Plant Derived Cyclopolypeptides: Targets for Drug Discovery

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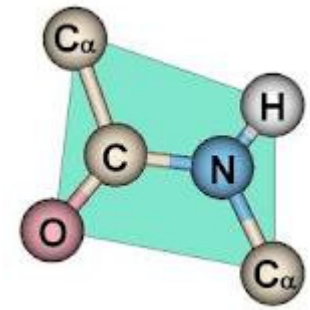


Peptide Bond…

Simplicity to complexity….

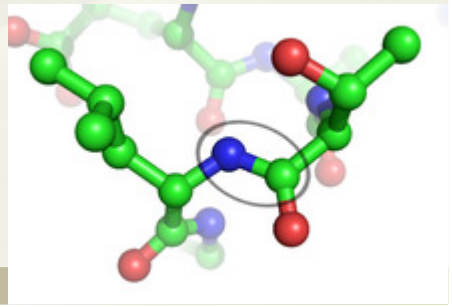
- A **peptide bond (amide bond)** is a covalent chemical bond formed between two molecules when the carboxyl group of one molecule reacts with the amino group of the other molecule, causing the release of a molecule of H_2O, and usually occurs between amino acids.
- Simplest amino acid: 'Glycine'
- Modified amino acids:

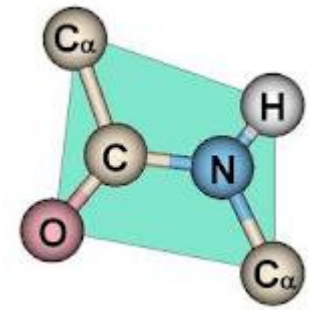
  Isoserine, Dehydrohomoalanine (Dhha),

  β-Hydroxy-p-bromophenylalanine,

  Chloroisoleucine,

  3-Hydroxy-3-methylproline





Peptide Bond...

Simplicity to complexity....

* Other moieties:

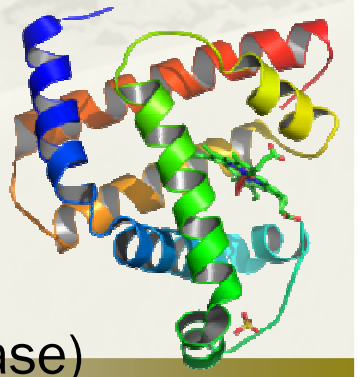
  a) Acids:- 4-amino-3,5-dihydroxyhexanoic acid (Adha), (2S,3R,5R)-3-amino-2,5-dihydroxy-8-phenyloctanoic acid (Ahoa), 3-amino-4-hydroxy-6-methyl-8-phenylocta-5,7-dienoic caid (AHMP).

  b) Heterocyclics:- thiazole (Tzl), oxazole (Ozl), methyloxazoline (mOzn), thiazoline (Tzn), 3-amino-6-hydroxy-2-piperidone (Ahp)

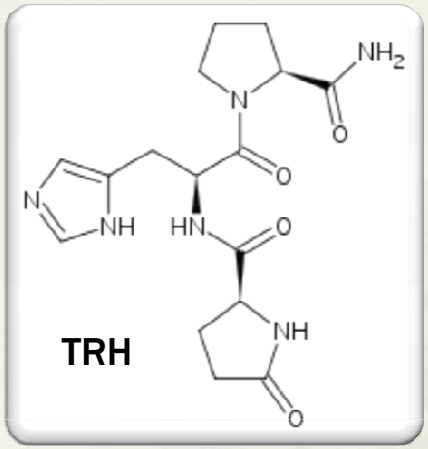
  c) With fatty acid acyl chains or even more complex 'with galactose bridges' and 'histidino-tyrosine moiety'.

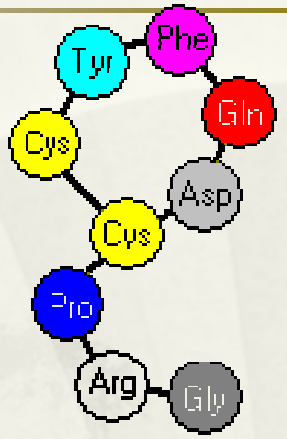
* Proteins (*actin, myocin, myoglobulin*) are polypeptides in folded form which function as enzymes (*Human glyoxalase I*), hormones (*TRH, vasopressin, insulin, gastrin*).

