1. Introduction and Outline

DNA (deoxyribonucleic acid) is the major target for many metal based antitumor drugs. The interactions between metal complexes and DNA are determined by the structural and kinetic properties of the metal complex, but also by its affinity towards the biopolymer caused by the structural and electrostatic characteristics of both, ligand and DNA.[1-5] Regarding kinetics, some metal coordinating compounds with relatively slow metal-ligand exchange rates, comparable to rates of cell division processes, are very active in killing cancer cells. The classical example is that of cisplatin, but also of other platinum based drugs. Improving the understanding of the antitumor action mechanism, as well as the development of new platinum based drugs with higher target specificity has always been considered a challenge for research.

Cisplatin (*cis*-diamminedichloroplatinum(II), cDDP, Platinol, Platinol-AQ) is one of the most successful metal-based anticancer drugs discovered almost 50 years ago.[6-8] Its main intracellular target is nuclear DNA. The antitumour activity derives from its capacity to form especially intrastrand crosslinks between two adjacent guanine or adenine-guanine bases.[9-13] The resulting DNA-GG/AG adducts induce disruption of the base pairing within the double helical structure with the bending of the helix with consequences on replication and transcription machinery (Figure 1.1).[14-18] Bent structures are recognized by proteins, such belonging to the high mobility group (HMG proteins), which shield the DNA from repair processes, and thus, leading to induction of apoptosis.[13,19-21]

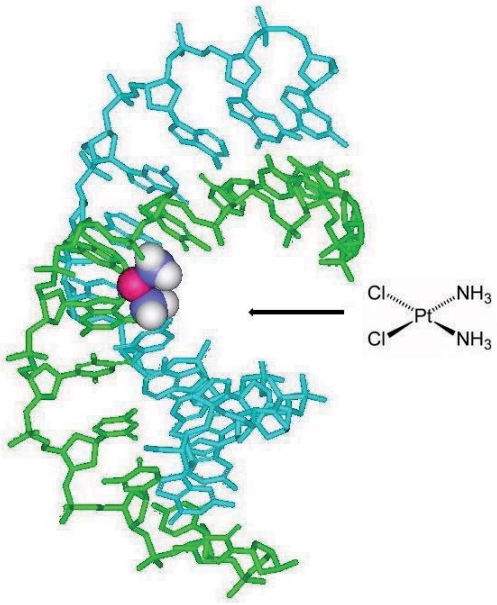

Figure 1.1 Cisplatin-GG-intrastrand DNA adduct.[11]

Modification and optimization of the anticancer drug cisplatin is still of interest with respect to enhanced selective cell targeting and DNA binding efficiency. Although novel platinum based anticancer drugs have been developed, their structural variation is rather limited.[22] Typically, a cationic metal complex or metabolite is used to enable electrostatic interaction with the polyanionic DNA backbone.[23-25] Association of such complexes to the DNA backbone facilitates covalent binding,[26-30] and thus, can be expected to improve the therapeutic efficacy. The lower *pH*- and the lower chloride concentration inside the cells (especially the cancer cells), compared to the blood stream, allow hydrolysis of cisplatin complexes in the cellular environment. This leads to formation of positively charged species with an increased affinity towards its cellular target.[31,32] Hence, the mechanistically importance of the charged metal complex is indicated.

Attractive approaches include modification of the platinum coordination sphere, as well as the design of hybrid molecules of the cisplatin binding moiety. In such approaches, peptide motifs seem to be promising candidates. Peptides with cell penetrating, directing or recognizing properties can be implemented.[33-37]

In this study, positively charged peptides were investigated with the potential of inducing DNA structural distortions, such as bending, caused by charge neutralization of the dsDNA helix.[38,39] Placement of fixed charges near the negatively charged DNA surface should induce bending through asymmetric reduction or enhancement of the inter-phosphate repulsive forces (responsible for the DNA stiffness). The resulting bent DNA is more likely to facilitate binding of cisplatin derivatives. The approach presented in this work for the design of cisplatin analogs with potentially improved reactivity and selectivity is based on platinum/peptide chimera.

Two different approaches were followed to combine DNA kinking with facilitated and specific covalent platinum binding: i) the use of *nonspecific* DNA binding peptides;[40] ii) the use of *specific* DNA binding peptides derived from proteins or natural products.[41]

The first approach makes use of platinum-peptide conjugates in which the cisplatin analogue is covalently linked to peptides that vary with respect to the number of positively charged amino acids. We anticipate that the presence of charged peptides should facilitate in particular DNA pre-association by adhesion at the DNA phosphodiester backbone, followed by bending of the DNA target (Figure 1.2). Bending of DNA is thought to be essential in the nucleobase platination mechanism since DNA is bent in the resulting covalently platinated product.[20]

