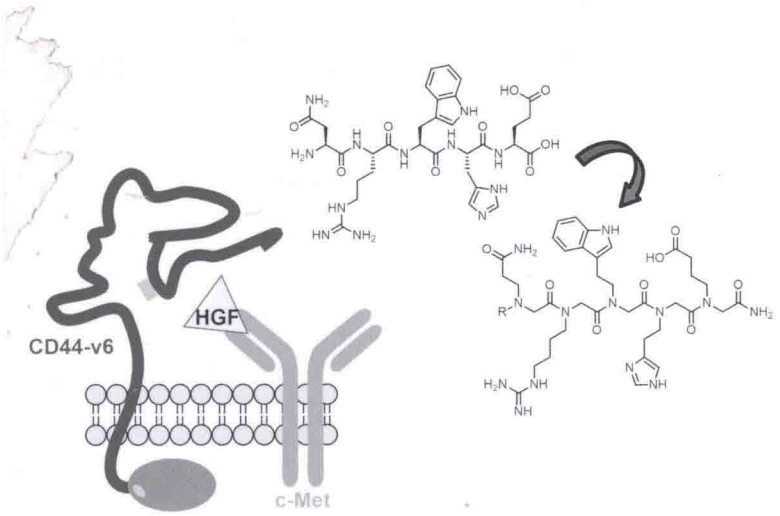
Carmen Cardenal Pac

Design, Synthesis and Evaluation of Highly Functionalized Peptoids as Antitumor Peptidomimetics

Beiträge zur organischen Synthese
Hrsg.: Stefan Bräse



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Design, Synthesis and Evaluation of Highly Functionalized Peptoids as Antitumor Peptidomimetics

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Die vorliegende Arbeit wurde in der Zeit vom 10. Oktober 2010 bis zum 06. November 2013 am Institut für Organische Chemie des Karlsruher Instituts für Technologie (KIT) unter der Leitung von Herrn Prof. Dr. Stefan Bräse durchgeführt.

Hiermit versichere ich, die vorliegende Arbeit selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet sowie die Zitate kenntlich gemacht zu haben.

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1. Abstract

Peptides and proteins are involved in a diverse range of essential processes in living organisms, and, therefore, they are of great interest for medical and biological applications. However, their fast degradation by proteases and, consequently, their poor bioavailability make them unsuitable as therapeutic agents. Peptoids are structural isomers of peptides with improved proteolytic resistance, good cell penetrating properties and easily accessible and derivatizable synthesis. Therefore peptoids are promising peptidomimetics for therapeutic applications.

The aim of this project was the design, synthesis and evaluation of peptoid analogs of short amino acid sequences with antitumor activity.

A small library of peptoids was synthesized mimicking a pentapeptide that inhibits ligand dependent Met activation by competing with the coreceptor function of v6-containing CD44. In addition to functional group modifications, derivatives with reduced flexibility could be obtained by incorporating α-chiral submonomers, peptide-peptoid hybrids or cyclization. Many of the synthesized peptoids were able to inhibit HGF induced Met activation and block scattering and migration of tumor cells like the original peptide. Comparison of the inhibitory ability of the peptide and the different peptoid analogs led to the establishment of some structure-activity relationships. Moreover, the peptoid that exhibited the strongest inhibitory effect *in vitro*, was also able to reduce the number of liver metastatic events and decrease angiogenesis *in vivo*, holding high potential for cancer therapy.

Peptoid analogs of a sequence of the BAG-1 protein inhibiting tumor cell growth in prostate cancer were also prepared. While addition of the peptoids alone was not able to reproduce the effect of the protein, the fact that rhodamine-labeled analogs were able to penetrate the cell and partially colocalized with the receptor, opens the way to the development of new inhibitors combined with targeting sequences.

Furthermore, a method for the synthesis of amide-containing submonomers as free bases and their incorporation into peptoid oligomers has been described. Different-length homo-oligomers of α-chiral amide residues were successfully synthesized for structural investigations *via* circular dichroism spectroscopy.