* The peptide bonds in proteins are metastable (*i.e.* in water, they break spontaneously, in living organisms, the process is facilitated by enzymes (protease/peptidase)

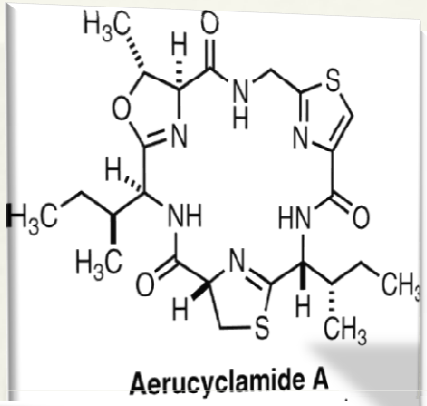


Peptidic structures…

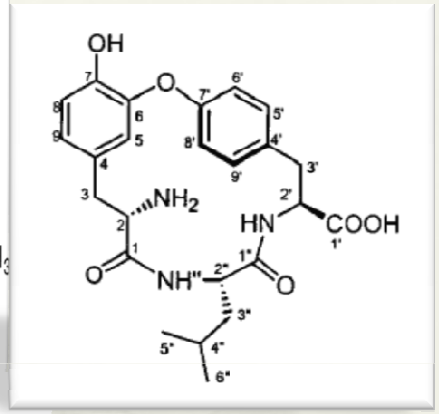




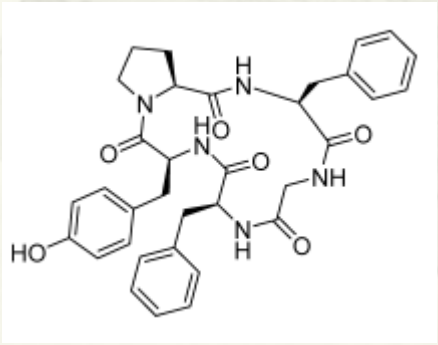
Antidiuretic hormone



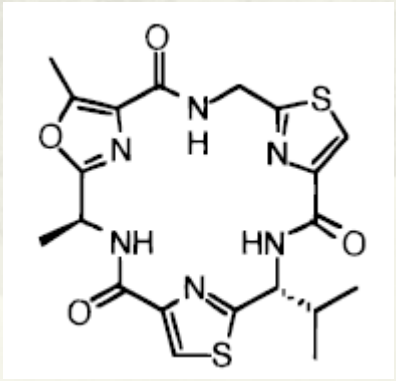
Aerucyclamide A



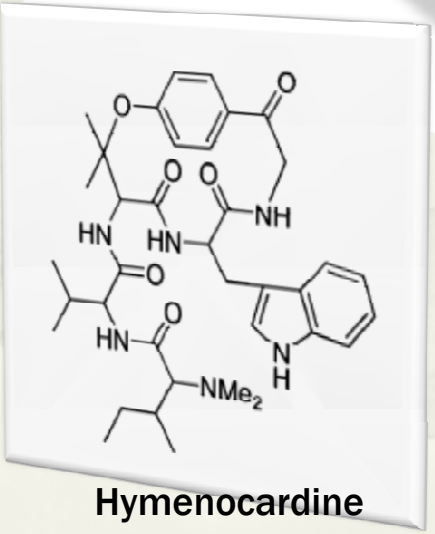
Renieramide



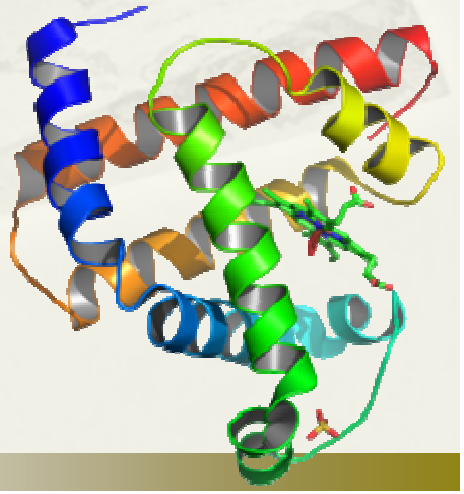
Longicalycinin A



Nostocyclamide



Hymenocardine



Preference of cyclic over linear peptides...

