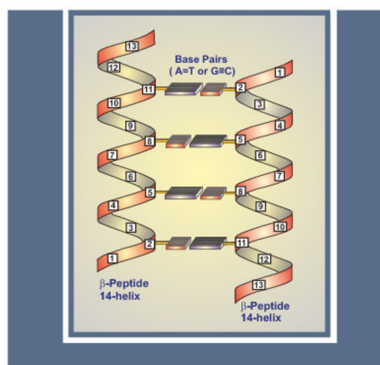
Pradip Chakraborty (Autor)

Design, Synthesis, and Structural Investigation of Nucleobase Functionalized β-Peptides

DESIGN, SYNTHESIS, AND STRUCTURAL INVESTIGATION OF NUCLEOBASE FUNCTIONALIZED β-PEPTIDES

Pradip Chakraborty



Cuvillier Verlag Göttingen

https://cuvillier.de/de/shop/publications/2517

Copyright:
Cuvillier Verlag, Inhaberin Annette Jentzsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen, Germany
Telefon: +49 (0)551 54724-0, E-Mail: info@cuvillier.de, Website: https://cuvillier.de

1 Introduction

In proteins three types of secondary structures helix, sheet, and turn are common motifs. The α-helix is one of the most fundamental secondary structures found in almost all naturally occurring proteins. The α-helical secondary structure was first described in 1951 by Pauling and Corey.[1] Helical protein motifs can be found in a large number of naturally occurring proteins. Furthermore, several helical proteins are directly involved in many important biological activities (Figure 1.1). The relationship between the secondary structures of a protein and its three-dimensional structure sometimes is not clearly understood because of the complexity of the protein structures. Despite extensive research in this field, the understanding of the pathway by which a linear polypeptide chain folds into its unique three dimensional structure remains one of the challenging tasks in the field of peptide research.

