CHAPTER 1
INTRODUCTION

1.1 Cancer problem

The world is facing a formidable health problem with cancer. In the USA, 30 % of the population suffers from some kind of cancer, while 20 % is killed by it like in some other western countries. South Africa is characterized by a particular spread of cancerous afflictions since the advent of HIV/AIDS. During the period before this event, the third cause of death among black adults was cancer, while it was the second cause among the remaining population. The rates for mesothelioma, cervical, oesophageal, and skin cancers in South Africa rank among the highest worldwide.

Without mentioning some other kinds of cancer like those of the lung, the prostate, the breasts etc…, affecting the South African population, it is convenient to notice the coincidence between high levels of life-time risk for females and males of various South African population groups with high age-standardized incidence rates. It is expected that the rapid urbanization and diet in South Africa could constitute factors coming to play in the cause and dissemination of cancer. The rapid spread of AIDS is another factor worthy of consideration as the syndrome is manifested by some kinds of cancer, so a special risk to develop early cancerous lesions will threaten immunodepressed patients; they will be a target for virus-related cancers.

1. 2 Cancer and HIV-AIDS

Patients affected by HIV infection, which is an immunodeficiency, are predisposed to the development of both opportunistic infections and neoplasms whose late manifestation is lymphoma. The appearing frequency of Hodgkin’s disease, non-Hodgkin’s lymphoma (NHL) and T-cell lymphoma

as different types of lymphoma is higher in individuals contracting HIV. However, a significant high rate has been noticed only for immunoblastic and primary central nervous system lymphoma. Burkitt's, immunoblastic and primary central nervous system lymphoma are associated with HIV.

The South African Cancer Association (Cansa) estimated in June 2012 that at least 100 000 people are diagnosed with cancer in the country every year, and this equates to a lifetime risk of one in six persons being diagnosed. The last report of the National Cancer Registry (NCR) of South Africa reports that before 2004, 55 772 people were diagnosed officially with cancer each year, but that many diagnoses from the private health sector are not included in this figure. Statistics from the Medical Research Council (MRC), which were last updated in 2010, show that lung cancer is responsible for the most deaths in both males and females, with breast cancer second. Other cancers that have been responsible for significant numbers of deaths in the country include oesophagial, cervical, liver, colorectal, and leukemia. However, compared with other countries, South Africa's cancer diagnostic rate is still fairly low, with the highest cancer rate for men and women found in Denmark. In 2008, about 326 people in 100 000 were diagnosed in that country, compared with South Africa's 202 in 100 000 for the same year with an emphasis on the increasing rate of cancers associated with HIV nationwide, e. g., Kaposi's sarcoma, a sort of cancer affecting a youth's skin and is the fourth-most common cancer in men and women aged from 15 to 29 years [4]. The rate of HIV-AIDS in the South African youth is stabilized by campaigns but still alarming. The report appears as a warning and these statistics show that the burden imposed on the National Health Services in controlling neoplasias or human suffering is staggering.

1.3 Objectives of the work

At the levels of the entire world in general, and each country in particular, the incidence of neoplasias entices the public health to consider cancer control as an economic issue and a high priority. Long ago, many scientists in medical research have been fighting against cancer as a burden. They have been using chemotherapy either *per se* or in combination with other treatment modalities. Much progress has been recorded in the chemotherapy of cancerous diseases but antitumor drugs in clinical use generally suffer from a series of deficiencies such as organ toxicity, lack of selectivity between normal and abnormal cells, reduced half-lives in circulation and the induction of resistance in the target cells. All these inconveniences make complete cure by chemotherapy alone very rare.

The first objective of this work was to focus on the issue of the lack of drug efficaciousness by building, with specific biomedical requirements, a carrier to act as a vehicle transporting the anticancer drug and ameliorating its pharmacokinetic utilization.

In the second objective, a designed water-soluble polymeric carrier was to be bioreversibly coupled with selected anticancer drug models. Contrary to the style commonly used in this laboratory, the anchoring process was extended to two drugs loaded to the same polymeric carrier expected to interact with nuclear DNA or proteinaceous constituents once in intracellular space.

The third objective was the biological activity evaluation of the synthesized polymer-drug conjugates and co-conjugates against the MCF-7 cell line. The motivation was to compare the potency of these conjugates with the free drugs presently in clinical use.

Cisplatin (Pt), methotrexate (MTX) and the organoiron ferrocene (Fc) were the three drugs to be investigated. Ferrocene and methotrexate were

selected for co-conjugation with Pt in an effort to provide macromolecular drugs for multidrug therapy.

After a literature review on the modalities of cancer treatment in chapter 2, the background of the macromolecular carriers and anticancer drug conjugates will be reviewed in chapter 3, followed by chapter 4, where the results will be discussed. Chapter 6 will draw a conclusion with future work, while experimental methods of the study will be described in chapter 5.

CHAPTER 2
OVERVIEW OF CANCER TREATMENT MODALITIES

Cancer can be treated by surgery, radiation, chemotherapy and immunotherapy. These four treatment modalities depend on the location and amount of disease as well as the health status of the patient. A particular accent will be put on chemotherapy as it is the field of application of our study. Generally, a cancer treatment is meant to kill or remove cancer cells by inhibiting the signal needed for their division, leading to their eventual death. The host immune system can also be stimulated after killing or removal of these cells.

Each anticancer drug has its own pharmacokinetic path and cancer treatments are often used in combination to exploit the potency of different drugs, either simultaneously or sequentially.

2.1 Surgery

Surgery is the first treatment until recently to be used against cancer. Advances in surgical techniques and a better understanding of the patterns of spread of individual cancers have allowed surgeons to perform successful resections for an increased number of patients by removing the primary tumors, but the treatment was ineffective for metastasized or disseminated tumors. However, the treatment of most tumors depends on the subsequent development of surgery, which also has an important role in diagnosing and staging of cancer. The different types of surgery, highly goal oriented are the following:

2.1.1 Curative surgery

Curative surgery is the technique mostly known. It is primarily applied when the chance to remove all the cancerous tissue is evident. Surgery is easier to handle when the tumor appears to be confined to one area. It is often combined with chemotherapy or radiation therapy, which can be practiced before or after operation. The failure of surgery alone to control local disease in patients with some forms of cancer led to the use, during the operation, of radiation therapy which is therefore termed intraoperative radiation therapy.

2.1.2. Cytoreductive and palliative therapy

This kind of surgery comes to play in some cases when removal of the entire tumor could be a tremendous problem to its related organ or its surrounding areas. The surgeon may therefor remove most of afflicted tissue as possible, and then apply radiation therapy or chemotherapy. Advanced cancer of ovary is the field of application of Cytoreductive (Debulking) surgery. Palliative surgery is not intended to cure the cancer; it can also be used to correct a problem that is causing discomfort or disability.

2.1.3 Diagnostic and staging surgery

Diagnostic surgery is the process of tissue sampling and is used to differenciate a cancer from another when tissues are observed through the microscope. The diagnosis is then confirmed only by cellular microscopy and staging surgery [5].

2.1.4 Prophylactic surgery

Also called preventive surgery, prophylactic surgery concerns the removal of a malignant body tissue. An entire organ can sometimes be removed when the development of cancer can be foreseen in an individual with an inherited

condition. A case to be mentioned is when a human female has a mutation in her DNA, in a breast cancer gene (*BRCA1* or *BRCA2*). This situation is generally inherited in the family and these genes account for approximately 5-10 % of all breast cancer cases [6] and 80 % of familial breast cancer cases [7, 8]. Such a female is a candidate for prophylactic mastectomy (breast removal).

2.1.5 Supportive and restorative surgery

It may happen sometimes that a vascular device such as a catheter port can be placed into a vein to help deliver chemotherapy treatments. This practice is generally combined with other modalities under supportive surgery and the consequence is a reduction of the number of needle sticks needed [9, 10].

2.2 Radiation therapy

Radiation is the second modality in cancer treatment. It is mostly applied when it comes to eliminate and stop the development of secondary tumors that could survive in the body after surgery. When it is used earlier, there is hope that the disease can be controlled and that the tumor can be shrunk. It's always an advice to handle radiation with precaution as radiation alters the genetic code which controls the cell growth and division. Cancer treatment uses mostly ionizing radiation, which when passing through tissues, generates ions and delocalize electrons from atomic species, which kill the cell or modifie the genetic code during interferences in the mitotic phase M of the cell cycle in the body. The efficacy of the therapy depends on the amount of energy spent, so this energy quantity can be measured according to the distance traveled by the radiation in the tissue. Photons (X-and γ-rays) and particulate radiations (electrons, neutrons, protons, α and β particles) are radiation energies. Neutron radiation is used for some cancers of the head, neck, and prostate. Figure 2.1 below depicts the cell cycle phases.

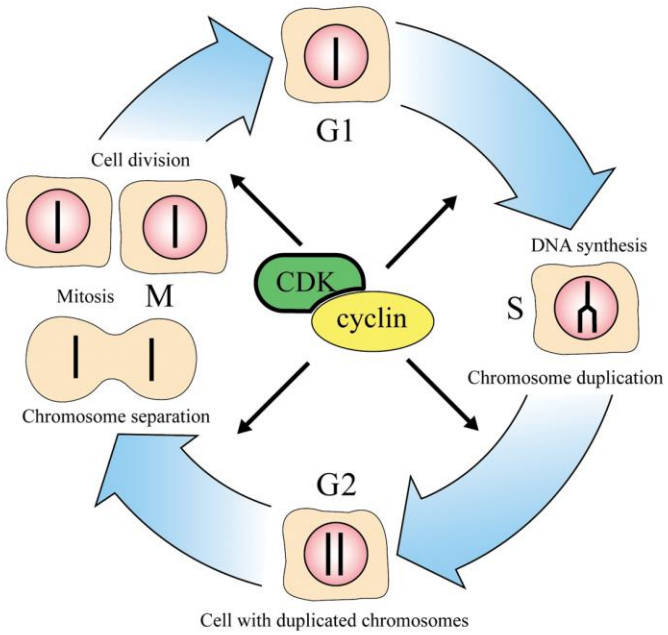


Figure 2.1 [233]: Cell cycle phases

G_1: RNA and protein synthesis phase.
G_2: Cell pre-splitting phase
S: DNA replicating phase
M or mitosis phase: The cell has actually split into two cells

2.3 Chemotherapy

Generally after surgery, residual and undetectable cancer cells frequently remain in the body. Chemotherapy can then be administered with a goal to inhibit and stop the development of such secondary tumors. It is, in the

simplest sense, the treatment of an ailment by chemicals especially by killing micro-organisms or cancerous cells. In popular usage, it refers to antineoplastic drugs used to treat cancer or the combination of these drugs into a cytotoxic standardized treatment regimen. The toxic effects of chemical agents on the cancerous cell for the most part involves interference with the cell's replication mechanism and, thus with intracellular nucleic acid synthesis and the ultimate role played by the nuclear DNA in mitosis. The effectiveness of the anticancer drugs is generally, although not exclusively, at an optimum level whenever the target tissue consists of rapidly dividing cells, as is generally the case in malignant systems. However, the human body contains a number of compartments in which normal, i.e., healthy cells are required to replicate at a high rate in order to fulfill their physiological role, e. g., the bone marrow, the linings of the gut and the urinary tract. It is, indeed, in these compartments where most of the systemically acting anticancer drugs exert their most undesirable toxic side effects, as the rapidly proliferating cells do not provide sufficiently long time intervals during which normal DNA repair mechanisms, mediated by selective repair enzymes, can remain operative. This results in the most common side effects of chemotherapy: myelosuppression, a decrease in production of blood cells, hence also immunosuppression, mucositis, an inflammation of the lining of the digestive track and alopecia which is a hair loss [11-13].

2.3.1 History of cancer chemotherapy

The first use of drugs to treat cancer was in the early 20th century although it was not originally intended for that purpose. Mustard gas was used as a chemical warfare agent during World War I and was studied further during World War II. During a military operation in World War II, a group of people were accidentally exposed to mustard gas and were later found to have very

low white blood cell counts. It was reasoned that an agent that damaged the rapidly growing white cells might have a similar effect on cancer. Therefore, in the 1940s, several patients with advanced lymphomas (cancer of certain white blood cells) were given the drug by the vein, rather than by breathing the irritating gas. Their improvement, although temporary, was remarkable. That experience led researchers to look for other substances that might have similar effects against cancer. As a result, many other drugs have been developed to treat cancer, and drug development since then has exploded into a multibillion-dollar industry, although the principles and limitations of chemotherapy discovered by the early researchers still apply [14,15].

2.3.2 Principles of cancer chemotherapy

Cancer is an uncontrolled growth of cells coupled with malignant behavior of invasion and metastasis. It is thought to be caused by the interaction between genetic susceptibility and environmental toxins. In the broad sense, most chemotherapeutic drugs work by impairing mitosis (cell division), effectively targeting fast-dividing cells. As these drugs cause damage to cells they are termed *cytotoxic*. Some drugs cause cells to undergo apoptosis, a self-programmed cell death. Scientists have yet to identify specific features of malignant and immune cells that would make them uniquely targetable. This means that other fast-dividing cells, such as those responsible for hair growth and for replacement of the intestinal epithelium, are also often affected. However, some drugs have better side effects than others, enabling doctors to adjust treatment regimens to the advantage of patients in certain situations. As chemotherapy affects cell division, tumors with high growth fractions such as acute myelogenous leukemia and the aggressive lymphomas, including Hodgkin’s disease, are more sensitive to chemotherapy, as a larger proportion of the targeted cells are undergoing cell division at any time.

Malignancies with slower growth rates, such as indolent lymphomas, tend to respond to chemotherapy much more modestly. Drugs affect younger tumors more effectively because mechanisms regulating cell growth are usually still preserved. With succeeding generations of tumor cells, differentiation is typically lost, growth becomes less regulated, and tumors become less responsive to most chemotherapeutic agents. Near the center of some solid tumors, cell division has effectively ceased, making them insensitive to chemotherapy. Another problem with solid tumors is the fact that the chemotherapeutic agent often does not reach the core of the tumor. Solutions to this problem include radiation therapy and surgery. Over time, cancer cells become more resistant to chemotherapy treatments. Recently, scientists have identified small pumps on the surface of cancer cell that actively move chemotherapy from inside the cell to the outside. Research on p-glycoprotein and other such chemotherapy efflux pumps, is currently ongoing. Medications to inhibit the function of p-glycoprotein are undergoing testing to enhance the efficacy of chemotherapy [16-20].

2.3.3 Treatment schemes of cancer by chemotherapy

There are a number of strategies in the administration of chemotherapeutic drugs either with a curative or with a palliative intent. Combined modalities chemotherapy is the use of drugs with other cancer treatments, such as radiation therapy or surgery, and most cancers are now treated in this way.

Combination therapy is a similar practice that involves treating a patient with a number of different drugs simultaneously. Each drug has its own pharmacokinetic path and combination chemotherapy presents advantages to minimize the chances of resistance developing to any of the treatment agents[21-25].

Neoadjuvant chemotherapy is a preoperative treatment where an initial treatment is designed to shrink the primary tumor, thereby rendering local therapy less destructive or more effective.

Adjuvant chemotherapy is a postoperative treatment that can be used when there is a little evidence of cancer presence recurring. This can reduce chances of developing resistance if the tumor develops. Adjuvant chemotherapy is often effective when the newly growing tumors are fast-dividing, and therefore very susceptible [26].

Palliative chemotherapy is given without curative intent, but simply to decrease tumor load and increase life expectancy. For these regimens, a better toxicity profile is generally expected.

All chemotherapy treatments require that the patient be capable of undergoing the treatment. Performance status is often used as a measure to determine whether a patient can receive chemotherapy, or whether dose reduction is required. Because only a fraction of the cells in a tumor die with each treatment, repeated doses must be administered to continue to reduce the size of the tumor. Current chemotherapy regimens apply drug treatment in cycles, with the frequency and duration of treatment limited by toxicity to the patient [27].

2.3.4 Types of anticancer agents

The majority of anticancer agents can be divided into alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors and other antitumor agents, according to the mechanism of cytotoxic activity and the cell cycle phase interference. Although on occasional instances, the mechanisms of cytotoxic activity associated with particular classes may

loosely coincide, in general, the members within a given class function by a mechanism particular to the class. All of these drugs affect cell division or DNA synthesis. Some newer agents do not directly interfere with DNA. These include monoclonal antibodies and the new tyrosine kinase inhibitors which directly target a molecular abnormality in certain types of cancer like chronic myelogenous leukemia and gastrointestinal stromal tumors. In addition, some drugs that modulate tumor cell behavior without directly attacking those cells may be used. Hormone treatments fall into this category. The anticancer activity is dependent on growth fraction (the fraction of cells actively dividing with respect to the entire population of viable cells), and mass doubling time (the time taken by a tumor to double in size). Tumors with high growth fraction are more susceptible to the cytotoxic effects of anticancer drugs than those with high percentage of dormant cells. Dividing-cells are drug-sensitive. Thus tumors with shorter mass doubling times are more amenable to treatment with drugs. As tumors get larger, the mass doubling time increases and the growth fraction decreases. Unfortunately, normal tissues with high growth fractions (bone marrow, oral and intestinal mucosa, hair follicles) are also damaged by anticancer drugs, and treatment with many of these drugs may produce bone marrow depression, gastrointestinal tact ulceration, and alopecia [28]. Another limiting factor to successful chemotherapy in malignant disease is tumor size. The amount of drugs penetrating into a solid tumor may not be sufficient to kill the cells. Also, most cells in a bulky tumor may be in a non-proliferative stage at the time of treatment and thus survive to re-establish the tumor mass. Thus, the longer a tumor has been present the greater is the likelihood that it has already metastasized. Therefore, for any tumor therapy to be completely effective the most invasive metastatic cells must be killed. Moreover, the response to certain cell phase-specific drugs depends on the percent of cells in a sensitive phase during the time of

exposure to pharmacologically effective concentrations of the drug. In general, for cycle-specific agents such exposure should be for at least two cell cycle times. Cells in the G_0 phase are, for the most part, refractory to chemotherapy. These cells may re-enter the cell cycle and result in disease recurrence. Most anticancer drugs are effective against cells in one particular phase of the cycle and have their greatest activity during S phase when cells are undergoing DNA synthesis.

Drug resistance is one of the most important problems encountered with cancer chemotherapy, and several different biochemical mechanisms by which tumor cells develop resistance to anticancer drugs have been identified. These include

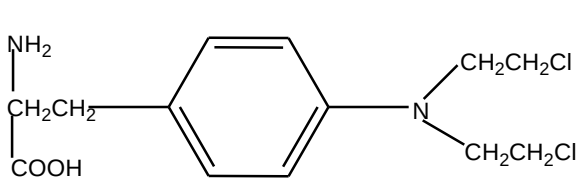
(a) Decreased intracellular drug levels resulting from increased drug efflux[29] or decreased inward .transport. Anthracyclines, dactinomycin, vinca alkaloids, and epidopodophyllotoxins are among the drugs becoming ineffective by this mechanism.
(b) Increased drug inactivation. Included in this group, are the alkylating agents, antimetabolites and bleomycin.
(c) Decreased conversion of drug to an active form. This mechanism is mostly common among the antimetabolites (5-FU, 6-MP, etc.) which must first be converted into nucleotide before therapeutic activity can be observed.
(d) Altered amount of target enzyme or receptor (gene amplification).

2.3.4.1 Alkylating drugs

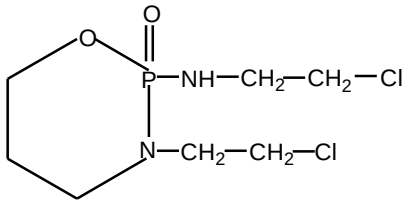
Alkylating agents are so named because of their ability to alkylate many nucleophilic functional groups under conditions present in cells. *Cisplatin* and

carboplatin, as well as oxaliplatin, are alkylating agents. These drugs are active against chronic leukemias, non-Hodgkin's lymphoma, Hodgkin's disease, multiple myeloma, and certain cancers of the lung, breast, and ovary. They impair cell function by forming covalent bonds with the amino, carboxyl, sulfhydryl, and phosphate group in biologically important molecules and act by forming strong electrophiles through the formation of carbenium ion intermediates. This results in the formation of covalent linkages by alkylating of various nucleophilic moieties. Other agents are mechlorethamine, cyclophosphamide, chlorambucil, ifosfamide. They work by chemically modifying a cell's DNA [30]. The chemotherapeutic and cytotoxic effects are directly related to the alkylating of DNA, mainly through the N-7 atom of guanine, although other moieties are also alkylated. The formation of one covalent bond with nucleophile can result in mutagenesis or teratogenesis, but the formation of two of these bonds through crosslinking can produce cytotoxicity. Bifunctional alkylating agents can undergo a second cyclization of the second side chain and form a covalent bond with another nucleophilic group. The second group can be N-7 of another guanine or some other nuleophilic moiety. These bifunctional alkylating agents such as nitrogen mustard react with another nucleophilic moiety resulting in the crosslinking of two nucleic acid chains or the linking of nucleic acid to a protein. This type of alteration causes a major disruption in nucleic acid function. Cytotoxicity of bifunctional alkylators correlates very closely with interstrand crosslinkage of DNA. Some of the cellular responses produced included cell-cycle blocking, DNA repair and apoptosis. The nucleophilic groups of proteins, RNA and many other molecules are potential targets of the alkylating agents which are generally considered to be cell-cycle phase non-specific and are mostly cytotoxic to rapidly proliferating cells. DNA alkylation occurs anytime in the cell cycle, but the biological consequences

are more severe during the replicating S phase. The biochemical mechanisms identified as a cause of resistance to the drugs include decreased cellular uptake of the drug, increased production of nucleophiles such as glutathione and increased repair of DNA. Of these, increased inactivation and decreased uptake of drug are of clinical importance [31-40]. Fig 2.2 below depicts the structures of two alkylating agents.



Melphalan



Ifosfamide

Figure 2.2 [31] : Sctructrures of two DNA alkylating agents

Nitrogen mustard: Also named mechlorethamine, it was the first alkylating agent to receive clinical trial and was found to produce responses in patients with lymphomas. This agent is highly reactive in aqueous solution and must be administered by IV injection. It also is effective as a topical solution for treatment of mycosis fungoides but produces hypersensitivity to its chloroethyl side-chain when used in this way. Nitrogen mustard penetrates cells through an active transport mechanism shared with the physiologic amine choline. Resistance to the agent is poorly understood; it is believed to

result from enhanced ability to repair DNA alkylation, but other mechanisms, such as defective transport or increased inactivation of the carbonium ion by enzymatic conjugation with intracellular sulfhydryl groups, may play a role. The primary toxicities of nitrogen mustard consist of myelosuppression, nausea, and vomiting. Minor cholinergic side-effects are present at high doses and include lacrimation, diarrhea, and diaphoresis. Because of the high chemical reactivity of this compound, it is a potent vesicant and causes severe local tissue injury when infiltrated into the skin [41]. Nitrogen mustard has been replaced to a great extent by more stable agents described below.

Cyclophosphamide: In an attempt to improve the selectivity of alkylating agents, cyclophosphamide was designed based on the fact that tumor cells posses a high concentration of enzyme activity that can cleave the N-P bond and liberate the potent phosphoramide mustard. In fact, activation of the drug is a multistep process. The first metabolite, hydroxycyclophosphamide, is produced by hepatic microsomal metabolism [41]. 4-OH cyclophosphamide reenters plasma and is transported to peripheral target tissues, where it crosses the cell membrane and undergoes sequential conversion to aldophosphamide and its ultimate active principles, phosphoramide mustard and acrolein. Aldophosphamide is also subject to inactivation by aldehyde dehydrogenase, an enzyme elevated in some resistant tumor cells [41]. Although phosphoramide is believed to be the primary active product, acrolein is a highly reactive compound capable of depleting glutathione and causing single-strand alkylation of DNA. Acrolein is excreted intact in the urine and has been implicated in the cystitis caused by cyclophosphamide. 4-Hydroperoxycyclophosphamide, a chemically stable form of 4-OH-cyclophosphamide, has been used for selecting purging of neoplastic cells

from bone marrow and appears to have much greater toxicity for tumor cells than for multipotent bone marrow progenitor cells [41].

Ifosfamide: Ifosfamide, a drug closely related to cyclophosphamide by virtue of its oxazaphosphorine ring structure, differs in its pattern of toxicity, causing less myelosuppression but dose-limiting cystitis. Like cyclophosphamide, it is activated by hepatic P-450 mixed-function oxidase, and alkylating metabolites are excreted in the urine. Sodium-2-mercapto-ethane sulfonate (MESNA) effectively prevents cystitis, even in patients with a history of cyclophosphamide-related cystitis. Other significant toxicities include cerebellar dysfunction, seizures, and altered mental status in as many as 30% of patients treated with high doses of ifosfamide (91.6 g/m^2/d for 5 days, or >g/day as a single dose) [42].

Melphalan: Melphalan, a phenylalanine derivative, was conceived as a compound that would localize preferentially in tumors, such as melanin-producing malignancies, that actively use phenylalanine or tyrosine. The resulting compound has a broad spectrum of antitumor activity similar to that of cyclophosphamide (lymphomas, breast and ovarian cancers, multiple myeloma) but has the added advantage of not causing hemorrhagic cystitis. Melphalan enters cells by active transport, using a high-affinity carrier-the “L” amino acid transport system, which also transports the amino acids leucine and glutamine. In some tumor cells, a second transport system (which also carries alanine, cysteine, and serine) promotes melphalan uptake but is less effective than the L system at high drug concentrations [43].

Chlorambucil: It is a close structural congener of melphalan and has similar stability in aqueous solution because of the electron withdrawing properties of its unsaturated ring. Chlorambucil is given orally and is a convenient

alkylating agent for treatment of malignancies such as chronic lymphatic leukemia (CLL), nodular lymphomas, or multiple myeloma, which require long-term management. It has predictable myelosuppressive effects on both granulocytes and platelets but few other side-effects. Like other alkylating agents, chlorambucil has been implicated in late occurrences of AML and in pulmonary fibrosis. Its pharmacokinetics is poorly understood, but the drug appears to be eliminated by metabolic transformation [44, 45].

Busulfan: Busulfan consists of two labile methane-sulphonate groups attached at opposite ends of a four-carbon alkyl chain. This compound is stable enough to allow oral administration but rapidly forms carbonium ions after systemic absorption through release of the methane-sulfonate group, leading to alkylation of DNA. Although the potential for interstrand cross-linkage exists in the bifunctional structure of busulfan, such cross-linkage has not been demonstrated [46].

Nitrosourea: The chloroethylnitrosoureas are highly lipid-soluble and chemically reactive compounds that are clinically reactive against the lymphomas, malignant melanomas, brain neoplasms, and gastrointestinal carcinomas. Many derivatives that incorporate this basic structure but differ in their lipid solubility, side-group substitution, and aqueous stability have been synthesized in an effort to improve their therapeutic index. Chlorozotocin, streptozotocin, and other glycosylated nitrosoureas have less bone marrow toxicity but have unproven clinical usefulness. Chemical decomposition of these compounds in aqueous solution yields two reactive intermediates, a chloroethyldiazohydroxide and an isocyanate. The former decomposes further to yield a reactive chloroethyl carbonium ion that forms a single-strand adduct with DNA and then, through a dehalogenation step, forms a second

reactive site and cross-links DNA. Thus cross-links are produced by both the monofunctional and bifunctional nitrosoureas [47, 48].

Cisplatin: *Cis*-(II) platinum diaminedichloride (*cis*-DDP) is the only heavy metal compound used as a cancer chemotherapeutic agent and has a spectrum of unique biologic effects. The biologic activity of platinum coordinate compounds was first recognized in 1965 [49, 50]. *Cis*-DDP subsequently entered clinical trials in 1971 and since then has become established as a highly effective drug for treatment of testicular tumors, ovarian carcinoma, bladder carcinoma, and neck cancer. The antitumor activity of *cis*-DDP is best understood in terms of its chemical properties in aqueous solution (Scheme 2.1, page 39). The tetravalent heavy metal platinum (Pt) binds two potential leaving groups, its chloride ions; in transposition to the chlorides are bound two NH groups in a firm linkage. Only the *cis*-dichloro structure is an active antitumor agent; the trans-DDP isomer lacks cytotoxic activity, possibly because of its inability to form stable intrastrand DNA cross-links [51]. Both chloride ions undergo a slow displacement by water, a process that may be accelerated in an environment of low chloride concentration (e.g. inside the cell or in urine), generating a positively charged, aquated complex. This activated complex then can interact with a nucleophilic site on DNA, RNA or protein to form bifunctional covalent links analogous to alkylating reactions. Favored sites of attack are the N^7 position of guanine and the N^3 position of cytosine [52]. A variety of bifunctional and monofunctional covalent bonds are possible, including intrastrand cross-links, interstrand links, and DNA-protein complexes [53]. The formation of intrastrand cross-links, a type of bond not formed by trans-DDP, may be an important feature of *cis*-DDP action, particularly those links that form between the N^7, N^1, or O^6 of one guanine base and the N^3 of the

neighboring cytosine. The consequences of cis-DDP attack on DNA include changes in DNA conformation and inhibition of DNA synthesis [54]. The formation of cross-links is a slow process that continues for hours after drug exposure and is opposed by enzymatic repair processes that excise and rebuild damaged segments of DNA and ultimately determine cytotoxicity [55, 56]. DNA cross-links may be prevented by preincubating the drug with thiourea, which combines readily with the aquated platinum binding sites [57]. Other thiols, including sodium thiosulfate, which decrease *cis*-DDP systemic toxicity, and diethyl dithiocarbamate, which specifically prevents cis-DDP renal toxicity, are potentially of clinical value, particularly in conjunction with intraperitoneal *cis*-DDP, or during high-dose *cis*-DDP therapy [58]. The cell-cycle dependence of *cis*-DDP is poorly understood. It appears that some cells are most sensitive to *cis*-DDP when exposed during the G_1 (intermitotic) phase of the cycle, possibly because of the delay in cross-link formation, which would then be maximal during the following S phase [59]. A delay in transit during S phase and the succeeding cell cycle is induced by drug treatment. Resistance to *cis*-DDP in some experimental studies has been linked to elevated levels of intracellular glutathione or the thiol-rich protein metallothionine. It is likely that the ability to prevent (through sulfhydryl reaction) or repair DNA cross-links plays an important role in determining sensitivity to this drug [56]. Platinum compounds do not share cross-resistance with nitrosoureas or classic alkylating agents in most experimental systems. Although specific processes that repair DNA-platinum adducts have not been identified, it has been possible to quantitate adduct formation with extreme sensitivity and to correlate their level in peripheral blood leucocytes with the dose of *cis*-DDP and response to treatment in patients with ovarian cancer [60]. These findings imply that pharmacogenetic or metabolic characteristics common to tumor and peripheral tissues determine response to *cis*-DDP.

2.3.4.2 Antibiotics

Antibiotics are present in small quantities in biological materials. They have been evolving from natural sources and many efforts are being done worldwide in order to synthesize and develop their relatives possessing particular structures. These structures, different in their mechanisms of action, are nonconvalent DNA-binding drugs and interfere with the synthesis, replication or transcription of proteins. So the analog structures can be intercalated between nuclear bases in the DNA double helix.

Antitumor antibiotics: They are characterized by a lack of specificity of antimicrobial antibiotics, and therefor produce a significant toxicity. They are isolated from natural sources and antibiotics and interact with DNA in a variety of different ways including intercalation, DNA strand breakage and inhibition of the enzyme topoisomerase II as they are noncovalent DNA-binding agents.

Bleomycin, one of the most unusual structures that has antitumor activity is a mixture of small molecular mass peptides (1500 Daltons) isolated from the fungus streptomyces verticullus. It is one of a family of antibiotic peptides possessing both antitumor and antimicrobial activity. The bleomycin mixture contains mostly the A_2 peptide, the unique pharmacologic properties of which have been characterized extensively. The structure of the A_2 compound consists of a DNA-binding fragment and an iron-binding portion located at the opposite end of the molecule. The primary action of bleomycin is to produce single-strand and double-strand breaks in DNA. The frequency of events leading to DNA breakage begins with the binding to DNA, preferentially to G-T or G-C sequences. Ferrous ion (Fe^{2+}), which is bound intimately to the

imidazole, pyrimidine, and other nitrogen-containing groups of bleomycin, undergoes spontaneous or enzymatic oxidation to the Fe^{3+} state. The electron that is liberated in this reaction is accepted by oxygen and forms active oxygen intermediates, such as the superoxide or hydroxyl radicals. These radicals, in turn, attack the 4'-H of deoxyribose, leading to cleavage of the sugar and release of its attached base, usually thymine, cytosine, or their propenal adducts. The action of bleomycin is specific for DNA and is not exerted against RNA. There appears to be some cytokinetic specificity to bleomycin cell kill. Cells in synchronized culture systems are most susceptible during the pre-mitotic phase or G_2 phase, or in the mitotic phase of the cell cycle. However, cells exposed during G_1 also are killed, and it is not known whether rapid cell division predisposes to cytotoxicity. The possibility of increasing cell kills by exposing cells during the G_2 phase has prompted trials of bleomycin administration by infusion.

The DNA lesions produced by bleomycin are visible as chromosomal breaks and deletions. It seems likely that repair processes play an important role in determining the lethality of these lesions because repair of potentially lethal damage occurs when cultured cells are exposed to these agents. There is indirect evidence that the same processes required to repair ionizing radiation damage also are used in bleomycin repair. The repair process is inhibited by calmodulin antagonists such as trifluoperazin. Glutathione enhances bleomycin cytotoxicity like misodinazole. Enhancement by glutathione likely relates to the need to recycle the Fe^{2+} state with each oxidation-reduction cycle [61].

Anthracyclines, as antitumor agents, are matched only by alkylating agents in terms of their clinical usefulness. Daunomycin and doxorubicin were the first anthracyclines in clinical use. They are antibiotics produced from

streptomyces species. These antibiotics are, in fact, part of a large group of highly colored bacterial products known as the rhodomycins. In general, these compounds, like daunomycin and doxorubicin, have a planar anthraquinone nucleus attached to an amino sugar. Within this group, or closely related to it, are compounds that have a wide range of biologic activity, which include antibacterial and antitumor agents. Daunorubicin is one of the most effective agents in the treatment of acute lymphocytic and myelocytic leukemia. On the other hand, doxorubicin is used to treat solid tumors, such as carcinomas of the breast, lung, thyroid, and ovary, and soft tissue sarcomas [62].

Mitomycin C is an antibiotic whose antitumor activity has been known a long time ago. Its primary clinical use has been for gastrointestinal sarcoma. This drug can be activated to an alkylating species through enzymatic reduction, mediated by cytochrom C reductase, xanthine oxidase, or cytochrom P-450 reductase, or can occur in an acid-catalyzed or base-catalyzed reaction in aqueous solution. Mitocyn C has been implicated as the cause of renal failure, often associated with microangiopathic hemolysis in the syndrome called the hemolytic-uremic syndrome [63].

Actinomycin D has an interesting structure. It is composed of a phenoxazone ring chromophore that gives a red color to the drug. Two identical polypeptides are bound to the chromophore. This antibiotic binds to DNA by intercalation, with phenoxazone ring inserted perpendicularly to the long axis of the DNA double helix and the polypeptide chains extending along the minor groove. Actinomycin D is a member of a large class of similar drugs that were first isolated from streptomyces species. It is the only member of the class to achieve significant clinical use. It is effective in the treatment of Wilm's tumor, Ewing's sarcoma, embryonal rhabdomyosarcoma, and

gestational choriosarcoma. Responses also are seen in testicular cancer, Kaposi's sarcoma, and lymphoma [64].

2.3.4.3 Antimetabolites

Antimetabolites are agents that, by virtue of structural similarity with physiologic intermediates, are accepted as substrates for vital biochemical reactions and thus interfere with required cell processes such as the production of nucleic acids. They work through a variety of mechanisms including competition for binding sites on enzymes and incorporation into nucleic acids. Antimetabolites inhibit the growth of the most rapidly proliferating cells in the body (e.g., bone marrow, gastrointestinal tract, etc.), and are divided into three categories: antifolates, purine analogs and pyrimidine antimetabolites [65].

1. Antifolates: Often called antifols, they are folic acid antagonists, cytotoxic drugs used as antineoplastic, antimicrobial, antiinflamatory, and immune-suppressive agents. Folate antagonists are thought to act as cytotoxic drugs by interfering with one or more biosynthetic steps involving folate coenzymes. A folate antagonist might function in one of several ways: by competing with folates for uptake into cells, by inhibiting the formation of folate coenzymes or by inhibiting one or more reactions that are mediated by folate coenzymes. Thus far, however, the clinically important antineoplastic folate analogs appear to work primarily by inhibiting dihydrofolate reductase (DHFR) [66].

(a) Methotrexate [67]

MTX is important within the context of this study as it is the second drug selected for co-conjugation with cisplatin after ferrocene.

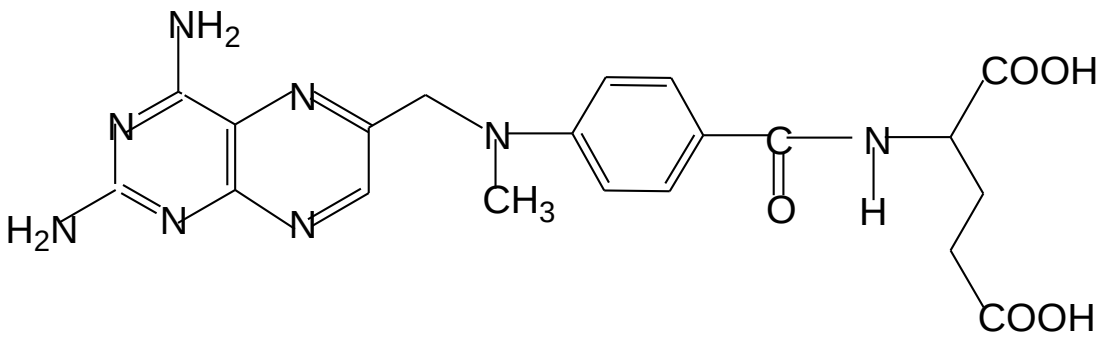


Figure 2.3 [67]: Structure of methotrexate

Historical overview: Though several folate antagoniosts have been developed, and several are now in clinical trial, methotrexate (MTX) is the antifolate with the most extensive history and a widest spectrum of use. The observation of serum folate deficiency (a vitamin B) among patients with acute leukemia prompted some investigators in the early 1940s [68] to postulate that acute leukemia might be the result of a deficiency of this vitamin. Specifically, megaloblasts resembling leukemia blasts predominate in the bone marrow of folate-deficient patients. The availability of crystalline

pteroylglutamic acid (PGA, folic acid), first isolated from spinach in 1941 and synthesized in 1945, prompted investigators to give folate to patients with leukemia. It was soon recognized that administration of this substance was not only ineffective but possibly even accelerated the course of the disease of patients with chronic myelocytic leukemia and acute leukemia. Efforts to treat these leukemias thus turned to creating a folate deficiency. Aminopterin (4-amino-PGA; AMT) produced temporary remissions in five of sixteen patients with acute leukemia. This demonstration was a landmark in cancer chemotherapy; it provided the first demonstration that an antimetabolite could be an effective antineoplastic agent. Since the initial study demonstrating the usefulness of AMT in the treatment of acute leukemia of childhood, there has been a sustained interest in, and a continued reevaluation of, this and other folate antagonists. In studies with mice bearing the L210 leukemia, methotrexate (4-amino-10-methyl PGA; amethopterin; MTX) was found to have a more favorable therapeutic index than AMT, and thus for the last 40 years, MTX has supplanted AMT in the clinic. However, it is of interest that these two drugs have not been compared in a clinical trial. The newer antifols are rationally designed analogs of folates or MTX. They have been synthesized either to overcome cellular resistance to MTX or to inhibit the metabolism of folate or folate-mediated reactions, instead of or in addition to that of DHFR. Some of these have been approved as antimicrobial or antineoplastic agents, and others are still in clinical trial.

- *Mechanism of action*: The prototype antifolate DHFR inhibitor is a 4-amino-substituted pterin compound. The substitution of an amino moiety for the 4-hydroxyl results in a folate analog with a thousand fold increase in affinity for DHFR. The Ki of MTX for DHFR is below 10^{-10} mol, well below the Km of the natural substrate dihydrofolate, which is

in the micromolar range. By stoichiometrically inhibiting DHFR, a key enzyme in the thymidylate cycle, MTX disrupts a critical step in the synthesis of deoxyribonucleic acid (DNA). In rapidly dividing cells, the inhibition of thymidylate biosynthesis leads to a decrease in thymidine biophosphate pools, a decrease in DNA synthesis, and eventually cell death. Inhibition of tetrahydrofolate formation leading to the inhibition of purine synthesis and rapid cell death has also been described as occurring in lymphoblasts treated with high doses of MTX. In recent years, the important role of polyglutamylation as a determinant of MTX sensitivity has been elucidated. A single enzyme, folylpolyglutamate synthetase (FPGS), appears to be responsible for adding glutamate residues in γ-carbonyl linkage to both folate coenzymes and MTX and other analogs with a glutamate moiety. This enzyme process, by which up to seven or eight additional glutamate molecules are added, serves to add both mass and negative charge to these molecules, thus markedly reducing their cell efflux. In addition, MTX polyglutamates can inhibit other folate dependant enzymes, including thymidilate synthase and 5-aminoimidazole-4-carboxamide ribonucleoside transformylase, enzymes involved in thymidylate and de novo purine synthesis respectively [69].

(*b*) *Aminopterin (AMT):* After producing remarkable, though transient responses in the 1940s, AMT was abandoned because MTX had more predictable toxicity and a better therapeutic index in a murine model. No randomized comparison between the two was ever performed in the clinic. Preclinical models show greater potency for AMT. Leukemic blasts from pediatric patients have been shown to accumulate AMT better than MTX, probably because AMT has a higher affinity for FPGS than does MTX. Given

the demonstrated importance of antifolate accumulation by malignant cells as a prognostic indicator, AMT has again entered clinical trials.

(*c*) *Folic acid*: It is pteroylglutamic acid consisting of pteridine that is linked to para-aminobenzoic acid (PABA) and glutamic acid.

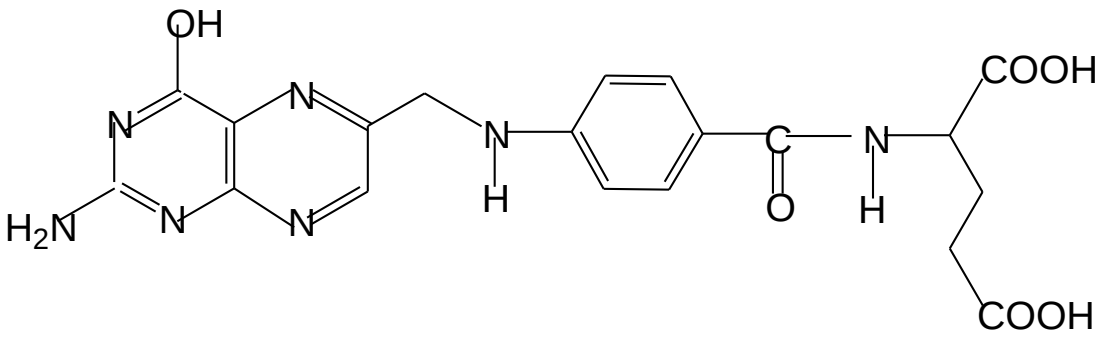


Figure 2.4: Structure of folic acid

Physiological role: As a pharmacological product, folic acid (FA) is a vitamin, a nutritional supplement, and a diagnostic aid in folate deficiency [70]. FA, referring to the folate form, is a well-known water-soluble vitamin of the B-complex. It is mainly evolved from natural sources, but as it is available in small quantities in biological material, worldwide efforts have focused on its development and synthesis. The pharmaceutical product is chemically synthesized, and the L-enantiomer is the biologically active form. Folic acid is involved in many metabolic mechanisms leading to the synthesis of DNA and normal erythropoiesis. In the intestinal cell, FA is mostly reduced to

tetrahydrofolate (H_4folate), the active form of this vitamin in a two-step reaction, which is catalyzed by the enzyme folate reductase. Inhibitors of this enzyme, like MTX, act as an antifolate. Figure 2.5 depicts the fate of folic acid in the biological environment.

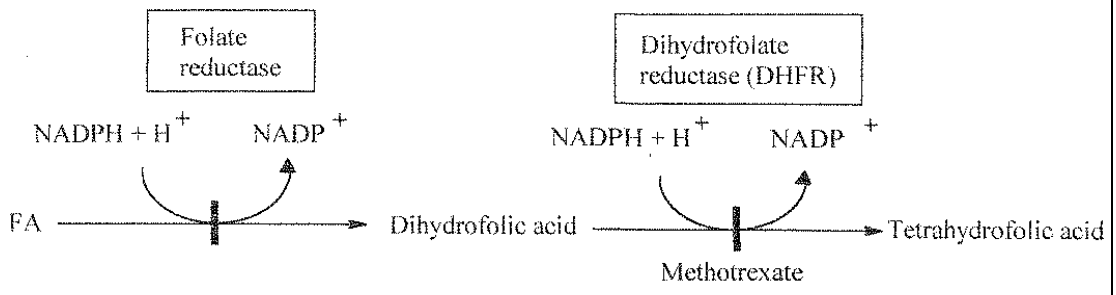


Figure 2.5 [233]: Fate of folic acid in the biological environment

H_4folate functions as a coenzyme, a carrier of various activated one-carbon units in the metabolic reactions. Folic acid and vitamin B_{12} metabolic path ways intersect at the conversion of homocysteine to methionine. FA has also proven to be completely ineffective [71] against any type of cancerous disease. However, it is also known to impact deeply on cancer development. Indeed, folate deficiency appears to play a crucial role in early cervical carcinogenesis by facilitating genetic modification at a fragile chromosomal site [72]. Also, the interest in folic acid has grown with the evidence that modest supplementation could prevent hyperhomocysteinemia, which is an independent risk factor to artherosclerotic cardiovascular disease. Folic acid

plays a crucial role in DNA synthesis, where it enables cells to replicate normally. This is particularly critical during foetal development. During pregnancy, especially the first semester, folic acid intake is important in preventing a wide range of birth defects, most notably neural tube defects. Deficiency in FA leads to anaemia. Indeed, normal physiologic changes in pregnancy affect the haemoglobin concentration, and there is a relative or absolute reduction. The most common anaemias during pregnancy are ion deficiency anaemia and folate deficiency macrocytic anaemia, which occur in women having inadequate diets and not receiving prenatal iron and folate supplements. In an attempt to assess the effects of iron and folate supplementation on haematological and biochemical parameters and on the outcome in pregnancy, Kulier et al. concluded that routine supplementation raises or maintains the iron, ferritin and folate levels in the serum and red cells, and results in substantial reduction of the proportion of women with haemoglobin below 10g/DL in late pregnancy [73]. In light of its physiological key role, FAO (Food and agricultural organization) and WHO recommended folic acid intakes in terms of free folate [74].

Mechanism of intracellular uptake: Folic acid is transported into cells either through a receptor-mediated endocytosis [75] facilated by the folate receptor, or with the help of carrier proteins, such as reduced folate carrier (RFC). The membrane-associated folate receptor is known to be overexpressed on the surface of a variety of human tumor cells, including cancers of the ovary, colon, kidney, uterus, testis, brain, lung, breast, and myelocytic blood cells, while it is highly restricted in most normal tissues [76]. Thus, when folic acid molecules are covalently linked to proteins, the folate-protein conjugate is internalized into cells via the receptor-mediated mechanism [77]. In this process, the ligand (folate)-bound receptor is sequestered in caveolae,

internalized into postcaveolar plasma vesicles, released from the receptor via an intavesicular reduction in pH, and subsequently transported into the cytoplasm. The ligand-free receptor is then recycled to the cell surface by reopening of the caveolae.

2. *Purine analog antimetabolites*: they are antimetabolites that mimic the structure of metabolic purines. Azathioprine is the main immunosuppressive cytotoxic substance. It is widely used in transplantations to control rejection reactions. It is nonenzymatically cleaved to 6-mercaptopurine that acts as a purine analogue and an inhibitor of DNA synthesis. By preventing the clonal expansion of lymphocytes in the induction phase of the immune response, it affects both the cell and the humoral immunity. It also successfully suppresses autoimmunity. Thioguanine is used to treat acute leukemias and remissions in acute granulocytic leukemias. Fludarabine inhibits function of multiple DNA polymerases, DNA primase, and DNA ligase I, and is S phase-specific (since these enzymes are highly active during DNA replication). Pentostatin and cladribine are adenosine analogs that are used primarily to treat hairy cell leukemia. The antipurines can both inhibit nucleotide and nucleic acid synthesis and be incorporated into nucleic acid. They function at multiple sites, and their cytotoxic activity is the result of combined effects on these different sites. Typically, 6-MP is first converted to 6-mercaptopurine ribose phosphate (6-MPRP), the nucleotide-active form that inhibits amidotransferase and dehydrogenase. Amidotransferase is the enzyme involved in the synthesis of the purine bases (adenine and guanine), while dehydrogenase is the key enzyme in guanine nucleotide biosynthesis. Additionally 6-MPRP can be incorporated into DNA and RNA, Thus forming a strand of nucleic acid with aberrant structure. This renders the resulting modified nucleic acids unable to direct proper protein synthesis.

Therapeutically, both drugs are primarily used in the treatment of leukemias. Side effects include myelosuppression, rash, nausea, vomiting, hepatotoxicity, and diarrhea. All these effects are the result of a rapid killing of rapidly dividing cells such as those found in the intestinal tract. The structures of these purines analogs are shown below.

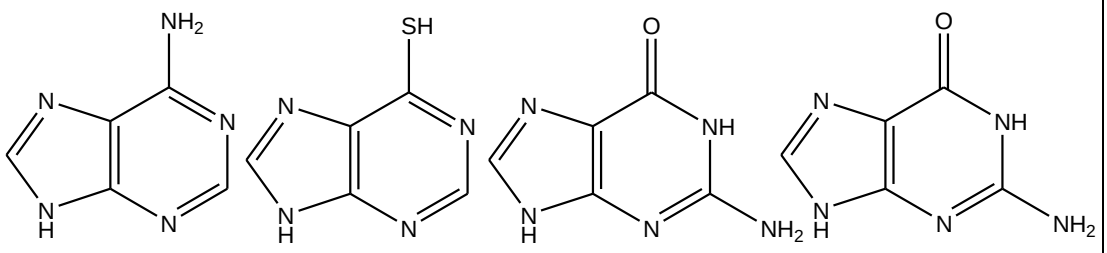


Figure 2.6: Structures of purine analog antimetabolites

3. *Pyrimidine antimetabolites*: Pyrimidine antimetabolites have also been used in the treatment of diseases as cancer, psoriasis, fungal infections and viral infections. The best characterized and most important representatives of this class are 5-fluorouracil (5-FU) and Ara-C.

