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Polymeric carriers in medicine

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CHAPTER 1

INTRODUCTION

1.1 Cancer overview

The term *cancer* is really a generic name for a group of more than 100 diseases that share a common type of disordered growth, and can affect any part of the body. It originates from the Latin word for crab, itself related to the Greek *karkinos* meaning both crab and cancer, from which the term *carcinoma* is derived. Since the original Indo-European root *kar* meant hard, it is evident that the name is suggestive of a hard mass that spreads like the claws of a crab. Galen[1] drew such an analogy nearly two thousand years ago, when he described cancer of the breast with its lateral prolongations of the tumor and the adjacent distended veins. Other terms used are malignant tumors and neoplasm. One defining feature of cancer is the rapid creation of abnormal cells which grow beyond their usual boundaries, and which can invade adjoining parts of the body and spread to other organs, a process referred to as metastasis. Metastases are the major cause of death from cancer.

Cancer is a leading cause of death on a global scale. From a total 58 million deaths worldwide in 2005, cancer accounts for 7.6 million (or 13%) of all deaths. The main types of cancer leading to overall cancer mortality are[2]:

- Lung (1.3 million deaths/year);
- Stomach (almost 1 million deaths/year);
- Liver (662,000 deaths/year) and

- Breast (502,000 deaths/year).

More than 70% of all cancer deaths in 2005 occurred in low and middle income countries. Deaths from cancer in the world are projected to continue rising, with an estimated 9 million people dying from cancer in 2015 and 11.4 million dying in 2030 [2].

The most frequent cancer types world wide are[2]:

- Among men (in order of number of global deaths): lung, stomach, liver, colorectal, esophagus and prostate.
- Among women (in order of number of global deaths): breast, lung, stomach, colorectal and cervical.

Cancer occurs because of changes of the genes responsible for cell growth and repair. These changes are the result of the interaction between genetic host factors and external agents which can be categorized as[3]:

- Physical carcinogens such as ultraviolet (UV) and ionizing radiation
- Chemical carcinogens such a asbestos and tobacco smoke
- Biological carcinogens such as - infections by virus (Hepatitis B Virus and liver cancer, Human Papilloma Virus (HPV) and cervical cancer) and bacteria (Helicobater pylori and gastric cancer) and parasites (schistosomiasis and bladder cancer); and
- Contamination of food by mycotoxins such as aflatoxins (products of Aspergillus fungi) causing liver cancer.

A novel factor now coming into play is the rapid spread of the human immunodeficiency virus HIV which weakens the immune system, putting immuno-depressed patients at high risk of developing early cancerous lesions and virus associated cancers such as Kaposi sarcoma and lymphomas[4,5].

The four basic types of cancer treatment include: surgery, a primary form of treatment and preferred for localized disease; radiotherapy, a procedure in which radiation is used to damage living cells; immunotherapy, aiming at stimulating and activating the patient's immune system to destroy cancer cells, and finally chemotherapy, which is the use of anticancer drugs to kill cancer cells at the microscopic level, but which like radiation may produce damage to normal tissues. Where a tumor is confined, not metastasized, surgery alone or combined with local radiation may be curative. For the majority of patients, however, physicians are forced to rely on chemotherapy either as primary treatment method or a means to attack residual diseases following surgery and / or radiation.[6]

Chemotherapy constitutes a major therapeutic approach for the treatment of cancer. It has played an important role in treating malignancies and solid tumors, though no effective chemotherapy is available for certain solid tumors and advanced tumors. Any effectiveness is diminished by drug resistance and drug toxicity, frequently leading to premature treatment discontinuation (see Chapter 2 for detailed discussion of drug deficiencies in cancer chemotherapy). This situation inspired researchers worldwide to explore the utilization of polymeric drugs and polymeric delivery systems as possible means of enhancing the efficiency of anti-tumor agents (see Chapter 2). Within the framework of macromolecular drug research in the School of Chemistry of the University of the Witwatersrand, this dissertation project represents a small contribution to the cancer drug development.

1.2 Aims of the study

It was the project's objective to synthesize macromolecular carriers specifically designed for bioreversible binding (conjugation) of medicinal

agents, specifically, anticancer drugs, for the purpose of overcoming toxicity and resistance problems and enhancing the agents' therapeutic effectiveness. It was a further objective to demonstrate the carrier's suitability for drug binding through conjugation with drug systems of the platinum complex type.

Experimentally the following goals were defined for the project:

1. Synthesis of special monomers to be incorporated into a polymer backbone and subsequent synthesis of water-soluble macromolecular carriers.
2. Synthesis of carrier-bound platinum drugs (conjugates).
3. Submission of a number of conjugates for biomedical assessment and toxicological tests.

CHAPTER 2

LITERATURE REVIEW

The topics covered in this chapter include cancer chemotherapy, polymer-drug conjugation, and drug systems investigated in this project. All these have been researched world-wide, and the number of publications on these topics is extremely large; therefore, only a small selected number of original papers have been cited in this section in favor of review articles.

2.1 Chemotherapeutic Treatment of Cancer

Cancer treatment research began with three major pieces of work. The first was the development of the principles of cancer surgery leading Halsted[7,8], in 1894, to propose *en block* resection as part of a cancer operation, particularly the radical mastectomy. At about the same time, Roentgen[9,10] discovered x-rays, which gave physicians a second means of treating localized cancer. The third advance had its roots in the work of Paul Ehrlich[11,12,13]. The use of rodent models for infectious diseases led George Clowes[11,12] to develop, in the early 1900s, inbred rodent lines that could carry transplanted rodent tumors. These models and others have since served for use as the testing ground for potential cancer chemotherapeutic agents.

Cancer chemotherapy is the treatment of malignant disease with drugs rather than radiation or surgical removal. Systematic therapy may be used alone, but is more often used in combination with surgery or radiotherapy as an adjuvant to locoregional treatment to enhance local control and attack potential sites of metastases[11,12].

Major chemotherapeutic drugs include the following: antimetabolites, alkylating agents, intercalating agents and antibiotics[11,12].

- ***Antimetabolites:*** These function at the level of DNA synthesis, interfering with the incorporation of nucleic acid bases (cytosine, thymine, adenine and guanine). There are two types of drugs: (1) drugs based on chemical modification of a nucleic acid so that the drug rather than the true nucleic acid is incorporated into the DNA, thereby preventing accurate replication of the complementary base sequence, *e.g.* 5-fluoro-uracil (5FU) or cytosine arabinoside; and (2) drugs based on inhibition of the reduction of folic acid from its inactive tetrahydrofolate form to the active tetrahydrofolate. *e.g. methotrexate*.

- ***Alkylating agents:*** These directly interfere with the DNA double strand of base pairs by chemically reacting with the structure, forming methyl cross-bridges. These then prevent the two DNA strands coming apart in mitosis to form daughter DNA fragments, and division therefore fails. Drugs in this group include *cyclophosphamide, chlorambucil, melphalan* and the *nitrosoureas.*

- ***Intercalating agents:*** These act in a similar way to the alkylating agents but, rather than directly forming cross-strands in the DNA molecule, they bind between the base pair molecules, e.g. binding adenine to thymine and cytosine to guanine, again preventing the DNA double strand from dividing in order to replicate and thereby stopping cell division. Platinum compounds (cisplatin and carboplatin) act by binding specifically to guanosine, forming DNA adducts that crosslink either within one DNA strand or across strands (*see section 2.3 for more details).*

- ***Antibiotics:*** These block cell growth by binding with DNA and interfering with DNA-dependent ribonucleic acid synthesis. The antibiotic in some way causes the double helix to unwind so that a space large enough for the insertion of molecules between the staked base pairs occurs; once intercalated in this way, the molecule is held in position by electrostatic interaction with the base pairs. When sufficient numbers of molecules have been inserted there is a distortion of the structure of DNA and a disturbance of its normal function. Drugs of this class include doxorubicin, daunomycin, actinomycin-D, bleomycin, mithramycin and mitomycin-C.

Chemotherapy constitutes an important cancer treatment modality, alone or in conjunction with other regimens. However, despite much progress in drug research, results on average have been highly discouraging.

Presently used anticancer drugs suffer from a multitude of severe pharmacological deficiencies, all of which continue to contribute to the limitations of effectiveness generally experienced in current clinical practice. Typical pharmacological shortcomings include:

(i) *Lack of cell specificity*, with ensuing drug distribution into both normal and transformed cells. In consequence, drug application will be excessively wasteful;
(ii) *Inadequate water solubility*, hampering swift and efficacious drug distribution in the body's aqueous fluid system, with resulting enhanced exposure to macrophage activity;
(iii) *Decreased serum half-life* as a consequence of catabolism, protein binding, capture by the reticuloendothelial system, or efficacious excretion mechanisms;
(iv) Monophasic *salt-like* or *charged structure*, inhibiting membrane penetration and cell entry through normal passive diffusion;

(v) *Excessive systemic toxicity*, which grossly diminishes therapeutic drug efficacy;
(vi) Lack of long-term efficaciousness because of inherent or induced *drug resistance*.

Both (v) and (vi) generally necessitate premature discontinuation of drug-specific therapy and, thus, tend to re-activate tumorous or metastatic growth. As an overall consequence of the cited drug deficiencies, periods of remission of most types of cancer by chemotherapy alone are still short, and complete cures are the exception rather than the rule.

Worldwide efforts in biomedical research are striving to overcome existing chemotherapeutic hurdles through the expediency of developing efficacious cancer-fighting tools, and it is precisely this global development drive in which macromolecular compounds are crucial participants and find their true and foremost destiny.

2.2 Polymer-Drug Conjugation Concept

In an effort to improve the outlook for cancer chemotherapy and raise the general drug performance level, advanced pharmaceutical research is focused not just on new drug development, but first and foremost on the design of efficacious drug delivery modalities capable of overcoming the resistance problem and rendering the drug system less toxic and more readily bioavailable. The last two decades have witnessed a worldwide surge of research activities toward this demanding goal. One of the most promising technologies emerging from those efforts is based on the polymer-drug conjugation concept, a strategy designed to take advantage of the pharmacokinetic peculiarities of macromolecular compounds.[14] In fact in

the early 1900s, Paul Ehrlich described drugs that could selectively be directed to their site of action as *the magic bullets*[15].

The concept of anchoring drugs to polymers has been successfully applied to a wide variety of drugs. Most applications include[16]:

- Enhancement of bioavailability and passage through various biological barriers
- Increased duration of pharmacological effects
- Decreased toxicity and adverse properties
- Increased site-specific delivery
- Improvement of stability and solubility properties.

The ideal drug carrier conjugate should conform to the following guidelines[16]:

- The drug release should be controlled and predictable
- The polymer-drug conjugate must be biodegradable, and the carrier and its degradation products should be non-immunogenic and non-toxic
- The drug-carrier should be stable during its shelf-life
- The drug-carrier should be simple and easy to manufacture

2.2.1 Natural Polymers

The targeting of drug to specific sites within the body is presently being studied, and it has been suggested that naturally occurring macromolecules can act as polymeric drug carriers. These include the polypeptides as discussed in Section 2.1. Some proteins, such as antibodies and glycoproteins, have intrinsic ability to deliver drugs actively to specific cells[17].

Although natural polymers can be used as drug carriers, they have some problems. Natural polymers such as starch or gelatin contain many biodegradable bonds, and in using them as carriers, the structures of these polymers have to be modified in order to reduce their rate of degradation[18]. Also they usually have a limited number of functional groups suitable for drug binding[19].

When considering the polypeptides, most of these are rapidly metabolized by proteolysis at most routes of administration[20]. They are, in general, non-lipophilic compounds showing poor biomembrane penetration characteristics, and they possess short biological half-lives because of rapid metabolism and clearance.

Liposomes are lipid vesicles of varying size and structural complexity within which a range of polar, non-polar and amphipathic drugs can be encapsulated. Liposomal formulations of the anti-tumor agents have shown great potential in overcoming deficiencies affiliated with these drugs. Liposomes are taken up extremely rapidly by phagocytic cells such as the liver, spleen and the avid reticulo-endothelial system, because of the tendency of their lipid bi-layers to interact with cellular surfaces[21,22]. The phagocytic uptake of liposomes by the reticulo-endothelial system almost precluded the liposome cancer therapy. However, a steric stabilization layer of a hydrophilic polymer on the liposome surface such as the frequently used polyethylene glycol, is responsible for a reduced uptake by cells of the reticulo-endothelial system which in turn results in prolonged circulation times as compared to non-coated liposomes. There are, however, some limitations associated with the use of polymers as liposome coatings. These polymers are generally not degradable by mammalian enzymes and upon uptake of the coated liposomes by cells, the polymer may accumulate and cause cell function impairement on the long term[23]. Polymer coatings may also hinder drug release and target cell interaction after liposome

localization in the target region therefore leading to a reduced therapeutic efficacy[24]. Another point of concern, which does not only apply to coated liposomes but also uncoated, is that liposomes have been reported to activate the complement system in preclinical studies[25]. In the clinical setting, adverse respiratory, hemodynamic and hematological changes may occur in patients upon complement activation and potentially lead to hypersensitivity reactions.

2.2.2 Synthetic Polymers

Synthetic polymers are advantageously used as drug carriers, since they are more susceptible to modifications than natural polymers. The advantage of the polymer synthesized is that it may contain the smallest number of bonds that are sensitive to enzymatic attack, and that scission of these bonds can be controlled by changing the structure, amount and the length of crosslinks[26,27].

The polymer-drug conjugates are designed according to a model proposed by Ringsdorf[28] in the mid 1970s (Figure 2.1), on the strength of which the notion has by now matured to a highly sophisticated pharmaceutical tool. It utilizes the advantageous pharmacokinetic behavior of polymeric compounds in the mammalian body, paired with their enormous compositional versatility allowing for near-limitless structural modifications of these large molecules. In practical terms, the technique comprises the bioreversible binding (*conjugation)* of a bioactive agent, typically an antitumor drug, to a water-soluble macromolecular carrier molecule designed and synthesized in strict conformance with pharmaceutical guidelines. Specifically, the carrier polymer will be composed of (1) subunits containing some molecular entity permitting facilitated cell entry, (2) other subunits equipped with intra- or extra-chain water-solubilizing groups, (3) still other units featuring a homing device capable of directing

the polymer-drug assembly, the *conjugate*, selectively to the target tissue, and (4) most importantly, units equipped with functional groups suitable for the critical conjugation step involving bioreversible drug binding to the polymer. The conjugate represents the prodrug, from which the active agent is hydrolytically or enzymatically released into the predestined biological environment, which, for anticancer action, are the cytoplasmic lysosomes and cell nuclei.

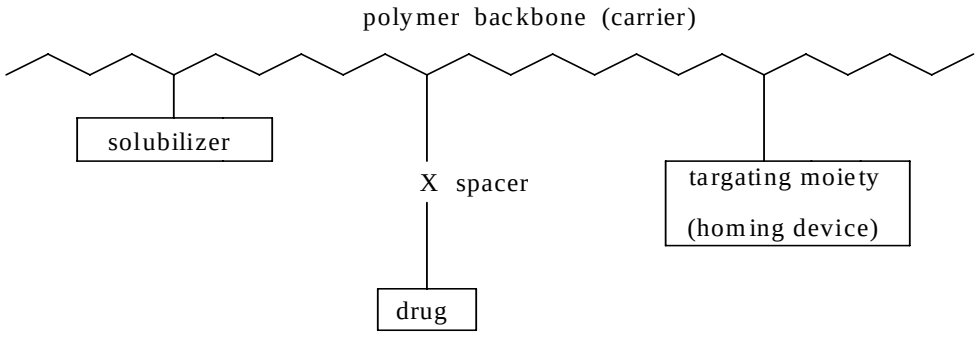


Figure 2.1: General structure of a macromolecular carrier as proposed by Ringsdorf[28]

For many years, the polymer research laboratory of this university has focused its work on synthesizing polymeric carriers for delivering drugs to tumor cells. The research has mostly concentrated on the synthesis of water-soluble carriers, such as polyaspartamides and polyamidoamines, and on their conjugation with a number of antineoplastic agents, as well as synthesizing suitable monomers for these polymers[29-44]. The value of polyaspartamides was first recognized by Drobnik[45] and colleagues in 1979 because of their biodegradability and solubility in the body, and Ferruti[46-48] and colleagues recognized the value of the polyamidoamines (see Chapter 3 for more details).

From studies done on polymeric drug carriers, it was noted that a water-soluble polymer-drug conjugate designed in full compliance with a variety of biomedical specifications will provide some or all of the following features exploited to pharmacological advantage:

- The possibility of introducing the drug, even if *per se* insoluble in water, as a solubilized species into the aqueous fluid system of central circulation for immediate dissipation;

- Drastically reduced toxic side effects because of the anchored nature of the drug in the initial state and its controlled, time-dependent release into the serum or, more appropriately for an anticancer agent, into the intracellular lysosomal environment;

- Facilitated cell entry by a pinocytotic mechanism, providing drug access to the nuclear target even for inherently polar or charged drug molecules, and compensating for accelerated drug efflux from drug-resistant cells;

- Enhanced drug affinity for the transformed cell as a consequence of the *enhanced permeation and retention* effect[49] or, simply, through the expediency of attaching to the conjugate an efficacious homing device, such as certain sugar moieties or, at the stage of ultimate perfection, selected monoclonal antibodies;

- Dramatically extended serum residence times as a result of temporary drug protection by the bulky carrier against premature enzymatic attack, catabolism, or simply capture by the reticuloendothelial system.

Taking advantage of the more favorable pharmacokinetics of macromolecules relative to nonpolymeric compounds, a nonpolymeric

biologically active agent is bioreversibly attached, *conjugated*, to water-soluble polymeric carrier synthesized in strict compliance with biomedical specifications. The water-soluble carrier component in this assembly functions as a vehicle transporting the medical agent. As depicted in Figure 2.2, the pro-drug (conjugate) travels essentially intact from central circulation through the various body compartments to, and into, the affected target where the bioactive agent is released for ultimate biological action[50].

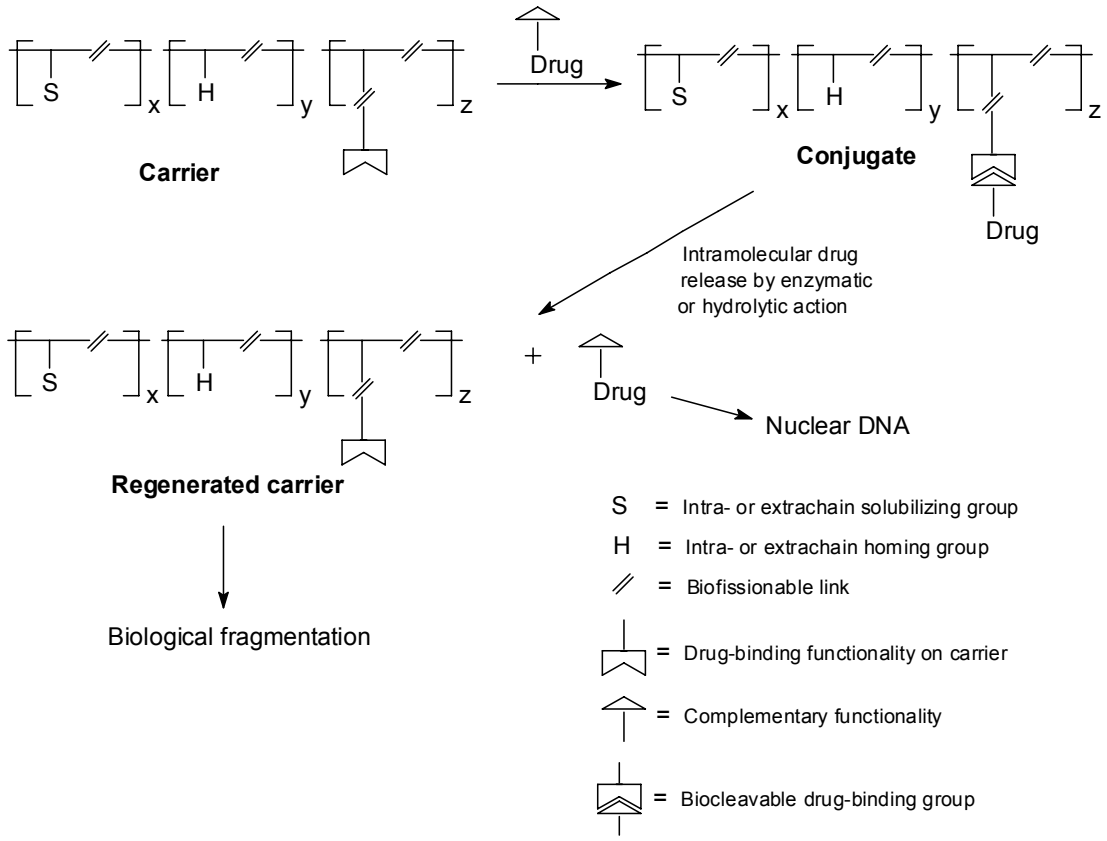


Figure 2.2: Flow chart describing drug conjugate pharmacokinetics[50].

2.3 The Drug Model

The application of inorganic chemistry to medicine is a rapidly developing field, and novel therapeutic and diagnostic metal complexes are having an impact on medical practice. The development of modern medicinal inorganic chemistry, stimulated by the discovery of *cisplatin*, has been facilitated by the inorganic chemist's extensive knowledge of the coordination and redox properties of metal ions. Metal centers, being positively charged, are favored to bind to negatively charged biomolecules; the constituents of proteins and nucleic acids offer excellent ligands for binding to metal ions. The pharmaceutical use of metal complexes therefore has excellent potential. In view of this, platinum compounds were the investigated drug systems in this project, and will be discussed in detail.

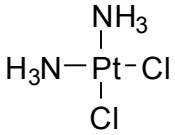
2.3.1 History of Platinum in Medicine

The successful development of metal-containing anticancer drugs clearly starts with *cis*-diamminedichloroplatinum(II), often referred to as *cisplatin*. The compound was first described in 1845 as Peyrone's salt[51,52], and its anticancer properties were not discovered until 1964. The antitumor activity of the platinum complexes was discovered as the result of a fortuitous observation by Barnett Rosenberg and coworkers[53,54] during a study of the effects of electrical current on growing bacteria. When alternating current was delivered through platinum electrodes to a growing bacterial culture, the bacterial cells stopped dividing and grew into long filaments. The cause of the inhibition of cell division was found to be *cis*-diamminedichloroplatinum (II), also known as *cis*-DDP and *cisplatin*.

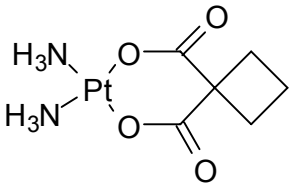
In the early 1970s, *cisplatin* went into clinical trials[42, 55-57] and was found to have significant antitumor activity against testicular cancer, lymphoma, squamous cell carcinoma of the head and neck, ovarian cancer, and

bladder cancer. Because of its significant therapeutic effect in these tumors and activity against a number of other solid tumors, it became the most frequently used antitumor agent. Because of the renal and neurotoxicities of *cisplatin*, there were intensive efforts to develop analogs with fewer of these toxicities. This work led to the development of carboplatin, which produces primarily hematopoietic toxicity and appears to have an antitumor effect similar to *cisplatin*[58-60] against the tumors against which it has been used.

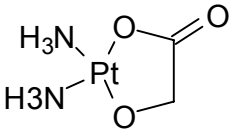
While thousands of *cisplatin* analogues have been synthesized and screened, only about 28 platinum compounds have entered clinical trials as anticancer agents. Of these only four are currently approved. Those approved are *cisplatin*, carboplatin, oxaliplatin, and nedaplatin. Only the first two are commercially available for general use in the treatment of cancer[51].



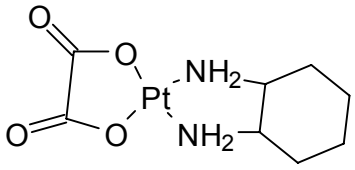
Cisplatin, cis-DDP
(*cis*-Diamminedichloroplatinum(II))



Carboplatin
(*cis*-Diammine-1,1-cyclobutane-dicarboxylato-platinum(II))



Nedaplatin
(*cis*-Diammineglycolatoplatinum (II))



Oxaliplatin
(Oxalatoplatinum(II))

Figure 2.3: *Cisplatin*, *Carboplatin, Nedaplatin* and *Oxaliplatin*

2.3.2 Chemistry

2.3.2.1 Covalent Bond character

Platinum is in the third row of transition metals in the periodic table and has eight electrons in the outer *d* shell. Palladium and Nickel, which occupy analogous positions in the second and first transition series, have similar configurations of outer electrons but little or no antitumor activity. Because the platinum atom has a much larger total number of electrons, however, the orbitals of its outer electrons are more polarizable, and bonds that are formed from these orbitals have more covalent character[61].