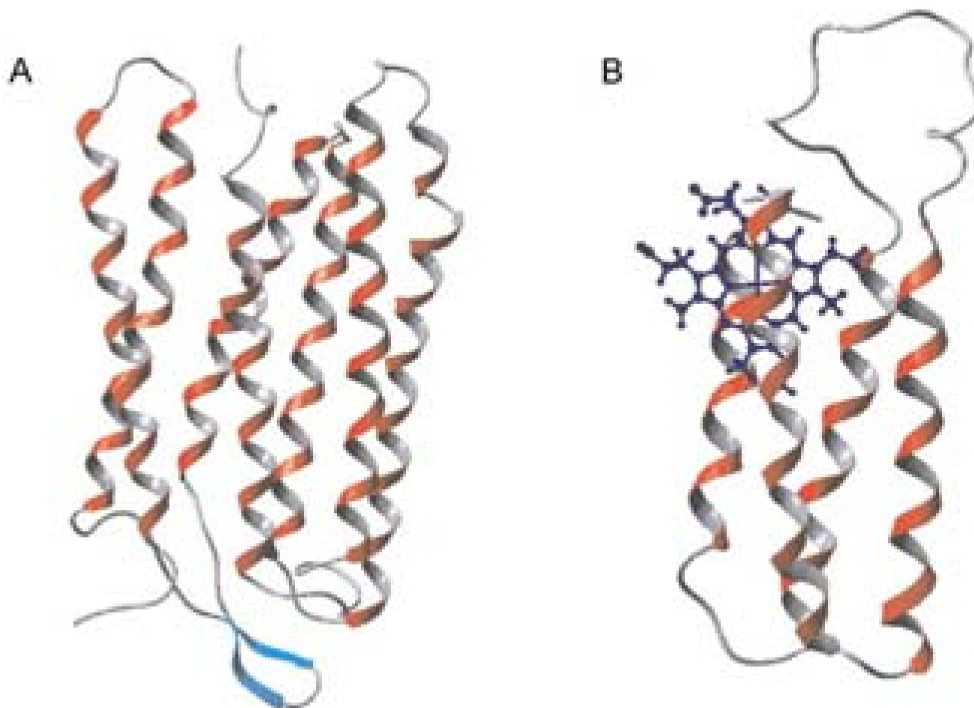


Figure 1.1: Helical proteins are responsible for many biological functions. A) *Bacteriorhodopsin* (PDB code 1KME) is a well known seven-helix bundle trans-membrane protein with short interconnecting loops which acts as a light-driven proton pump in *Halobacterium salinarum*;[2] B) *Cytochrome b-562* (PDB code 1QPV) is a four-helix bundle heme binding protein containing a non-covalently bound iron-protoporphyrin IX.[3]

De novo protein design has shown to be a powerful tool which provides the opportunity to understand the mechanism of protein folding, structure, and function.[4,5] This method involves the design of a sequence that is intended to fold into a predetermined three dimensional structure comparable to that of the natural protein. The *de novo* protein design also provides an opportunity to investigate the structure-activity relationship between proteins or between proteins and nucleic acids. The construction of protein-like folding motifs as structurally stable scaffolds for the introduction of functional properties represents a major goal in protein design. For any protein to fold into predetermined tertiary structures, the amino acid side chains should utilize various non-covalent forces including hydrophobic, van der Waals, and electrostatic interactions to organize in a compact stable conformation. As a long term goal, this approach could even be applied for the construction of several helical well-defined tertiary structures. Till date the design is based on the use of α-peptides.[5,6] But several other unnatural molecules are known to adopt well-defined secondary structures.[10] Especially, oligomers composed of β-amino acids (β-peptides) are interesting as very stable helices that could be obtained even with six to seven residues.[7] A β-peptide 14-helix contains approximately three residues per turn, which results in the orientation of every third residue on the one side of the helix. Such a 14-helix might be suitable to build an artificial helical organization by noncovalent interactions. However, use of hydrophobic interactions is not suitable for the β-peptide 14-helix association since 14-helices can not form a wide hydrophobic face. Therefore, an alternative non-covalent interaction force has to be considered. In this regard, hydrogen bonding interactions of nucleobases are very interesting and this force was used by the nature successfully to assemble double strands of nucleic acids DNA and RNA.[8] Nucleobase pairing is a powerful tool in the preparation of complex molecular architecture in the field of supramolecular chemistry and molecular recognition. The use of nucleobase pairing should offer the possibility to construct a well-defined β-peptide tertiary architecture. Therefore, covalent functionalization at the side chains of β-amino acids with nucleobases is necessary for the non-covalent assembly of the β-peptide helices to organize in a well-defined tertiary structure.