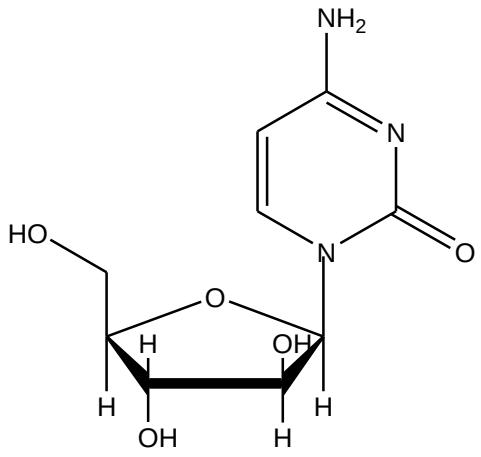
   In contrast to MTX that inhibits indirectly the enzyme thymidylate synthetase through inhibition of dihydrofolate reductase, 5-FU is a direct inhibitor of this key enzyme. In this inhibition process, 5-FU is first converted to the nucleotide 5'-FUMP, which, after several different pathways, can either be incorporated into RNA or converted to the

deoxyribonucleotide (F-dUMP). The inhibition of the enzyme thymidylate synthetase by F-dUMP leads to delection of TTP, a necessary constituent of DNA, resulting in cell death.

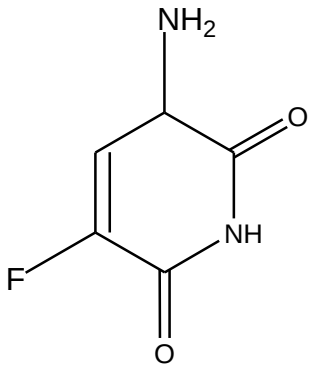
The major biochemical mechanisms of resistance associated with the use of 5-FU include decreased conversion on the nucleotide form and increased break down of the nucleotide. 5-FU is used in the treatment of several common solid tumors. It is partially effective against metastatic carcinomas of breast and the gastrointestinal tract. Combination with leucovorin has been very successful as the leucovorin enhances formation of the ternary complex. The toxicities of this drug are administration mode-dependent, and anorexia and nausea are among the earliest observed symptoms.

In its mechanism of action, cytosine arabinose (Ara-C) is first converted to the monophosphate nucleotide (AraCMP) by deoxycytidine kinase. The monophosphate then reacts with appropriate kinases to form the Ara-C di-and triphosphate nucleotide (AraCTP), which, on accumulation, causes inhibition of the DNA chain elongation when Ara-C is incorporated at the terminal position of a growing DNA chain. Unlike other metabolites, the effects of Ara-C are directed exclusively towards DNA, AND it has little or no effect on RNA synthesis or function. Ara-C is primarily used either alone or in combination with daunorubicin for the treatment of acute myelocytic leukemia due to its potent myelosuppressive action. It has occasionally been used to treat acute lymphocytic leukemia, and in high doses for non-Hodgkin’s lymphoma and chronic myelocytic leukemia. The principal toxicity is bone marrow depression, which is manifested by granulocytopenia and thrombocytopenia. Other toxicities include oral ulceration, nausea, vomiting and diarrhoea, and peripheral neurotoxicity

with high dose therapy. Figure 2.7 depicts the structures of both pyrimidine antimetabolites.



Cytosine arabinose (**Ara-C**)



5-Fluorouracil (**5-FU**)

Figure 2.7: Structures of pyrimidine antimetabolites

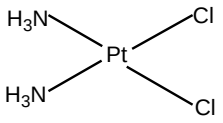
2.3.4.4 Other chemotherapeutic drugs: Anticancer agents are numerous. They vary in their mechanisms of action and frequently also in their pharmacokinetic pathways. Some of them could be mentioned as hormonally acting or hormone-blocking agents, intercalating agents of the platinum type, photodynamically activated drugs, hypoxia-selective cytotoxins and radiation sensitizers. One could cite as well the enzyme-type agents like L-asparaginase and the organoiron drug of the ferrocene-type, then the mitotic-type inhibitors such as vinblastine, vincristine, and vinorebline, which are active specifically during the phase M of the cell cycle.

These drugs have in common a cytotoxic effect, which results from interaction with endocytic nuclear material.

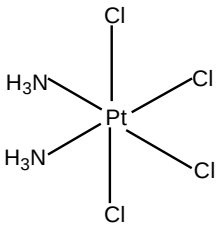
a.Platinum analogs

1. History

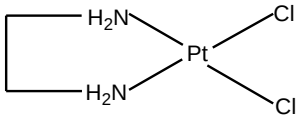
In 1965 Rosenberg [78] noted the similarity of mitotic spindles in dividing cells to the lines of force in a magnetic field. This observation suggested the possible effect of magnetic or electric fields on cell growth, and this led to a series of experiments in which he investigated these effects. He found that cell growth continued but that cell division was inhibited. It was established that this effect was not due to the electric current but rather to the electrolysis products of the platinum electrodes used in the experiments. Further investigation identified the compounds shown in figure 2.8. It was noted that only *cis*-forms were active, while the *trans*-form failed to produce this effect [79].



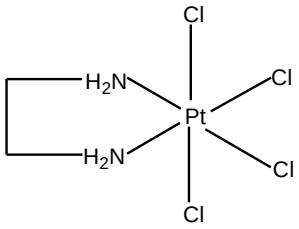
cis-Diaminedichloroplatinum (ll)
(cisplatin)



cis-Diaminetetrachloroplatinum (lV)



Dichloroethylenediamineplatinum (ll)



Tetrachloroethylenediamineplatinum (lV)

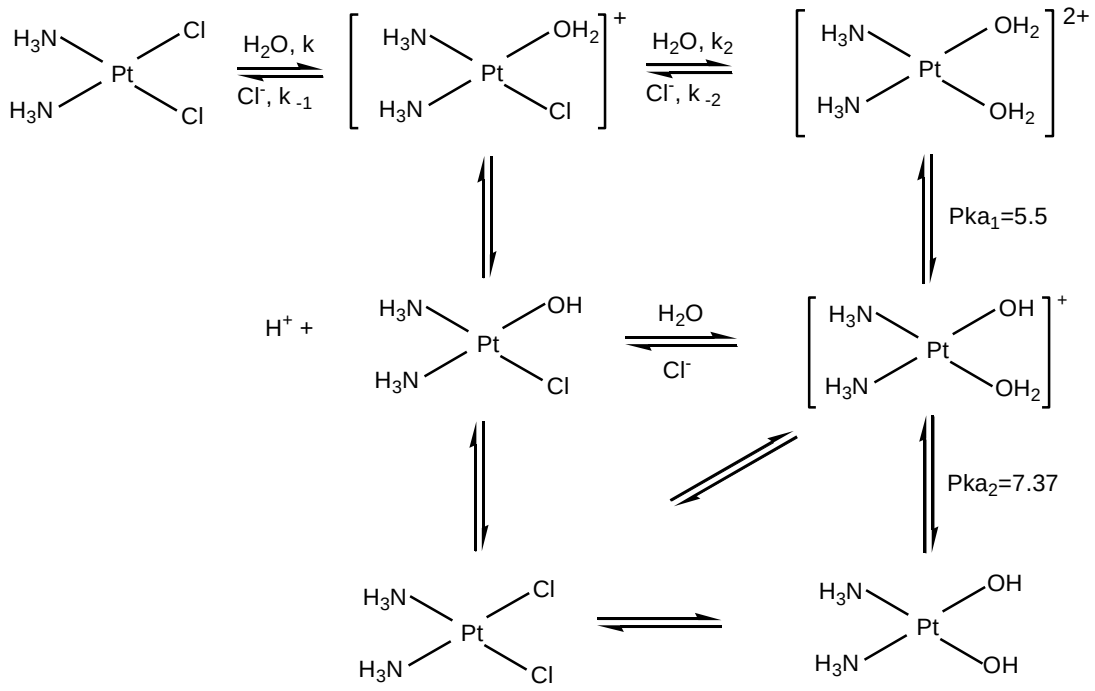
Figure 2.8 [234]: Exemplifying platinum coordination complexes showing anticancer activity

Cis-platinum(ll) diaminedichloride (*cis*-DDP) and related compounds were found to possess profound anticancer activity [80], and shortly afterwards Hill found these compounds to be active against cancer in human patients [81]. Clinical trials on *cisplatin* ensued in 1976 [82], cumulating in FDA approval (USA) in 1979. Today the platinum coordination complexes, especially those

of the *cisplatin* type, represent the most important group of agents in use for cancer treatment.

2. Aquation chemistry

Cisplatin itself is not the active drug species but may be considered as a prodrug. It has been shown that there is an apparent lack of sensitivity of about 2 hours after drug administration [83, 84]. This is the result of the Pt aquation chemistry. Although it is possible that a chloride ligand in cisplatin might in some instances be displaced in a reaction with macromolecule, it is generally agreed that the more usual path is by way of an initial aquation reaction in which a chloride ion is replaced by a water molecule. The aquation reaction is driven by the high concentration of water in the tissues and occurs in a stepwise fashion, as summarized in Scheme 2.1



Scheme 2.1: The aqueous chemistry of *cisplatin*

In blood plasma, the high chloride concentration of 100 mM would keep the *cisplatin* in the unchanged and relatively inactive dichloro form. This form may react to some degree with sulfhydryl groups of plasma proteins. The neutral free dichloro form could enter cells by a passive diffusion. In the cytoplasm, the relatively low chloride concentration of approximately 4 mM would favor the aquation reaction that would yield the highly reactive aqua species, whose ionic charge may retard exit from the cell. The mechanism of antitumor action is not completely understood, but is generally believed that the monoaqua complex (Scheme 2.1) represents the ultimate cytotoxic agent that reacts with the target DNA [85].

3. Reaction with DNA

Cisplatin can bind to all DNA bases, but in intact DNA there appears to be preferential binding to the N7 positions of guanine and adenine [86]. The cytotoxicity of *cisplatin* against cells in culture has been found to be related to the total platinum binding to DNA. The platinum can interact with DNA in three possible modes as shown in figure 2.9: (a) DNA protein crosslinking, b) interstrand crosslinking or c) intrastrand crosslinking.

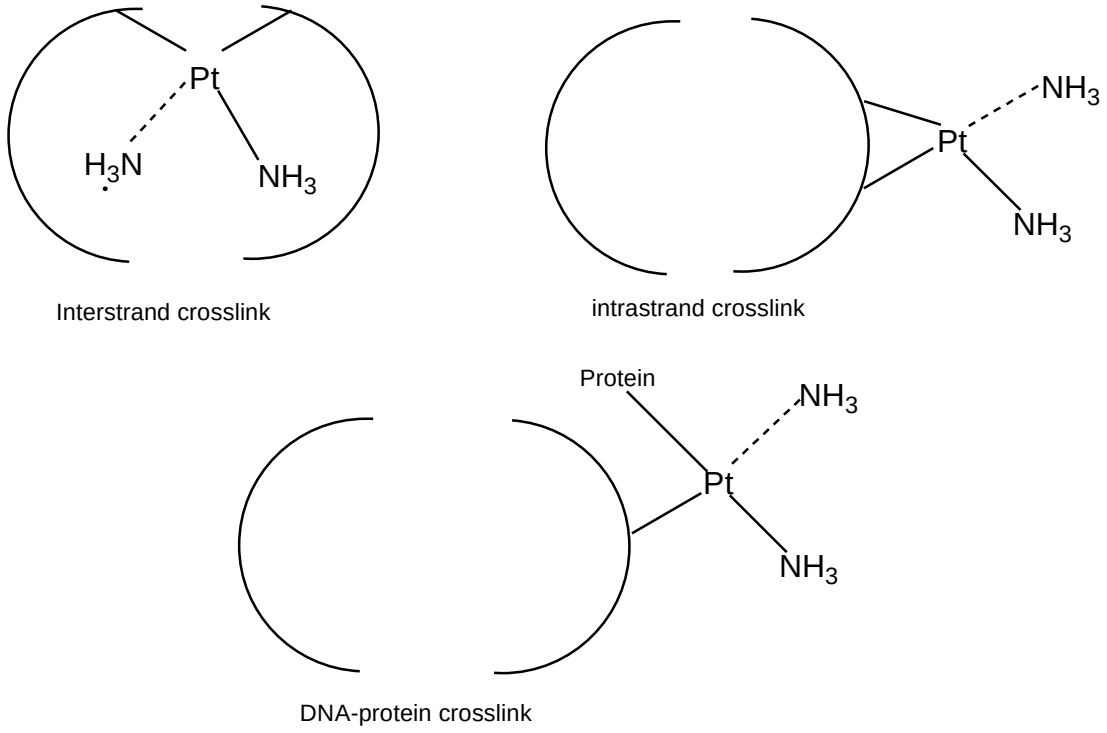


Figure 2.9 [236]: (a) DNA-protein, Inter-(b) and intrastrand (c) crosslinking of DNA with *cisplatin*

The mechanism by which DNA adducts lead to cell death is uncertain. DNA-protein crosslinking has been shown to arise mainly due to the *trans* isomer (which shows no chemotherapeutic activity [87]). The *cis* isomer contributes only 0.15% [88] of the total Pt-DNA-protein adducts formed in mammalian cells. This essentially eliminates the DNA-protein crosslink as the molecular mechanism of action.

Studies indicate that the *cisplatin*-DNA interstrand crosslink, which occurs predominantly between two guanine N7 atoms on opposite strands [89], accounts for only 1% [89] of total Pt-DNA adducts shortly after treatment. Statistically, this is too low to account for the antineoplastic activity of *cisplatin*[90, 91].

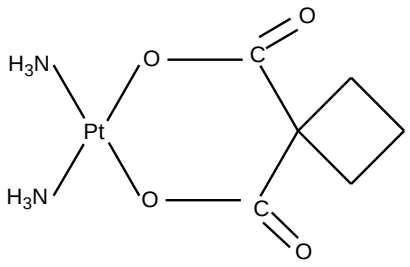
Intrastrand crosslinking of DNA by the platinum is the most abundant type of Pt-DNA bonds and appears to be the most probable cause for inhibition of DNA replication. There are two important types of intrastrand crosslinks, namely crosslink between two adjacent guanine bases separated by a third base. Recent results suggest that the *cisplatin*-DNA adducts can be accommodated in the double helix, causing only a minute localized disruption of the helix that cannot be recognized by any repair enzymes [92]. In contrast, the disruption caused by the inactive *trans* isomer are more profound and easily recognized and repaired [92]. Although DNA damage has been the intracellular effect most related to *cisplatin*-induced cell death, other effects may contribute to side effects and to the toxicity to specific tissues.

4. Second generation platinum drugs

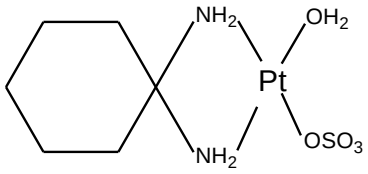
The parent platinum drug is very effective against certain cancer types, however owing to its severe toxicity and limitations in clinical use, a world-

wide research effort was launched to overcome these drawbacks. This resulted in the second generation of platinum drugs. Numerous platinum complexes have now been synthesized and tested for antineoplastic activity. These complexes are characterized by a similar clinical spectrum of activity, but have reduced nephrotoxicity and increased myelotoxicity [93]. The second generation of platinum drugs induced two fundamental differences: 1) in some, but not all, the no-leaving amine ligands became more complex than simple amines, diamines or ammonia, and 2) the leaving groups were changed from chloro, mostly, to some carboxylato ligand. The reduced nephrotoxicity [94] may stem from excretion of the more inert platinum complex in its unreactive form as well as an improvement in the drug's aqueous solubility. This improvement in solubility enhances the efficacy of the drug administration and pharmacokinetics of active compound.

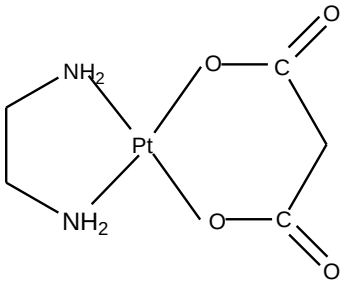
While some individual complexes have shown improved activity toward some tumor lines, only a few (notably *carboplatin*) are significantly more active than the original complex [95]. Their lack of cross-resistance with *cisplatin* resistant tumor lines, though, is of great importance, and investigation of new *cisplatin* analogues is proceeding on a continuing basis.



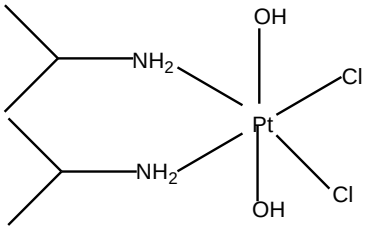
Carboplatin



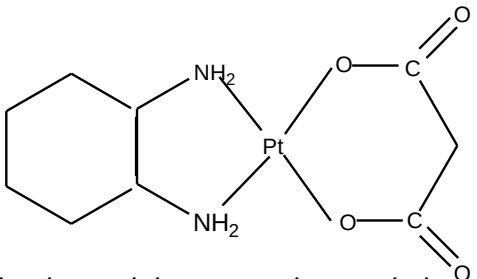
Spiroplatin



Malonatoplatin



Iproplatin



Diaminocyclohexanemalonatoplatinum

Figure 2.10 [234]: Second generation platinum drugs

In the common belief that the two tightly bound amine ligands, as represented by the *cisplatin* example, are required for carcinostatic activity, scant attention has been paid in cancer chemotherapy research to coordination compounds comprising a monoamineplatinum structural unit. The monoamine complexes reported to posses antiproliferative properties feature anion structures of the type $[PtCl_3(NH_2\text{-}R)]^-$ R=H, alkyl) [96, 97]. As salt-like compounds such complexes cannot efficiently penetrate cell membranes by the passive diffusion mechanism generally utilized by non-polar neutral compounds. Transmembrane transport is a necessary requirement for antiproliferative effectiveness of most known anticancer drugs, which act upon the nuclear DNA of the affected cell and, for this reason, must cross into intracellular space. The salt-like nature of the typical monoamine platinum complexes may therefore have been considered a convincing argument against their inclusion in major *in vitro* and in *vivo* screening projects.

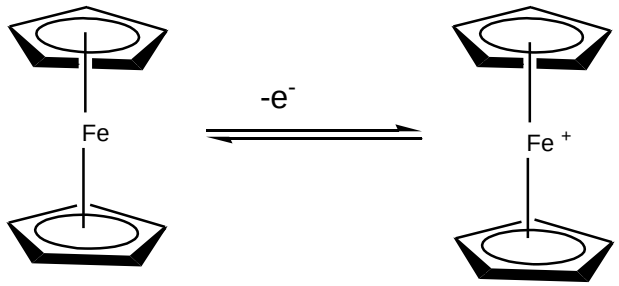
b. The ferrocene drug system

There has been considerable interest and increased research activity in developing other transition metal compounds as anticancer drugs with less toxic effects than the platinum-based drugs because of their serious side effects, including renal impairment, neurotoxicity and troubles in hearing. From these transitional metal compounds, metallocenes and metallocene dihalides proved to be particularly active against several kinds of tumors [98], and, though their activity seems to follow a different mechanistic path from those of *cisplatin*, these two drug types have proved to target intacellular DNA.

Ferrocene was the first metallocene to be discovered by Wilkinson [99] who proposed its particular structure in the early 1950s. Since then, the study of

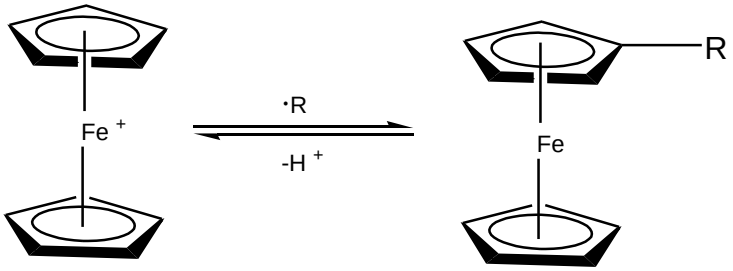
ferrocenes has grown fast due to their highly promising antiproliferative activity against various murine and human cancer lines, and also for their unusual stability due to the sandwich structure conferred by the binding of the iron center by two aromatic cyclopentadienyl rings.

1. Reactions in the biological environment: The ferrocene complex displays outstanding oxidation-reduction behavior. Through a one-electron transfer reversible reaction (Scheme 2.4), the complex converts to ferrocenium ion, a free radical of high stability. The mechanism has some implications in the biological realm as electron transfer and free-radical reactions play a vital role in biological processes, which gives a major interest to the ferricenium-ferrocene system in both biochemical and biomedical research. Many investigations dealing with the biological behavior and functioning of ferrocene compounds have been reported. It has been noticed that under enzymatic control, ferroce is oxidized by hydrogen peroxide while ferricenium ion is reduced by NADH and metalloproteins. Moreover, ferricenium ion reacts with the biologically important superoxide anion radical, leading to a regeneration of ferrocene and dioxygen (Schem 2.6). A reverse electron transfer reaction, resulting in oxidation of the ferrocene complex to its ferricenium salt, occurs with ferrocenylcarboxylates in their interaction with the highly reactive hydroxyl radical, transforming the latter to a harmless hydroxyl anion (Scheme 2.7). Scheme 2.4-2.7 below gathers the different reactions of the ferrocene complex in the biological environment.

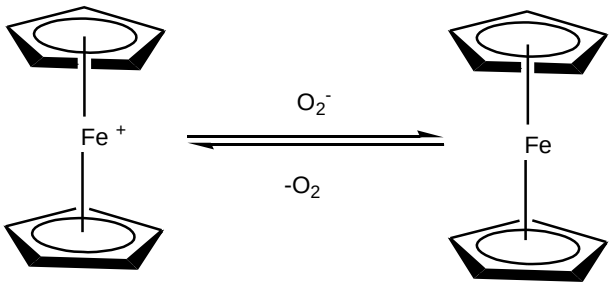


1 **1+**

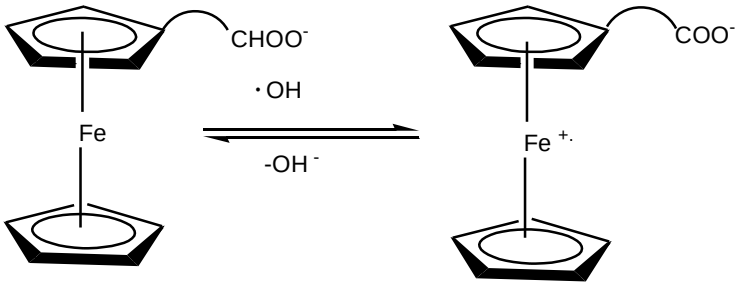
Scheme 2.4



Scheme 2.5



Scheme 2.6



Scheme 2.7

Free radical chemistry plays a vital role in cancer generation and in various phases of growth and control of neoplasia. Effective inhibition and detoxification processes in the cancerous organism may well be involved in the aforementioned superoxide and free radical-scavenging reactions. A deactivating recombination of ferrocene in its oxidized state with free-radical form of ribonucleotide reductase, an important enzymatic link in DNA synthesis, may represent another potential contribution to the inhibition of the cell's proliferation process. The preparation of numerous ferricenium compounds and their evaluation for antiproliferative activity against ascetic murine tumors and several human tumor clonogenic cultures are reported in review articles [100, 101]. For those salts tested that were insoluble like the ferrocene parent itself, no activity was revealed in the screens. In contrast, ferricenium salts comprising the picrate, trichloroferrate (lll), μ-oxo-bis(trichloroferrate (lll)), trichoroacetate, or chloride counter ions, were found to be active, and they all showed good water solubility with high saturation limits. Under optimal conditions, in the Ehrlich ascites screening test, best

results (cure rates of 100%) were displayed by the picrate and the trichloroacetate salts. A moderate activity was even detected with ferrocenylacetic cid, a water-soluble derivative of unoxidized ferrocene. Ferricenium salts, dissolved in aqueous medium at physiological pH (7.4), are unstable, and this would indicate short half lives in *vivo* of such compounds in the central circulation system for effective survival *en route* to the target tissue. However, the results drawn from ferrocenylacetic acid behavior suggest that the administration of a ferrocene compound *in vivo* in the ferricenium state may not be necessary. The oxidation-reduction equilibrium distribution of ferricenium and ferrocene species in any body compartment is likely to be solely under the control of the biological environment [102], *i.e*. pH and enzymatic activity in that compartment, and not of the oxidation state in which the ferrocene compound was initially administered. Thus, the biological effects encountered *in vitro* should also be shown *in vivo* irrespective of the compounds' initial oxidation state, the only prerequisite being water solubility of the compound, which should be highly sufficient for rapid dissolution and dissipation in the aqueous fluid system. Unlike the platinum-based compounds, the cytotoxic activity of ferrocene compounds, namely ferricenium salts, is probably not based on their direct binding to DNA, but on their ability to regenerate oxygen-active species which induce oxidative DNA damage.

Interest in the antitumor activity of ferrocene derivatives, usually associated with ferricenium compounds, recently increased when it was observed that ferrocene itself has a marked antitumor effect in experiments with tumor-bearing mice. Indeed, Kovjazin and *al.* [103], investigating the antitumor properties of ferrocene, conducted *in vivo* experiments in mice bearing established lung metastases of B-16 melanoma. The results unexpectedly

showed that ferrocene possessed outstanding antitumor effects. This was attributed to its immune stimulatory potential which was induced by lymphocyte activation. The Figure 2.11 below depicts the proposed mechanism of ferrocene action [103].

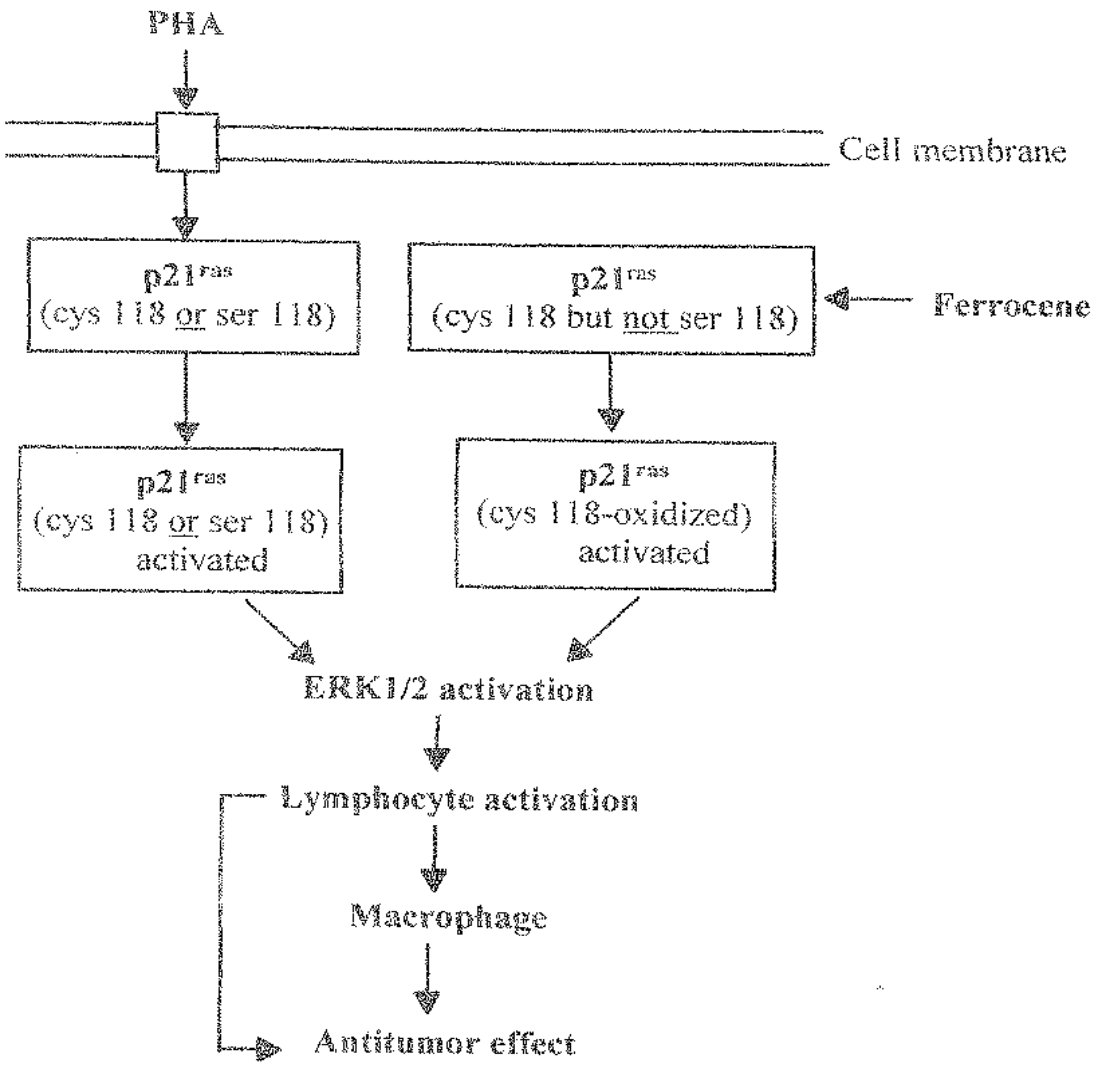


Figure: 2.11 [103] : behavior of ferrocene in the physiologic environment

2. 4. Combination therapy

As a strategy to produce additive and synergetic effects without enhancing overall drug toxicity, combination therapy, of fundamental importance and involving multidrug administration, is being used with the specific goal to broaden the activity spectrum through the use of two or more agents functioning by different mechanisms and exerting different toxicity effects. Methotrexate is commonly used in combination with 5-fluorouracil and cyclophosphamide in the adjuvant treatment of breast cancer, while co-administration of doxorubicin, bleomycin, and vinscristine has been used as therapy for AIDS-related Kaposi's sarcoma and other germ cell neoplasms[104].

2.5 Immunotherapy

Immunotherapy is the fifth modality, much still in its infancy and aiming at stimulating the host's own immune system. It is sometimes used by itself, but often as an adjuvant (along with or after another type of therapy) to add to the cancer effects of the main therapy. Immunotherapy is described as a biological response modifier or a biological therapy. Although the thought of using one's own immune system to fight cancer is appealing, immunotherapy currently has a small role in the treatment of common types of cancer. In general, immunotherapy is most likely to be effective when treating small cancers, and will probably be less effective for more advanced stages of disease. In the presence, or after removal of cancer cells, this immunotherapy either stimulates the host's own immune system, and this is known as active specific immunotherapy [105] (*e.g.* cancer [106] and dendritic vaccines) or uses the immune system components such as antibodies created outside, and is known as passive immunotherapy (e.g. naked [107] and conjugated monoclonal antibody therapy). The most promising tumor cell vaccines are currently

under study in clinical trials against several cancer malignancies including melanoma [108], kidney, ovarian, breast [109], colorectal and lung cancer, as well as leukemia.

2.6 Antiangiogenesis therapy

Antiangiogenesis therapy is the sixth modality, which, in cancer treatment, refers to the use of drugs or other substances to stop tumors from developing new vessels, leading to tumor death [110]. Most antiangiogenic drugs work by preventing the first step in the making of new blood vessels. They present two main advantages over chemotherapeutic drugs: Firstly, they are non-toxic to normal cells, and therefore can be applied over a long period without interruption, thus leading to higher effectiveness, and secondly, since they do not lead to cell resistance, they could be active for much longer periods of time.

2.7 Photodynamic therapy

Also called photoradiation therapy, phototherapy or photochemotherapy, photodynamic therapy (PDT) is a treatment that combines a light source and a photosensitizing agent (a light-activated drug) to destroy cancer cells. The mechanism of action is described as follows: On exposure to light, the photosensitizer agent reacts with oxygen to from chemicals that destroy cancerous cells. The use of PDT is limited by the depth of penetration of light in the tissue. Therefore, PDT is mainly used to treat areas on or just under the skin, or in the lining of internal organs.

2.8 Gene therapy

Gene therapy consists of inserting a specific gene into cells to restore a missing function, or to give cells s new function, as cancer is the result of a cell's genetic mutations. It is one of the modalities for cancer treatment still in its infancy and is used in a variety of ways in clinical trials. These compromise the addition of functioning genes to cells with abnormal or missing genes, block genes from making cancer cells resistant to chemotherapy or add genes to tumor cells for easy detection and elimination by the host's immune system. Adding genes to immune system cells, and stopping genes from contributing to angiogenesis (blood vessels formation), or adding angiogenesis inhibitor genes to cancer cells also helps.

Gene therapy meets an obstacle in finding the appropriate vectors for the gene delivery. Many vectors are under investigation. Of these, viruses are known to be extremely efficient in the delivery of foreign genes into cells and tissues [111], as well as the non-viral vectors such as cationic liposomes and cationic polymers [112, 113].

CHAPTER 3
MACROMOLECULAR CARRIERS AND POLYMERIC ANTICANCER DRUG CONJUGATES

3.1 Background and literature review

The unsuccessful research for effective and non-toxic new drugs and, on the other hand, new knowledge of biological events around and inside the tissue, gave rise to the use of therapeutic systems to obtain a controlled drug release as a new and growing approach in cancer studies[114,115]. In this new method, the active agent, after administration, is distributed in the entire body and reaches not only the target cells or tissues but also interacts with healthy cells. This leads to peripheral toxicities and low therapeutic efficiency, and prompts the search for novel strategies. Many drug delivery systems have been explored, including drug-antibody conjugates (immunoconjugates), conjugates obtained by linking drugs to natural or synthetic polymers (macromolecular prodrugs)[116-118], vesicular and particulate systems (lyposomes [119], nanoparticles [120], microparticles for regional therapy [121]) and polymeric implants [122]. Unfortunately some of these systems have often not lived up to early expectations. In the case of immunoconjugates, the inefficiency was mainly due to the limited access of these relatively big molecules into tumor mass, the heterogeneity of the tumor cell and the different humoral response among patients [123]. Therefore, a strong interest was developed in the potentially promising systems, and one of these is based on water-soluble carriers covalently linked to the drug through a biodegradable spacer.

3.2 Polymers as drug carriers.

Many efforts have been focused on finding more potent and more selective compounds for treating cancer. Despite this, advances have been disappointing, and many researchers would contend that there is already a plethora of chemicals on the market that have the potential to be antitumor agents. Their limitation can be viewed as the inability to deliver them properly. The objective of optimized drug therapy is to maximize the therapeutic effect of the drug while minimizing any side effect and adverse effects. In order to achieve this, the drug has to be made available at the site of action and its access to non-target tissue limited.

The need for targetable drug carriers has long been realized. Paul Ehrlich coined the well known phrase “the magic bullet” when describing drugs which may be selectively directed to their specific site of action [124]. Conjugating antitumor drugs to polymers (so called *Polymer Therapeutics* [125]) is providing a novel and effective class of anticancer agents.

There are principally three approaches to the design of pharmacological polymers. One approach is to prepare a polymerizable monomeric drug and then homopolymerize or copolymerize it with other monomers. Surgically implantable polymer matrices loaded with chemotherapeutic agents provide another approach to drug delivery [126,127]. The matrix is loaded with the desired agent and then implanted within the tumor activity. The matrix then releases its drug load over a period determined by the characteristics of the polymer. During the last 20 years research on drug carriers has been focused on the encapsulation of biologically active agents in solid release devices, liposomes and nanoparticles. In a third approach an active drug species may be complexed or covalently bound to a preformed polymer. It was Ringsdorf [128] in the mid-seventies who first proposed using water-soluble polymers as drug carriers. One of the most promising approaches adopted in present

biological research toward overcoming the problems associated with current chemotherapy involves the reversible coupling of toxic, water-soluble or excessively labile drugs to suitably designed macromolecular carrier molecules.

3.2.1 Requirements for polymeric drug carrier

These requirements include hydrosolubility, biodegradability, biocompatibility and chemical composition.

3.2.1.1. Water-solubility

Good solubility in aqueous media is a necessary prerequisite for the polymer-drug conjugate. The presence of intrachain or extrachain hydrophilic entities capable of undergoing effective hydration is therefore required. The amide groups, which are very important linkages in a carrier polymer, as well as the presence of hydroxyl and amine terminals are of excellent utility for imparting solubility in aqueous media. The possibility of including charged species also leads to improved solubility of the polymer. The use of poly-(ethylene glycol) (PEG) has also found increasing use in the field of polymer therapeutics [129-131]. Poly(ethylene glycol) is a polyether-diol with a general structure HO(-CH_2CH_2O-)$_n$H. They are neutral, non-toxic, hydrophilic polymers with each ethylene oxide segment able to bind approximately three molecules of water.[132] The addition of PEGs either as part of the polymer backbone or as side groups imparts excellent solubility to the polymer and offers other exceptional advantages to be discussed later.

3.2.1.2 Degradability

Degradation of polymers within an organism is a vital aspect when one assesses the functional capabilities of potential carriers. Synthetic polymers

with a carbon-carbon backbone are generally not biodegradable. If they do degrade at all, degradation is so slow; it may be considered negligible [133]. This leads to problems during elimination in the “spent” state, especially if their molecular masses exceed the kidney threshold (30000-50000). If a carrier has been designed so as to contain biofissionable links, it will undergo degradation either to a limited extent giving rise to lower-molecular-mass products or, alternatively, generating monomeric constituents upon complete destruction of the carrier. Such links perform two major functions, determining the rate of the drug release from the polymers as well as controlling the long-term fate of the polymeric carrier.

The development of targetable lysosomotropic drug carrier systems is critically dependent on the various linkages, as this determines the timing of the release rate of the drug from the carriers. If the bonds are cleaved before the conjugate arrives at its destination, the drug will be released prematurely making the carrier redundant. If on the other hand the linkages are too stable, the drug will not be released, and consequently, there will be no pharmacological benefit. Linkages therefore have to be chosen so as to be stable during the transit period, but susceptible to some form of hydrolysis at the target cell so as to liberate the pharmacologically active agent.

The ultimate destination of a polymeric anticancer drug carrier is generally the lysosomal compartment of the cell. These lysosomal compartments contain some seventy hydrolytic enzymes [134] capable of digesting all naturally occurring macromolecules that they would normally expect to encounter. This suggests the need for enzymatically cleavable links to be present in a macromolecular carrier system, i. e. the synthetic polymer should have all the beneficial structural linkages such as peptide or saccharide bonds.

In order for an enzyme to be active, it must be able to undergo a number of interactions with the substrate leading to the formation of an enzyme-substrate complex. Therefore the degradable sequence of an artificial substrate must display the characteristics which in some way resemble those of the physiologically active substrate [135]. To increase the biodegradability of a polymeric carbon backbone, it is thus necessary to equip it with peptide, saccharide or nucleotide sequences. This can be achieved by linking relatively short synthetic polymer chains with each other using bonds susceptible to enzymatic attack, or by choosing or designing a polymeric backbone that already contains the required linkage as part of it inherent composition, e.g. polyamides. Correct design of the polymer will then allow the rate of scission of these bonds and hence the control of rate of drug release.

3.2.1.3 Immunogenicity

Non-immunogenicity of potential macromolecular drug carriers is one of the key requirements for their successful use in the biological environment. As optimal compatibility of the polymer with its working environment is a crucial biological requirement, the polymer must not be provocative to its surrounding in any way. Premature destruction of the carrier by attack from the host defense mechanisms would render the drug delivery system useless.

As mentioned earlier, PEG's have found increasing use in the field of biomedical chemistry as a mean to modify anticancer drugs in order to improve their solubility and decrease the immunogenic effects of the free drug [129,130]. Although the polyether is not degraded by mammalian enzymes [136], once the PEG is in aqueous solution, it is heavily hydrated, highly mobile and excludes other polymers including proteins and nucleic acids [131]. As a

consequence PEG's are nontoxic and non immunogenetic, and molecules coupled with PEG become essentially nontoxic and non immunogenic. It has been repeatedly demonstrated that covalent attachment of the multiple strands of the PEG to proteins produces conjugates with dramatically reduced immunogenicity and antigenicity [136]. PEG also has the lowest levels of protein or cellular adsorption of any known polymer [137].

3.2.1.4 Drug anchoring

Biologically active drug species can be bound to the polymer either by covalent or ionic bonds, or by dipole (Van der Waals) forces. Covalent forces are regarded as the important bonds of synthetic soluble polymers. In cases where binding occurs other than through covalent fixation, slight changes in the shape of a polymer coil may result when the polymer passes from one body compartment to another. This can lead to dramatic changes in the biological activity. The drug can either be part of the polymer backbone, or bound to a side chain.

When bound to the polymer backbone, most drugs show a reduced or zero biological activity [138] and need to be liberated by complete breakdown of the polymer. If the drug is bound to the polymer via a spacer, which is significantly remote from the main chain, then the active agent can be more readily liberated through increased steric accessibility by enzymes in the target compartment. This, however, is only exploitable if the free drug can be liberated from the spacer or, if the spacer is irreversibly bound, it must not interfere with the biological activity of the attached agent. Another advantage of using side chains is the ability to "tailor-making" them according to the characteristics required. In this manner specific solubility can be achieved by including functionalities on the spacer. Linkages may be incorporated

allowing various hydrolysis rates, thereby controlling the rate of drug delivery at the target site and regulating the lifespan of the carrier. To facilitate the release of the drug, the spacer must be attached to both the drug and the macromolecular backbone by covalent bonds of limited stability in a biological environment [139].

3.2.2 Natural polymers as drug carriers

On account of easy availability and biocompatibility, natural polymers have an advantage. Although their preparation may be restricted by the need for several purification steps, they induce a high immunogenicity. Many of them have been identified and have been or are being used as possible drugs or possess an intrinsic anticancer activity. Naturally occurring polymers such as albumin, BSA, Chitins, and dextrans have been successfully conjugated to doxorubicin [140,141] and mitomicin [142], respectively. In general, these polymers are biodegradable owing to their natural origin and they will be excreted from the bloodstream by natural catabolic mechanisms. However, their use as drug carriers is often limited. Indeed, the substitution of natural macromolecules with covalently linked low-molecular-mass drug molecules generally results in the hindering of the host's ability to enzymatically degrade the polymer carrier effectively [143]. The loss of biodergradation availability into easily eliminated fragments leads to inhibition of elimination from the body.

Some synthetic macromolecules, including polylysine (PLL Mw = $5x10^6$) [144], diethylaminoethyl-dextran (DEAE-dextran (Mw = $2x10^6$), and poly (Arg-Gly-Asp) (Mw = 10 000) [145], have shown intrinsic anticancer activity. They are termed a *polymer drug*, and their mechanism of action is either the direct action upon the tumor cell, or the simulation of the host's immune system [146]. Some cytokines, topoisomerase inhibitors, monoclonal antibodies, thymic

hormones, cell growth inhibitors and enzymes [147] are among the natural and biological macromolecules possessing anticancer activity. The main problem associated with the administration of such macromolecules is their short intravascular half-life, immunogenicity, and sometimes poor solubility. Their modification with synthetic macromolecules dramatically increases their therapeutic effectiveness.

3.2.3 Synthetic polymers as drug carriers

Synthetic polymers are advantageously used as drug carriers since they are clearly more susceptible to modifications than natural macromolecules. The preparation of the ideal polymeric carrier is a complicated task requiring optimization of a number of properties of the polymer that are important from a biological standpoint. These properties include molecular mass, biodegradability, toxicity and the possibility for drug attachment/release as well as for targeting.

The molecular mass is an important factor in the determination of the biological activity of the overall activity of a polymer [128]. For example, the rate of elimination of the polymer from the blood stream, the deposition in organs as well as the rate of uptake into cells by pinocytosis, are influenced by both molecular mass and mass distribution. Since synthetic polymers are polydisperse, not only the average molecular mass, but the distribution is important. Hespe and coworkers [148] demonstrated that two polymers of similar molecular mass but different distributions exhibited different periods of detection in organs after intravenous administration, although their rates of excretion were similar. Analogous results were also observed with vinylic polymers, where it was shown that higher-molecular mass fractions are more toxic than their low-molecular-mass counterparts [149].

Tacticity has also shown to have an effect on the biological properties of a carrier. Muck and coworkers [150, 151] showed that isotactic polymers exhibited considerably higher antiviral activity than the atactic polymers of the same type. These physical properties are of extreme importance to synthetic polymers that do not contain bonds which are degradable in the organism.

Ringsdorf's [152] original concept of a linear polymer composed of subunits bearing solubilizing groups capable of drug binding, and still other subunits comprising a homing device, provides the most relevant guidelines to the design and synthesis of a potential water-soluble carrier molecule.

3.2.3.1 Amino acid polymers and copolymers

In recent years, intensive studies of poly (amino acids) have been performed; motivated by the definite advantages these polymers have over other macromolecules generally used as drug carriers. Indeed, because of the proteinaceous structure imparted to them by the protein-like amide linkage, one assumes their easy cleavage into amino acids (in the body), which, being non-toxic, would be used as cellular nutrients. The first poly(amino acid) whose plasma expander property was investigated was poly(glutamic acid). However, the results were not encouraging, the compound proved to be inefficient [153] and toxic [154]. The major cause of this was that the physiological pH and the net charge exhibited were too high, and this was confirmed by the lower toxicity observed with poly(glutamic acid-co-lysine) [155]. Therefore, the interest in the preparation of uncharged poly(amino acids) as plasma expander rose. This was achieved by blocking the carboxylic side group through amine linkage by an amino alcohol whose hydroxyl groups render the polymer water-soluble and at the same time eliminate the electrostatic

interaction with cells and other components of the organism. The resulting poly([N^5-(2-hydroxyethyl)-L-glutamine] (PHEG) proved to be more efficient, non-toxic, and non-immunogenic on animal testing [156] but from an economic viewpoint its large-scale production was found to be costly [157]. In this respect, the synthesis of analogous derivatives of poly(aspartic acid) lent itself as an alternative. These derivatives are widely used, and exhibit special properties, such as biocompatibility, biodegradability, and non-toxicity. The potential of plasma expander properties of poly(aspartic acids) was revealed in 1974 [158] by Antony and co-workers on their study of poly-α,β-(2-hydroxyethyl)-DL-aspartamide (PHEA), and more recently with α,β-polyasparthydrazide (PAHy) [159]. Many model polymeric drugs have been reported, such as PAHy-Ofloxacin [159], and PHEA-L-dopa [160]. The pulmonary adsorption kinetics of a single molecular-mass distribution of fluorophore-labeled-PHEA, a hydrophilic and biocompatible synthetic polypeptide studied in isolated perfused rat lung [161], was also reported. Poly(aspartic acid)-derived *cisplatin* conjugates were prepared and in *vitro* cytotoxicity was studied [162]. More recently [163], the synthesis and biopharmaceutical characterization of four new PHEA-based polymers, which bear PEG as pendant groups, confirmed the retention of the suitable properties previously observed with PHEA, namely, biodegradability, solubility, multifunctionality, and biocompatibility.

3.2.3.2 Poly(amidoamines)

Poly(amidoamines) (PAAs) belong to the family of polymers characterized by the regular arrangement of amido groups and secondary (or tertiary) amines along the macromolecular chain. Pioneered by Ferruti [164], they are synthesized by polyaddition of primary monoamines or bis(secondary amines) to bisacrylamides. Since they bind tightly to heparin-adsorbing surface for medical devices (heparin renders the device non-thrombogenic) [165]. PAAs

were later proposed as particularly promising drug [165] and DNA [166] delivery carriers owing to their water-solubility [165], biodegradability [167], conformation - changing ability with pH [168], and potentially low toxicity compared with other synthetic polymers, e. g. poly-L-lysine [167]. Furthermore, PAAs are also known to possess structure-toxicity relationships [167]. They have both aminic and amidic sites in their backbones, and the polymers prepared, in general, are selected to incorporate several different variables to reinforce the aforementioned physicochemical properties. Using the *pKa* values of different PAAs, Ferruti demonstrated the basicity of the aminic nitrogen atoms of each repeating unit to be independent of the degree of protonation of the whole molecule. Thus, the main influence of polymer basicity is governed by the aminic moiety [165]; therefore several different monoamines were incorporated. The purpose of monoamine incorporation was to ensure firstly higher basicity, as the mechanism of a macromolecule cell entry, endocytosis, exposes it to pH changes, from pH 7.4 extracellular to pH 5.5-6.5 within the endosomal-lysosomal system [169], and, secondly, increased water-solubility. More recent review articles from Ferruti's laboratory cover the synthesis of primary amine-functionalized PAAs [170], the biomedical applications [171], and the correlation between physicochemical and biological properties [172] of the PAAs.

3.2.4 Advantages of drug carriers and their mechanism of action

When an ideal polymer-drug conjugate is administered intravenously or intraperitoneally, the performance of the free drug is enhanced firstly by carrying the drug, even if inherently hydrophobic and insoluble in water, immediately into the aqueous phase of the central circulatory system or intraperitoneal cavity for rapid dissipation. While in transit, the drug will also benefit from temporary protection against enzyme attack and serum protein

binding, thereby prolonging the lifetime of the conjugate in the central circulation.

While most antitumor agents are low-molecular-mass compounds that more or less rapidly penetrate all tissues by passing across the cell membrane, the polymer conjugate can only gain entry to the cell by endocytosis. There are two different types of endocytotic uptake of material by cells, phagocytosis and pinocytosis. Phagocytosis describes the capture of particulate material (usually >1 μm in diameter) by macrophages. In contrast to pinocytosis, phagocytosis requires attachment of the macromolecules to the cell membrane to trigger the engulfment.

Pinocytosis describes the invagination of the cell membrane to form smaller membrane-bound vacuoles or vesicles which, during their formation, capture extracellular fluid, all solutes dissolved therein and any material adherent to the infolding surface. Pinocytosis is a phenomenon common to most, if not all cell types, and it appears to be an ongoing event with no obvious rate control mechanism [173].