The special configuration depends on whether the oxidation state of platinum is +2 or +4; these oxidation states are usually designated as Pt(II) or Pt(IV), respectively. Because the bonds are fixed and not readily interchangeable, the complexes can have distinct isomers, such as *cis*- and *trans*-Pt(II)$(NH_3)_2Cl_2$ (Figure 2.3). The *cis* isomer is the antitumor drug *cisplatin*. The importance of steric conformation is highlighted by the fact that the *trans* isomer has virtually no antitumor activity.



Figure 2.4: *Cis*- and *Trans*- Platin(II) Isomers

Some platinum bonds, such as those to nitrogen, are essentially irreversible under physiologic conditions, while others, such as the chloride-platinum(II) bond in *cisplatin*, are more labile[61].

2.3.2.2 Displacement Reactions

Displacement reactions can occur in Pt(II) complexes in which one or both ligands are displaced by a competing nucleophile. The biologic actions of Pt(II) complexes are due to displacement reactions, which cause the platinum to become stably bound to DNA, RNA, proteins, or other critical biomolecules[62]. The stability of the binding of different ligands to Pt(II) varies greatly. Although the binding to sulfur or nitrogen sites is essentially irreversible under physiologic conditions, the stability of binding to halogens is much lower, in the order $I^- > Br^- > Cl^-$, and the stability of binding H_2O is weaker still[63].

The dissociation of sulfur or nitrogen ligands is generally negligible under physiologic conditions. The aquo (H_2O) ligand dissociates rapidly, whereas chloride dissociates more slowly. The dissociation rate also can be influenced by the identity of the other ligands in the complex, especially by the ligand located *trans* to the dissociating ligand[52,63]. The tendency of a ligand to leave the complex correlates inversely with the affinity for binding to platinum (II) and increases in the order:

$CN^- > NH_3 \approx OH^- > I^- > SCN^- > Br^- > Cl^- \approx$ Caboxylate $> H_2O > NO_3^-, ClO_4^-$.

2.3.2.3 Reactions in Water and Biologic Fluids

The pharmacologic behavior of *cisplatin* is in part determined by its reactions in water, which are summarized in Figure 2.5.

In blood plasma, the high chloride concentration of approximately 100 mM would keep *cisplatin* predominantly in the uncharged and relatively unreactive dichloro form. This form may react to some degree with sulfhydryl groups of plasma proteins. The free dichloro form could enter cells by passive diffusion. In the cytoplasm, the relatively low chloride

concentration of approximately 4 mM would favor the aquation reaction, which would yield high reactive species whose ionic charge may retard exit from the cell[52,64]. Thus, the active form within the cell is believed to be the monohydrated structure, $[Pt(NH_3)_2(OH_2)Cl]+$, the preferred extracellular species contains two *cis*-oriented leaving groups that are normally chloride ligands[52]. Before the molecule enters the cell, it will be electrically neutral due to the high concentration of chloride ion in blood.

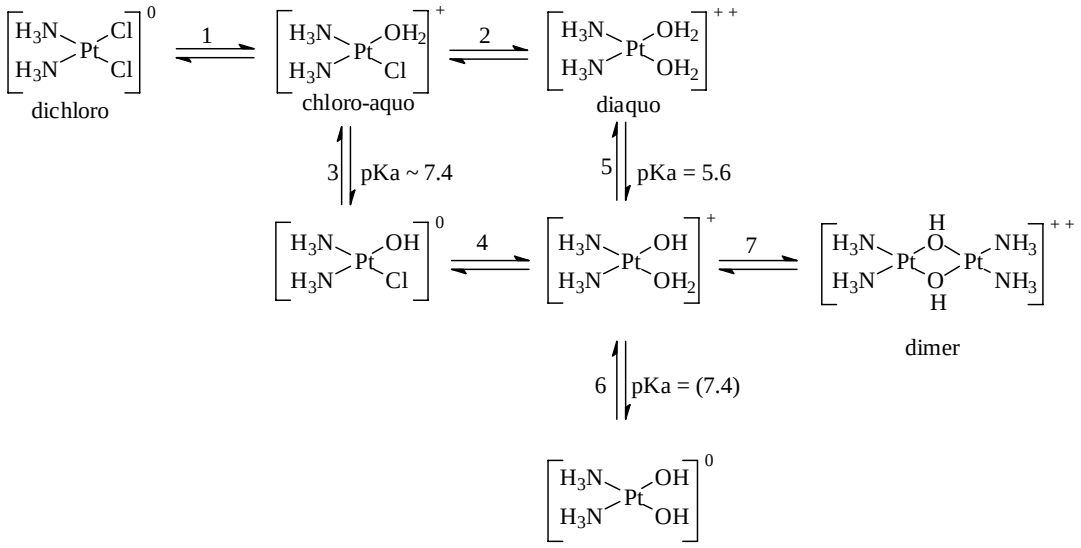


Figure 2.5: Aquation and hydrolysis equilibria of *cisplatin*

2.3.2.4 Reaction with DNA

The mechanism of platinum drugs action generally involves hydrolytic dissociation of the leaving groups in lysosomal compartment in intracellular space, leading to aqua-complexed intermediate. This highly reactive compound is implicated as the active drug species, generally interacting with the cell's nuclear DNA. The intra- and interstrand cross-links or DNA-protein crosslink (Figure 2.6) formed in such interactions create lesions in

the double helix, which cannot be repaired in time prior to division of the rapidly proliferating cancer cell. As a result, the cells, unable to survive with such lesions in its nuclear material, will die[52,64].

Cisplatin has been noted to bind to all DNA bases, but there seems to be preferential binding to the N-7 positions of guanine and adenine bases, and N-3 position of cytosine and uracil. Kinetic studies indicate that *cisplatin* cross-links to DNA through two successive pseudo first order reaction steps. In the first step, *cisplatin* is hydrolyzed in aqueous medium to the intermediate mono-aqua platinum complex. The intermediate migrates to the most favorable binding site of the DNA helix and binds to the N-7 atom of the guanine through displacement of water. The second chloro ligand is displaced by water in the second step, and the final step is the closure of the chelate complex[64-70].

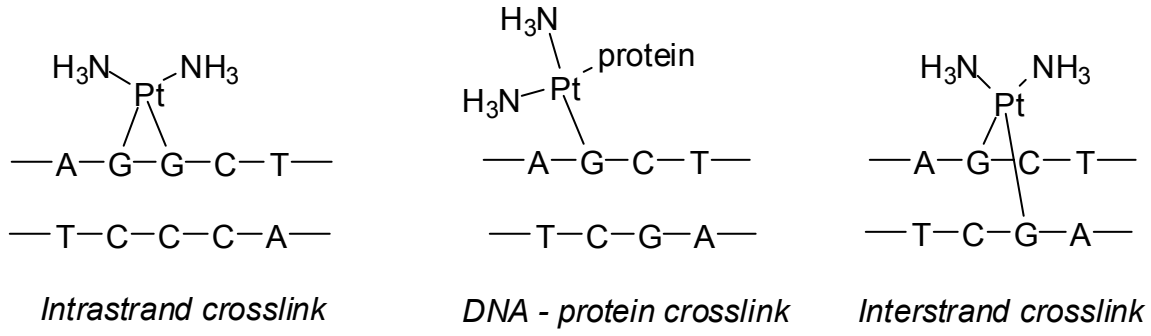


Figure 2.6: General crosslinked forms of DNA from reaction with *cisplatin.*

2.3.2.5 Toxicity of Cisplatin

Since the discovery by Rosenberg[53,54] that *cisplatin* is an effective anticancer drug, large synthesis and evaluation programs have been aimed at the creation of *cisplatin* derivatives that show greater and more widespread activity against cancer but with lowered toxicity. Despite much

progress in drug research, results on average have been highly discouraging. The major toxic manifestations of *cisplatin* include pronounced gastrointestinal problems such as acute nausea, vomiting, and diarrhea; occasional liver dysfunction; myelosuppression involving anemia, leucopenia, and thrombocytopenia; nephrotoxicity, and less frequently cases of immunosuppression, hypomagnesia, hypocalcemia, and cardiotoxicity. The most serious side effect is damage to the kidney. Much of the administered *cisplatin* is filtered out of the body within a few hours, exposing the kidneys to bursts of high concentrations of platinum. The rapid rate at which the kidneys filter the platinum from the blood is believed to be responsible for the kidney problems. Another problem is the cumulative and irreversible hearing loss experienced first in the 4000 – 8000 Hz range and then later in the 1000 – 4000 Hz range[52,64,71-73].

CHAPTER 3

RESULTS AND DISCUSSION

In accordance with the specific experimental goals outlined in chapter one, this dissertation project was carried out within the framework of the following work phases:

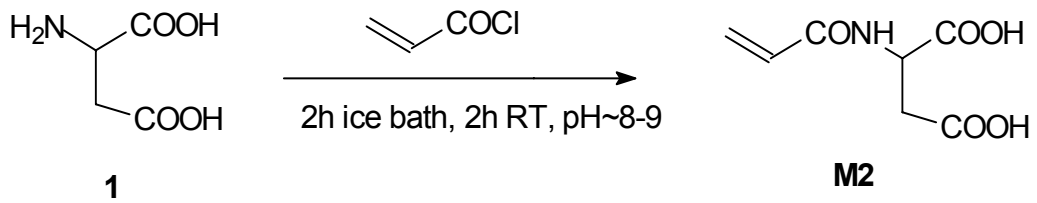
Phase 1: Synthesis of special monomers to be incorporated into the polymer backbone and subsequent synthesis of water-soluble macromolecular carriers. Preparative methods developed in previous projects and improved where necessary were used in this investigation.

Phase 2: Conjugation of the carriers obtained in Phase 1 with platinum containing drug.

Phase 3: Submission of a number of conjugates for *in vitro* biomedical assessment and toxicological tests.

3.1 Synthesis of Monomers

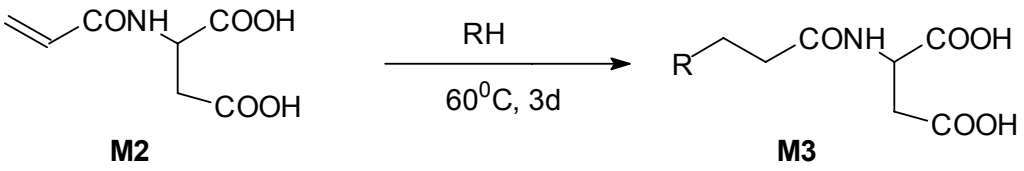
3.1.1 Synthesis of amine-functionalized aspartic acid derivatives



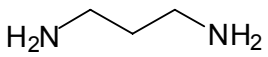
1 **M2**

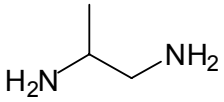
Scheme 1: Synthesis of acryloylaspartic acid derivative **M2**

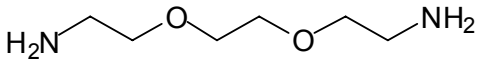
The aspartic acid derivative was a two step reaction. In the first step the reaction involved the synthesis of acryloylaspartic acid monomer under very mild conditions. Aspartic acid was reacted with acryloyl chloride (Scheme1) at 0–5^{0}C then raised to RT. The ice bath temperature was necessary during the process to avoid any polymerization of the double bond since the reaction is exothermic. The pH of the solution was critically maintained at ~8–9. Both acidic and more basic conditions would have resulted in the displacement of the chloride ion by water forming an inert carboxylic acid group, or the hydrolysis of the product, forming acrylic acid and aspartic acid. The experimental conditions and ^{1}H NMR data are summarized in Tables 3.1 and 3.2.



M2 **M3**

M3a : RH = 

M3b : RH = 

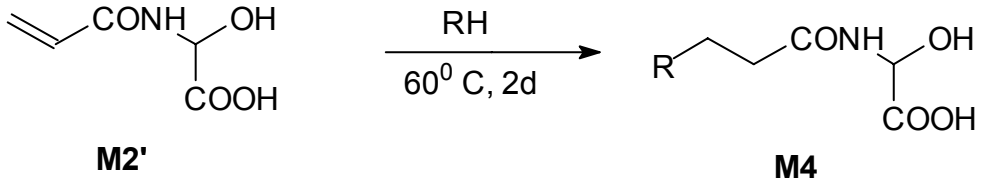
M3c : RH = 

Scheme 2: *Synthesis of macromonomers aspartic acid derivatives*

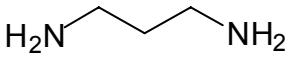
Key monomers N-(4,8-diaza-octanoyl)aspartic acid (**M3a**), N-(4,7-diaza-6(5)-methyl-heptanoyl)aspartic acid (**M3b**) and N-(4,13-diaza-7,10-dioxa-tridecanoyl)aspartic acid (**M3c**) were obtained by Michael addition of diamines across the active double bond in acryloylaspartic acid and different

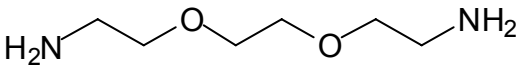
diamines such as 1,3-diaminopropane (PDA), 1,2-diaminopropane (1,2-PDA) and 2,2'-(ethylenedioxy)diethylamine (EDDA), at 60^0C (scheme 2).

3.1.2 Synthesis of amine-functionalized glycolic acid derivatives



M2' **M4**

M4a : RH = 

M4b : RH = 

Scheme 3: *Synthesis macromonomers glycolic acid derivatives*

Glycolic acid derivatives N-(4,8-diaza-octanoyl)amidoglycolic acid (**M4a**) and N-(4,13-diaza-7,10-dioxa-tridecanoyl)amidoglycolic acid (**M4b**) were synthesized as depicted in scheme 3, by treatment of 2-acrylamidoglycolic acid monohydrate **M2'** with PDA or EDDA via Michael addition to the activated double bond. The experimental conditions and ^{1}H NMR data are summarized in Tables 3.1 and 3.2.

Table 3.1: *^{1}H NMR of **M2, M3a, M3b, M3c, M4a** and **M4b***

Monomer Designation	Assignment	^{1}H NMR[a,b]		
		Shift range δ(ppm)	Proton count	
			Found	Expected
M2	**CH_2** (Asp) **CH** (Asp) CH_2**CH**CONH **CH_2**CHCONH	2.4 – 2.2 4.2 – 4.1 5.5 – 5.4 6.1 – 5.8	2 1 0.9 1.9	2 1 1 2
M3a	NH_2CH_2**CH_2**CH_2NH NH_2**CH_2**CH_2**CH_2**NH**CH_2**; **CH_2** (Asp); **CH_2**CONH **CH** (Asp)	1.7 – 1.5 2.9 – 2.4 4.5 – 4.4	2.3 10.4 1	2 10 1
M3b	CH**CH_3** NH_2**CH_2CH**(CH_3)NH**CH_2**; **CH_2** (Asp); **CH_2**CONH **CH** (Asp)	1.1 – 1.0 2.9 – 2.4 4.5 – 4.4	3.3 9.2 1	3 9 1
M3c	NH_2**CH_2**$CH_2OCH_2CH_2OCH_2$**CH_2**NH-**CH_2**; **CH_2**CONH; **CH_2** (Asp) NH_2CH_2**CH_2**O**CH_2CH$_2$**O**CH_2**CH_2NH **CH** (Asp)	2.9 – 2.4 3.7 – 3.5 4.5 – 4.4	9.6 7.2 1	10 8 1
M4a	NH_2CH_2**CH_2**CH_2NH NH**CH_2** NH_2**CH_2**CH_2**CH_2**NH **CH_2**CONH	1.7 – 1.5 2.5 – 2.4 2.6 – 2.5 2.9 – 2.8	2 2.4 3.2 2.1	2 2 4 2
M4b	NH_2**CH_2**CH_2OCH_2 **CH_2**CONH; OCH_2**CH_2**NH**CH_2** NH_2CH_2**CH_2**O**CH_2CH$_2$**O**CH_2**CH_2NH	2.5 – 2.4 2.9 – 2.8 3.7 – 3.5	2 6.1 8.4	2 6 8

[a] *In D_2O, pD 10 – 11. Chemical shifts, δ (ppm), referenced against internal sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate*

[b] *Integration error limit ± 15%*

Table 3.2: *Summary of experimental data for Monomers **M2, M3a, M3b, M3c, M4a** and **M4b***

Monomer Designation	Molar ratio[a]							Experimental Conditions[c]	Yield %
	Aspartic acid	Acryloyl Chloride	M2	M2'	PDA[b]	1.2-PDA[b]	EDDA[b]		
M2	1.0	1.2	-	-	-	-	-	2h, 0-5^0 C; 2h, RT	88.0
M3a	-	-	1.0	-	1.2	-	-	3d, 60^0 C	81.4
M3b	-	-	1.0	-	-	1.2	-	3d, 60^0 C	67.4
M3c	-	-	1.0	-	-	-	-	3d, 60^0 C	60.2
M4a	-	-	-	1.0	1.2	-	1.2	3d, 55^0 C	59.1
M4b	-	-	-	1.0	-	1.2	-	3d, 55^0 C	36.2

[a] *Base molar for aspartic acid, **M2** and **M2'** respectively*

[b] *PDA: 1,3-diaminopropane; EDDA: 2,2'-(ethylenedioxy)diethylamine; 1,2-PDA: 1,2-diaminopropane*

[c] *Ice bath = 0-5^0 C; RT: room temperature*

3.2 Synthesis of Macromolecular Carriers

With the program's synthetic operations predominantly in the area of carrier polymers, the research was directed principally toward the polymers' main chain and side group design in strict accordance with biomedically prescribed specifications, and toward the development of the kind and sequence of chemical reaction steps required to reduce these designs to practice. The following features were incorporated into the product polymers:

(i) A nontoxic chain construction amenable to biodegradation to ensure catabolic elimination of the polymer upon drug release.

(ii) A highly flexible backbone and the presence of intrachain-type or side group-attached solubilizing groups to ensure conjugate solution in aqueous media required for rapid dissipation in the central circulation system.

(iii) The presence of functional groups as binding sites to ensure proper drug attachment and subsequent release in the biological environment.

(iv) The presence of cationic functions to cause facilitated cell entry by endocytosis, thus circumventing potential problems caused by drug polarity or ionicity. This mode of entry may assume unique importance where drug resistance has been developed by the target cells.

(v) Delayed and controlled drug release involving the polymer-drug connective link, thereby ensuring restriction of drug serum conjugate concentration well within pharmacologically dictated limits with concomitant reduction of organ toxicity. Features are also built into the molecule that provides conjugate stability in the plasma and drug release upon endocytotic cell entry.

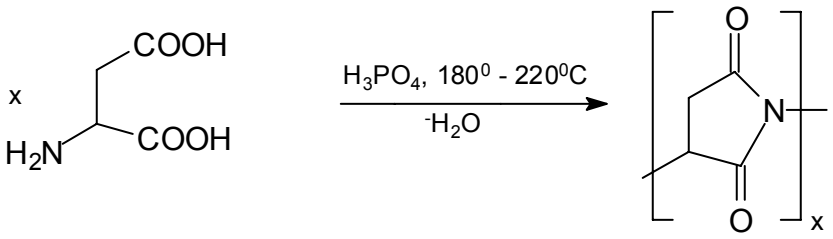
(vi) The incorporation of poly(ethylene oxide) chain segments or side chains will provide temporary conjugate protection from enzymatic attack and protein binding while in transit. This leads to reduced renal clearance and prolonged serum life time with substantially enhanced drug effectiveness.

3.2.1 Polyaspartamides

In the early 'Seventies Drobnick[45] *et al* recognized the potential value of polysuccinimide-derived aspartamide polymers as drug carriers. For a number of years the polymer research laboratory of this university has made use of these macromolecular carriers for a number of antineoplastic drug systems. Accordingly a major portion of the polymers synthesized in this project was based on this class.

3.2.1.1 Poly-DL-Succinimide

Polycondensation of DL-aspartic acid[74] **1** in the presence of orthophosphoric acid at high temperature (180^0–220^0 C) yielded poly-DL-succinimide **2** (PSI), (poly[2,5-dioxopyrrolidine-1,3-diyl]) as indicated in Scheme 4. Poly-DL-succinimide **2** was subsequently subjected to carbodiimide-mediated chain extension. The representative polymer had an inherent viscosity of 53 ml/g. In the past an inherent viscosity ranging from 25–45 mL/g has been reported by other workers of the same laboratory.

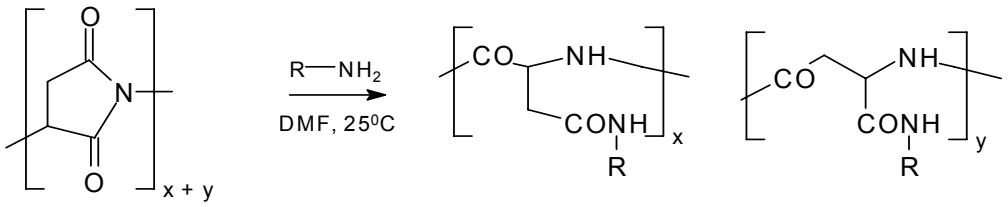


1 **2**

Scheme 4: *Polycondensation of DL-aspartic acid*.

3.2.1.2 Poly-α,β-DL-aspartamides

Polyaspartamides are readily prepared from poly-DL-succinimide **2** by aminolytic ring opening carried out under carefully controlled conditions in anhydrous, dipolar aprotic medium, such as N,N-dimethylformamide (DMF), giving rise to a mixture of D- and L- enantiomeric forms, consisting of both α- and β- peptidic repeat units[65] (Scheme 5); only α peptide forms are shown for simplication, throughout this dissertation work.

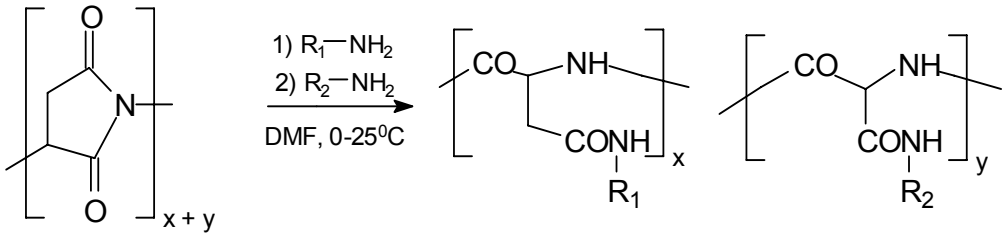


(α peptide) **(β peptide)**

Scheme 5: *Synthesis of Poly-α,β-DL-aspartamide*

The aminolytic ring-opening reaction of polysuccinimide gives rise to the generation of the ultimate polyaspartamides through a stepwise, sequential addition of two amine nucleophile reactants, NH_2-R_1 and NH_2-R_2, in

predetermined stoichiometric feed ratios. Occasionally a third amine, NH_2-R_3, may be employed in this operation. The resultant copolyaspartamides are composed of randomly distributed aspartamide repeat units bearing N-attached substituents (R_1, R_2 etc.) as introduced by the amine nucleophiles chosen. Scheme 6 depicts this sequence of ring opening steps.



Scheme 6: *Aminolytic ring opening reaction in polysuccinimide*

In general, R_1, is chosen to represent a hydrosolubilizing moiety (e.g. hydroxyl or tertiary amine), and R_2 represents a drug-anchoring (e.g. carboxyl, a primary or secondary amine) functionality.

The preferential use of poly-α,β-DL-aspartamide in this project is motivated by the following considerations:

(i) The molecular mass, which on average ranges from about 25000 to 35000, is well within a desirable range that is sufficiently low to suppress inherent polymer toxicity, and still high enough to retard renal excretion.

(ii) The intrachain amide groups allow a gradual backbone cleavage for ease of catabolic elimination of the spent polymer. The L-type polymers are very vulnerable to enzyme attack and consequently undergo rapid degradation, while D-type polymers are known to be more resistant to the enzymatic attack. The presence of D-

configurated CH groups and β-peptide units in the chain thus prevents the so called “unzipping” of the chain from its terminals brought about by the exopeptidases[45] class of enzymes, whose function is to cleave the terminal residues from polypeptides. It is therefore ensured that fragmentation of the polymer is retarded.

(iii) Polyaspartamides are essentially non-toxic, and the immunogenicity of these synthetic peptide-type polymers is expected to be distinctly lower than that of high molecular proteinaceous biopolymers. Furthermore, since one half of the final backbone degradation product is the essential amino acid, L-aspartic acid, one would expect this to be a “body-friendly” catabolite.