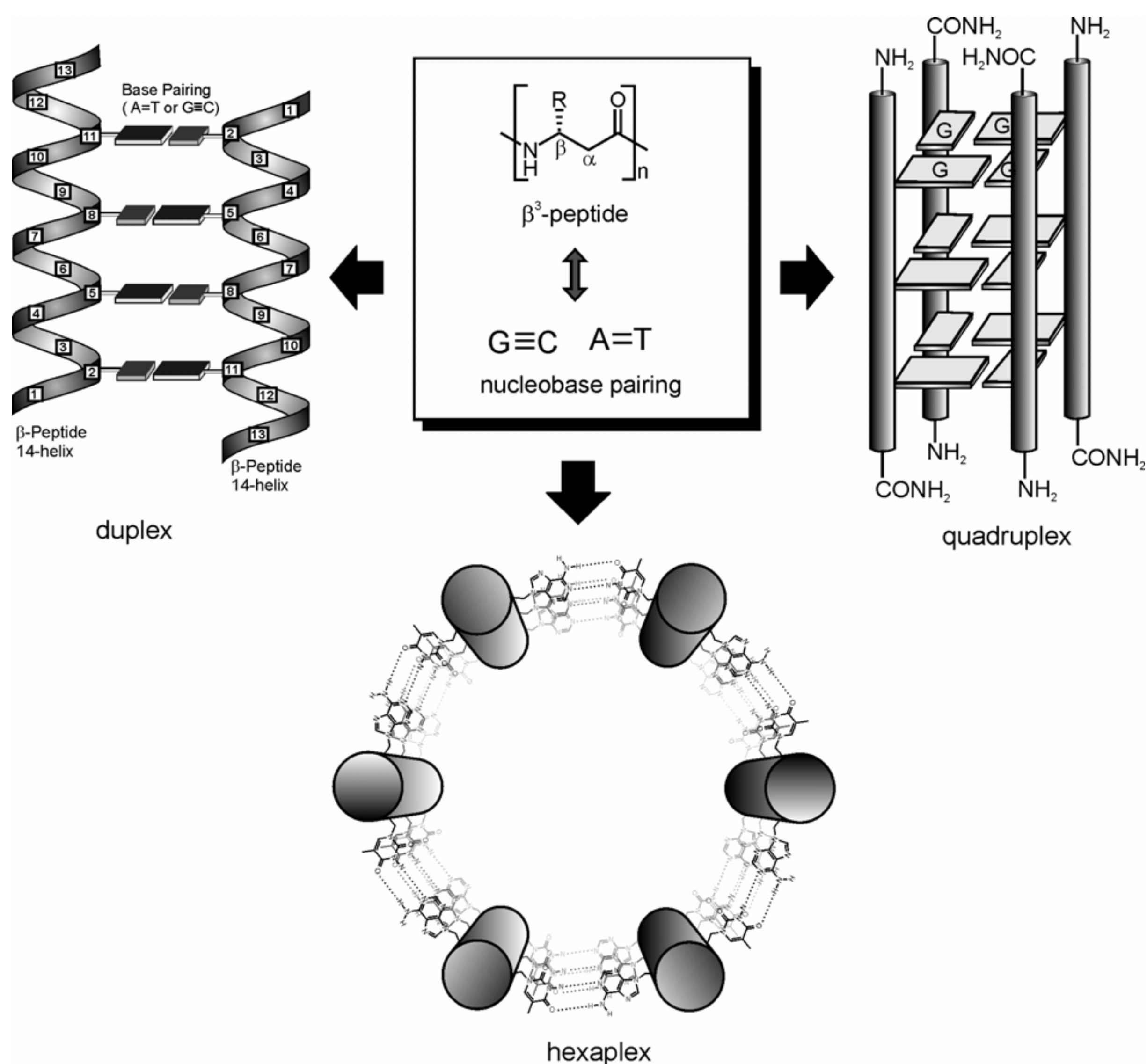


Figure 1.2: Model for the association of β-peptide helices by nucleobase pairing. Nucleobase-functionalized β-peptides could be organized in well-defined duplex, quadruplex, and hexaplex structures.

Functionalization of every third side chain of a β-peptide 14-helix by a nucleobase results in a linear orientation of the nucleobases on the same side of the helix (Figure 1.2). Such a design should be ideal to recognize an additional 14-helix with a complementary nucleobase sequence to form a dimeric or a tetrameric helical structure (Figure 1.2). Similarly, a β-peptide 14-helix with two different base sequences on the two sides of the same helix may form a hexameric architecture (Figure 1.2).

The aim of the current study is to construct well-defined complex helical architecture based on nucleobase functionalized β-peptides and to investigate their specificity and stability (Figure 1.2). In the current report the following tasks will be reported:

- Development of a new synthetic route towards adenine and 7-deazaguanine functionalized nucleo-β-amino acids.
- Design and synthesis of novel β-peptides functionalized with all four canonical nucleobases and their association behavior in solution
- The structural investigation of hydrogen-bonding complexes of β-peptides (Figure 1.2) by temperature dependent UV spectroscopy, circular dichroism spectroscopy, and mass spectrometry.