Although linear peptides are associated with pharmacological activities but cyclic peptides dominate over them due to the facts that :

1) Inherent flexibility of linear peptides lead to different conformations which can bind to more than one receptor molecules, resulting in undesirable adverse effects.

2) Cyclization of peptides reduces the degree of freedom for each constituent within the ring and thus substantially leads to reduced flexibility, increased potency and selectivity of cyclic peptides.

Isolation sources…

1) Marine sponges –*Jaspis sp., Hymenacidon sp., Microscleroderma sp., Discodermia sp., Theonella sp.*

2) Marine mollusks - *Elysia rufescens*

3) Fungi - *Petriella sordida, SANK 17397*

4) Bacteria - *Rhodococcus sp., Halobacillus litoralis*

5) Cyanobacteria - *Tolypothrix byssoidea, Hassallia sp.*

6) Hyphomycetes – *Clavariopsis aquatica*

7) Plants – *Pseudostellaria heterophylla*

Bioactivity of Peptides...

Pharmacological aspects....

* ***Cytotoxic activity*** against various *cell lines*.

  e.g. Cycloxazoline, Stylostatin 1, Discokiolides, Discodermins, Phakellistatins, Aciculitins, Axinellins, Tasiamide etc.

* ***Antifungal activity*** against *Candida* and *Cryptococcus sp*.

  e.g. Jasplakinolide, Hymenamides, Aciculitins, Tolybyssidins, Halolitoralins, Arborcandins, Rhodopeptins etc.

* ***Antimalarial activity***

  e.g. Carmabin A, Dragomabin, Dragonamide A

* ***Anti-HIV activity***

  e.g. Circulins, Cycloviolins, Palicourein

* ***Immunosuppressive activity***

  e.g. Cyclolinopeptides, Schnabepeptide

Bioactivity of Cyclopeptides…

Pharmacological aspects….

* *Cyclooxygenase inhibitory activity*

  e.g. Dichotomins D, F-G, Cycloleonuripeptide D

* *Tyrosinase inhibitory activity*

  e.g. Pseudostellarins

* *Antibacterial activity*

  e.g. Verrucamides A-D, *Abyssenine C, Mucronine F-H, Discarine A, B, Scutianine B, Condaline A, Amphibine H, Nummularine B, R, Rugosanine A*

* *Antimycobacterial activity*

  e.g. Ziziphine N, Q,

* *Anti-ACE and Anti-renin activity*

  e.g. Lyciumin A, B

Mechanism of action…

Pharmacological aspects….

* Cyclopeptides act as *cytotoxics* by **inducing apoptosis** especially by binding to highly tyrosine-phosphorylated IFG-1 receptors. Antagonism of transport proteins such as Pgp and MRP-1 may be the other vital mechanism of action of cytotoxic cyclopeptides.
* Cyclopeptides act as *antifungals* by a novel mechanism comprising **inhibition of cell wall biosynthesis.** These peptidic congeners non-competitively inhibit the enzyme β-(1,3)-D-glucan synthase which forms stabilizing glucan polymers in fungal cell wall. Another sensitive target enzyme is ionositol phosphorylceramide synthase (IPC synthase) which is essential for fungal sphingolipid biosynthesis.

Cyclopeptides in clinical trials...

* Kahalalide F: Phase III clinical trial

* COR-1: Phase 1 clinical trial

Synthesis of Cyclopolypeptides

* Solid Phase Peptide Synthesis
* Solution Phase Peptide Synthesis

Cyclopeptides Synthesized by Our Research Group

1. **Cyclotetrapeptide** [Dahiya and Gautam, *Chin. J. Chem.* 2011, 29(9), 1911-6.] [Wiley, IF: 0.755]
2. **Cyclomontanin D** [Dahiya and Gautam, *Afr. J. Pharm. Pharmacol.* 2011, 5(3), 447-53.] [IF: 0.839]
3. **Cordyhetapeptide B** [Dahiya and Gautam, *Bull. Pharm. Res. 2011,* 1(1), 1-10.] [UIF: 0.735]
4. **Cyclotetrapeptide** [Dahiya and Gautam, *Mar. Drugs 2011,* 9(1), 71-81.] [MDPI, IF: 3.854]
5. **Gypsin D** [Dahiya and Gautam, *Am. J. Sci. Res. 2010,* 11, 150-8.]