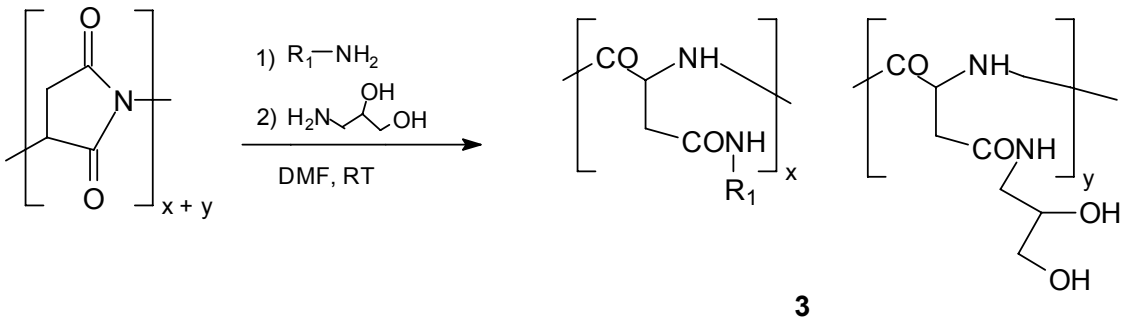
(iv) The subunits bearing the hydrosolubilizing group R_1 can be introduced in excess (x > y), as this will provide an effective insulation of the R_2-modified subunits from each other and, thus, will reduce the risk of intramolecular interaction of neighboring drug species.

(v) The introduction of tertiary amine functions in R_1 may confer the special functions of pinocytotic cell entry and target cell affinity.

a. Polyaspartamide Carriers Bearing a Dihydroxyl functionality Side Chain as the Drug Anchoring Site

The following series of copolymers (Scheme 7) were synthesized under experimental conditions similar to previous work performed in this laboratory, with preparative methods improved where necessary.
The side chains represented by R_1 in this category are derived either from 3-(dimethylamino)propylamine (DMP), 2-(dimethylamino)ethylamine (DMEA),

2-(diethylamino)ethylamine (DEEA) or 2-(2-aminoethoxy)ethanol (AEE) in accordance with the structures in Scheme 4. In agreement with the general principle outlined before, DMP, DMEA, DEEA and AEE were used as major reactants (x > y). The aminolysis is a two-step reaction, which generally occurs at room temperature. In the first step, a given amount of R_1-NH_2 was allowed to react with polysuccinimide (**2**) for a specified time, generally ~5 hours. Thereafter, in the second step, the dihydroxyl-functionalized unit, 3-amino-1,2-propanediol (APD), was added in excess to the resulting solution and the reaction allowed to continue for a specified time, ~48 hours, at ambient temperature. The relative amount of APD (three times the stoichiometric amount) was found necessary to achieve the desired percentage incorporation with complete ring opening and substitution of the remaining succinimide units of the substrate polymer.



3

3a(80): R_1 = NMe_2 x/y = 4

3b(80): R_1 = NMe_2 x/y = 4

3c(80): R_1 = NEt_2 x/y = 4

3d(80): R_1 = O OH x/y = 4

3a(90): R_1 = NMe_2 x/y = 9

3b(90): R_1 = NMe_2 x/y = 9

3d(90): R_1 = O OH x/y = 9

Scheme 7: *Synthesis of APD-derived polyaspartamide carriers*

In order to avoid unwanted hydrolytic ring opening resulting in the generation of free carboxylic acid side groups, both steps were performed under strictly anhydrous conditions, and N,N'-dimethylformamide (DMF) was the solvent of choice for this purpose. The polymers were routinely obtained by precipitation, aqueous dialysis in membranes of 12 000 and 25 000 molecular mass cut-off limit, and ultimately isolated by freeze-drying as water-soluble solids possessing inherent viscosities in the range of 7 to 34 mL/g. For copolymer **3** types, various molar feed ratios were used. The

experimental details and viscosity results are presented in Table 3.3, and ^{1}H NMR data are presented in Table 3.4. The ^{1}H NMR data were in agreement with the structures indicated in Scheme 7.

Table 3.3: *Summary of experimental data Polyaspartamide Carriers Bearing a Dihydroxyl-functionalized Side Chain as the Drug Anchoring Site*

Polymer Designation	Molar Ratios[a]						Reaction time & Temperature[b]		Yield %	η_{inh} [c] mL/g	x/y
	PSI 2	DMP[d]	DMEA[d]	DEEA[d]	AEE[d]	APD[d]	1st Step	2nd Step			
3a(80)	1.00	0.80	-	-	-	0.60	5h, RT	48h, RT	63.2	15.3	4
3b(80)	1.00	-	0.80	-	-	0.60	5h, RT	48h, RT	58.6	7.4	4
3c(80)	1.00	-	-	0.80	-	0.60	5h, RT	48h, RT	51.1	10.9	4
3d(80)	1.00	-	-	-	0.80	0.60	5h, RT	48h, RT	42.7	7.2	4
3a(90)	1.00	0.90	-	-	-	0.30	5h, RT	48h, RT	48.5	34.2	9
3b(90)	1.00	-	0.90	-	-	0.30	5h, RT	48h, RT	46.4	18.4	9
3d(90)	1.00	-	-	-	0.90	0.30	5h, RT	48h, RT	38.3	22.1	9

[a] *Base molar **PSI 2***

[b] *RT: room temperature*

[c] *At 30.0^{0}C ± 0.5^{0}C in dist H_2O; conc = 0.2mg/100mL*

[d] *DMP: 3-(dimethylamino)propylamine; DMEA: 2-(dimethylamino)ethylamine; DEEA: 2-(diethylamino)ethylamine; AEE: 2-(2-aminoethoxy)ethanol; APD: 3-Amino-1,2-propanediol*

Table 3.4: *^{1}H NMR of copolyaspartamides **3a(80), 3b(80), 3c(80), 3d(80), 3a(90), 3b(90)** and **3d(90)***

Polymer Designation	Assignment	^{1}H NMR		
		Shift range δ(ppm)	Proton count	
			Found	Expected
3a(80)	$CONHCH_2\mathbf{CH_2}CH_2NMe_2$	1.7 – 1.5	8	8
	$N\mathbf{Me_2}$	2.3 – 2.1	24.4	24
	$\mathbf{CH_2}NMe_2$	2.4 – 2.3	9.3	8
	$\mathbf{CH_2}CONH$	2.8 – 2.6	10.2	10
	$CONH\mathbf{CH_2}$	3.3 – 3.1	9.8	10
	$\mathbf{CH}(OH)\mathbf{CH_2}OH$	3.7 – 3.5	2.9	3
3b(80)	$N\mathbf{Me_2}$	2.3 – 2.1	24	24
	$\mathbf{CH_2}NMe_2$	2.4 – 2.3	9.0	8
	$\mathbf{CH_2}CONH$	2.8 – 2.6	10.6	10
	$CONH\mathbf{CH_2}$	3.3 – 3.1	11.2	10
	$\mathbf{CH}(OH)\mathbf{CH_2}OH$	3.7 – 3.5	2.8	3
3c(80)	$CH_2N(CH_2\mathbf{CH_3})_2$	1.1 – 0.9	24	24
	$\mathbf{CH_2}N(\mathbf{CH_2}CH_3)_2$	2.7 – 2.5	24.3	24
	$\mathbf{CH_2}CONH$	2.9 – 2.7	8.8	10
	$CONH\mathbf{CH_2}$	3.4 – 3.2	10.1	10
	$\mathbf{CH}(OH)\mathbf{CH_2}OH$	3.8 – 3.4	3.4	3
3d(80)	$\mathbf{CH_2}CONH$	2.9 – 2.6	10	10
	$CONH\mathbf{CH_2}$	3.4 – 3.3	10.6	10
	$\mathbf{CH_2O CH_2CH_2}OH$; $\mathbf{CH}(OH)\mathbf{CH_2}OH$	3.8 – 3.5	30	27
3a(90)	$CONHCH_2\mathbf{CH_2}CH_2NMe_2$	1.7 – 1.5	18	18
	$N\mathbf{Me_2}$	2.3 – 2.1	53.4	54
	$\mathbf{CH_2}NMe_2$	2.4 – 2.3	19.1	18
	$\mathbf{CH_2}CONH$	2.8 – 2.6	20.3	20
	$CONH\mathbf{CH_2}$	3.3 – 3.1	20.3	20
	$\mathbf{CH}(OH)\mathbf{CH_2}OH$	3.7 – 3.5	3.2	3
3b(90)	$N\mathbf{Me_2}$	2.3 – 2.1	54	54
	$\mathbf{CH_2}NMe_2$	2.4 – 2.3	19.1	18
	$\mathbf{CH_2}CONH$	2.8 – 2.6	23.1	20
	$CONH\mathbf{CH_2}$	3.3 – 3.1	22.4	20
	$\mathbf{CH}(OH)\mathbf{CH_2}OH$	3.7 – 3.5	2.9	3
3d(90)	$\mathbf{CH_2}CONH$	2.9 – 2.6	20	20
	$CONH\mathbf{CH_2}$	3.4 – 3.3	20	20
	$\mathbf{CH_2O CH_2CH_2}OH$; $\mathbf{CH}(OH)\mathbf{CH_2}OH$	3.8 – 3.5	56	57

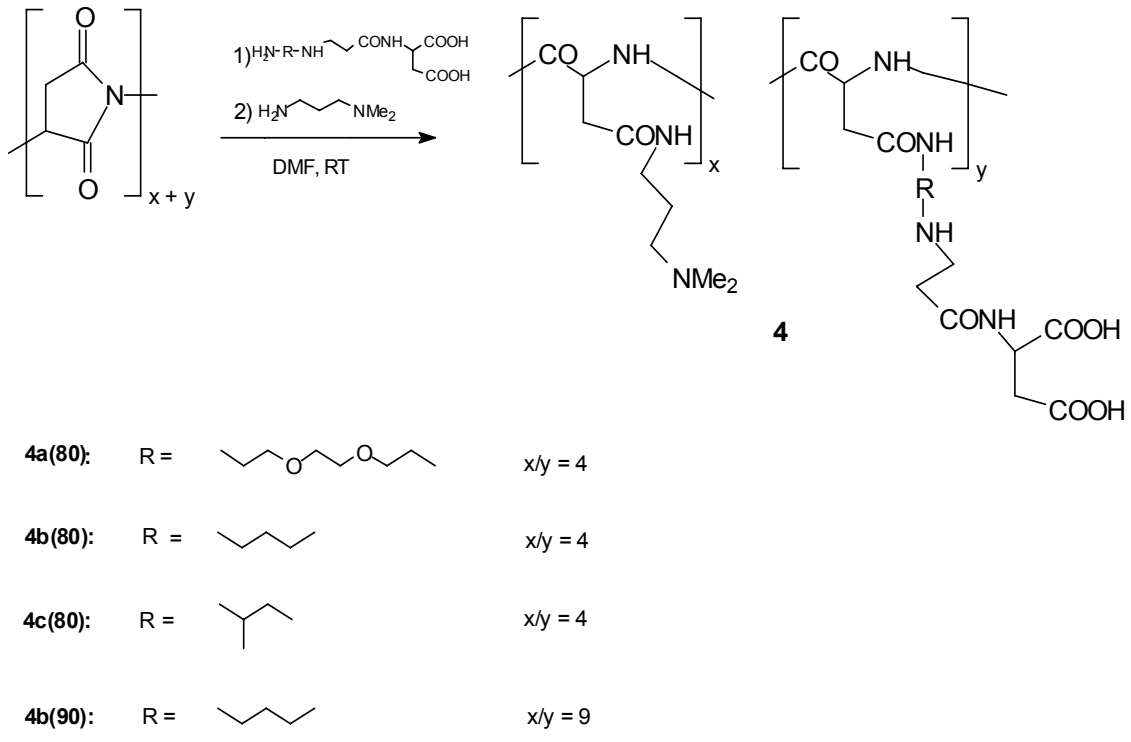
[a] *In D_2O, pD 10 – 11. Chemical shifts, δ (ppm), referenced against internal sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate;*

[b] *Integration error limit ± 15%*

b. Polyaspartamide carriers bearing dicarboxylato and hydroxylatocarboxylato functionalities

The same procedure as described for copolymers **3a(80), 3b(80), 3c(80), 3d(80), 3a(90), 3b(90)** and **3d(90)** was used for the preparation of the copolyaspartamide carriers **4a(80)**, **4b(80)**, **4c(80)** and **4b(90)** (Scheme 8) and **5a(80), 5b(80), 5c(80)** and **5a(90)** (Scheme 9), but the first step involved the reaction between the poly-DL-succinimide **2** with the drug-binding group in the presence of triethylamine for a specified time, typically 3 d. In the second step, a given amount of the hydrosolubilizing group was added to the suspension, and the reaction completed after a specific time, typically 1 d. This category of reactions was performed at room temperature. The polymeric products were isolated as completely water-soluble solids by a succession of operations including precipitation with an adequate non-solvent, aqueous dialysis, and freeze-drying. The polymeric products were obtained in typical yields of 18 – 31%, and possessed inherent viscosities in the range of 10 – 68 mL/g (see Table 3.5 and Table 3.7).

For Scheme 8, 3-(dimethylamino)propylamine (DMP) is used as solubilizing group, while the dicarboxyl groups introduced by N-(4,8-diaza-octanoyl)aspartic acid (**M3a**), N-(4,7-diaza-6(5)-methyl-heptanoyl)-aspartic acid (**M3b**) and N-(4,13-diaza-7,10-dioxa-tridecanoyl)aspartic acid (**M3c**), act as drug-binding site. The hydrosolubilizing tertiary amine functionality may also confer the special functions of pinocytotic cell entry and target cell affinity. The analysis of the NMR data indicated that the incorporation of the aspartic acid into the polymer backbone was enhanced by the utilization of aspartic acid derivatives. Table 3.5 and Table 3.6 summarize the experimental conditions, viscosity and ^{1}H NMR data.



Scheme 8: *Synthesis of Dicarboxylato-functionalized polyaspartamide carriers*

Table 3.5: *Summary of experimental data for polyaspartamide carriers bearing a dicarboxylato-terminated side chain as the drug binding site*

Polymer Designation	Molar Ratios[a]					Reaction time & Temperature[b]		Yield %	η_{inh}[c] mL/g	x/y
	PSI 2	M3a	M3b	M3c	DMP	1st Step	2nd Step			
4a(80)	1.00	-	-	0.40	0.80	3d, RT	1d, RT	25.6	12.35	4
4b(80)	1.00	0.40	-	-	0.80	3d, RT	1d, RT	31.0	16.8	4
4c(80)	1.00	-	0.40	-	0.80	3d, RT	1d, RT	18.9	14.9	4
4b(90)	1.00	0.20	-	-	0.90	3d, RT	1d, RT	22.8	10.4	9

[a] *Base molar **PSI 2***

[b] *RT: room temperature*

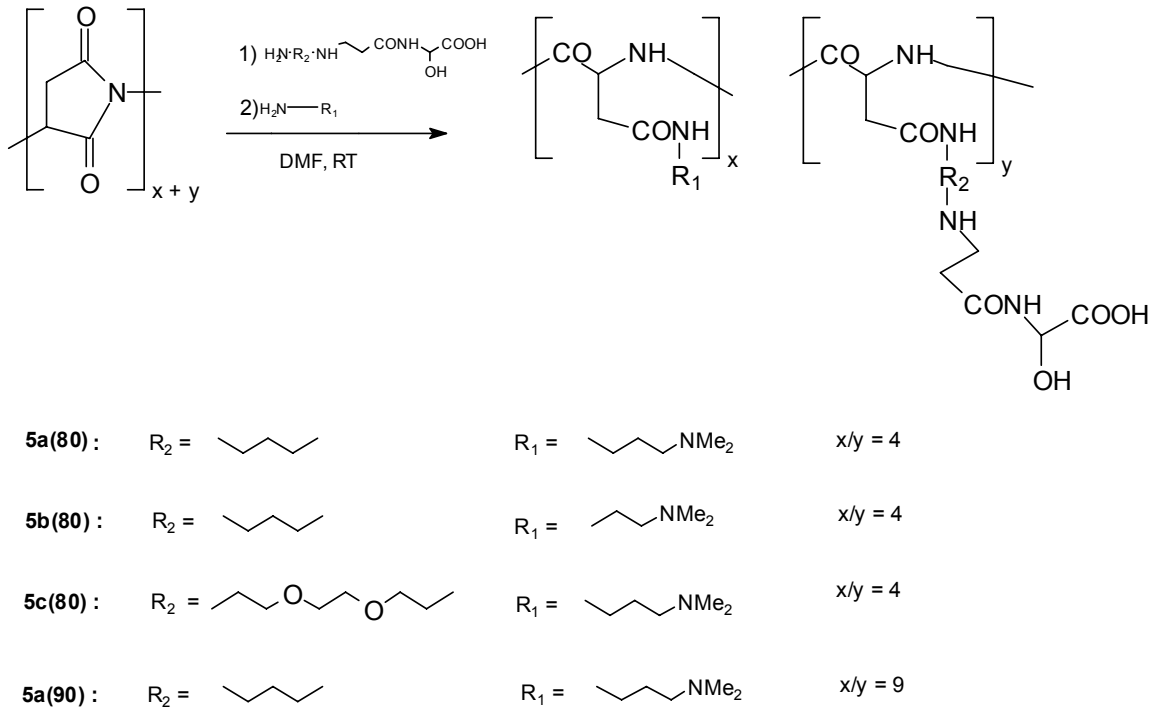
[c] *At 30.0^0C ± 0.5^0C in dist H_2O; conc = 0.2mg/100mL*

Table 3.6: *^{1}H NMR of copolyaspartamides **4a(80)**, **4b(80)**, **4c(80)** and **4b(90)***

Polymer Designation	Assignment	^{1}H NMR		
		Shift range δ(ppm)	Proton count	
			Found	Expected
4a(80)	$CONHCH_2\mathbf{CH_2}CH_2NMe_2$ $N\mathbf{Me_2}$ $\mathbf{CH_2}NMe_2$; $\mathbf{CH_2}NH\mathbf{CH_2}$ $\mathbf{CH_2}CONH$; $\mathbf{CH_2}$ (Asp) $CONH\mathbf{CH_2}$ $\mathbf{CH_2}O\mathbf{CH_2}\mathbf{CH_2}O\mathbf{CH_2}$	1.8 – 1.6 2.3 – 2.1 2.4 – 2.3 2.9 – 2.5 3.3 – 3.0 3.8 – 3.6	8 22.8 8 17.8 9.2 5.9	8 24 12 14 10 8
4b(80)	$CONHCH_2\mathbf{CH_2}CH_2NMe_2$; $CH_2\mathbf{CH_2}CH_2NH$ $N\mathbf{Me_2}$ $\mathbf{CH_2}NMe_2$; $\mathbf{CH_2}NH\mathbf{CH_2}$ $\mathbf{CH_2}CONH$; $\mathbf{CH_2}$ (Asp) $CONH\mathbf{CH_2}$	1.7 – 1.5 2.3 – 2.1 2.4 – 2.3 2.8 – 2.5 3.3 – 3.0	10 25.6 10 12.5 10	10 24 12 14 10
4c(80)	$CH\mathbf{CH_3}$ $CONHCH_2\mathbf{CH_2}CH_2NMe_2$ $N\mathbf{Me_2}$ $\mathbf{CH_2}NMe_2$; $\mathbf{CH_2}NH\mathbf{CH_2}$ $\mathbf{CH_2}CONH$; $\mathbf{CH_2}$ (Asp) $CONH\mathbf{CH_2}$	1.1 – 1.0 1.7 – 1.5 2.3 – 2.1 2.4 – 2.3 2.9 – 2.4 3.3 – 3.0	3 10.9 31.5 11.1 23.6 10.7	3 8 24 11 14 10
4b(90)	$CONHCH_2\mathbf{CH_2}CH_2NMe_2$; $CH_2\mathbf{CH_2}CH_2NH$ $N\mathbf{Me_2}$ $\mathbf{CH_2}NMe_2$; $\mathbf{CH_2}NH\mathbf{CH_2}$ $\mathbf{CH_2}CONH$; $\mathbf{CH_2}$ (Asp) $CONH\mathbf{CH_2}$	1.7 – 1.5 2.3 – 2.1 2.4 – 2.3 2.9 – 2.4 3.3 – 3.0	20 56.2 20 35 20	20 54 22 24 20

[a] *In D_2O, pD 10 – 11. Chemical shifts, δ (ppm), referenced against internal sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate*

[b] *Integration error limit ± 15%*



Scheme 9: *Synthesis of Carboxylatohydroxylato-functionalized polyaspartamide carriers*

In scheme 9, the side chains represented by R_2 in this category are derived either from 3-(dimethylamino)propylamine (DMP), or from 2-(dimethylamino)ethylamine (DMEA) used as the hydrosolubilizing groups, and the drug anchoring site is introduced by N-(4,8-diaza-octanoyl)amidoglycolic acid (**M4a**) or N-(4-13-diaza-7,10-dioxa-tridecanoyl)amidoglycolic acid (**M4b**). In agreement with the general principle outlined before, DMP and DMEA are used as major reactants (x > y). The experimental variables, viscosity and ^{1}H NMR data are summarized in Tables 3.7 and 3.8.

Table 3.7: *Summary of experimental data for polyaspartamide carriers bearing a hydroxylatocarboxylato-terminated side chain as the drug binding site*

Polymer Designation	Molar Ratios[a]					Reaction time & Temperature		Yield	η_{inh}[b] mL/g	x/y
	PSI 2	DMP	DMEA	M4a	M4b	1st Step	2nd Step			
5a(80)	1.00	0.80	-	0.40	-	3d, RT	1d, RT	27.3	68.2	4
5b(80)	1.00	-	0.80	0.40	-	3d, RT	1d, RT	21.7	21.8	4
5c(80)	1.00	0.80	-	-	0.40	3d, RT	2d, RT	29.8	14.5	4
5a(90)	1.00	0.90	-	0.20	-	3d, RT	1d, RT	19.2	19.6	9

[a] *Base molar **PSI 2***

[b] *At 30.0^{0}C ± 0.5^{0}C in dist H_2O; conc = 0.2mg/100mL*

Table 3.8: *^{1}H NMR of copolyaspartamides **5a(80), 5b(80), 5c(80)** and **5a(90)***

Polymer Designation	Assignment	^{1}H NMR		
		Shift range δ(ppm)	Proton count	
			Found	Expected
5a(80)	$CONHCH_2\mathbf{CH_2}CH_2NMe_2$; $CH_2\mathbf{CH_2}CH_2NH$	1.7 – 1.5	10	10
	$N\mathbf{Me_2}$	2.3 – 2.1	26	24
	$\mathbf{CH_2}NMe_2$; $\mathbf{CH_2}NH\mathbf{CH_2}$	2.4 – 2.3	11	12
	$\mathbf{CH_2}CONH$	2.9 – 2.5	10.8	12
	$CONH\mathbf{CH_2}$	3.3 – 3.0	9.8	10
5b(80)	$CONHCH_2\mathbf{CH_2}CH_2NH$	1.7 – 1.5	2	2
	$N\mathbf{Me_2}$	2.3 – 2.1	25.6	24
	$\mathbf{CH_2}NMe_2$; $\mathbf{CH_2}NH\mathbf{CH_2}$	2.4 – 2.3	13.2	12
	$\mathbf{CH_2}CONH$	2.9 – 2.5	11.5	12
	$CONH\mathbf{CH_2}$	3.3 – 3.0	9.4	10
5c(80)	$CH_2\mathbf{CH_2}CH_2NMe_2$	1.7 – 1.5	8	8
	$CH_2N\mathbf{Me_2}$	2.3 – 2.2	21.6	24
	$\mathbf{CH_2}N\ Me_2$; $\mathbf{CH_2}NH\mathbf{CH_2}$	2.4 – 2.3	9	12
	$\mathbf{CH_2}CONH$	2.9 – 2.5	12.6	12
	$CONH\mathbf{CH_2}$	3.3 – 3.1	8.9	10
	$\mathbf{CH_2}O\mathbf{CH_2}\mathbf{CH_2}O\mathbf{CH_2}$	3.7 – 3.5	7.3	8
5a(90)	$CONHCH_2\mathbf{CH_2}CH_2NMe_2$; $CH_2\mathbf{CH_2}CH_2NH$	1.7 – 1.5	20	20
	$N\mathbf{Me_2}$	2.3 – 2.1	59	54
	$\mathbf{CH_2}NMe_2$; $\mathbf{CH_2}NH\mathbf{CH_2}$	2.4 – 2.3	20.5	22
	$\mathbf{CH_2}CONH$	2.9 – 2.5	21.8	22
	$CONH\mathbf{CH_2}$	3.3 – 3.0	19.3	20

[a] *In D_2O, pD 10 – 11. Chemical shifts, δ (ppm), referenced against internal sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate*

[b] *Integration error limit ± 15%*

3.2.2 Polyamidoamines

Polyamidoamines are synthetic polymers characterized by the presence of amido and tertiary amino groups regularly arranged along the macromolecular chain. The addition of amine nucleophiles across an activated double bond (Michael addition) was utilized by Ferruti[46,47] and colleagues for the preparation of polymeric compounds possessing both amide and tertiary amine functions in the chain. Using equimolar quantities of bisacrylamides such as ethylenebisacrylamide and primary amines as comonomers, they prepared numerous linear macromolecules in which pairs of amide functionality and a tertiary amine functionality appear along the polymeric backbone.