Kurzzusammenfassung

Peptide und Proteine sind in lebenden Organismen an einer Vielzahl von essentiellen Prozessen beteiligt und sind daher von großem Interesse für medizinische und biologische Anwendungen. Allerdings sind sie aufgrund ihres schnellen Abbaus durch Proteasen und der daraus folgenden schlechten Bioverfügbarkeit ungeeignet um als therapeutische Wirkstoffe eingesetzt zu werden. Peptoide sind strukturelle Isomere der Peptide und zeichnen sich besonders durch ihre höhere Stabilität gegenüber enzymatischem Abbau, ihre guten Zell penetrierenden Eigenschaften, sowie ihre einfache und derivatisierbare Synthese aus. Daher sind Peptoide äußerst vielversprechende Peptidomimetika für therapeutische Anwendungen.

Das Ziel dieses Projekts war es Peptoid-Analoga von kurzen Aminosäure-Sequenzen mit Antitumor-Aktivität zu entwickeln, zu synthetisieren und zu evaluieren.

Im ersten Projekt wurde eine kleine Peptoid-Bibliothek synthetisiert, welche ein Pentapetid nachahmt. Dieses Pentapeptid fungiert als Inhibitor für die Liganden-abhängige Aktivierung von Met, indem es mit der Korezeptor-Funktion von v6-haltigem CD44 konkurriert. Neben Peptoiden mit modifizierten funktionellen Gruppen wurden auch Derivate mit reduzierter Flexibilität erhalten. Dies konnte durch die Einführung von α-chiralen Submonomeren, die Darstellung von Peptid-Peptoid-Hybriden oder durch Cyclisierung erreicht werden. Viele der dargestellten Peptoide besaßen die gleiche Aktivität wie das ursprüngliche Peptid und konnten die HGF-induzierte Met-Aktivierung verhindern, sowie das Streuen und die Migration von Tumor-Zellen blockieren. Durch einen Vergleich der inhibierenden Wirkung des Peptids und der unterschiedlichen Peptoid-Analoga konnten einige Struktur-Aktivitätsbeziehungen aufgestellt werden. Außerdem konnte das Peptoid, welches *in vitro* die stärkste inhibierende Wirkung besaß, *in vivo* sowohl die Anzahl der Lebermetastasen reduzieren als auch die Angiogenese verringern. Dieses Peptoid besitzt somit ein hohes Potential für die Krebs-Therapie.

Weiterhin wurden auch Peptoid-Analoga von einer Sequenz des BAG-1 Proteins, welches das Wachstum von Tumor-Zellen in Prostata-Krebs inhibiert, dargestellt. Die Peptoide waren jedoch alleine nicht dazu in der Lage den Effekt des Proteins zu reproduzieren. Allerdings konnten Rhodamin-markierte Peptoid-Analog die Zelle penetrieren und kolokalisierten zum Teil mit dem Rezeptor. Dies ermöglicht die Entwicklung von neuen Inhibitoren, welche mit zielspezifischen Sequenzen kombiniert werden.

Zudem wurde eine Methode entwickelt, welche die Synthese von Amid-haltigen Submonomeren als freie Basen und deren Einführung in Peptoid-Oligomere ermöglicht. Für Struktur-Untersuchungen mittels Zirkulardichroismus-Spektroskopie wurden unterschiedlich lange Homo-Oligomere mit α-chiralen Amid-Resten dargestellt.

2. Introduction

Peptides perform a wide variety of essential functions in living organisms with great specificity and precision. Their diverse roles include hormones, toxins, enzymes, antibodies, receptors, neurotransmitters, structural proteins, etc.[1]

However, the use of peptides for biological and therapeutical applications is limited, mainly due to their sensitivity to proteolytic degradation, which results in poor bioavailability. For this reason there is a strong interest in developing compounds that mimic the structure and/or function of peptides and proteins overcoming their drawbacks.