Figure 3.1 shows the events which occur during and after uptake of macromolecules by pinocytosis (The general fate of phagocytosis is the same). Subsequent to the membrane invagination, the newly formed vesicle pinches off and migrates into the cell cytoplasm. Pinocytic vessicles may fuse with each other giving rise to larger vessicles or fuse with primary or secondary lysosomes, where the hydrolysis of the captured material proceeds. The low-molecular-mass products liberated pass through the lysosomal membrane into the cytoplasm for reutilization or removal from the cell. Non-biodegradable macromolecules accumulate within lysosomes and are released slowly by exocytosis or as a consequence of cell death. The

pinocytic mode of entry may assume unique importance where drug resistance has developed by the target cells, counteracting the detrimental drug efflux mechanism largely responsible for the resistance built-up.

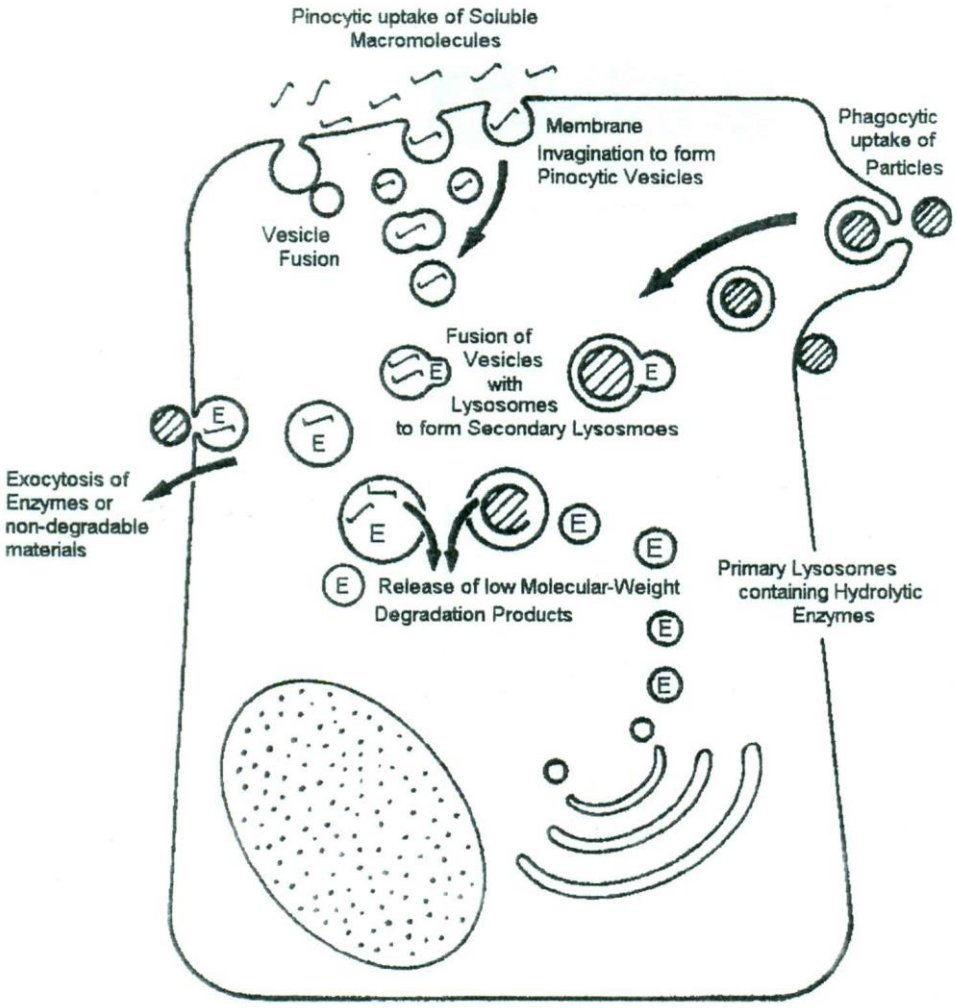


Figure 3.1[234]: Uptake of macromolecules by pinocytosis

Polymer conjugates, in common with macromolecules in general, also tend to accumulate in solid tumors. This phenomenon was discovered by Maeda [174] and is known as the enhanced permeability and retention (EPR) effect

(Figure 3.2). In order for a growing tumor to survive, it has to establish its blood supply. The tumor vasculature is uniquely different from that of normal tissue in that it is more permeable to circulating macromolecules.

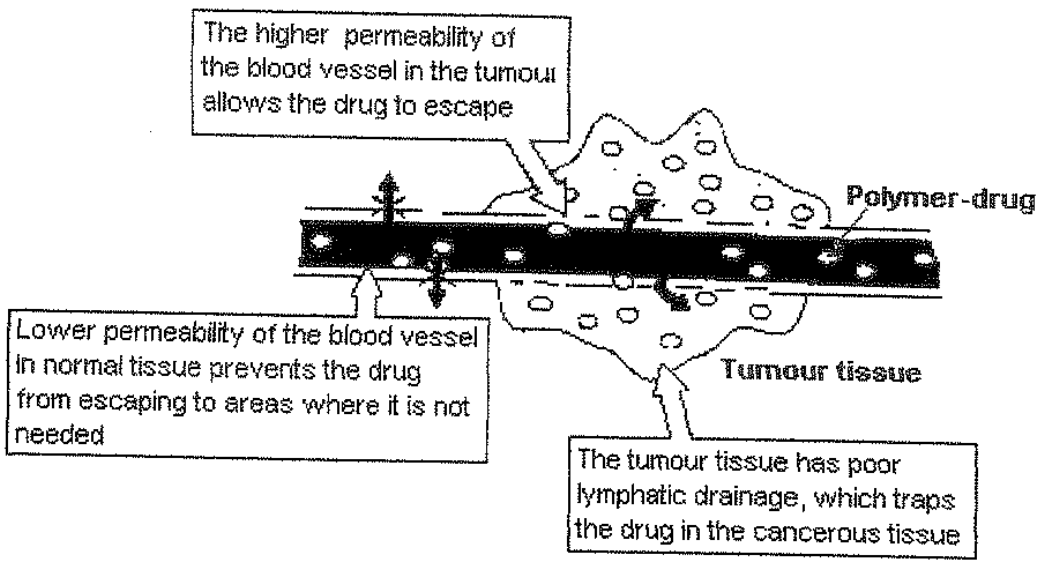


Figure 3.2 [234]**:** The EPR effect

In addition, tumor tissue has no lymphatic drainage, so the macromolecules leaving the blood vessels are not returned to the circulation very quickly. Together these factors allow conjugate concentrations in tumor tissue to reach levels several orders of magnitude higher than would normally be seen after intravenous administration of the free drug.

3.2.5 Polymer-drug conjugation

The drug systems submitted to investigation in the framework of this project have been conjugated to various polymeric carriers and the conjugation which is achieved by formation of their biocleavable ester or amide bond is extensively reported.

3.2.5.1 Polymer-Folic acid anchoring

The potential use of cellular nutrients as mediators of macromolecular and colloidal particle uptake is appealing, since receptor-mediated endocytosis is a cellular process designed for transporting critical molecules across the plasma membrane into the cytoplasm [175]. Rapidly dividing cells express high affinities for folic acid because folate is an essential factor in purine, nucleotide and DNA synthesis. Hence, the possibility exists of utilizing this path way for promoting folate-linked molecules or colloidal particles to enter gastrointestinal (GI) epithelia. And, by virtue of its ability to be taken up by folate receptor overexpressed on certain tumor cells, folic acid has been widely investigated as a targeting molecule for anticancer drug delivery. Proper synthesis procedures have been pointed out to link to drug carriers to produce targeting drug delivery system. The folic acid molecule possesses two carboxyl groups, termed α- and γ-, which can act as handles for covalent attachment. However, according to literature, there is a stronger affinity of folate towards its receptor when linked via the γ-carboxyl group, whereas its α-carboxyl derivatives are not readily recognized [176]. Folate-induced receptor-mediated endocytosis has been extensively exploited to facilitate entry of anchored drugs [177], antibodies [178], imaging agents [179], liposomes [180] or macromolecules [181], and proteins into cells. Folate-conjugated proteins present the advantages of conceivably contacting and binding to all cells in a

culture medium simultaneously. This avoids membrane damage or alteration as the macromolecular uptake occurs through a natural vitamin endocytosis pathway. Unlike hormone- or virus-mediated endocytosis, folate uptake occurs in all dividing cells, at reasonably rats, and folate is deposited into cytosolic rather than lysosomal compartments.

3.2.5.2 Polymer-MTX conjugation

In an attempt to improve its therapeutic index, including the site-specific targeting or providing controlled release, MTX has been conjugated to biopolymers like mono- and poly-clonal antibodies [182, 183], serum albumins [184, 185], neoglycoproteins [186], chitosan [187]. The conjugation of MTX to synthetic polymers has been extensively reported [188-190]. These reports include investigations from this laboratory. Indeed, following Ringsdorf's model of macromolecule prodrugs, MTX was conjugated to various polymeric carriers. In several projects polyaspartamides were conjugated with MTX through tethers containing either ester or amide groups as biofissionable sites. MTX was also conjugated to polyamidoamines with cleavable hydroxamide and carboxamide links in the connecting spacer [188, 189].

3.2.5.3 Polymer-ferrocene conjugation

From previous considerations, our research was led to the strategy of conjugating the inherently hydrophobic ferrocene complex reversibly to water-soluble macromolecular carriers. The reversible anchoring of the complex to the polymeric carrier can lead to considerably enhanced therapeutic effectiveness of the therapeutic agent, as crucially important factors combined to increase bioavailability at the target site while reducing toxicity and risk of resistance build-up. Following Ringsdorf's model of macromolecular prodrugs, the ferrocene complex was conjugated to various polymeric carriers in

Neuse's laboratory. Conjugation was achieved with ferrocenylcarboxylic acids and either amine-functionalized carriers leading to amide-bonded [191] ferrocene conjugates, or hydroxyl-functionalized carriers affording ester-bonded [192] ferrocene conjugates. A recent article from the same laboratory reported the cytotoxic activity of macromolecular ferrocene conjugates against Colo 320 DM human colon cancer line [193]. In the present study, 4-ferrocenylbutanoic acid was used as ferrocene complex for anchoring purpose.

3.2.5.4 Polymer-platinum conjugation

As mentioned in chapter one, the concept of binding (anchoring) medicinal agents to polymeric carriers for enhancement of their therapeutic effectiveness has established itself in recent years as an intimate component of advanced drug research. Such macromolecularization of cytotoxic drugs could affect their distribution and facilitate slow and continuous release while ensuring better stability of the active substance and decreasing its toxicity.

Schechter and coworkers have demonstrated that *cisplatin* can form pharmacologically active complexes with polyanions [194, 195]. In their work, *cisplatin* and the diaqua species was allowed to interact with carboxymethyldextran (CM-dex) in water. The low-molecular-mass platinum compound was then removed by dialysis. The platinum(ll) compounds form complexes with the carboxylic groups. These complexes are reported to be water-soluble, stable in aqueous and physiological conditions and biologically active, inhibiting both *in vitro* and *in vivo* tumor growth. A comparison of a number of complexes prepared under various conditions of platinum-CM-dex ratios and temperature or time of interaction showed that there were no significant differences among them, and that activity was dictated by the

amount of platinum in the complex. Schechter *et al* also used polycarboxylic poly(amino acids) [196, 197]as potential carriers for platinum(ll) analogues, and found them to be much less active than the free drug and the CM-dex complexes, but very much less toxic. *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* [198] 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(ll) complexes cannot efficiently penetrate cell membrane by a passive diffusion mechanism generally utilized by the non polar, neutral compounds. In comparison with a conventional low-molecular-mass 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 [199-201, 202] and pinocytic cell entry.

It is evident from the foregoing 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 [203-205], 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 [202]. Also noteworthy are the investigations by Ohya's group [198], Duncan's team [206], and most notably, Schechter's laboratory [207]. Initially water-soluble, physically isolated and analytically characterized polymer-platinum conjugates have predominantly been the domain of the polymer research group in this department.

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 carrier.

3.2.6 Multidrug conjugation

During the past decade, many research projects have focused on the sequential and simultaneous delivery of drug combinations to reduce the side effects associated with the systemic delivery of anticancer agents [208]. Several drugs have been found to amplify the anticancer activity of others [209]. This synergistic effect can potentially lead to reduced doses for each drug administered [210]. Hence, the simultaneous administration of several drugs could reduce the side effects caused by the high doses of single drug, and could prevent the development of multi-drug resistance (MDR) [211].

An alternative approach to the systemic delivery of antineoplastic agents is the localized release from a polymer. Various strategies can lead to the simultaneous and targeted delivery of a combination of anticancer drugs, and the use of hydrogels constitutes one of them. Bouchir et *al*. [212] incorporated three model drugs, namely, methotrexate (antimetabolite), doxorubicin (anthracycline), and mitoxantrone (anthracenedione antibiotic) into cross-linked oxidized alginate hydrogel and successfully released them from the polymer. The model antineoplastic agents were loaded into the hydrogel *via* three different mechanisms. Methotrexate was incorporated within the pores of the hydrogel and was released by diffusion in the surrounding medium. Doxorubicin was covalently attached to the polymer backbone *via* hydrolytically labile linker and was released following the chemical hydrolysis of the linker. Mitoxantrone was ionically complexed to the polymer and was released after dissociation of the complex. Rao and co-worker [213] also

reported the simultaneous incorporation and controlled release of methotrexate and *cisplatin* using the hydrogel approach. Another possible route for the simultaneous delivery of anticancer compounds is the co-conjugation of two or more of these agents to a single polymeric carrier *via* biofissionable linkages. Reports covering this approach are rare. However, the literature extensively reported the targeted delivery of a drug system in which a targeting moiety together with drug is anchored to the same polymer backbone *via* biofissionable linkages [214, 215].

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Polymeric Drug Carriers

4.1.1 Introduction

This work had three major objectives: the synthesis of water-soluble macromolecular structures to serve as drug carriers, the bioreversible binding of selected anticancer drug models with these carriers, and the biological activity evaluation of the resulting conjugates. In agreement with the necessary conditions for any macromolecule intended to serve in the biomedical field (Section 3.2.1), an attempt was made to design and synthesize several polymeric carrier structures and their derivatives as the number of their naturally occurring counterparts is limited. This offers the advantage to impart the desired functional groups for the accomplishment of our goals. The polymeric carriers used for the anchoring of selected drug models, all taken from the large group of polyamides whose representative figure appears below, are broken in two categories. The first one is represented by the wide aliphatic polyaspartamide type with subunits randomly distributed along the chain, and the second is represented by the family of aliphatic polyamidoamines obtained by a Michael polyaddition process of methylenebisacrylamide (MBA) as the monomer, also with subunits randomly distributed along the chain.

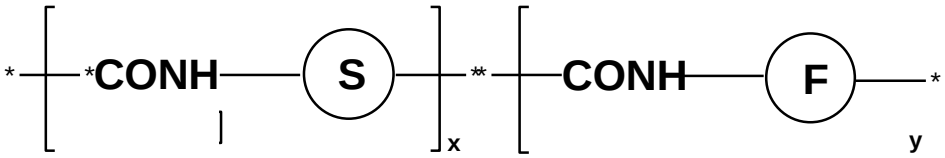


Figure 4.1: Structure of polyamide carrier

In figure 4.1, **S** represents a hydrosolubilizing group located on the main or the side chain, with the goal to provide water-solubility to the ultimate polymer-drug conjugate. **S** may also be a tertiary amine. **F** is an extra- or intra-chain functional group, a primary amine providing a reversible-drug anchoring site. This amino-group leads to polymer-drug binding *via* amide bond formation. In the presence of enzymes, the amide bond has the ability to undergo biofission in the cellular compartments enclosing the lysosomes, which results in a drug release from the cytoplasm. According to the need, additional subunits of required structures may be incorporated, to afford tailor-made polymers that are equipped with all necessary functions. This allows efficacious administration and enhanced pharmacokinetics of the anticancer drug.

All the drug systems selected in this project are hydrophobic. Hence, the polymeric carriers were designed with a good stoichiometric control of solubilizing moieties in order to provide a balance against the load of these inherently hydrophobic drugs. The carriers designed, all linear, had various side chains but similar drug anchoring capacity to the respective different primary amino group. The side chains were tertiary amine-terminated. The following considerations could explain our choice of the tertiary amine groups mentioned above:

(a) The range of the mass-average molecular mass, while chain length-dependant, is generally in the range of 20 000-30 000 and sufficiently low to suppress inherent polymer toxicity, but still high enough to retard renal clearance. The conjugates, therefore, enter cells easily owing to the *enhanced permeability and retention* (EPR) effect.

(b) The intrachain amide groups assist in gradual backbone cleavage for ease of catabolic elimination of the polymer in the ‘spent” state. The stereoisomeric “scrambling” of any peptide units in the backbone chain impedes unduly rapid α-peptidase-mediated “unzipping”, ensuring this fragmentation to be an appropriately retarded process.

(c) When we consider the polyamides mentioned in this work with the characteristics previously described, they are essentially non-toxic, and their immunogenicity is expected to be appreciably lower in comparison with that of commonly occurring high-molecular-mass proteinaceous biopolymers.

(d) The S-modified subunits can be easily introduced as majority components (x>y), thus ensuring effective insulation of the F-modified subunits from each other. This will reduce the risk of intramolecular interaction of adjacent conjugate drug species.

(e) Tertiary amine functions can be introduced as side groups to provide the special functions of adsorptive pinocytotic cell entry and target cell affinity.

(f) While inherently water-soluble, with S comprising selected amine or hydroxyl functions, the polyamides can be made to acquire additional solubility in methanolic medium. This can be achieved by incorporation of poly- (ethylene oxide) (PEO) side chains as additional solubilizing groups. This added solubility feature will be advantageous in follow-up reactions in alcoholic media.

(g) When synthesized from amino-acid monomers, the polyamide carrier will provide a vitally required nutrient for the rapidly growing cell tissue, and hence may be preferentially taken up by the cancerous cell.

The option of polymeric carrier bearing an extra-chain functional group for drug species anchoring was adopted in this project. The adoption was based on a commonly observed phenomenon that fissionable groups as main-chain components are generally less prone to cleavage than the same type of groups located more accessibly in side chains.

Two classes of polyamide conforming to the basic carrier model (Fig 4.1), namely, polyaspartamide (PAsA) and poly(amidoamines) (PAAs) were used for drug conjugation by amide bond formation between the carriers and the carboxylic acid-functionalized drugs. The primary amine functional groups of these polymers were introduced as terminals in component **F**. The subunits incorporating this binding site form 20 to 40 mol- % of the chains, leaving 80 and 60 mol-% of **S-**labeled subunits of the *tert*-amine types for hydrosolubilization. The choice of the compositional specifications was based on the necessity to restrict drug loading to levels commensurate with retention of water-solubility of the resulting conjugates. Indeed, experiments with a carrier containing larger mole percentage of **F**-labelled subunits had previously demonstrated the need for utilization of a disproportionately large excess of drug in the feed for complete drug incorporation. In addition, the coupling products so obtained displayed a propensity for branching and crosslinking with concomitantly decreasing solubility once they had been isolated in the solid state. All polymeric carriers were fractionated by dialysis to remove constituents with molecular mass substantially below 25 000.

4.1.2 Polyaspartamide carriers (PAsA)

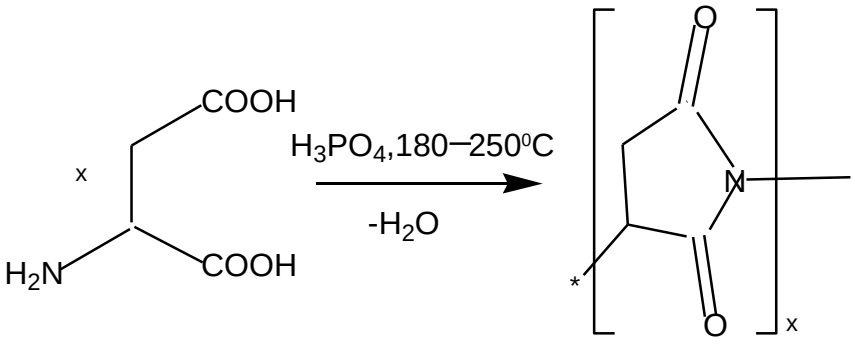
The potential value of polysuccinimide-derived aspartamide polymeric carriers for biomedicinal agents was revealed by Drobnik [216], and his proposal was subsequently translated in this laboratory to the reality of a class of eminently functional carriers for a variety of antineoplastic drug systems, that include those utilized in our investigation. The polyaspartamides were prepared from polysuccinimide.

4.1.2.1 Synthesis of polysuccinimide (PSI)

The literature reported two different methods for the synthesis of polysuccinimide (PSI). One of these approaches is the polymerization of N-carboxyanhydride of α-aminoacid in general (NCA method) [217]. This NCA method was found to be disadvantageous both in cost and production as the pendant reactive groups carried by the amino acid had to be protected before polymerization, and then deprotected under harsh conditions to give the poly-(amino acid). In addition, the management of large amounts of phosgene, diphosgene or triphosgene, reagents widely used for the synthesis of N-carboxyanhydride, raise complex safely problems in a large-scale plant. The other method, known as the Neri method, on the contrary is reported to deliver high molecular mass PSI in one step [218].

For the reasons named above, The Neri method was the approach adopted for the synthesis of PSI from DL-aspartic acid as shown in Scheme 4.1. The crude polymer was treated with dicyclohexylcarbodiimide (DCC) coupling agent for further chain extension purposes. A representative polymer had a relative viscosity of 135 mL/g in DMF at 30 °C, which corresponds to a molecular mass average of 32 000 [234]. On the ^{1}H NMR spectrum of PSI in DMSO-$d_{6,}$ a large resonance, due to the methane proton (NCO-CH), is

observed at 5.2 ppm, while methylene protons (CH_2) appear as two peacks of roughly equal intensity at 3.3 and 2.6 ppm.

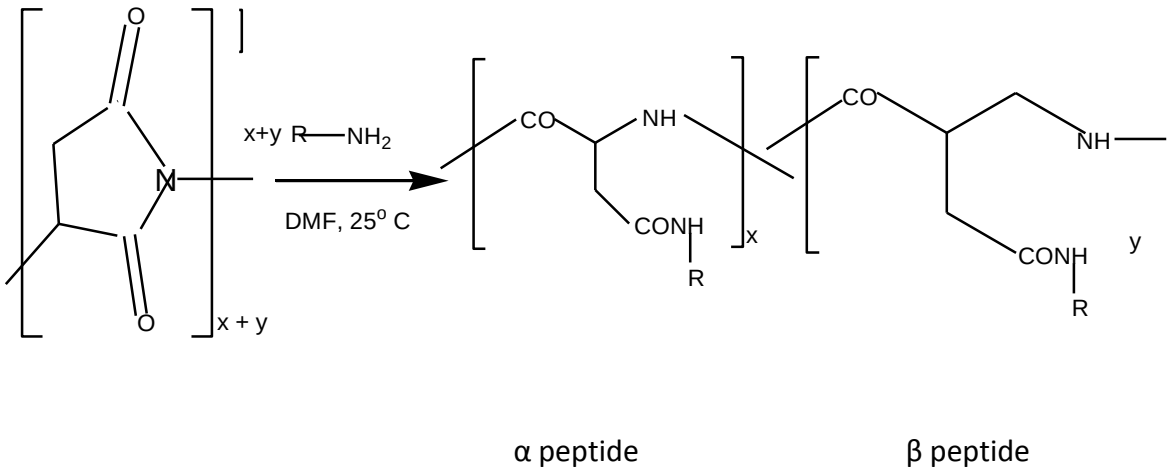


Scheme 4.1: Synthesis of *polysuccinimide*

4.1.2.2 Preparation of homopoly(α, β-DL-aspartamides)

Polyaspartamides are generally prepared from poly-DL-succinimide by an aminolytic ring-opening process in anhydrous, dipolar aprotic medium, such as N,N-dimethylformamide (DMF) at 25°C. The product is racemic and possesses both α- and β-peptidic repeat units. Scheme 4.2 shows the preparation of homopoly(α,β-DL-aspartamides) by the poly-DL-succinimide aminolytic ring-opening process using one type of amine reactant. For convenience, only the α-form of polyaspartamide will be depicted in further schematic diagrams.

While D-type polymers are resistant to enzymatic cleavage, L-type polymers are more prone to rapid enzymatic degradation. Therefore, a backbone structure of the DL-racemic mixture will ensure retarded enzymatic cleavage and ultimate backbone degradation as a result of hydrolytic cleavage.



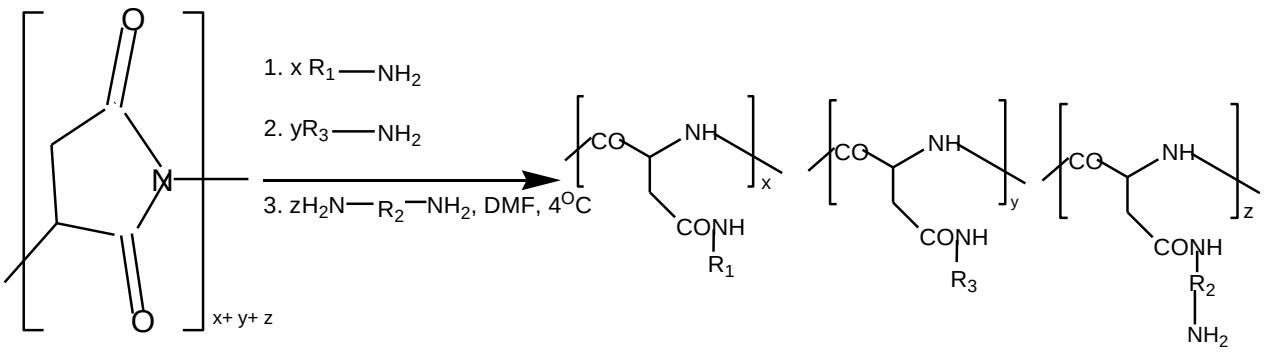
Scheme 4.2: Synthesis of Poly- α, β-DL-aspartamide

4.1.2.3 Preparation of copolyaspartamides (CoPAs)

From previous experience gained in this laboratory, it has been established that aminolytic ring-opening of polysuccinimide can lead to the generation of copolyaspartamides in given stoichiometric feed ratios. Thus H_2N-R_1 and H_2N-R_2-NH_2 were used sequentially for that purpose, leading to the formation of polyaspartamides bearing two side chains pendant to the main chain. One side chain is equipped with a tertiary amine group generated by the reaction of PSI with H_2N-R_1. This tertiary amine plays the role of a solubilizing group. The second side chain, arising from the second step in the ring opening process with the diamine H_2N-R_2-NH_2 as input, is a primary amine covalently attached to the main chain. This side chain bears the unit R_2 as a spacer segment and has a drug-anchoring function at its terminal primary amine. In

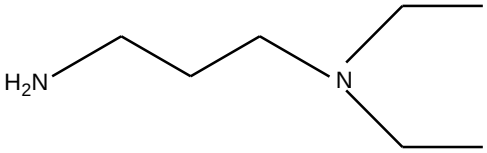
the framework of this project, a series of copolyaspartamides of the general type depicted in Scheme 4.3 were prepared as drug carriers. They arise from a sequential ring opening of PSI in three steps by R_1-NH_2, H_2H-R_2-NH_2 and R_3-NH_2 under an appropriate stochiometry and reaction condition control, contrary to the general habit where the reaction proceeds in two steps. In this new method, the first step of the ring opening process will provide the solubilizing group, a tertiary amine with R_1-NH_2, while R_3-NH_2 and H_2N-R_2-NH_2 will each provide a drug binding site respectively at the second and third step of the ring opening process. The diamine (H_2N-R_2-NH_2) could, by its nucleophilic difunctionality, lead to irreversible crosslinking during the aminolytic ring-opening process. However, the operational conditions developed in this study led to complete PSI ring opening with no evidence of irreversible crosslinking.

The adsorptive endocytotic cell entry of a prodrug [219] is influenced deeply by its ability to endergo protonation in physiological conditions (pH=7.4). All the copolyaspartamides synthesized in this study are containing tertiary amine-terminated side chains.

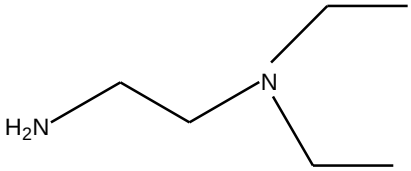


Scheme 4.3: Preparation of copolyaspartamides

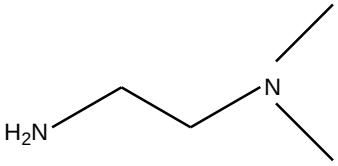
In Scheme 4.3, R_1 represents an aliphatic tertiary amine like diethylaminoethylamine (DEE), dietylaminopropylamine (DEP), dimethylaminoethylamine (DMEA), aromatic-like aminopropylimidazole (API) and cyclic-like aminopropylmorpholine (APM) and aminoethylmorpholine (AEM). These monomers are used as solubilizing groups and their structures appear in figure 4.2. R_2 is a spacer attached to a primary amine drug binding site able to react with a carboxylic group of any drug. In this project the use of propylenediamine, ethylenediamine and diethylenetriamine (figure 4.3) could provide such spacers, and R_3 is another spacer, a 1,2 dihydroxylated monoamine for platinum binding (conjugation) through chelation. Two monomers with such functionalities have been selected for the synthesis of our copolyaspartamide carriers: 3-aminopropyl 1,2 diol (APD) and noradrenaline (Norad), and their structures are depicted in figure 4.4.



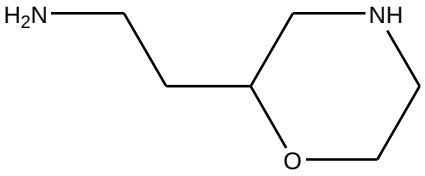
N,N-diethylaminopropylamine



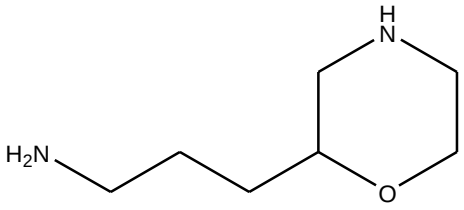
N,N-diethylaminoethylamine



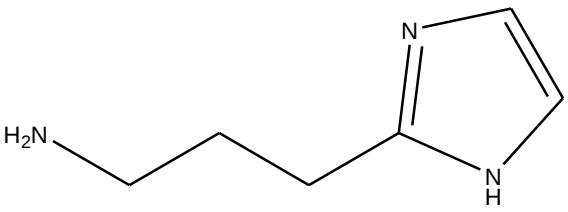
N,N-dimethylaminoethylamin



aminoethylmorpholine



aminopropylmorpholine



amninopropylimidazole

Figure 4.2: Structures of aliphatic tertiary amine, cyclic and aromatic amine solubilizing groups

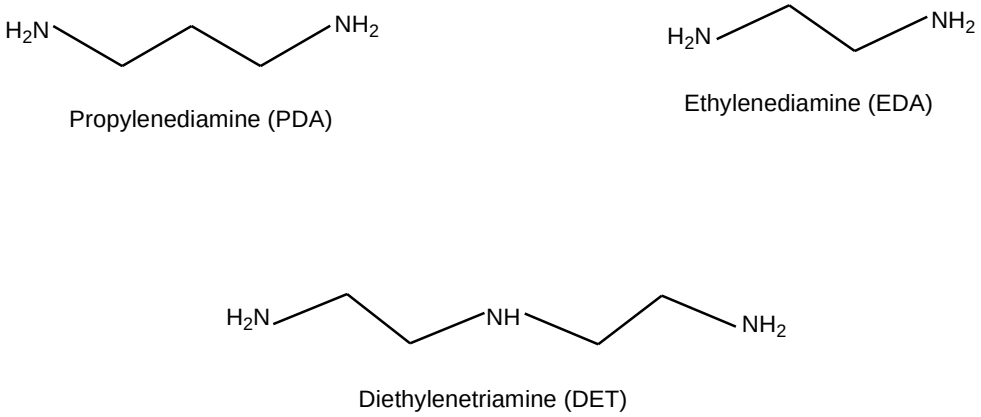
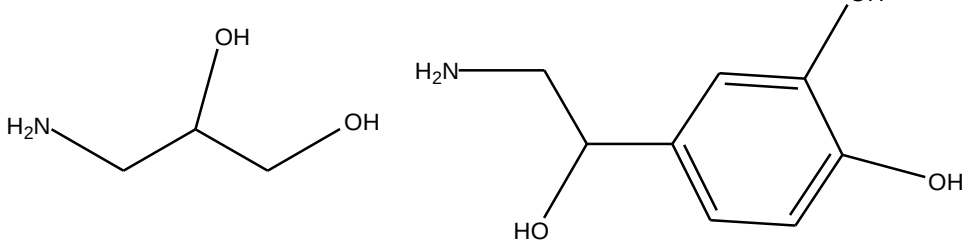


Figure 4.3: structures of primary amines yielding the spacer R_2 In scheme 4.3

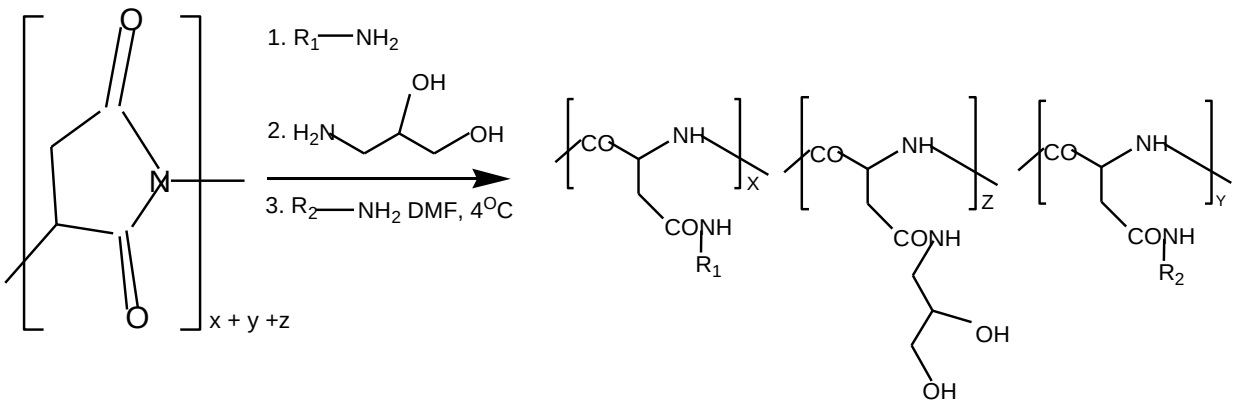


aminopropyl-1,2-diol noradrenaline

Figure 4.4: Structures of 1,2 dihydroxylated monoamine monomers

The group of copolyaspartamides involved in this work has been split into two parts: the copolyaspartamides with 3-aminopropyl-1,2-diol (APD) and those with noradrenaline (Norad) for platinum binding.

1. Copolyaspartamides containing APD: they have been synthesized according to scheme 4.4 and their composition appears in table 4.1 for carriers with aliphatic solubilizing groups and in table 4.4 for carriers with cyclic and aromatic solubilizing groups.



Scheme 4.4: symthesis of copolyaspartamides containing 3-aminopropyl-1,2-diol

In Scheme 4.4, R_1 represents an aliphatic tertiary amine like diethylaminoethylamine (DEE), dietylaminopropylamine (DEP), dimethylaminoethylamine (DMEA), aromatic-like aminopropylimidazole (API) and cyclic-like aminopropylmorpholine (APM) and aminoethylmorpholine (AEM). These monomers are used as solubilizing groups and their structures

appear in figure 4.2. R_2 is a spacer attached to a primary amine drug binding site able to react with a carboxylic group of any drug

Table 4.1: Copolyaspartamides with APD as platinum binding site and aliphatic solubilizing groups

Carriers	R_1	R_2	R_3	x: y: z
K1(60: 20: 20)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	3: 1: 1
K2(60: 20: 20)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	3: 1: 1
K3(80: 10: 10)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	8: 1: 1
K4(80: 10: 10)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	8: 1: 1
K5(80: 10: 10)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	8: 1: 1
K6(60: 20: 20)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	3: 1: 1
K7(80: 10: 10)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	8: 1: 1
K8(60: 20: 20)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	3: 1: 1
K9(60: 20: 20)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	3: 1: 1
K10(80: 10: 10)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	8: 1: 1

In Table 4.1, the ratios in parentheses represent the molar percentage of the respective constituents in the chains of these polymers and in all subsequent

carriers and conjugates, while the ratio x: y: z represents these percentages normalized to y=z=1. All these copolyaspartamides were synthesized under experimental conditions similar to those used in previous work from our laboratory, improved in some cases. The side-chains represented by R_1 in this table are derived either from 2-diethylaminoethylamine (DEE), 3-diethylaminopropylamine (DEP), or 2-dimethylaminoethylamine (DMEA). Those represented by R_2 are derived either from 1,3-diaminopropene (PDA), ethylenediamine (EDA) or diethylenetriamine (DET) in reference to the Scheme 4.4 while R_3 is derived from APD. The general principle adopted from previous experience allowed the use of DEP, DEE and DMEA and all the solubilizing groups in the next reactions of this work as major reactants ($x > y = z$) at room temperature in the first step of the PSI aminolytic ring opening. The diamines PDA, EDA and DET were used in a same ratio as APD. This achievement was fulfilled by using APD in the second step under slight excess (1.2 times the stoichiometric amount), and then the mixture was added to the diamine (PDA, EDA or DET) in excess (three times the stoichiometric amount) at ice bath temperature. This reaction continued over night at the same temperature, then at ambient temperature for 48 h. This strategy of using reagents in excess, also valid in all synthesis of our copolyaspartamide carriers, was necessary for the incorporation of the desired percentage of the functionality in each polymeric chain with complete ring-opening and substitution of the remaining succinimide units of the substrate polymer. Because the solvent N,N'–dimethylformamide (DMF) was used in all the syntheses of our carriers, the work was performed under strictly anhydrous conditions, hence avoiding unwanted ring opening, resulting in generation of free carboxylic acid side groups in all steps. The polymers were obtained after precipitation. They were submitted to an aqueous dialysis in membranes of 12 000 and 25 000 molecular cut-off limits, and then isolated by freeze-drying. The products were water-soluble solids with an inherent

viscosity varying from 11 to 29 mL/g. Table 4.2 shows the experimental results, while ^{1}H NMR and viscosity data appear in Table 4.3.

Table 4.2: Summary of preparative data for copolyaspartamide carriers with APD as platinum binding site and aliphatic solubilising groups

Reactants in feed (mol-%)[a]			Products	Reactions condition 1)24h, RT ; (2) 48h, RT ; 3)12h, 0°C then 24h RT	Copolyaspartamide carriers	
R_1-NH_2	R_3-NH_2	H_2N- R_3-NH_2	Mole ratio x: y: z [b]		Yield (%)[d]	Designation
DEE (60)	APD (24)	PDA (60)	3: 1: 1	"	71	K1(60: 20: 20)
DEP (60)	APD (24)	PDA (60)	3: 1: 1	"	52	K2(60: 20: 20)
DMEA (80)	APD (12)	PDA (30)	8: 1: 1	"	56	K3(80: 10: 10)
DEE (80)	APD (12)	EDA (30)	8: 1: 1	"	50	K4(80: 10: 10)
DEP (80)	APD (12)	EDA (30)	8: 1: 1	"	67	K5(80: 10: 10)
DEP (60)	APD (24)	EDA (60)	3: 1: 1	"	70	K6(60: 20: 20)
DMEA (80)	APD (12)	EDA (30)	8: 1: 1	"	70	K7(80: 10: 10)
DEE (60)	APD (24)	DET (60)	3: 1: 1	"	51	K8(60: 20: 20)
DEP (60)	APD (24)	DET (60)	3: 1: 1	"	53	K9(60: 20: 20)
DMEA (80)	APD (12)	DET (30)	8: 1: 1	"	53	K10(80: 10: 10)

[a] *Parenthetic numbers denote the number of moles of amino compound per 100 base moles of poly-DL-succinimide*
DEE: 2-diethylaminoethylamine, DEP: 3-diethylaminopropylamine, DMEA: 2-dimethylaminoethylamine, APD: 3-Amino-1, 2-propanediol, EDA: Ethylenediamine, PDA: 1, 3-Diaminopropane, DET: DiethyleneTriamine
[b] *Mole ratios of hydrosolubilizing to the two drug-anchoring groups*
[c] *RT= room temperature; reaction step sequence in parentheses.*
[d] *Polymer yield after ultimate (12 000 molecular weight cut-off) dialysis.*

Table 4.3: ^{1}H NMR and viscometric results for copolyaspartamides with APD for platinum binding and aliphatic solubilizing groups

Copolyaspartamide carriers				Number of protons counted [d] (expected)[e] Chemical shift (ppm)				
Designation	η_{inh} mL/g [a]	x: y: z [b]	Base molecular Weight [c]	δ.4.75-4.5 [f]	δ. 3.75-3.5	δ. 3.4-3.0	δ. 2.9-2.0	δ. 1.8-1.5
K1(60:20:20)	15	3: 1: 1	998	5(5)	3(3)	11(10)	28(30)	2(2)
K2(60:20:20)	17	3: 1: 1	984	5(5)	3(3)	10(10)	27(30)	8(8)
K3(80:10:10)	24	8: 1: 1	2092	11(10)	3(3)	21(20)	84(86)	2(2)
K4(80:10:10)	28	8: 1: 1	2175	11(10)	3(3)	19(20)	66(70)	-
K5(80:10:10)	27	8: 1: 1	2161	10(10)	3(3)	18(20)	67(70)	2(2)
K6(60:20:20)	12	3: 1: 1	1027	5(5)	3(3)	8(8)	27(30)	-
K7(80:10:10)	27	8: 1: 1	2204	9(10)	3(3)	18(18)	83(86)	-
K8(60:20:20)	12	3: 1: 1	914	6(5)	3(3)	9(10)	31 (32)	-
K9(60:20:20)	12	3: 1: 1	900	5(5)	3(3)	8(8)	31(32)	-
K10(80:10:10)	26	8: 1: 1	1868	10(10)	3(3)	21(20)	85(88)	-

[a] *At 30.0± 0.5° C, in deionized H_2O; concentration c= 2 mg/mL.*

[b] *Mole ratio (found) of hydrosolubilizing to drug-anchoring groups after ^{1} H NMR integration.*

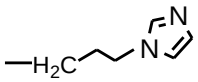
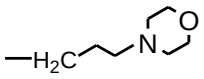
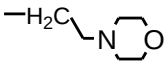
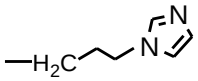
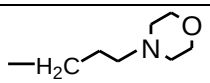
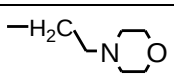
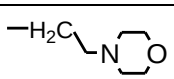
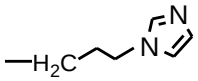
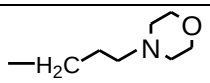
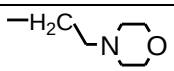
[c] *Molecular mass of the simplest* recurring *unit (normalized to y=z=1) rounded off to the nearest integer.*

[d] *In D_2O, pH 10-11, chemical shifts, δ/ppm, referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limit ± 12%. Protons are calculated (figures rounded off to the nearest integer) on basis of found x:y:z ratios.*

[e] *Expected count for composition in accordance with recurring unit (see b).*

[f] *Assignment: δ/ppm: **4.75-4.5** (CH Asp); **3.75-3.5** (CH_2OH; CHOH); **3.4-3.0** (CONH-CH2); **2.9-2.0 (**CH_2-N(CH_3) **1.8-1.5 (**$CH_2CH_2CH_2$)*

Table 4.4: composition of copolyaspartamides bearing APD as platinum binding site with cyclic and aromatic solubilizing groups

Carriers	R_1	R_2	R_3	x: y: z
K11(80: 10: 10)		$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	8: 1: 1
K12(60: 20: 20)		$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	3: 1: 1
K13(80: 10: 10)		$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	8: 1: 1
K14(60: 20: 20)		$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	3: 1: 1
K15(60: 20: 20)		$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	3: 1: 1
K16(80: 10: 10)		$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	8: 1: 1
K17(60: 20: 20)		$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	3: 1: 1
K18(80: 10: 10)		$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	8: 1: 1
K19(60: 20: 20)		$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	3: 1: 1
K20(60: 20: 20)		$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	3: 1: 1

In Table 4.4 shown, all the copolyaspartamides cited were synthesized under experimental conditions similar to those used for carriers presented in table 4.1. However, the time was extended to 48 h in all the next reactions using the same kind of reagents for the first step, which is an aminolytic ring opening with cyclic and aromatic solubilizing groups. This time extension is motivated by the lower reactivity of these cyclic and aromatic amines when compared to the aliphatic ones. The side chains represented by R_1 in this table are derived either from aminopropylimidazole (API), 3-aminopropylmorpholine (APM), or 2-aminoethylmorpholine (AEM). Those represented by R_2 are derived either from

1,3-diaminopropane (PDA), ethylenediamine (EDA) or diethylenetriamine (DET) in reference to the scheme 4.4 while R_3 is derived from APD. We could achieve the desired percentage of each reagent with complete ring opening and substitution of the remaining succinimide units of the substrate polymer.

The same principle of collection of polymers applies to all the synthetic steps described in this work. It involves a precipitation followed by an aqueous dialysis in membranes of 12 000 and 25 000 molecular mass-cut-off limits and then isolation by freeze-drying. The products were water-soluble solids with an inherent viscosity varying from 13 to 34 mL/g. Table 4.5 shows the experimental results, while ^{1}H NMR and viscosity data appear in Table 4.6.

Table 4.5: Summary of preparative data for copolyaspartamide carriers with APD as platinum binding site bearing cyclic and aromatic solubilising groups

Reactants in feed (mol-%) [a]			Products	Reactions conditions [c]	Copas carriers	
R_1-NH_2	R_3-NH_2	H_2N-R_2-NH_2	Mole ratio [b] x : y : z	1)24h, RT ; (2) 48h, RT ; 3)12h, 0°C then 24h RT	Yield (%)[d]	Designation
API (80)	APD (12)	PDA (30)	8: 1: 1	"	67	K11(80: 10: 10)
APM (60)	APD (24)	PDA (60)	3: 1: 1	"	65	K12(60: 20: 20)
AEM (80)	APD (12)	PDA (30)	8: 1: 1	"	54	K13(80: 10: 10)
API (60)	APD (24)	EDA (60)	3: 1: 1	"	52	K14(60: 20: 20)
APM (60)	APD (24)	EDA (60)	3: 1: 1	"	51	K15(60: 20: 20)
AEM (80)	APD (12)	EDA (30)	8: 1: 1	"	57	K16(80: 10: 10)
AEM (60)	APD (24)	EDA (60)	3: 1: 1	"	50	K17(60: 20: 20)
API (80)	APD (12)	DET (30)	8: 1: 1	"	73	K18(80: 10: 10)
APM (60)	APD (24)	DET (60)	3: 1: 1	"	59	K19(60: 20: 20)
AEM (60)	APD (24)	DET (60)	3: 1: 1	"	60	K20(60: 20: 20)

[a] Parenthetic numbers denote the number of moles of amino compound per 100 base moles of poly-DL-succinimide
AEM: 4- (2-aminoethyl)-morpholine, API: 1- (3-aminopropyl)-imidazol, APM: 4- (3-aminopropyl) morpholine, APD: 3-amino-1, 2-propanediol,EDA: ethylenediamine, PDA: 1, 3-diaminopropane, DET: diethyleneTriamine

[b] Mole ratios of hydrosolubilizing to the two drug-anchoring groups

[c] RT= room temperature; reaction step sequence in parentheses.

[d] Polymer yield after ultimate (12 000 molecular weight cut-off) dialysis.

Table 4.6: ^{1}H NMR and viscometric results for copolyaspartamides with APD for platinum binding bearing cyclic and aromatic solubilizing groups

Copolyaspartamide carriers				Number of protons counted[d] (expected)[e] Chemical shift (ppm)				
Designation	η_{inh} mL/g [a]	x:y:z [b]	Base molecular Weight [c]	δ.8.5-4.0 [f]	δ. 3.75-3.5	δ. 3.4-3.0	δ. 2.9-2.0	δ. 1.9-1.4
K11(80: 10: 10)	32	8:1: 1	2135	32(34)	3(3)	22(20)	20(22)	31(34)
K12(60: 20: 20)	15	3: 1: 1	1011	4(5)	3(3)	19(22)	10(12)	15(14)
K13(80: 10: 10)	29	8: 1: 1	2164	11(10)	3(3)	48(52)	19(22)	19(18)
K14(60: 20: 20)	13	3: 1: 1	1082	15(14)	3(3)	11(10)	9(12)	11.2(12)
K15(60: 20: 20)	15	3: 1: 1	1068	6(5)	3(3)	23(22)	11(12)	14(12)
K16(80: 10: 10)	34	8: 1: 1	2316	9(10)	3(3)	46(52)	20(22)	18(18)
K17(60: 20: 20)	13	3: 1: 1	1097	5(5)	3(3)	20(22)	13(12)	6.4(6)
K18(80: 10: 10)	32	8: 1: 1	2175	35(34)	3(3)	18(20)	23(26)	23.8(22)
K19(60: 20: 20)	14	3: 1: 1	1026	5(5)	3(3)	21(22)	14(16)	9(12)
K20(60: 20: 20)	13	3: 1: 1	1069	5(5)	3(3)	19(22)	15(16)	7.2(6)

[a] *At 30.0± 0.5° C, in deionized H_2O; concentration c= 2 mg/mL.*

[b] *Mole ratio (found) of hydrosolubilizing to drug-anchoring groups after ^{1} H NMR integration.*

[c] *Molecular weight of the simplest recurring unit (normalized to y=z=1) rounded off to the nearest integer.*

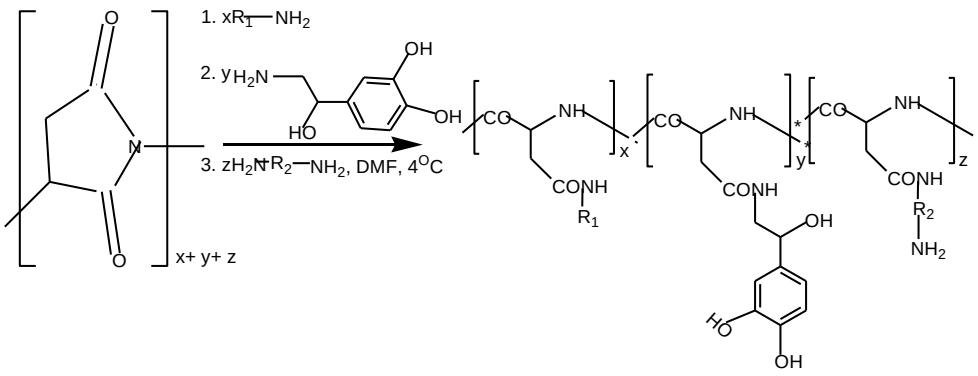
[d] *In D_2O,* pH *10-11, chemical shifts, δ/ppm, referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limit ± 12%*

Protons are calculated (figures rounded off to the nearest integer) on basis of found x:y:z ratios.