2 Artificial helical secondary structures

2.1 Foldamers

In nature there are only three major biopolymer backbones and they are proteins, DNA and RNA that fold into well-defined secondary structures.[8] Such specific and well-defined structures are responsible for many crucial biological functions. The biological evaluation of protein folding is not clearly understood. Therefore, there is need to design various artificial polymers that fold into well-defined secondary structures. Over the years chemists have contributed substantial efforts to generate unnatural oligomeric sequences and synthetic macromolecules that take on well-defined conformations in solution. However, the principles of protein design are not restricted to the use of α-peptides, but can be extended to any polymer with a tendency to fold into the periodic and compact structures referred as foldamers.

A foldamer is defined as an oligomer that folds into a conformationally ordered state in solution, a structure that is stabilized by a collection of noncovalent interactions between nonadjacent monomeric units.[9,10] There are two major types of foldamers: single-stranded foldamers and multiple-stranded foldamers. Recently, several peptidomimetic foldamers have attracted a lot of attention because of their unique conformations and interesting bioactivities.[10] However, the foldamers that fold into the helical secondary structures are commonly observed. A variety of synthetic polymers such as β-peptides,[9,15] γ-peptides,[11] δ-peptides,[12] peptiods,[13] and other synthetic molecules[14] have shown to adopt well-defined secondary structures. These unnatural polymers may provide an excellent medium for the design of biomimetic structures with practical applications in the field of pharmaceuticals and material science.

2.2 β-Peptides

The family of β-peptides is a major class of foldamers studied over the years due to their unique folding behavior in solution.[7,15] In comparison to α-peptides, β-peptides are more flexible due to the presence of an additional methylene group. Over the decades various synthetic oligomers with conformations similar to natural peptides and proteins have been studied extensively to increase our understanding of protein folding and stability.[10] Especially, β-peptides have received considerable attention by many research groups due to their structural diversities.[7,15,16,17]

At least 15-20 α-amino acids are required to form a stable α-helical secondary structure. Considering the higher conformational flexibility of β-peptides, one might expect that even a longer backbone would be required to obtain a stable helical secondary structure. However, it was shown that β-peptides composed of as few as six β-amino acid residues form surprisingly stable helices.[15] The β-peptide oligomers are resistant towards enzymatic degradation and have little tendency for the H/D exchange of the central amide protons.[18] Recently, β-peptides are shown to have versatile biological activity.[19] Furthermore, it was shown that β-amino acids have a significant potential to function as powerful peptidomimetics.[20] In the last decade several research groups have contributed in this field and have concluded that short β-peptide oligomers form surprisingly stable helices in solution and in solid state. Seebach's group has focused on the β-peptides composed of conformationally flexible acyclic β-amino acids.[15,21] Whereas, Gellman and coworkers have focused on conformationally constrained cyclic residues with limited degree of freedom about the C_α–C_β bond.[7]

The conformation of β-peptides can be analyzed in terms of the main chain torsional angles, which are assigned as ω, θ, ϕ, and ψ (Figure 2.1A) according to the convention of Balaram.[22] Folded helical or turn-like conformations of β-peptides require a gauche conformation about the θ torsion angle defined by the C_2-C_3 bond. The substitution pattern at position 2 and 3 influence the torsional angles and thereby determine the nature of the overall secondary structure. β-Peptide oligomers that adopt helical conformations are composed of C_2 and/or C_3 substituted β-amino acids.