The reactions are preferably done in aqueous solutions at room temperature. Using bisacrylamides and primary amines as comonomers, Ferruti's group obtained polymers characterized by the presence of only one tertiary amine segment for each pair of amine groups[46,47]. Some of these polymers possessed heparin-complexing or antimicrobial properties[47,75], and several types showed activity as inhibitors of secondary (metastatic) growth of certain murine tumor lines[76], suggesting interesting applications in the biomedical field.

In the ongoing investigation of macromolecular carrier systems possessing drug-binding functions, this group of polymers which falls into the category of polyamidoamines, was further developed in the polymer research laboratory of this University[77,78]. It is characterized by the presence in the backbone of both amide links and tertiary amino groups, and comprising glycolic acid and carboxylic acid groups as side chain terminals.

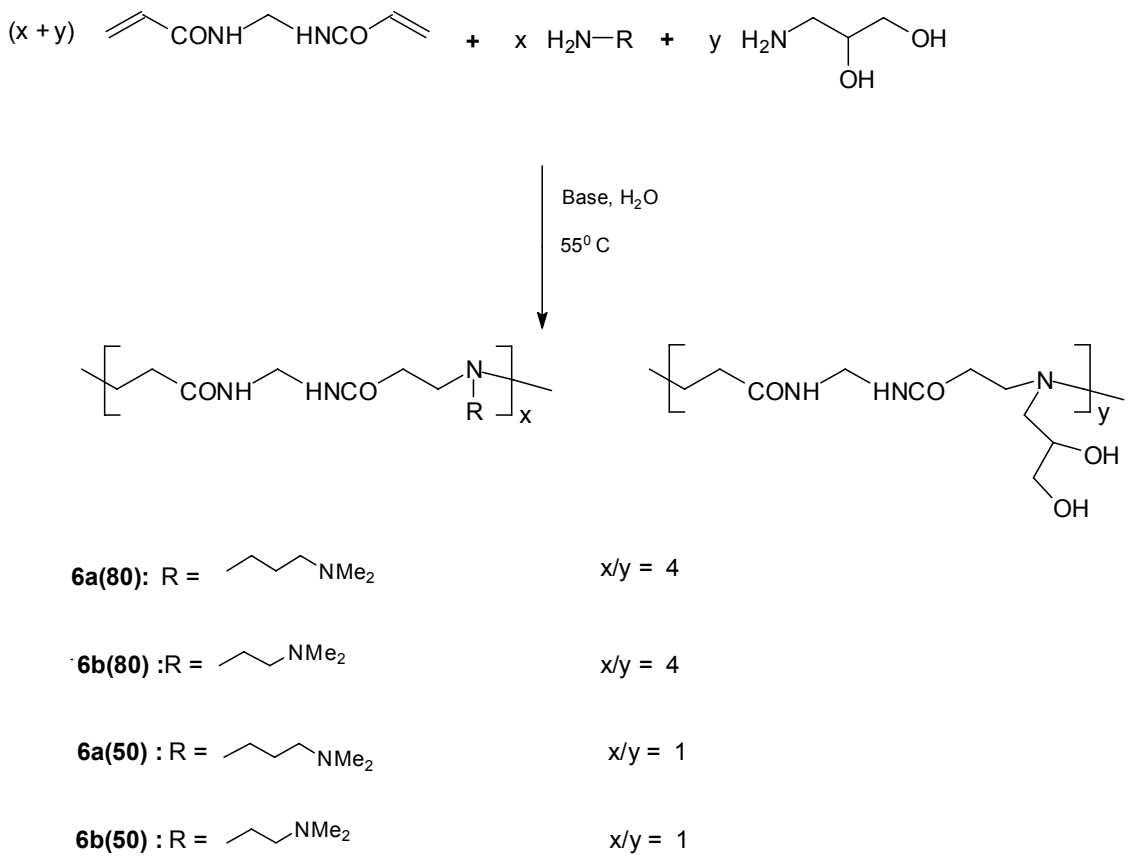
3.*2.2.1 Polyaddition reaction of methylenebisacrylamide with primary amine*

Experimental conditions similar to previous work performed in this laboratory[77] were used for this class of carrier polymers (see schemes 10 – 12). Methylenebisacrylamide (MBA), DL-aspartic acid (Asp), 3-amino-1,2-propanediol (APD), 3-(dimethylamino)propylamine (DMP), 2-(methoxy)ethylamine (MEA), ethanolamine (EA), 2-(dimethylamino)ethylamine (DMEA), N-(4,8-diaza-octanoyl)-amidoglycolic acid (**M4a**) and N-(4-13-diaza-7,10-dioxa-tridecanoyl)amidoglycolic acid (**M4b**) were used as comonomers for the synthesis of polyamidoamines **6a(80) – 8b(90)**.

In order to extend the spacing between functionalized groups and the enhancement of the solubility of the polymer in aqueous media, DMP, MEA, EA and DMEA were incorporated in the main chain, while DL-aspartic acid, APD, N-(4,8-diaza-octanoyl)amidoglycolic acid (**M4a**) and N-(4-13-diaza-7,10-dioxa-tridecanoyl)amidoglycolic acid (**M4b**) provided the ligands for drug anchoring. The polyaddition reactions were performed in aqueous medium, with reaction time of 3 days at 50 - 55^0 C. The products were isolated by freeze-drying after dialysis in 12 000 molecular weight cut-off membranes, as water soluble solids with inherent viscosities ranging from 4 to 22 mL/g.

Polyamidoamines **6a(80), 6b(80), 6a(50)** and **6b(50)** are shown in Scheme 10. Their synthesis involved the addition of the primary amine introduced by 3-amino-1,2-propanediol (APD), as the drug biding site, to methylenebisacrylamide (MBA) in the first step, followed by the addition of the hydrosolubilizing groups exemplified by the *tert*-amine terminals introduced by 3-(dimethylamino)propylamine (DMP) or 3-(dimethylamino)ethylamine (DMEA). Different feed ratios of comonomers were used. The carriers of this class were obtained as water-soluble solids

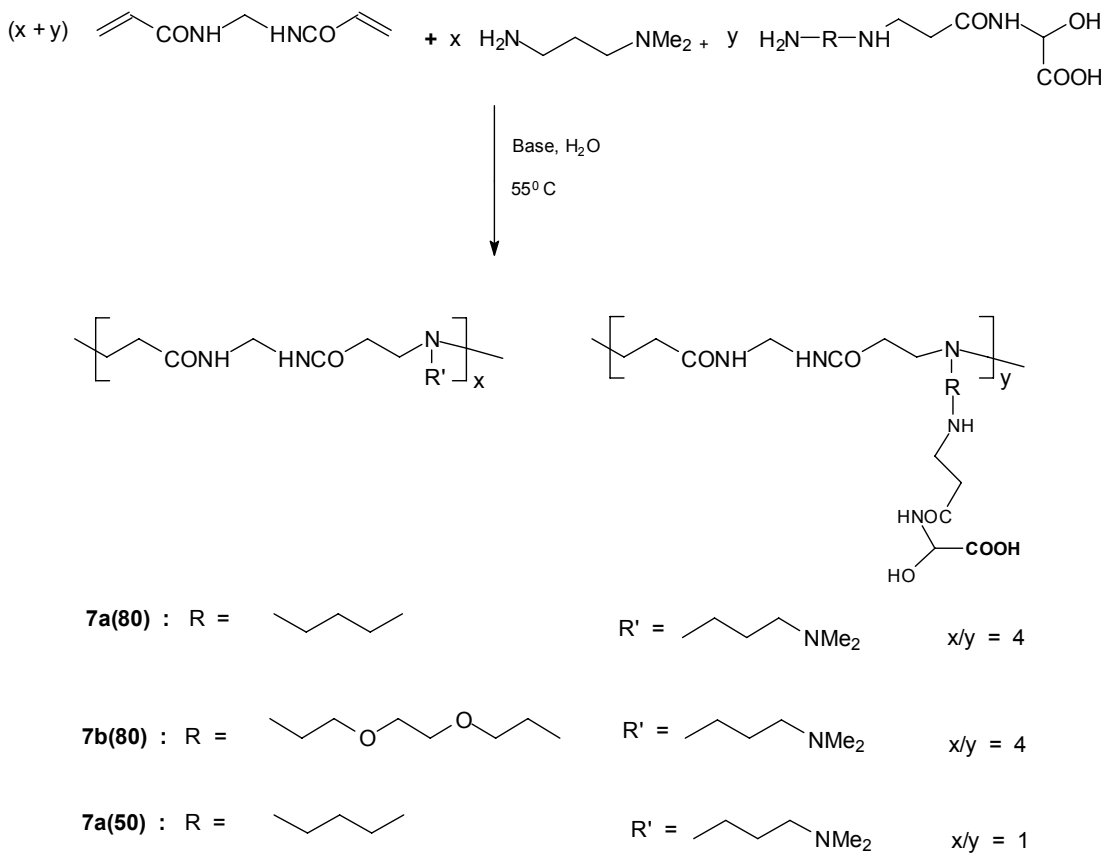
with inherent viscosities ranging from 12–22 mL/g. The experimental variables, viscosity and ^{1}H NMR data are summarized in Tables 3.9 and 3.10 respectively.



Scheme 10: *Synthesis of polyamidoamines* ***6a(80), 6b(80), 6a(50), 6b(50)***

Scheme 11 represents the synthesis of polyamidoamines **7a(80), 7b(80)** and **7a(50)** with N-(4,8-diaza-octanoyl)amidoglycolic acid (**M4a**) and N-(4-13-diaza-7,10-dioxa-tridecanoyl)amidoglycolic acid (**M4b**) containing the drug anchoring functionality, and the hydrosolubilizing unit represented by the *tert*-amine terminals derived from 3-(dimethylamino)propylamine (DMP). Different feed ratios of comonomers were used and the polyamidoamines

were obtained after routine work-up as water-soluble solids. The experimental conditions, viscosity and ^{1}H NMR data are summarized in Tables 3.9 and 3.10 respectively.



Scheme 11: *Synthesis of polyamidoamines* ***7a(80), 7b(80)*** *and* ***7a(50)***.

Table 3.9: *Preparative data of polyamidoamines* ***6a(80) – 6b(50)*** *and* ***7a(80) – 7a(50)***

Polymer Designation	Molar Ratios [a]						Reaction time & Temperature		Yield %	η_{inh} [b] mL/g	x/y
	MBA [c]	DMP	DMEA	APD	M4a	M4b	1st Step	2nd Step			
6a(80)	1.00	0.80	-	0.21	-	-	1d, 50^0 C	2d, 55^0 C	42.7	22.6	4
6b(80)	1.00	-	0.80	0.21	-	-	1d, 50^0 C	2d, 55^0 C	39.9	19.6	4
6a(50)	1.00	0.50	-	0.505	-	-	1d, 50^0 C	2d, 55^0 C	44.5	17.2	1
6b(50)	1.00	-	0.50	0.505	-	-	1d, 50^0 C	2d, 55^0 C	37.8	12.4	1
7a(80)	1.00	0.80	-	-	0.22	-	1d, 50^0 C	2d, 55^0 C	41.3	9.3	4
7b(80)	1.00	0.80	-	-	-	0.22	1d, 50^0 C	2d, 55^0 C	45.6	7.5	4
7a(50)	1.00	0.50	-	-	0.22	-	1d, 50^0 C	2d, 55^0 C	39.7	11.3	1

[a] *Base molar* for ***MBA***

[c] *MBA: Methylenebisacrylamide*

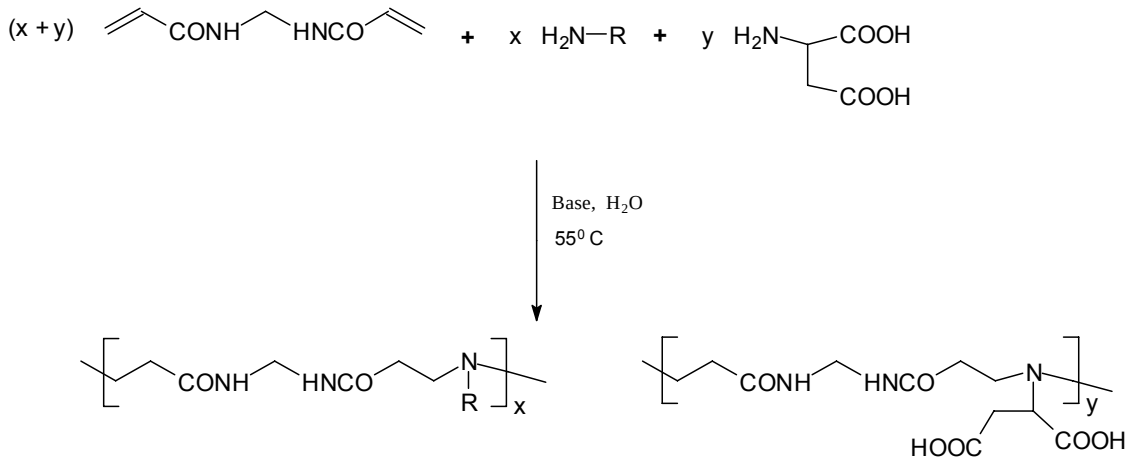
[b] *At 30.0^0C ± 0.5^0C in dist H_2O; conc = 0.2mg/100mL*

Table 3.10: *^{1}H NMR of polyamidoamines **6a(80) – 6b(50)** and **7a(80) – 7a(50)***

Polymer Designation	Assignment	^{1}H NMR		
		Shift range δ(ppm)	Proton count	
			Found	Expected
6a(80)	$CH_2\mathbf{CH_2}CH_2NMe_2$ $CH_2N\mathbf{Me_2}$ $\mathbf{CH_2CH_2}CONH$, $N\mathbf{CH_2}CH_2\mathbf{CH_2}NMe_2$ $CH_2\mathbf{CH_2}(OH)\mathbf{CH_2}OH$ $CONH\mathbf{CH_2}NHCO$	1.9 – 1.7 2.5 – 2.3 3.1 – 2.5 3.9 – 3.4 4.6 – 4.5	8 20 56 3 10.4	8 24 58 3 10
6b(80)	$CH_2N\mathbf{Me_2}$ $\mathbf{CH_2CH_2}CONH$, $N\mathbf{CH_2}CH_2\mathbf{CH_2}NMe_2$ $CH_2\mathbf{CH_2}(OH)\mathbf{CH_2}OH$ $CONH\mathbf{CH_2}NHCO$	2.5 – 2.3 3.2 – 2.6 3.9 – 3.4 4.6 – 4.5	23.4 60.6 3 10.2	24 58 3 10
6a(50)	$CH_2\mathbf{CH_2}CH_2NMe_2$ $CH_2N\mathbf{Me_2}$ $\mathbf{CH_2CH_2}CONH$, $N\mathbf{CH_2}CH_2\mathbf{CH_2}NMe_2$ $CH_2\mathbf{CH_2}(OH)\mathbf{CH_2}OH$ $CONH\mathbf{CH_2}NHCO$	1.9 – 1.7 2.5 – 2.3 3.1 – 2.5 3.9 – 3.4 4.6 – 4.5	2 7 20.4 3 4	2 6 22 3 4
6b(50)	$CH_2N\mathbf{Me_2}$ $\mathbf{CH_2CH_2}CONH$, $N\mathbf{CH_2}CH_2\mathbf{CH_2}NMe_2$ $CH_2\mathbf{CH_2}(OH)\mathbf{CH_2}OH$ $CONH\mathbf{CH_2}NHCO$	2.5 – 2.3 3.2 – 2.6 3.9 – 3.4 4.6 – 4.5	8 19.8 3 4.2	6 22 3 4
7a(80)	$CH_2\mathbf{CH_2}CH_2NMe_2$, $CH_2\mathbf{CH_2}CH_2NH$ $CH_2N\mathbf{Me_2}$ $\mathbf{CH_2CH_2}CONH$; $N\mathbf{CH_2}CH_2\mathbf{CH_2}NMe_2$; $\mathbf{CH_2}N\ \mathbf{CH_2}$ $CONH\mathbf{CH_2}NHCO$	1.7 – 1.5 2.3 – 2.2 2.8 – 2.3 4.6 – 4.5	10 25.6 62.5 10	10 24 64 10
7b(80)	$CH_2\mathbf{CH_2}CH_2NMe_2$ $CH_2N\mathbf{Me_2}$ $\mathbf{CH_2CH_2}CONH$; $N\mathbf{CH_2}CH_2\mathbf{CH_2}NMe_2$; $\mathbf{CH_2}N\ \mathbf{CH_2}$ $\mathbf{CH_2}O\mathbf{CH_2CH_2}O\ \mathbf{CH_2}$ $CONH\mathbf{CH_2}NHCO$	1.7 – 1.5 2.3 – 2.2 2.8 – 2.3 3.7 – 3.6 4.6 – 4.5	8 24 62.7 7.4 9.2	8 24 64 8 10
7c(50)	$CH_2\mathbf{CH_2}CH_2NMe_2$, $CH_2\mathbf{CH_2}CH_2NH$ $CH_2N\mathbf{Me_2}$ $\mathbf{CH_2CH_2}CONH$; $N\mathbf{CH_2}CH_2\mathbf{CH_2}NMe_2$; $\mathbf{CH_2}N\ \mathbf{CH_2}$ $CONH\mathbf{CH_2}NHCO$	1.7 – 1.5 2.3 – 2.2 2.8 – 2.3 4.6 – 4.5	4 5.2 27 3.8	4 6 28 4

[a] *In D_2O, pD 10 – 11. Chemical shifts, δ (ppm), referenced against internal sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate*
[b] *Integration error limit ± 15%*

Scheme 12 represents the synthesis of polyamidoamines **8a(80), 8b(80)** and **8b(90)** with the drug anchoring site derived from aspartic acid, and the solubilizing groups represented by the tert-amine, neutral methoxy and the hydroxyl terminals derived from 3-(dimethylamino)propylamine (DMP) and 2-(methoxy)ethylamine (MEA). Various molar feed rations were used. The experimental conditions, viscosity and ^{1}H NMR data are found in Table 3.11 and Table 3.12.



8a(80) : R = NMe_2 x/y = 4

8b(80) : R = OMe x/y = 4

8b(90) : R = OMe x/y = 9

Scheme 12: *Synthesis of polyamidoamines **8a(80), 8b(80)** and **8b(90)***

Table 3.11: *Preparative data of polyamidoamines **8a(80), 8b(80)** and **8b(90)***

Polymer Designation	Molar Ratios [a]					Reaction time & Temperature		Yield %	η_{inh} [b] mL/g	x/y
	MBA	DMP	MEA [c]	EA [c]	Asp [c]	1st Step	2nd Step			
8a(80)	1.00	0.80	-	-	0.20	1d, 50^0 C	3d, 55^0 C	41.3	6.4	4
8b(80)	1.00	-	0.80	-	0.20	1d, 50^0 C	3d, 55^0 C	37.5	5.1	4
8b(90)	1.00	-	0.90	-	0.10	1d, 50^0 C	3d, 55^0 C	39.1	4.6	9

[a] *Base molar* for ***MBA***

[b] *At 30.0^0C ± 0.5^0C in dist H_2O; conc = 0.2mg/100mL*

[c] *MEA: 2-(methoxy)ethylamine; EA: Ethanolamine; Asp: DL-Aspartic acid*

Table 3.12: *^{1}H NMR of polyamidoamines* ***8a(80); 8b(80), 8a(90) and 8b(90)***

Polymer Designation	Assignment	^{1}H NMR		
		Shift range δ(ppm)	Proton count	
			Found	Expected
8a(80)	CH_2**CH_2**CH_2NMe_2	1.7 – 1.5	8	8
	CH_2N**Me_2**	2.3 – 2.2	23.4	24
	CH_2N(**CH_2**)(**CH_2**); **CH_2**NMe_2	2.6 – 2.3	40.5	36
	CH_2CONH; **CH_2** (Asp)	2.9 – 2.7	21.2	22
	CONH**CH_2**NHCO; **CH** (Asp)	4.7 – 4.5	11.5	11
8b(80)	**CH_2**N(**CH_2**)(**CH_2**)	2.5 – 2.3	19.2	28
	CH_2CONH; **CH_2** (Asp)	2.9 – 2.6	25.5	22
	O**Me**	3.4 – 3.3	12	12
	CH_2OMe	3.6 – 3.5	7.9	8
	CONH**CH_2**NHCO; **CH** (Asp)	4.7 – 4.5	9.3	11
8a(90)	CH_2**CH_2**CH_2NMe_2	1.7 – 1.5	18	18
	CH_2N**Me_2**	2.3 – 2.2	57.6	54
	CH_2N(**CH_2**)(**CH_2**); **CH_2**NMe_2	2.6 – 2.3	80.4	76
	CH_2CONH; **CH_2** (Asp)	2.9 – 2.7	40.8	42
	CONH**CH_2**NHCO; **CH** (Asp)	4.7 – 4.5	20.4	22
8b(90)	**CH_2**N(**CH_2**)(**CH_2**)	2.5 – 2.3	42	42
	CH_2CONH; **CH_2** (Asp)	2.9 – 2.6	53	58
	O**Me**	3.4 – 3.3	27	27
	CH_2OMe	3.6 – 3.5	17.5	18
	CONH**CH_2**NHCO; **CH** (Asp)	4.7 – 4.5	18	22

[a] *In D_2O, pD 10 – 11. Chemical shifts, δ (ppm), referenced against internal sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate*
[b] *Integration error limit ± 15%*

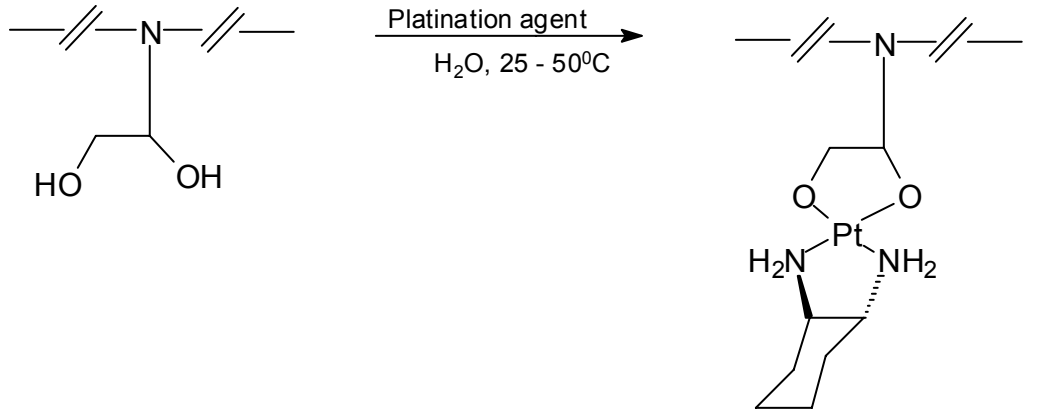
3.3 Polymer-Platinum Anchoring

Since the discovery of the antitumor properties of *cisplatin*, many metal complexes have been tested for this activity, especially platinum(II) compounds[79,80]. Cisplatin is one of the most potent antitumor platinum complexes and is widely used in the treatment of various solid tumors. However, renal clearance of *cisplatin*[81] is quite fast, and its accumulation in kidney causes severe renal toxicities. By varying the amine ligands of the cisplatin it was found that *cis*-dichloroplatinum(II) complexes containing *trans*-1,2-diaminocyclohexane (DACH) also showed a very high antitumor activity. As salt-like compounds, such complexes cannot efficiently penetrate cell membranes by the passive diffusion mechanism generally utilized by the nonpolar, neutral compounds. In comparison with a conventional low-molecular-weight drug, a macromolecular prodrug, that is a polymer-drug conjugate, can be expected to overcome such serious problems by improving the body distribution of antitumor agent through the enhanced permeability and retention effect[50,51,52,82] and pinocytotic cell entry.

