.86 (s, 6 H, 6 NH, D_2O exchangeable, sec. amide), 7.25 (m, 4 H, 2 aromatic H (C_3 and C_5, L-Phe), 7.20 (m, 4 H, 2 aromatic H (C_2 and C_6,, L-Phe), 7.12 (m, 2 H, aromatic H, C_4 , L-Phe), 3.91 (t, 2 H, $NHCHCH_2Phe$), 3.61 (t, 1H, $NHCH_2CH_2CH_2CH_2CHNH$, α- CH, Lys, methino), 3.93 (s, 2H, $CONHNH_2$), 3.37 (s , 4 H, 2 CH_2, $NHCH_2CO$, α Gly), 3.00 (dd, 4 H, $2CH_2$, CH_2Phe, methylene), 2.49 (m, 2 H, $NHCH_2CH_2CH_2CH_2CHNH$, ε-CH_2, Lys, methylene), 1.31- 1.00 (m, 6 H, 3 CH_2, $NHCH_2CH_2CH_2CH_2CHNH$, γ-, δ-, -β CH_2, Lys). *MS(m/z, %):* 699 (M^++1,1.53%), 623 (1.62), 527 (2.04%), 439 (2.54%), 400 (2.07%), 327 (4.72%), 301 (5.20%), 260 (1.89%), 227 (8.55%), 194 (26.46%), 163 (57.27%), 156 (100%), 148 (27.32%), 104 (18.95%), 90 (17.17), 84 (31.35%), 76 (85.53%), 67 (6.94%), 50 (1.28). *Molecular formula (M.wt.):* $C_{36}H_{42}N_8O_7$ (698.8) Calculated *analysis (%):* C 61.88, H 6.06, N, 16.04; Found *(%):* C 61.96, H, 6.09, N, 15.95.

19: Yield: 70 %; m.p. 236-238 ^{o}C. $[\alpha]_{D}^{25}$ = -5.6 (C = 0.03, MeOH). *IR (cm^{-1}):* 3395 (NH stretching), 2946 (CH, aromatic), 2836 (CH aliphatic), 1644, 1451 and 1244 (C=O amide I, II and III, respectively), *^{1}H-NMR (δ, ppm):* 8.82 (s, 1H, $CONHNH_2$), 8.54 (m, 1 H, aromatic H) (C_2), 8.29 (m, 2 H, aromatic H,C_4, C_6), 8.24- 7.89 (s, 4 H, 4 NH, D_2O exchangeable, sec. amide), 7.49 (m, 1H, aromatic H) (C_5), 4.05 (t, 1H, CH_2CH_2CHNH, α- CH, Lys), 3.95 (s , 8H, $4CH_2$ ($2NHCH_2CO$, α- Gly), 3.58- 3.46 (s,4H, $2NCH_3CH_2CO$, α Sar, methylene), 3.33 (s, 2H, $CONHNH_2$), 3.22 (s, 6H, $2CH_3$, NCH_3), 2.90 (m, 2H,$NHCH_2CH_2CH_2CH_2CHNH$, ε-CH_2, Lys, methylene), 1.59- 1.51 (m , 6H, 3CH2, $NHCH_2CH_2CH_2CH_2CHNH$, γ-, -δ, β- CH_2, Lys), *^{13}C-NMR (δ, ppm):* 23.20 ($CH_2CH_2CH_2CH_2CH$, δ CH_2, Lys), 29.50 ($CH_2CH_2CH_2CH_2CH$, γ -CH_2, Lys), 30.8 ($CH_2CH_2CH_2CH_2CH$, β -CH_2, Lys), 40.03, 40.1 ($2NCH_3$), 54, 52.20 ($2NCH_3COCH_2$, α- Sar), 54.2 ($NHCH_2CH_2CH_2CH_2CHNH$, ε CH_2, Lys, methylene), 59.9 ($2NHCOCH_2$, α- Gly), 60.5 ($NHCH_2CH_2CH_2CH_2CHNH$, α-CH, Lys), 120.3 (aromatic $C_{2, 5}$), 134 (aromatic $C_{4, 6}$), 134.10 (aromatic, $C_{1, 3}$), 156.20 (2 NHCOPhe, 2 NCH_3CO CH_2, 2 NHCO CH_2), 173.60 ($CONHNH_2$). *MS (m/z %):* 546 (M^+, 37.12), 407 (34.45), 302 (34.11), 274 (34.11), 255 (37.12), 196 (34.11), 149 (40.80), 126 (45.82), 108 (39.46), 91 (43.48), 76 (35.45), 64 (100), 55 (23.08), 51 (4.01). *Molecular formula (M.wt.),* $C_{24}H_{34}N_8O_7$ (546.6): *Calculated analysis (%):* C, 52.74, H, 6.27, N, 20.50; *Found (%):* C, 52.83, H, 6.30, N, 20.56.

Conclusion

Cyclopeptides of the structure: *Cyclo*-[N$^{\alpha}$-isophthaloyl-bis-(Gly-Amino Acid)-L-Lys] esters, acids or hydrazides, appeared promising, as cytotoxic, namely, anticancer candidates. Further profound biological, particularly, conventional anticancer investigations, on experimental animal models, seem worthy to be realized.

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تشييد ببتيدات حلقية جديدة: 1- تشييد مشتقات ببتيديه خماسية الحلقة لأيزوفثالويل [متماثل (جليسين - حمض أمينى) – ل . ليزين] ذات نشاط متوقع كمضادات للسرطان

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يهدف البحث إلى تصميم وتشييد وتعريف الشكل الجزيئى لبعض المشتقات الببتيدية الجديدة خماسية الحلقة , ذات التركيب : " أيزوفثالويل [*متماثل* (جليسين- حمض أمينى) – ل.ليزين]" والمتوقع لها سمية وتأثير مضاد لخلايا السرطانات البشرية .

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