Cyclopolypeptides Synthesized by Our Research Group

6. **Cycloheptapeptide** [Dahiya and Gautam, *Mar. Drugs 2010,* 8(8), 2384-94.] [MDPI, IF: 3.854]
7. **Annomuricatin B** [Dahiya et al., *Z. Naturforsch.* 2009, 64b(2), 237-44.] [IF: 0.864]
8. **Cyclopolypeptide** [Dahiya et al., *Chem. Pharm. Bull. 2009,* 57(2), 214-7.] [IF: 1.592]
9. **Hirsutide** [Dahiya et al., *Monatsh. Chem. 2009,* 140(1), 121-7.] [Springer, IF: 1.532]
10. **Cyclopolypeptide** [Dahiya, *J. Iran. Chem. Soc. 2008,* 5(3), 445-52.] [Springer, IF: 1.689]
11. **Cyclohexapeptide** [Dahiya, *Chem. Pap. 2008,* 62(5), 527-35.] [Springer, IF: 1.096]
12. **Psammosilenin A** [Dahiya, *Arch. Pharm. Chem. Life Sci. 2008,* 341(8), 502-9.] [Wiley, IF: 1.708]
13. **Cyclohexapeptide** [Dahiya and Kumar, *J. Zhejiang Univ. Sci. B. 2008,* 9(5), 391-400.] [Springer, IF: 1.099]

Synthesis of a Cyclohexapeptide

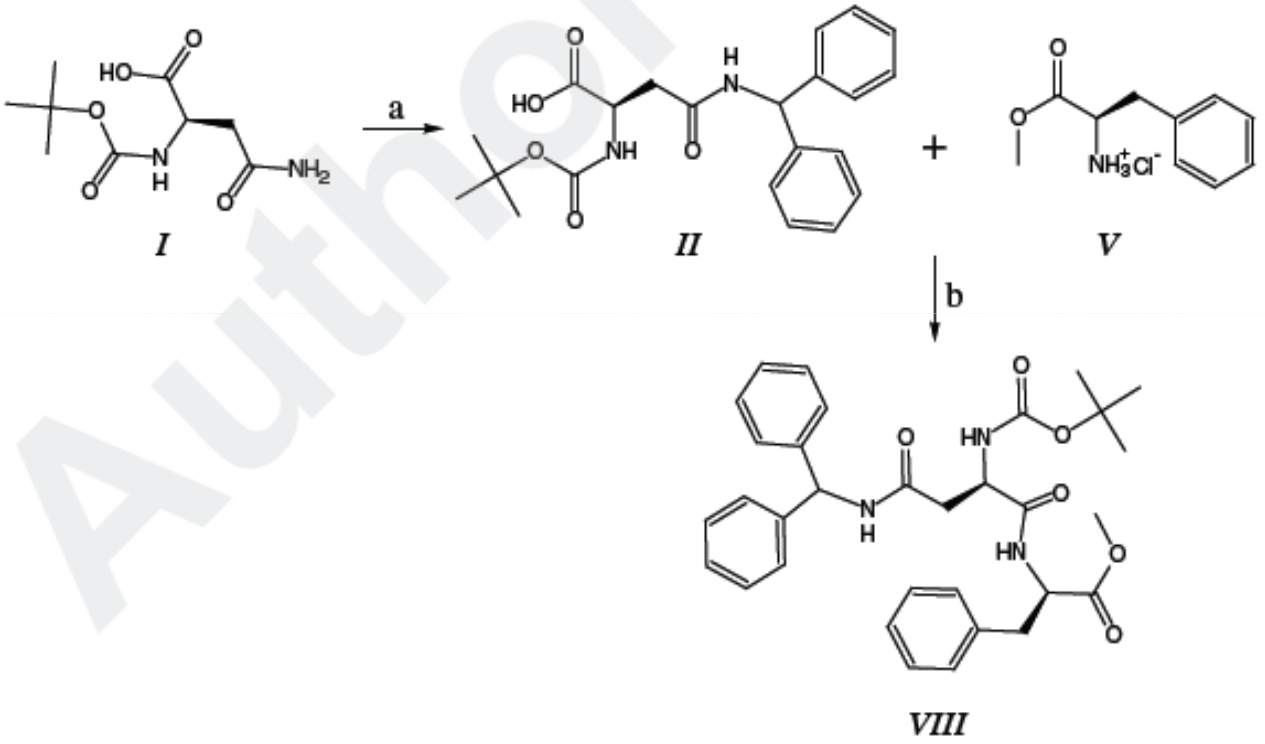


Fig. 1. Synthesis of dipeptide unit *VIII*. Reaction conditions: a) diphenylmethanol, AcOH, H_2SO_4, room temperature, 12 h; b) DCC, TEA, DCM, room temperature, 24 h.