[e] *Expected count for composition in accordance with recurring unit (see b).*

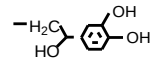
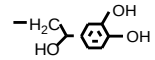
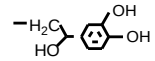
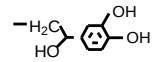
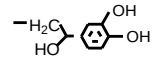
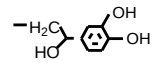
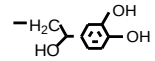
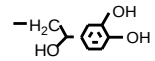
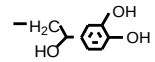
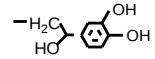
[f] *Assignment: δ/ppm*

1. Copolyaspartamides containing noradrenaline as platinum-binding site: Scheme 4.5 shows their synthesis while Tables 4.7 and 4.10 show their compositions.



Scheme 4.5: copolyaspartamide containing noradrenaline

Table 4.7: copolyaspartamides with noradenaline as platinum-binding site and aliphatic solubilizing groups

Carriers	R_1	R_2	R_3	x: y: z
L1(80: 10: 10)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_3-$		8: 1: 1
L2(60: 20: 20)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_3-$		3: 1: 1
L3(80: 10: 10)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_3-$		8: 1: 1
L4(60: 20: 20)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2-$		3: 1: 1
L5(80: 10: 10)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2-$		8: 1: 1
L6(80: 10: 10)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_2-$		8: 1: 1
L7(60: 20: 20)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$		3: 1: 1
L8(60: 20: 20)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$		3: 1: 1
L9(80: 10: 10)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$		8: 1: 1
L10(60: 20: 20)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$		3: 1: 1

All these copolyaspartamides were synthesized under experimental conditions similar to those used for carriers presented in table 4.1. However in the preparation of this series of carriers, the time was extended to 48 h for the second step which is an aminolytic ring opening with noradrenaline (NOR) as an aromatic dihydroxylated amine present in a salt form of D,L-noradrenaline hydrochloride. It had thus to be precipitated from its salt by addition to the mixture, of triethylamine (TEA). This time extension is motivated by the lower reactivity of aromatic compounds when compared to the aliphatic ones. The side chains represented by R_1 in this table are derived either from 2-diethylaminoethylamine (DEE), 3-diethylaminoethylamine (DEP), or 2-

dimethylaminoethylamine (DMEA). Those represented by R_2 are derived either from 1,3-diaminopropene (PDA), ethylenediamine (EDA) or diethylenetriamine (DET) in reference to the scheme 4.5 while R_3 is derived from D,L noradrenaline. All the reagents presented in the table above were used in the same stoichiometric ratios as in the foregoing. The solvent used was N,N'–dimethylformamide. It was chosen for the same reasons as mentioned before. The polymers were obtained by the same methods previously presented, while their viscosities appeared in the range between 11 and 31 mL/g as shown in Tables 4.8 and 4.9.

Table 4.8: Summary of preparative data for copolyaspartamide carriers with Noradrenaline as platinum binding site bearing aliphatic solubilising groups

Reactants in feed (mol-%)[a]			Products	Reactions conditions [c]	Copolyaspartamide carriers	
R_1-NH_2	R_3-NH_2	H_2N-R_2-NH_2	Mole ratio [b] x : y : z	(1) 24h, RT ; (2) 48h, RT ; (3) 12h, 0^oC then 24h RT	Yield (%)[d]	Designation
DEE (80)	NOR(12)	PDA (30)	8: 1: 1	"	67	L1(80: 10: 10)
DEP (60)	NOR(24)	PDA (60)	3: 1: 1	"	65	L2(60: 20: 20)
DMEA (80)	NOR(12)	PDA (30)	8: 1: 1	"	54	L3(80: 10: 10)
DEE (60)	NOR(24)	EDA (60)	3: 1: 1	"	52	L4(60: 20: 20)
DEP (80)	NOR(12)	EDA (30)	8: 1: 1	"	51	L5(80: 10: 10)
DMEA (80)	NOR(12)	EDA (30)	8: 1: 1	"	57	L6(80: 10: 10)
DEE (60)	NOR(24)	DET (60)	3: 1: 1	"	58	L7(60: 20: 20)
DEP (60)	NOR(24)	DET (60)	3: 1: 1	"	59	L8(60: 20: 20)
DMEA (80)	NOR(12)	DET (30)	8: 1: 1	"	55	L9(80: 10: 10)
DMEA (60)	NOR(24)	DET (60)	3: 1: 1	"	60	L10(60: 20: 20)

[a] *Parenthetic numbers denote the number of moles of amino compound per 100 base moles of poly-DL-succinimide*
DEE: 2diethylaminoethylamine, DEP: 3-diethylaminopropylamine, DMEA: 2-dimethylaminoethylamine, NO:= D, L-noradrenaline
EDA: ethylenediamine, PDA: 1, 3-diaminopropane, DET: diethylenetriamine
[b] *Mole ratios of hydrosolubilizing to the two drug-anchoring groups*
[c] *RT= room temperature; reaction step sequence in parentheses.*
[d] *Polymer yield after ultimate (12 000 molecular mass-cut-off) dialysis*

Table 4.9: ^{1}H NMR and viscometric results for Copolyaspartamides with noradrenaline for platinum-binding And aliphatic solubilizing groups

Copolyaspartamide carriers				Number of protons counted[d] (expected)[e] Chemical shift (ppm)				
Designation	η_{inh} mL/g [a]	x: y:z [b]	Base molecular mass [c]	δ.8.5-4.5 [f]	δ. 3.75-3.5	δ. 3.4-3.0	δ. 2.9-2.0	δ. 1.8-0.9
L1(80: 10: 10)	30	8: 1: 1	2141	13(16)	1(1)	22(20)	65(70)	54(50)
L2(60: 20: 20)	12	3: 1: 1	906	7(10)	1.2(1)	11(10)	27(30)	22(20)
L3(80: 10: 10)	29	8: 1: 1	2170	12(16)	1.1(1)	19(20)	82(86)	1.7(2)
L4(60: 20: 20)	11	3: 1: 1	1118	8(10)	1.1(1)	8(10)	25(28)	-
L5(80: 10: 10)	30	8: 1: 1	2238	13.6(16)	0.9(1)	18(20)	65(68)	-
L6(80: 10: 10)	29	8: 1: 1	2282	14.2(16)	1.2(1)	22(20)	81(84)	-
L7(60: 20: 20)	13	3: 1: 1	992	7.4(10)	1(1)	8(10)	31(34)	16(18)
L8(60: 20: 20)	13	3: 1: 1	978	7.2(10)	1.1(1)	7(10)	30(34)	18.6(20)
L9(80: 10: 10)	30	8: 1: 1	1946	14(16)	1.2(1)	22(20)	83(88)	2(2)
L10(60: 20: 20)	12	3: 1: 1	1021	8.6(10)	0.9(1)	8(10)	35(38)	2(2)

[a] At 30.0± 0.5° C, in deionized H_2O; concentration c= 2 mg/mL.

[b] Mole ratio (found) of hydrosolubilizing to drug-anchoring groups after [1] H NMR integration.

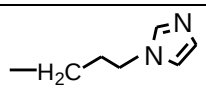
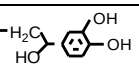
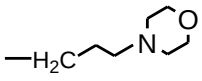
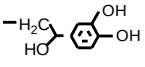
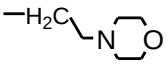
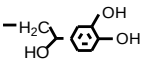
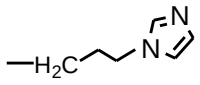
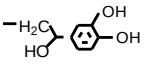
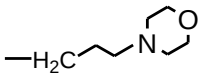
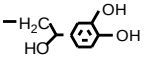
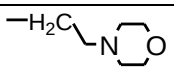
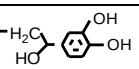
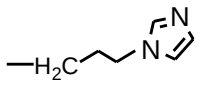
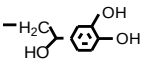
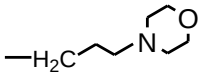
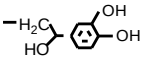
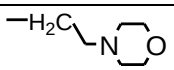
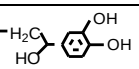
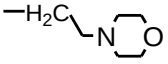
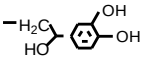
[c] Molecular mass of the simplest recurring unit (normalized to y=z=1) rounded off to the nearest integer.

[d] In D_2O, pH 10-11, chemical shifts, δ/ppm, referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limit ± 12%. Protons are calculated (figures rounded off to the nearest integer) on basis of found x:y:z ratios.

[e] Expected count for composition in accordance with recurring unit (see b).

[f] Assignment: δ/ppm:

Table 4.10: copolyaspartamides with noradrenaline for platinum-binding, bearing cyclic and aromatic solubilizing groups

Carriers	R1	R2	R3	x: y: z
L11(60: 20: 20)		-$(CH_2)_3$-		3: 1: 1
L12(80: 10: 10)		-$(CH_2)_3$-		8: 1: 1
L13(60: 20: 20)		-$(CH_2)_3$-		3: 1: 1
L14(80: 10: 10)		-$(CH_2)_2$-		8: 1: 1
L15(60: 20: 20)		-$(CH_2)_2$-		3: 1: 1
L16(80: 10: 10)		-$(CH_2)_2$-		8: 1: 1
L17(80: 10: 10)		-$(CH_2)_2NH(CH_2)_2$-		8: 1: 1
L18(80: 10: 10)		-$(CH_2)_2NH(CH_2)_2$-		8: 1: 1
L19(80: 10: 10)		-$(CH_2)_2NH(CH_2)_2$-		8: 1: 1
L20(60: 20: 20)		-$(CH_2)_2NH(CH_2)_2$-		3: 1: 1

In this table, the ratios in parentheses denote the molar percentage (x: y: z) of the respective constituents in the chains of these polymers and in all subsequent carriers and conjugates. All these copolyaspartamides were synthesized under experimental conditions similar to those used for carriers presented in table 4.9, except that in this case, the time was extended to 48 h for the first step which is an aminolytic ring opening ring opening with cyclic and aromatic solubilizing groups. This extension is motivated by the lower reactivity of cyclic and aromatic compounds when compared to the aliphatic

ones. The side chains represented by R_1 in this table are derived either from aminopropylimidazole (API), 3-aminopropylmorpholine (APM), or 2-aminoethylmorpholine (AEM). Those represented by R_2 are derived either from 1,3-diaminopropene (PDA), ethylenediamine (EDA) or diethylenetriamine (DET) in reference to the scheme 4.5 while R_3 is derived from NOR. The general principle adopted from previous experiences allowed the use of all the reagents, respectively, in the same stoichiometric ratios and conditions as before. The same methods of purification were applied, and the products were collected with viscosities varying from 10 to 28 mL/g. Table 4.11 depicts the experimental conditions, while viscosities and ^{1}H NMR data appear in Table 4.12.

Table 4.11: Summary of preparative data for copolyaspartamide carriers with noradrenaline as platinum-binding Site bearing cyclic and aromatic solubilising groups

Reactants in feed (mol-%)[a]			Products	Reaction conditions [c]	Copolyaspartamide carriers	
R_1-NH_2	R_3-NH_2	H_2N-R_2-NH_2	Mole ratio [b] x : y : z	(1) 24h, RT ; (2) 48h, RT ; (3) 12h, 0°C then 24h, RT	Yield (%)[d]	Designation
API (60)	NOR(24)	PDA (60)	3: 1: 1	"	61	L11(60: 20: 20)
APM (80)	NOR(12)	PDA (30)	8: 1: 1	"	65	L12(80: 10: 10)
AEM (60)	NOR(24)	PDA (60)	3: 1: 1	"	59	L13(60: 20: 20)
API (80)	NOR(12)	EDA (30)	8: 1: 1	"	71	L14(80: 10: 10)
APM (60)	NOR(24)	EDA (60)	3: 1: 1	"	51	L15(60: 20: 20)
AEM (80)	NOR(12)	EDA (30)	8: 1: 1	"	57	L16(80: 10: 10)
API (80)	NOR(12)	DET (30)	8: 1: 1	"	73	17(80: 10: 10)
APM (80)	NOR(12)	DET (30)	8: 1: 1	"	58	L18(80: 10: 10)
AEM (80)	NOR(12)	DET (30)	8: 1: 1	"	55	L19(80: 10: 10)
AEM (60)	NOR(24)	DET (60)	3: 1: 1	"	60	L20(60: 20: 20)

[a] Parenthetic numbers denote the number of moles of amino compound per 100 base moles of poly-DL-succinimide AEM: 4- (2-aminoethyl)-morpholine, API: 1- (3-aminopropyl)-imidazol, APM: 4- (3-aminopropyl) morpholine, NOR: D, L-noradrenaline, EDA: ethylenediamine, PDA: 1, 3-diaminopropane, DET: diethylenetriamine

[b] Mole ratios of hydrosolubilizing to the two drug-anchoring groups

[c] RT= room temperature; reaction step sequence in parentheses.

[d] Polymer yield after ultimate (12 000 molecular mass cut-off) dialysis.

Table 4.12: ^{1}H NMR and viscometric results for copolyaspartamides with Noradrenaline for platinum binding, bearing cyclic and aromatic solubilizing groups

Copolyaspartamide carriers				Number of protons counted[d] (expected)[e] Chemical shift (ppm)				
Designation	η_{inh} mL/g [a]	x: y: z [b]	Base molecular mass [c]	δ.8.5-4.5 [f]	δ.3.75-3.5	δ.3.4-3.0	δ.2.9-2.0	δ.1.8-0.9
L11(60: 20: 20)	12	3: 1: 1	1103	15(17)	1.1(1)	8.8(10)	10(12)	12.5(14)
L12(80: 10: 10)	27	8: 1: 1	2199	11(13)	22(25)	49(52)	20.4(22)	30(34)
L13(60: 20: 20)	12	3: 1: 1	1132	8.8(8)	11(10)	19(22)	10(12)	7.5(8)
L14(80: 10: 10)	27	8: 1: 1	2365	29(32)	1.2(1)	18.6(20)	21(24)	31(34)
L15(60: 20: 20)	11	3: 1: 1	1146	7.2(8)	11(10)	19(22)	16(14)	11(14)
L16(80: 10: 10)	27	8: 1: 1	2394	9.2(8)	22(25)	30(32)	23.6(22)	6.4(6)
L17(80: 10: 10)	28	8: 1: 1	2253	30(33)	0.8(1)	19(22)	21(24)	33(36)
L18(80: 10; 10)	25	8: 1: 1	2239	15(13)	22(25)	47(52)	26(24)	32(34)
L19(80: 10: 10)	23	8: 1: 1	2282	11(13)	23(25)	49(52)	21(24)	16(18)
L20(60: 20: 20)	11	3: 1: 1	1147	8(8)	9(10)	9(2 2)	13(14)	7.4(8)

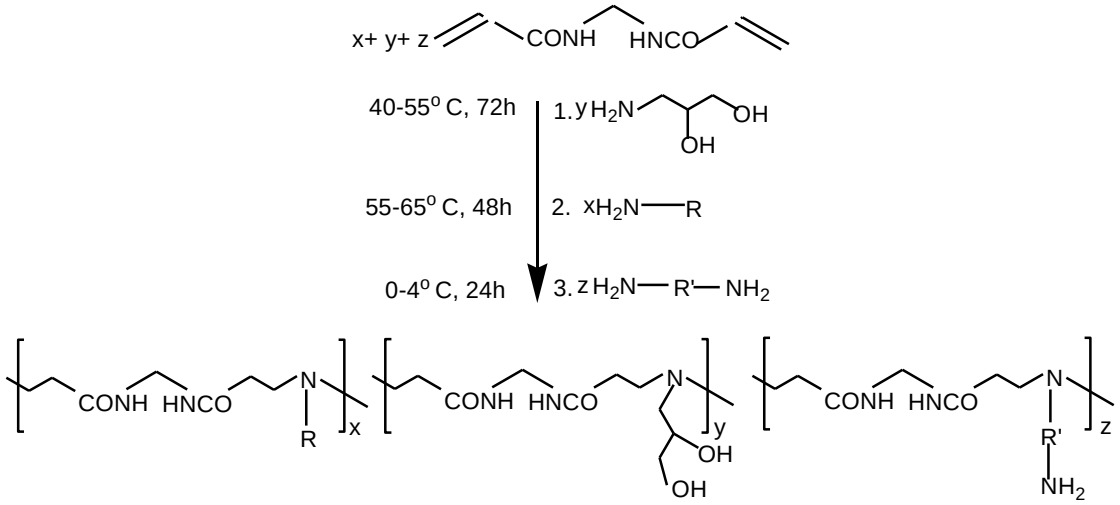
[a] At 30.0± 0.5° C, in deionized H_2O; concentration c= 2 mg/mL.
[b] Mole ratio (found) of hydrosolubilizing to drug-anchoring groups after ^{1}H NMR integration.
[c] Molecular mass of the simplest recurring unit (normalized to y=z=1) rounded off to the nearest integer.
[d] In D_2O, pH 10-11, chemical shifts, δ/ppm, referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limit ± 12%. Protons are calculated (figures rounded off to the nearest integer) on basis of found x:y:z ratios.
[e] Expected count for composition in accordance with recurring unit (see b). [f] *Assignment: δ/ppm*

4.1.3 Poly (amidoamine) carriers (Polyam)

The study of poly (amidoamines) was pioneered by Ferruti [1]. Interest in this study was prompted by the need for polymeric carriers providing primary amino groups as side chain terminals for drug anchoring while possessing complete solubility in both aqueous and organic media, and more critically in methanolic media. Therefore bisacrylamide-derived polymers containing various solubilising groups as side-chain or main-chain constituents were synthesised by a Michael addition mechanism. These chains were in addition to the short side chains which possess primary amine functionality as the drug anchoring site.

The bifunctional acrylic acid derivative used in this work is methylenebisacrylamide. It has been used in earlier [2] and recent [3] investigations by this laboratory. It had to be copolymerized in various feed ratios with most of the functionalized monoamines and diamines used for the synthesis of polyaspartamides and under conditions described in schemes 4.6 and 4.7 and specified in more detail in the experimental section. The only difference with the synthesis of copolyaspartamides is that one of the two dihydroxylated monomers (noradrenaline) has been replaced by aspartic acid, a dicarboxylated monomer, because it is soluble in H_2O, the reaction solvent and because the two carboxyl functionalities in geometry 1,2 offer a possibility of platinum conjugation through chelation. Thus the polyamidoamines presented in this chapter have been grouped in two parts: those using APD and those using D,L aspartic acid (Aspac) for platinum binding.

1. Polyamidoamines containing APD: they were synthesized according to scheme 4.6, and their composition appears in Table 4.13 for carriers with aliphatic solubilizing groups and in Table 4.16 for carriers with cyclic and aromatic solubilizing groups.



Scheme 4.6: synthesis of polyamidoamine carriers containing APD

Table 4.13: composition of copolyamidoamines carriers bearing APD and aliphatic solubilizing groups

Carriers	R_1	R_2	R_3	x: y: z
M1(60: 20: 20)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	3:1: 1
M2(80: 10: 10)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	8: 1: 1
M3(60: 20: 20)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	3: 1: 1
M4(80: 10: 10)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	8: 1: 1
M5(60:20: 20)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	3: 1: 1
M6(80: 10: 10)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	8: 1: 1
M7(60: 20: 20)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	3: 1: 1
M8(80: 10: 10)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	8: 1: 1
M9(80: 10: 10)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	8: 1: 1
M10(60: 20: 20)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	3: 1: 1

In this table, the ratios in parentheses denote the molar percentage (x: y: z) of the respective constituents in the chains of these polymers and in all subsequent carriers and conjugates. All the polyamidoamines presented here were synthesized under experimental conditions previously described in publications from our laboratory in reference to scheme 4. 6. The reaction proceeded in three steps in aqueous medium. Methylenebisacrylamide (MBA), 2-diethylaminoethylamine (DEEA), 3-diethylaminopropylamine (DEP), 2-dimethylaminoethylamine (DMEA), 1,3-diaminopropane (PDA), ethylene diamine (EDA), diethylenetriamine (DET) and 3-amino-1,2-propanediol (APD) were used as comonomers for the synthesis of polyamidoamines M1 (80: 10: 10) - M10 (3: 1: 1).

With a major goal to enhance the solubility of our polymers in aqueous medium and in order to extend the spacing between functional groups, DEE, DEP and DMEA were incorporated in the main chain during the first step, while APD used in the second step and the diamines PDA, EDA and DET used in the third step provided ligands for platinum and other drug anchoring. The polyaddition went over 5 days at 55-60^{o}C. The products were isolated by freeze-drying after dialysis in membranes with a 12 000 molecular mass cut-off as water-soluble solids, whose inherent viscosities ranged from

5 to 19 mL/g and appear in Table 4.15 together with ^{1}H NMR structural data, while experimental conditions and stoichiometric ratios are shown in Table 4.14.

Table 4.14: Summary of preparative data for Copolyamidoamine carriers with APD as platinum-binding site bearing aliphatic solubilising groups

Reactants in feed (mol-%)[a]			Products	Reaction conditions [c]	Copolyamidoamine carriers	
R_1-NH_2	R_3-NH_2	H_2N-R_2-NH_2	Mole ratio [b] x : y : z	1)24h, RT ; (2) 48h, RT ; (3) 12h, 0°C then 24h RT	Yield (%)[d]	Designation
DEE (60)	APD (24)	PDA (60)	3: 1: 1	"	61	M1(60: 20: 20)
DEP (80)	APD (12)	PDA (30)	8: 1: 1	"	65	M2(80: 10: 10)
DMEA (60)	APD (24)	PDA (60)	3: 1: 1	"	59	M3(60: 20: 20)
DEE (80)	APD (12)	EDA (30)	8: 1: 1	"	71	M4(80: 10: 10)
DEP (60)	APD (24)	EDA (60)	3: 1: 1	"	51	M5(60: 20: 20)
DMEA (80)	APD (12)	EDA (30)	8: 1: 1	"	57	M6(80: 10: 10)
DEE (60)	APD (24)	DET (60)	3: 1: 1	"	58	M7(60: 20: 20)
DEP (80)	APD (12)	DET (30)	8: 1: 1	"	58	M8(80: 10: 10)
DMEA (80)	APD (12)	DET (30)	8: 1: 1	"	55	M9(80: 10: 10)
DMEA (60)	APD (24)	DET (60)	3: 1: 1	"	60	M10(60:20:20)

[a] *Parenthetic numbers denote the number of moles of amino compound per 100 base moles of poly-DL-succinimide*
DEE= 2-diethylaminoethylamine, DEP= 3diethylaminopropylamine, DMEA= 2-dimethylaminoethylamine, APD= 3-amino-1,2-propanediol, EDA= ethylenediamine, PDA= 1, 3-diaminopropane, DET= diethylenetriamine
[b] *Mole ratios of hydrosolubilizing to the two drug-anchoring groups*
[c] *RT= room temperature; reaction step sequence in parentheses.*
[d] *Polymer yield after ultimate (12 000 molecular mass cut-off) dialysis.*

Table 4.15: ^{1}H NMR and viscometric results for Copolyamidoamines carriers with APD for platinum-binding and aliphatic solubilizing groups

Copolyamidoamine carriers				Number of protons counted[d] (expected)[e] Chemical shift (ppm)				
Designation	η_{inh} mL/g [a]	x: y: z [b]	Base molecular Weight [c]	δ.4.7-4.5 [f]	δ. 3.9-3.5	δ. 3.1-3.0	δ. 2.9-2.0	δ. 1.8-0.9
M1(60:20:20)	7	3: 1: 1	1283	8(10)	3(3)	11(10)	107(100)	35(38)
M2(80: 10: 10)	19	8: 1: 1	2619	17(20)	3.2(3)	18(20)	118(128)	60(66)
M3(60: 20: 20)	7	3: 1: 1	1326	9(10)	3.2(3)	7.8(10)	105(114)	2(2)
M4(80: 10: 10)	18	8: 1: 1	2265	21(20)	3(3)	17(20)	119(126)	43(48)
M5(60: 20: 20)	8	3: 1: 1	1311	9(10)	3.1(3)	9(10)	94(102)	21(24)
M6(80: 10: 10)	19	8: 1: 1	2294	22(20)	3.1(3)	17(20)	131(142)	-
M7(60: 20: 20)	5	3: 1: 1	1199	9.3(10)	2.8(3)	8.7(10)	102(104)	34(38)
M8(80: 10: 10)	18	8: 1: 1	2395	17(20)	2.8(3)	17(20)	126(132)	61(66)
M9(80: 10: 10)	18	8: 1: 1	2438	18(20)	3.1(3)	18(20)	133(148)	2.2(2)
M10(60: 20: 20)	8	3: 1: 1	1228	8(10)	3.1(3)	11(10)	109(116)	2(2)

[a] At 30.0± 0.5° C, in deionized H_2O; concentration c= 2 mg/mL.

[b] Mole ratio (found) of hydrosolubilizing to drug-anchoring groups after ^{1}H NMR integration.

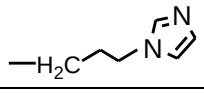
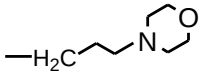
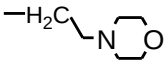
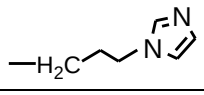
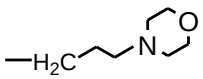
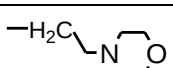
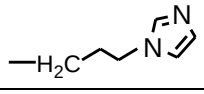
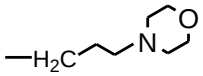
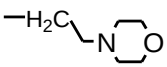
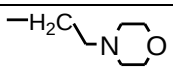
[c] Molecular mass of the simplest recurring unit (normalized to y=z=1) rounded off to the nearest integer.

[d] In D_2O, pH 10-11, chemical shifts, δ/ppm, referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limit ± 12%. Protons are calculated (figures rounded off to the nearest integer) on basis of found x:y:z ratios.

[e] Expected count for composition in accordance with recurring unit (see b).

[f] Assignment: δ/ppm:

Table 4.16: composition of copolyamidoamine carriers bearing APD with cyclic and aromatic solubilizing groups

Carriers	R_1	R_2	R_3	x: y: z
M11(80: 10: 10)		-$(CH_2)_3$-	-$CH_2CHOHCH_2OH$	8: 1: 1
M12(60: 20: 20)		-$(CH_2)_3$-	-$CH_2CHOHCH_2OH$	3: 1: 1
M13(80: 10: 10)		-$(CH_2)_3$-	-$CH_2CHOHCH_2OH$	8: 1: 1
M14(80: 10: 10)		-$(CH_2)_2$-	-$CH_2CHOHCH_2OH$	8: 1: 1
M15(60: 20: 20)		-$(CH_2)_2$-	-$CH_2CHOHCH_2OH$	3: 1: 1
M16(80: 10: 10)		-$(CH_2)_2$-	-$CH_2CHOHCH_2OH$	8: 1: 1
M17(60: 20: 20)		-$(CH_2)_2NH(CH_2)_2$-	-$CH_2CHOHCH_2OH$	3: 1: 1
M18(80: 10: 10)		-$(CH_2)_2NH(CH_2)_2$-	-$CH_2CHOHCH_2OH$	8: 1: 1
M19(80: 10: 10)		-$(CH_2)_2NH(CH_2)_2$-	-$CH_2CHOHCH_2OH$	8: 1: 1
M20(60: 20: 20)		-$(CH_2)_2NH(CH_2)_2$-	-$CH_2CHOHCH_2OH$	3: 1: 1

Taking into account that the ratios in parentheses play the same role described before, we synthesized the polyamidoamines of this table under experimental conditions previously reported in this work in reference to scheme 4.6, except that the aliphatic tertiary amines 2-diethylaminoethylamine (DEEA), 3-diethylaminopropylamine (DEP), and 2-dimethylaminoethylamine (DMEA) were replaced by the aromatic monomer API, and the two cyclic APM and AEM. The polyaminoamines were collected as white solid products with inherent viscosities

varying from 5 to 23 mL/g as shown together with ^{1}H NMR data in Table 4.18, while Table 4.17 shows experimental conditions.

Table 4.17: Summary of preparative data for copolyamidoamine carriers with APD as platinum-binding site bearing cyclic and aromatic solubilising groups

Reactants in feed (mol-%)[a]			Products	Reaction conditions [c]	Copolyamidoamine carriers	
R_1-NH_2	R_3-NH_2	H_2N-R_2-NH_2	Mole ratio [b] x : y : z	(1) 24h, RT ; (2) 48h, RT ; (3) 12h, 0°C then 24h RT	Yield (%)[d]	Designation
API (80)	APD (12)	PDA (30)	8: 1: 1	"	67	M11(80: 10: 10)
APM (60)	PD (24)	PDA (60)	3: 1: 1	"	65	M12(60: 20: 20)
AEM (80)	APD (12)	PDA (30)	8: 1: 1	"	54	M13(80: 10: 10)
API (80)	PD (12)	EDA (30)	8: 1: 1	"	52	M14(80: 10: 10)
APM (60)	APD (24)	EDA (60)	3: 1: 1	"	51	M15(60: 20: 20)
AEM (80)	APD (12)	EDA (30)	8: 1: 1	"	50	M16(80: 10: 10)
API (60)	APD (24)	DET (60)	3: 1: 1	"	73	M17(60: 20: 20)
APM (80)	PD (12)	DET (30)	8: 1: 1	"	59	M18(80: 10: 10)
AEM (80)	APD (12)	DET (30)	8: 1: 1	"	55	M19(80: 10: 10)
AEM (60)	PD (24)	DET (60)	3: 1: 1	"	60	M20(60: 20: 20)

[a] Parenthetic numbers denote the number of moles of amino compound per 100 base moles of poly-DL-succinimide
AEM= 4- (2-Aminoethyl)-morpholine, API= 1- (3-Aminopropyl)-imidazol, APM= 4- (3-Aminopropyl) morpholine, APD= 3-Amino-1, 2-propanediol, EDA=Ethylenediamine
Ethylenediamine, PDA= 1, 3-Diaminopropane, DET= DiethyleneTriamine
[b] Mole ratios of hydrosolubilizing to the two drug-anchoring groups
[c] RT= room temperature; reaction step sequence in parentheses.
[d] Polymer yield after ultimate (12 000 molecular weight cut-off) dialysis.

Table 4.18: ^{1}H NMR and viscometric results for copolyamidoamines with APD for platinum binding bearing Cyclic and aromaticsolubilizing groups

copolyamidoamine carriers				Number of protons counted[d] (expected)[e] Chemical shift (ppm)				
Designation	η_{inh} mL/g [a]	x: y: z [b]	Base molecular mass[c]	δ.8.5-4.5 [f]	δ. 3.9-3.5	δ. 3.4-3.0	δ. 2.9-2.0	δ. 1.8-0.9
M11(80: 10: 10)	19	8: 1: 1	2705	41(44)	3.2(3)	17.7(20)	75(82)	30(34)
M12(60: 20: 20)	6	3: 1: 1	1296	8(10)	10(12)	20(22)	37(40)	12(14)
M13(80: 10: 10)	22	8: 1: 1	2734	18(20)	24(27)	48(52)	82(86)	17(18)
M14(80: 10: 10)	23	8: 1: 1	2857	41(40)	3.1(3)	18(20)	90(98)	29(34)
M15(60: 20: 20)	5	3: 1: 1	1353	8(10)	10(12)	19(22)	37(40)	10(12)
M16(80: 10: 10)	19	8: 1: 1	2886	17(20)	2.8(3)	47(52)	75(80)	14(16)
M17(60: 20; 20)	7	3: 1: 1	1325	16(19)	2.9(3)	8(10)	41(44)	11(14)
M18(80: 10; 10)	22	8: 1: 1	2731	17(20)	23(27)	45(52)	76(82)	29(34)
M19(80: 10: 10)	23	8: 1: 1	2774	18(20)	25(27)	48(52)	79(82)	15(18)
M20(60: 20: 20)	5	3: 1: 1	1354	8(10)	10(12)	20(22)	39(42)	7(8)

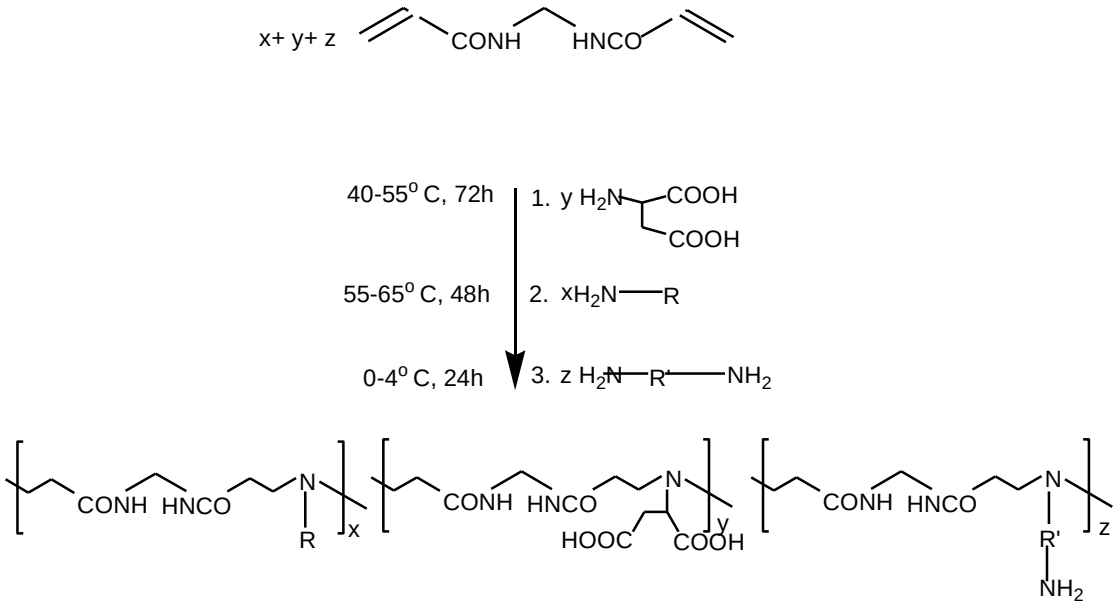
[a] At 30.0± 0.5° C, in deionized H_2O; concentration c= 2 mg/mL.[b] Mole ratio (found) of hydrosolubilizing to drug-anchoring groups after [1] H NMR integration. [c] Molecular mass of the simplest recurring unit (normalized to y=z=1) rounded off to the nearest integer.

[d] In D_2O, pH 10-11, chemical shifts, δ/ppm, referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limit ± 12%. Protons are calculated (figures rounded off to the nearest integer) on basis of found x:y:z ratios.

[e] Expected count for composition in accordance with recurring unit (see b).

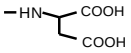
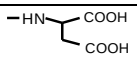
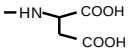
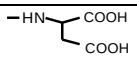
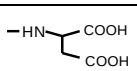
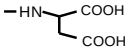
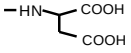
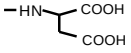
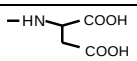
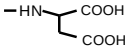
[f] Assignment: δ/ppm:

1. Copolyamidoamines containing D,L aspartic acid (Asp): Scheme 4.7 shows their synthesis. Table 4.19 shows the composition of copolyamidoamines with aliphatic solubilizing groups while those with cyclic and aromatic solubilizing groups appear in Table 4.22.



Scheme 4.7: synthesis of polyamidoamines bearing D,L-aspartic acid

Table 4.19: Copolyamidoamines with D,L-aspartic acid, bearing aliphatic solubilizing groups

Carriers	R_1	R_2	R_3	x: y: z
N1(80: 10: 10)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_3-$		8: 1: 1
N2(60: 20: 20)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_3-$		3: 1: 1
N3(60: 20: 20)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_3-$		3: 1: 1
N4(80: 10: 10)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_3-$		8: 1: 1
N5(60: 20: 20)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2-$		3: 1: 1
N6(80: 10: 10)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2-$		8: 1: 1
N7(60: 20: 20)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_2-$		3: 1: 1
N8(80: 10: 10)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$		8: 1: 1
N9(60: 20: 20)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$		3: 1: 1
N10(80: 10: 10)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$		8: 1: 1

In this table, the ratios in parentheses denote the molar percentage (x: y: z) of the respective constituents in the chains of these polymers and in all subsequent carriers and conjugates. All these copolyaspartamides were synthesized under experimental conditions similar to those used for carriers presented in Table 4.13, except that in this new synthesis referred to Scheme 4.7, APD was replaced by D,L-aspartic acid. The side chains represented by R_1, all derived from aliphatic solubilizing groups, are: DEE, DEP and DMEA. Those represented by R_2 are derived either from 1,3-diaminopropane, ethylenediamine or diethylenetriamine in reference to the Scheme 4.7, while R_3 is derived from Asp, which was used in the first step of the reaction. The conditions used were of 72 hours at 40-55° C in a slight excess (1.5 of the required stoichiometric amount) of reactant. The general principle adopted from previous

experiences allowed the use of DEE, DEP and DMEA as major reactants (x > y = z) at a temperature of 55-65° C for two days in the second step of the reaction. The diamines PDA, EDA and DET were used in the same ratio as Asp. This achievement was fulfilled by adding the mixture from the first two steps to the diamine (PDA, EDA or DET) in excess (three times the stoichiometric amount) at ice bath temperature. This reaction continued over night at the same temperature, then at ambient temperature for 48 hours. These ratios were necessary for the incorporation of the desired percentage of each reagent The product was a water-soluble solid with an inherent viscosity varying from 13 to 34 mL/g. Table 4.20 shows the experimental and viscosity results while ^{1}H NMR data appear in Table 4.21.

Table 4.20: Summary of preparative data for copolyamidoamines carriers with D, L aspartic acid as platinum binding site bearing aliphatic solubilizing groups

Reactants in feed (mol-%)[a]			Products	Reaction conditions [c]	Copolyamidoamine carriers	
R_1-NH_2	R_3-NH_2	H_2N-R_2-NH_2	Mole ratio [b] x : y : z	24h, RT ; (2) 48h, RT ; (3) 12h, 0°C then 24h RT	Yield (%)[d]	Designation
DEE (80)	Asp (12)	PDA (30)	8: 1: 1	"	67	N1(80: 10: 10)
DEE (60)	Asp (24)	PDA (60)	3: 1: 1	"	61	N2(60: 20: 20)
DEP (60)	Asp (24)	PDA (60)	3: 1: 1	"	65	N3(60: 20: 20)
DMEA (80)	Asp (12)	PDA (30)	8: 1: 1	"	54	N4(80: 10: 10)
DEE (60)	Asp (24)	EDA (60)	3: 1: 1	"	52	N5(60: 20: 20)
DEP (80)	Asp (12)	EDA (30)	8: 1: 1	"	51	N6(80: 10: 10)
DMEA (60)	Asp (24)	EDA (60)	3: 1: 1	"	50	N7(60: 20: 20)
DEE (80)	Asp (12)	DET (30)	8: 1: 1	"	73	N8(80: 10: 10)
DEP (60)	Asp (24)	DET (60)	3: 1: 1	"	59	N9(60: 20: 20)
DMEA (80)	Asp (12)	DET (30)	8: 1: 1	"	55	N10(80: 10: 10)

[a] *Parenthetic numbers denote the number of moles of amino compound per 100 base moles of poly-DL-succinimide*
DEE= 2-diethylaminoethylamine, DEP= 3-diethylaminopropylamine, DMEA= 2-dimethylaminoethylamine, Asp= D, L- aspartic acid, EDA= ethylenediamine, PDA= 1, 3-diaminopropane, DET= diethyleneTriamine
[b] *Mole ratios of hydrosolubilizing to the two drug-anchoring groups*
[c] *RT= room temperature; reaction step sequence in parentheses.*
[d] *Polymer yield after ultimate (12 000 molecular mass cut-off) dialysis.*

Table 4.21: ^{1}H NMR and viscosimetric results for copolyamidoamines with Asp for platinum-binding bearing aliphatic solubilizing groups

Copolyamidoamine carriers				Number of protons counted[d] (expected)[e] Chemical shift (ppm)				
Designation	H_{inh} mL/g [a]	x: y: z [b]	Base molecular mass[c]	δ.4.7-4.5 [f]	δ. 3.75-3.5	δ. 3.1-2.9	δ. 2.8-2.	δ. 1.8-0.9
N1(80: 10: 10)	33	8: 1: 1	2691	19(23)	-	17(18)	123(132)	45(50)
N2(60: 20: 20)	15	3: 1: 1	1341	10(13)	-	7(8)	56(62)	18(20)
N3(60: 20: 20)	13	3: 1: 1	1327	10(13)	-	7(8)	54(62)	23(26)
N4(80: 10: 10)	34	8: 1: 1	2720	20(23)	-	16(18)	142(148)	. 7(2)
N5(60: 20: 20)	14	3: 1: 1	1382	12(13)	-	7(8)	55(62)	16(18)
N6(80: 10: 10)	34	8: 1: 1	2309	20(23)	-	16(18)	127(132)	44(48)
N7(60: 20: 20)	16	3: 1: 1	1411	11(13)	-	7.5(8)	58(62)	1.8(2)
N8(80: 10: 10)	32	8: 1: 1	2467	21(23)	-	15(18)	131(134)	46(50)
N9(60: 20: 20)	15	3: 1: 1	1243	11(13)	-	7(8)	59(64)	22(26)
N10(80: 10: 10)	34	8: 1: 1	2496	2(02)3	-	16(18)	144(150)	2.2(2)

[a] At 30.0± 0.5° C, in deionized H_2O; concentration c= 2 mg/mL.

[b] Mole ratio (found) of hydrosolubilizing to drug-anchoring groups after [1] H NMR integration.

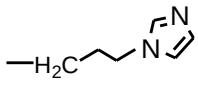
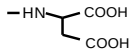
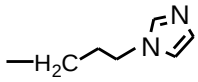
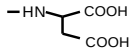
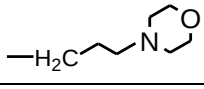
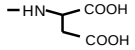
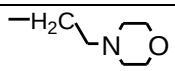
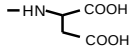
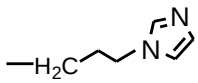
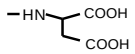
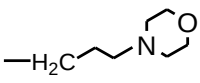
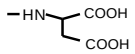
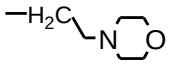
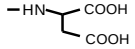
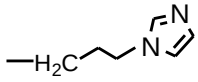
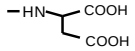
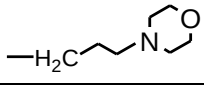
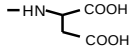
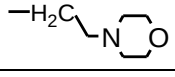
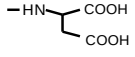
[c] Molecular MASS of the simplest recurring unit (normalized to y=z=1) rounded off to the nearest integer.

[d] In D_2O, pH 10-11, chemical shifts, δ/ppm, referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limit ± 12%. Protons are calculated (figures rounded off to the nearest integer) on basis of found x/y ratios.

[e] Expected count for composition in accordance with recurring unit (see b). [f] Assignment: δ/ppm

As mentioned before, Table 4.22 below depicts the composition of polyamidoamines with aspartic acid for platinum binding, bearing cyclic and aromatic solubilizing groups.

Table 4.22: Polyamidoamines with Aspac, bearing cyclic and aromatic solubilizing groups

Carriers	R1	R2	R3	x: y: z
N11(80: 10: 10)		-$(CH_2)_3$-		8: 1: 1
N12(60: 20: 20)		-$(CH_2)_3$-		3: 1: 1
N13(60: 20: 20)		-$(CH_2)_3$-		3: 1: 1
N14(80: 10: 10)		-$(CH_2)_3$-		8: 1: 1
N15(60: 20: 20)		-$(CH_2)_2$-		3: 1: 1
N16(80: 10: 10)		-$(CH_2)_2$-		8: 1: 1
N17(60: 20: 20)		-$(CH_2)_2$-		3: 1: 1
N18(80: 10: 10)		-$(CH_2)_2NH(CH_2)_2$-		8: 1: 1
N19(60: 20: 20)		-$(CH_2)_2NH(CH_2)_2$-		3: 1: 1
N20(80: 10: 10)		-$(CH_2)_2NH(CH_2)_2$-		8: 1: 1

All these polyamidoamines were synthesized under experimental conditions similar to those used for carriers presented in table 4.19, except that in this synthesis, the solubilizing groups are either cyclic (AEM and APM) or aromatic (API).

The products were water-soluble solids with inherent viscosities varying from 10 to 29 mL/g. Table 4.23 shows the experimental and viscosity results, while ^{1}H NMR data appear in Table 4.24.

Table 4.23: Summary of preparative data for copolyaspartamide carriers with Aspartic acid as platinum Binding site, bearing cyclic and aromatic solubilising groups

Reactants in feed (mol-%)[a]			Products	Reaction conditions [c]	Copolyamidoamine carriers	
R_1-NH_2	R_3-NH_2	H_2N-R_2-NH_2	Mole ratio [b] x : y : z	(1) 24h, RT ; (2) 48h, RT ; (3) 12h, 0^oC then 24h RT	Yield (%)[d]	Designation
API (80)	Asp (12)	PDA (30)	8: 1: 1	"	67	N11(80: 10: 10)
API (60)	Asp (24)	PDA (60)	3: 1: 1	"	61	N12(60: 20: 20)
APM (60)	Asp (24)	PDA (60)	3: 1: 1	"	65	N13(60: 20: 20)
AEM (80)	Asp (12)	PDA (30)	8: 1: 1	"	54	N14(80: 10: 10)
API (60)	Asp (24)	EDA (60)	3: 1: 1	"	52	N15(60: 20: 20)
APM (80)	Asp (12)	EDA (30)	8: 1: 1	"	51	N16(80: 10: 10)
AEM (60)	Asp (24)	EDA (60)	3: 1: 1	"	50	N17(60: 20: 20)
API (80)	Asp (12)	DET (30)	8: 1: 1	"	73	N18(80: 10: 10)
APM (60)	Asp (24)	DET (60)	3: 1: 1	"	59	N19(60: 20: 20)
AEM (80)	Asp (12)	DET (30)	8: 1: 1	"	55	N20(80: 10: 10)

[a] *Parenthetic numbers denote the number of moles of amino compound per 100 base moles of poly-DL-succinimide*
AEM= 4- (2-aminoethyl)-morpholine, API= 1- (3-aminopropyl)-imidazol, APM= 4- (3-aminopropyl) morpholine, Asp= D, L- Aspartic acid, EDA= ethylenediamine, PDA= 1, 3-diaminopropane, DET= diethyleneTriamine
[b] *Mole ratios of hydrosolubilizing to the two drug-anchoring groups*
[c] *RT= room temperature; reaction step sequence in parentheses.*
[d] *Polymer yield after ultimate (12 000 molecular weight cut-off) dialysis.*

Table 4.24: ^{1}H NMR and viscometric results for coplyaspartamidess with APD for platinum-binding and aliphatic solubilizing groups

Copolyamidoamine carriers				Number of protons counted[d] (expected)[e] Chemical shift (ppm)				
Designation	η_{inh} (mL/g)[a]	x:y:z [b]	Base molecular mass[c]	δ.8.5-4.5 [f]	δ. 3.75-3.5	δ. 3.4-3.0	δ. 2.9-2.0	δ. 1.8-1.4
N11(80:10:10)	28	8: 1: 1	2763	42(45)	-	18(20)	81(84)	31(34)
N12(60:20:20)	12	3: 1: 1	1368	19(20)	-	8(10)	42(44)	12(14)
N13(60:20:20)	11	3: 1: 1	1354	9.6(11)	8(9)	19(22)	41(44)	12(14)
N14(80:10:10)	29	8: 1: 1	2792	19(21)	22(24)	48(52)	81(84)	31(34)
N15(60:20:20)	11	3: 1: 1	1425	18(20)	-	9(10)	41(44)	10(12)
N16(80:10:10)	28	8: 1: 1	2901	18(21)	22(24)	47(52)	79(82)	22(24)
N17(60:20:20)	10	3: 1: 1	1454	9(11)	8(9)	20(22)	40(44)	7(8)
N18(80:10:10)	29	8: 1: 1	2803	42(45)	-	18(20)	83(86)	32(34)
N19(60:20:20)	12	3: 1: 1	1369	10(11)	10(9)	20(22)	43(46)	12(14)
N20(80:10:10)	26	8: 1: 1	2832	19(21)	21(24)	49(52)	82(86)	31(34)

[a] At 30.0± 0.5° C, in deionized H_2O; concentration c= 2 mg/mL.

[b] Mole ratio (found) of hydrosolubilizing to drug-anchoring groups after ^{1} H NMR integration.

[c] Molecular mass of the simplest recurring unit (normalized to y=z=1) rounded off to the nearest integer.

[d] In D_2O, pH 10-11, chemical shifts, δ/ppm, referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limit ± 12%. Protons are calculated (figures rounded off to the nearest integer) on basis of found x:y:z ratios.

[e] Expected count for composition in accordance with recurring unit (see b).