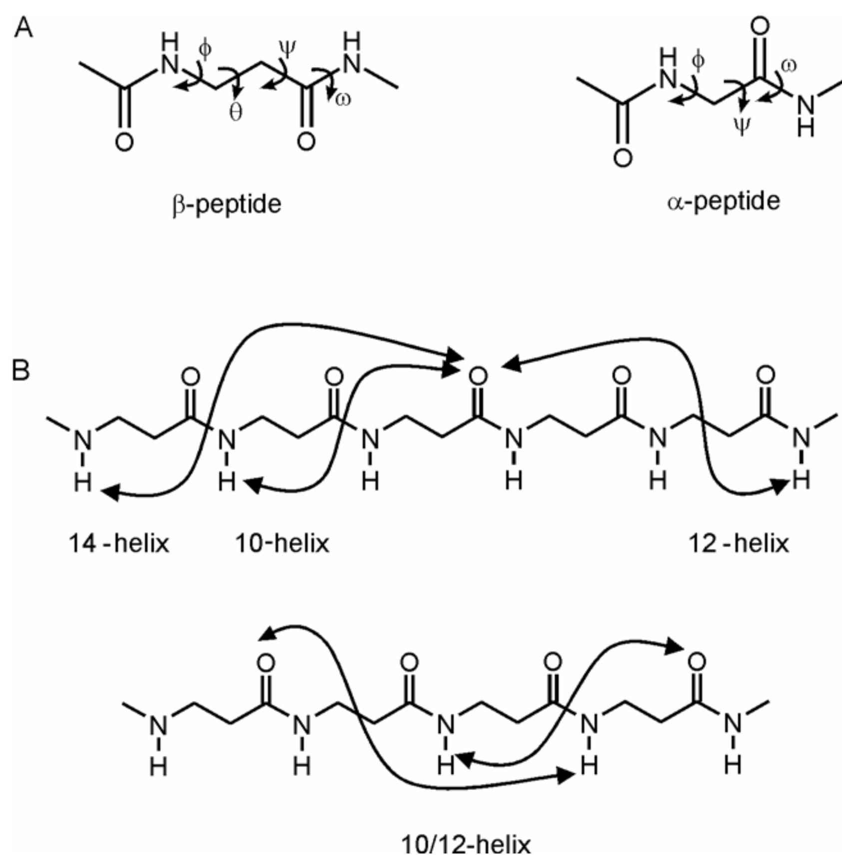


Figure 2.1: A) The main chain torsional angles of a β-peptide and an α-peptide; B) nomenclature for β-peptide helices based on different possible hydrogen-bonding patterns.[10]

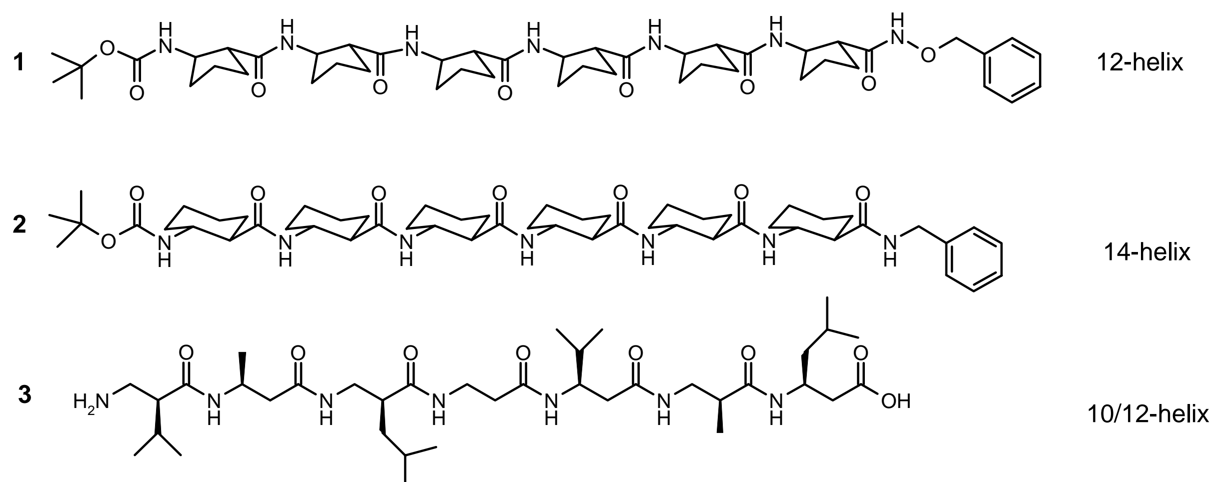


Figure 2.2: β-Peptides **1-3** adopt different helical conformations (12-helix, 14-helix and 10/12-helix) in solution.