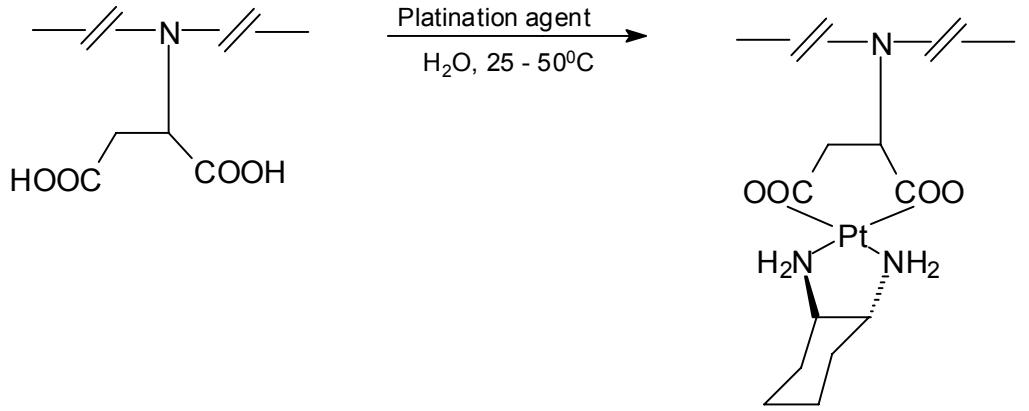
It is evident from the forgoing that the drugs based on platinum should lend themselves as outstanding candidates for polymer anchoring, *i.e.* the bioreversible conjugation with suitably designed carrier polymers. The literature indeed provides a plethora of illustrating examples of research in this field. By far the most significant work, published in numerous papers dating back to 1983[83,84,85], has been performed in Carraher's laboratory, and this researcher has presented a comprehensive and proficiently written account of his work and other publications in the field[52]. Also noteworthy are the investigations by Ohya' group[81], Duncan's team[86], and most notably, Schechter's laboratory[87]. Initially water-soluble, physically isolated and analytically characterized polymer-platinum conjugates have predominantly been the domain of the polymer research group in this Department. These

conjugates were based on polyaspartamide carrier structures and typically embodied a drug-anchoring design featuring Pt bonding through ethylenediamine chelation, the ligand being attached to short polymer side chains *via* biofissionable amide links. High activity against HeLa cells as well as against explanted human cancers of the liver and lung, paired with excellent cell specificity, was demonstrated *in vitro* for these Pt-conjugates[27,88-94]. These encouraging earlier results provided the impetus for the present project.

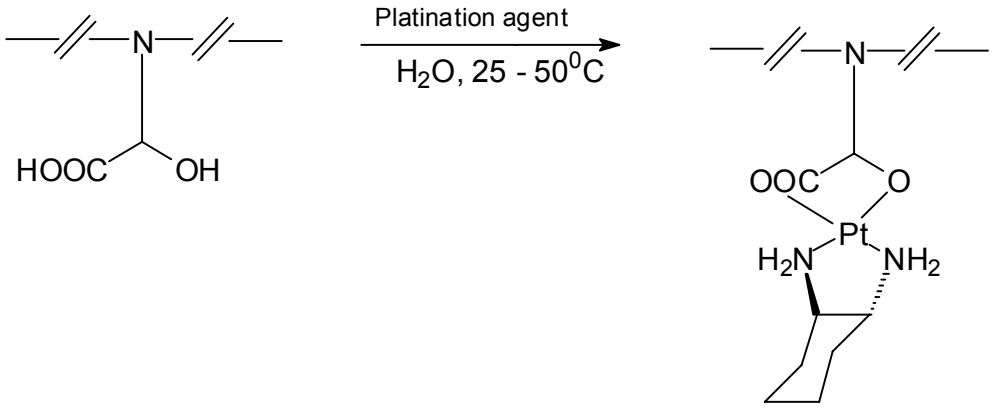
The controlled release of platinum agents from carriers depends critically on the type and structure of the metal ligands chosen to serve as the connecting link to the carrier. In this project, platinum complexes anchored *via* the leaving group ligands, namely dihydroxylato (model 1), dicarboxylato (model 2) and carboxylatohydroxylato (model 3) ligands are investigated (Figure 3.1). The choice of these ligands was motivated by their ability to chelate the platinum agent upon coordination, forming five- or seven-membered rings stable enough to survive in central circulation. From the ultimate conjugate, the release will occur by hydrolytic cleavage of oxygen-metal bonds at low pH, freeing the *trans*-1,2-diaminocyclohexaneplatinum(II) as an aquated species. The platination agent, *trans*-1,2-diaminocyclohexanediaquaplatinum(II) dinitrate (DACH-Pt), was prepared by a literature procedure[95]. As depicted in Scheme 13, tetrachloroplatinum(II) salt in aqueous solution was treated with *trans*-1,2-diaminocyclohexane, followed by chloro-aqua ligand exchange in the presence of silver nitrate.



Model 1

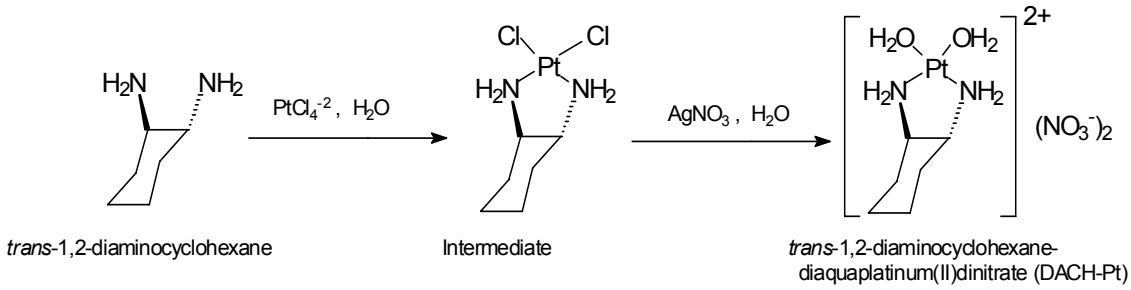


Model 2



Model 3

Figure 3.1: *Models of platinum-conjugates via chelation*



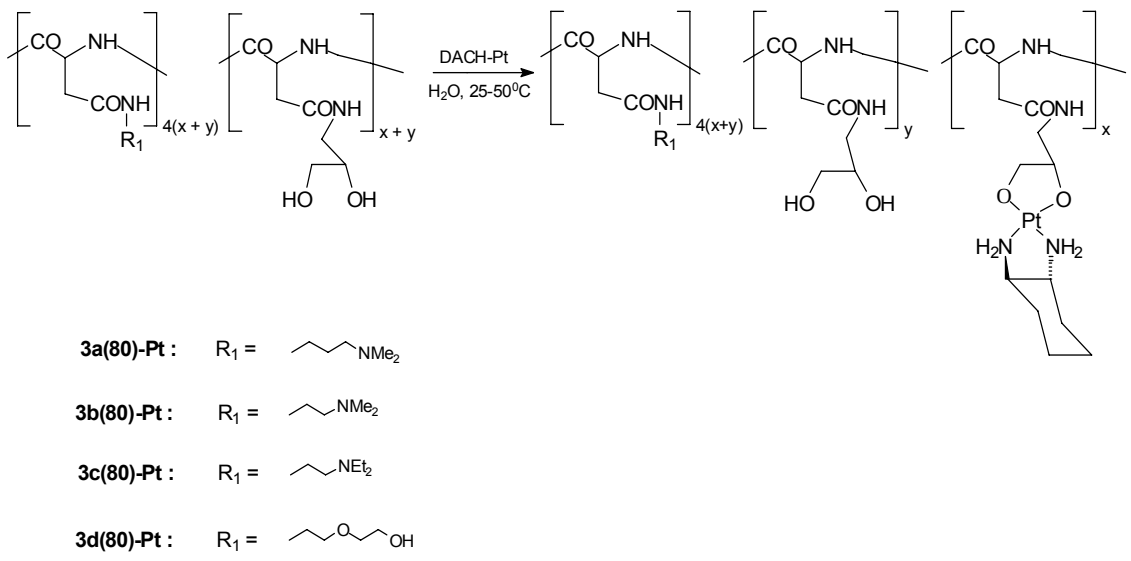
trans-1,2-diaminocyclohexane Intermediate *trans*-1,2-diaminocyclohexane-diaquaplatinum(II)dinitrate (DACH-Pt)

Scheme 13: *Synthesis of DACH-Pt as platination agent*

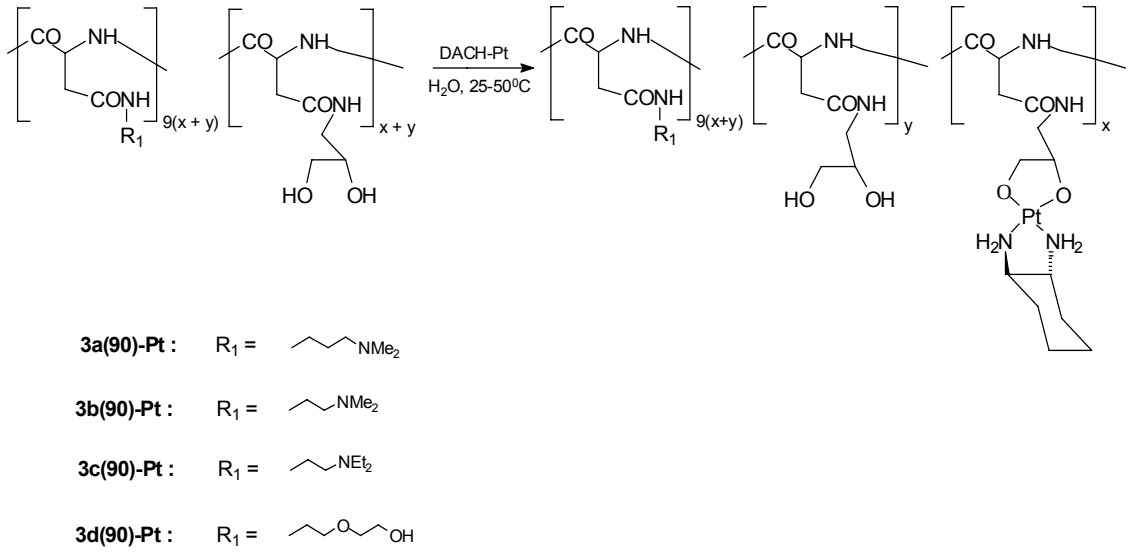
3.3.1 Polymer-Platinum Anchoring *via* Dihydroxylato Ligand

Conjugates **3a(80)-Pt – 3d(80)-Pt, 3a(90)-Pt – 3d(90)-Pt, 6a(80)-Pt, 6b(80)-Pt, 6a(90)-Pt,** and **6b(90)-Pt** were derived from carriers **3a(80) – 3d(80), 3a(90) – 3d(90), 6a(80), 6b(80), 6a(90)** and **6b(90)**. The carriers were treated with *trans*-1,2-diaminocyclohexanediaquaplatinum(II) dinitrate (DACH-Pt) in aqueous medium, according to schemes 14, 15 and 16 under controlled experimental conditions of time, temperature and acidity. In order to suppress the hydrolysis of the carrier catalysed by lower pH or hydroxo bridging which causes insolubility of the final conjugate at high pH, the pH was carefully adjusted and maintained in the range of the range of pH ~ 5.5 – 6. The conjugates were routinely filtered and dialyzed for 2 d in Spectra/Por 4. Freeze-drying of the retentate afforded the water-soluble solids with inherent viscosities of 5 – 12 ml/g. The platinum content was calculated for the structures normalized to y = 1, and cases where platinum content was higher than calculated, suggested that there is some unbound platinum salt. As a general trend, poor platinum content was observed with carriers of molar feed ratio 9 to 1, solubilizing group to drug binding site, when containing tert-amine side group functionality. This suggested that there is less binding site available. However, when the solubilizing group was the 2-(2-aminoethoxy)ethanol (AEE), providing an hydroxyl side group terminal, high platinum incorporation was observed. This suggested that there is a poor discrimination between the drug binding group and the

solubilizing group during platination process, leading to partial solubilizing-group platination. High platinum content was obtained in both polyaspartamide and polyamidoamine carriers with a molar feed ratio of 4 to 1, solubilizing group to drug binding site, when platinum was chelated to dicarboxylato ligand. When platinum was chelated to dihydroxylato ligand, high platinum content was obtained with polyaspartamide carriers, while the carboxylatohydroxylato ligand did not generally give good results. These findings suggested that conjugates are stable in both polyaspartamide and polyamidoamine carriers when platinum is chelated *via* dicarboxylato ligand in contrast to platinum chelated to dihydroxylato or carboxylatohydroxylato ligands. The molar feed ratio, experimental conditions, viscosities, and platinum content data are summarized in Table 3.13.



Scheme 14: *Synthesis of conjugates* ***3a(80)-Pt – 3d(80)-Pt***



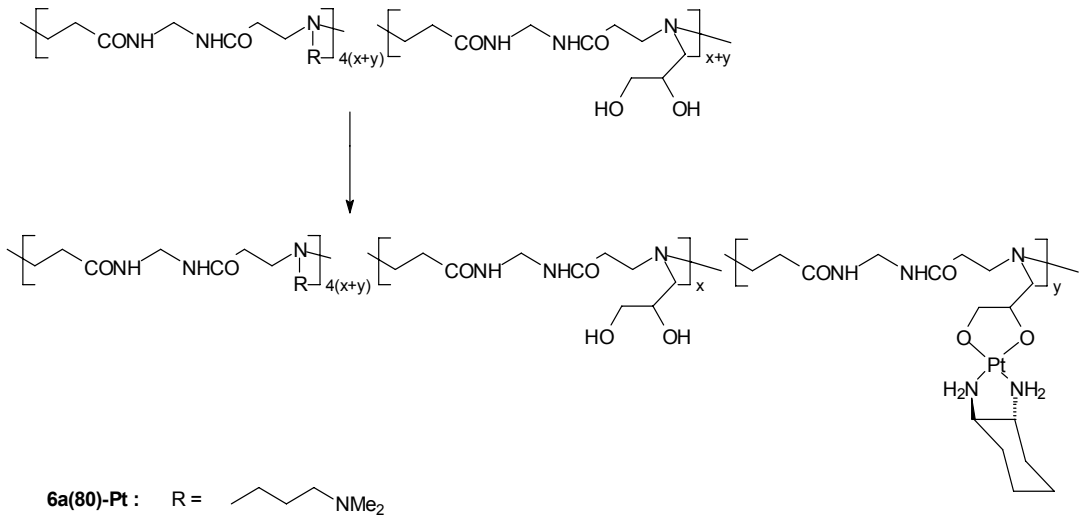
3a(90)-Pt : R_1 = NMe_2

3b(90)-Pt : R_1 = NMe_2

3c(90)-Pt : R_1 = NEt_2

3d(90)-Pt : R_1 = O OH

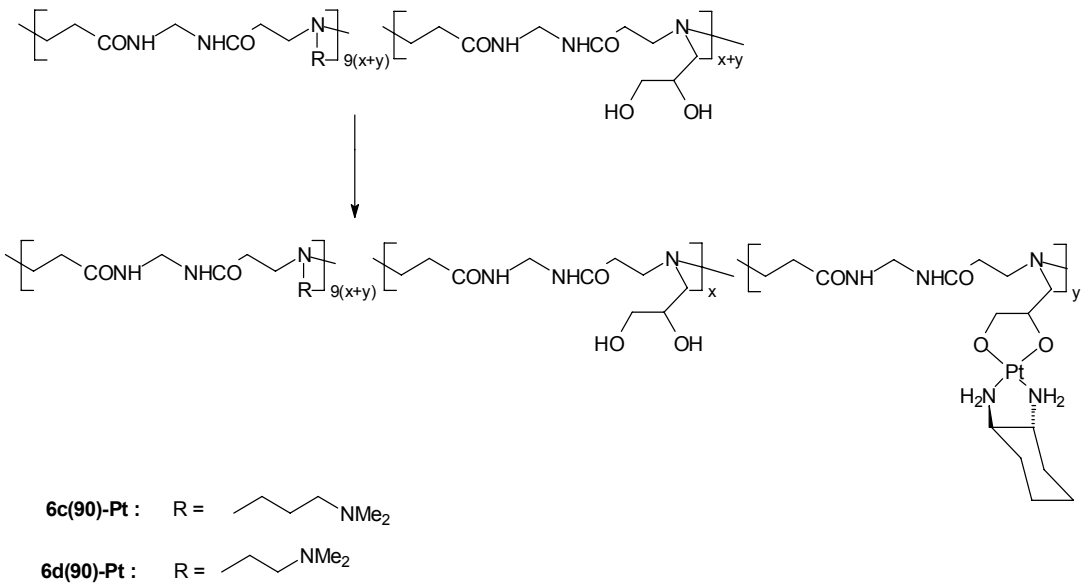
Scheme 15: *Synthesis of conjugates **3a(90)-Pt – 3d(90)-Pt***



6a(80)-Pt : R = NMe_2

6b(80)-Pt : R = NMe_2

Scheme 16: *Synthesis of conjugates **6a(80)-Pt** and **6b(80)-Pt***



Scheme 17: *Synthesis of conjugates* ***6c(90)-Pt*** *and* ***6d(90)-Pt***

Table 3.13: *Preparative and Analytical data of platinum-conjugates, Pt chelated via dihydroxylato ligand*

Conjugate Designation	Molar Ratios		Reaction time & Temperature		Yield %	Base Molecular Mass[a]	η_{inh}[b] mL/g	x/y[c]	Pt %	
	Carrier	DACH-Pt	Fist Step	Second Step					Found[d]	Calcd[e]
3a(80)-Pt	1.0	1.1	3d, RT	2h, 50^0C	56.3	1292.3	5.8	3.7	15.25	15.1
3d(80)-Pt	1.0	1.1	3d, RT	2h, 50^0C	48.1	1360.9	7.3	1.1	14.11	15.0
3a(90)-Pt	1.0	1.1	3d, RT	2h, 50^0C	10.7	14261.9	9.1	7.2	1.18	8.5
3d(90)-Pt	1.0	1.1	3d, RT	2h, 50^0C	37.9	2377.1	12.4	1.03	8.15	8.4
6a(80)-Pt	1.0	1.1	3d, RT	2h, 50^0C	38.1	3580.8	7.9	13.9	0.92	12.3
6b(50)-Pt	1.0	1.1	3d, RT	2h, 50^0C	12.3	4678.2	12.1	9.5	2.57	24.5

[a] *Molecular mass of the simple recurring unit; structures normalized to y = 1*

[b] *At 30.0^0 C ± 0.5^0 C in distilled water; conc = 2mg/mL*

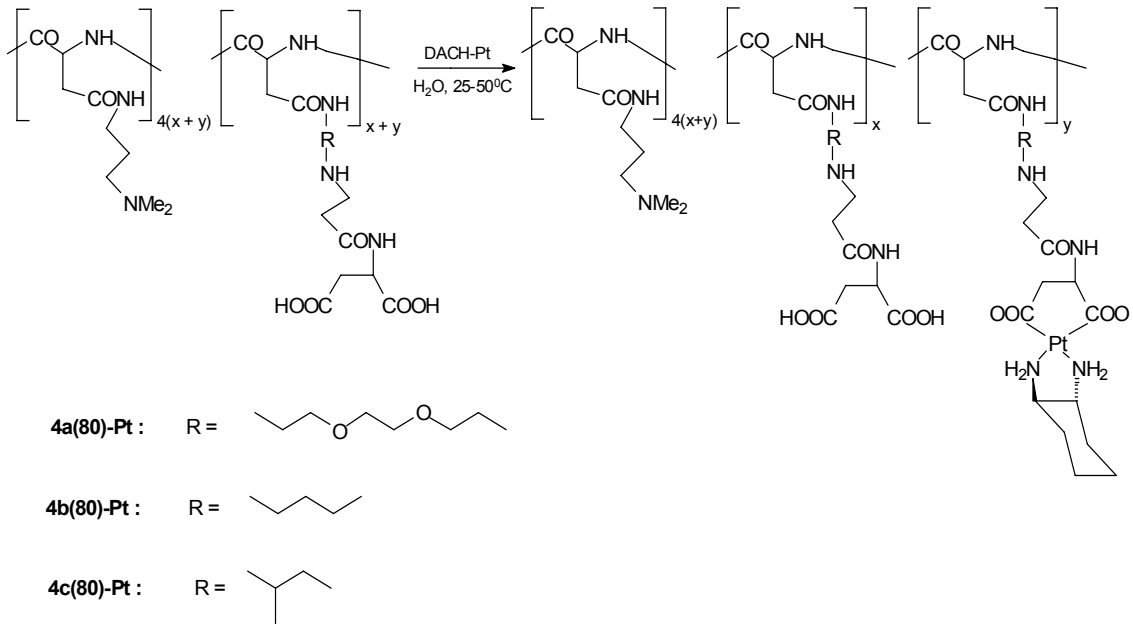
[c] *Calculated from analytically determined Pt content*

[d] *Pt content (%) obtained by atomic absorption analysis performed by an outside laboratory*

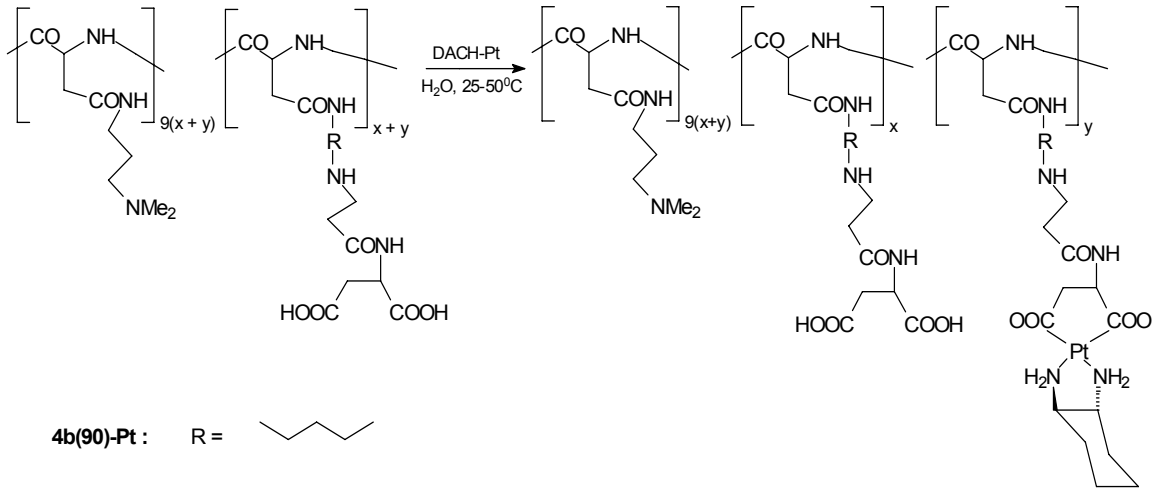
[e] *Pt content for complete incorporation of the drug*

3.3.2 Polymer-Platinum Anchoring *via* Dicarboxylato Ligand

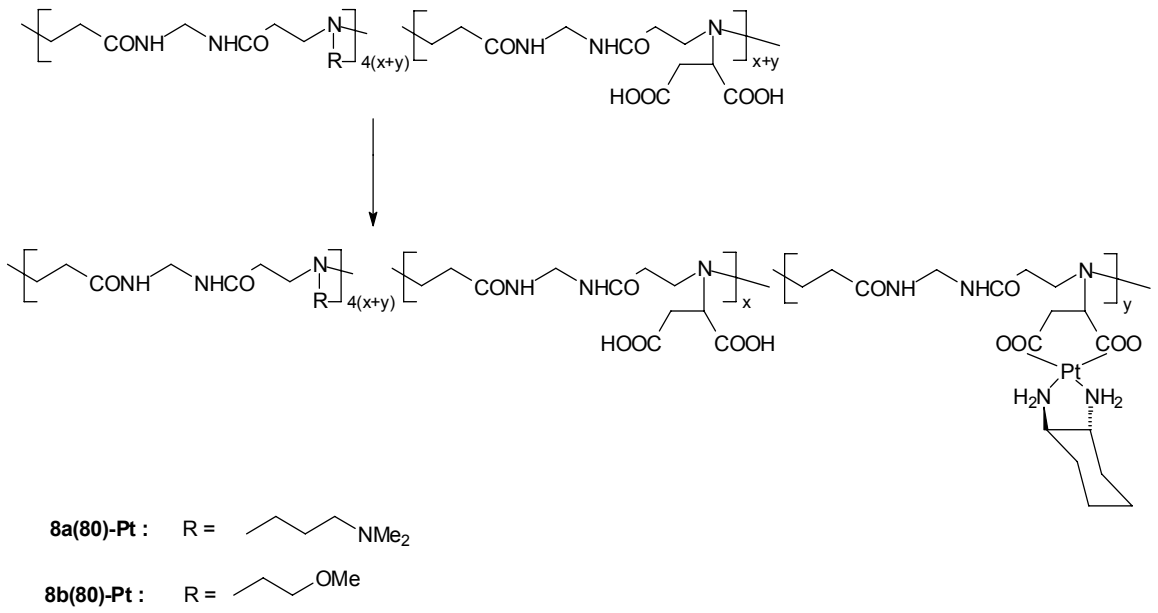
Conjugates **4a(80)-Pt – 4c(80)-Pt, 4b(90)-Pt,** and **8a(80)-Pt – 8c(80)-Pt** were obtained from carriers **4a(80) – 4c(80), 4b(90),** and **8a(80) – 8b(80)**. As depicted in schemes 17, 18 and 19, carriers **4a(80) – 4c(80), 4b(90),** and **8a(80) – 8b(80)** were treated with DACH-Pt in aqueous medium under controlled experimental conditions of time, temperature and acidity. No modification in the work-up steps was observed, this was done as discussed in the foregoing paragraph. The experimental conditions, viscosities, and platinum content data are summarized in Table 3.14.