[f] Assignment: δ/ppm:

4.2 Polymer-drug conjugates

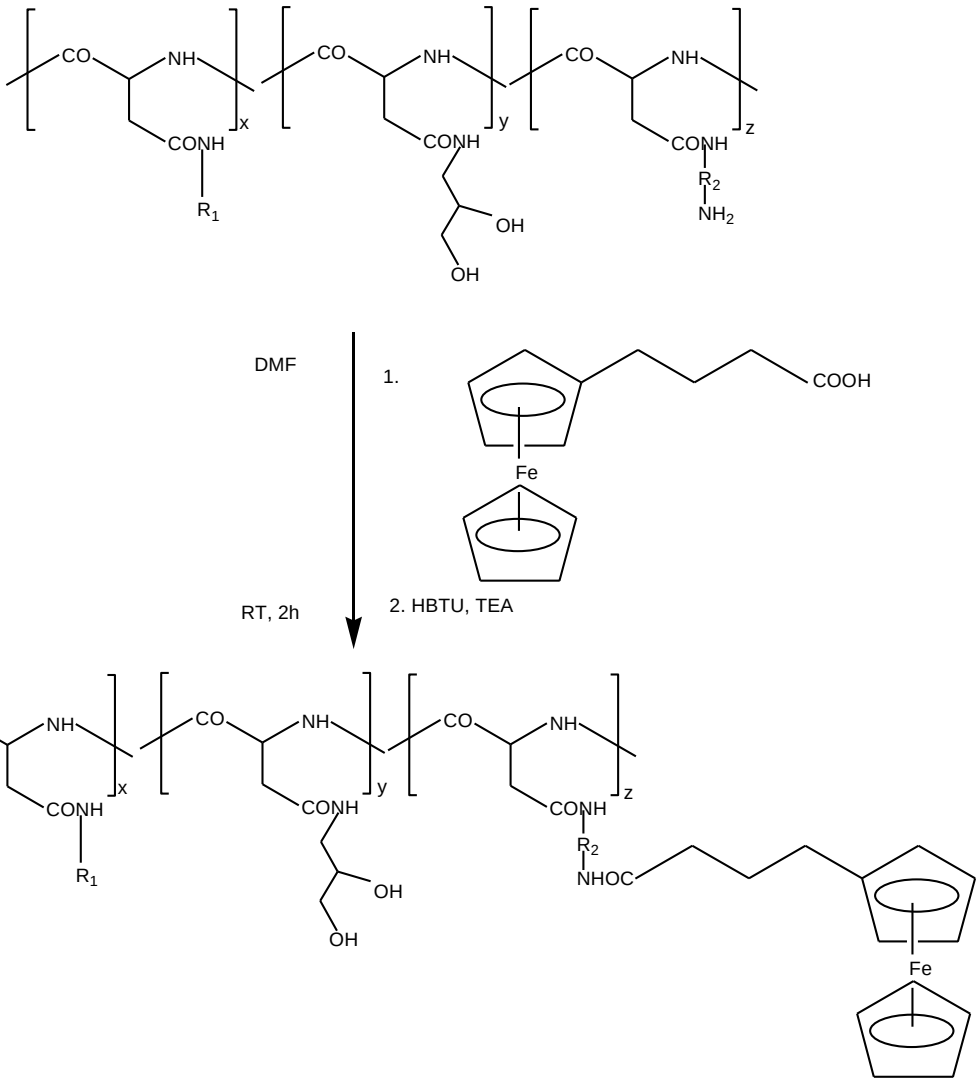
Each polyamide carrier involved in this work has been designed with a possibility to the anchoring of the following three selected drugs: platinum, methotrexate and ferrocene. Platinum was polymer-bound (conjugated) through chelation with carrier-attached pairs of carboxyl or hydroxyl functionalities, acting as leaving group ligands upon metal coordination. Methotrexate and ferrocene were attached to our polymeric structures by the expediency of amide bonds mediated by a coupling agent (HBTU). In respect to the title of this work, (macromolecular platinum-based anticancer agents) platinum was the drug common to all our synthesized anticancer agents, and the second drug imparted in the conjugate was either methotrexate or ferrocene, hence the resulting co-conjugates should enclose two different drugs. Both the copolyaspartamides and polyamidoamines belonging to the big group of our polyaspartamides have been involved in the conjugation work. From the three drugs selected in this study, ferrocene is the most demanding for its oxidative chemical property, followed by methotrexate.The anchoring of both of these two drugs goes through various steps, which is not the case with platinum as it is the less demanding with a straight forward anchoring technique.

4.2.1 Polymer-ferrocene conjugates

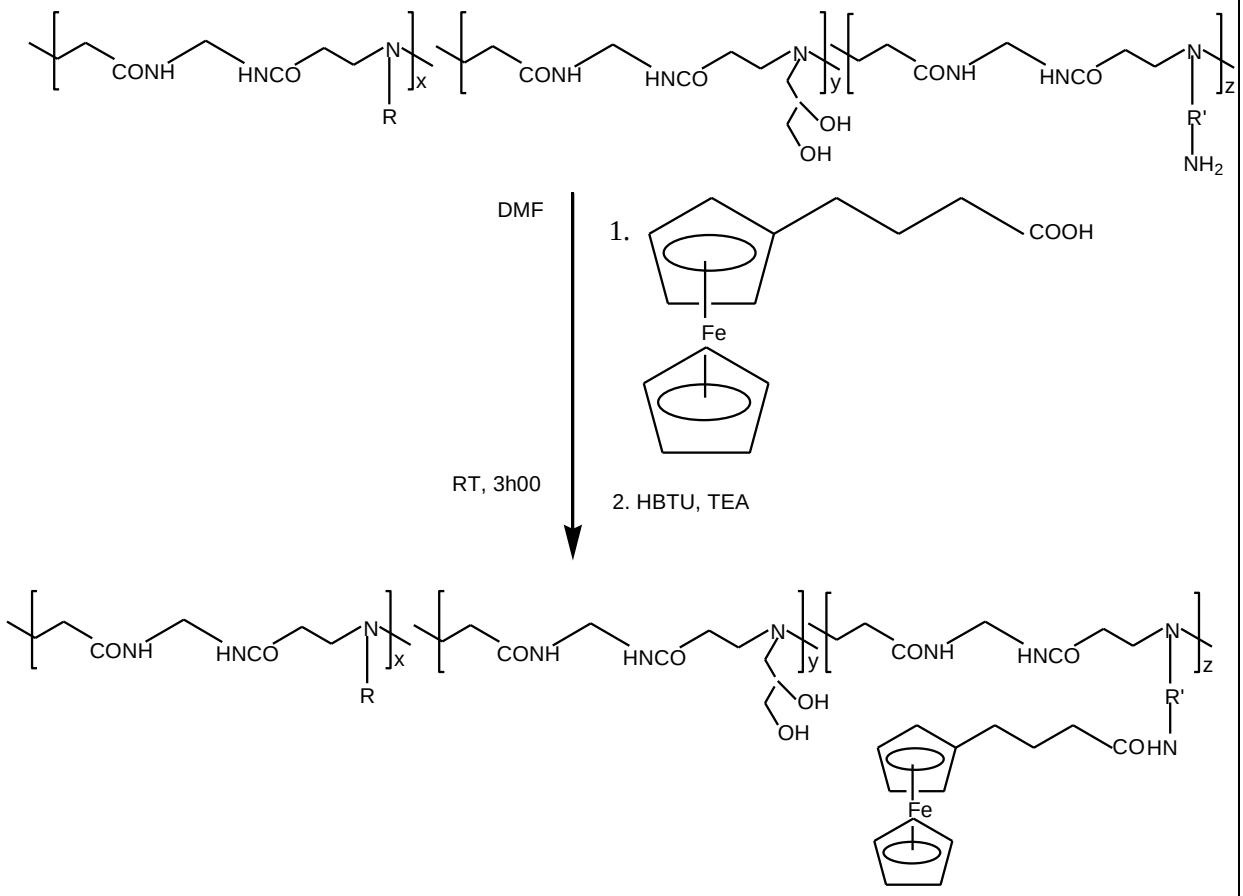
Various examples concerning ferrocene anchoring, involving the covalent reversible attachment of the carrier through amide bond formation are available in the literature. In this work, the ferrocene derivative of our study was 4-ferrocenylbutanoic acid. Swarts and co-workers [220] reported that this drug presents a low reduction potential, which motivates its choice. Its preparative method is also described in the literature [221]. This study presents

ferrocene conjugates of both our copolyaspartamide and polyamidoamine carriers featuring primary amino side chain terminals with biofissionable amide link. In previous studies, this laboratory has been investigating two different ferrocene anchoring methods. The first one involves a direct acid-amine coupling with mediation of *2*(*1H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium fluorophosphates (HBTU) as coupling agent, while the second method involves the coupling *via* N-succinimidyl-4-ferrocenylbutanoate as an active N-succinimide ester of the ferrocene compound. This active ester is prepared from the 4-ferrocenylbutanoic acid and N-hydroxysuccinimide (HSU) in the presence of dicyclohexylcarbodiimide (DCC) in ethyl acetate medium.

In this work, we are describing the first method which proceeds in DMF as solvent for copolyaspartamide (Scheme 4.8) and in aqueous medium for polyamidoamine-based conjugates (Scheme 4.9). The resulting conjugates were obtained after a treatment of primary amine-functionalized carriers of both copolyaspartamide and polyamidoamine types with 1.4 molar equivalents of the ferrocenylbutanoic acid in the presence of 1.3 equivalents of HBTU and 2 equivalents of organic base (triethylamine, TEA) at pH 7 or higher as shown in Schemes 4.8 and 4.9



Scheme 4.8: Coupling of copolyaspartamide carriers with ferrocene



Scheme 4.9: Coupling of copolyamidoamine carriers with ferrocene

In all conjugates prepared according to the two schemes above, primary amine functional groups have been completely acylatated over 2h. The resulting structure was a water-soluble conjugate, precipitated from reaction medium with a mixture of hexane-acetone in the ratio of 2:1 for copolyaspartamide conjugates and with acetone for polyamidoamine conjugates, then purified by exclusion chromatography and exhaustive dialysis before freeze-drying in the solid state. The series of

copolyaspartamide carriers from K11 (80: 10: 10) to K20 (60: 20: 20) and the series of copolyamidoamine carriers from M11 (80: 10: 10) to M20 (60: 20: 20) previously presented in this work were selected for this achievement. Composition data for copolysapartamide-based ferrocene conjugates are presented in Table 4.25 while the experimental conditions are depicted in Table 4.26. The ^{1}H NMR data and viscometric results are summarized in Table 4.27. The analytical data are presented in Table 4.28, whereas for polyamidoamine-based conjugates, they are given in Tables 4.29, 4.30, 4.31 and 4.32.

Table 4.25: Composition of copolyaspartamide-based ferrocene conjugates prepared by HBTU-mediated coupling method

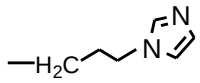
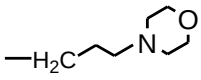
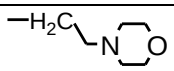
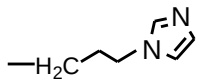
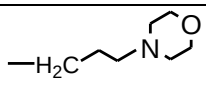
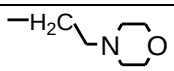
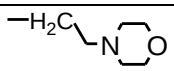
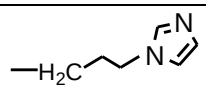
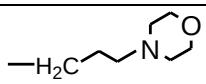
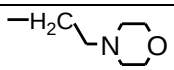
Carriers	R_1	R_2	R_3	Conjugates designation
K11 (80: 10: 10)		-$(CH_2)_3$-	-$CH_2CHOHCH_2OH$	**K11-Fc**
K12 (60: 20: 20)		-$(CH_2)_3$-	-$CH_2CHOHCH_2OH$	**K12-Fc**
K13 (80: 10: 10)		-$(CH_2)_3$-	-$CH_2CHOHCH_2OH$	**K13-Fc**
K14 (60: 20: 20)		-$(CH_2)_2$-	-$CH_2CHOHCH_2OH$	**K14-Fc**
K15 (60: 20: 20)		-$(CH_2)_2$-	-$CH_2CHOHCH_2OH$	**K15-Fc**
K16 (80: 10: 10)		-$(CH_2)_2$-	-$CH_2CHOHCH_2OH$	**K16-Fc**
K17 (60: 20: 20)		-$(CH_2)_2$-	-$CH_2CHOHCH_2OH$	**K17-Fc**
K18 (80; 10: 10)		-$(CH_2)_2NH$-$(CH_2)_2$-	-$CH_2CHOHCH_2OH$	**K18-Fc**
K19 (60; 20: 20)		-$(CH_2)_2NH$-$(CH_2)_2$-	-$CH_2CHOHCH_2OH$	**K19-Fc**
K20 (60: 20: 20)		-$(CH_2)_2NH$-$(CH_2)_2$-	-$CH_2CHOHCH_2OH$	**K20-Fc**

Table 4.26: Experimental data for the synthesis of copolyaspartamide-based ferrocene conjugates

Reactants in feed				**Reactions Conditions**[b]	**Fc (HBTU) conjugates**	
Carrier designation	**Coupling agent**	**Medium**	**Carrier:drug:coupling agent ratio (**mol-%) [a]		**Yield** (%) [c]	**Designation**
K11 (80: 10: 10)	HBTU	DMF	1: 1.2: 1.1	2h at RT	63	**K11-Fc**
K12 (60: 20: 20)	"	"	"	"	56	**K12-Fc**
K13 (80: 10: 10)	"	"	"	"	54	**K13-Fc**
K14 (60: 20: 20)	"	"	"	"	58	**K14-Fc**
K15 (60: 20: 20)	"	"	"	"	61	**K15-Fc**
K16 (80: 10: 10)	"	"	"	"	61	**K16-Fc**
K17 (60: 20: 20)	"	"	"	"	60	**K17-Fc**
K18 (80; 10: 10)	"	"	"	"	57	**K18-Fc**
K19 (60; 20: 20)	"	"	"	"	61	**K19-Fc**
K20 (60: 20: 20)	"	"	"	"	58	**K20-Fc**

[a] *Molar ratio of carrier repeating unit to Fc to coupling agent.*

[b] *RT= room temperature.*

[c] *Conjugate yield after size exclusion chromatography and (12 000 molecular weight cut-off) dialysis.*

Table 4.27: [1] H NMR and viscometric data for copolyaspartamide-based ferrocene conjugates

Conjugate designation	**η** $_{inh}$ mL/g [a]	**x: y: z** [b]	**Base molecular mass** [c]	% **Fe** Calcd [d]	% **Fe** NMR[e]	**Protons counted** [f] (expected) [g] **chemical shift** (ppm)	
						δ. 4.5-4.1 [h]	δ.1.9-1.4
K11-Fc	28	8: 1: 1	2389	2.3	2.2	8.5(9)	33(36)
K12-Fc	16	3: 1: 1	1336	4.2	4.1	8.8(9)	17(16)
K13-Fc	28	8: 1: 1	2429	2.3	2.2	8.5(9)	21(20)
K14-Fc	16	3: 1: 1	1265	4.4	4.4	9(9)	13.2(14)
K15-Fc	17	3: 1: 1	1322	4.2	4.2	9(9)	16(14)
K16-Fc	29	8: 1: 1	2415	2.3	2.3	8.9(9)	20(20)
K17-Fc	18	3: 1: 1	1280	4.4	4.4	9(9)	8.4(8)
K18-Fc	29	8: 1: 1	2418	2.3	2.2	8.6(9)	25.8(24)
K19-Fc	17	3: 1: 1	1351	4.1	4.0	8.7(9)	11(14)
K20-Fc	17	3: 1: 1	1323	4.2	4.2	9(9)	9.2(8)

[a] At 30±0.5°C, in deionized H_2O; concentration c=2mg/mL

[b] Mole ratio (found) of hydrosolubilizing to platinum to drug anchoring groups after ^{1}H NMR integration.

[c] Molecular weight, actual (in parenthesis, calculated for 100% acylation)

[d] Derived from 100% acylation

[e] Derived from H NMR spectrum (error limit± 12%).

[f] In D_2O, p H 10-11,chemical shifts, δ/ppm,referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limit± 12%. Protons are calculated for the structural representations in Table 4.27.

[g] Expected count for composition in accordance with recurring unit (see b)

[h] Proton assignement, δ/ppm:

Table 4.28: Summary of analytical data for copolyaspartamide-based ferroce conjugates

Conjugate designation	**η_{inh}** (mL/g)[a]	**% Fe** Calcd [b]	**% Fe** NMR [c]	**NH_2 acylation** % NMR [d]
K11-Fc	28	2.3	2.2	95
K12-Fc	16	4.2	4.1	98
K13-Fc	28	2.3	2.2	94
K14-Fc	16	4.4	4.4	100
K15-Fc	17	4.2	4.2	100
K16-Fc	29	2.3	2.3	99
K17-Fc	18	4.4	4.4	100
K18-Fc	29	2.3	2.2	96
K19-Fc	17	4.1	4.0	97
K20-Fc	17	4.2	4.2	100

[a] *At 30±0.5°C, in deionized H_2O; concentration c=2mg/mL*
[b] *Derived from 100% acylation*
[c] *Derived from ^1H NMR spectrum (error limit± 12%)*
[d] *Ratio of c to b.*

Table 4.29: Composition of copolyamidoamine-ferrocene conjugates prepared by HBTU-mediated coupling method

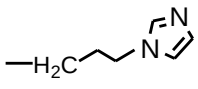
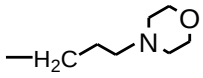
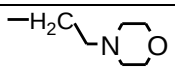
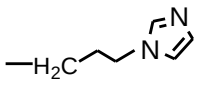
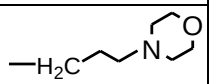
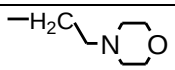
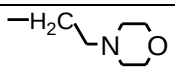
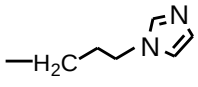
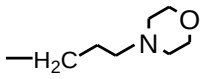
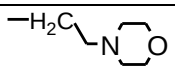
Carriers	R_1	R_2	R_3	Conjugate designation
M11 (80: 10: 10)		$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	**M11-Fc**
M12 (60: 20: 20)		$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	**M12-Fc**
M13 (80: 10: 10)		$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	**M13-Fc**
M14 (80: 10: 10)		$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**M14-Fc**
M15 (60: 20: 20)		$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**M15-Fc**
M16 (80: 10: 10)		$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**M16-Fc**
M17 (60: 20: 20)		$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**M17-Fc**
M18 (80: 10: 10)		$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**M18-Fc**
M19 (80: 10: 10)		$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**M19-Fc**
M20 (60: 20: 20)		$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**M20-Fc**

Table 4.30: Experimental data for the synthesis of copolyamidoamine-based ferrocene conjugates

Reactants in feed				**Reaction Conditions**[b]	**Fc (HBTU) conjugates**	
Carrier designation	**Coupling agent**	**Medium**	**Carrier:drug:coupling agent ratio (**mol-%) [a]		**Yield** (%) [c]	**Designation**
M11 (80: 10: 10)	HBTU	DMF	1: 1.2: 1.1	2h at RT	63	**M11-Fc**
M12 (60: 20: 20)	"	"	"	"	56	**M12-Fc**
M13 (80: 10: 10)	"	"	"	"	54	**M13-Fc**
M14 (80: 10: 10)	"	"	"	"	58	**M14-Fc**
M15 (60: 20: 20)	"	"	"	"	61	**M15-Fc**
M16 (80: 10: 10)	"	"	"	"	61	**M16-Fc**
M17 (60: 20: 20)	"	"	"	"	60	**M17-Fc**
M18 (80; 10: 10)	"	"	"	"	57	**M18-Fc**
M19 (80: 10: 10)	"	"	"	"	61	**M19-Fc**
M20 (60: 20: 20)	"	"	"	"	58	**M20-Fc**

[a] *Molar ratio of carrier repeating unit to Fc to coupling agent.*
[b] *RT= room temperature.*
[c] *Conjugate yield after size exclusion chromatography and (12 000 molecular weight cut-off) dialysis.*

Table 4.31: ^{1}H NMR and viscometric data for Polyamidoamine-based ferrocene conjugates

Conjugate designation	**η_{inh}** (mL/g)[a]	**x: y: z** [b]	**Base molecular mass** [c]	% **Fe** Calcd [d]	% **Fe** NMR [e]	**Protons counted** [f] (expected) [g] **chemical shift** (ppm)	
						δ.4.5-4.1 [h]	δ.1.8-0.9
M11-Fc	19	8: 1: 1	2959	1.9	1.7	8(9)	30(34)
M12-Fc	13	3: 1: 1	1621	3.5	3.1	8(9)	37(40)
M13-Fc	20	8: 1: 1	2999	1.9	1.6	8(9)	7(18)
M14-Fc	19	8: 1: 1	2945	1.9	1.9	9(9)	29(34)
M15-Fc	12	3: 1: 1	1607	3.5	3.3	8.5(9)	10(12)
M16-Fc	19	8: 1: 1	2985	1.9	1.8	8.7(9)	14(16)
M17-Fc	12	3: 1: 1	1593	3.5	3.3	8.5(9)	11(14)
M18-Fc	20	8: 1: 1	3140	1.8	1.7	8.6(9)	29(34)
M19-Fc	21	8: 1: 1	3028	1.8	1.7	8.3(9)	15(18)
M20-Fc	13	3: 1: 1	1608	3.5	3.3	8.6(9)	7(8)

[a] *At 30.0± 0.5° C, in deionized H_2O; concentration c= 2 mg/mL.*
[b] *Mole ratio (found) of hydrosolubilizing to drug-anchoring groups after ^{1}H NMR integration.*
[c] *Molecular mass of the simplest recurring unit (normalized to x>y=z=1) rounded off to the nearest integer.*
[d] *Derived from 100% acylation*
[e] *Derived from ^{1}H NMR spectrum (error limit± 12%)*
[f] *In D_2O, p H 10-11,chemical shifts, δ/ppm,referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limit± 12%. Protons are calculated for the structural representations in Table 4.27.*
[g] *Expected count for composition in accordance with recurring unit (see b)*
[h] *Proton assignement, δ/ppm:*

Table 4.32: Summary of analytical data for copolyamidoamine-based ferroce conjugates

Conjugate designation	**η_{inh}** (mL/g) [a]	**% Fe** Calcd [b]	**% Fe** NMR [c]	**NH_2 acylation** % NMR [d]
M11-Fc	19	1.9	1.7	89
M12-Fc	13	3.5	3.1	90
M13-Fc	20	1.9	1.6	88
M14-Fc	19	1.9	1.9	100
M15-Fc	12	3.5	3.3	95
M16-Fc	19	1.9	1.8	97
M17-Fc	12	3.5	3.3	95
M18-Fc	20	1.8	1.7	96
M19-Fc	21	1.8	1.7	92
M20-Fc	13	3.5	3.3	96

[a] *At 30±0.5°C, in deionized H_2O; concentration c=2mg/mL*
[b] *Derived from 100% acylation*
[c] *Derived from ^1H NMR spectrum (error limit± 12%)*
[d] *Ratio of c to b.*

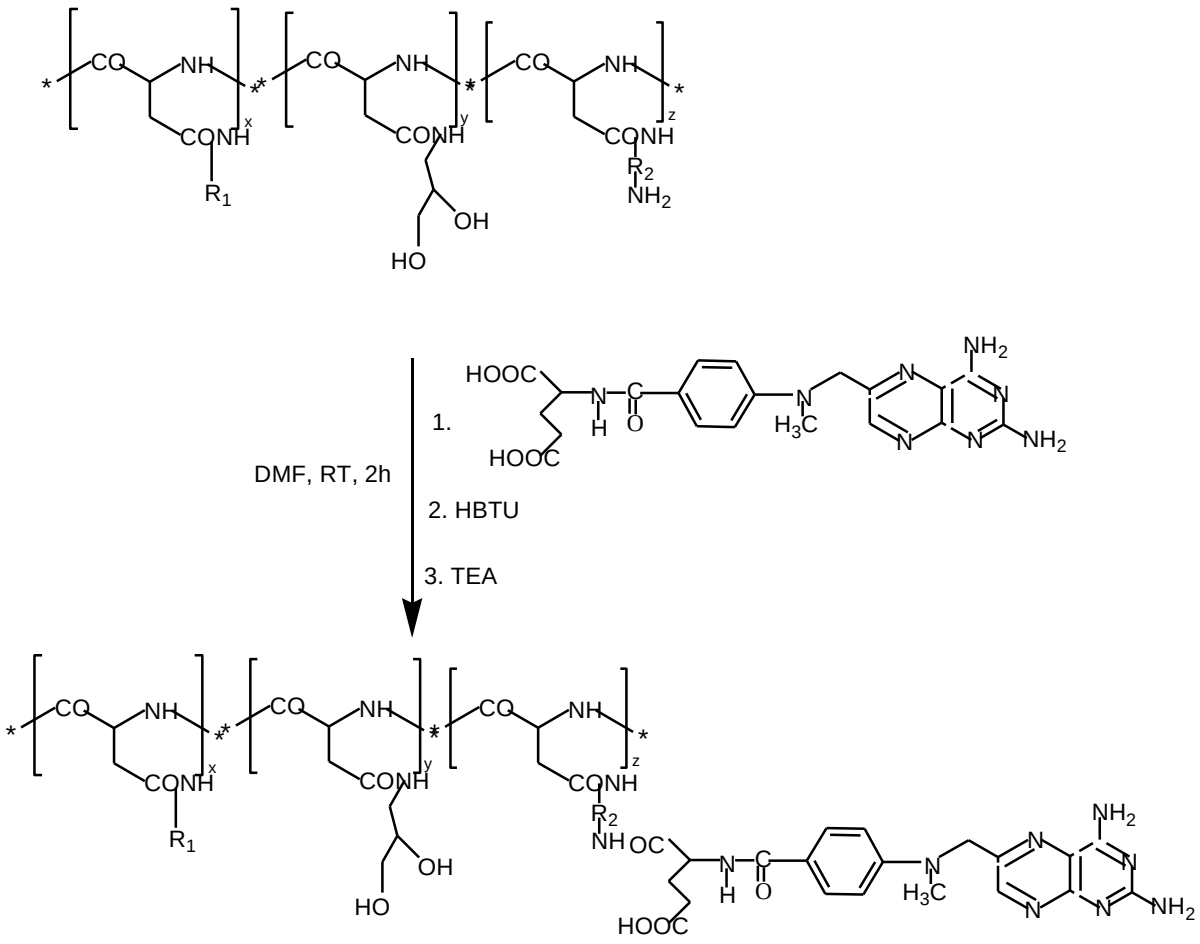
4.2.2 Polymer-methotrexate conjugates

Numerous examples concerning MTX anchoring are available in the literature. They all result in an amide bond formation after a covalent reversible attachment of MTX to the carrier. One can anchor MTX to a carrier with the aid of a water-soluble carbodiimide. The carbodiimide commonly used in previous works from this laboratory is 1-ethyl-3-(3'-dimethylaminopropyl) carbodiimide hydrochloride [222, 223]. It has been serving in the coupling process of proteinaceous carriers with MTX in aqueous phase. A second coupling method concerns the use of an active N-succinimide ester, prepared *in situ* while the reaction is taking place in anhydrous medium such as N,N'-dimethylformamide. This method presents, due to the presence of bifunctionally active drug molecules, the inconvenience to cause gradual crosslinking of the conjugate when the coupling agent is used in higher mole ratios. Therefore, the preferred method used here is the direct acid-amine coupling in DMF through the intermediacy of HBTU (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate). In this MTX coupling work, the carriers were those selected from copolyaspartamides (K11-K20) and copolyamidoamines (M11-M20) for the coupling of ferrocene in section 4.2.1, with the same organic base (TEA), coupling agent (HBTU), molar ratio MTX: NH_2 (1.2) and solvent (DMF). These experimental conditions allowed for the achievement of a percentage of MTX incorporation in the carrier approaching 100%, if not 100% in the majority of cases. It is important to note that though the structure of MTX presents two carboxylic groups, the literature reports that during the binding process, preference is given to the α-carboxyl group as the most reactive [224]. Although steric factors seem to suggest a different order of reactivity of the two carboxyls of MTX, the literature reports once again suggest that during the formation of the complex MTX-dihydrofolate

reductase, the bond engages the drug's α-carboxyl group [225]. With all these considerations, we focused our attention to the synthesis of copolyaspartamide- and copolyamidoamine-based MTX conjugates.

4.2.2.1 Copolyaspartamide-based MTX conjugates

They were prepared according to Scheme 4.10 by a direct acid-base coupling, HBTU mediated process. The experimental section describes largely the procedure leading to their synthesis. Carriers K1-K10 previously presented in this work were involved in this anchoring task. The resulting conjugates were all water-soluble, had an average yield (in %) varying from 52-57% and an inherent viscosity varying from 24 to 39 mL/g. Table 4.33 shows their composition, while Tables 4.34 and 4.35 present, respectively, their experimental data and their ^{1}H NMR data and viscosity results. Their analytical data appear in Table 4.36.



Scheme 4.10: Synthesis of copolyaspartamide-MTX conjugates

Table 4.33: Composition of copolyaspartamide-MTX conjugates prepared by HBTU-mediated coupling method

Carriers	R_1	R_2	R_3	Conjugate designation
K1(60: 20: 20)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	**K1-MTX**
K2(60: 20: 20)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	**K2-MTX**
K3(80: 10: 10)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	**K3-MTX**
K4(80: 10: 10)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**K4-MTX**
K5(80: 10: 10)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**K5-MTX**
K6(60: 20: 20)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**K6-MTX**
K7(80: 10: 10)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**K7-MTX**
K8(60: 20: 20)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**K8-MTX**
K9(60: 20: 20)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**K9-MTX**
K10(80:10:10)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**K10-MTX**

Table 4.34: Experimental data for the synthesis of Copolyaspartamide-based MTX conjugates

Reactants in feed				**Reaction Conditions**[b]	**MTX (HBTU) conjugates**	
Carriers designation	**Coupling agent**	**Medium**	**Carrier:drug:coupling agent ratio (**mol-%) [a]		**Yield** (%) [c]	**Designation**
K1(60: 20: 20)	HBTU	DMF	1: 1.2: 1.1	2h at RT	55	**K1-MTX**
K2(60: 20: 20)	"	"	"	"	57	**K2-MTX**
K3(80: 10: 10)	"	"	"	"	54	**K3-MTX**
K4(80: 10: 10)	"	"	"	"	52	**K4-MTX**
K5(80: 10: 10)	"	"	"	"	52	**K5-MTX**
K6(60: 20: 20)	"	"	"	"	57	**K6-MTX**
K7(80: 10: 10)	"	"	"	"	55	**K7-MTX**
K8(60: 20: 20)	"	"	"	"	52	**K8-MTX**
K9(60: 20: 20)	"	"	"	"	53	**K9-MTX**
K10(80: 10: 10)	"	"	"	"	54	**K10-MTX**

[a] *Molar ratio of carrier repeating unit to Fc to coupling agent.*
[b] *RT= room temperature.*
[c] *Conjugate yield after size exclusion chromatography and (12 000 molecular weight cut-off) dialysis.*

Table 4.35: ^{1}H NMR and viscometric data for copolyaspartamide-based MTX conjugate

Conjugate designation	η_{inh} (mL/ g) [a]	**x: y: z** [b]	**Base molecular mass** [c]	% **MTX** Cald [d]	% **MTX** NMR [e]	**Protons counted** [f] (expected) [g] **chemical shift** (ppm)	
						δ.8.5-6.5 [h]	δ. 1.8-1.5
K1-MTX	24	(3: 1: 1)	1437	30.4	30.4	5(5)	4(4)
K2-MTX	27	(3: 1: 1)	1478	29.6	29.6	5(5)	10(10)
K3-MTX	38	(8: 1: 1)	2276	19.2	19.2	5(5)	4(4)
K4-MTX	39	(8: 1: 1)	2486	17.6	17.2	4.9(5)	2(2)
K5-MTX	36	(8: 1: 1)	2598	16.8	16.8	5(5)	2(2)
K6-MTX	24	(3: 1: 1)	1464	29.9	28.7	4.8(5)	2(2)
K7-MTX	35	(8: 1: 1)	2262	19.3	19.3	5(5)	2(2)
K8-MTX	25	(3: 1: 1)	1463	29.9	29.9	5(5)	1 9(20)
K9-MTX	25	(3: 1: 1)	1506	28.9	28.7	4.9(5)	24(26)
K10-MTX	37	(8: 1: 1)	2305	19.0	18.6		

[a] *At 30.0± 0.5° C, in deionized H_2O; concentration c= 2 mg/mL.*

[b] *Mole ratio (found) of hydrosolubilizing to drug-anchoring groups after [1] H NMR integration.*

[c] *Molecular mass of the simplest recurring unit (normalized to x>y=z=1) rounded off to the nearest integer.*

[d] *Derived from 100% acylation*

[e] *Derived from ^{1}H NMR spectrum (error limit± 12%)*

[f] *In D_2O, p H 10-11,chemical shifts, δ/ppm,referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limit± 12%. Protons are calculated for the structural representations in Table 4.27.*

[g] *Expected count for composition in accordance with recurring unit (see b)*

[h] *Proton assignement, δ/ppm:*

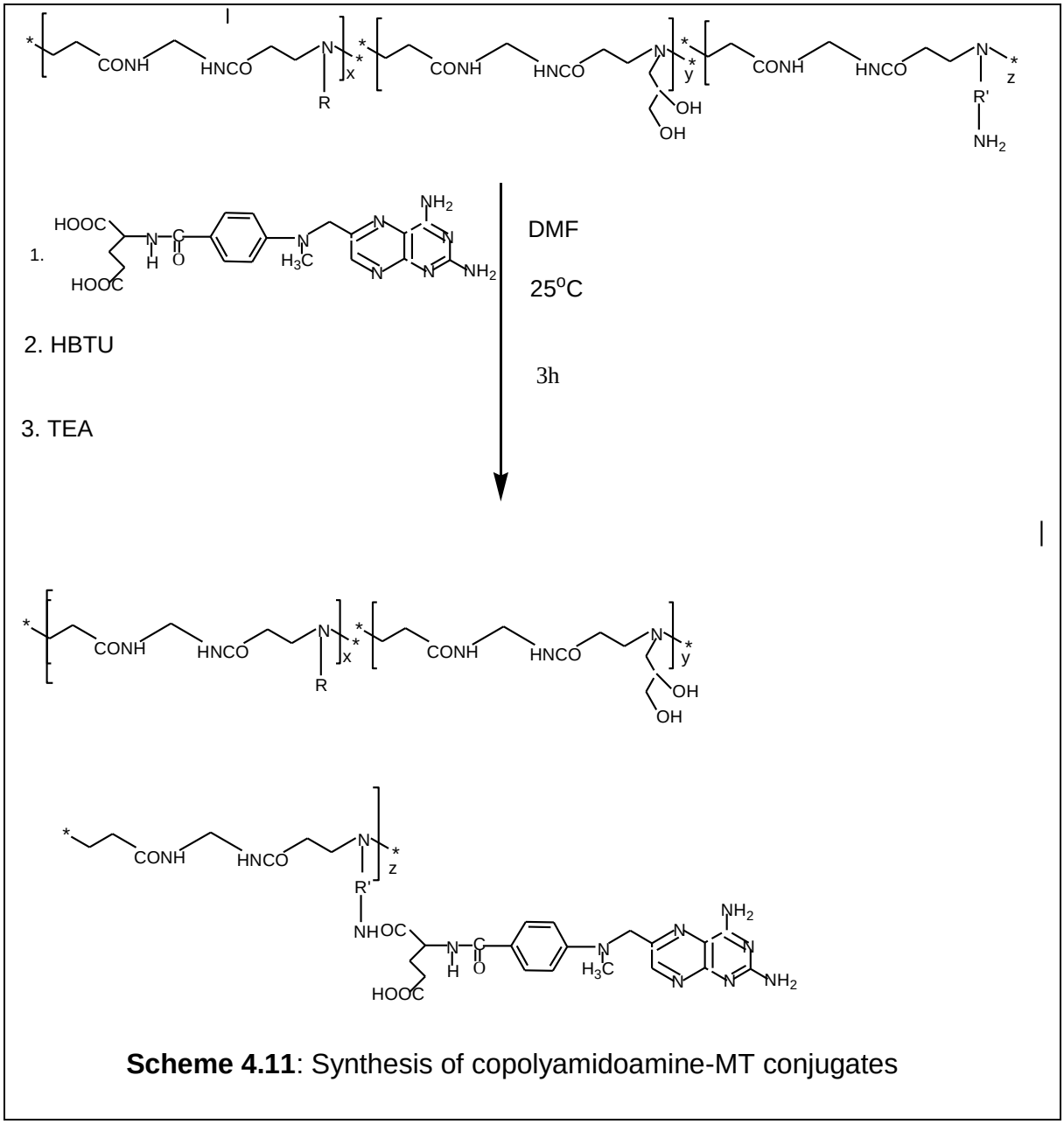
Table 4.36: Summary of analytical data for copolyaspartamide-based MTX conjugates

Conjugate designation	**η** $_{inh}$ (mL/g) [a]	**% MTX** Calcd [b]	**% MTX** NMR [c]	**NH_2 acylation** % NMR [d]
K1-MTX	24	30.4	30.4	100
K2-MTX	27	29.6	29.6	100
K3-MTX	38	19.2	19.2	100
K4-MTX	39	17.6	17.2	98
K5-MTX	36	16.8	16.8	100
K6-MTX	24	29.9	28.7	96
K7-MTX	35	19.3	19.3	100
K8-MTX	25	29.9	29.9	100
K9-MTX	25	28.9	28.7	99
K10-MTX	37	19.0	18.6	98

[a] *At 30±0.5°C, in deionized H_2O; concentration c=2mg/mL*
[b] *Derived from 100% acylation*
[c] *Derived from ^1H NMR spectrum (error limit± 12%)*
[d] *Ratio of c to b.*

4.2.2.2 Polyamidoamine-based MTX conjugates

Scheme 4.11 depicts their synthetic procedure, which is given in more detail in the experimental chapter of this study. Polyamidoamine carriers M1-M10 previously mentioned were selected for this purpose. The resulting conjugates are all water-soluble and appeared in a mass percentage going from 32 to 39% and an inherent viscosity varying from 12 to 23 mL/g. Tables 4.37 and 4.38 present, respectively, their composition and experimental results. Their ^{1}H NMR data and viscosities are shown in Table 4.39, while their analytical data appear in Table 4.4



Scheme 4.11: Synthesis of copolyamidoamine-MT conjugates

Table 4.37: Composition of copolyamidoamine-MTX conjugates prepared by HBTU-mediated coupling method

Carriers	R_1	R_2	R_3	Conjugate designation
M1(60:20:20)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	**M1-MTX**
M2(80:10:10)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	**M2-MTX**
M3(60:20:20)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_3-$	$-CH_2CHOHCH_2OH$	**M3-MTX**
M4(80:10:10)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**M4-MTX**
M5(60:20:20)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**M5-MTX**
M6(80:10:10)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**M6-MTX**
M7(60:20:20)	$-CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**M7-MTX**
M8(80:10:10)	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**M8-MTX**
M9(80:10:10)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**M9-MTX**
M10(60:20:20)	$-CH_2CH_2N(CH_3)_2$	$-(CH_2)_2NH-(CH_2)_2-$	$-CH_2CHOHCH_2OH$	**M10-MTX**

Table 4.38: Experimental data for the synthesis of copolyamidoamine-based MTX conjugates

Reactants in feed				Reaction Conditions[b]	MTX (HBTU) conjugates	
Carriers designation	**Coupling agent**	**Medium**	**Carrier:drug:coupling agent ratio (**mol %) [a]		**Yield** (%) [c]	**Designation**
M1(60: 20: 20)	HBTU	DMF	1: 1.2: 1.1	2h at RT	35	**M1-MTX**
M2(80: 10: 10)	"	"	"	"	37	**M2-MTX**
M3(60: 20: 20)	"	"	"	"	38	**M3-MTX**
M4(80: 10: 10)	"	"	"	"	37	**M4-MTX**
M5(60: 20: 20)	"	"	"	"	32	**M5-MTX**
M6(80: 10: 10)	"	"	"	"	32	**M6-MTX**
M7(60: 20: 20)	"	"	"	"	38	**M7-MTX**
M8(80: 10: 10)	"	"	"	"	39	**M8-MTX**
M9(80: 10: 10)	"	"	"	"	34	**M9-MTX**
M10 (60: 20: 20)	"	"	"	"	36	**M10-MTX**

[a] *Molar ratio of carrier repeating unit to Fc to coupling agent.*
[b] *RT= room temperature.*
[c] *Conjugate yield after size exclusion chromatography and (12 000 molecular weight cut-off) dialysis.*

Table 4.39: ^{1}H NMR and viscometric data for copolyamidoamine-based MTX conjugates

Conjugate designation	**η_{inh}** (mL*l*g) [a]	**x: y: z** [b]	**Base molecular mass** [c]	% **MTX** Calcd [d]	% **MTX** NMR [e]	**Protons counted** [f] (expected) [g] **chemical shift** (ppm) δ.8.5-6.5 [h]	δ. 1.8-0.9
M1-MTX	12	3: 1: 1	1720	25.4	22.8	4.5(5)	37(40)
M2-MTX	23	8: 1: 1	2702	16.1	14.4	4.5(5)	62(68)
M3-MTX	12	6: 1: 1	1635	26.7	24.1	9(5)	4(4)
M4-MTX	21	8: 1: 1	3056	14.3	13.4	4.7(5)	45(52)
M5-MTX	11	6: 1: 1	1748	25.0	23.0	4.6(5)	25(2 8)
M6-MTX	23	8: 1: 1	2832	15.4	14.2	4.7(5)	2
M7-MTX	12	6: 1: 1	1763	24.8	23.8	4.8(5)	36(40)
M8-MTX	21	8: 1: 1	2731	16.0	14.6	4(5)	63(68)
M9-MTX	22	8: 1: 1	2875	15.2	14.0	4.6(5)	4.2(4)
M10-MTX	12	6: 1: 1	1665	26.2	23.9	4(5)	4(4)

[a] *At 30.0± 0.5° C, in deionized H_2O; concentration c= 2 mg/mL.*
[b] *Mole ratio (found) of hydrosolubilizing to drug-anchoring groups after ^{1} H NMR integration.*
[c] *Molecular mass of the simplest recurring unit (normalized to x>y=z=1) rounded off to the nearest integer.*
[d] *Derived from 100% acylation*
[e] *Derived from ^{1}H NMR spectrum (error limit± 12%)*
[f] *In D_2O, p H 10-11,chemical shifts, δ/ppm,referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limit± 12%. Protons are calculated for the structural representations in Table 4.27.*
[g] *Expected count for composition in accordance with recurring unit (see b)*
[h] *Proton assignement, δ/ppm:*

Table 4.40: Summary of analytical data for polyamidoamine-based MTX conjugates

Conjugate designation	η_{inh} (mL/g) [a]	**% MTX** Calcd [b]	**% MTX** NMR [c]	**NH_2 acylation** % NMR [d]
M1-MTX	12	25.4	22.8	90
M2-MTX	23	16.1	14.4	89
M3-MTX	12	26.7	24.1	90
M4-MTX	21	14.3	13.4	94
M5-MTX	11	25.0	23.0	92
M6-MTX	23	15.4	14.2	94
M7-MTX	12	24.8	23.8	96
M8-MTX	21	16.0	14.6	91
M9-MTX	22	15.2	14.0	92
M10-MTX	12	26.2	23.9	91

[a] *At 30±0.5°C, in deionized H_2O; concentration c=2mg/mL*
[b] *Derived from 100% acylation*
[c] *Derived from ^1H NMR spectrum (error limit± 12%)*
[d] *Ratio of c to b.*

4.2.3 Polymer platinum co-conjugates

The antitumor activity of many metal complexes, notably the platinum (ll) compounds, has been tested after the antitumor activity of *cisplatin* was established [226, 227]. Cisplatin has since been much used as the most potent platinum complex against a variety of solid tumors despite its fast renal clearance and toxicity generated by an accumulation in the kidney [198]. Cis-dichloroplatinum (ll) complexes are obtained by varying the amine ligand of cisplatin and have shown very high antitumor activity. Among them is mentioned *trans*-1,2-diaminocyclohexane (DACH). Such complexes, because of their salt-like state cannot cross cell membranes by a passive diffusion mechanism like nonpolar and neutral compounds. Polymer-drug conjugates, also known as prodrugs, have proved more efficient to circumvent such deficits by comparison with low-molecular-mass clinically used drugs as they show an improvement in body distribution of the antitumor agent through pinocytotic cell entry as well as an enhanced permeability and retention effect [199, 200, 201,202] . All these statements lead to the conclusion that platinum-based drugs are the best candidates for polymer anchoring with many examples available in the literature. By far the most significant work, published in numerous papers dating back to 1983 [203, 204, 205], 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 [201]. The investigations of Schechter's laboratory [207], the Ohya' [198] and Duncan's team [206] deserve to be mentioned. The anchoring of platinum to a macromolecular carrier can be established in many different ways, according to the type of ligand or metal ligand serving as the connecting link to the polymeric carrier. In this laboratory, water-soluble platinum-based conjugate polymers were synthesized and analytically characterized as in the past. These polymers featured

polyaspartamide structures and their bond to Pt was established through chelation by ethylenediamine as a ligand attached to the polymeric side chain *via* a biofissionable amide link [228, 229] allowing for hydrolytic and enzymatic cleavage in the lysosomal compartment, resulting in liberation of the active cis-diamineplatinum side-chain component. These previously synthesized platinum conjugates demonstrated an *in vitro* high activity and cell specificity against a variety of cell lines, namely HeLa and explanted human cancers cells of the liver and lung.

The findings above inspired the present study. In this work, platinum complexes anchored via dihydroxylato ligands as leaving group have been investigated (Figure 4.5).