Synthesis of tetrapeptide unit

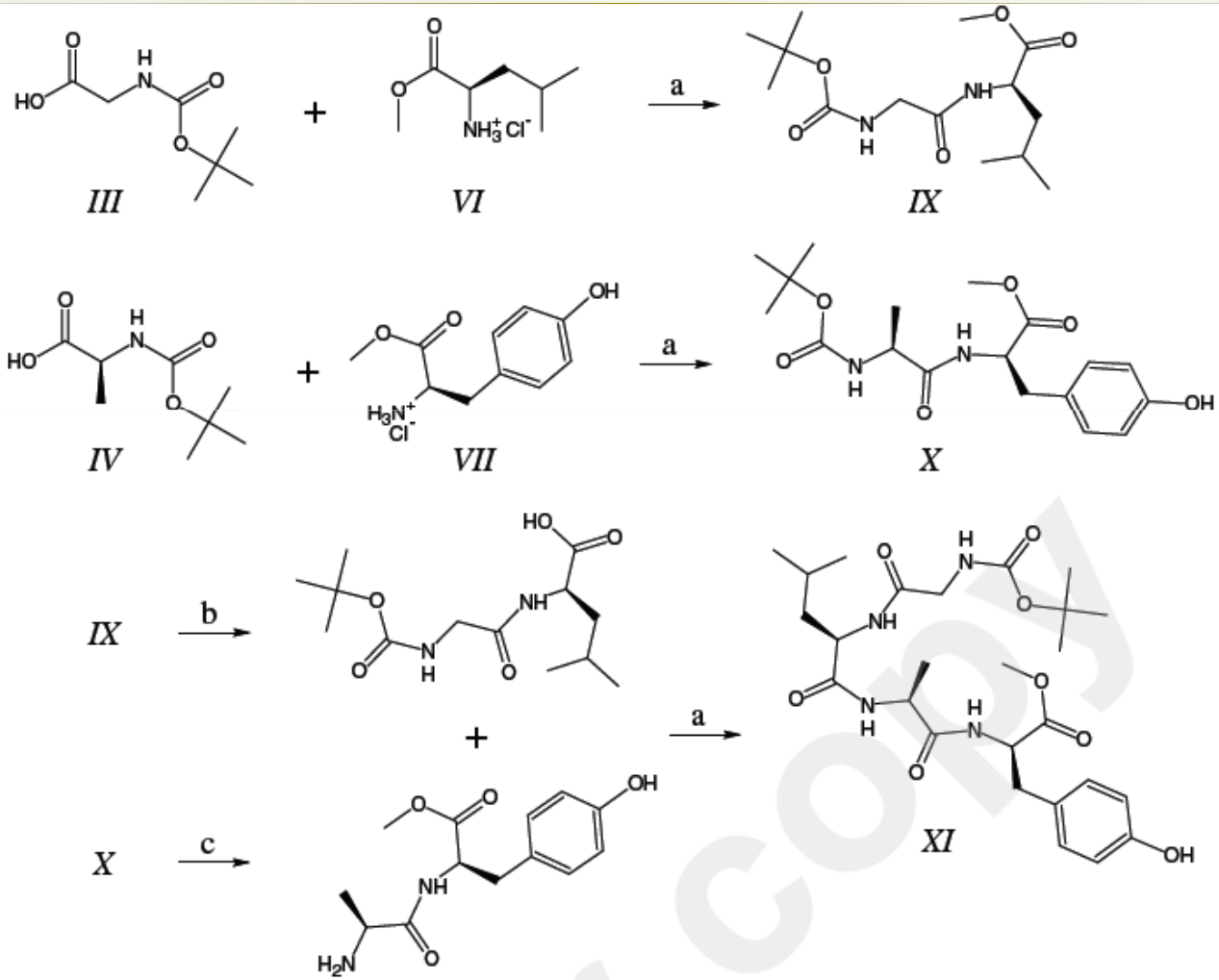
