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Synthesis of new hybrid cell penetrating peptides-medical drugs molecules

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In 1988 the remarkable ability of a peptide to traverse a cell's plasma membrane independent of a membrane receptor was revealed. They cause changes in the membranes leading to their permeability. This type of peptide molecules is called cell penetrating peptides (CPP). One of these peptides is sweet arrow peptide (SAP). Herein, we report for the synthesis of four analogues of SAP. A new interesting strategy for synthesis of Asn and Gln containing peptides starting from Glu and Asp and using Rink amide resin was applied.

Keywords: cell penetrating peptides, SAP analogues, CPP-medical drug hybrid molecules

INTRODUCTION

As it is well know the main problem with medical drugs absorption in human body is their hydrophilicity. Moreover, cell wall and membrane of the pathogen microorganisms and the membrane of eukaryotic cells and organelles, represent a major barrier to hydrophilic substances to penetrate into the cell.

The process of introducing drugs into cells has always proved a major challenge for research scientists and for the pharmaceutical industry. The cell membrane is selectively permeable and supports no generic mechanism for their uptake. A drug must be either highly lipophilic or very small to stand a chance of cellular internalization. These restrictions mean that the repertoire of possible drug molecules is limited. The existing methods for delivery of macromolecules, such as viral vectors and membrane perturbation techniques, can result in high toxicity, immunogenicity and low delivery yield. However, in 1988 the remarkable ability of a peptide to traverse a cell's plasma membrane independent of a membrane receptor was revealed. They cause changes in the membranes leading to their permeability. This type of peptide molecules is called cell penetrating peptides (CPP) [1].

CPPs have the ability to enter cells independent of a membrane receptor, and they show no cell-type specificity. They are small (10–30 residues in length), often positively charged sequences of amino acids. CPPs facilitate cellular uptake of various molecular cargo (from small chemical molecules to nanosize particles and large fragments of DNA). The "cargo" is associated with the peptides either through chemical linkage via covalent bonds or through non-covalent interactions. The function of the CPPs are to deliver the cargo into cells, a process that commonly occurs through endocytosis with the cargo delivered to the endosomes of living mammalian cells. CPPs hold great potential as *in vitro* and *in vivo* delivery vectors for use in research and medicine. Remarkable is the extremely low toxicity that these peptides show. Their current application in medical practice is limited due to lack of cell selectivity in delivery of goods and lack of understanding of how their utilization. However, the CPP have found many applications in medicine as suppliers of medicines to treat various diseases including cancer, inhibitors of viruses, as well as in diagnosis, as transporters of contrast agents for cellular "labeling".

Three different mechanism of cellular uptake were revealed [2]:

- direct penetration
- translocation through the formation of a transitory structures
- translocation mediated by the formation of inverted micelles

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But today, it is thought that each CPP expresses its own preferred mechanism of uptake. CPPs typically could be 2 different types:

- with amino acid composition containing a high relative abundance of positively charged amino acids such as lysine or arginine;
- or have sequences that contain an alternating pattern of polar/charged amino acids and non-polar, hydrophobic amino acids.

These two types of structures are referred to as polycationic or amphipathic, respectively.

A well know member of second group peptides, with proven membrane-penetrating properties, is so called "sweet arrow peptide" (SAP) $(VRLPPP)_3$ described by Fernandez-Carneado et al. in 2004 [3].

Herein, we report on the synthesis of four SAP analogues replacing Leu residue with Asp, Glu, Asn and Gln in order to introduce in the final molecule a specific functions we need to obtain hybrid structures CPP-medical drug.

RESULTS AND DISCUSSION

Our attention was attracted by SAP because it has relatively simple and easily realizable synthetically primary structure consisting of 6 amino acid residues.

We chose to replace Leu residue of SAP with the amino acids Asp, Glu, Asn and Gln and to synthesise the following products:

H-Val-Arg-Asp (drug molecule)-Pro-Pro-Pro-OH;

H-Val-Arg-Glu (drug molecule)-Pro-Pro-Pro-OH;

H-Val-Arg-Asn-Pro-Pro-Pro-drug molecule;

H-Val-Arg-Gln-Pro-Pro-Pro-drug molecule.

