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Macromolecular derivatives of methotrexate and ferrocene as potential prodrugs in cancer chemotherapy

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CHAPTER 1: INTRODUCTION

1.1 What is Cancer?

Cancer (medical term: malignant neoplasm) is a class of diseases in which a group of cells display *uncontrolled growth* (division beyond the normal limits), *invasion* (intrusion on and destruction of adjacent tissues), and sometimes *metastasis* (spread to other locations in the body via lymph or blood).

These three malignant properties of cancer differentiate them from benign tumors, which are self-limited in their growth and do not invade or metastasize[1].

Cancer is the general name for a group of more than 100 diseases in which cells in part of the body begin to grow out of control. Although there are many kinds of cancer, they all start because abnormal cells grow out of control[2].

One defining feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs[3].

Untreated cancers can cause serious illness and even death[2.]

1.2 Cancer problems

Cancer knows no borders and it is the second leading cause of death in developed countries and is among the three leading causes of death for adults in developing countries.

12, 5% of all deaths are caused by cancer and this is more than the percentage of deaths caused by HIV/AIDS, tuberculosis, and malaria put together.

Cancer is a public health problem worldwide, it affects all people: the young and the old, the rich and poor, men, women and children[4].

Cancer may affect people at all ages, even fetuses, but risk for the common varieties tends to increase with age. According to the American Cancer Society (ACS), 7.6 million people died from cancer in the world during 2007. Apart from humans, forms of cancer may affect other animals and plants[1].

Cancer represents a tremendous burden on patients, families, and societies and it is still increasing, particularly in developing countries.

In USA, for example, one in every three adults currently alive will contract some form of cancer, and every fifth will succumb to it. Indeed, South African males have a life time risk of 1 in 4 of developing cancer, while South African females have a lifetime risk of 1 in 6 of developing cancer.[5]

The age, race, gender and socio-economic status also play an important part in determining the prevalence of particular cancers.

The cancers affecting South African women, in order of prevalence, are: Cervical, breast, colorectal, lung, and oesophageal cancer. The risk of cervical cancer is one in 29, while that of breast cancer is one in 31.

The significant trend is that cervical cancer has overtaken breast cancer. Cervical cancer comprises 13, 4% of all cancers among women from 15 to 29 years old, with young black women being particularly vulnerable.

Prostate, lung, oesophageal, bladder and colorectal cancer rate among the top five cancers affecting all South African men. The leader, prostate cancer, has risen from a risk of one in 31 to one in 24.

Statistics for lung cancer and oesophageal cancer, both fuelled by smoking, show a slight increase, with the incidence of lung cancer much higher among men than women, and among white and colored men than black men[6]. Almost seven million people die each year of cancer, and many of these deaths can be avoided if appropriate measures are put in place to prevent, early detect, cure and care.

By applying existing knowledge and promoting evidence-based actions in cancer control, we will turn this truth into reality for all people everywhere.

The causes of cancer are complex and appear to be multifactorial[7,8], with varying degrees of morphologic disorientation, aggressive growth and invasion with ultimate destruction of the normal cell population. Extensive literature search has also revealed that cancer may be caused apparently by almost limitless variety of physical, chemical and biological agents in which life style; nutrition, food additives, pesticides, ultraviolet radiation and variety of other environmental factors have been directly implicated[8,9,10,11].

Some kinds of cancer are caused by things people do. Smoking can cause cancers of the lungs, mouth, throat, bladder, kidneys and several other organs, as well as heart disease and stroke. Drinking a lot of alcohol has also been shown to increase a person's chance of getting cancer of the mouth, throat, and some other organs. This is especially true if the person drinks and smokes.

Radiation (x-rays) can cause cancer. But the x-rays used by the doctor or dentist are safe. Too much exposure to sunlight without any protection can cause skin cancer.

In many cases, the exact cause of cancer remains a mystery. We know that certain changes in our cells can cause cancer to start, but we do not yet know exactly how this happens. Scientists world-wide are style studying this problem[12].

The four basic types of cancer treatment include: surgery, a primary form of treatment and preferred for localized disease; radiotherapy, a procedure in which radiation is used to damage living cells; immunotherapy, a form of therapy in which the host's immune system is activated, and finally chemotherapy, which is the use of anticancer drugs to kill cancer cells at microscopic level, but which like radiation may produce damage to normal tissues.

Chemotherapy constitutes an important cancer treatment approach alone, or in combination with other treatments regimens. It has played a significant role in treating malignancies and solid tumors, though no effective chemotherapy is available for certain solid tumors and advanced tumors.