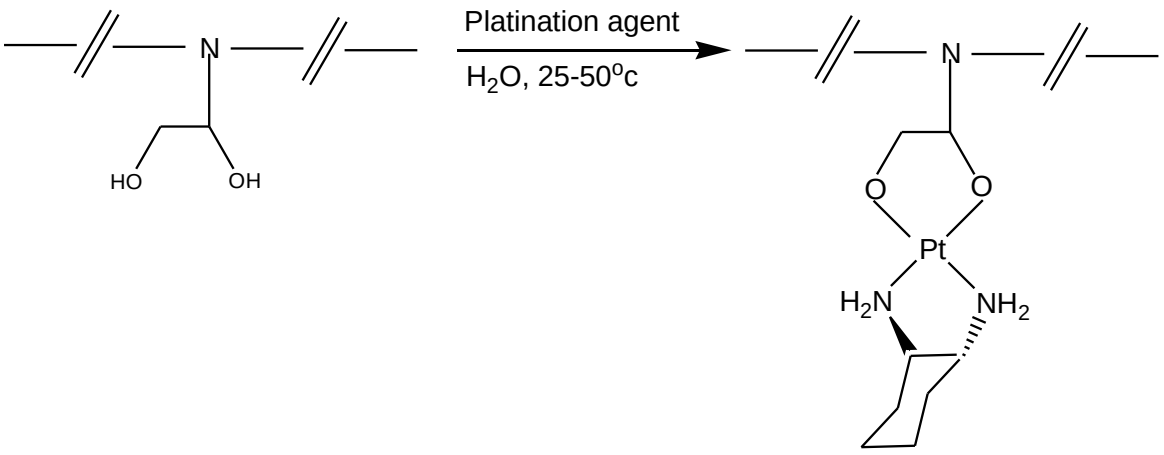
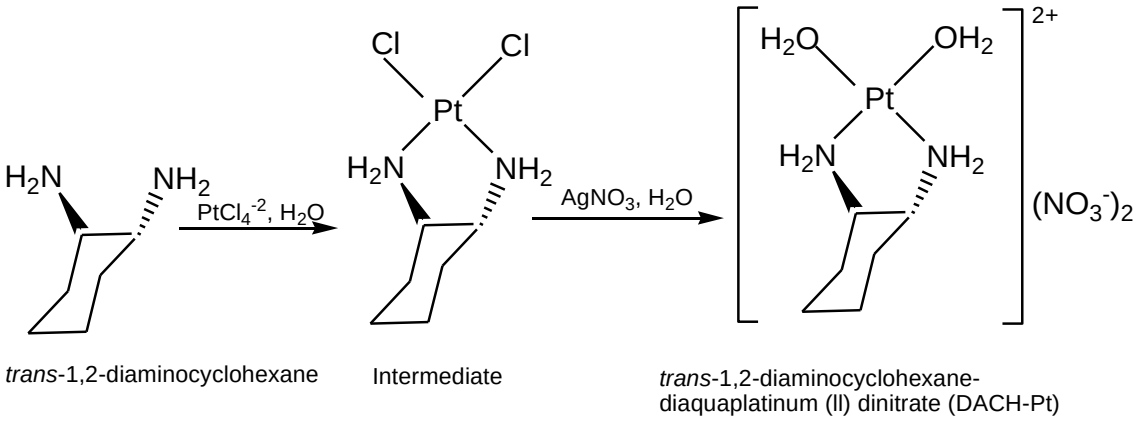


Figure 4. 5: Model of platinum-conjugate via dihydroxylato-chelation

The preference for this ligand is justified by its ability to chelate the platinum agent through a five-membered ring chelate upon coordination [230]. This ring has proved stable enough to survive in the central circulation. From the ultimate conjugate, platinum liberation will occur by hydrolytic cleavage of oxygen-metal bonds at a lower pH, freeing the trans-1,2-diaminecyclohexaneplatinum (ll) as an aquated species. The platination agent used was trans-1,2-diaminecyclohexanediaquaplatinum (ll) dinitrate (DACH-Pt), prepared according to a literature procedure [231] by treating tetrachloroplatinum-(ll) salt in aqueous solution with trans-1,2-diaminocyclohexane, followed by chloro-aqua ligand exchange in the presence of silver nitrate as shown in Scheme 4.12



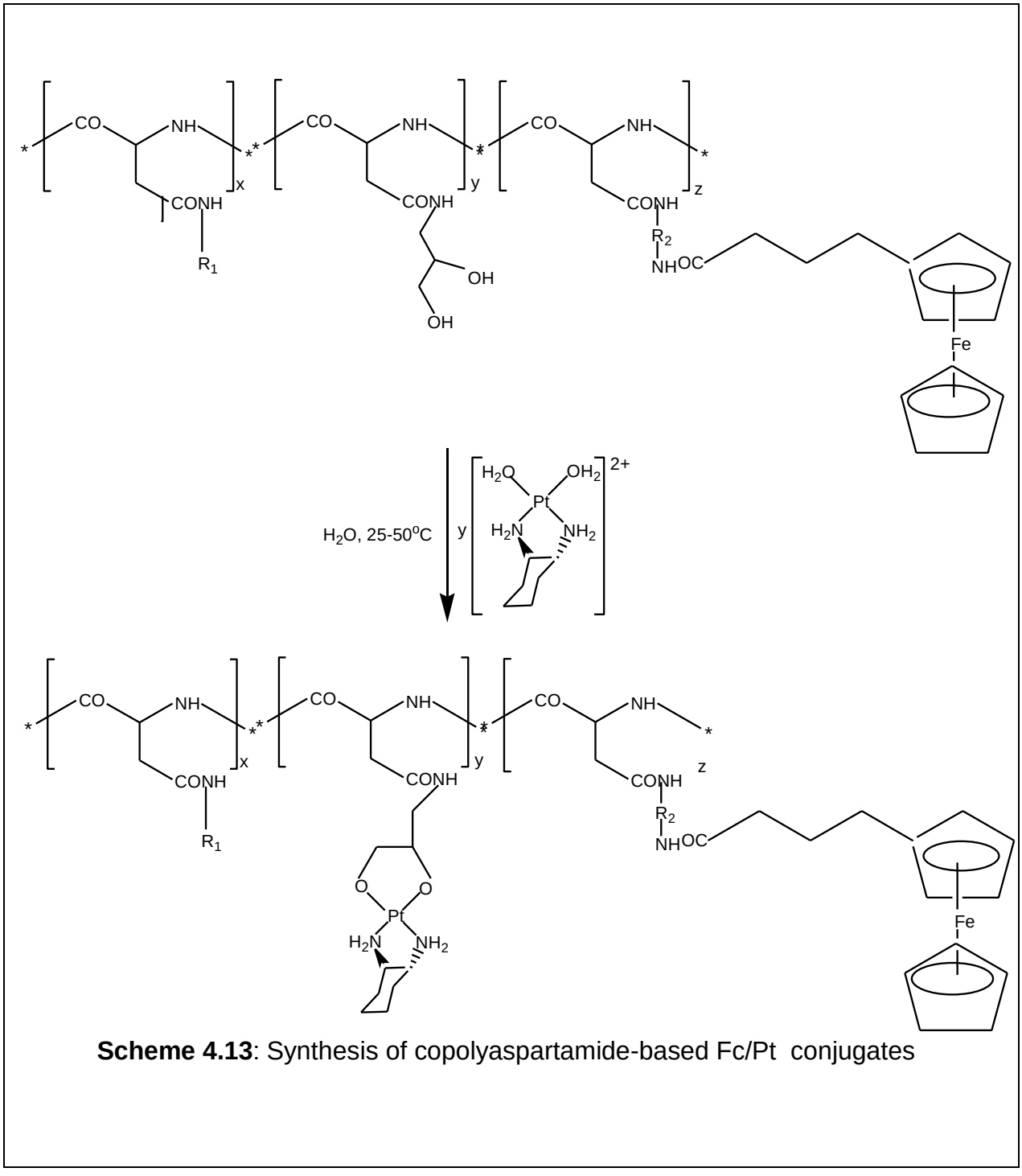
Scheme 4.12: Synthesis of DACH-Pt as platination agent

One of the tasks assigned to this work was polymer multidrug conjugation. Platinum was anchored to ferrocene-based conjugates on one side and to MTX-based conjugates on the other side, resulting in respective co-conjugates. The conjugates selected for the achievement of this goal were those synthesized and described in Sections 4. 2. 1 and 4. 2. 2. This approach to co-drug conjugation features the exploitation of the potency of two different drugs attached to the same polymeric carrier, each drug having its own pharmacokinetic path in the killing mechanism. This results in the advantage of an additive effect of the two drugs from a same carrier and circumvents the space shortage due to the two different carriers bearing each drug during the bioevaluation work. As with conjugation, the co-conjugation work discribed in this study gave way to the formation of an amide

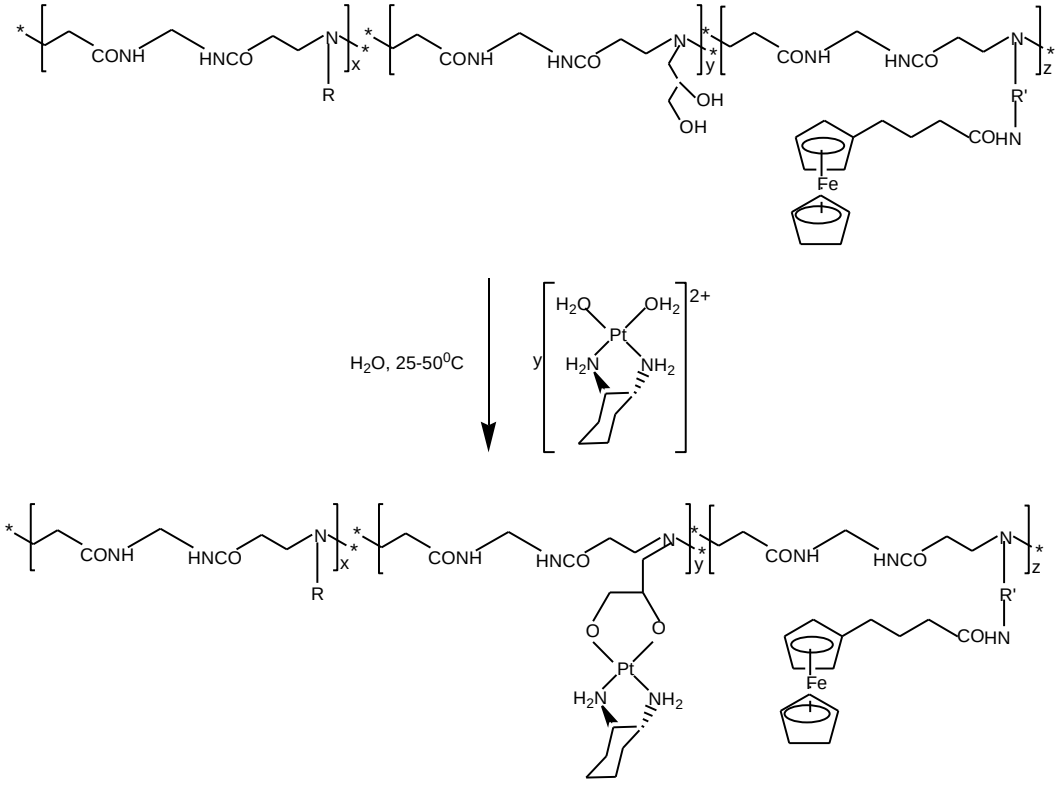
bond resulting from the reaction of the drug's acid group with the carrier's amine function. In all synthetic work concerning platinum, a strict control of pH (5.5-6), temperature (3 days at 25°C, then 6 h at 50°C), and time was used. A lower pH would result in carrier hydrolysis while a higher one would be the consequence of hydroxobridging with a result of final co-conjugate non-solubility.

4.2.3.1 Synthesis of polymeric Fc/Pt co-conjugates

Both copolyaspatamide- and polyamidoamine ferrocene-based conjugates were involved in this synthesis, as shown in Schemes 4.13 and 4.14.



Scheme 4.13: Synthesis of copolyaspartamide-based Fc/Pt conjugates



Scheme 4.14: Synthesis of copolyamidoamine-based Fc/Pt conjugates

Platinum and iron being two metals with different redox potentials, a question whether platinum would oxidize iron or not was our concern as the anchoring reaction would take place in aqueous medium. A blank test consisting in submission of selected polyaspartamide and polyamidoamine carriers as well as their ferrocene conjugates to the platinum anchoring conditions, that is in aqueous medium for 3 days at room temperature and 6 hours at 50-55°C with the pH kept at

5.5-6 (HCl and NaOH), was therefor performed and showed a prevalence of our carriers and conjugates, giving way to a safe co-conjugation work.

4.2.3.1.1 Preparation of copolyaspartamide-based Fc/ Pt co-conjugates

Copolyaspartamide-ferrocene conjugates K11-Fc, K12-Fc, K13-Fc, K14-Fc, K15-Fc, K16-Fc, K17-Fc, K18-Fc, K19-Fc and K20-Fc were involved in this synthesis. All subsequent co-conjugates were water-soluble, with molar feed ratios, experimental conditions and viscosities summarized in Table 4.41, while H NMR and metal content data are presented in Table 4.42.

Table 4.41: Experimental data for the synthesis of copolyaspartamide-based Fc/Pt co-conjugates

Co-conjugate designation	Molar ratios		Reaction time & temperature		Yield [a] %	Base Molecular Mass [b]	η_{inh} [c] mL/g	x:y:z [d]	Conjugate designation
	Conjugate	DACH-Pt	First step	Second step					
K11-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	26.8	2577	33	8: 1: 1	**K11-Fc**
K12-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	17.5	1523	18	3: 1: 1	**K12-Fc**
K13-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	11.4	2617	30	8: 1: 1	**K13-Fc**
K14-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	36.0	1453	19	3: 1: 1	**K14-Fc**
K15-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	31.6	1510	20	3: 1: 1	**K15-Fc**
K16-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	29.4	2603	31	8: 1: 1	**K16-Fc**
K17-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	37.2	1468	20	3: 1: 1	**K17-Fc**
K18-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	28.7	2606	23	8: 1: 1	**K18-Fc**
K19-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	29.6	1539	19	3: 1: 1	**K19-Fc**
K20-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	32.0	1511	20	3: 1: 1	**K20-Fc**

[c] Conjugate yield after size exclusion chromatography and (12 000 molecular weight cut-off) dialysis.
[b] Molecular mass of the simple recurring unit; structures normalized to x>y=z=1
[c] At 30.0°C ± 0.5°C in distilled water, conc 2mg/mL
[d] Mole ratio (found) of hydrosolubilizing to drug-anchoring groups after platination

Table 4.42: ^{1}H NMR, viscosities and metal content of copoyaspartamide-based Fc/Pt co-conjugates

Co-conjugates designation	η_{inh} [a] (mL*/*)	% **Pt** [b] cald	**% Pt** [c] found	% **Fe** [d] Cald	% **Fe** [e] NMR	**Protons counted** [f] (expected) [g] **chemical shift** (ppm) δ.4.5-4.1 [h]	δ.1.9-1.4
K11-Fc/Pt	33	3.03	2.97	2.3	2.2	8.6(9)	33(36)
K12-Fc/Pt	18	5.01	6.14	4.2	4.1	8.8(9)	17(16)
K13-Fc/Pt	30	2.99	2.51	2.3	2.2	8.5(9)	21(20)
K14-Fc/Pt	19	5.39	5.00	4.4	4.4	9(9)	13.2(14)
K15-Fc/Pt	20	5.16	3.76	4.2	4.2	9(9)	16(14)
K16-Fc/Pt	31	3.00	5.34	2.3	2.3	8.9(9)	20(20)
K17-Fc/Pt	20	5.31	4.62	4.4	4.4	9(9)	8.4(8)
K18-Fc/Pt	23	3.00	11.33	2.3	2.2	8.6(9)	25.8(24)
K19-Fc/Pt	19	5.07	4.85	4.1	4.0	8.7(9)	11(14)
K20-Fc/Pt	20	5.16	4.74	4.2	4.2	9(9)	9.2(8)

[a] *At 30±0.5°C, in deionized H_2O; concentration c=2mg/mL*

[b] *Calculated from analytically determined Pt content*

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

[d] *Derived from 100% acylation*

[e] *Derived from ^{1}H NMR spectrum (error limit± 12%)*

[f] *In D_2O, p H 10-11,chemical shifts, δ/ppm,referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limit± 12%. Protons are calculated for the structural representations in Table 4.27.*

[g] *Expected count for composition in accordance with recurring unit (see b)*

[h] *Proton assignement, δ/ppm:*

4.2.3.1.2 Preparation of copolyamidoamine Fc/Pt co-conjugates

Copolyamidoamines M11-Fc, M12-Fc, M13-Fc, M14-Fc, M15-Fc, M16-Fc, M17-Fc, M18-Fc, M19-Fc and M20-Fc were selected for this purpose, and the synthesis gave water-soluble co-conjugates whose compositions, experimental data and viscosities are depicted in Table 4.43, while ^{1}H NMR and metal content data are presented in Table 4.44.

Table 4.43: Experimental data for the synthesis of polyamidoamine-based Fc/Pt co-conjugates

Co-conjugate designation	Molar ratios		Reaction time & temperature		Yield % [a]	Base Molecular Mass [b]	η_{inh} [c] mL/g	x:y:z [d]	Conjugate designation
	Conjugate	DACH-Pt	First step	Second step					
M11-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	2.4	3147	21	8: 1: 1	**M11-Fc**
M12-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	16.3	1809	16	3: 1: 1	**M12-Fc**
M13-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	26.8	3183	23	8: 1: 1	**M13-Fc**
M14-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	30.7	3133	20	8: 1: 1	**M14-Fc**
M15-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	26.6	1795	14	3: 1: 1	**M15-Fc**
M16-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	22.4	3173	21	8: 1: 1	**M16-Fc**
M17-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	20.0	1781	15	3: 1: 1	**M17-Fc**
M18-Fc/Pt	1.0	1,4	3d,RT	6h, 50°C	24.2	3328	22	8: 1: 1	**M18-Fc**
M19-Fc/Pt	1.0	1,4	3d,RT	6h, 50°C	30.6	3216	23	8: 1: 1	**M19-Fc**
M20-Fc/Pt	1.0	1.4	3d,RT	6h, 50°C	26.0	1796	16	3: 1: 1	**M20-Fc**

[a] Co-conjugate yield after size exclusion chromatography and (12 000 molecular weight cut-off) dialysis.
[b] Molecular mass of the simple recurring unit; structures normalized to x>y=z=1
[c] At 30.0°C ± 0.5°C in distilled water, conc 2mg/mL
[d] Mole ratio (found) of hydrosolubilizing to drug-anchoring groups after platination

Table 4.44: ^{1}H NMR, viscometric data and metal content for polyamidoamine-based Fc/Pt co-conjugates

Co-conjugates designation	**η_{inh}** [a] (mL/g)	**% Pt** [b] Calcd	**% Pt** [c] Found	% **Fe** [d] Cald	% **Fe** [f] NMR	**Protons counted** [f] (expected) [g] **chemical shift** (ppm) δ.4.3-4.1[h]	δ. 1.8-0.9
M11-Fc/Pt	21	2.48	2.12	1.9	1.7	8.1(9)	32(36)
M12-Fc/Pt	16	4.31	3.67	3.5	3.1	8(9)	39(42)
M13-Fc/Pt	23	2.45	2.31	1.9	1.6	8.1(9)	19(20)
M14-Fc/Pt	20	2.49	2.16	1.9	1.9	8.5(9)	31(36)
M15-Fc/Pt	14	4.35	4.07	3.5	3.3	8.3(9)	12(14)
M16-Fc/Pt	21	2.46	1.98	1.9	1.8	8.5(9)	16(18)
M17-Fc/Pt	15	4.38	3.97	3.5	3.3	8.6(9)	13(16)
M18-Fc/Pt	22	2.34	2.71	1.8	1.7	8.2(9)	31(36)
M19-Fc/Pt	23	2.43	2.45	1.8	1.7	8.3(9)	17(20)
M20-Fc/Pt	16	4.34	4.20	3.5	3.3	8.2(9)	9(10)

[a] *At 30±0.5°C, in deionized H_2O; concentration c=2mg/mL*

[b] *Calculated from analytically determined Pt content*

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

[d] *Derived from 100% acylation*

[e] *Derived from ^{1}H NMR spectrum (error limit± 12%)*

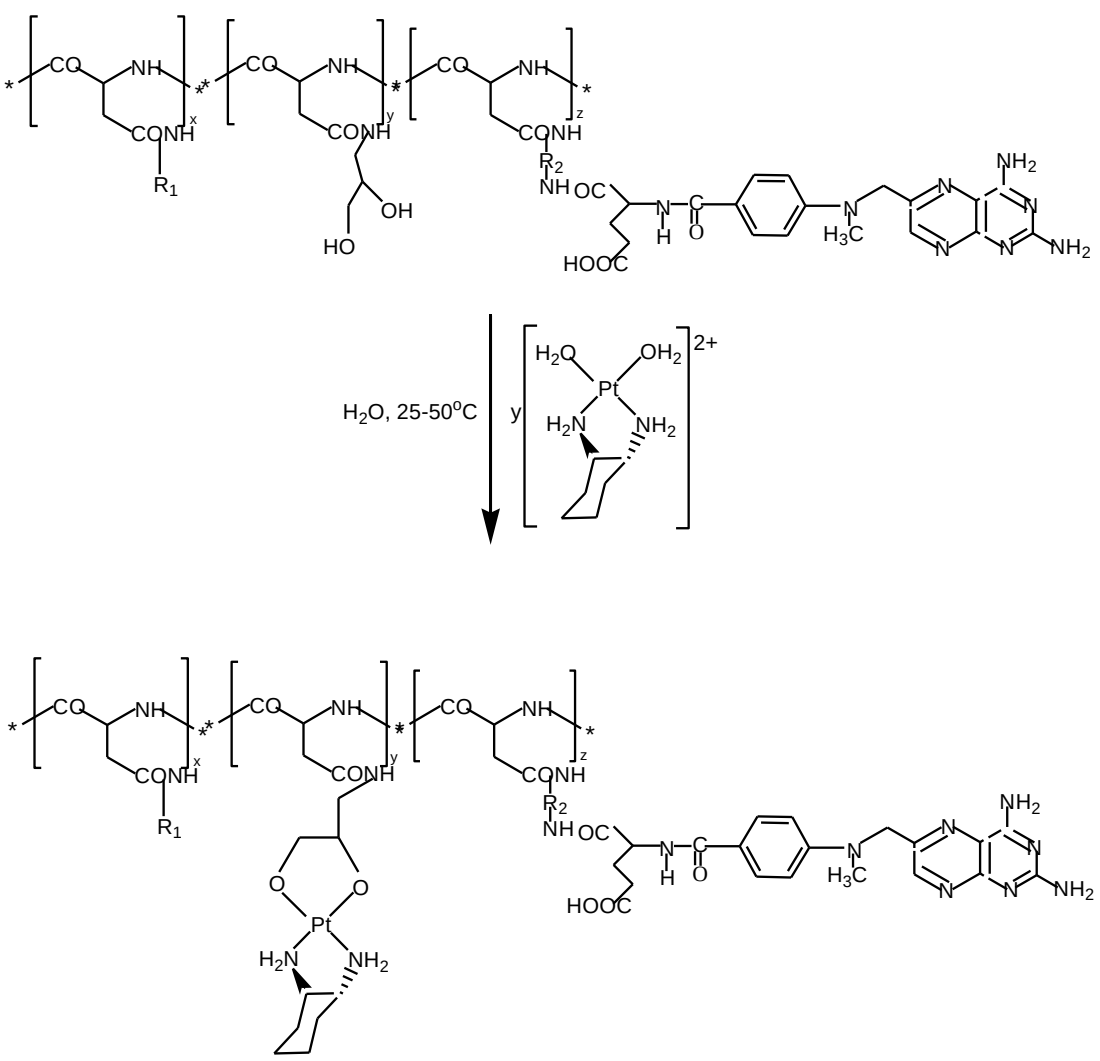
[f] *In D_2O, p H 10-11,chemical shifts, δ/ppm,referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limit± 12%. Protons are calculated for the structural representations in Table 4.27.*

[g] *Expected count for composition in accordance with recurring unit (see b)*

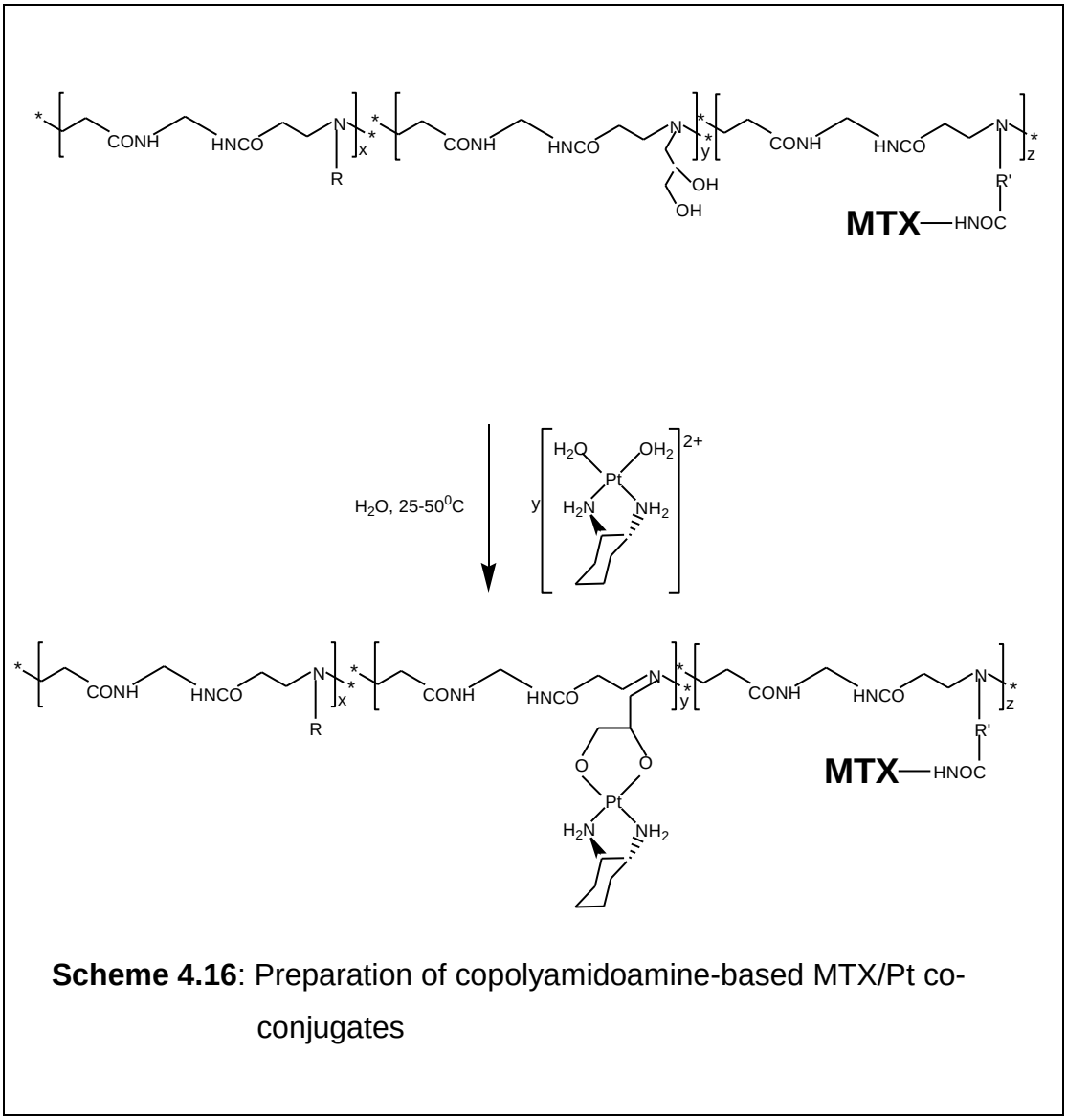
[h] *Proton assignement, δ/ppm:*

4.2.3.2 Synthesis of polymeric MTX/Pt co-conjugates

In this section, Pt was anchored to MTX-containing copolyaspartamide and copolyamidoamine conjugates previously described for the generation of subsequent co-conjugates according to scheme 4.15 for copolyaspartamide- and 4.16 for copolyamidoamine-based co-conjugates. The blank test performed in section 4.2.3.1 was our source of inspiration though not necessary as no risk of oxidation between Pt and MTX was feared in this synthesis. A strict control of pH (5.5-6) with universal indicator was applied during the platination process. The temperature and the time were also regulary controlled.



Scheme 4.15: Preparation of copolyaspartamide-based MTX/Pt co-conjugates



Scheme 4.16: Preparation of copolyamidoamine-based MTX/Pt co-conjugates

4.2.3.2.1 Preparation of copolyaspartamide-based MTX/Pt co-conjugates

Copolyaspartamide-MTX conjugates K1-MTX, K2-MTX, K3-MTX, K4-MTX, K5-MTX, K6-MTX, K7-MTX, K8-MTX, K9-MTX and K10-MTX were involved in this synthesis. All subsequent Pt/MTX co-conjugates were water-soluble with molar feed ratios, experimental conditions and viscosities summarized in Table 4.45, while ^{1}H NMR and metal content data are presented in Table 4.46.

Table 4.45: Experimental data for the synthesis of copolyaspartamide-based MTX/Pt co-conjugates

Co-conjugate designation	Molar ratios		Reaction time & temperature		Yield % [a]	Base Molecular Mass [b]	η_{inh} [c] (mL/g)	x:y:z [d]	Conjugate designation
	Conjugate	DACH-Pt	First step	Second step					
K1-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	22.0	1625	26	3: 1: 1	**K1-MTX**
K2-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	31.0	1666	30	3: 1: 1	**K2-MTX**
K3-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	29.4	2464	40	8: 1: 1	**K3-MTX**
K4-MTX/Pt	1.0	1.4	3d,RT	6h,50°C	28.7	2674	42	8: 1: 1	**K4-MTX**
K5-MTX/Pt	1.0	1.4	3d,RT	6h,50°C	30.6	2786	38	8: 1: 1	**K5-MTX**
K6-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	26.6	1652	26	3: 1: 1	**K6-MTX**
K7-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	28.0	2450	37	8: 1: 1	**K7-MTX**
K8-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	30.3	1651	27	3: 1: 1	**K8-MTX**
K9-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	24.0	1694	27	3: 1: 1	**K9-MTX**
K10-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	24.0	2493	38	8: 1: 1	**K10-MTX**

[a] Co-conjugate yield after size exclusion chromatography and (12 000 molecular weight cut-off) dialysis.
[b] Molecular mass of the simple recurring unit; structures normalized to x>y=z=1
[c] At 30.0°C ± 0.5°C in distilled water, conc 2mg/mL
[d] Mole ratio (found) of hydrosolubilizing to drug-anchoring groups after platination

Table 4.46: ^{1}H NMR, viscometric data and drug content for copolyaspartamide-based MTX/Pt conjugates

Co-conjugates designation	**η_{inh}** [a] (mL/g)	**% Pt** [b] Calcd	**% Pt** [c] Found	**%MTX** [d] Calcd	**%MTX** [e] NMR	**Protons counted** [f] (expected) [g] chemical shift (ppm)	
						δ.8.5-6.5 [h]	δ.1.8-1.5
K1-MTX/Pt	26	4.80	5.03	30.4	30.4	5(5)	4(4)
K2-MTX/Pt	30	4.68	4.85	29.6	29.6	5(5)	10(10)
K3-MTX/Pt	40	3.17	3.04	19.2	19.2	5(5)	4(4)
K4-MTX/Pt	42	2.92	2.78	17.6	17.2	4.9(5)	2(2)
K5-MTX/Pt	38	2.80	3.11	16.8	16.8	5(5)	2(2)
K6-MTX/Pt	26	4.72	4.01	29.9	28.7	4.8(5)	2(2)
K7-MTX/Pt	37	3.18	3.52	19.3	19.3	5(5)	2(2)
K8-MTX/Pt	27	4.72	4.16	29.9	29.9	5(5)	19(20)
K9-MTX/Pt	27	4.60	4.22	28.9	28.7	4.9(5)	24(26)
K10-MTX/Pt	38	3.13	3.37	19.0	18.6	4.9(5)	2(2)

[a] *At 30±0.5°C, in deionized H_2O; concentration c=2mg/mL*

[b] *Calculated from analytically determined Pt content*

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

[d] *Derived from 100% acylation*

[e] *Derived from ^{1}H NMR spectrum (error limit± 12%)*

[f] *In D_2O, p H 10-11,chemical shifts, δ/ppm,referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limit± 12%. Protons are calculated for the structural representations in Table 4.27.*

[g] *Expected count for composition in accordance with recurring unit (see b)*

[h] *Proton assignement, δ/ppm:*

4.2.3.2.2 Preparation of copolyamidoamine-based MTX/Pt co-conjugates

Polyamidoamines M1-MTX, M2-MTX, M3-MTX, M4-MTX, M5-MTX, M6-MTX, M7-MTX, M8-MTX, M9-MTX and M10-MTX were selected for this purpose and the synthesis gave water-soluble Pt/MTX co-conjugates, whose compositions, experimental data and viscosities are depicted in Table 4.47, while ^{1}H NMR and metal content data are presented in Table 4.48.

Table 4.47: Compositions, experimental data and viscosities of polyamidoamine-based MTX/Pt co-conjugates

Co-conjugate designation	Molar ratios		Reaction time & temperature		Yield [a] %	Base [b] Molecular Mass	η_{inh} [c] mL/g	x:y:z [d]	Conjugate designation
	Conjugate	DACH-Pt	First step	Second step					
M1-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	27.7	1906	15	3: 1: 1	**M1-MTX**
M2-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	35.3	2890	28	8: 1: 1	**M2-MTX**
M3-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	25.4	1823	14	3: 1: 1	**M3-MTX**
M4-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	22.2	3244	24	8: 1: 1	**M4-MTX**
M5-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	30.0	1936	14	3: 1: 1	**M5-MTX**
M6-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	30.5	3020	25	8: 1: 1	**M6-MTX**
M7-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	24.6	1951	15	3: 1: 1	**M7-MTX**
M8-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	24.4	2919	23	8: 1: 1	**M8-MTX**
M9-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	29.6	3063	24	8: 1: 1	**M9-MTX**
M10-MTX/Pt	1.0	1.4	3d,RT	6h, 50°C	26.4	1853	15	3: 1: 1	**M10-MTX**

[a] *Co-conjugate yield after size exclusion chromatography and (12 000 molecular weight cut-off) dialysis.*

Table 4.48: ^{1}H NMR, viscometric data and drug contents for copolyamidoamine-based MTX/Pt co-conjugates

Co-conjugate designation	**η_{inh}** [a] (mL/g)	**% Pt** [b] Calcd	**% Pt** [c] Found	% **MTX** [d] Calcd	% **MTX**[e] NMR	**Protons counted** [f] (expected) [g] **chemical shift** (ppm)	
						δ.8.5-6.5 [h]	δ. 1.8-0.9
M1-MTX/Pt	15	4.09	4.24	25.4	22.8	4.5(5)	37(40)
M2-MTX/Pt	28	2.70	2.93	16.1	14.4	4.5(5)	62(68)
M3-MTX/Pt	14	4.29	4.28	26.7	24.1	4.5(5)	4(4)
M4-MTX/Pt	24	2.40	2.17	14.3	13.4	4.7(5)	45(50)
M5-MTX/Pt	14	4.03	4.00	25.0	23.0	4.6(5)	23(26)
M6-MTX/Pt	25	2.58	2.74	15.4	14.2	4.7(5)	2(2)
M7-MTX/Pt	15	4.00	3.77	24.8	23.8	4.8(5)	36(40)
M8-MTX/Pt	23	2.67	2.31	16.0	14.6	4(5)	63(68)
M9-MTX/Pt	24	2.55	2.93	15.2	14.0	4.6(5)	4.2(4)
M10-MTX/Pt	15	4.21	4.42	26.2	23.9	4(5)	4(4)

[a] *At 30±0.5°C, in deionized H_2O; concentration c=2mg/mL*

[b] *Calculated from analytically determined Pt content*

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

[d] *Derived from 100% acylation*

[e] *Derived from ^{1}H NMR spectrum (error limit± 12%)*

[f] *In D_2O, p H 10-11,chemical shifts, δ/ppm,referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate;*

4.3 Cell culture testing

The synthetic work described in the previous sections of this chapter gave rise to the availability of carriers of the copolyaspartamide and copolyamidoamine types. These carriers were used in the synthesis of copolyaspartamide and copolyamidoamine-based Fc and MTX conjugates. *Cisplatin* was anchored to these conjugates in multidrug conjugation, giving Fc/Pt and MTX/Pt-containing copolyaspartamide and copolyamidoamine co-conjugates. One co-conjugate selected in each class was evaluated in cell culture test against the MCF-7 cell line of the human breast cancer by the School of Pharmacy and Pharmacology, Faculty of Health Science, University of the Witwatersrand. This cell line is reputed to have a high resistance to ferrocene, metotrexate and *cisplatin* when compared to other cell lines which are more sensible, previously used in this laboratory and which, unfortunately, were not available for this test. Activities of free drugs, carriers and conjugates were also evaluated for reference and comparison to those of the co-conjugates. Because of the notorious high resistance of the cell line used, better results can be expected with other cell lines, like CEM/S, CEM/E, HeLa and Colo in publications fromthis thesis to come later. The cytotoxic activities, which appear together with cell viabilities in Table 4.49 below, were expressed in terms of IC_{50} values (drug concentration required to retain 50 % of cell viability relative to drug-free control). From the copolyaspartamides, the co-conjugate K2-Fc/Pt with its parents, conjugate K2-Fc and carrier K2 (60: 20: 20), were selected for evaluation, as for co-conjugate K12-MTX/Pt with its parents, conjugate K12-MTX and carrier K12 (60: 20: 20). From the polyamidoamines, the co-conjugate M2-Fc/Pt, its

parents, conjugate M2-Fc and carrier M2 (80: 10: 10) were selected, as for conjugate M12-MTX/Pt with its parents, conjugate M12-MTX and carrier M12 (60: 20: 20). The free drugs evaluated were *cisplatin* (Pt), ferrocenylbutanoic acid (Fc) and methotrexate (MTX). The performance of the Fc containing homoconjugates was referenced against that of free Fc used as control, while the activity of free MTX served as reference for MTX containing homoconjugates. As all the co-conjugates were supposed to contain Pt, their activities were referenced against that of *cisplatin*. The results of this cell culture testing are compiled in Table 4.50.

Table 4.49: Antiproliferative activities of free drugs, copolyaspartamide, and copolyamidoamine homo- and co-conjugates

Designation	**IC_{50}**					**% cell viability**
	µg Fc/mL	µg MTX/mL	µg Pt/mL	µg Fc/Pt/mL	µg MTX/Pt/mL	100 µg/mL
Fc	67.3					48.9
MTX		87.6				82.7
Pt			103.2			69.7
K2 (60: 20: 20)						
K2-Fc/Pt				43.1		54.1
K12 (60:20:20)						
K12-MTX/Pt					7.8	25.4
M2 (80: 10: 10)						
M2-Fc/Pt				50. 9		45.6
M12 (80: 10: 10)						
M12-MTX/Pt					20.7	40.4

The table shows a superiority of all free drugs' IC_{50} values over those of co-conjugates, which means a best MCF-7 cell lines' killing power of co-conjugates over free drugs as confirmed in previous theses, dissertations and publications from the Polymer Research Group.

When comparing the IC_{50} values of the 3 drugs used (Fc, MTX and Pt), Fc appears with the best performance (67.33), followed by MTX (87.64) and Pt (103.23).

From all co-conjugates investigated, the outstanding two IC_{50} values appear with co-conjugates K12 MTX/Pt (7.815) and M12 MTX/Pt (20.72), both containing MTX/Pt. K12 MTX/Pt is a copolyaspartamide with a molar mass lower than that of M12 MTX/Pt, a copolyamidoamine-based polymer; a steric hindrance might be expected in the targeting process of the MCF-7 cell lines by M12 MTX/Pt and that could explain its lower performance, comparing to that of K12 MTX/Pt. The co-conjugates K2 Fc/Pt and M2 Fc/Pt display, respectively, 43.13 and 50.86 as IC_{50} values, which are lower but not very critical, comparing to their MTX/Pt-based counterparts. A good performance was expected from the two Fc-containing co-conjugates, with regard to the good IC_{50} value shown by free Fc over MTX *and cisplatin*. We suspect a further and progressive oxidation of Fc by Pt in co-conjugates bearing the two metals, though a preliminary test confirming the possibility of coexistence was performed before anchoring these metals to a same polymeric carrier. In fact, the biological test took place more than six months after the co-conjugation process. The same way that MTX/Pt-containing copolyaspartamide described in this section showed better performance over its copolyamidoamine counterpart, copolyaspartamide-based Fc/Pt co-conjugate

showed better performance over copolyamidoamine-based one. The same reason of steric hindrance as in copolyamidoamine-based MTX/Pt co-conjugates is valid. The order of viscosities between all co-conjugates was in line with that of their molar masses.

CHAPTER 5
EXPERIMENTAL

5. 1 General procedures

Melting points were determined in sealed capillary tubes.^{1}H NMR spectra were obtained at 300 and 400 MHz in D_2O solution. Chemical shifts, δ in ppm, were referenced against sodium 3-(trimethylsilyl)-2,2,3,3-d4-propionate, and to eliminate potential protonation effects, sample solution pH values were adjusted to 10-11 with sodium hydroxide where applicable.

Dialysis was performed using cellulose membrane spectra (Spectrum Industries, Los Angeles, CA), with a molecular cut-off limit of 12000-14000. Distilled water was used as the dialysis phase. Freeze-drying of polymer and conjugate solutions was performed in a VIRTIS bench-Top 3 freeze-drier at -30°C and a pressure of 0.5 Torr. The freeze-dried polymer was post-dried in a SARTORIUS Thermo Control Infrared drying apparatus and kept in a dessicator. Analytical samples were dried using Abderhalden apparatus, and calcium chloride ($CaCl_2$) was used as the drying agent. UV/VIS spectroscopy was performed in a HITACHI 2000 spectrophotometer at a scan speed of 400nm/min; both analytical methods were performed in the School of Chemistry, Wits University. Flash chromatography was performed using basic alumina as solid support. The aprotic solvent, N,N-dimethylformamide (DMF) was redistilled under nitrogen gas and reduced pressure, and kept over molecular sieves A. Microanalytical platinum determinations were performed at CHEMTECH Laboratory Services by EPA method 200.7. Inherent viscosities, η_{inh} were

determined at 30 ± 0.5°C in Cannon Fenske tubes. Deionized water wasused as solvent; the concentration was c = 0.2 g/mL, and the results are given in units of mL/g. The pH was measured using universal indicator.

5. 2 Reagents, solvents and monomeric reactants

The aprotic solvent, N,N-dimethylformamide (DMF), distilled under reduced pressure in a faint stream of N_2, and kept over molecular sieves A. All reactions involving its use were performed under anhydrous conditions. Distilled H_2O was used for dialysis operations.

The hydroxyamines, mono- and diamines, and methoxyamines were of commercial grade (Fluka Chemie AG, Aldrich Chemie G.m.b.H), and used as received. These included: 1,3-propylenediamine (PDA); diethylenetriamine (DET); ethylediamine (EDA); 3-(N,N-dimethylamino)propylamine (DMP); 3-(N,N-dimethylamino)ethylamine (DMEA); 2-(N,N- diehylamino)ethylamine (DEE); 3-(N, N-diethylamino)propylamine (DEP); API, APM, AEM and 3-amino-1, 2 propanediol (APD) were also of commercal grade (Across Organics) and used as received, and so was glacial acetic acid (Merk).

Solvents acetone (Me_2CO), diethyl ether (Et_2O), and hexane (Hex) were used as upplied. Ethylacetate was kept dry over $CaCl_2$.

Methylenebisacrylamide (MBA) was recrystallized in 2,6-di-*tert*-butyl-p-cresol (Fluka Chemie AG) before use. D,L-aspartic acid; N, N'- dicyclohexylcarbodiimide (DCC); 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium fluorophosphate

(HBTU) and triethylamine (TEA) (Fluka Chemie AG, Aldrich Chemie G.m.b.H) were also used as delivered.

Folic acid (FA) was purchased from Across Organics and predried in an Aberhalden tube for 24h at 40^{o}C. Methotrexate (MTX) purchased from Sigma, as sodium salt-like solution was precipitated with HCl, then dried like folic acid, before use for binding.

5.3 Experimental Procedures

5.3.1 Preparation of polymeric carriers

5.3.1.1 Copolyaspartamide (Copas) carriers

Poly-DL,succinimide (PSI) was prepared according to the method of Neri, previously and commonly used in our research group. A mixture of D,L aspartic acid (50 g) and orthophosphoric acid (25 g) was heated at reduced pressure in an oil bath at 250 °C. The expansion (polymerization) was controlled with N_2, while H_2O was circulating in the condenser. Upon expansion, the temperature was brought to 180 °C for 2 h.The beige solid was then washed with H_2O until the pH was 6. This method gave the PSI an inherent viscosity of 34 mL/g.

Copolyaspartamide carriers were prepared by following the general procedure in use in the Wits Polymer Research Group (PRG) with slight modifications in the reaction time and in the order of addition of different reactants.

5.3.1.1.1 Synthesis of Copolyaspartamides with APD as platinum binding site and

aliphatic solubilizing groups

For the preparation of copolyaspartamide **K1 (60:20:20),** (PSI) (1.94 g, 20 mmol) was dissolved in 2 mL of DMF with stirring until dissolution. The solubilizing agent 2-(N,N-diethylamino)ethylamine (DEE) (12 mmol) dissolved in 10mL of DMF was added before saturation with N_2 then stirring for 8h at RT. APD (4 mmol) predisolved in 5mL of DMF was added to the mixture before flushing N_2 while stirring carried on for overnight.

The mixture was added to the diamine, 1,3-propylenediamine (PDA) (8 mmol), predisolved in 8 mL of DMF, at 0°C before being flushed with N_2 and stirred for overnight at that temperature, then at RT for 24 h.

After concentration at 70^{o}C under vacuum on rotary evaporation to half volume, the polymer was precipitated with 100 mL of a mixture of Et_2O-Hex (2:1), and then thoroughly washed with hot toluene, before hot acetone to eliminate the unreacted amines. The polymer was dissolved in 50 mL of distilled water with a pH adjusted to 7-8 with HCl, before purification by a two-step dialysis procedure in Spectra/Por 4 tubing for 48 h against distilled water. During the last 6 h of dialysis, the pH was elevated to 9 with aqueous ammonia to avoid any protonation before freeze-drying. The retentate was collected at 71% in mass of product against mass of reactants as beige, water-soluble solid with an inherent viscosity (η_{inh}) of 15 mL/g and a calculated molar mass (repeating unit) of 998 g/mol (Table 4.3 row 1).

For **K1 (60:20:20):** ^{1}NMR (D_2O), δ/ppm: 4.75-4.5: 5H (5H: C*H* asp); 3.75-3.5: 3H (3H: CHO*H*-C*H*$_2$OH); 3.4-3.0: 11H (1OH: CONHC*H*$_2$), 2.9-2.0: 28H (30H: C*H*$_2$NC*H*$_2$, C*H*$_2$CONH, C*H*$_2$NH); 1.8-1.5: 2H (2H: CH_2-C*H*$_2$-CH_2)

For the synthesis of copolyaspartamides **K6 (60: 20: 20), K8 (60: 20: 20)** and **K9 (60: 20: 20),** the same procedure as for **K1 (60: 20: 20)** was used except that 3-diethylaminopropylamine (DEP) was used as solubilizing group for **K6 (60: 20: 20**) and **K9 (60: 20: 20);** ethylene diamine (EDA) was used as diamine for **K6 (60: 20**: **20)** and **K8 (60: 20: 20);** while the diamine used in **K9 (60: 20: 20)** was diethylene triamine (DET). For **K6 (60: 20: 20**), the procedure resulted in a white water-souble carrier collected in 70% yield (mass of product over mass of reactants) with an inherent viscosity of 12 mL/g and a calculated molar mass of 1027 g/mol. For **K8 (60: 20: 20),** the carrier was collected at 51% in mass, with an inherent viscosity of 12 mL/g and a molar mass (repeating unit) of 914 g/mol. While for **K9 (60: 20: 20),** the carrier was afforded in 53% yield with a viscosity and calculated molar mass of 12 mL/g and 900 g/mol, respectively.

For **K6 (60: 20: 20):** ^{1}NMR (D_2O), δ/ppm 4.75-4.5: 5H (5H: C*H* asp); 3.75-3.5: 3H (3H: C*H*OH-C*H*$_2$OH); 3.4-3.0: 8H (8H: CONH-C*H*$_2$); 2.9-2.0: 30H (32H: C*H*$_2$CONH; C*H*$_2$N-CH_2, C*H*$_2$-NH_2)

For **K8 (60: 20: 20):** ^{1}NMR (D_2O), δ/ppm 4.75-4.5: 5H (5H: C*H* asp); 3.75-3.5: 3H (3H: C*H*OH-C*H*$_2$OH); 3.4-3.0: 9H (10H: CONH-C*H*$_2$); 2.9-2.0: 31H (32H: C*H*$_2$CONH; C*H*$_2$N-CH_2, C*H*$_2$-NH_2, C*H*$_2$-NH)

For **K9 (60: 20: 20):** ^{1}NMR (D_2O), δ/ppm 4.75-4.5: 5H (5H: C*H* asp); 3.75-3.5: 3H (3H: C*H*OH-C*H*$_2$OH); 3.4-3.0: 8H (8H: CONH-C*H*$_2$); 2.9-2.0: 31H (32H: -CH-C*H*$_2$CONH; C*H*$_2$N-CH_2, C*H*$_2$-NH_2, C*H*$_2$-NH)

For the preparation of copolyaspartamide **K3 (80: 10: 10),** 20 mmol (1.94g) of PSI was dissolved in DMF (20mL). 2-Dimethylaminoethylamine (16mmol) predissolved in 10 mL of DMF was added to the above solution and stirred for 8 h once the reaction was flushed with nitrogen. 3-Amino-1, 2-propanediol (12mmol) predissolved in 10 mL of DMF was then added to the mixture, followed by nitrogen flushing. Upon stirring for 24 h, the clear solution was added dropwise to 1, 3-Diaminopropane (12 mmol) predissolved in 10 mL of DMF at 0°C and stirred at that temperature overnight, followed by 24 h at room temperature. The remaining steps in the process were conducted as for carrier **K1 (60: 20: 20).** The product, collected in 52% yield by mass was a white water-soluble solid with an inherent viscosity of 24 mL/g and a calculated molar mass (repeating unit) of 2092 g/mol.

For **K3 (80: 10: 10):** ^{1}NMR (D_2O), δ/ppm: 4.75-4.5: 11H (10H: C*H* asp); 3.75-3.5: 3H (3H: CHO*H*-C*H*$_2$OH); 3.4-3.0: 21H (2OH: CONHC*H*$_2$), 2.9-2.0: 84H (86H: C*H*$_2$NC*H*$_2$, CH-C*H*$_2$CONH, C*H*$_2$NH); 1.8-1.5: 2H (2H: CH_2-C*H*$_2$-CH_2).

Carriers **K4 (80: 10: 10)**, **K5 (80: 10: 10)**, **K7 (80: 10: 10) and K10 (80: 10: 10)** were prepared by the same procedure as for K3 (80: 10: 10), except that the DMEA of **K3 (80: 10: 10)** was replaced in carrier **K4 (80: 10: 10)** with 2-diethylaminoethylamine (DEEA) and 3-diethylaminopropylamine (DEP) in **K5 (80: 10: 10)**. In **K4 (80: 10: 10)**, **K5 (80: 10: 10)** and **K7 (80: 10: 10)**, PDA was replaced

with ethylenediamine (EDA), while the DMEA was replaced with diethylenetriamine (DET) in **K10 (80: 10: 10)**. After concentration, precipitation, dialysis and freeze-drying as described for **K3 (80: 10: 10)**, water-soluble yellowish solids were collected with the following respective yield percentages, inherent viscosities and calculated molar masses(repeating unit): 50%, 28 mL/g and 2175g/mol for **K4 (80: 10: 10)**; 67%, 27 mL/g and 2161 g/mol for **K5 (80: 10: 10)**; 70%, 27 mL/g and 2204g/mol for **K7 (80: 10: 10)**; and 53%, 26mL/g and 1868g/mol for **K10 (80: 10: 10)**.

For **K4 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 4.75-4.5: 11H (10H: C*H* asp); 3.75-3.5: 3H (3H: CHO*H*-CH_2OH); 3.4-3.0: 19H (2OH: CONHCH_2), 2.9-2.0: 66H (70H: CH_2NCH_2, CH-CH_2CONH, CH_2NH)

For **K5 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 4.75-4.5: 10H (10H: C*H* asp); 3.75-3.5: 3H (3H: CHO*H*-CH_2OH); 3.4-3.0: 18H (2OH: CONHCH_2), 2.9-2.0: 67H (70H: CH_2NCH_2, CH-CH_2CONH, CH_2NH); 1.8-1.5: 2H (2H: CH_2-CH_2-CH_2)

For **K7 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 4.75-4.5: 9H (10H: C*H* asp); 3.75-3.5: 3H (3H: CHO*H*-CH_2OH); 3.4-3.0: 18H (2OH: CONHCH_2), 2.9-2.0: 83H (86H: CH_2NCH_2, CH-CH_2CONH, CH_2NH)

For **K10 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 4.75-4.5: 10H (10H: C*H* asp); 3.75-3.5: 3H (3H: CHO*H*-CH_2OH); 3.4-3.0: 21H (2OH: CONHCH_2), 2.9-2.0: 85H (88H: CH_2NCH_2, CH-CH_2CONH, CH_2NH)

5. 3. 1. 1. 2. Synthesis of copolyaspartamides bearing APD as platinum binding site with cyclic and aromatic solubilizing groups

Copolyaspartamides **K11 (80: 10: 10)** to **K20 (60: 20: 20)** were prepared using the same procedure as for **K1 (60: 20: 20)** to **K10 (80: 10: 10),** with the exception of some modifications in the structure of solubilizing group and in the reaction time of the second step of aminolytic ring-opening, which was prolonged to 24 h instead of 8 h. In **K11 (80: 10: 10), K13 (80: 10: 10), K16 (80: 10: 10)** and **K18 (80: 10: 10)**, the solubilizing groups were respectively 1-(3-aminopropyl)-imidazol (API), 4-(2-aminoethyl)-morpholine (AEM), 4-(2-aminoethyl)-morpholine (AEM) and 1-(3-aminopropyl)-imidazol (API). For the preparation of each of these carriers, 18 mmol of the solubilizing group dissolved in 10 mL of DMF was added to a solution of PSI (20 mmol, 1.94 g) that was predissolved in 20 mL of DMF. Once flushed with N_2, the solution was stirred for 24 h. APD (6 mmol) predissolved in 10 mL of DMF was added to the clear solution before being flushed with N_2 and stirred for 24 h. The mixture was then added to the drug binding site diamine dropwise at 0°C, and stirred overnight at the same temperature, and then for 24 h at RT. For **K11 (80: 10: 10)** and **K13 (80: 10: 10)**, the diamine was PDA; for **K16 (80: 10:** 10), EDA and for **K18 (80: 10: 10)**, DET. All these carriers were water-soluble solids with respective yields, inherent viscosities and calculated molar masses(repeating unit): for **K11 (80: 10: 10)**: 67%, 32 mL/g and 2135 g/mol; for **K13 (80: 10: 10)**: 54%, 32 mL/g and 2164 g/mol; for **K16 (80: 10: 10)**: 51%, 34 mL/g and 2316 g/mol and for **K18 (80: 10: 10)**: 73%, 32 mL/g and 2175 g/mol.