2.1 Peptidomimetics

Peptidomimetics were initially defined by Giannis and Kolter as "compounds that imitate or block the biological effect of a peptide at the receptor level".[2] One year later Gante extended the definition to include both peptide function and structure: "a peptidomimetic is defined as a substance having a secondary structure as well as other structural features analogous to that of the original peptide, which allows it to displace the original peptide from receptors or enzymes".[3]

An ideal peptidomimetic should be resistant to proteolytic degradation, improve the oral bioavailability of the original peptide, and increase its effectivity and selectivity, reducing possible side-effects.[3]

Most peptidomimetics are oligomers derived from natural peptides with modifications in the backbone and/or in the side-chains. In addition, there are also non-oligomeric peptidomimetic compounds that have no apparent structural similarity with the original peptide. These are usually discovered by random screening of natural products and compound libraries. A classical example is morphine (**1**) that binds an opioid receptor, mimicking the peptide β-endorphin (composed of 31 amino acids), and has long been used as an analgesic (Figure 1).[2] Rational design and computer modeling have also led to the development of therapeutically relevant peptidomimetics like the HIV-protease inhibitor Ritonavir (**2**) (Figure 1).[4-5]

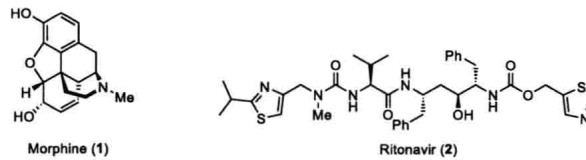

Figure 1: Examples of non-oligomeric peptidomimetic drugs.

A commonly used strategy for side-chain modification is the replacement of natural by unnatural amino-acids. For example, switching phenylalanine for the more sterically demanding diphenylalanine led to a potent angiotensin II agonist.[6]

Modification of the peptide backbone has afforded many types of functional peptidomimetics. Figure 2 depicts the most representative examples conserving the amide linkage.

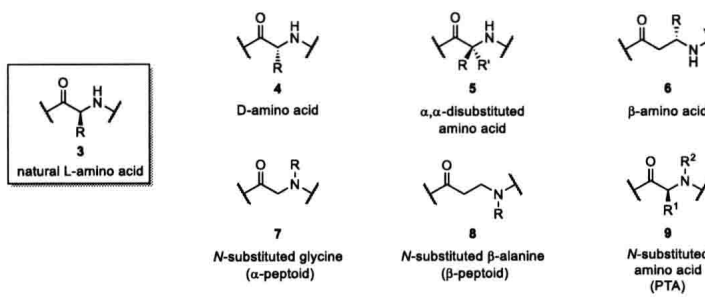

Figure 2: L-Amino acid (**3**) and derived monomer units with modified backbones.

Peptides assembled from D-amino acids (**4**) exhibit a higher proteolytic stability than their natural analogs and have shown promising results in immunological applications.[7] The desire to create peptide analogs with reduced flexibility led to the development of α,α-disubstituted amino acids (**5**). Thanks to the extra substitution at the α-carbon, these oligomers were able to adopt new secondary structures.[8] β-Peptides (**6**), first described by Seebach and coworkers,[9] differ from α-peptides in the presence of an extra methylene unit in their backbone. Despite their increased flexibility, β-peptides are able to form stable secondary structures and a number of biological activities have been reported. They hold special promise in the treatment of autoimmune diseases.[10] The formal shift of the side-chain from the α-carbon to the amide nitrogen afforded new types of peptidomimetics: α- and β-peptoids (**7** and **8**). α-Peptoids have attracted a great amount of interest, probably due to their easily accessible synthesis. Key aspects of α-peptoid research have been summarized in the

following chapters. β-peptoids have been less investigated but several applications as antimicrobial agents have been reported.[11] Recently, Gao and Kodadek described the synthesis of a combinatorial library of peptide tertiary amides (PTAs) (**9**).[12] These conformationally constrained oligomers show great potential as protein ligands.