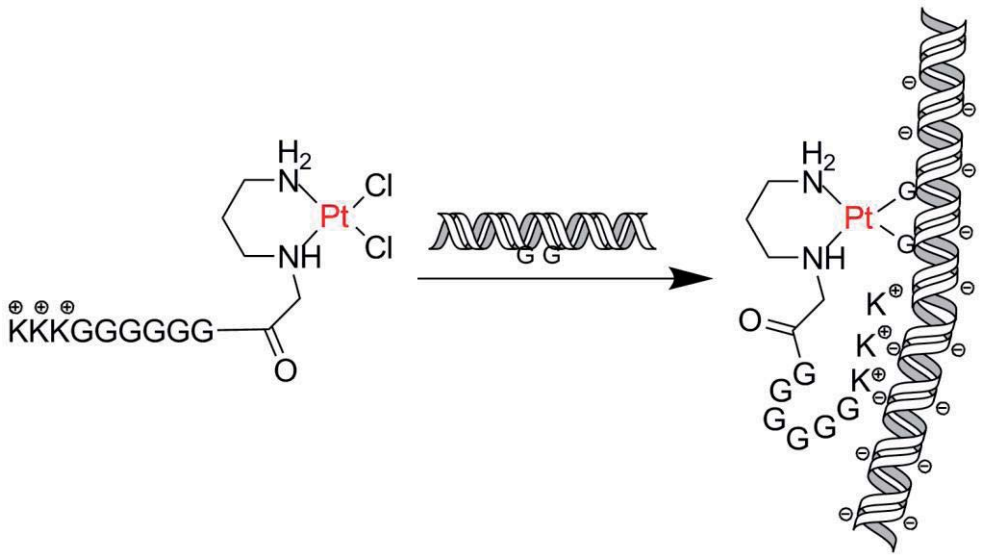

Figure 1.2 Representative example of cisplatin analog based on *nonspecific* DNA binding platinum/peptide conjugate.

In the second approach, the Integration Host Factor (IHF) cocrystal structure with double stranded DNA[42-44] (Figure 1.3, left) serves as a lead concept for designing peptides that not only bind to DNA, but also induce bending.[41] This approach uses the DNA bending mimic of IHF in combination with a covalently bound platinum binding site as cisplatin analogue. It is assumed for this construct to provide a double effect by influence on both, DNA binding ability and specificity.

IHF is a small heterodimeric protein that functions as an architectural factor in many cellular processes of prokaryotes. It consists of an α-and β-subunit, that specifically bind to the minor groove of DNA and bend the double strand by about 180°.[43,44] The stacking interactions between the DNA bases are interrupted by intercalation of proline residues situated at the tip of the two flexible β-ribbon arms. The bend is stabilized by interactions with the positively charged α-helical body of the protein due to its contents of basic amino acids, such as arginine or lysine.[45]

The chosen IHF mimicking peptide (Figure 1.3, right) includes a small lysine dendrimer which is linked to a cyclopeptide core, responsible for specific DNA recognition in the minor groove, followed by bending of the double strand.[41] Because of the high nett positive charges, the lysine dendrimer imitates the body of

the protein and stabilizes bending by electrostatic interactions. More precisely, the second strategy involves incorporation of newly synthesized artificial amino acids with a suitable donor atom pattern for platinum coordination, in the linker region of the IHF mimicking peptide, followed by platination.

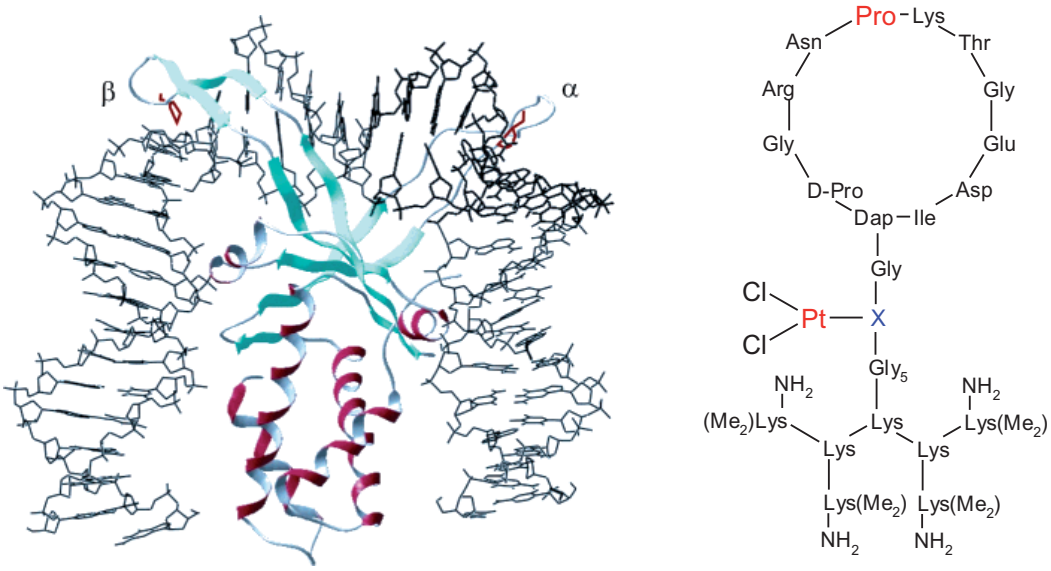

Figure 1.3 DNA-IHF cocrystal structure (left).[44] Schematic representation of *platinated IHF mimicking peptide* (right) with DNA interacting residues (red) and unnatural amino acids containing the platinum coordinating site (blue).

In the current study are discussed the following tasks:

- DNA-metal complex interaction, in particularly the case of cisplatin.
- The design, synthesis and DNA binding studies of cisplatin analogs modified with nonspecifically DNA binding peptides.
- The design, synthesis and DNA binding studies of cisplatin analogs modified with specifically DNA binding peptides derived from IHF.