Several helical secondary structures are known for β-peptides including the 14-helix, 12-helix, 10/12-helix, and 10-helix. The nomenclature of the β-peptide helices is based

on the pattern of hydrogen bonding and the number of atoms in the hydrogen-bonded rings as shown in Figure 2.1B. For example β-peptide **1** folds in a 12-helical conformation, β-peptide **2** in a 14-helical conformation, and β-peptide **3** is known to fold into a 10/12-helical conformation (Figure 2.2). Although helical conformations are most common structural motifs for α-peptides, they are significantly different from β-peptide helices. A comparison of an α-helix with the β-peptide helices is represented in Figure 2.3.

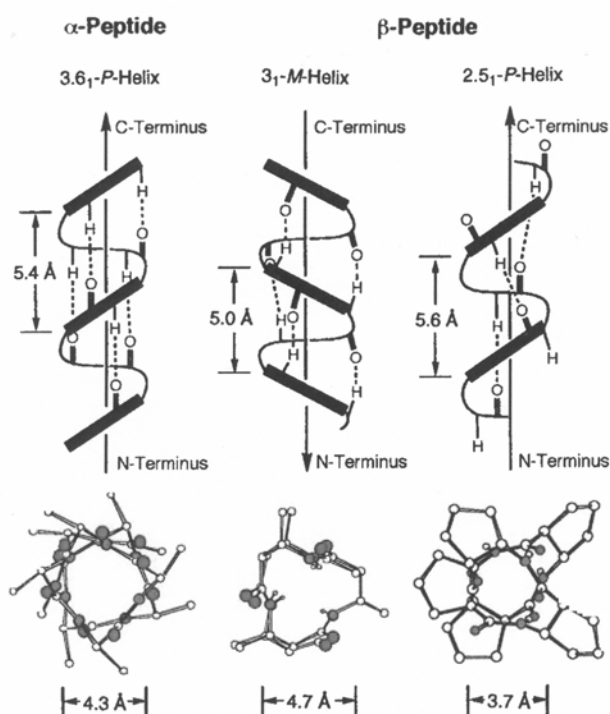


Figure 2.3: Schematic representation of three helices: regular α-helix, 3_1-helix (14-helix) and β-peptide 2.5_1-helix (12-helix). This figure shows the polarities, pitches and diameters of the helices and their views along the axis.[15]

All three helical structures differ from each other significantly by polarities with respect to their *C*- and *N*-termini, radius, and number of residues per turn. The 14-helical structure is stabilized by hydrogen bonding between an amide proton (N-H) at position *i* and a main chain carbonyl group (C=O) at position *i+2*, forming a series of intercatenated 14-membered rings (Figure 2.1). The overall structure of the 14-helix differs from that of the α-helix in many aspects like radius, polarity, and overall dipole. The net dipole of the 14-helix is opposite to that of an α-helix as the amide carbonyl

and NH groups project towards the *N*- and *C*-terminus, respectively. Furthermore, a β-peptide 14-helix has approximately three residues per turn, which positions the side chains of every third residue on the same side of the helix with linear orientation. Gellman and coworkers have shown that the short β-peptide oligomer **2** composed of *trans*-2-aminocyclohexanecarboxylic acid (ACHC) adopts a 14-helical conformation in solid state and in solution.[23,24]