Scheme 18: *Synthesis of conjugates **4a(80)-Pt, 4b(80)-Pt** and **4c(80)-Pt***



Scheme 19: *Synthesis of conjugate **4b(90)-Pt***



Scheme 20: *Synthesis of conjugates **8a(80)-Pt, 8b(80)-Pt** and **8c(80)-Pt***

Table 3.14: *Preparative and Analytical data of platinum-conjugates, Pt chelated via dicarboxylato ligand*

Conjugate Designation	Molar Ratios		Reaction time & Temperature		Yield %	Base Molecular Mass [a]	η_{inh} [b] mL/g	x/y [c]	Pt %	
	Carrier	DACH-Pt	Fist Step	Second Step					Found [d]	Calcd [e]
4a(80)-Pt	1.0	1.1	3d, RT	2h, 50^0C	42.7	1823.9	7.3	1.2	10.28	12.7
4b(80)-Pt	1.0	1.1	3d, RT	2h, 50^0C	49.1	1555.2	9.7	1.1	12.3	13.3
4c(80)-Pt	1.0	1.1	3d, RT	2h, 50^0C	54.3	2412.3	6.4	1.8	7.3	13.3
4b(90)-Pt	1.0	1.1	3d, RT	2h, 50^0C	21.6	9750.2	5.9	4.4	1.8	7.9
8a(80)-Pt	1.0	1.1	3d, RT	2h, 50^0C	52.6	1619.8	11.3	1.0	12.33	12.0
8b(80)-Pt	1.0	1.1	3d, RT	2h, 50^0C	44.5	1511.5	8.1	1.0	16.46	12.9

[a] *Molecular mass of the simple recurring unit; structures normalized to y = 1*

[b] *At 30.0^0 C ± 0.5^0 C in distilled water; conc = 2mg/mL*

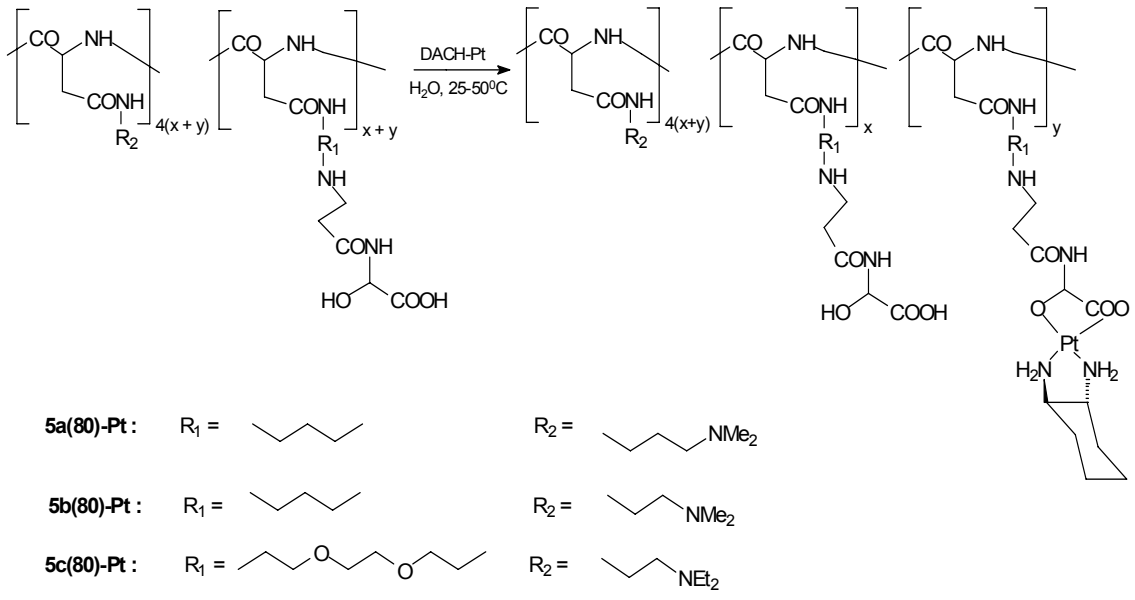
[c] *Calculated from analytically determined Pt content*

[d] *Pt content (%) obtained by atomic absorption analysis performed by an outside laboratory*

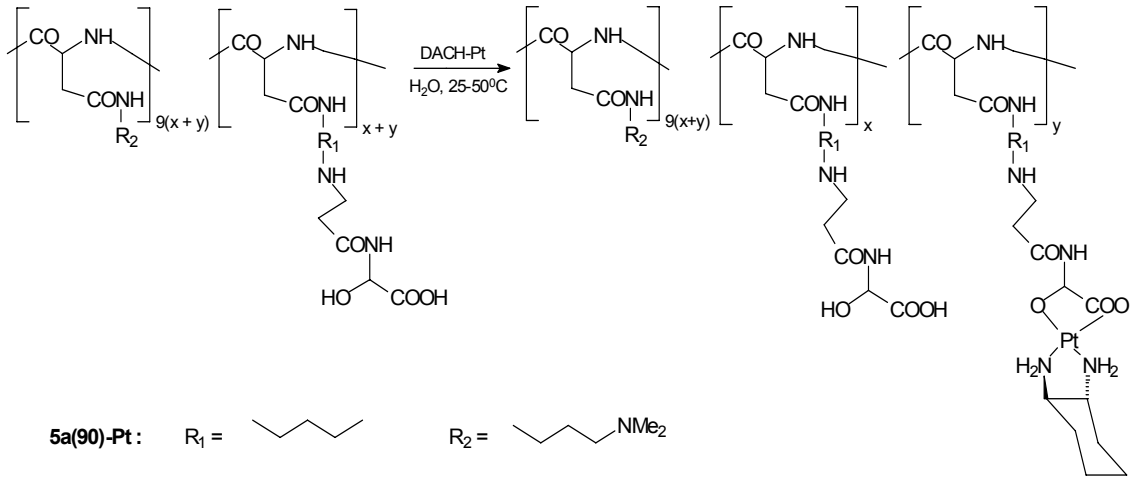
[e] *Pt content for complete incorporation of the drug*

3.3.3 Polymer-Platinum Anchoring *via* Carboxylatohydroxylato Ligand

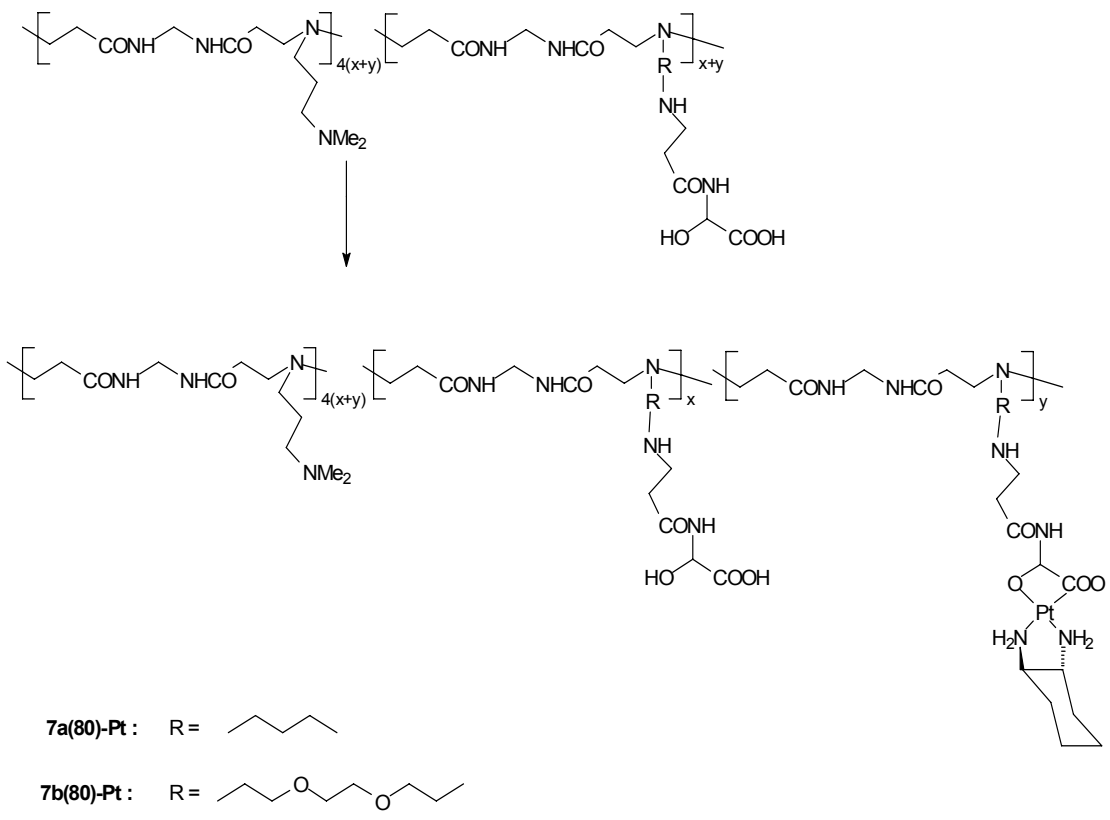
Conjugates **5a(80)-Pt – 5c(80)-Pt, 5a(90)-Pt, 7a(80)- Pt** and **7b(80)-Pt** were derived from carriers **5a(80) – 5c(80), 5a(90), 7a(80)** and **7b(80)**. The preparation of this class of conjugates (schemes 20, 21 and 22) followed the same procedure as described in the foregoing experiment. The conjugates were isolated as water-soluble solid with inherent viscosities of 7 – 14 mL/g. The molar feed ratio, experimental conditions, viscosities, and platinum content data are summarized in Table 3.15.



Scheme 21: *Synthesis of conjugates **5a(80)-Pt, 5b(80)-Pt** and **5c(80)-Pt***



Scheme 22: *Synthesis of conjugate **5a(90)-Pt***



Scheme 23: *Synthesis of conjugates **7a(80)-Pt** and **7b(80)-Pt***

Table 3.15: *Preparative and Analytical data of platinum-conjugates, Pt chelated via carboxylatohydroxylato ligand*

Conjugate Designation	Molar Ratios		Reaction time & Temperature		Yield %	Base Molecular Mass [a]	η_{inh} [b] mL/g	x/y [c]	Pt %	
	Carrier	DACH-Pt	Fist Step	Second Step					Found [d]	Calcd [e]
5a(80)-Pt	1.0	1.4	3d, RT	2h, 50^0C	39.8	2255.3	12.1	1.8	7.75	13.7
5b(80)-Pt	1.0	1.4	3d, RT	2h, 50^0C	9.6	10059.5	9.4	8.7	1.64	14.3
5a(90)-Pt	1.0	1.4	3d, RT	2h, 50^0C	37.8	12026.9	7.2	5.7	1.45	8.3
7a(80)-Pt	1.0	1.4	3d, RT	2h, 50^0C	9.2	13318.1	8.5	9.2	1.24	11.4

[a] *Molecular mass of the simple recurring unit; structures normalized to y = 1*

[b] *At 30.0^0 C ± 0.5^0 C in distilled water; conc = 2mg/mL*

[c] *Calculated from analytically determined Pt content*

[d] *Pt content (%) obtained by atomic absorption analysis performed by an outside laboratory*

[e] *Pt content for complete incorporation of the drug*

3.4 Bio-evaluation in cell culture tests: Preliminary results

Four conjugates (**4b(80)-Pt, 4b(90)-Pt, 5a(80)-Pt** and **5a(90)-Pt**) together with their corresponding carriers (**4b(80), 4b(90), 5a(80)** and **5a(90)**) were submitted to a collaborating institution (Department of Pharmacology, University of Pretoria) for biomedical evaluation and toxicological tests.

The above samples were bio-evaluated *in vitro* against the HeLa (Human adenocarcinoma of the cervix), Colo 320 DM (Human colon cancer) and MCF 7 (Human breast cancer) cancer cell lines. The HeLa line is sensitive to most anticancer drugs, whereas Colo 320 DM is considered multidrug-resistant. The results in Table 3.16 are given in terms of IC_{50}, the drug concentration required to achieve 50% cell killing relative to drug-free control. Comparative data on free *cisplatin* are also included in the table as a non-polymeric standard.

4b(80)-Pt and **4b(90)-Pt** are polymeric chelates, in which the Pt atom is anchored through two carboxylato ligand system, while **5a(80)-Pt** and **5a(90)-Pt** are anchored through a carboxylatohydroxylato ligand system. From the data obtained, **4b(80)-Pt** and **5a(80)-Pt** gave best results in the tests against HeLa, Colo 320, MCF7 and lymphocytes, with **4b(90)-Pt** the worst performer. In fact, the HeLa column of the data indicates the **4b(80)-Pt** and **5a(80)-Pt** to be some thousand and hundred times more active, respectively, when compared to free *cisplatin*. Although a considerably larger number of samples of each type of anchoring system will be required to arrive at an accurate figure, it is obvious even from these limited data that the polymeric dicarboxylato-chelated platinum and carboxylatohydroxylato-chelated platinum compounds represent a sizeable advantage over that of free *cisplatin.* As far as the respective carriers are concerned in the

lymphocyte tests, **4b(80), 5a(80)** and **5a(90)** are best, showing lowest toxicity, where as **4b(90),** again, is the worst performer.

Table 3.16: *Antiproliferative activity of platinum conjugates and toxicity of carriers*

	Designation	%Pt [b]	IC_{50}, (µg Pt/mL)[a]				
			HeLa	Colo320	MCF 7	Primary Resting lymphocites	Primary PHA stimulated lymphocites
Conjugate	**4b(80)-Pt**	5.74	0.000044	0.02	0.006	7.06	8.254
	4b(90)-Pt		0.0097	2.149	3.959	3.001	0.4338
	5a(80)-Pt	6.19	0.000173	0.037	0.007	10.382	8.896
	5a(90)-Pt		0.00022	2.557	8.976	10.967	10.54
Carrier	**4b(80)**	-	-	-	-	>50	>50
	4b(90)	-	-	-	-	4.386	6.728
	5a(80)	-	-	-	-	>50	19.128
	5a(90)	-	-	-	-	14.021	13.095
Free drug	*Cisplatin*		0.029667	0.172	0.159	14.907	1.779

[a] IC_{50} *defined here as the drug concentration, in µg Pt/mL, required to achieve 50% cell killing relative to a drug-free control. For the platinum-free carriers, masses equivalent to the respective conjugates were utilized, given in µg/mL.*

[b] *Platinum content, by mass, found.*

CHAPTER 4

EXPERIMENTAL

4.1 General Procedures

^{1}H NMR spectra (300 MHz or 400 MHz unless otherwise specified) were taken on D_2O solutions. Chemical shifts, δ in ppm, were given relative to sodium 3-(trimethylsilyl)-2,2,3,3-d_4- propionate. Integration error limits 12 – 15%. In order to eliminate protonation effects, spectral sample solutions were adjusted to pD 10 – 11 with NaOH.

Inherent viscosities, η_{inh}, were determined at 30.0 ± 0.5^{0}C in Cannon-Fenske tubes. Deionized water was used as solvent; the concentration was c = 0.2 g/100 mL, and the results, averages of three determinations, are given in units of mL/g. Dialysis was performed with the aid of cellulose membranes (Spectrum Industry Inc., Los Angeles, USA) of type Spectra/Por 4 (12 000 – 14 000 molecular mass cut-off limit), and type Spectra/Por 6 (25 000 molecular mass cut-off limit), against several batches of distilled water.

For freeze drying operations, a Virtis Bench Top 3 freeze drier (-40^{0}C, 10 – 15 Pa) was used. The freeze-dried polymer samples were post-dried in an infrared drying apparatus and kept in a desiccator. Analytical samples of the conjugates were normally dried for 2 days at 55 – 60^{0}C under reduced pressure in an Abderhalden apparatus ($CaCl_2$ as a drying agent). The platinum determinations were undertaken at De Bruyn Spectroscopic Solutions, Bryanston, Johannesburg. The analysis was performed by ICP – OES using matrix matched standards. Yttrium (Y) was used as internal standard to compensate for viscosity effects.

4.2 Solvents and Reagents

Distilled water was used for all preparative work. The reaction solvent, N,N'-dimethylformamide (DMF), was distilled under reduced pressure in a faint stream of N_2, with a fore-run of around 10% being discarded, and was dried over molecular sieves 4Å. All other solvents were laboratory grade, received from commercial sources.

The monomeric reagents, 2-acrylamidoglycolic acid monohydrate (Acraga) and acryloyl chloride, were used as received (Fluka Chemie). The Hydroxyamines and diamines, chemical grade (Aldrich Chemie, Fluka AG), were also used as received. These included: 3-amino-1,2-propanediol (APD), 2-(2-aminoethoxy)ethanol (AEE), ethanolamine (EA), 2-(methoxy)ethylamine (MEA), methylenebisacrylamide (MBA), DL-aspartic acid, N,N'-dicyclohexylcarbodiimide (DCC), 3-(dimethylamino)propylamine (DMP), 2-dimethylaminoethylamine (DMEA), 2-diethylaminoethylamine (DEEA), 1,3-diaminopropane (PDA), 1,2- diaminopropane (1,2-PDA), and 2,2-(ethylenedioxy)-diethylamine (EDDA).
Potassium tetrachloroplatinate (K_2PtCl_4) was in part donated by the Western Platinum Refinery and by Johnson Matthey.

4.3 Experimental procedures

4.3.1 Preparation of Monomers

Monomer M2: Acryloyl chloride (0.38 mL, 4.7 mmol) in CH_2Cl_2 (2 mL) was added drop-wise to an aqueous solution of DL-aspartic acid (0.502 g, 3.9 mmol), Na_2CO_3 (0.415 g, 3.9 mmol), and hydroquinone (5 mg) with rapid stirring. The pH of the solution was maintained at pH~8 – 9 with the drop-wise addition of a 2 M solution of Na_2CO_3. The solution was stirred for 2 h at

ice bath temperature and then another 2 h at RT. The solution was acidified to pH~3 – 4 with conc. HCl and then extracted with ethyl acetate. Several crops were collected and in total yield of 0.643 g (88%); mp 163 – 165^{0}C.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 2.4 – 2.2, 2H (2H, **CH_2** (Asp)); 4.2 – 4.1, 1H (1H, **CH** (Asp)); 5.5 – 5.4, 0.9H (1H, CH_2**CH**CONH); 6.1 – 5.8, 1.9H (2H, **CH_2**CHCONH).

Monomer M3a (*N-(4,8-diaza-octanoyl)aspartic acid*): 1,3-diaminopropane (PDA) (0.25 mL, 2.92 mmol) was added to **M2** (0.500 g, 2.7 mmol) and Na_2CO_3 (0.285 g, 2.7 mmol) in H_2O (1 mL). The solution was stirred in the incubator for 3d at 60^{0}C. The solution was concentrated on a rotary-evaporator and then washed with boiling toluene several times and re-washed with hot acetone. Thereafter it was slurried in MeOH (3mL) and acidified to pH~3 with conc. HCl. The solution was concentrated on rotary-evaporator and re-dissolved in MeOH (8 mL). The product was collected as crystallized crops in a total yield of 0.799 g (81.4 %).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7 – 1.5, 2.3H (2H, NH_2CH_2**CH_2**CH_2NH); 2.9 – 2.4, 10.4H (10H, **CH_2** (Asp); **CH_2**CONH; NH_2**CH_2**CH_2**CH_2**NH**CH_2**); 4.5 – 4.4, 1H (1H, **CH** (Asp)).

Monomer M3b (*N-(4,7-diaza-6(5)-methyl-heptanoyl)aspartic acid*): To an aqueous solution of **M2** (0.723 g, 3.9 mmol) in H_2O (1 mL) was added Na_2CO_3 (0.41 g, 3.9 mmol) and 1,2-diaminopropane (1,2PDA) (0.36 mL, 4.2 mmol) and stirred in the incubator for 3 d at 60^{0}C. The work-up was similar to the work-up of **M3a**. The yield of the product was 0.957 g (67.4%).
^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.1 – 1.5, 3.3H (3H, CH**CH_3**); 2.9 – 2.4, 9.2H (9H, **CH_2** (Asp); **CH_2**CONH; NH_2**CH_2CH**(CH_3)NH**CH_2**); 4.5 – 4.4, 1H (1H, **CH** (Asp)).

Monomer M3c (*N-(4,13-diaza-7,10-dioxa-tridecanoyl)aspartic acid*): 2,2-(ethylenedioxy)diethylamine (EDDA) (0.95 mL, 6.45 mmol) was added to an aqueous solution of **M2** (1.0 g, 5.35 mmol) and Na_2CO_3 (0.57 g, 5.35 mmol) in H_2O (1.5 mL) and stirred in the incubator for 3 d at 60^0C. The work-up was analogous to the work-up of **M3a.** The yield of the product was 1.516 g (60.2 %).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 2.9 – 2.4, 9.6H, (10H, NH_2**CH_2**$CH_2OCH_2CH_2OCH_2$**CH_2**NH-**CH_2**; **CH_2**CONH; **CH_2** (Asp)); 3.7 – 3.5, 7.2H (8H, NH_2CH_2**CH_2**O**CH_2CH_2**O**CH_2**CH_2NH); 4.5 – 4.4, 1H (1H, **CH** (Asp)).

Monomer M4a (*N-(4,8-diaza-octanoyl)amidoglycolic acid*): To an aqueous solution of 2-acrylamidoglycolic acid monohydrate **M2'** (Acraga) (1.0 g, 6.14 mmol) in H_2O (1 mL) was added Na_2CO_3 (0.65 g, 6.14 mmol) and PDA (0.52 mL, 6.26 mmol). The solution was stirred in the incubator for 3 d at 55^0C. The solution was concentrated on a rotary-evaporator and then washed with boiling toluene several times and re-washed with hot acetone. Thereafter it was slurried in MeOH (3mL) and acidified to pH~3 with conc. HCl. The solution was concentrated on rotary-evaporator and re-dissolved in MeOH (8 mL). The product was collected as crystallized crops in a total yield of 0.973 g (59.1 %).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7 – 1.5, 2H (2H, NH_2CH_2**CH_2**CH_2NH); 2.5 – 2.4, 2.4H (2H, NH**CH_2**); 2.6 – 2.5, 3.2H (4H, NH_2**CH_2**CH_2**CH_2**NH); 2.9 – 2.8, 2.1H (2H, **CH_2**CONH).

Monomer M4b (*N-(4,13-diaza-7,10-dioxa-tridecanoyl)amidoglycolic acid*): EDDA (0.92 mL, 6.26 mmol) was added to an aqueous solution of **M2'** (1.0

g, 6.14 mmol) and Na_2CO_3 (0.65 g, 6.14 mmol) in H_2O (1 mL) and stirred in the incubator for 3 d at 55^0C. The work-up was similar to the work-up of **M4a**. The product was collected as crystallized crops in a total yield of 0.765g (36.2 %).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 2.5 – 2.4, 2H (2H, NH_2**CH_2**CH_2OCH_2); 2.9 – 2.8, 6.2H (6H, **CH_2**CONH; **CH_2**NH**CH_2**); 3.7 – 3.5, 8.4 (8H, NH_2CH_2**CH_2**O**CH_2CH_2**O**CH_2**CH_2NH).

4.3.2 Preparation of polymeric carriers

4.3.2.1 Poly-DL-succinimide (PSI) 2

Polysuccinimide (PSI) **2** was prepared by the method of Neri and Antoni[73]. This method involved the polymerization of DL-aspartic acid in orthophosphoric acid at high temperature. In order to achieve chain extension, the end carboxylic groups were coupled with N,N'-dicyclohexylcarbodiimide (DCC). The products of approximately equal viscosity were pooled from several runs and thoroughly mixed to give a master batch with inherent viscosity, η_{inh}, (determined in DMF) of 54 mL/g.