These amino acids were chosen because of its adequate structure to provide us the necessary additional functional groups in their side chains for binding of the drug molecules. This decision was also framed by the idea of using two strategies for the synthesis of CPP-drug molecule. For the synthesis of aim CPP we used conventional SPPS (solid phase peptide synthesis) by Fmoc-strategy.

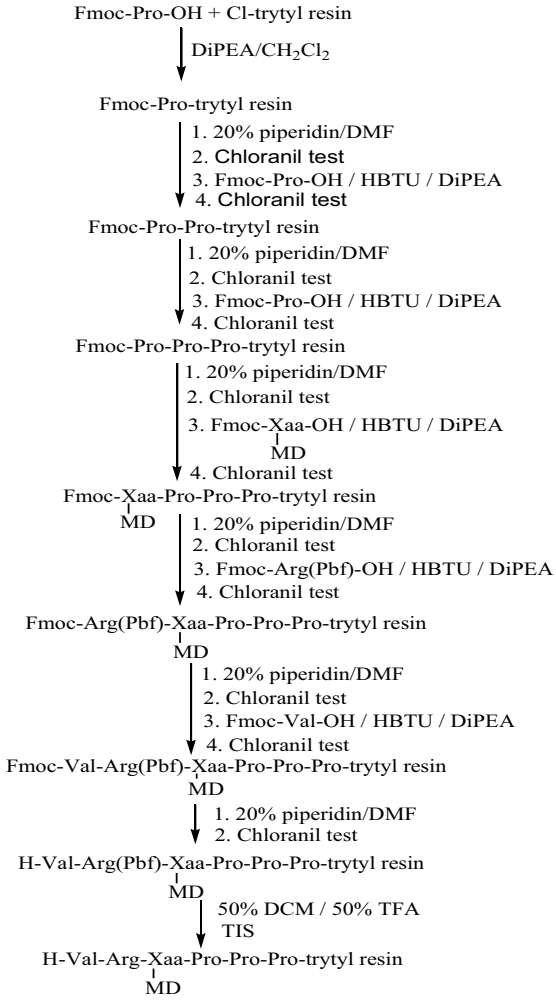
Here drug molecule was previously linked to the carboxyl function of the side chain of Asp or Glu. For this purpose, two acidic amino acids were previously protected on their $^{\alpha}NH_2$-group with Fmoc-group and $^{\alpha}COOH$ group as allyl esters. This type of esters became very useful in recent years due to their specific properties. They are completely stable in acidic and alkaline hydrolysis, unlike other ester groups such as OMe, OEt, OBzl and OBut. On every stage, this function can be successfully unblocked by treatment with tetrakis / Pd, allowing the extension of the main chain. At the beginning Wang resin was used as solid phase carrier. After connecting the second Pro residue during the reaction of deblocking of Fmoc-function, we found that the obtained dipeptide Pro-Pro is cleaved from the resin. The literature data shown that during the synthesis of difficult sequences to the C-terminal peptides as a Pro-Pro, Val-Pro, Tyr-Pro and others in the process of unblocking of Fmoc-group in alkaline medium using Wang-resin the diketopiperazines are formed [4]. The authors recommend using of chlorotrytyl resin for this type of sequences. That's why we changed our strategy and used cholotrytyl resin for synthesis of target peptides. Thus, we synthesize the first two peptide H-Val-Arg-Asp(drug molecule)-Pro-Pro-Pro-OH and H-Val-Arg-Glu(drug molecule)-Pro-Pro-Pro-OH. Compounds were synthesized according to the following scheme:

Strategy I

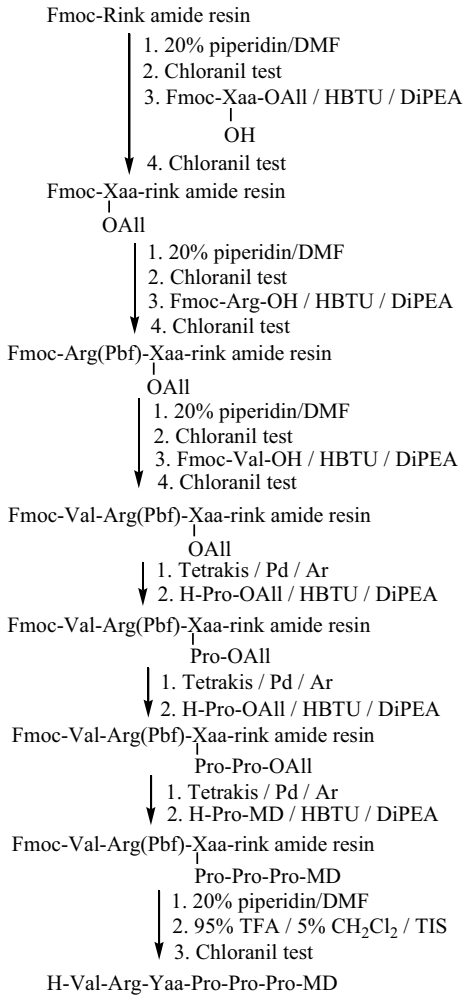
Since in our opinion the incorporation of drug molecule in the middle of the peptide fragment could lead to distortions of secondary and tertiary structure of the peptide, and hence the loss of its membrane penetrating properties, we decided to realize the synthesis of these peptides, but linking drug molecules to their C-terminus. For this purpose, was used strategy II.

Strategy II

Again, we replaced the amino acid Leu in the structure of SAP with Gln and Asn using an original strategy for the synthesis of Gln and Asn – containing peptides previously created by us, assuming adequately protected Glu and Asp and connect them to the Rink amide resin [5]. For this purpose the connection of peptide and resin is made by COOH functions in a side chain of Glu and Asp. Typical of Rink amide resin is that the final peptide unblocking leads to the amides obtaining. Thus using Glu and Asp as starting amino acids, in the final peptides they become to Gln and Asn. Reactions were conducted according to the following scheme 2:



Scheme 1. SPPS of peptides H-Val-Arg-Asp(drug molecule)-Pro-Pro-Pro-OH and H-Val-Arg-Glu(drug molecule)-Pro-Pro-Pro-OH, where Xaa = Asp or Glu and MD = medical drug



Scheme 2. Synthesis of H-Val-Arg-Asn-Pro-Pro-Pro-drug molecule and H-Val-Arg-Gln-Pro-Pro-Pro-drug molecule, where Yaa = Asn or Gln, and MD = medical drug

CONCLUSION

We synthesized 4 new peptides analogues of SAP with potential cell penetrating properties. A new interesting strategy for synthesis of Gln and Asn containing peptides starting form Glu and Asp and using Rink amide resin was applied for the synthesis of two of the target peptides. The biological investigations on the cell penetrating properties of newly synthesized peptides are in the progress.

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СИНТЕЗ НА НОВИ ХИБРИДНИ МОЛЕКУЛИ ВКЛЮЧВАЩИ МЕМБРАННО ПРОНИКВАЩИ ПЕПТИДИ И ЛЕКАРСТВЕНИ МОЛЕКУЛИ

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(Резюме)

През 1988 е отрито забележителното свойство на група пептиди да преминават през клетъчната мембрана, независимо от мембранните рецептори. Те предизвикват промени в мембраната, водещи до поява на пропускливост. Тези молекули са наречени мембранно проникващи пептиди (CPP). Един представител на тази група съединения е т.нар. „sweet arrow” пептид (SAP). В тази работа ние докладваме синтезата на 4 аналога на SAP. Описани са и някои интересни подходи за синтез на пептиди включващи Asn и Gln изхождайки от съответстващите им киселини Glu и Asp, свързани към Rink амиден твърдофазен носител.