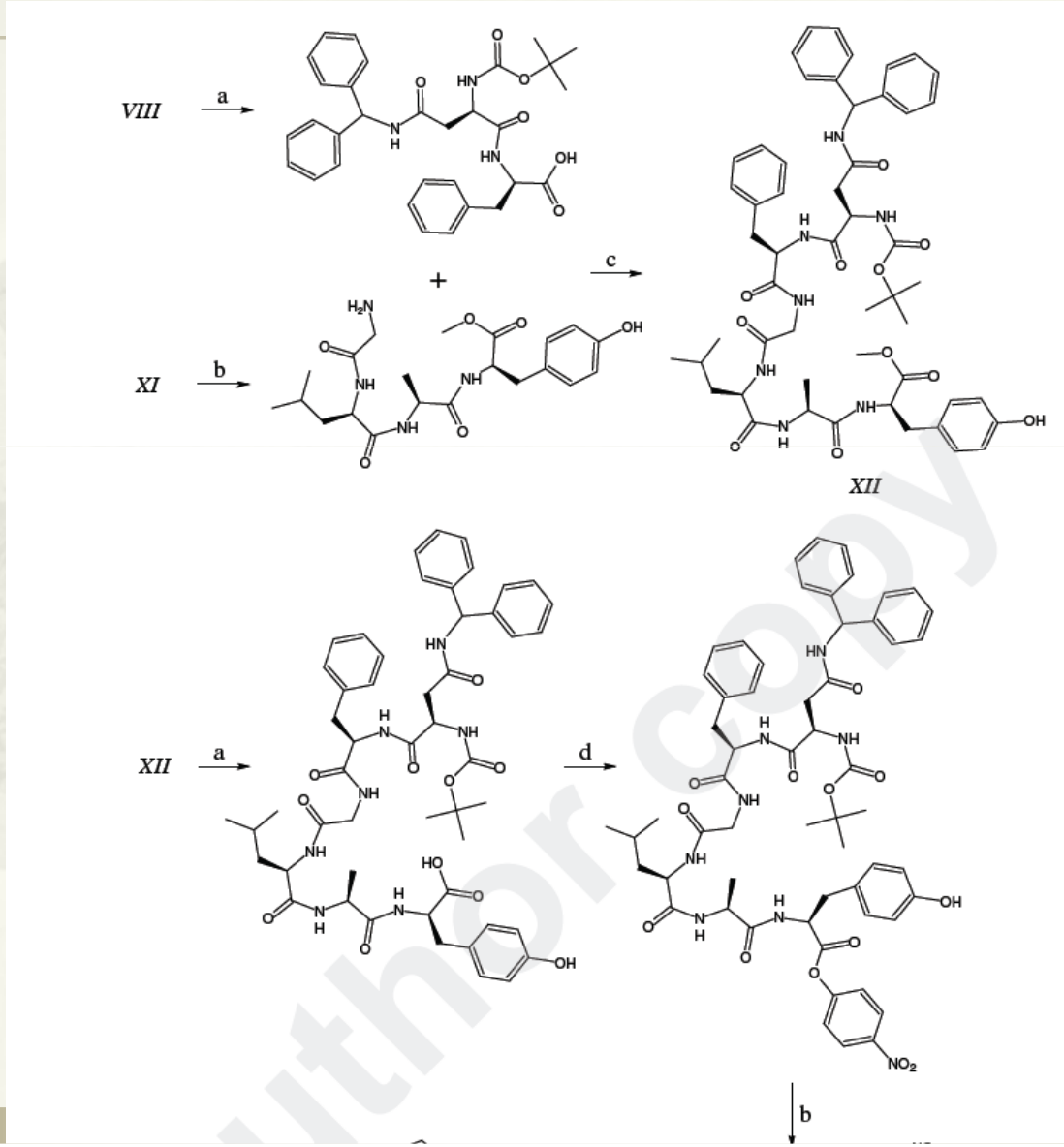