For **K11 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 32H (34H: C*H* asp, *H* API); 3.75-3.5: 3H (3H: CHO*H*-C*H*$_2$OH); 3.4-3.0: 22H (20H: CONHC*H*$_2$), 2.9-2.0: 20H (22H: $CH_2CH_2CH_2$, CH-C*H*$_2$CONH,), 1.9-1.4: 31H (34H: CH_2-C*H*$_2$-CH_2)

For **K13 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 9H (10H: C*H* asp,); 3.75-3.5: 3H (3H: CHO*H*-C*H*$_2$OH); 3.4-3.0: 48H (52H: CONHC*H*$_2$, *H AEM*), 2.9-2.0: 19H (22H: CH_2CH CH_2, CH-C*H*$_2$CONH,), 1.9-1.4: 19H (18H: CH_2-C*H*$_2$-CH_2)

For **K16 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 9H (10H: C*H* asp); 3.75-3.5: 3H (3H: CHO*H*-C*H*$_2$OH); 3.4-3.0: 46H (52H: CONHC*H*$_2$, *H* AEM), 2.9-2.0: 19H (22H: $CH_2CH_2CH_2$, CH-C*H*$_2$CONH,), 1.9-1.4: 31H (34H: CH_2-C*H*$_2$-CH_2)

For **K18 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 35H (34H: C*H* asp, *H* API); 3.75-3.5: 3H (3H: CHO*H*-C*H*$_2$OH); 3.4-3.0: 18H (20H: CONHC*H*$_2$), 2.9-2.0: 23H (26H: $CH_2CH_2CH_2$, CH-C*H*$_2$CONH, CH_2CH_2NH, $CH_2CH_2NH_2$), 1.9-1.4: 23H (22H: CH_2-C*H*$_2$-, $CH_2CH_2CH_2$)

In **K12 (60: 20: 20)**, **K15 (60: 20: 20)** and **K19 (60: 20: 20)**, the solubilizing group was 4-(3-aminopropyl)-morpholine (APM) whilst for **K14 (60: 20: 20)** it was 1-(3-aminopropyl)-imidazol (API) and for **K17 (60: 20: 20)** and **K20 (60: 20: 20)**, it was 4-(2-aminoethyl)-morpholine (AEM). For the preparation of each of these carriers, 16 mmol of the solubilizing group dissolved in 10 mL of DMF was added to a solution of PSI (20 mmol, 1.94g) predissolved in 20 mL of DMF before flushing with N_2 and stirred for 24 h. APD (6 mmol) predissolved in 10 mL of DMF was added to the clear solution before being flushed with N_2 stirred for 24 h. The mixture was then added to the drug binding site diamine dropwise at 0°C and stirred overnight

at that temperature, and then for 24 h at RT. For **K12 (60: 20: 20)**, the diamine was PDA; for **K14 (60: 20: 20), K15 (60: 20: 20)** and **K17 (60: 20: 20)**, EDA and for **K18 (60: 20: 20)** and **K20 (60: 20: 20)**, DET. All these carriers were water-soluble solids with respective yield percent, inherent viscosities and calculated molar masses (repeating unit) determined as: for **K12 (60: 20: 20)**: 65%, 15mL/g and 1011 /mol; for **K14 (60: 20: 20)**: 52%, 13 **K17 (60: 20: 20)**mL/g and 1082 g/mol; for **K15 (60: 20: 20)**: 51%, 15 mL/g and 1068 g/mol; for: 50%, 13 mL/g and 1097 g/mol; for **K19 (60: 20: 20)**: 59%, 14mL/g and 2135 g/mol and for **K20 (60: 20: 20)**: 60%, 13mL/g and 1069 g/mol.

For **K12 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 5H (5H: C*H* asp, *H*); 3.75-3.5: 3H (3H: CHO*H*-C*H*$_2$OH); 3.4-3.0: 19H (22H: CONHC*H*$_2$), 2.9-2.0: 10H (12H: CH-C*H*$_2$CONH,), 1.9-1.4: 15H (14H: CH_2-C*H*$_2$-CH_2, CH_2-C*H*$_2$-C*H*$_2$-APM)

For **K14 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 32H (34H: C*H* asp, *H* API); 3.75-3.5: 3H (3H: CHO*H*-C*H*$_2$OH); 3.4-3.0: 22H (20H: CONHC*H*$_2$), 2.9-2.0: 10H (12H: CH_2 C*H*$_2$ C*H*$_2$, CH-C*H*$_2$CONH), 1.9-1.4: 11.2H (12H: CONH-CH_2-C*H*$_2$-C*H*$_2$-API)

For **K15 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 6H (5H: C*H* asp); 3.75-3.5: 3H (3H: CHO*H*-C*H*$_2$OH); 3.4-3.0: 23H (22H: CONHC*H*$_2$), 2.9-2.0: 11H (12H: CH-C*H*$_2$CONH), 1.9-1.4: 11.2H (12H: CH_2-C*H*$_2$-C*H*$_2$-APM)

For **K17 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 6 (5H: C*H* asp); 3.75-3.5: 3H (3H: CHO*H*-C*H*$_2$OH); 3.4-3.0: 20H (22H: CONHC*H*$_2$, *H* AEM), 2.9-2.0: 13H (12H: CH-C*H*$_2$CONH, CH_2-C*H*$_2$-,), 1.9-1.4: 6.4H (6H: CH_2-C*H*$_2$-CH_2-)

For **K19 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 32H (34H: C*H* asp); 3.75-3.5: 3H (3H: CHO*H*-C*H*$_2$OH); 3.4-3.0: 21H (22H: CONHC*H*$_2$), 2.9-2.0: 14H (16H: CH-C*H*$_2$CONH,), 1.9-1.4: 9H (12H: CH_2-C*H*$_2$-CH_2, CH_2-C*H*$_2$-C*H*$_2$-APM)

For **K20 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 5H (5H: C*H* asp); 3.75-3.5: 3H (3H: CHO*H*-C*H*$_2$OH); 3.4-3.0: 19H (22H: CONHC*H*$_2$, *H* AEM), 2.9-2.0: 15H (16H: --*CH*$_2$ C*H*$_2$-NH_2, CH_2 C*H*$_2$-NH-, CH-C*H*$_2$CONH), 1.9-1.4: 7.2H (6H: CH_2-C*H*$_2$-CH_2).

5. 3. 1. 1. 3 Synthesis of copolyaspartamides with noradenaline as platinum Binding site and aliphatic solubilizing groups

Copolyaspartamides **L1 (80: 10: 10)** to **L10 (60: 20: 20)** were prepared using the same procedure as for **K1 (60: 20: 20)** to **K10 (80: 10: 10)**, except that a variation in the structure of dihydroxylated platinum binding site led to the replacement of 3-amino-1, 2-propanediol (APD) by noradrenaline (Norad), an aromatic compound.

Polymers **L1 (80: 10: 10), L3 (80: 10: 10), L5 (80: 10: 10), L6 (80: 10: 10)** and **L9 (80: 10: 10)** were synthesized using the same mole ratios for solubilizing groups, platinum binding sites and diamine as for carrier **K3 (80: 10: 10)**. All these carriers were water-soluble **L1 (80: 10: 10)** solids with respective yield percentages, inherent viscosities and calculated molar masses(repeating unit), respectively, determined as for **L1 (80: 10: 10)**,: 65%, 15 mL/g and 1011 g/mol; for, 67%, 30 mL/g and 2141 g/mol, for **L3 (80: 10: 10)**, 54%, 27 mL/g and 2170 g/mol; for **L5 (80: 10: 10)**, 52%, 11 mL/g and 1118 g/mol; for **L6 (80: 10: 10)**, 51%, 29 mL/g and 2282 g/mol and for **L9 (80: 10: 10)**, 55%, 30 mL/g and 1946 g/mol.

For **L1 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 16H (13H: C*H* asp, *H aromatic*); 3.75-3.5: 1H (1H: CH_2-*CH*-OH); 3.4-3.0: 22H (20H: CONHC*H_2*), 2.9-2.0: 65H (70H: -CHC*H_2*CONH, CH_2-C*H_2*-N-(C*H_2*CH_3)$_2$), 1.8-0.8: 54H (50H: CH_2-C*H_2*-CH_2, N-CH_2-$(CH_3)_2$)

For **L3 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 12H (16H: C*H* asp, *H aromatic*); 3.75-3.5: 1.1H (1H: -CH_2C*H*OH); 3.4-3.0: 19H (20H: CONHC*H_2*), 2.9-2.0: 82H (86H: CH-C*H_2*CONH), 1.8-0.8: 1.7H (2H: CH_2-C*H_2*-CH_2)

For **L5 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 13.6H (13H: C*H* asp, *H aromatic*); 3.75-3.5: 0.9H (1H: -CH_2C*H*OH); 3.4-3.0: 18H (20H: CONHC*H_2*),

For **L6 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 14.2H (16H: C*H* asp); 3.75-3.5: 1.2H (1H: -CH_2C*H*OH); 3.4-3.0: 22H (20H: CONHC*H_2*), 2.9-2.0: 81H (84H: -N(C*H_3*)$_2$, -CH-CH_2CONH, -C*H_2*N)

For **L9 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 14H (16H: C*H* asp, *H aromatic*); 3.75-3.5: 1.2H (1H: -CH_2C*H*OH); 3.4-3.0: 22H (20H: CONHC*H_2*), 2.9-2.0: 83H (88H: CH-C*H_2*CONH, N(C*H_3*)$_2$, CH_2*NH*, C*H_2*NH_2), 1.8-0.8: 2H (2H: NHC*H_2*CH_2)

5. 3. 1. 1. 4 Copolyaspartamides with noradrenaline for platinum binding, bearing cyclic and aromatic solubilizing groups

Copolyaspartamides **L11 (80: 10: 10)** to **L20 (60: 20: 20)** were prepared using the same procedure as for **K11 (80: 10: 10)** to **K20 (60: 20: 20)**, except that a variation in the structure of dihydroxylated platinum binding site led to the

replacement of 3-amino-1, 2-propanediol (APD) with noradrenaline (Norad), an aromatic compound. Each of these polymers was synthesized according to the required stoichiometric ratios as specified for carriers **K11 (80: 10: 10)** to **K20 (60: 20: 20)**. All the synthesized copolyaspartamides obtained were water-soluble solids. Their respective yield percentages, inherent viscosities and calculated molar masses (repeating unit) are given below.

For **L11 (60: 20: 20)**: 61%, 12 mL/g and 1103 g/mol; for **L12 (80: 10: 10)**, 65%, 27 mL/g and 2199 g/mol; for **L13 (60: 20: 20)**: 59%, 12 mL/g and 1132 g/mol; for **L14 (80: 10: 10)**: 71%, 27 mL/g and 2365 g/mol; for **L15 (60: 20: 20)**: 51%, 11 mL/g and 1146 g/mol and for L16 (80: 10: 10): 57%, 27 mL/g and 2394 g/mol; for **L17 (80: 10: 10)**: 73%, 28 mL/g and 2253 g/mol; for **L18 (80: 10: 10)**: 58%, 25 mL/g and 2239 g/mol; for **L19 (80: 10: 10)**: 55%, 23 mL/g and 2282 g/mol; for **L20 (60: 20: 20):** 60%, 11 mL/g and 1147 g/mol.

For L11 (60: 20: 20): ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 15H (17H: C*H* asp*, H* aromatic); 3.75-3.5: 1.1H (1H: -CH_2C*H*OH); 3.4-3.0: 8.8H (10H: CONHC*H_2*), 2.9-2.0: 20.4H (22H: CH-C*H_2*CONH, -CH_2-C*H_2*NH_2), 1.8-0.9: 30H (34H: CH_2C*H_2*CH_2-APM, CH_2C*H_2*CH_2)

For **L12 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 11H (13H: C*H* asp, *H aromatic*); 3.75-3.5: 22H (25H: -CH_2C*H*OH, C*H* APM); 3.4-3.0: 49H (52H: CONHC*H_2*, C*H_2* APM), 2.9-2.0: 83H (88H: CH-C*H_2*CONH, N(C*H_3*)$_2$, CH_2*NH*, C*H_2*NH_2), 1.8-0.9: 2H (2H: NHC*H_2*CH_2)

For **L13 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 8.8H (8H: C*H* asp, *H aromatic*); 3.75-3.5: 11H (10H: -CH_2C*H*OH, C*H* AEM); 3.4-3.0: 19H (20H: CONHC*H_2*, C*H_2* AEM), 2.9-2.0: 10H (12H: CH-C*H_2*CONH, N(C*H_3*)$_2$, C*H_2*NH_2), 1.8-0.9: 7.5H (8H: CH_2-AEM, CH_2C*H_2*CH_2)

For **L14 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 29H (32H: C*H* asp*, H* aromatic); 3.75-3.5: 1.2H (1H: -CH_2C*H*OH); 3.4-3.0: 18.6H (20H: CONHC*H_2*), 2.9-2.0: 21H (24H: CH-C*H_2*CONH, -CH_2-C*H_2*NH_2), 1.8-0.9: 31H (34H: CH_2C*H_2*CH_2-APM, CH_2C*H_2*CH_2)

For **L15 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 7.8H (8H: C*H* asp, *H aromatic*); 3.75-3.5: 11H (10H: -CH_2C*H*OH, C*H* APM); 3.4-3.0: 19H (22H: CONHC*H_2*, C*H_2* APM), 2.9-2.0: 16H (14H: CH-C*H_2*CONH, N(C*H_3*)$_2$, CH_2*NH*, C*H_2*NH_2), 1.8-0.9:11H (14H: NHC*H_2*CH_2)

For **L16 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 9.2H (8H: C*H* asp, *H aromatic*); 3.75-3.5: 22H (25H: -CH_2C*H*OH, C*H* AEM); 3.4-3.0: 30H (32H: CONHC*H_2*, C*H_2* AEM), 2.9-2.0: 23.6H (22H: CH-C*H_2*CONH, N(C*H_3*)$_2$, C*H_2*NH_2), 1.8-0.9: 6.4H (6H: CH_2-AEM, CH_2C*H_2*CH_2)

For **L17 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 30H (33H: C*H* asp*, H* aromatic); 3.75-3.5: 0.8H (1H: -CH_2C*H*OH); 3.4-3.0: 19H (22H: CONHC*H_2*), 2.9-2.0: 21H (22H: CH-C*H_2*CONH, -CH_2-C*H_2*NH_2), 1.8-0.9: 33H (36H: CH_2C*H_2*CH_2-API, CH_2C*H_2*CH_2)

For **L18 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 15H (33H: C*H* asp, *H aromatic*); 3.75-3.5: 22H (25H: -CH_2C*H*OH, C*H* APM); 3.4-3.0: 47H (52H: CONHCH_2, CH_2 APM), 2.9-2.0: 26H (24H: CH-CH_2CONH, N(CH_3)$_2$, CH_2*NH*, C$H_2$$NH_2$), 1.8-0.9: 32H (34H: C$H_2CH_2$-APM)

For **L19 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 11H (13H: C*H* asp, *H aromatic*); 3.75-3.5: 23H (25H: -CH_2C*H*OH, C*H* AEM); 3.4-3.0: 49H (52H: CONHCH_2, CH_2 AEM), 2.9-2.0: 21H (24H: CH-CH_2CONH, N(CH_3)$_2$, C$H_2$$NH_2$), 1.8-0.9: 16H (18H: CH_2-AEM)

For **L19 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 11H (13H: C*H* asp, *H aromatic*); 3.75-3.5: 23H (25H: -CH_2C*H*OH, C*H* AEM); 3.4-3.0: 49H (52H: CONHCH_2, CH_2 AEM), 2.9-2.0: 21H (24H: CH-CH_2CONH, N(CH_3)$_2$, C$H_2$$NH_2$), 1.8-0.9: 16H (18H: CH_2-AEM)

For **L20 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm: 8.4-4.0: 8H (8H: C*H* asp, *H aromatic*); 3.75-3.5: 9H (10H: -CH_2C*H*OH, C*H* AEM); 3.4-3.0: 19H (22H: CONHCH_2, CH_2 AEM), 2.9-2.0: 13H (14H: CH-CH_2CONH, N(CH_3)$_2$, C$H_2$$NH_2$), 1.8-0.9: 9.4H (8H: CH_2-AEM)

5. 3. 1. 2: Synthesis *of polyamidoamine carriers*

5. 3. 1. 2.1 Preparation of polyamidoamine carriers with APD as platinum binding site bearing aliphatic solubilising groups

Carrier M1 (60: 20: 20): MBA (3. 082 g, 20 mmol) was dissolved in hot H_2O (16 mL). After cooling at RT, APD (3. 5 mmol: 0.319 g) in H_2O (3 mL) was added and the suspension, after saturation with N_2, was heated for 24 h in the incubator at 50°C. With all components dissolved, DEEA (12 mmol) was added to the clear solution, which was re-saturated with N_2 and heated at 50°C in the incubator for 48 h. After cooling at RT, the clear solution was added dropwise to PDA (12 mmol: 0.89 g) at ice bath temperature, while stirring went for overnight at that temperature then at RT for 24 h. The solution was concentrated on rotating evaporator to 6 mL, bath temperature 50°C, and then precipitated with ethanol: hexane (2: 1, 20 mL). The precipitate was washed with hot acetone several times, then dissolved in distilled H_2O (15 mL) and the pH of the solution adjusted to 8 with conc. HCl. After dialysis for 48 h in 12000-14000 cut membrane tubing against changing of distilled water, the solution was freeze dried, post-dried and collected in a yield range of 61%, η_{inh}= 7 mL/g and a molar mass (repeating unit) of 1283 g/mol.

For **M1 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 8H (10H: CONHC*H*$_2$HNCO); 3.9-3.5: 3H (3H: C*H*OH-C*H*$_2$OH); 3.1-3.0: 11H (1OH: NC*H*$_2$C*H*$_2$), 2.9-2.0: 107H (100H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$ CH_2N), 1.8-0.9: 35H (38H: CH_2CH_3, $CH_2CH_2CH_2$).

Carrier M2 (80: 10: 10): Following the same procedure as before, except that DEP was used in place of DEEA. After the solution was worked up, it was collected at 65 % in yield, η_{inh}= 19 mL/g and a molar mass (repeating unit) of 2619 g/mol.

For **M2 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 17H (20H: CONHC*H*$_2$HNCO); 3.9-3.5: 3.2H (3H: C*H*OH-C*H*$_2$OH); 3.1-3.0:

18H (2OH: NC*H*$_2$C*H*$_2$), 2.9-2.0: 118H (128H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$ CH$_2$N, C*H*$_2$NC*H*$_2$, CH$_2$NH$_2$), 1.8-0.9: 60H (66H: CH$_2$C*H*$_3$, CH$_2$C*H*$_2$CH$_2$).

Carrier M3 (60: 20: 20): Same procedure as the forgoing. DMEA was used as solubilizing group. The yield percent was 59 %, η_{inh}= 7 mL/g, and the molar mass(repeating unit) was 1326 g/mol.

For **M3 (60: 20: 20)**: ^{1}NMR (D$_2$O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 9H (10H: CONHC*H*$_2$HNCO); 3.9-3.5: 3.2H (3H: C*H*OH-C*H*$_2$OH); 3.1-3.0: 7.8H (10H: NC*H*$_2$C*H*$_2$), 2.9-2.0: 105H (114H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$ CH$_2$N, C*H*$_2$NC*H*$_3$), 1.8-0.9: 2H (2H: CH$_2$C*H*$_2$CH$_2$).

Carrier M4 (80: 10: 10): Following the same procedure as for **Carrier M1 (60: 20: 20)**, except that EDA was used in place of PDA. After the solution was worked up, it was collected at 71 % in yield, η_{inh}= 18 mL/g and a molar mass (repeating unit) of 2265 g/mol.

For **M4 (80: 10: 10)**: ^{1}NMR (D$_2$O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 21H (20H: CONHC*H*$_2$HNCO); 3.9-3.5: 3H (3H: C*H*OH-C*H*$_2$OH); 3.1-3.0: 17H (2OH: NC*H*$_2$C*H*$_2$), 2.9-2.0: 119H (126H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$ CH$_2$N), 1.8-0.9: 43H (48H: CH$_2$C*H*$_3$, CH$_2$C*H*$_2$CH$_2$).

Carrier M5 (60: 20: 20): Following the same procedure as for **Carrier M2 (80: 10: 10)**, except that EDA was used in place of PDA. After the solution was worked up, it was collected at 51 % in yield, η_{inh}= 8 mL/g and a molar mass (repeating unit) of 1311 g/mol.

For **M5 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 9H (10H: CONHC*H*$_2$HNCO); 3.9-3.5: 3.2H (3H: C*H*OH-C*H*$_2$OH); 3.1-3.0: 9H (1OH: NC*H*$_2$C*H*$_2$), 2.9-2.0: 94H (102H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$ CH_2N, C*H*$_2$NC*H*$_2$, CH_2NH_2), 1.8-0.9: 21H (24H: CH_2C*H*$_3$).

Carrier M6 (80: 10: 10): Following the same procedure as for **Carrier M3 (60: 20: 20)**, except that EDA was used in place of PDA. After the solution was worked up, it was collected in 57 % yield, η_{inh}= 19 mL/g and a molar mass (repeating unit) of 2294 g/mol.

For **M6 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 22H (20H: CONHC*H*$_2$HNCO); 3.9-3.5: 3.1H (3H: C*H*OH-C*H*$_2$OH); 3.1-3.0: 17H (20H: NC*H*$_2$C*H*$_2$), 2.9-2.0: 131H (142H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$, CH_2N, C*H*$_2$NC*H*$_3$).

Carrier M7 (60: 20: 20): Following the same procedure as for **Carrier M1 (60: 20: 20)**, except that DET was used in place of PDA. After the solution was worked up, it was collected in 58 % yield, η_{inh}= 5 mL/g and a molar mass (repeating unit) of 1199 g/mol.

For **M7 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 9.3H (10H: CONHC*H*$_2$HNCO); 3.9-3.5: 3H (3H: C*H*OH-C*H*$_2$OH); 3.1-3.0: 8.7H (1OH: NC*H*$_2$C*H*$_2$), 2.9-2.0: 102H (104H: C*H*$_2$C*H*$_2$CONH, HNCOCH2, C*H*$_2$NH, C*H*$_2$$NCH_2CH_3$, C*H*$_2$NC*H*$_2$), 1.8-0.9: 34H (38H: CH_2C*H*$_3$).

Carrier M8 (80: 10: 10): Following the same procedure as before, except that DET was used in place of PDA. After the solution was worked up, it was collected in 58 % yield, η_{inh}= 18 mL/g and a molar mass (repeating unit) of 2395 g/mol.

For **M8 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 17H (20H: CONHC*H*$_2$HNCO); 3.9-3.5: 2.8H (3H: C*H*OH-C*H*$_2$OH); 3.1-3.0: 17H (2OH: NC*H*$_2$C*H*$_2$), 2.9-2.0: 126H (132H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$ CH_2N, C*H*$_2$NC*H*$_2$, CH_2NH_2), 1.8-0.9: 61H (66H: CH_2CH_3, $CH_2CH_2CH_2$).

Carrier M9 (80: 10: 10): Following the same procedure as for **M3 (60: 20: 20)**, except that DET was used in place of PDA. After the solution was worked up, it was collected in 55 % yield, η_{inh}= 18 mL/g and a molar mass (repeating unit) of 2438 g/mol.

For **M9 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 18H (20H: CONHC*H*$_2$HNCO); 3.9-3.5: 3.1H (3H: C*H*OH-C*H*$_2$OH); 3.1-3.0: 18H (20H: NC*H*$_2$C*H*$_2$), 2.9-2.0: 133H (148H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$C*H*$_2$ C*H*$_2$$NH_2$, C*H*$_2$$NH_2$, C*H*$_2$NC*H*$_3$), 1.8-0.9: 2. 2H (2H: NHC*H*$_2$).

Carrier M10 (60: 20: 20): Following the same procedure as for **M3 (60: 20: 20)**, except that DET was used in place of PDA. After the solution was worked up, it was collected in 20 % yield, η_{inh}= 8 mL/g and a molar mass (repeating unit) of 1228 g/mol.

For **M10 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 8H (10H: CONHC*H*$_2$HNCO); 3.9-3.5: 3.1H (3H: C*H*OH-C*H*$_2$OH); 3.1-3.0:

11H (10H: NC*H*$_2$C*H*$_2$), 2.9-2.0: 109H (116H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$C*H*$_2$ C*H*$_2$NH$_2$, C*H*$_2$NH$_2$, C*H*$_2$NC*H*$_3$), 1.8-0.9: 2H (2H: NHC*H*$_2$).

5. 3. 1. 2. 2 Preparation of polyamidoamine carriers with APD as platinum binding site, bearing cyclic and aromatic solubilizing groups

Carrier M11 (80: 10: 10): MBA (3. 082 g, 20 mmol) was dissolved in hot H_2O (16 mL). After cooling at RT, APD (2 mmol: 0.182 g) in H_2O (3 mL) was added, and the suspension, after saturation with N_2, was heated for 24 h in the incubator at 50°C. With all components dissolved, API (16 mmol) was added to the clear solution which was re-saturated with N_2 and heated at 50°C in the incubator for 48 h. After cooling at RT, the clear solution was added dropwise to PDA (6 mmol, 0.445 g) at 0˚C and stirred overnight at that temperature, then at RT for 24 h. The solution was concentrated on rotating evaporator to 6 mL, bath temperature 50°C, and then precipitated with ethanol: hexane (2: 1, 20 mL). The precipitate was washed with hot acetone several times, then dissolved in distilled H_2O (15 mL) and the pH of the solution adjusted to 8 with conc. HCl. After dialysis for 48 h in 12000-14000 cut membrane tubing against changing of distilled water, the solution was freeze dried, post-dried and collected in a yield range of 67%, η_{inh}= 19 mL/g and a molar mass (repeating unit) of 2705 g/mol.

^{1}NMR (D_2O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 41H (44H: CONHC*H*$_2$HNCO, *H* API); 3.9-3.5: 3.2H (3H: CHO*H*-C*H*$_2$OH); 3.4-3.0: 17H (20H:

NC*H*$_2$C*H*$_2$), 2.9-2.0: 75H (82H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, *CH*$_2$NH$_2$), 1.8-0.9: 30H (34H: CH$_2$-C*H*$_2$-CH$_2$, CH$_2$CH$_2$-API)

Carrier M12 (60: 20: 20): Following the same procedure as before, except that APM was used in place of API. After the solution was worked up, it was collected in 65 % yield, η_{inh}= 6 mL/g and a molar mass (repeating unit) of 1296 g/mol.

For **M12 (60: 20: 20)**: ^{1}NMR (D$_2$O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 8H (10H: CONHC*H*$_2$HNCO, *H* API); 3.9-3.5: 10H (12H: CHO*H*-C*H*$_2$OH, *H* APM); 3.4-3.0: 20H (22H: NC*H*$_2$CH$_2$, *H* APM), 2.9-2.0: 37H (40H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, *CH*$_2$NH$_2$), 1.8-0.9: 12H (14H: CH$_2$-C*H*$_2$-CH$_2$)

Carrier M13 (80: 10: 10): Same procedure as the forgoing. AEM was used as solubilizing group. The yield was 54 %, η_{inh}= 22 mL/g and a molar mass (repeating unit) of 2734 g/mol.

For **M13 (80: 10: 10)**: ^{1}NMR (D$_2$O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 18H (20H: CONHC*H*$_2$HNCO, *H* AEM); 3.9-3.5: 24H (27H: CHO*H*-C*H*$_2$OH, *H* AEM); 3.4-3.0: 48H (52H: NC*H*$_2$CH$_2$, *H* AEM), 2.9-2.0: 82H (86H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, *CH*$_2$NH$_2$), 1.8-0.9: 17H (18H: CH$_2$-C*H*$_2$-CH$_2$, CH$_2$-C*H*$_2$-AEM)

Carrier M14 (80: 10: 10): Following the same procedure as for **Carrier M11 (80: 10: 10)**, except that EDA was used in place of PDA. After the solution was worked up, it was collected in 52 % yield, η_{inh}= 23 mL/g and a molar mass (repeating unit) of 2857 g/mol.

For **M14 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 41H (40H: CONHC*H*$_2$HNCO, *H* API); 3.9-3.5: 3.1H (3H: CHO*H*-C*H*$_2$OH); 3.4-3.0: 18H (20H: NC*H*$_2$CH$_2$), 2.9-2.0: 90H (98H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, *CH*$_2$NH$_2$), 1.8-0.9: 29H (24H: CH$_2$-C*H*$_2$-CH$_2$, CH$_2$CH$_2$-API)

Carrier M15 (60: 20: 20): Following the same procedure as for **Carrier M12 (80: 10: 10)**, except that EDA was used in place of PDA. After the solution was worked up, it was collected in 51 % yield, η_{inh}= 5 mL/g and a molar mass (repeating unit) of 1353 g/mol.

For **M15 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 8H (10H: CONHC*H*$_2$HNCO, *H* API); 3.9-3.5: 10H (12H: CHO*H*-C*H*$_2$OH, *H* APM); 3.4-3.0: 19H (22H: NC*H*$_2$CH$_2$, *H* APM), 2.9-2.0: 37H (40H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, *CH*$_2$NH$_2$), 1.8-0.9: 10H (12H: CH$_2$-C*H*$_2$-CH$_2$)

Carrier M16 (80: 10: 10): Following the same procedure as for **Carrier M13 (80: 10: 10)**, except that EDA was used in place of PDA. After the solution was worked up, it was collected in 50 % yield, η_{inh}= 19 mL/g and a molar mass (repeating unit) of 2886 g/mol.

For **M16 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 17H (20H: CONHC*H*$_2$HNCO, *H* AEM); 3.9-3.5: 32H (35H: CHO*H*-C*H*$_2$OH, *H* AEM); 3.4-3.0: 47H (52H: NC*H*$_2$CH$_2$, *H* AEM), 2.9-2.0: 75H (80H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, *CH*$_2$NH$_2$), 1.8-0.9: 14H (16H: CH$_2$-C*H*$_2$-CH$_2$, CH$_2$-C*H*$_2$-AEM)

Carrier M17 (60: 20: 20): Following the same procedure as for **Carrier M11 (80: 10: 10)**, except that DET was used in place of PDA. After the solution was worked up, it was collected in 73 % yield, η_{inh}= 7 mL/g and a molar mass (repeating unit) of 1325 g/mol.

For **M17 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 16H (19H: CONHC*H*$_2$HNCO, *H* API); 3.9-3.5: 2.9H (3H: CHO*H*-C*H*$_2$OH); 3.4-3.0: 8H (10H: NC*H*$_2$CH$_2$), 2.9-2.0: 41H (44H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, *CH*$_2$NH$_2$), 1.8-0.9: 11H (14H: CH$_2$-C*H*$_2$-CH$_2$, CH$_2$CH$_2$-API)

Carrier M18 (80: 10: 10): Following the same procedure as before, except that DET was used in place of PDA. After the solution was worked up, it was collected in 59 % yield, η_{inh}= 22 mL/g and a molar mass (repeating unit) of 2731 g/mol.

For **M18 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 17H (20H: CONHC*H*$_2$HNCO, *H* API); 3.9-3.5: 23H (27H: CHO*H*-C*H*$_2$OH, *H* APM); 3.4-3.0: 45H (52H: NC*H*$_2$CH$_2$, *H* APM), 2.9-2.0: 76H (82H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, *CH*$_2$NH$_2$), 1.8-0.9: 11H (14H: CH$_2$-C*H*$_2$-CH$_2$)

Carrier M19 (80: 10: 10): Following the same procedure as for **M13 (80: 10: 10)**, except that DET was used in place of PDA. After the solution was worked up, it was collected in 55 % yield, η_{inh}= 23 mL/g and a molar mass (repeating unit) of 2774 g/mol.

For **M19 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 18H (20H: CONHC*H*$_2$HNCO, *H* AEM); 3.9-3.5: 25H (27H: CHO*H*-C*H*$_2$OH,

H AEM); 3.4-3.0: 48H (52H: NC*H*$_2$CH_2, *H* AEM), 2.9-2.0: 79H (82H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, *C*H_2NH_2), 1.8-0.9: 15H (18H: CH_2-C*H*$_2$-CH_2, CH_2-C*H*$_2$-AEM)

Carrier M20 (60: 20: 20): Following the same procedure as for **M19 (80: 10: 10)**. After the solution was worked up, it was collected in 60 % yield, η_{inh}= 5 mL/g and a molar mass (repeating unit) of 1354 g/mol.

For **M20 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 8H (10H: CONHC*H*$_2$HNCO, *H* AEM); 3.9-3.5: 10H (12H: CHO*H*-C*H*$_2$OH, *H* AEM); 3.4-3.0: 20H (22H: NC*H*$_2$CH_2, *H* AEM), 2.9-2.0: 39H (42H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, *C*H_2NH_2), 1.8-0.9: 7H (8H: CH_2-C*H*$_2$-CH_2, CH_2-C*H*$_2$-AEM)

5. 3. 1. 2.3 Preparation of copolyamidoamine carriers with Asp as platinum binding site, bearing aliphatic solubilising groups

Carrier N1 (80: 10: 10): MBA (3.082 g, 20 mmol) was dissolved in hot distilled H_2O (16 mL). The clear solution allowed cooling at RT before the addition of DL-aspartic acid (0.293 g, 2.2 mmol) and Na_2CO_3 (0.233 g, 2.2 mmol), dissolved in H_2O (10 mL). After saturation with N_2 and stirring for 24 h in the incubator at 60°C, DEEA (16 mmol) dissolved in H_2O (10 mL) was added and the clear solution allowed to stir for 48 h at 60°C in the incubator. After cooling at RT, the solution was added dropwise to PDA (6 mmol, 0.445 g) dissolved in H_2O (10 mL) then flushed with N_2. With all the components dissolved, the solution was stirred at RT for 24 h, then concentrated on rotating evaporator to 5 mL at 50°C bath temperature, then precipitated with 25 mL of a mixture of ethanol: hexane (2: 1). The precipitate was washed with acetone, dissolved in H_2O and dilalysed for 48 h in Spectra/Por 4

tubing after pH adjustement to 8 with conc HCl. Dialysis occurred against distilled water before freeze drying and collection of the whitish solid in a yield range of 67 %, η_{inh}= 33 mL/g and calculated molar mass (repeating unit) of 2691 g/mol.
For **N1 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 19H (21H: CONHC*H*$_2$HNCO, NC*H*CH_2 $(COOH)_2$); 3.1-2.9: 17H (18H: NC*H*$_2$C*H*$_2$), 2.8-2.0: 123H (132H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$, C*H*$_2$$NH_2$, C*H*$_2$COOH, CH_2C*H*$_2$N(C*H*$_2$$CH_3)_2$), 1.8-0.9: 45H (50H: NCH_2C*H*$_3$, CH_2C*H*$_2$$CH_2$).

Carrier N1 (80: 10: 10): This carrier was prepared by the same procedure leading to **N1 (80: 10: 10)**, except that 12 mmol of DEEA, 4.4 mmol of DL-aspartic acid (0.586 g) and 12 mmol of PDA (0.89 g) were used. The solid product was collected in 61% yield, η_{inh}=15 mL/g and calculated molar mass (repeating unit) =1341 g/mol.

For **N1 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 10H (11H: CONHC*H*$_2$HNCO, NC*H*CH_2 $(COOH)_2$); 3.1-2.9: 7H (8H: NC*H*$_2$C*H*$_2$), 2.8-2.0: 56H (62H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$, C*H*$_2$$NH_2$, C*H*$_2$COOH, CH_2C*H*$_2$N(C*H*$_2$$CH_3)_2$), 1.8-0.9: 18H (20H: NCH_2C*H*$_3$, CH_2C*H*$_2$$CH_2$).

Carrier N3 (60: 20: 20): By the same procedure leading to **N2 (60: 20: 20),** except that DEP was used in place of DDEA. 65% of the product was collected with η_{inh}=13 mL/g and calculated molar mass (repeating unit) of 1327 g/mol.

For **N3 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 10H (11H: CONHC*H*$_2$HNCO, NC*H*CH_2 $(COOH)_2$); 3.1-2.9: 7H (8H:

NC*H*$_2$C*H*$_2$), 2.8-2.0: 54H (62H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$, C*H*$_2$NH$_2$, C*H*$_2$COOH, CH$_2$C*H*$_2$N(C*H*$_2$CH$_3$)$_2$), 1.8-0.9: 23H (26H: NCH$_2$C*H*$_3$, CH$_2$C*H*$_2$CH$_2$).

Carrier N4 (80: 10: 10) was prepared by the same procedure leading to **N1 (80: 10: 10)**, except that DMEA was used in place of DEEA. 54% of the product was collected with η_{inh}=34 mL/g and calculated molar mass (repeating unit) of 2720 g/mol.

For **N4 (80: 10: 10):** ^{1}NMR (D$_2$O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 20H (21H: CONHC*H*$_2$HNCO, NC*H*CH$_2$ (COOH)$_2$); 3.1-2.9: 16H (18H: NC*H*$_2$C*H*$_2$), 2.8-2.0: 142H (148H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$, C*H*$_2$NH$_2$, C*H*$_2$COOH, CH$_2$C*H*$_2$N(C*H*$_2$CH$_3$)$_2$), 1.8-0.9: 1.7H (2H: CH$_2$C*H*$_2$CH$_2$).

Carrier N5 (60: 20: 20) was prepared by the same procedure as for **N2 (60: 20: 20)**, except that EDA was used in place of PDA. Yield =52 %, η_{inh}=14 mL/g and calculated molar mass (repeating unit) =1382 g/mol.

For **N5 (60: 20: 20)**: ^{1}NMR (D$_2$O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 12H (11H: CONHC*H*$_2$HNCO, NC*H*CH$_2$ (COOH)$_2$); 3.1-2.9: 7H (8H: NC*H*$_2$C*H*$_2$), 2.8-2.0: 55H (62H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$, C*H*$_2$NH$_2$, C*H*$_2$COOH, CH$_2$C*H*$_2$N(C*H*$_2$CH$_3$)$_2$), 1.8-0.9: 16H (18H: NCH$_2$C*H*$_3$, CH$_2$C*H*$_2$CH$_2$).

Carrier N6 (80: 10: 10): By the same procedure as for **N4 (80: 10: 10)**, except that DEP and EDA were used respectively in places of DMEA and PDA. Yield =51 %, η_{inh}=34 mL/g and calculated molar mass (repeating unit) =2309 g/mol.

For **N6 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 20H (21H: CONHC*H*$_2$HNCO, NC*H*CH_2 $(COOH)_2$); 3.1-2.9: 16H (18H: NC*H*$_2$C*H*$_2$), 2.8-2.0: 127H (132H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$, C*H*$_2$$NH_2$, C*H*$_2$COOH, CH_2C*H*$_2$N(C*H*$_2$$CH_3$)$_2$), 1.8-0.9: 44H (48H: NCH_2C*H*$_3$, CH_2C*H*$_2$$CH_2$).

Carrier N7 (60: 20: 20): By the same procedure leading to **N2 (60: 20: 20)**, except that DMEA and EDA were used respectively in places of DEEA and PDA. 50 % of the product was collected with η_{inh}=16 mL/g and calculated molar mass (repeating unit) of 1411 g/mol.

For **N7 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 11H (11H: CONHC*H*$_2$HNCO, NC*H*CH_2 $(COOH)_2$); 3.1-2.9: 7.5H (8H: NC*H*$_2$C*H*$_2$), 2.8-2.0: 58H (62H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$, C*H*$_2$$NH_2$, C*H*$_2$COOH, CH_2C*H*$_2$N(C*H*$_2$$CH_3$)$_2$.

Carrier N8 (80: 10: 10): By the same procedure leading to **N1 (80: 10: 10),** except that DET was used in place of PDA. 73 % of the product was collected with η_{inh}=32 mL/g and calculated molar mass (repeating unit) of 2467 g/mol.

For **N8 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 21H (23H: CONHC*H*$_2$HNCO, NC*H*CH_2 $(COOH)_2$); 3.1-2.9: 15H (18H: NC*H*$_2$C*H*$_2$), 2.8-2.0: 131H (134H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$, C*H*$_2$$NH_2$, C*H*$_2$COOH, CH_2C*H*$_2$N(C*H*$_2$$CH_3$)$_2$), 1.8-0.9: 46H (50H: NCH_2C*H*$_3$, CH_2C*H*$_2$$CH_2$).

Carrier N9 (60: 20: 20) was prepared by the same procedure as for **N2 (60: 20: 20)**, except that DET was used in place of PDA. 59 % of the product was collected with η_{inh}=15 mL/g and calculated molar mass (repeating unit) of 1243 g/mol.

For **N9 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 11H (13H: CONHC*H*$_2$HNCO, NC*H*CH_2 $(COOH)_2$); 3.1-2.9: 7H (8H: NC*H*$_2$C*H*$_2$), 2.8-2.0: 59H (64H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$, C*H*$_2$$NH_2$, C*H*$_2$COOH, CH_2C*H*$_2$N(C*H*$_2$$CH_3)_2$), 1.8-0.9: 22H (26H: NCH_2C*H*$_3$, CH_2C*H*$_2$$CH_2$).

Carrier N10 (80: 10: 10) was prepared by the same procedure leading to **N4 (80: 10: 10)**, except that DET was used in place of PDA. 55 % of the product was collected with η_{inh}=34 mL/g and calculated molar mass (repeating unit) of 2496 g/mol.

For **N10 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.75-4.5: 20H (23H: CONHC*H*$_2$HNCO, NC*H*CH_2 $(COOH)_2$); 3.1-2.9: 16H (18H: NC*H*$_2$C*H*$_2$), 2.8-2.0: 144H (150H: C*H*$_2$C*H*$_2$CONH, HNCOC*H*$_2$, C*H*$_2$$NH_2$, C*H*$_2$COOH, CH_2C*H*$_2$N(C*H*$_2$$CH_3)_2$), 1.8-0.9: 2.2H (2H: CH_2C*H*$_2$$CH_2$).

5. 3. 1. 2. 4 Preparation of polyamidoamine carriers with Asp as platinum binding Site, bearing cyclic and aromatic solubilizing groups

Carrier N11 (80: 10: 10): MBA (3.082 g, 20 mmol) was dissolved in hot distilled H_2O (16 mL). The clear solution allowed cooling at RT before the addition of DL-aspartic acid (0.293 g, 2.2 mmol) and Na_2CO_3 (0.233 g, 2.2 mmol) dissolved in H_2O (10 mL). After saturation with N_2 and stirring for 24 h in the incubator at 60°C,

API (16 mmol) dissolved in H_2O (10 mL) was added and the clear solution was stirred for 48 h at 60°C in the incubator. After cooling at RT, the solution was added dropwise to PDA (6 mmol, 0.445 g) dissolved in H_2O (10 mL) then flushed with N_2. With all the components dissolved, the solution was stirred at RT for 24 h, and then concentrated on rotating evaporator to 5 mL at 50°C bath temperature, then precipitated with 25 mL of a mixture of ethanol: hexane (2: 1). The precipitate was washed with acetone, dissolved in H_2O and dilalysed for 48 h in Spectra/Por 4 tubing after pH adjustement to 8 with conc HCl. Dialysis occurred against distilled water before freeze drying and collection of the whitish solid in a yield range of 62 %, η_{inh}= 28 mL/g and calculated molar mass (repeating unit) of 2763 g/mol.
For **N11 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 42H (45H: CONHC*H*$_2$HNCO, NC*H* $(COOH)_2$ *H* API);); 3.4-3.0: 18H (20H: NC*H*$_2$C*H*$_2$), 2.9-2.0: 81H (84H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, $CHCH_{2(}COOH)_2$, *CH*$_2$$NH_2$), 1.8-1.4: 31H (34H: CH_2-C*H*$_2$-CH_2, C*H*$_2$C*H*$_2$-API).

Carrier N12 (60: 20: 20): This carrier was prepared by the same procedure leading to **N11 (80: 10: 10)**, except that 12 mmol of API, 4.4 mmol of DL-aspartic acid (0.586 g) and 12 mmol of PDA (0.89 g) were used. The solid product was collected in a yield range of 60% , η_{inh}=15 mL/g and calculated molar mass (repeating unit) =1341 g/mol.

For **N12 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 19H (20H: CONHC*H*$_2$HNCO, NC*H* $(COOH)_2$ *H* API);); 3.4-3.0: 8H (10H: NC*H*$_2$C*H*$_2$), 2.9-2.0: 42H (44H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, $CHCH_{2(}COOH)_2$, *CH*$_2$$NH_2$), 1.8-1.4: 12H (14H: CH_2-C*H*$_2$-CH_2, CH_2CH_2-API).

Carrier N13 (60: 20: 20): By the same procedure leading to **N12 (60: 20: 20),** except that APM was used in place of API. 65% of the product was collected with η_{inh}=11 mL/g and calculated molar mass (repeating unit) of 1354 g/mol.

For **N13 (60: 20: 20)**: ^{1}NMR (D_2O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 9.6H (11H: CONHC*H*$_2$HNCO, NC*H* $(COOH)_2$); 3.75-3.5: 8H (9H: *H* APM); 3.4-3.0: 8H (9H: *H* APM), 2.9-2.0: 41H (44H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, CHC*H*$_2$$(COOH)_2$, *CH*$_2$$NH_2$), 1.8-1.4: 12H (14H: CH_2-C*H*$_2$-CH_2, CH_2CH_2-APM).

Carrier N14 (80: 10: 10) was prepared by the same procedure leading to **N11 (80: 10: 10)**, except that AEM was used in place of API. 52% of the product was collected with η_{inh}=29 mL/g and calculated molar mass (repeating unit) of 2792 g/mol.

For **N14 (80: 10: 10)** ^{1}NMR (D_2O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 19H (21H: CONHC*H*$_2$HNCO, NC*H* $(COOH)_2$); 3.75-3.5: 8H (9H: *H* AEM); 3.4-3.0: 48H (52H: NC*H*$_2$CH, *H* AEM), 2.9-2.0: 81H (84H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, CHC*H*$_2$$(COOH)_2$, *CH*$_2$$NH_2$), 1.8-1.4: 14H (16H: CH_2-C*H*$_2$-CH_2).

Carrier N15 (60: 20: 20) was prepared by the same procedure as for **N12 (60: 20: 20)**, except that EDA was used in place of PDA. 54 % of the product was collected with η_{inh}=11 mL/g and calculated molar mass (repeating unit) of 1425 g/mol.

For **N15 (60: 20: 20)** ^{1}NMR (D_2O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 18H (20H: CONHC*H*$_2$HNCO, NC*H* $(COOH)_2$ *H* API);); 3.4-3.0: 9H (10H:

NC*H*$_2$CH$_2$), 2.9-2.0: 41H (44H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, CHCH$_2$(COOH)$_2$, *CH*$_2$NH$_2$), 1.8-1.4: 10H (12H: CH$_2$CH$_2$-API).

Carrier N16 (80: 10: 10): By the same procedure as for **N14 (80: 10: 10)**, except that APM and EDA were used respectively in places of AEM and PDA.54 % of the product was collected with η_{inh}=28 mL/g and calculated molar mass (repeating unit) of 2901 g/mol.

For **N16 (80: 10: 10)**: ^{1}NMR (D$_2$O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 18H (21H: CONHC*H*$_2$HNCO, NC*H* (COOH)$_2$); 3.75-3.5: 22H (24H: *H* APM); 3.4-3.0: 47H (52H: NC*H*$_2$CH$_2$, *H* APM), 2.9-2.0: 79H (82H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, CHC*H*$_2$(COOH)$_2$, *CH*$_2$NH$_2$), 1.8-1.4: 30H (32H: CH$_2$-C*H*$_2$-CH$_2$).

Carrier N17 (60: 20: 20): By the same procedure leading to **N12 (60: 20: 20)**, except that AEM and EDA were used respectively in places of API and PDA. 50 % of the product was collected with η_{inh}=10 mL/g and calculated molar mass (repeating unit) of 1454 g/mol.

For **N17 (60: 20: 20)**: ^{1}NMR (D$_2$O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 9H (11H: CONHC*H*$_2$HNCO, NC*H* (COOH)$_2$); 3.75-3.5: 8H (9H: *H* AEM); 3.4-3.0: 20H (22H: NC*H*$_2$CH, *H* AEM), 2.9-2.0: 40H (44H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, CHC*H*$_2$(COOH)$_2$, *CH*$_2$NH$_2$), 1.8-1.4: 5H (6H: CH$_2$-C*H*$_2$-CH$_2$).

Carrier N18 (80: 10: 10): By the same procedure leading to **N11 (80: 10: 10),** except that DET was used in place of PDA. 63 % of the product was collected with η_{inh}=29 mL/g and calculated molar mass (repeating unit) of 2803 g/mol.

For **N18 (80: 10: 10)**: ^{1}NMR (D_2O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 42H (45H: CONHC*H*$_2$HNCO, NC*H* $(COOH)_2$ *H* API);); 3.4-3.0: 18H (20H: NC*H*$_2$C*H*$_2$), 2.9-2.0: 83H (86H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, CHC*H*$_2$$(COOH)_2$, *CH*$_2$$NH_2$), 1.8-1.4: 30H (32H: CH_2CH_2-API).

Carrier N19 (60: 20: 20) was prepared by the same procedure as for **N12 (60: 20: 20)**, except that DET was used in place of PDA. 59 % of the product was collected with η_{inh}=12 mL/g and calculated molar mass (repeating unit) of 1369 g/mol.

For **N19 (60: 20: 20)** ^{1}NMR (D_2O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 10H (11H: CONHC*H*$_2$HNCO, NC*H* $(COOH)_2$); 3.75-3.5: 8H (9H: *H* APM); 3.4-3.0: 20H (22H: NC*H*$_2$C*H*$_2$, *H* APM), 2.9-2.0: 43H (46H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, CHC*H*$_2$$(COOH)_2$, *CH*$_2$$NH_2$), 1.8-1.4: 11H (12H: CH_2-C*H*$_2$-C*H*$_2$).

Carrier N20 (80: 10: 10) was prepared by the same procedure leading to **N14 (80: 10: 10)**, except that DET was used in place of PDA. 52 % of the product was collected with η_{inh}=26 mL/g and calculated molar mass (repeating unit) of 2832 g/mol.

For **N20 (80: 10: 10)** ^{1}NMR (D_2O), δ/ppm, (expected proton count in parentheses): 8.5-4.5: 19H (21H: CONHC*H*$_2$HNCO, NC*H* $(COOH)_2$); 3.75-3.5: 21H (24H: *H* AEM); 3.4-3.0: 49H (52H: NC*H*$_2$CH, *H* AEM), 2.9-2.0: 82H (86H: HNCOC*H*$_2$C*H*$_2$, C*H*$_2$-C*H*$_2$CONH, CHC*H*$_2$$(COOH)_2$, *CH*$_2$$NH_2$), 1.8-1.4: 31H (32H: CH_2-C*H*$_2$-CH_2).