Oligomeric peptidomimetics where the amide bond is replaced by an isostere have also been explored. Some examples are peptidosulfonamides,[13] urea-peptide hybrids,[14] and triazol peptidomimetics.[15-16]

2.2 Peptoids

Peptoids, oligomers of *N*-substituted glycines, are a prominent class of peptidomimetics, pioneered by Bartlett and coworkers in 1992.[17] The word "peptoid" had been previously suggested by Farmer and Ariëns to refer to substances that could mimic the biological activity of a peptide but were structurally different.[18] Bartlett *et al.* specifically applied the term to *N*-substituted glycine oligomers, as it is commonly used today. Peptoids are synthetic regioisomers of peptides in which the typical amino acid side-chain at the α-carbon has been formally shifted to the amide nitrogen (Figure 2). This structural change improves their stability against enzymatic degradation[19] as well as their membrane-permeability,[20] both desirable characteristics for therapeutic applications.

Unlike peptides, peptoids do not contain stereogenic centers in their backbone. This feature simplifies their synthesis avoiding racemization and epimerization problems. Moreover peptoids are unable to form hydrogen bonds along their backbone, which are responsible for the stabilization of peptide secondary structures. There is a considerable amount of ongoing research about the tridimensional structure of peptoids and how it affects their activity. A few strategies have been developed to favor specific structures and will be discussed in Chapter 2.2.2.

The main advantage of peptoids with respect to other types of peptidomimetics is their ease of synthesis on solid support *via* the submonomer method developed by Zuckermann and coworkers.[21] This modular approach allows the incorporation of a wide diversity of functionalities and can be applied to the synthesis of libraries and high-throughput screening methods.

2.2.1 Peptoid synthesis

Peptoids are usually synthesized on solid-phase, a strategy developed by Merrifield in 1963 for the synthesis of peptides.[22] Here, the first building block (in this case the monomer at the *C*-terminus) is covalently attached to a solid support (typically a cross-linked polystyrene resin) by a linker that can be selectively cleaved at the end of the synthesis. The desired molecule is assembled by a series of heterogeneous reactions alternated with washing steps. Reagents can be used in excess, which may lead to higher yields, and are easily removed by filtration at the end of each reaction, together with unbound by-products. Thanks to the simplicity of operation solid-phase synthesis (SPS) can be easily automated.[23] After cleavage from the resin the final products are usually purified by HPLC (High Performance Liquid Chromatography).

Two common acid-labile linkers are depicted in Figure 3. After acidic cleavage the Rink-amide linker leaves an amide group at the *C*-terminus, whereas the chlorotrityl chloride linker leaves a carboxylic acid, which can be further modified e.g. *via* cyclization or bioconjugation. This second linker can also be cleaved under mild conditions (1,1,1,3,3,3-hexafluoroisopropanol, HFIP) avoiding removal of acid-labile protecting groups.

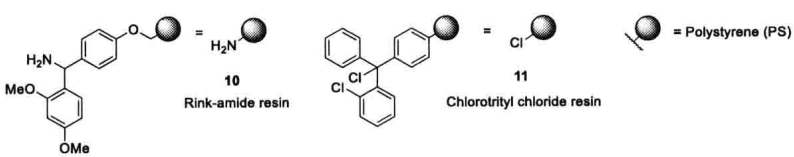

Figure 3: Chemical structures of the Rink-amide- (**10**) and chlorotrityl- (**11**) functionalized resins for solid-phase synthesis.

In addition to the traditional solid-phase synthesis on polystyrene resins, peptides and peptoids have also been synthesized on cellulose membranes (SPOT synthesis).[24-25] This support is particularly suitable for the screening of combinatorial libraries, since several biological tests can be performed directly on the membrane. There are also examples of peptoid synthesis in solution.[26-27]

On solid-phase, peptoid oligomers can be synthesized *via* two main approaches known as monomer and submonomer methods.