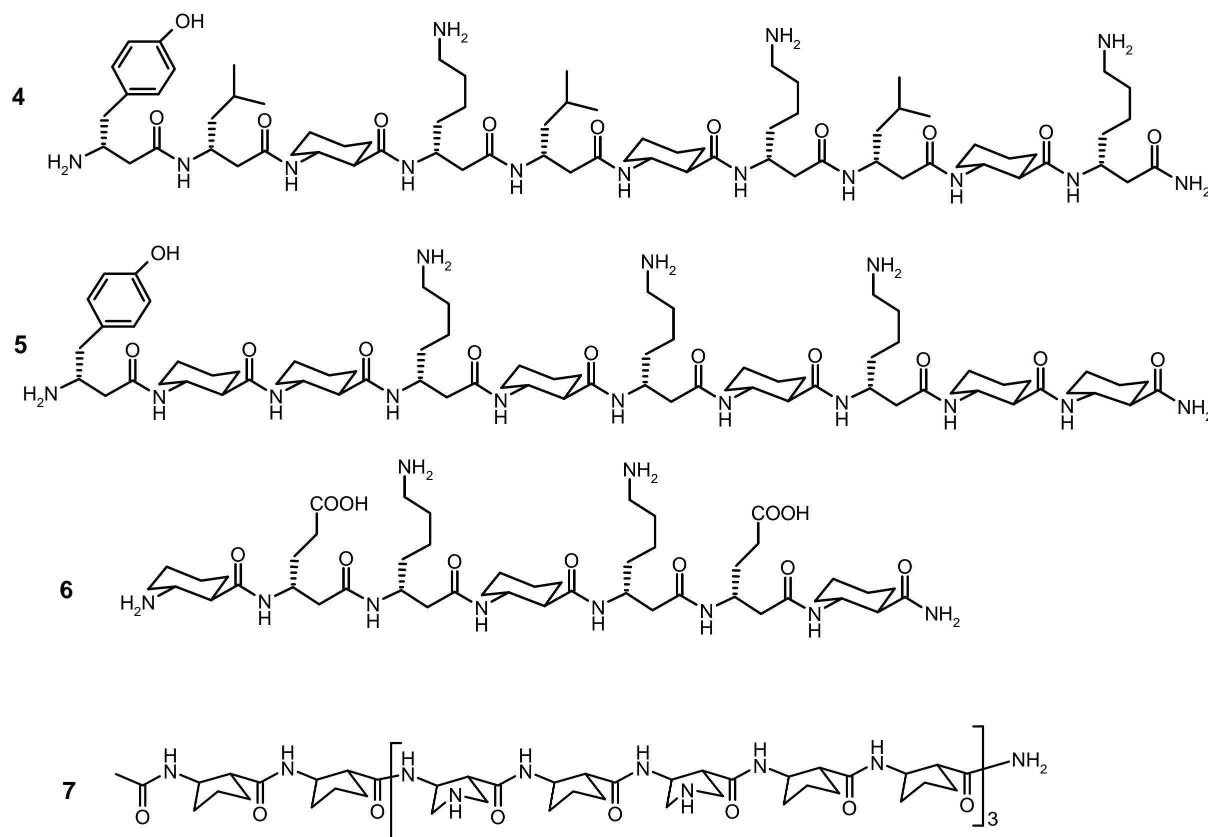


Figure 2.4: β-Peptides **4-7** were designed with conformationally constrained cyclic β-amino acids (ACHC and APC), which adopt a helical conformation in solution.

The CD spectra of several β-peptides, which adopt left-handed 14-helices, show a maximum near 195 nm and a minimum near 215 nm (and *vice-versa* for the right-handed 14-helices). The intensity of the CD-spectrum of the 14-helix depends on the chain length and helix propensity. As chain length increases, the intensity of the CD signal also increases.[25] So far a variety of β-peptides are known to fold into the 14-helical conformation. Amphiphilic β-peptides **4** and **5** composed of conformationally constrained ACHC and acyclic β-amino acids fold into a 14-helical