4.3.2.2 Synthesis of poly-α,β-DL-aspartamides

Carrier 3a(80): The designation **3a(80)** indicates a (80:20) mole % ratio of DMP to APD in the polymer product. 3-dimethylaminopropyl-amine (DMP) (1.88 mL, 16 mmol) in N,N'-dimethylformamide (DMF) (15 mL) was added drop-wise, with rapid stirring, to polysuccinimide **2** (1.961 g, 20 mmol) dissolved in DMF (15 mL). The solution was saturated with N_2 and stirred for 5 h at room temperature (RT). Then 3-amino-1,2-propanediol (APD) (1.093

g, 12 mmol) in DMF (5 mL) was added to the stirred solution. The solution re-saturated with N_2 and stirred for 2 d at RT. The solution was concentrated on rotary-evaporator (bath 60-65^{0}C) to approximately 10 mL. The product was obtained by precipitation with diethyl ether (Et_2O):Hexane (2:1, 20 mL), and the precipitate washed with hot acetone and redissolved in H_2O (15 mL). The pH was adjusted from pH~12 to pH~7.5 with conc. HCl. The solution was dialyzed successively for 2 d in Spectra/Por 4 and another 2 d in Spectra/Por 6 tubing. The water-soluble polymer was then freeze-dried, post-dried, and kept in a desiccator. The yield of the post-dried polymer was 2.490 g (63.2 %), and η_{inh} = 15.3 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7 – 1.5, 8H (8H, CONHCH$_2$**CH$_2$**CH$_2$NMe$_2$); 2.3 – 2.1, 24.4H (24H, N**Me$_2$**); 2.4 – 2.3, 9.3H (8H, **CH$_2$**NMe$_2$); 2.8 – 2.6, 10.2H (10H, **CH$_2$**CONH); 3.3 – 3.1, 9.8H (10H, CONH**CH$_2$**); 3.7 – 3.5, 2.9H (3H, **CH**(OH)**CH$_2$**OH).

Carrier 3b(80): Same procedure as in carrier **3a(80)** was used. 3-dimethylaminoethylamine DMEA (1.75 mL, 14.4 mmol) in DMF (5 mL) was added to polysuccinimide **2** (1.765 g, 18 mmol) dissolved in DMF (15 mL). The solution saturated with N_2 and stirred for 5 h, thereafter APD (0.984 g, 10.8 mmol) in DMF (5 mL) was added, the solution re-saturated with N_2 and stirred for 2d at RT. This gave the copolymer **3b(80)** in a yield of 1.960 g (58.6 %), and η_{inh} = 7.4 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 2.3 – 2.1, 24H (24H, N**Me$_2$**); 2.4 – 2.3, 9.0H (8H, **CH$_2$**NMe$_2$); 2.8 – 2.6, 10.6H (10H, **CH$_2$**CONH); 3.3 – 3.1, 11.2H (10H, CONH**CH$_2$**); 3.7 – 3.5, 2.8H (3H, **CH**(OH)**CH$_2$**OH).

Carrier 3c(80): By a similar procedure as above 2-(diethylamino)ethylamine DEEA (2.27 mL, 16 mmol) in DMF (5 mL) was added to polysuccinimide **2** (1.961 g, 20 mmol) dissolved in DMF (15 mL). The solution was saturated with N_2 and stirred for 5h, thereafter APD (1.093 g, 12 mmol) in DMF (5 mL) was added, the solution re-saturated with N_2 and stirred for 2 d at RT. This resulted in the copolymer **3c(80)** in a yield of 2.128 g (51.1 %), and η_{inh} = 10.9 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.1 – 0.9, 24H (24H, $CH_2N(CH_2\mathbf{CH_3})_2$); 2.7 – 2.5, 24.3H (24H, $\mathbf{CH_2}N(\mathbf{CH_2}CH_3)_2$); 2.9 – 2.7, 8.8H (10H, $\mathbf{CH_2}$CONH); 3.4 – 3.2, 10.1H (10H, CONH$\mathbf{CH_2}$); 3.8 – 3.4, 3.5H (3H, **CH**(OH)$\mathbf{CH_2}$OH).

Carrier 3d(80): By the procedure leading to copolymer **3a(80)**, polysuccinimide **2** (1.883 g, 19.2 mmol) in DMF (15 mL), was treated with 2-(2-aminoethoxy)ethanol (AEE) (1.62 mL, 15.36 mmol). APD (1.050 g, 11.52 mmol) in DMF (5 mL) was added to the solution and the resultant solution was treated and worked up as before, giving 1.635g (42.7 %) of **3d(80)** as a water soluble solid. η_{inh} = 7.2 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 2.9 – 2.6, 10H (10H, $\mathbf{CH_2}$CONH); 3.4 – 3.3, 10.6H (10H, CONH$\mathbf{CH_2}$); 3.8 – 3.5, 30H (27H, $\mathbf{CH_2}O\mathbf{CH_2}\mathbf{CH_2}$OH; **CH**(OH)$\mathbf{CH_2}$OH).

Carrier 3a(90): Following the same procedure as above, polysuccinimide **2** (1.765g, 18 mmol) in DMF (15 mL) was treated with DMP (2.03 mL, 16.2 mmol) in DMF (5 mL) in the first step, and APD (0.492 g, 5.4 mmol) in DMF (5 mL) in the second step, and this resulted in a water-soluble polymer, **3a(90)**, in a yield of 1.729 g (48.5 %). η_{inh} = 34.2 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses):1.7 – 1.5, 18H (18H, CONHCH$_2$**CH$_2$**CH$_2$NMe$_2$); 2.3 – 2.1, 53.4H (54H, N**Me$_2$**); 2.4 – 2.3, 19.1H (18H, **CH$_2$**NMe$_2$); 2.8 – 2.6, 20.3H (20H, **CH$_2$**CONH); 3.3 – 3.1, 20.3H (20H, CONH**CH$_2$**); 3.7 – 3.5, 3.2H (3H, **CH**(OH)**CH$_2$**OH).

Carrier 3b(90): By a similar procedure as above, DMEA (1.79 mL, 16.34 mmol) in DMF (5 mL) was added to polysuccinimide **2** (1.781g, 18.16 mmol) dissolved in DMF (15 mL). The solution saturated with N_2 and stirred for 5 h, thereafter APD (0.496 g, 5.45 mmol) in DMF (5 mL) was added, the solution re-saturated with N_2, and stirred for 2 d at RT. This resulted in the copolymer **3b(90)** in a yield of 1.563 g (46.4 %). η_{inh} = 18.4 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 2.3 – 2.1, 54H (54H, N**Me$_2$**); 2.4 – 2.3, 19.1H (18H, **CH$_2$**NMe$_2$); 2.8 – 2.6, 23.1H (20H, **CH$_2$**CONH); 3.3 – 3.1, 22.4H (20H, CONH**CH$_2$**); 3.7 – 3.5, 2.9H (3H, **CH**(OH)**CH$_2$**OH).

Carrier 3d(90): Following the same procedure as above, polysuccinimide **2** (2.00 g, 20.4 mmol) in DMF (15 mL) was treated with AEE (1.930 g, 18.36 mmol) in DMF (5 mL) in the first step, and APD (0.558 g, 6.12 mmol) in DMF (5 mL) in the second step and this resulted in a water-soluble polymer, **3d(90)**, in a yield of 1.568 g (38.3 %). η_{inh} = 22.1 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 2.9 – 2.6, 20H (20H, **CH$_2$**CONH); 3.4 – 3.3, 20H (20H, CONH**CH$_2$**); 3.8 – 3.5, 56H (57H, **CH$_2$**O**CH$_2$CH$_2$**OH; **CH**(OH)**CH$_2$**OH).

Carrier 4a(80): To the stirred solution of polysuccinimide **2** (1.02 g, 10.4 mmol) in DMF (15 mL), was added was added **M3c** (1.395 g, 4.16 mmol) in DMSO (2 mL) and trimethylamine (Et_3N) (0.59 mL, 4.16 mmol). The resulting suspension, saturated with N_2, was stirred for 3 d at RT. DMP (1.04 mL, 8.32 mmol) dissolved in DMF (5 mL), was added drop-wise with stirring, and upon re-saturation with N_2 the suspension was stirred for another 1 d at RT. The contents were filtered and the filtrate was concentrated by volume reduction to ca. ~5 mL under reduced pressure at 55 – 60^0C, and the polymeric product was precipitated with Et_2O:hexane (2:1, 15 mL). Upon washing with hot toluene then hot acetone, the residue was dissolved in H_2O (10 mL) and the pH was adjusted from pH~12 to pH~8 with conc. HCl. The solution was dialyzed for 2 d in Spectra/Por 4 and another 2 d in Spectra/Por 6 tubing. Freeze-drying of the retentate and IR post-drying gave 0.655 g (25.6 %) of water-soluble **4a(80)**. η_{inh} = 12.35 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.8 – 1.6, 8H (8H, CONHCH$_2$**CH$_2$**CH$_2$NMe$_2$); 2.3 – 2.1, 22.8H (24H, N**Me$_2$**); 2.4 – 2.3, 8H (12H, **CH$_2$**NMe$_2$; **CH$_2$**NH**CH$_2$**); 2.9 – 2.5, 17.8H (14H, **CH$_2$**CONH; **CH$_2$** (Asp)); 3.3 – 3.0, 9.2H (10H, CONH**CH$_2$**); 3.8 – 3.6, 5.9H (8H, **CH$_2$**O**CH$_2$CH$_2$**O**CH$_2$**).

Carrier 4b(80): By the procedure leading to **4a(80)**, this carrier was prepared from polysuccinimide **2** (0.92g, 9.38 mmol) dissolved in DMF (15 mL), treated with **M3a** (0.980 g, 3.75 mmol) in DMSO (2 mL), and Et_3N (0.53 mL, 3.75 mmol). DMP (0.94 mL, 7.51 mmol) dissolved in DMF (5 mL) was then added to the suspension, and the mixture treated and worked up as before, giving 0.672 g (31.0 %) of **4b(80)** as a water-soluble solid. η_{inh} = 16.8 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7 – 1.5, 10H (10H, CONHCH$_2$**CH$_2$**CH$_2$NMe$_2$; CH$_2$**CH$_2$**CH$_2$NH); 2.3 – 2.1, 25.6H (24H, N**Me$_2$**); 2.4 – 2.3, 10H (12H, **CH$_2$**NMe$_2$; **CH$_2$**NH**CH$_2$**); 2.8 – 2.5, 12.5H (14H, **CH$_2$**CONH; **CH$_2$** (Asp)); 3.3 – 3.0, 10H (10H,CONH**CH$_2$**).

Carrier 4c(80): Following the same procedure as above, polysuccinimide **2** (0.96 g, 9.79 mmol) in DMF (15 mL), was treated with **M3b** (1.023 g, 3.916 mmol) in DMSO (2 mL), and Et_3N (0.52 mL, 3.916 mmol). DMP (0.98 mL, 7.83 mmol) in DMF (5 mL) was then added, and mixture was treated and worked up as before, giving 0.427 g (18.9 %) of **4c(80)** as a water-soluble solid. η_{inh} = 14.9 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.1 – 10, 3H (3H, CH**CH$_3$**); 1.7 – 1.5, 10.9H (8H, CONHCH$_2$**CH$_2$**CH$_2$NMe$_2$); 2.3 – 2.1, 31.5H (24H, N**Me$_2$**); 2.4 – 2.3, 11.1H (11H, **CH$_2$**NMe$_2$; **CH$_2$**NH**CH$_2$**); 2.9 – 2.4, 23.6H (14H, **CH$_2$**CONH; **CH$_2$** (Asp)); 3.3 – 3.0, 10.7H (10H, CONH**CH$_2$**).

Carrier 4b(90): By an analogous procedure described in the forgoing experiment, polysuccinimide **2** (1.0 g, 10.20 mmol) in DMF (15 mL), was treated with **M3a** (0.533 g, 2.04 mmol) in DMSO (1 mL) and Et_3N (0.29 mL, 2.04 mmol). DMP (1.15 mL, 9.18 mmol) in DMF (5 mL) was then added, and mixture was treated and worked up as before, giving 0.500 g (22.8 %) of **4b(90)** as a water-soluble solid. η_{inh} = 10.4 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7 – 1.5, 20H (20H, CONHCH$_2$**CH$_2$**CH$_2$NMe$_2$; CH$_2$**CH$_2$**CH$_2$NH); 2.3 – 2.1, 56.2H (54H, N**Me$_2$**); 2.4 – 2.3, 20H (22H, **CH$_2$**NMe$_2$; **CH$_2$**NH**CH$_2$**); 2.9 – 2.4, 35H (24H, **CH$_2$**CONH; **CH$_2$** (Asp)); 3.3 – 3.0, 20H (20H, CONH**CH$_2$**).

Carrier 5a(80): To the stirred solution of polysuccinimide **2** (1.20 g, 12.23 mmol) in DMF (15 mL), was added **M4a** (1.073 g, 4.89 mmol) in DMSO (2 mL) and Et_3N (0.69 mL, 4.89 mmol). The suspension was saturated with N_2 and stirred for 3d at RT. DMP (1.0 mL, 9.784 mmol) in DMF (5 mL) was added drop-wise, and the suspension re-saturated with N_2 and stirred for one more day at RT. The contents were filtered and the filtrate was concentrated by volume reduction to ca. ~5 mL under reduced pressure at 55 – 60^0C, and the polymeric product was precipitated with Et_2O:hexane (2:1, 15 mL). Upon washing with hot toluene then hot acetone, the residue was dissolved in H_2O (10 mL) and the pH was adjusted from pH~12 to pH~8 with conc. HCl. The solution was dialysed for 2 d in Spectra/Por 4 and another 2 d in Spectra/Por 6 tubing. Freeze-drying of the retentate and IR post-drying gave 0.744 g (27.3 %) of water-soluble **5a(80)**. η_{inh} = 68.2 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7 – 1.5, 10H (10H, CONHCH$_2$**CH$_2$**CH$_2$NMe$_2$; CH$_2$**CH$_2$**CH$_2$NH); 2.3 – 2.1, 26H (24H, N**Me$_2$**); 2.4 – 2.3, 11H (12H, **CH$_2$**NMe$_2$; **CH$_2$**NH**CH$_2$**); 2.9 – 2.5, 10.8H (12H, **CH$_2$**CONH); 3.3 – 3.0, 9.8H (10H, CONH**CH$_2$**).

Carrier 5b(80): By the procedure leading to **5a(80)**, this carrier was prepared from polysuccinimide (1.0 g, 10.20 mmol) dissolved in DMF (15 mL), treated with **M4a** (0.895, 4.08 mmol) in DMSO (2 mL), and Et_3N (0.56 mL, 4.08 mmol). DMEA (0.89 mL, 8.16 mmol) dissolved in DMF (5 mL) was then added to the suspension, and the mixture treated and worked up as before, giving 0.468 g (21.7 %) of **5b(80)** as a water-soluble solid. η_{inh} = 21.8 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7 – 1.5, 2H (2H, CONHCH$_2$**CH$_2$**CH$_2$NH); 2.3 – 2.1, 25.6H (24H, N**Me$_2$**); 2.4 – 2.3,

13.2H (12H, **CH_2**NMe_2; **CH_2**NH**CH_2**); 2.9 – 2.5, 11.5H (12H, **CH_2**CONH); 3.3 – 3.0, 9.4H (10H, CONH**CH_2**).

Carrier 5c(80): Following the same procedure as above, polysuccinimide **2** (1.20 g, 12.23 mmol) in DMF (15 mL), was treated with **M4b** (1.376 g, 4.89 mmol) in DMSO (2 mL), and Et_3N (0.69 mL, 4.89 mmol). DMP (1.15 mL, 9.18 mmol) in DMF (5 mL) was then added, and mixture was treated and worked up as before, giving 0.912 g (29.8 %) of **5c(80)** as a water-soluble solid. η_{inh} = 14.5 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7 – 1.5, 8H (8H, CH_2**CH_2**CH_2NMe_2); 2.3 – 2.2, 21.6H (24H, CH_2N**Me_2**); 2.4 – 2.3, 9H (12H, **CH_2**NMe_2; **CH_2**NH**CH_2**); 2.9 – 2.5, 12.6H (12H, **CH_2**CONH); 3.3 – 3.1, 8.9H (10H, CONH**CH_2**); 3.7 – 3.5, 7.3H (8H, **CH_2**O**CH_2****CH_2**O**CH_2**).

Carrier 5a(90): By an analogous procedure described in the forgoing experiment, polysuccinimide **2** (1.0 g, 10.20 mmol) in DMF (15 mL), was treated with **M4a** (0.448 g, 2.04 mmol) and Et_3N (0.28 mL, 2.04 mmol). DMP (1.15 mL, 9.18 mmol) in DMF (5 mL) was then added, and mixture was treated and worked up as before, giving 0.413 g (19.2 %) of **5a(90)** as a water-soluble solid. η_{inh} = 19.6 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7 – 1.5, 20H (20H, CONHCH_2**CH_2**CH_2NMe_2; CH_2**CH_2**CH_2NH); 2.3 – 2.1, 58.1H (54H, N**Me_2**); 2.4 – 2.3, 20.5H (22H, **CH_2**NMe_2; **CH_2**NH**CH_2**); 2.9 – 2.2, 21.8H (22H, **CH_2**CONH); 3.3 – 3.0, 19.3H (20H, CONH**CH_2**).

4.3.2.3 Synthesis of Polyamidoamines

Carrier 6a(80): MBA (1.541 g, 10 mmol) was dissolved in hot H_2O (8 mL), allowed to cool down to RT, then followed by the addition of APD (0.182 g, 2.0 mmol) in H_2O (1mL), The suspension was saturated with N_2 and left in the incubator for 1 d at 50^0C. With all components dissolving, DMP (1.0 mL, 8.0 mmol) was added to the solution, re-saturated with N_2 and allowed to react in the incubator for 2 d at 55^0C. The solution was concentrated on roti evaporator to around 3 mL, bath temperature 50^0C, then precipitated with Ethanol:Hexane (2:1, 15 mL). The precipitate was washed with hot acetone several times, then dissolved in distilled H_2O (10 mL) and the pH of the solution adjusted to pH~ 8 with conc. HCl. The solution dialyzed for 48 hrs in pore spectra/Por 4 against changing of distilled water. The solution was freeze dried, and post dried was in the yield of 1.085 g (42.7 %). η_{inh} = 22.6 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses):1.9 – 1.7, 8H (8H, CH_2**CH_2**CH_2NMe_2); 2.5 – 2.3, 20H (24H, CH_2N**Me_2**); 3.1 – 2.5, 56H (58H, **CH_2CH_2**CONH, N**CH_2**CH_2**CH_2**NMe_2); 3.9 – 3.5, 3H (3H, CH_2**CH_2**(OH)**CH_2**OH); 4.6 – 4.5, 10.4H (10H, CONH**CH_2**NHCO).

Carrier 6b(80): Following the same procedure as above, APD (0.182 g, 2.0 mmol) in H_2O (1 mL) was added to MBA (1.541 g, 10 mmol) in H_2O (8 mL). The suspension was saturated with N_2 and left in the incubator for 1 d at 50^0C. With all components dissolving, DMEA (0.88 mL, 8.0 mmol) was added to the solution, re-saturated with N_2 and allowed to react in the incubator for another 2 d at 55^0C. The solution was treated and worked up as before, giving 0.969 g (39.9 %) of **6b(80)** as a water soluble-solid. η_{inh} = 19.6 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 2.5 – 2.3, 23.4H (24H, $CH_2N\mathbf{Me_2}$); 3.2 – 2.6, 60.6H (58H, $\mathbf{CH_2CH_2}$CONH, $N\mathbf{CH_2}CH_2\mathbf{CH_2}NMe_2$); 3.9 – 3.4, 3H (3H, $CH_2\mathbf{CH_2}(OH)\mathbf{CH_2}OH$); 4.6 – 4.5, 10.2H, (10H, CONH$\mathbf{CH_2}$NHCO).

Carrier 6a(50): By an analogous procedure described in the forgoing experiment, MBA (1.541 g, 10 mmol) in H_2O (8 mL), was treated with APD (0.456 g, 5.0 mmol) in H_2O (1.5 mL) and allowed to react for 1 d. With all components dissolving, DMP (0.63 mL, 5.0 mmol) was then added and the solution allowed to react for another 2 d. The solution was treated and worked up as before, giving 0.969 g (39.9 %) of **6a(50)** as a water-soluble solid. η_{inh} = 19.6 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.9 – 1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NMe_2$); 2.5 – 2.3, 7H (6H, $CH_2N\mathbf{Me_2}$); 3.1 – 2.5, 20.4H (22H, $\mathbf{CH_2CH_2}$CONH, $N\mathbf{CH_2}CH_2\mathbf{CH_2}NMe_2$); 3.9 – 3.4, 3H (3H, $CH_2\mathbf{CH_2}(OH)\mathbf{CH_2}OH$); 4.6 – 4.5, 4H (4H, CONH$\mathbf{CH_2}$NHCO).

Carrier 6b(50): By the procedure leading to **6a(80)**, this carrier was prepared from MBA (1.541 g, 10 mmol) in H_2O (8 mL), treated with APD (0.456 g, 5 mmol) in H_2O (2 mL) for 1d in the incubator. With all components dissolving, DMEA (0.55 mL, 5 mmol) was then added to the solution. The solution was treated and worked up as before, giving 0.921 g (37.8 %) of **6b(50)** as a water-soluble solid. η_{inh} = 12.4 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 2.5 – 2.3, 8H (6H, $CH_2N\mathbf{Me_2}$); 3.2 – 2.6, 19.8H (22H, $\mathbf{CH_2CH_2}$CONH, $N\mathbf{CH_2}CH_2\mathbf{CH_2}NMe_2$); 3.9 – 3.4, 3H, (3H, $CH_2\mathbf{CH_2}(OH)\mathbf{CH_2}OH$); 4.6 – 4.5, 4.2H (4H, CONH$\mathbf{CH_2}$NHCO).

Carrier 7a(80): A mixture of MBA (1.541 g, 10 mmol), **M4a** (0.439 g, 2 mmol), and Na_2CO_3 (0.212 g, 2.0 mmol) in H_2O (8 mL) was saturated with N_2 and left in the incubator for 1d at 50^0C. DMP (0.67 mL, 8.0 mmol) was added to the resultant solution, then re-saturated with N_2 and left in the incubator for another 2 d at 55^0C. The solution was concentrated on roti evaporator to around 3 mL, bath temperature 50^0C, then precipitated with Ethanol:Hexane (2:1, 15 mL). The precipitate was washed with hot acetone several times, then dissolved in distilled H_2O (10 mL) and the pH of the solution adjusted to pH~ 8 with conc. HCl. The solution was dialyzed for 48 h in spectra/Por 4 against distilled water. The solution was freeze dried, and the product was post-dried and isolated in a yield of 1.155 g (41.3 %). η_{inh} = 9.3 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7 – 1.5, 10H (10H, CH_2**CH_2**CH_2NMe_2, CH_2**CH_2**CH_2NH); 2.3 – 2.2, 25.6H (24H, CH_2N**Me_2**); 2.8 – 2.3, 64H (62.5H, **CH_2CH_2**CONH; N**CH_2**CH_2**CH_2**NMe_2; **CH_2**N**CH_2**); 4.6 – 4.3, 10H (10H, CONH**CH_2**NHCO).

Carrier 7b(80): By the procedure leading to **7a(80)**, this carrier was prepared from MBA (1.541 g, 10 mmol) in H_2O (8 mL) treated with **M4b** (0.563 g, 2.0 mmol) and Na_2CO_3 (0.212 g, 2.0 mmol) for 1 d at 50^0C. DMP (0.67 mL, 8.0 mmol) was then added and the solution left in the incubator for another 2 d at 55^0C. The solution was treated and worked up as before, giving 1.224 g (45.6 %) of **7b(80)** as a water soluble solid. η_{inh} = 7.5 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7 – 1.5, 8H (8H, CH_2**CH_2**CH_2NMe_2); 2.3 – 2.2, 24H (24H, CH_2N**Me_2**); 2.8 – 2.3, 62.7H (64H, **CH_2CH_2**CONH; N**CH_2**CH_2**CH_2**NMe_2; **CH_2**N **CH_2**); 3.7 – 3.6, 7.4H (8H, **CH_2**O**CH_2CH_2**O **CH_2**); 4.6 – 4.5, 9.2H (10H, CONH**CH_2**NHCO).