Fig. 2. Synthesis of tetrapeptide unit *XI*. Reaction conditions: a) DCC, TEA, DCM, room temperature, 24 h; b) LiOH, THF/H_2O ($\varphi_r = 1:1$), room temperature, 1 h; c) TFA, $CHCl_3$, room temperature, 1 h.

Synthesis of linear hexapeptide unit



Cyclization of linear hexapeptide unit

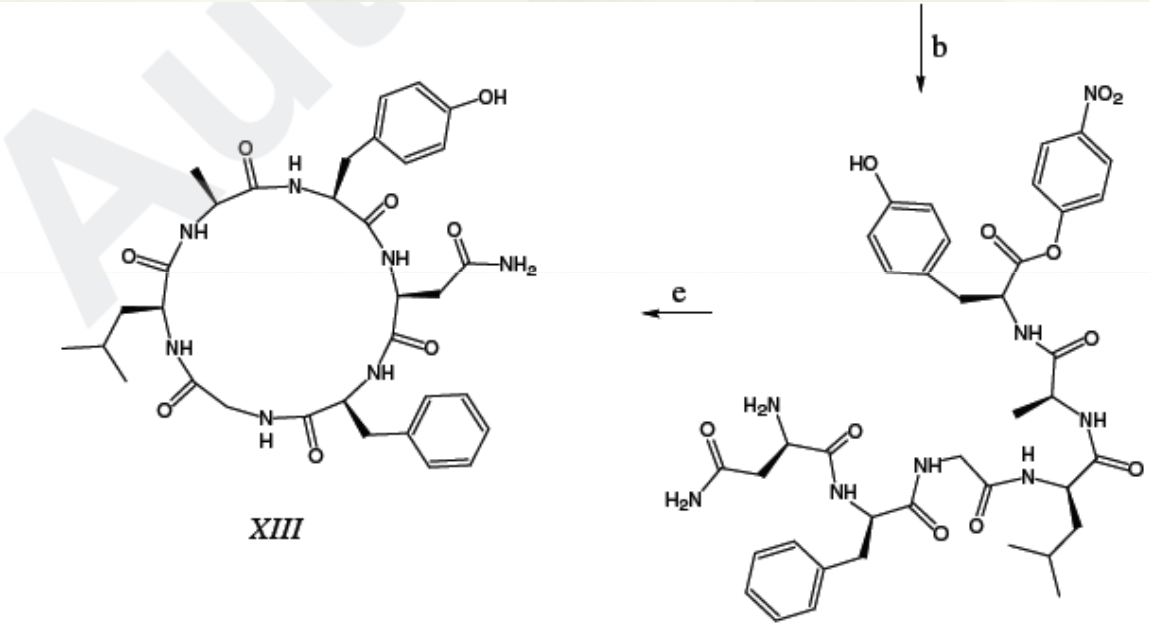


Fig. 3. Synthetic pathway for cyclic hexapeptide – dianthin A (*XIII*). Reaction conditions: a) LiOH, THF/H_2O ($\varphi_r = 1:1$), room temperature, 1 h; b) TFA, $CHCl_3$, room temperature, 1 h; c) DCC, TEA, DCM, room temperature, 24 h; d) DCC, pnp, room temperature, 12 h; e) TEA or NMM or pyridine, $CHCl_3$, 0 °C, 7 days.

Controlled Delivery of Peptides/Proteins

- Although many peptide/protein like products are generally designed for parenteral administration, some other noninvasive routes have also been used. *e.g. desmopressin* is delivered nasally and *deoxyribonuclease* by inhalation. Although peptides and proteins are generally orally inactive, cyclosporine is an exception.
- In order to design and develop long-acting, more effective peptide/protein drugs, the controlled release mechanisms and effective parameters need to be understood.
- Various peptide/protein delivery systems includes biodegradable and nondegradable **microspheres**, microcapsules, nanocapsules, injectable implants, diffusion-controlled hydrogels and other hydrophilic systems, microemulsions and multiple emulsions, and the use of iontophoresis or electroporation etc.

Materials Used to Prepare Microspheres for Controlled Delivery of Peptide and Proteins