5. 3. 2 Preparation of conjugates

In this work, the anchoring method of ferrocene and methotrexate to polymeric carriers (copolyaspartamides and copolyamidoamines) was achieved by amidation promoted by HBTU as coupling agent. This amidation is a result of the reaction between polymeric carrier's amine functional group and the carboxylic acid functional group of the monomeric drug.

5. 3. 2. 1. Preparation of copolyaspartamide-based ferrocene conjugates

Conjugate K11-Fc: 0.102 mmol (0.2 g) of carrier **K11 (80: 10: 10)** was dissolved in 7 mL of DMF, while 4-ferrocenylbutanoic acid (34 mg, 0.12 mmol) was dissolved together with HBTU (42 mg, 0.11 mmol) in 2 mL of DMF and stirred for 1 h at RT with direct light protection in another flask. The latter solution was added to the first, followed by the addition of TEA (21 mg, 0.204 mmol). After saturation with N_2, the brownish solution was stirred for 2 h at RT with protection against light. The conjugate was then precipitated with 30 mL of hexane-acetone (2:1), isolated by centrifugation and dissolved in 10 mL of H_2O, while the pH was adjusted from 9 to 10 with NaOH. The product was then purified by size exclusion on 2.5 x 30 cm column chromatography eluted with distilled H_2O and packed with Sephadex G-25. After the column, the pH was readjusted at 7 with glacial acetic acid to prevent any

hydrolysis, then ascorbic acid was added to preclude ferrocene oxidation to ferrocenium salt. The solution was dialysed for 48 h against distilled H_2O in 12000-14000 cut membrane tubing and freeze-dried to give a brown, water-soluble solid conjugate **K11-Fc,** collected in a yield range of 63 %, with η_{inh}=28 mL/g and calculated molar mass (repeating unit) of 2389 g/mol. % Fe: 2.3 % (calculated from NMR). 2.2 % (calculated from molar mass).

For **K11-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 8H (9H: C*H* ferrocene); 1.8-0.9: 33H (36H: $CH_2CH_2CH_2$).

Conjugate K12-Fc: The same procedure as for **K11-Fc** was used, except that 0.102 mmols (0.136 g) of carrier **K12 (60: 20: 20)** was used, giving a brownish water-soluble conjugate collected in 56 % yield, with η_{inh}=16 mL/g and calculated molar mass (repeating unit) =1336 g/mol. % Fe: 4.2 % (calculated from NMR). 4.1 % (calculated from molar mass).

For **K12-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 8.8H (9H: C*H* ferrocene); 1.8-0.9: 17H (16H: $CH_2CH_2CH_2$)

Conjugate K13-Fc: The same procedure as for **K11-Fc** was used, except that 0.102 mmols (0.25g) of carrier **K13 (80: 10: 10)** was used, giving a brownish water-soluble conjugate collected in 54 % yield, with η_{inh}=28 mL/g and calculated molar mass of 2429 g/mol. % Fe: 2.3 % (calculated from NMR). 2.2 % (calculated from molar mass).

For **K13-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 8.5H (9H: C*H* ferrocene); 1.8-0.9: 21H (20H: $CH_2CH_2CH_2$)

Conjugate K14-Fc: The same procedure as for **K11-Fc** was used, except that 0.102 mmols (0.13g) of carrier **K14 (60: 20: 20)** was used, giving a brownish water-soluble conjugate collected in 58 % yield, with η_{inh}=16 mL/g and calculated molar mass of 1265 g/mol. % Fe: 4.4 % (calculated from NMR). 4.4 % (calculated from molar mass).

For **K14-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 9H (9H: C*H* ferrocene); 1.8-0.9: 13.2H (14H: $CH_2CH_2CH_2$)

Conjugate K15-Fc: The same procedure as for **K11-Fc** was used, except that 0.102 mmols (0.13g) of carrier **K15 (60: 20: 20)** was used, giving a brownish water-soluble conjugate collected in 61 % yield, with η_{inh}=17 mL/g and calculated molar mass 1322 g/mol. % Fe: 4.2 % (calculated from NMR). 4.2 % (calculated from molar mass).

For **K15-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 9H (9H: C*H* ferrocene); 1.8-0.9: 16H (14H: $CH_2CH_2CH_2$)

Conjugate K16-Fc: The same procedure as for **K11-Fc** was used, except that 0.102 mmols (0.25g) of carrier **K16 (80: 10: 10)** was used, giving a brownish water-soluble conjugate collected in 61 % yield, with η_{inh}=29 mL/g and calculated molar mass of 2415 g/mol. % Fe: 2.3 % (calculated from NMR). 2.3 % (calculated from molar mass).

For **K16-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 8.9H (9H: C*H* ferrocene); 1.8-0.9: 8.4H (8H: $CH_2CH_2CH_2$)

Conjugate K17-Fc: The same procedure as for **K11-Fc** was used, except that 0.102 mmols (0.13g) of carrier **K17 (60: 20: 20)** was used, giving a brownish water-soluble conjugate collected in 60 % yield, with η_{inh}=18 mL/g and calculated molar mass of 1280 g/mol. % Fe: 4.4 % (calculated from NMR). 4.3 % (calculated from molar mass).

For **K17-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 9H (9H: C*H* ferrocene); 1.8-0.9: 8.4H (8H: $CH_2CH_2CH_2$)

Conjugate K18-Fc: The same procedure as for **K11-Fc** was used, except that 0.102 mmols (0.25g) of carrier **K18 (80: 10: 10)** was used, giving a brownish water-soluble conjuate collected in 57% yield, with η_{inh}=29 mL/g and calculated molar mass of 2418 g/mol. % Fe: 2.3 % (calculated from NMR). 2.2 % (calculated from molar mass).

For **K18-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 8.6H (9H: C*H* ferrocene); 1.8-0.9: 25.8H (24H: $CH_2CH_2CH_2$)

Conjugate K19-Fc: The same procedure as for **K11-Fc** was used, except that 0.102 mmols (0.14g) of carrier **K19 (60: 20: 20)** was used, giving a brownish water-soluble conjugate collected in 61 % yield, with η_{inh}=17 mL/g and calculated molar mass of 1351 g/mol. % Fe: 4.1 % (calculated from NMR). 4.0 % (calculated from molar mass).

For **K19-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 8.7H (9H: C*H* ferrocene); 1.8-0.9: 11H (14H: $CH_2CH_2CH_2$)

Conjugate K20-Fc: The same procedure as for **K11-Fc** was used, except that 0.102 mmols (0.13g) of carrier **K20 (60: 20: 20)** was used, giving a brownish water-soluble conjugate collected in 58 % yield, with η_{inh}=17 mL/g and calculated molar mass (repeating unit) of 1323 g/mol. % Fe: 4.2 % (calculated from NMR). 4.2 % (calculated from molar mass).

For **K20-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 9H (9H: C*H* ferrocene); 1.8-0.9: 9.2H (8H: $CH_2CH_2CH_2$)

5. 3. 2. 2 Polyamidoamine-based ferrocene conjugates

Conjugate M11-Fc: 0.102 mmol (0.27g) of carrier **M11 (80: 10: 10)** was dissolved in 7 mL of DMF while 4-ferrocenylbutanoic acid (34 mg, 0.12 mmol) was dissolved together with HBTU (42 mg, 0.11 mmol) in 2 mL of DMF and stirred for 1 h at RT with direct light protection in another flask. The later solution was added to the first, followed by the addition of TEA (21 mg, 0.204 mmol). After saturation with N_2, the yellowish solution was stirred for 2 h at RT with protection against light. The conjugate was then precipitated with 30 mL of acetone, isolated by centrifugation and dissolved in 10 m L of H_2O while the pH was adjusted from 9 to 10 with NaOH. The product was then purified by size exclusion on 2.5 x 30 cm column chromatography eluted with distilled H_2O and packed with Sephadex G-25. After

the column, the pH was readjusted at 7 with glacial acetic acid to prevent any hydrolysis, then ascorbic acid was added to preclude ferrocene oxidation to ferrocenium salt. The solution was dialysed for 48 h against distilled H_2O in 12000-14000 cut membrane tubing and freeze-dried to give a brown, water-soluble solid conjugate **M11-Fc,** collected in 63 % yield, with η_{inh}=19 mL/g and calculated molar mass (repeating unit) of 2959 g/mol. % Fe: 1.7 % (calculated from NMR), 1.9 % (calculated from molar mass).

For **M11-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 8H (9H: C*H* ferrocene); 1.8-0.9: 30H (34H: $CH_2CH_2CH_2$)

Conjugate M12-Fc: The same procedure as for **M11-Fc** was used, except that 0.102 mmols (0.13g) of carrier **M20 (60: 20: 20)** was used, giving a brownish water-soluble conjugate collected in 56 % yield, with η_{inh}=13 mL/g and calculated molar mass (repeating unit) of 1621 g/mol. % Fe: 3.1 % (calculated from NMR), 3.5 % (calculated from molar mass).

For **M12-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 8H (9H: C*H* ferrocene); 1.8-0.9: 37H (40H: $CH_2CH_2CH_2$)

Conjugate M13-Fc: The same procedure as for **M11-Fc** was used, except that 0.102 mmols (0.28g) of carrier **M13 (80: 10: 10)** was used, giving a brownish water-soluble conjugate collected in 54 % yield, with η_{inh}=20 mL/g and calculated molar mass (repeating unit) of 2999 g/mol. % Fe: 1.6 % (calculated from NMR), 1.9 % (calculated from molar mass).

For **M13-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 8H (9H: C*H* ferrocene); 1.8-0.9: 17H (18H: $CH_2CH_2CH_2$)

Conjugate M14-Fc: The same procedure as for **M11-Fc** was used, except that 0.102 mmols (0.29g) of carrier **M14 (80: 10: 10)** was used, giving a brownish water-soluble conjugate collected in 58 % yield, with η_{inh}=19 mL/g and calculated molar mass (repeating unit) of 2945 g/mol. % Fe: 1.9 % (calculated from NMR), 1.9 % (calculated from molar mass).

For **M14-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 9H (9H: C*H* ferrocene); 1.8-0.9: 29H (34H: $CH_2CH_2CH_2$)

Conjugate M15-Fc: The same procedure as for **M11-Fc** was used, except that 0.102 mmols (0.14g) of carrier **M15 (60: 20: 20)** was used, giving a brownish water-soluble conjugate collected in 61 % yield, with η_{inh}=12 mL/g and calculated molar mass (repeating unit) of 1607 g/mol. % Fe: 3.3 % (calculated from NMR), 3.5 % (calculated from molar mass).

For **M15-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 8.5H (9H: C*H* ferrocene); 1.8-0.9: 10H (12H: $CH_2CH_2CH_2$)

Conjugate M16-Fc: The same procedure as for **M11-Fc** was used, except that 0.102 mmols (0.29g) of carrier **M6 (60: 20: 20)** was used, giving a brownish water-soluble conjugate collected in 61 % yield, with η_{inh}=19 mL/g and calculated molar mass=2985 g/mol. % Fe: 1.8 % (calculated from NMR), 1.9 % (calculated from molar mass).

For **M16-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 8.7H (9H: C*H* ferrocene); 1.8-0.9: 14H (16H: $CH_2CH_2CH_2$)

Conjugate M17-Fc: The same procedure as for **M11-Fc** was used, except that 0.102 mmols (0.14g) of carrier **M17 (60: 20: 20)** was used, giving a brownish water-soluble conjugate collected in 60 % yield, with η_{inh}=12 mL/g and calculated molar mass of 1593 g/mol. % Fe: 3.3 % (calculated from NMR), 3.5 % (calculated from molar mass).

For **M17-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 8.5H (9H: C*H* ferrocene); 1.8-0.9: 11H (14H: $CH_2CH_2CH_2$)

Conjugate M18-Fc: The same procedure as for **M11-Fc** was used, except that 0.102 mmols (0.28g) of carrier **M18 (80: 10: 10)** was used, giving a brownish water-soluble conjugate collected in 57 % yield, with η_{inh}=20 mL/g and calculated molar mass of 3140 g/mol. % Fe: 1.7 % (calculated from NMR), 1.8 % (calculated from molar mass).

For **M18-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 8.6H (9H: C*H* ferrocene); 1.8-0.9: 29H (34H: $CH_2CH_2CH_2$)

Conjugate M19-Fc: The same procedure as for **M11-Fc** was used, except that 0.102 mmols (0.28g) of carrier **M19 (80: 10: 10)** was used, giving a brownish water-soluble conjugate collected in 61 % yield, with η_{inh}=21 mL/g and calculated molar mass of 3028 g/mol. % Fe: 1.7 % (calculated from NMR), 1.8 % (calculated from molar mass).

For **M19-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 8.3H (9H: C*H* ferrocene); 1.8-0.9: 15H (18H: $CH_2CH_2CH_2$)

Conjugate M20-Fc: The same procedure as for **M11-Fc** was used, except that 0.102 mmols (0.14g) of carrier **M20 (60: 20: 20)** was used, giving a brownish water-soluble conjugate collected in 58 % yield, with η_{inh}=13 mL/g and calculated molar mass (repeating unit) of 1608 g/mol. % Fe: 3.3 % (calculated from NMR), 3.5 % (calculated from molar mass).

For **M20-Fc**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 4.5-4.1: 8.6H (9H: C*H* ferrocene); 1.8-0.9: 7H (8H: $CH_2CH_2CH_2$)

5. 3. 2. 3 Copolyaspartamide-based MTX conjugates

Conjugate K1-MTX: 0.102 mmol (102mg) of carrier **K1 (60: 20: 20)** was dissolved in 7 mL of DMF with stirring for 1 h. MTX (0.122 mmol, 56mg) was dissolved in 4 mL of DMF in another flask with slight warming during 1 h. After total dissolution of all reagents and cooling at RT, the 2 solutions were mixed, then HBTU (43mg, 0.112mmol) dissolved in 1mL of DMF was added dropwise, followed by TEA (28 µl, 0. 204mmol) while stirring carried on for 2 h. The conjugate was then worked-up by using the standard procedure having led to MTX conjugates to afford a yellow water-soluble powder in 55% yield, with η_{inh}=24 mL/g and calculated molar mass (repeating unit) of 1437 g/mol. % MTX: 30.4 % (calculated from NMR). 30.4 % (calculated from molar mass).

^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 5H (5H: aromatic); 1.8-1.5: 4H (4H: $CH_2CH_2CH_2$).

Conjugate K2-MTX: The same procedure as for **K1-MTX** was used, except that 0.102 mmols (0.10g) of carrier **K2 (60: 20: 20)** was used, giving a yellowish water-soluble conjugate collected in 57 % yield, with η_{inh}=27 mL/g and calculated molar mass of 1478 g/mol. % MTX: 29.6 % (calculated from NMR), 29.6 % (calculated from molar mass).

^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 5H (5H: aromatic); 1.8-1.5: 4H (4H: $CH_2CH_2CH_2$).

Conjugate K3-MTX: The same procedure as for **K1-MTX** was used, except that 0.102 mmols (0.21g) of carrier **K3 (80: 10: 10)** was used, giving a yellowish water-soluble conjugate collected in 54 % yield, with η_{inh}=38 mL/g and calculated molar mass of 2276 g/mol. % MTX: 19.2 % (calculated from NMR), 19.2 % (calculated from molar mass).

^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 5H (5H: aromatic); 1.8-1.5: 4H (4H: $CH_2CH_2CH_2$).

Conjugate K4-MTX: The same procedure as for **K1-MTX** was used, except that 0.102 mmols (0.22g) of carrier **K4 (80: 10: 10)** was used, giving a yellowish water-soluble conjugate collected in 57 % in yield, with η_{inh}=27 mL/g and calculated molar mass of 1478 g/mol. % MTX: 17.2 % (calculated from NMR), 17.6 % (calculated from molar mass).

^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 4.9H (5H: aromatic); 1.8-1.5: 2H (2H: $CH_2C\mathit{H}_2CH_2$

Conjugate K5-MTX: The same procedure as for **K1-MTX** was used, except that 0.102 mmols (0.22g) of carrier **K5 (80: 10: 10)** was used, giving a yellowish water-soluble conjugate collected in 52 % yield, with η_{inh}=36 mL/g and calculated molar mass of 2598 g/mol. % MTX: 16.8 % (calculated from NMR), 16.8 % (calculated from molar mass).

^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 5H (5H: aromatic); 1.8-1.5: 2H (2H: $CH_2C\mathit{H}_2CH_2$).

Conjugate K6-MTX: The same procedure as for **K1-MTX** was used, except that 0.102 mmols (0.10g) of carrier **K6 (60: 20: 20)** was used, giving a yellowish water-soluble conjugate collected in 57 % yield, with η_{inh}=24 mL/g and calculated molar mass of 1464 g/mol. % MTX: 28.7 % (calculated from NMR), 29.9 % (calculated from molar mass).

^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 4.8H (5H: aromatic); 1.8-1.5: 2H (2H: $CH_2C\mathit{H}_2CH_2$).

Conjugate K7-MTX: The same procedure as for **K1-MTX** was used, except that 0.102 mmols (0.22g) of carrier **K7 (80: 10: 10)** was used, giving a yellowish water-soluble conjugate collected in 55 % yield, with η_{inh}=35 mL/g and calculated molar mass of 2262 g/mol. % MTX: 19.3 % (calculated from NMR), 19.3 % (calculated from molar mass).

^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 5H (5H: aromatic); 1.8-1.5: 2H (2H: $CH_2C\mathit{H}_2CH_2$).

Conjugate K8-MTX: The same procedure as for **K1-MTX** was used, except that 0.102 mmols (0.09g) of carrier **K8 (60: 20: 20)** was used, giving a yellowish water-soluble conjugate collected in 52 % yield, with η_{inh}=25 mL/g and calculated molar mass of 1463 g/mol. % MTX: 29.9 % (calculated from NMR), 29.9 % (calculated from molar mass).

^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 5H (5H: aromatic); 1.8-1.5: 19H (20H: $CH_2C\mathit{H}_2CH_2$).

Conjugate K9-MTX: The same procedure as for **K1-MTX** was used, except that 0.102 mmols (0.09g) of carrier **K9 (60: 20: 20)** was used, giving a yellowish water-soluble conjugate collected in 53 % yield, with η_{inh}=25 mL/g and calculated molar mass of 1506 g/mol. % MTX: 28.7 % (calculated from NMR), 28.9 % (calculated from molar mass).

^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 4.9H (5H: aromatic); 1.8-1.5: 24H (26H: $CH_2C\mathit{H}_2CH_2$).

Conjugate K10-MTX: The same procedure as for **K1-MTX** was used, except that 0.102 mmols (0.10g) of carrier **K10 (80: 10: 10)** was used, giving a yellowish water-soluble conjugate collected in 54 % yield, with η_{inh}=37 mL/g and calculated molar mass (repeating unit) of 2305 g/mol. % MTX: 18.6 % (calculated from NMR), 19.0 % (calculated from molar mass).

^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 4.9H (5H: aromatic); 1.8-1.5: 2H (2H: $CH_2CH_2CH_2$).

5. 3. 2. 4 Ployamidoamine-based MTX conjugates

For the preparation of polyamidoamine-based MTX conjugates investigated in this work, the same procedure used for copolyaspartamide-based MTX conjugates was still valid.

Conjugate M1-MTX: 0.102 mmol (131 mg) of carrier **M1 (60: 20: 20)** was dissolved in 7 mL of DMF with stirring for 1 h. MTX (0.122 mmol, 56 mg) was dissolved in 4 mL of DMF in another flask with slight warming during 1 h. After total dissolution of all reagents and cooling at RT, the 2 solutions were mixed, then HBTU (43mg, 0.112mmol) dissolved in 1 mL of DMF was added dropwise, followed by TEA (28 µL, 0. 204mmol) while stirring carried on for 2 h. The conjugate was then worked-up by using the standard procedure having led to ferrocene conjugates to afford a yellow water-soluble powder in 35% yield, with η_{inh}=12 mL/g and calculated molar mass (repeating unit) of 1720 g/mol. % MTX: 22.8 % (calculated from NMR). 25.4 % (calculated from molar mass).

For **M1-MTX**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 4.5H (5H: aromatic); 1.8-1.5: 37H (40H: $CH_2CH_2CH_2$).

Conjugate M2-MTX: The same procedure as for **M1-MTX** was used, except that 0.102 mmols (0.27g) of carrier **M2 (80: 10: 10)** was used, giving a yellowish water-

soluble conjugate collected in 37 % yield, with η_{inh}=23 mL/g and calculated molar mass of 2619 g/mol. % MTX: 14.4 % (calculated from NMR), 16.1 % (calculated from molar mass).

For **M2-MTX**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 4.5H (5H: aromatic); 1.8-1.5: 62H (68H: $CH_2C\mathit{H}_2CH_2$).

Conjugate M3-MTX: The same procedure as for **M1-MTX** was used, except that 0.102 mmols (0.14g) of carrier **M3 (60: 20: 20)** was used, giving a yellowish water-soluble conjugate collected in 38 % yield, with η_{inh}=12 mL/g and calculated molar mass of 1635 g/mol. % MTX: 24.1 % (calculated from NMR), 26.7 % (calculated from molar mass).

For **M3-MTX**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 4.9H (5H: aromatic); 1.8-1.5: 4H (4H: $CH_2C\mathit{H}_2CH_2$).

Conjugate M4-MTX: The same procedure as for **M1-MTX** was used, except that 0.102 mmols (0.22g) of carrier **M4 (80: 10: 10)** was used, giving a yellowish water-soluble conjugate collected in 37 % yield, with η_{inh}=21 mL/g and calculated molar mass of 1478 g/mol. % MTX: 13.4 % (calculated from NMR), 14.3 % (calculated from molar mass).

For **M4-MTX**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 4.7H (5H: aromatic); 1.8-1.5: 45H (52H: $CH_2C\mathit{H}_2CH_2$

Conjugate M5-MTX: The same procedure as for **M1-MTX** was used, except that 0.102 mmols (0.13g) of carrier **M5 (60: 20: 20)** was used, giving a yellowish water-

soluble conjugate collected in 32 % yield, with η_{inh}=11 mL/g and calculated molar mass of 1748 g/mol. % MTX: 23.0 % (calculated from NMR), 25.0 % (calculated from molar mass).

For **M5-MTX**: (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 4.6H (5H: aromatic); 1.8-1.5: 25H (28H: $CH_2C\mathit{H}_2CH_2$).

Conjugate M6-MTX: The same procedure as for **M1-MTX** was used, except that 0.102 mmols (0.23g) of carrier **M6 (80: 10: 10)** was used, giving a yellowish water-soluble conjugate collected in 32 % yield, with η_{inh}=23 mL/g and calculated molar mass of 2832 g/mol. % MTX: 14.2 % (calculated from NMR), 15.4 % (calculated from molar mass).

M6-MTX: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 4.6H (5H: aromatic); 1.8-1.5: 2H (2H: $CH_2C\mathit{H}_2CH_2$).

Conjugate M7-MTX: The same procedure as for **M1-MTX** was used, except that 0.102 mmols (0.12g) of carrier **M7 (60: 20: 20)** was used, giving a yellowish water-soluble conjugate collected in 38 % yield, with η_{inh}=12 mL/g and calculated molar mass=1763 g/mol. % MTX: 23.8 % (calculated from NMR), 24.8 % (calculated from molar mass).

For **M7-MTX**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 4.8H (5H: aromatic); 1.8-1.5: 36H (40H: $CH_2C\mathit{H}_2CH_2$).

Conjugate M8-MTX: The same procedure as for **M1-MTX** was used, except that 0.102 mmols (0.24g) of carrier **M8 (80: 10: 10)** was used, giving a yellowish water-

soluble conjugate collected in 39 % yield, with η_{inh}=21 mL/g and calculated molar mass=2731 g/mol. % MTX: 14.6 % (calculated from NMR), 16.0 % (calculated from molar mass).

For **M8-MTX**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 4H (5H: aromatic); 1.8-1.5: 63H (68H: $CH_2C\mathit{H}_2CH_2$).

Conjugate M9-MTX: The same procedure as for **M1-MTX** was used, except that 0.102 mmols (0.25g) of carrier **M9 (80: 10: 10)** was used, giving a yellowish water-soluble conjugate collected in 34 % yield, with η_{inh}=22 mL/g and calculated molar mass=2875 g/mol. % MTX: 14.0 % (calculated from NMR), 15.2 % (calculated from molar mass).

For **M9-MTX**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 4.6H (5H: aromatic); 1.8-1.5: 4.2H (4H: $CH_2C\mathit{H}_2CH_2$).

Conjugate M10-MTX: The same procedure as for **M1-MTX** was used, except that 0.102 mmols (0.23g) of carrier **M10 (60: 20: 20)** was used, giving a yellowish water-soluble conjugate collected in 36 % yield, with η_{inh}=12 mL/g and calculated molar mass (repeating unit) =1665 g/mol. % MTX: 23.9 % (calculated from NMR), 26.2 % (calculated from molar mass).

For **M10-MTX**: ^{1}NMR (D_2O), δ/ppm: (expected proton count in parentheses): 8.5-6.5: 4H (5H: aromatic); 1.8-1.5: 4H (4H: $CH_2C\mathit{H}_2CH_2$).

5. 3. 3 Preparation of platinum co-conjugates

5. 3. 3. 1 Preparation of the platination agent (DACH-Pt)

The platination agent DACH-Pt(NO_3)$_2$ (trans-1, 2-diaminocyclohexanediaquaplatinum (ll) dinitrate) was prepared according to scheme 4.12 by a literature method [232].

Anal. % Pt: 40.4 % (Calcd. for $C_6H_{18}N_4O_8Pt$: 469.3), 40.7 % (Found).

5. 3. 3.2 Copolyaspartamide-based ferrocene/platinum co-conjugates

Co-conjugate K11-Fc/Pt: 0.305 mmol (0.73 g) of conjugate **K11-Fc** was dissolved in 5 mL of H_2O and saturated with N_2. DACH-Pt (0.366 mmol, 0.172 g) dissolved in 5 mL of H_2O was added to the above mixture before saturation with N_2, while stirring carried on for 72 h d at RT, under protection against direct light and strict control of pH (5.5-6) with Hcl and NaOH. The mixture was stirred for 2 h at 50°C before filtration and dialyzed for 24 h in 12000-14000 cut membrane tubing. The retentate was then freeze-dried to give a brown water-soluble powder collected in 26.8 % yield, η_{inh}= 33 mL/g and a molar mass of 2577 g/mol.

For **K11-Fc/Pt**: Anal. % Pt: 3.03 % (Calcd.), 2.97 % (Found).

Co-conjugate K12-Fc/Pt: The same procedure as for **K11- Fc/Pt** was used, except that 0.305 mmol (0.40g) of conjugate **K12-Fc** was used, giving a brown water-soluble powder collected in 17.5 % yield, η_{inh}= 18 mL/g and a molar mass of 1523 g/mol.

For **K12-Fc/Pt**: Anal. % Pt: 5.01 % (Calcd.), 6.14 % (Found).

Co-conjugate K13-Fc/Pt: The same procedure as for **K11-Fc/Pt** was used, except that 0.305 mmol (0.74g) of conjugate **K13-Fc** was used, giving a brown water-soluble powder collected in 11.4 % yield, η_{inh}= 30 mL/g and a molar mass of 2617 g/mol.

For **K13-Fc/Pt**: Anal. % Pt: 2.99 % (Calcd.), 2.51 % (Found).

Co-conjugate K14-Fc/Pt: The same procedure as for **K11-Fc/Pt** was used, except that 0.305 mmol (0.39g) of conjugate **K14-Fc** was used, giving a brown water-soluble powder collected in 36.0 % yield, η_{inh}= 19 mL/g and a molar mass of 1453 g/mol.

For **K14-Fc/Pt**: Anal. % Pt: 5.39 % (Calcd.), 5.00 % (Found).

Co-conjugate K15- Fc/Pt: The same procedure as for **K11-Fc/Pt** was used, except that 0.305 mmol (0.40g) of conjugate **K15-Fc** was used, giving a brown water-soluble powder collected in 31.6 % yield, η_{inh}= 20 mL/g and a molar mass of 1510 g/mol.

For **K15- Fc/Pt**: Anal. % Pt: 5.16 % (Calcd.), 3.76 % (Found).

Co-conjugate K16-Fc/Pt: The same procedure as for **K16-Fc/Pt** was used, except that 0.305 mmol (0.74g) of conjugate **K16-Fc** was used, giving a brown water-soluble powder collected in 29.4 % yield, η_{inh}= 31 mL/g and a molar mass of 2603 g/mol.

For **K16-Fc/Pt**: Anal. % Pt: 3.0 % (Calcd.), 5.34 % (Found).

Co-conjugate K17-Fc/Pt: The same procedure as for **K11-Fc/Pt** was used, except that 0.305 mmol (0.40g) of conjugate **K17-Fc** was used, giving a brown water-soluble powder collected in 37.2 % yield, η_{inh}= 20 mL/g and a molar mass of 1468 g/mol.

For **K17-Fc/Pt**: Anal. % Pt: 5.31 % (Calcd.), 4.62 % (Found).

Co-conjugate K18-Fc/Pt: The same procedure as for **K11-Fc/Pt** was used, except that 0.305 mmol (0.74g) of conjugate **K18-Fc** was used, giving a brown water-soluble powder collected in 28.7 % yield, η_{inh}= 23 mL/g and a molar mass of 2606 g/mol.

For **K18-Fc/Pt**: Anal. % Pt: 3.0 % (Calcd.), 4.3 % (Found).

Co-conjugate K19-Fc/Pt: The same procedure as for **K11-Fc/Pt** was used, except that 0.305 mmol (0.41g) of conjugate **K19-Fc** was used, giving a brown water-soluble powder collected in 29.6 % yield, η_{inh}= 19 mL/g and a molar mass of 1539 g/mol.

For **K19-Fc/Pt**: Anal. % Pt: 5.07 % (Calcd.), 4.85 % (Found).

Co-conjugate K20-Fc/Pt: The same procedure as for **K11-Fc/Pt** was used, except that 0.305 mmol (0.40g) of conjugate **K20-Fc** was used, giving a brown water-soluble powder collected in 32.0 % yield, η_{inh}= 20 mL/g and a molar mass of 1511 g/mol.

For **K20-Fc/Pt**: Anal. % Pt: 5.16 % (Calcd.), 4.74 % (Found).

5. 3.3. 3 Polyamidoamine-based ferrocene/platinum co-conjugates

Co-conjugate M11-Fc/Pt: 0.305 mmol (0.90 g) of conjugate **M11-Fc** was dissolved in 5 mL of H_2O and saturated with N_2. DACH-Pt (0.366 mmol: 0.172 g) dissolved in 5 mL of H_2O was added to the above mixture before saturation with N_2, while stirring carried on for 72 h d at RT under protection against direct light and strict control of pH (5.5-6) with HCl and NaOH. The mixture was stirred for 2 h at 50°C before filtration and dialyzed for 24 h in 12000-14000 cut membrane tubing. The retentate was then freeze-dried to give a brown water-soluble powder collected in 24.0 % yield, η_{inh}= 21 mL/g and a molar mass of 3147 g/mol.

For **M11-Fc/Pt**: Anal. % Pt: 2.48 % (Calcd.), 2.12 % (Found).

Co-conjugate M12-Fc/Pt: The same procedure as for **M11-Fc/Pt** was used, except that 0.305 mmol (0.49g) of conjugate **M12-Fc** was used, giving a brown water-soluble powder collected in 16.3 % yield, η_{inh}= 16mL/g and a molar mass of1809 g/mol.

For **M12-Fc/Pt**: Anal. % Pt: 4.31 % (Calcd.), 3.67 % (Found).

Co-conjugate M13-Fc/Pt: The same procedure as for **M11- Fc/Pt** was used, except that 0.305 mmol (0.91g) of conjugate **M13-Fc** was used, giving a brown water-soluble powder collected in 26.8 % yield, η_{inh}=23 mL/g and a molar mass of 3183 g/mol.

For **M13- Fc/Pt**: Anal. % Pt: 2.45 % (Calcd.), 2.31 % (Found).

Co-conjugate M14- Fc/Pt: The same procedure as for **M11-Fc/Pt** was used, except that 0.305 mmol (0.90g) of conjugate **M14-Fc** was used, giving a brown water-soluble powder collected in 30.7 % yield, η_{inh}= 20mL/g and a molar mass of 3133 g/mol.

For **M14- Fc/Pt**: Anal. % Pt: 2.49 % (Calcd.), 2.16 % (Found).

Co-conjugate M15- Fc/Pt: The same procedure as for **M11-Fc/Pt** was used, except that 0.305 mmol (0.49g) of conjugate **M15-Fc** was used, giving a brown water-soluble powder collected in 26.6 % yield, η_{inh}= 14mL/g and a molar mass of 1795 g/mol.

For **M15-Fc/Pt**: Anal. % Pt: 4.35 % (Calcd.), 4.07 % (Found).

Co-conjugate M16-Fc/Pt: The same procedure as for **M11-Fc/Pt** was used, except that 0.305 mmol (0.91g) of conjugate **M16-Fc** was used, giving a brown water-soluble powder collected in 22.4 % yield, η_{inh}= 21mL/g and a molar mass of 3173 g/mol.

For **M16-Fc/Pt**: Anal. % Pt: 2.46 % (Calcd.), 1.98 % (Found).

Co-conjugate M17-Fc/Pt: The same procedure as for **M11-Fc/Pt** was used, except that 0.305 mmol (0.49g) of conjugate **M17-Fc** was used, giving a brown water-soluble powder collected in 20.0 % yield, η_{inh}= 15mL/g and a molar mass of 1781 g/mol.

For **M17-Fc/Pt**: Anal. % Pt: 4.38 % (Calcd.), 3.97 % (Found).

Co-conjugate M18-Fc/Pt: The same procedure as for **M11-Fc/Pt** was used, except that 0.305 mmol (0.96g) of conjugate **M18-Fc** was used, giving a brown water-soluble powder collected in 24.2 % yield, η_{inh}= 22mL/g and a molar mass of 3328 g/mol.

For **M18-Fc/Pt**: Anal. % Pt: 2.34 % (Calcd.), 2.71 % (Found).

Co-conjugate M19-Fc/Pt: The same procedure as for **M11-Fc/Pt** was used, except that 0.305 mmol (0.92g) of conjugate **M19-Fc** was used, giving a brown water-soluble powder collected in 30.6 % yield, η_{inh}= 23mL/g and a molar mass of 3216 g/mol.

For **M19-Fc/Pt**: Anal. % Pt: 2.43 % (Calcd.), 2.45 % (Found).

Co-conjugate M20-Fc/Pt: The same procedure as for **M11-Fc/Pt** was used, except that 0.305 mmol (0.49g) of conjugate **M20-Fc** was used, giving a brown water-soluble powder collected in 26.0 % yield, η_{inh}= 16mL/g and a molar mass of 1796 g/mol.

For **M20-Fc/Pt**: Anal. % Pt: 4.34 % (Calcd.), 3.50 % (Found).

5. 3. 3. 4 Copolyaspartamide-based MTX/Pt co-conjugates

Co-conjugate K1-MTX/Pt: 0.305 mmol (0.44g) of conjugate **K1-MTX** was dissolved in 5 mL of H_2O and saturated with N_2. DACH-Pt (0.366 mmol: 0.172 g) dissolved in 5 mL of H_2O was added to the above mixture before saturation with N_2, while stirring carried on for 72 h at RT under protection against direct light and strict control of pH (5.5-6) with HCl and NaOH. The mixture was stirred for 2 h at 50°C before filtration and dialyzed for 24 h in 12000-14000 cut membrane tubing. The retentate was then freeze-dried to give a brown water-soluble powder collected in 22.0 % yield, η_{inh}= 26 mL/g and a molar mass of 1625 g/mol.

Anal. % Pt: 4.80 % (Calcd.), 5.03 % (Found).

Co-conjugate K2-MTX/Pt: The same procedure as for **K1-MTX/Pt** was used, except that 0.305 mmol (0.45g) of conjugate **K2-MTX** was used, giving a brown water-soluble powder collected in 31.0 % yield, η_{inh}= 30 mL/g and a molar mass of 1666 g/mol.

For **K2-MTX/Pt**: Anal. % Pt: 4.68 % (Calcd.), 4.85 % (Found).

Co-conjugate K3-MTX/Pt: The same procedure as for **K1-MTX/Pt** was used, except that 0.305 mmol (0.69g) of conjugate **K3-MTX** was used, giving a brown

water-soluble powder collected in 29.4% yield, η_{inh}= 40mL/g and a molar mass of 2464 g/mol.

For **K3-MTX/Pt**: Anal. % Pt: 3.17 % (Calcd.), 3.04 % (Found).

Co-conjugate K4-MTX/Pt: The same procedure as for **K1-MTX/Pt** was used, except that 0.305 mmol (0.76 g) of conjugate **K4-MTX** was used, giving a brown water-soluble powder collected in 28.7 % yield, η_{inh}= 42mL/g and a molar mass of 2674 g/mol.

For **K4-MTX/Pt**: Anal. % Pt: 2.92 % (Calcd.), 2.78 % (Found).

Co-conjugate K5-MTX/Pt: The same procedure as for **K1-MTX/Pt** was used, except that 0.305 mmol (0.79g) of conjugate **K5-MTX** was used, giving a brown water-soluble powder collected in 30.6 % yield, η_{inh}= 38mL/g and a molar mass of 2786 g/mol.

For **K5-MTX/Pt**: Anal. % Pt: 2.86 % (Calcd.), 3.11 % (Found).

Co-conjugate K6-MTX/Pt: The same procedure as for **K1-MTX/Pt** was used, except that 0.305 mmol (0.45g) of conjugate **K6-MTX** was used, giving a brown water-soluble powder collected in 26.6 % yield, η_{inh}= 26mL/g and a molar mass of 1652 g/mol.

For **K6-MTX/Pt**: Anal. % Pt: 4.72 % (Calcd.), 4.01 % (Found).

Co-conjugate K7-MTX/Pt: The same procedure as for **K1-MTX/Pt** was used, except that 0.305 mmol (0.69g) of conjugate **K7-MTX** was used, giving a brown

water-soluble powder collected in 28.0 % yield, η_{inh}= 37mL/g and a molar mass of 2450 g/mol.

For **K7-MTX/Pt**: Anal. % Pt: 3.18 % (Calcd.), 3.52 % (Found).

Co-conjugate K8-MTX/Pt: The same procedure as for **K1-MTX/Pt** was used, except that 0.305 mmol (0.g) of conjugate **K8-MTX** was used, giving a brown water-soluble powder collected in 30.3 % yield, η_{inh}= 27 mL/g and a molar mass of 1651 g/mol.

For **K8-MTX/Pt**: Anal. % Pt: 4.72 % (Calcd.), 4.16 % (Found).

Co-conjugate K9-MTX/Pt: The same procedure as for **K1-Pt/MTX** was used, except that 0.305 mmol (0.46g) of conjugate **K9-MTX** was used, giving a brown water-soluble powder collected in 24.0 % yield, η_{inh}= 27mL/g and a molar mass of 1694 g/mol.

Anal. % Pt: 4.60 % (Calcd.), 4.22 % (Found).

Co-conjugate K10-Pt/MTX: The same procedure as for **K1-Pt/MTX** was used, except that 0.305 mmol (0.70g) of conjugate K10-MTX was used, giving a brown water-soluble powder collected in 24.0 % yield, η_{inh}= 38mL/g and a molar mass of 2493 g/mol.

For **K9-Pt/MTX**: Anal. % Pt: 3.13 % (Calcd.), 3.37 % (Found).

5. 3. 3. 5 Copolyamidoamine-based MTX/Pt co-conjugates

Co-conjugate M1-MTX/Pt: 0.305 mmol (0.52g) of conjugate **M1-MTX** was dissolved in 5 mL of H_2O and saturated with N_2. DACH-Pt (0.366 mmol, 0.172 g) dissolved in 5 mL of H_2O was added to the above mixture before saturation with N_2, while stirring carried on for 72 h d at RT under protection against direct light and strict control of pH (5.5-6) with HCl and NaOH. The mixture was stirred for 2 h at 50°C before filtration and dialyzed for 24 h in 12000-14000 cut membrane tubing. The retentate was then freeze-dried to give a brown water-soluble powder collected in 35.0 % yield, η_{inh}= 15 mL/g and a molar mass of 1906 g/mol.

For **M1-MTX/Pt**: Anal. % Pt: 4.09 % (Calcd.), 4.24 % (Found).

Co-conjugate M2-MTX/Pt: The same procedure as for **M1-MTX/Pt** was used, except that 0.305 mmol (0.82g) of conjugate **M2-MTX** was used, giving a brown water-soluble powder collected in 35.3 % yield, η_{inh}= 28 mL/g and a molar mass of 2890 g/mol.

For **M2-MTX/Pt**: Anal. % Pt: 2.70 % (Calcd.), 2.93 % (Found).

Co-conjugate M3-MTX/Pt: The same procedure as for **M1-MTX/Pt** was used, except that 0.305 mmol (0.50g) of conjugate **M3-MTX** was used, giving a brown water-soluble powder collected in 25.4 % yield, η_{inh}= 14mL/g and a molar mass of 1823 g/mol.

For **M3-MTX/Pt**: Anal. % Pt: 4.29 % (Calcd.), 4.28 % (Found).

Co-conjugate M4-MTX/Pt: The same procedure as for **M1-MTX/Pt** was used, except that 0.305 mmol (0.93g) of conjugate **M4-MTX** was used, giving a brown water-soluble powder collected in 22.2 % yield, η_{inh}= 24mL/g and a molar mass of 3244 g/mol.

For **M4-MTX/Pt**: Anal. % Pt: 2.40 % (Calcd.), 2.17 % (Found).

Co-conjugate M5-MTX/Pt: The same procedure as for **M1-MTX/Pt** was used, except that 0.305 mmol (0.53 g) of conjugate **M5-MTX** was used, giving a brown water-soluble powder collected in 30.0 % yield, η_{inh}= 14 mL/g and a molar mass of 1936 g/mol.

For **M5-MTX/Pt**: Anal. % Pt: 4.03 % (Calcd.), 4.00 % (Found).

Co-conjugate M6-MTX/Pt: The same procedure as for **M1-MTX/Pt** was used, except that 0.305 mmol (0.86g) of conjugate **M6-MTX** was used, giving a brown water-soluble powder collected in 30.5 % yield, η_{inh}= 25 mL/g and a molar mass of 3020 g/mol.

For **M6-MTX/Pt**: Anal. % Pt: 2.58 % (Calcd.), 2.74 % (Found).

Co-conjugate M7-MTX/Pt: The same procedure as for **M1-MTX/Pt** was used, except that 0.305 mmol (0.54g) of conjugate **M7-MTX** was used, giving a brown water-soluble powder collected in 24.6 % yield, η_{inh}= 15mL/g and a molar mass of 1951 g/mol.

For **M7-MTX/Pt**: Anal. % Pt: 4.00 % (Calcd.), 3.77 % (Found).

Co-conjugate M8-MTX/Pt: The same procedure as for **M1-MTX/Pt** was used, except that 0.305 mmol (0.83g) of conjugate **M8-MTX** was used, giving a brown water-soluble powder collected in 24.4 % yield, η_{inh}= 23mL/g and a molar mass of 2919 g/mol.

For **M8-MTX/Pt**: Anal. % Pt: 2.67 % (Calcd.), 2.31 % (Found).

Co-conjugate M9-MTX/Pt: The same procedure as for **M1-MTX/Pt** was used, except that 0.305 mmol (0.88g) of conjugate **M9-MTX** was used, giving a brown water-soluble powder collected in 29.6 % yield, η_{inh}= 24 mL/g and a molar mass of 3063 g/mol.

For **M9-MTX/Pt**: Anal. % Pt: 2.55 % (Calcd.), 2.93 % (Found).

Co-conjugate M10-MTX/Pt: The same procedure as for **M1-MTX/Pt** was used, except that 0.305 mmol (0.51g) of conjugate **M10-MTX** was used, giving a brown water-soluble powder collected 26.4 % yield, η_{inh}= 15mL/g and a molar mass of 1853 g/mol.

For **M10-MTX/Pt**: Anal. % Pt: 4.21 % (Calcd.), 4.42 % (Found).

CHAPTER 6
CONCLUSION AND FUTURE WORK

Clinically used anticancer drugs suffer from a severe pharmacological deficiency, which includes poor water-solubility, lack of cell specificity and, more critically, a tendancy to induce drug resistance. Because of these short comings, drug-specific therapy should be discontinuated, with a consequence of tumorous or metastatic growth re-activation. Advanced technology has proved that the technique of binding reversibly (anchoring) a drug to a water-soluble macromolecular carrier, resulting in a polymer-drug system (conjugate) has emerged as a highly promising strategy to overcome these deficiencies and thus enhance the overall therapeutic effectiveness of anticancer drugs.

This research project was assigned three objectives. The first one was the synthesis of water-soluble macromolecular compounds to serve as carriers transporting the drug to the cancerous cells. The anchoring of methotrexate and the organoiron compound ferrocene to these carriers, resulting in methotraxte and ferrocene conjugates to which was bound *cisplatin*, resulting in ferrocene/platinum and methotrexate/platinum co-conjugates was the second objective of the work. In

the third objective, the work focused on the biological evaluation of the antiproliferative activity of the synthesized conjugates and co-conjugates.

These objectives were achieved by the synthesis of two major classes of polymeric carriers: the coplyaspartamide and the copolyamidoamine types. In contrast to the model commonly used in this laboratory, involving buildIng polymeric carriers with one hydrosolubilizing group and one drug binding moiety, the careful design of the carriers used in this project allowed the imparting of one hydrosolubilizing group and two drug binding moieties to the carrier. The first site was used for platinum binding and the other for either methotrexate, or ferrocene coupling, using HBTU as coupling agent. All water-soluble copolyaspartamides, synthesized in DMF by aminolytic ring opening of polysuccinimide and characterized with inherent viscosities in the range 12-32 mL/g, were obtained in 50-73 % yield. Their molar masses were controlled by size exclusion aqueous dialysis which gave polymeric carriers large enough to retard renal clearance and small enough to prevent inherent polymer toxicity. Copolyamidoamine carriers, also designed with one hydrosolubilizing group and two drug binding moieties, were water-soluble. They were synthesized in H_2O by a Michael polyaddition process of methylenebisacrylamide. Their sizes were also controlled by aqueous dialysis for a low inherent toxicity and slow renal clearance. They were collected in 50-73 % yield, with inherent viscosities in the range 5-33 mL/g.

Both types of carriers were used for binding with the organoiron ferrocene under mediation of HBTU as coupling agent. The one step conjugation process was a reaction of the free organoiron's carboxylic acid with the carrier's primary amine, resulting in the formation of a biofissionable amide bond. The conjugates, purified by size exlusion on column chromatography, then by aqueous dialysis before freeze-drying, were characterized by yield of 54-63 % yield, inherent viscosities of 16-29 mL/g, conjugation extents of 94-100 %, metal incorporations by mass of 2.2-4.4 % and molar masses of 1265-2389 g/mol (repeating unit) for copolyaspartamide-based ferrocene conjugates. The copolyamidoamine-based ferrocene conjugates were collected in 54-61 % yield with inherent viscosities of 12-20 mL/g, binding extents of 88-100 %, metal incorporations by mass of 1.6-3.5 % and molar masses of 1593-3140 g/mol. All ferrocene conjugates of this synthesis were water-soluble. In both types of conjugates, viscosities increase with chains lengths.

Methotrexate was also anchored to both types of carriers. As for ferrocene, the one step conjugation process involved the reaction of the carrier's free amine with one of the drug's free carboxylic acids in DMF. The coupling agent was HBTU. Column chromatography and aqueous dialysis allowed size exlusion and purification of MTX conjugates, which were all water soluble. Copolyaspartamide-based MTX conjugates were collected in 52-57 % yield, with inherent viscosities of 24-39 mL/g, conjugation extents of 96-100 %, drug incorporations by mass of 16.8-30.4 % and molar masses (repeating unit) of 1437-2598 g/mol. The copolyamidoamine-based

MTX conjugates were collected in 32-39 % yield with inherent viscosities of 11-23 mL/g, conjugation extents of 89-96 %, drug incorporations by mass of 14.0-26.7 % and molar masses (repeating unit) of 1635-3056 g/mol. It can also be seen that in both types of conjugates, viscosities increase with chain length.

Cisplatin is one of the platinum coordination complexes important for cancer treatment. Its poor solubility makes it an ideal candidate for the test of antiproliferative potenty of conjugates in which this metal is present. MTX and ferrocene conjugates produced in this work were coupled with *cisplatin via* dihydroxylato ligand. The reaction took place in H_2O between the two hydroxyls of the carrier and *trans*-1,2-diaminocyclohexane-diaquaplatinum (ll) dinitrate (DACH-Pt), the platination agent, under a strict control of pH. The reaction resulted in water-soluble Fc/Pt and MTX/Pt-containing copolyaspartamide and copolyamidoamine co-conjugates. The platinum content in copolyaspartamide-based Fc/Pt co-cojugates was found to be 2.51-11.33 %, while in copolyamidoamine counterparts, it was lower at 2.12-4.20 %. For co-conjugates containing MTX/Pt, the metal contents were in the ranges of 16.8-30.4 % in coplyaspartamides and again lower in copolyamidoamines, 2.17-4.42 %. These findings are in agreement with speculations from previous studies that when platinum is anchored to a copolyaspartamide and a polyamidoamine of the same composition, the metal content is always higher in polyaspartamide than in polyamidoamine. The order of molar masses between all co-conjugates of the same type is in line with that of their viscosities.

For the cell culture testing, selected co-conjugates as well as the three free drugs ferrocene, methotrexate and *cisplatin* were submitted to an antiproliferative activity evaluation against the MCF-7 cell line, using established protocols. The results obtained show that the co-conjugates were more active against the selected line compared to the free drugs, and in some cases, they were even better than the average of the two constitutive free drugs. Given the high resistance of the cell line used, better performances are expected with more sensitive cell lines.

In the future, we intend to submit more co-conjugates and more sensitive cell lines in order to study the trend of variability of the activities. We also suspect that the *in vitro* and *in vivo* evaluation of co-conjugates with dicarboxylato and carboxylatohydroxylato ligands for platinum binding, as well as diamines other than diaminopropane (PDA) for MTX and Fc binding would be useful ways.

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APPENDIX