Carrier 7a(50): Following the same procedure as above, **M4a** (1.10 g, 5.0 mmol) and Na_2CO_3 (0.530 g, 5.0 mmol) were added to MBA (1.541 g, 10 mmol) in H_2O (8 mL). The mixture was saturated with N_2 and left in the incubator for 1 d at 50^0C. With all components dissolving, DMP (1.14 mL, 8.0 mmol) was added to the solution, re-saturated with N_2 and allowed to react in the incubator for another 2 d at 55^0C. The solution was treated and worked up as before, giving 1.25 g (39.7 %) of **7a(50)** as a water soluble solid. η_{inh} = 11.3 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7 – 1.5, 4H (4H, CH_2**CH_2**CH_2NMe_2, CH_2**CH_2**CH_2NH); 2.3 – 2.2, 5.2H (6H, CH_2N**Me_2**); 2.8 – 2.3, 27H (28H, **CH_2CH_2**CONH; N**CH_2**CH_2**CH_2**NMe_2; **CH_2**N **CH_2**); 4.6 – 4.5, 3.8H (4H, CONH**CH_2**NHCO).

Carrier 8a(80): MBA (1.541 g, 10 mmol) was dissolved in hot distilled H_2O (8 mL), allowed to cool to ambient and followed by the addition of DL-Aspartic acid (0.293 g, 2.2 mmol) and Na_2CO_3 (0.233 g, 2.2 mmol). The solution was saturated with N_2 and left in the incubator for 1 d at 60^0C. DMP (1.0 mL, 8.0 mmol) was added to the solution and then re-saturated with N_2 and left in the incubator for another 2 d at 65^0C. The solution was concentrated on roti evaporator to around 3 mL, (bath temperature 50^0C), then precipitated with Ethanol:Hexane (2:1, 15 mL). The precipitate was washed with hot acetone several times and then dissolved in distilled H_2O (15 mL). The pH of the solution was adjusted to pH~8 with conc. HCl, and the solution was dialyzed for 48 h in Spectra/Por 4 tubing against distilled water. The solution was freeze dried, and the product post dried; it was isolated in a yield of 1.084 g (0.41 %). η_{inh} = 6.4 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses):1.7 – 1.5, 8H (8H, CH_2**CH_2**CH_2NMe_2); 2.3 – 2.2, 23.4H (24H, CH_2N**Me_2**); 2.6 – 2.3,

40.5H (36H, **CH_2**N(**CH_2**)(**CH_2**); **CH_2**NMe_2); 2.9 – 2.7, 21.5H (22H, **CH_2**CONH; **CH_2** (Asp)); 4.7 – 4.5, 11.5H (11H, CONH**CH_2**NHCO; **CH** (Asp)).

Carrier 8b(80): Following the same procedure as above, MBA (1.541 g, 10 mmol) in H_2O (8 mL), was treated with DL-Aspartic acid (0.293 g, 2.2 mmol) and Na_2CO_3 (0.233g, 2.2 mmol) for 1 d in the incubator at 60^0C. MEA (0.70 mL, 8.0 mmol) was then added, and the solution allowed to react for anther 2 d at 65^0C. The solution was treated and worked up as before, giving 0.903 g (37.5 %) of **8b(80)** as a water-soluble solid. η_{inh} = 5.1 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses):2.5 – 2.3, 19.2H (28H, **CH_2**N(**CH_2**)(**CH_2**)); 2.9 – 2.6, 25.5H (22H, **CH_2**CONH; **CH_2** (Asp)); 3.4 – 3.3, 12H (12H, O**Me**); 3.6 – 3.5, 7.9H (8H, **CH_2**OMe); 4.7 – 4.5, 9.3H (11H, CONH**CH_2**NHCO; **CH** (Asp)).

Carrier 8a(90): By the procedure leading to **8a(80)**, this carrier was prepared from MBA (1.541 g, 10 mmol) in H_2O (8 mL) treated with DL-Aspartic acid (0.147 g, 1.1 mmol) and Na_2CO_3 (0.117 g, 1.1 mmol) for 1 d in the incubator at 60^0C. DMP (1.13 mL, 9.0 mmol) was then added and the solution left in the incubator for another 2 d at 65^0C. The solution was treated and worked up as before, giving 0.851 g (32.8 %) of **8a(90)** as a water-soluble solid. η_{inh} = 7.8 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7 – 1.5, 18H (18H, CH_2**CH_2**CH_2NMe_2); 2.3 – 2.2, 57.6H (54H, CH_2N**Me_2**); 2.6 – 2.3, 80.4H (76H, **CH_2**N(**CH_2**)(**CH_2**); **CH_2**NMe_2); 2.9 – 2.7, 40.8H (42H, **CH_2**CONH; **CH_2** (Asp)); 4.7 – 4.5, 20.4H (22H, CONH**CH_2**NHCO; **CH** (Asp)).

Carrier 8b(90): By an analogous procedure as described in the forgoing experiment, MBA (1.541 g, 10 mmol) in H_2O (8 mL), was treated with DL-Aspartic acid (0.147 g, 1.1 mmol) and Na_2CO_3 (0.117 g, 1.1 mmol) for 1 d in the incubator at 60^0C. MEA (0.79 mL, 9.0 mmol) was then added and the solution left in the incubator for another 2 d at 65^0C. The solution was treated and worked up as before, giving 0.919 g, (39.1 %) of **8b(90)** as a water-soluble solid. η_{inh} = 4.6 mL/g.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 2.5 – 2.3, 42H (42H, **CH_2**N(**CH_2**)(**CH_2**)); 2.9 – 2.6, 53H (58H, **CH_2**CONH; **CH_2** (Asp)); 3.4 – 3.3, 27H (27, O**Me**); 3.6 – 3.5, 17.5H (18H, **CH_2**OMe); 4.7 – 4.5, 18H (22H, CONH**CH_2**NHCO; **CH** (Asp)).

4.3.3 Preparation of polymeric platinum conjugates

4.3.3.1 Preparation of DACH-Pt

The DACH-Pt$(NO_3)_2$ platination agent, *trans*-1,2-diaminocyclohexanediaquaplatinum(II) dinitrate (Scheme 13), was prepared by a literature method[95]. Anal. Calcd. for $C_6H_{18}N_4O_8Pt$ (469.3): Pt, 41.6 %; found: Pt, 41.8 %.

4.3.3.2 Polyaspartamides-platinum anchoring

Conjugate 3a(80)-Pt: To the N_2-saturated solution of carrier **3a(80)** (0.30 g, 0.305 mmol) in H_2O (5 mL), DACH-Pt (0.172 g, 0.366 mmol) in H_2O (5 mL) was added. Upon re-saturation with N_2, the solution was light protected, and stirred for 3 d at RT with the pH of the solution maintained at ~5.5 - 6 and another 2 h at 50^0C. The solution was filtered and dialyzed for 2 d in

Spectra/Por 4 tubing. The retentate was freeze-dried, to give 0.22 g (56.3 %) of water-soluble solid **3a(80)-Pt**. η_{inh} = 5.8 mL/g.

Anal. Calcd. for $(C_{49}H_{92}N_{16}O_{12}Pt)_n$ $(1292.3)_n$: Pt, 15.1 %; found: Pt, 15.25 %.

Conjugate 3b(80)-Pt: By the same preceding procedure as described in the foregoing experiment, carrier **3b(80)** (0.32 g, 044 mmol) in H_2O (5 mL) was used instead of carrier **3a(80)**, and DACH-Pt (0.194 g, 0413 mmol) in H_2O (5mL) was added to the stirred polymeric solution. The solution was treated and worked up as before, giving conjugate **3b(80)-Pt** as a water-soluble solid in a yield of 0.197 g (46.3 %). η_{inh} = 11.2 mL/g.

Conjugate 3c(80)-Pt: By an analogous procedure as described in the forgoing experiment, carrier **3c(80)** (0.296 g, 0.284 mmol) was used instead of carrier **3a(80)** and DACH-Pt (0.160g, 0.341 mmol). The solution was treated and worked up as before, giving conjugate **3c(80)-Pt** as a water-soluble solid in a yield of 0.196 g (51.1 %). η_{inh} = 8.5 mL/g.

Conjugate 3d(80)-Pt: Conjugate **3d(80)-Pt** was obtained by the same procedure as described in the forgoing experiment. A solution of carrier **3d(80)** (0.316 g, 0.317 mmol) was treated with DACH-Pt (0.179 g, 0.380 mmol) and resulted in a water-soluble conjugate **3d(80)-Pt** in a yield of 0.21 g (48.1 %). η_{inh} = 7.3 mL/g.

Anal. Calcd. for $(C_{47.5}H_{84.4}N_{12.1}O_{21.3}Pt)_n$ $(1360.9)_n$: Pt, 15.0 %; found: Pt, 14.11 %.

Conjugate 3a(90)-Pt: To a stirred aqueous solution (5 mL) of carrier **3a(90)** (0.300 g, 0.151 mmol), saturated with N_2, light protected, was added DACH-Pt (0.085 g, 0.181 mmol). The solution was re-saturated with N_2, stirred for 3d at RT and then stirred for another 2 h in the incubator at 50^0C. During the process, the pH was carefully monitored and maintained at ~5.5 - 6. The routinely filtered solution was dialyzed in Spectra/Por 4 tubing for 2d, and the conjugate isolated as a water-soluble solid by freeze-drying in a yield of 0.231 g (10.7 %). η_{inh} = 9.1 mL/g.

Anal. Calcd. for $(C_{634.5}H_{1190.4}N_{209.1}O_{157.1}Pt)_n$ $(14261.9)_n$: Pt, 8.5 %; found: Pt, 1.18 %.

Conjugate 3b(90)-Pt: By the same preceding procedure as described in the forgoing experiment, carrier **3b(90)** (0.284 g, 0.153 mmol) in H_2O (5 mL) was used instead of carrier **3a(90)**, and DACH-Pt (0.086 g, 0.184 mmol) in H_2O (5 mL) was added to the stirred polymeric solution. The solution was treated and worked up as before, giving conjugate **3b(90)-Pt** as a water-soluble solid in a yield of 0.130 g (39.4 %). η_{inh} = 11.6 mL/g.

Conjugate 3c(90)-Pt: Conjugate **3c(90)-Pt** was obtained by the same procedure as described in the forgoing experiment. A solution of carrier **3c(90)** (0.300 g, 0.142 mmol) was treated with DACH-Pt (0.080 g, 0.171 mmol) and resulted in a water-soluble conjugate **3c(90)-Pt** in a yield of 0.148 g (43.1 %). η_{inh} = 9.8 mL/g.

Conjugate 3d(90)-Pt: By an analogous procedure as described in the forgoing experiment, carrier **3d(90)** (0.320 g, 0.159 mmol) was used instead of carrier **3a(90)** and DACH-Pt (0.090 g, 0.191 mmol). The solution was

treated and worked up as before, giving conjugate **3d(90)-Pt** as a water-soluble solid in a yield of 0.177 g (46.9 %). η_{inh} = 12.4 mL/g.

Anal. Calcd. for $(C_{87.5}H_{154.3}N_{22.6}O_{41.2}Pt)_n$ $(2377.1)_n$: Pt, 8.43 %; found: Pt, 8.15 %.

Conjugate 4a(80)-Pt: The light protected solution (5 mL) of carrier **4a(80)** (0.314 g, 0.255 mmol) was saturated with N_2, then treated with a solution of DACH-Pt (0.144 g, 0.306 mmol) dissolved in H_2O (5 mL). The solution was stirred for 3 d at RT, the pH monitored and maintained at ~5.5 – 6. The solution was further stirred for 2 h at 50^0C, and during the heating process, the pH was carefully monitored and maintained at ~5.5. The solution was filtered and dialyzed for 2 d in Spectra/Por 4 tubing against regular changing of distilled water. The retentate was freeze-dried, to give 0.199 g (42.7 %) of water-soluble, solid **4a(80)-Pt**. η_{inh} = 7.3 mL/g.

Anal. Calcd. for $(C_{71.4}H_{130.4}N_{21.7}O_{21}Pt)_n$ $(1823.9)_n$: Pt, 12.7 %; found: Pt, 10.28 %.

Conjugate 4b(80)-Pt: By the same preceding procedure as described in the forgoing experiment, carrier **4b(80)** (0.296 g, 0.256 mmol) in H_2O (5 mL) was used instead of carrier **4a(80)**, and DACH-Pt (0.144 g, 0.307 mmol) in H_2O (5 mL) was added to the stirred polymeric solution. The solution was treated and worked up as before, giving conjugate **4b(80)-Pt** as a water-soluble solid in a yield of 0.196 g (49.1 %). η_{inh} = 9.7 mL/g.

Anal. Calcd. for $(C_{60}H_{109.2}N_{19.3}O_{16.2}Pt)_n$ $(1555.2)_n$: Pt, 13.3 %; found: Pt, 12.3 %.

Conjugate 4c(80)-Pt: Conjugate **4c(80)-Pt** was obtained by the same procedure as described in the forgoing experiment. A solution (5 mL) of carrier **4c(80)** (0.250 g, 0.216 mmol) was treated with DACH-Pt (0.122 g, 0.259 mmol) and resulted in a water-soluble conjugate **4c(80)-Pt** in a yield of 0.283 g (54.3 %). η_{inh} = 6.4 mL/g.

Anal. Calcd. for $(C_{97.1}H_{176}N_{31.2}O_{27.3}Pt)_n$ $(2412.3)_n$: Pt, 13.3 %; found: Pt, 7.3 %.

Conjugate 4b(90)-Pt: To a stirred aqueous solution (5 mL) of carrier **4b(90)** (0.306 g, 0.142 mmol), saturated with N_2, light protected, was added DACH-Pt (0.080 g, 0.170 mmol). The solution was re-saturated with N_2, stirred for 3 d at RT and then stirred for another 2 h in the incubator at 50^0C. During the process, the pH was carefully monitored and maintained at ~5.5 - 6. The routinely filtered solution was dialyzed in Spectra/Por 4 tubing for 2 d, and the conjugate isolated as a water-soluble solid by freeze-drying in a yield of 0.299 g (21.6 %). η_{inh} = 5.9 mL/g.

Anal. Calcd. for $(C_{423}H_{780}N_{138.1}O_{109.7}Pt)_n$ $(9750.2)_n$: Pt, 7.9 %; found: Pt, 1.8 %.

Conjugate 5a(80)-Pt: To the N_2-saturated solution (5 mL) of carrier **5a(80)** (0.312 g, 0.280 mmol), DACH-Pt$(NO_3)_2$ (0.158 g, 0.336 mmol) in H_2O (5 mL) was added. Upon re-saturation with N_2, the solution was light protected, and stirred for 3 d at RT with the pH of the solution maintained at ~5.5 - 6 and another 2 h at 50^0C. During the heating period, the pH was carefully monitored, and maintained at ~5.5. The solution was filtered and dialyzed for 2 d in Spectra/Por 4 tubing. The retentate was freeze-dried, to give 0.251 g (39.8 %) of water-soluble, solid **5a(80)-Pt**. η_{inh} = 12.1 mL/g.

Anal. Calcd. for $(C_{90}H_{166}N_{30}O_{24.5}Pt)_n$ $(2255.3)_n$: Pt, 13.7 %, found: Pt, 7.75 %.

Conjugate 5b(80)-Pt: Conjugate **5b(80)-Pt** was obtained by the same procedure as described in the forgoing experiment. A solution (5 mL) of carrier **5b(80)** (0.292 g, 0.276 mmol) was treated with DACH-Pt (0.155 g, 0.331 mmol). The solution was treated and worked up as before, resulting in a water-soluble conjugate **5b(80)-Pt** in a yield of 0.266 g (9.6 %). η_{inh} = 9.4 mL/g.

Anal. Calcd. for $(C_{412.8}H_{753}N_{150.2}O_{127.8}Pt)_n$ $(10059.5)_n$ Pt (calc.) 14.3 %, Pt (found) 1.64 %.

Conjugate 5c(80)-Pt: By the same preceding procedure as described in the forgoing experiment, carrier **5c(80)** (0.300 g, 0.257 mmol) in H_2O (5 mL) was used instead of carrier **5a(80)**, and DACH-Pt (0.145 g, 0.308 mmol) in H_2O (5 mL) was added to the stirred polymeric solution. The solution was treated and worked up as before, giving conjugate **5c(80)-Pt** as a water-soluble solid in a yield of 0.142 g (37.8 %). η_{inh} = 14.6 mL/g.

Conjugate 5a(90)-Pt: Conjugate **5a(90)-Pt** was obtained by the same procedure as described in the forgoing experiment. A solution (5 mL) of carrier **5a(90)** (0.305 g, 0.146 mmol) was treated with DACH-Pt (0.082 g, 0.175 mmol) and resulted in a water-soluble conjugate **5a(90)-Pt** in a yield of 0.201 g (11.2 %). η_{inh} = 7.2 mL/g.

Anal. Calcd. for $(C_{522.7}H_{973.2}N_{174.2}O_{133.4}Pt)_n$ $(12026.9)_n$: Pt, 8.3 %; found: Pt, 1.45 %.

4.3.3.3 Polyamidoamines-platinum anchoring

Conjugate 6a(80)-Pt: To the N_2-saturated and light protected solution of carrier **6a(80)** (0.450g, 0.354 mmol) in H_2O (5 mL), DACH-Pt (0.200g, 0.425 mmol) in H_2O (5 mL) was added. Upon re-saturation with N_2, the solution was stirred for 3 d at RT with the pH of the solution maintained at ~5.5 - 6 and another 2 h at 50^0C. During the heating period, the pH was carefully monitored, and maintained at ~5.5. The solution was filtered and dialyzed for 2 d in Spectra/Por 4 tubing. The retentate was freeze-dried, to give 0.414 g (31.9 %) of water-soluble, solid **6a(80)-Pt**. η_{inh} = 7.9 mL/g.

Anal. Calcd. for $(C_{157.8}H_{313}N_{51.7}O_{28.2}Pt)_n$ $(3580.8)_n$: Pt, 12.3 %; found: Pt, 4.70 %.

Conjugate 6b(80)-Pt: By an analogous procedure as described in the forgoing experiment, carrier **6b(80)** (0.420 g, 0.346 mmol) was used instead of carrier **6a(80)** and DACH-Pt (0.195 g, 0.415 mmol). The solution was treated and worked up as before, giving conjugate **6b(80)-Pt** as a water-soluble solid in a yield of 0.223 g (41.3 %). η_{inh} = 6.1 mL/g.

Conjugate 6a(50)-Pt: To a stirred, light protected, aqueous solution (5 mL) of carrier **6a(50)** (0.432 g, 0.861 mmol), saturated with N_2, was added DACH-Pt (0.485 g, 1.033 mmol). The solution was re-saturated with N_2, stirred for 3 d at RT and then stirred for another 2 h in the incubator at 50^0C. During the process, the pH was carefully monitored and maintained at ~5.5 - 6. The routinely filtered solution was dialyzed in Spectra/Por 4 tubing for 2 d, and the conjugate isolated as a water-soluble solid by freeze-drying in a yield of 0.279 g (37.5 %). η_{inh} = 8.2 mL/g.

Conjugate 6b(50)-Pt: Conjugate **6b(50)-Pt** was obtained by the same procedure as described in the forgoing experiment, carrier **6b(50)** was used instead of carrier **6a(50).** A solution (5 mL) of carrier **6b(50)** (0.420 g, 0.862 mmol) was treated with DACH-Pt (0.485 g, 1.031 mmol) and resulted in a water-soluble conjugate **6b(50)-Pt** in a yield of 0.530 g (12.3 %). η_{inh} = 12.1 mL/g.

Anal. Calcd. for $(C_{206}H_{402.4}N_{68.7}O_{40.1}Pt)_n$ $(4678.2)_n$: Pt, 24.5 %; found: Pt, 2.57 %.

Conjugate 7a(80)-Pt: The light protected solution (5 mL) of carrier **7a(80)** (0.451 g, 0.323 mmol) was saturated with N_2, then treated with a solution of DACH-Pt (0.182 g, 0.390 mmol) dissolved in H_2O (5 mL). The solution was re-saturated with N_2 and stirred for 3 d at RT. The pH monitored and maintained at ~5.5 - 6. The solution was further stirred for 2 h at 50^0C, and during the heating process, the pH was carefully monitored and maintained at ~5.5. The solution was filtered and dialyzed for 2 d in Spectra/Por 4 tubing against regular changing of distilled water. The retentate was freeze-dried, to give 0.395 g (9.2 %) of water-soluble, solid **7a(80)-Pt**. η_{inh} = 8.5 mL/g.

Conjugate 7b(80)-Pt: Conjugate **7b(80)-Pt** was obtained by the same procedure as described in the forgoing experiment. A solution (5 mL) of carrier **7b(80)** (0.435 g, 0.324 mmol) was treated with DACH-Pt (0.182 g, 0.389 mmol) and resulted in a water-soluble conjugate **7b(80)-Pt** in a yield of 0.215 g (40.3 %). η_{inh} = 11.8 mL/g.

Conjugate 8a(80)-Pt: To the N_2-saturated and light protected solution (5 mL) of carrier **8a(80)** (0.450 g, 0.343 mmol), DACH-Pt (0.193 g, 0.412

mmol) in H_2O (5 mL) was added. Upon re-saturation with N_2, the solution was stirred for 3 d at RT with its pH maintained at ~5.5 - 6, and another 2 h at 50^0C. During the heating period, the pH was carefully monitored, and maintained at ~5.5. The solution was filtered and dialyzed for 2 d in Spectra/Por 4 tubing. The retentate was freeze-dried, to give 0.292 g (52.6 %) of water-soluble, solid **8a(80)-Pt**. η_{inh} = 11.3 mL/g.

Anal. Calcd. for $(C_{65}H_{125}N_{21}O_{14}Pt)_n$ $(1619.8)_n$: Pt, 12.0 %; found: Pt, 12.33 %.

Conjugate 8b(80)-Pt: By an analogous procedure as described in the forgoing experiment, carrier **8b(80)** (0.450 g, 0.374 mmol) was used instead of carrier **8a(80)** and DACH-Pt (0.211 g, 0.449 mmol). The solution was treated and worked up as before, giving conjugate **8b(80)-Pt** as a water-soluble solid in a yield of 0.251 g (44.5 %). η_{inh} = 8.1 mL/g.

Anal. Calcd. for $(C_{57}H_{105}N_{17}O_{18}Pt)_n$ $(1511.5)_n$: Pt, 12.9 %; found: Pt, 16.46 %.

CHAPTER 5

CONCLUSION

The treatment of malignancies with carcinostatic drugs, per *se* or in combination with other modalities, has proved its value over many decades of clinical administration. However, despite much progress in drug research in recent years, results on average have been highly discouraging. Presently used anticancer drugs suffer from multitude of severe pharmacological deficiencies, such as, lack of cell specificity, inadequate water solubility, decreased serum half-life, salt-like or charged structure, excessive systemic toxicity, and induced resistance, which continue to contribute to the limitations of effectiveness generally experienced in current clinical practice. In an effort to increase the effectiveness of chemotherapy, several new alleys of development are being pursued world-wide, and this project is intended to contribute to this goal.

With the program's synthetic operations predominantly in the area of carrier polymers, the research protocol was directed principally toward the polymers' main chain and side group design in accordance with biomedically prescribed specifications. The synthetic methodology, based in part on experience gained in previous programs, included polyaspartamide formation by aminolytic ring opening in polysuccinimide, and Michael-type addition polymerization using activated bisacryloyl monomers. Three different types of carriers were investigated: (1) carriers modified with dicarboxyl functionalities; (2) carriers modified with carboxylhydroxyl functionalities; (3) carriers modified with dihydroxyl functionalities. These three types of functionalities were incorporated in polymer backbones as side chain for platinum chelation. The first and main step of the project, utilized the synthesis of key monomers derived from aspartic and glycolic acid. The preparation of these monomers and subsequent carriers were

synthesized under critically controlled conditions of pH, temperature, time, and choice of solvents.

The carriers obtained were platinated by treatment with *trans*-1,2-diaminocyclohexane-diaquaplatinum(II) dinitrate in aqueous solution under carefully controlled conditions elaborated in previous work.

Some selected conjugates were evaluated in cell culture tests probing their cell-killing activities against human cancer lines, HeLa, Colo 320 DM and MCF 7, using established protocols. The results obtained show that the conjugates were more active against the selected lines compared to free *cisplatin*.

In future work, a broader number of conjugates should be synthesized with special emphasis on variation of side groups, to be evaluated toxicologically and in both *in vitro* and *in vivo* screens for their antiproliferative activity against different cancer lines. The results will serve as a basis for more advanced follow-on investigations.

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APPENDIX

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