Material	Degradation mechanism	Biodegradation	Active substance
Natural			
Starch	Amylase	Biodegradable	Insulin
Alginate	pH, enzymes	Biodegradable	Protein
Chitin	pH, enzymes	Biodegradable	Bovine serum albumin
Chitosan	pH, enzymes	Biodegradable	Antigens, Bovine serum albumin, salmon calcitonin
Collagen/gelatin	Collegenase	Biodegradable	Hydroxyapatite
Corn protein(zein)	Enzymes	Biodegradable	Ivermectin
Cross linked albumin	Enzymes	Biodegradable	Virus antigen
Hydroxyapatite	Dissolves by the time	Biodegradable	Bone morphogenic protein, Recombinant human glucocerebrosidase
Hyaluronik asit		lable	Bovine serum albumin
Azo-cross-linked copolymer of styrene and HEMA coated particles	Reduction of azo bonds by microflora in large intestine	Partially degradable	Insulin and vasopresin
Maleic anhydride/poly (N- isopropylacrylamide) hybrid hydrogels	Enzymes	Partially degradable	Dextran
Hydrogels	Hydrolysis	Biodegradable	Peptides, proteins
Poly sebacic anhydrides	Hydrloysis	Biodegradable	Rhodamin B
Polyesters/poly lactides	Ester hydrolysis by esterases	Biodegradable	Somatostatin anoloques
Polyorthoesters	Hydrolysis	Biodegradable	Bovine serum albumin
Polycarbonates	Hydrolysis	Biodegradable	Dopamine
Poly lactic acid / glycolic acid (PLGA)	Hydrolysis	Biodegradable	Leuprolide acetate, goserelin acetate, triptorelin, integrilin, insulin
Polycaprolactones	Hydrolysis	Biodegradable	Bovine serum albumin, insulin, nerve growth factor
Poly etilen oksit/amino acids	Enzymes	Biodegradable	Poly(L-aspartic acid), Plasmid , DNA, Cyclophosphamide
Polyphosphazenes	Hydrolysis, dissolution	Biodegradable	Naproxen, Bovine serum albumin

Methods Used for Preparation of Polymeric Nanocapsules

Polymer	Drug	Size (nm)	Preparation method
PLGA	Insulin	>1 μm	Phase inversion
PLGA	Hemagglutinin	250	Multiple emulsion
PLA	Tetanus toxoid	200	Multiple emulsion
PLA	PDGFR β tyrphostin inhibitor	125	Solvent displacement

Marketed Formulations of Proteins Based on Biodegradable Microspheres

Drug	Trade name	Company	Route	Application
Leuprolide acetate	Lupron Depot®	Takeda-Abott	3 months depot suspention	Prostate cancer
Recombinant human growth hormone	Nutropine Depot®	Genentech-Alkermes	Monthly S/C injection	Growth hormone deficiency
Goserelin acetate	Zoladex®	I.C.I.	S/C İmplant	Prostate cancer
Octreotide acetate	Sandostatin LAR® depot	Novartis	Injectable S/C suspension	GH suppression, anticancer
Triptorelin	Decapeptyl®	Debiopharm	Injectable depot	LHRH agonist
Recombinant bovine somatropin	Posilac®	Monsanto	Injectable depot, oil based injection	To increase milk production in cattle

Advantages of Controlled Delivery of Peptide and Protein Drugs

- Controlled drug delivery is delivery of drug at a rate or to a location determined by needs of the body or disease state over a specified or extended period of time during the therapy
- Conventional drug therapy requires periodic doses of therapeutic agents and some solubility problems can be seen in conventional formulations
- Controlled delivery and the formulation can provide maximum stability, activity and bioavailability
- Controlled delivery of peptide and protein drugs provides improved efficiency, reduced toxicity and improved patient convenience

Examples and Application of Peptides and Proteins in Clinical Use or Undergoing Clinical Trial

Therapeutic peptide or protein	Application
Tissue necrosis factor	Carcinoma
Proleukin	Carcinoma
y-Interferon	Carcinoma
Epidermal growth factor	Wound healing
Transforming growth factors	Wound healing
Fibroblast growth factor	Wound healing
Insulin-like growth factors	Wound healing
Hirudin	Fibrinolytic
Tissue plasminogen activator	Fibrinolytic
Streptokinase	Fibrinolytic
Erythropoietin	Erythropoieais stimulation
Factor VIII	Haemophilia
Factor IX	Christmas disease
Triproamylin	Glucose regulation
Insulin	Glucose regulation
Somatostatm	Glucose regulation
Proinsulin	Glucose regulation
α-Interferon	Viral diseases/hairy cell leukemia
β-Interferon	Multlple sclerosis
Glucocerebrosidase	Gaucher'disease
Cerezyme	Type I Gaucher's disease
Pulmozyme	Cystic fibrosis
Calcitoninh	Bone disease
Oxytocin	Labour induction
Growth hormone	Short stature
α1 Antitrypsin (aat)	aat deficiency
Superoxide dismutase	Respiratory disorders

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THANKS !!!

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