1.3 Aims of the project

Present-day clinical chemotherapeutic treatment modalities are unsatisfactory. Cure rates are low and relapses frequent, largely for reasons of excessive systemic toxicity and induction of drug resistance. The project aims at the provision of anticancer drug conjugates featuring enhanced therapeutic effectiveness through the expediency of incorporating a water-soluble polymeric carrier to which the drug model is bioreversibly attached. This conjugation strategy provides a prodrug from which the bioactive species

is liberated in biological environment, generally the nucleus of transformed (i.e. cancerous) cells. The fundamentally altered pharmacokinetic path of the drug so conjugated leads to grossly reduced systemic toxicity and other drug-induced detrimental side effects, thus enhancing patient compliance and life expectancy.

The macromolecular conjugates developed in this project contain the antifolate drug, methotrexate, and the organoiron compound, ferrocene, both individually and in co-conjugated form.

Experimentally, the following specific goals making up the primary objective were defined for the project:

1. Synthesis of water-soluble macromolecular carriers.
2. Synthesis of carrier-bound methotrexate (conjugates)
3. Synthesis of carrier-bound ferrocene (conjugates)
4. Synthesis of carrier-bound platinum drugs (conjugates)
5. Synthesis of carrier-bound methotrexate-ferrocene (co-conjugates)
6. Provision of representative samples to collaborating outside laboratories for biomedical activity assessment and toxicological tests.

CHAPTER 2: LITERATURE REVIEW

The topics covered in this chapter include causes of cancer, different treatments of cancer with accent put on chemotherapy, polymer-drug conjugation, and the main drug model used in this dissertation. They have been in the center of extensive research world-wide, and the numbers of publications on these subjects are extremely large. Therefore, only a small number of original papers will be cited in favor of review articles.

2.1 Causes of cancer

The causes of cancer may actually be: -carcinogens, age, genetic make up, immune system, diet, day-to-day environment, viruses and others.

2.1.1 Carcinogens

A "carcinogen" is something that can help to cause cancer. Tobacco smoke is powerful carcinogen. But not every-one who smokes gets lung cancer. So there must be other factors at work[13]. Smoking increases a person's chance of getting the disease.

Cancer pathogenesis is traceable back to DNA mutations that impact cell growth and metastasis. Substances that cause DNA mutations are known as mutagens, and mutagens that cause cancers are known as carcinogens[1].

2.1.2 Age

Although cancer strikes both young and old, it is primarily a disease of aging. In the United States, 50 percent of all malignancies and 67 percent of cancer deaths occur in persons over the age of sixty-five. That's currently one American in eight; by the 2030, it's expected to be one in five[14].

Most types of cancer become more common as we get older. This is because the changes that cause a cell to become cancerous in the first place take place a long time to develop. There have to be a number of changes to the genes within a cell before it turns into a cancer cell. The changes can happen by accident when the cell is dividing or they can happen because the cell has been damaged by carcinogens and the damage is then passed on to future daughter cells when that cell divides. The longer we live, the more time there is for us to accumulate these genetic mistakes in our cells[13].

2.1.3 Genetic make up

There have to be a number of genetic mutations within a cell before it become cancerous. Sometimes we are born with one of these mutations already. This does not mean we will get cancer. But with one mutation from the outset, it makes it more likely statistically that we will, and this is called by doctors, genetic predisposition.

The BRCA1 and BRCA2 breast cancer genes are examples of genetic predisposition. Women who carry one of these faulty genes have a higher chance of developing breast cancer than women who do not[13].

2.1.4 The immune system

People who have problems with their immune systems are more likely to get some forms of cancer. This group includes people who: - have had organ transplants and take drugs to suppress their immune systems to stop organ rejection. - have AIDS.- are born with rare medical syndromes which affect their immunity[13].

HIV is associated with number of malignancies, including Kaposi's sarcoma, non-Hodgkin's lymphoma, and HPV associated malignancies such as anal cancer and cervical cancer. AIDS defining illnesses have long included these diagnoses. The increased incidence of malignancies in HIV patients points to the break-down of immune surveillance as a possible etiology of cancer[1].

Chronic infections or transplanted organs can continually stimulate cells to divide. This continual cell division means that immune cells are more likely to acquire mutations and develop into lymphomas.

2.1.5 Diet

Cancer experts estimate that changes to our diet could prevent about one in three cancers in UK.

People who do not eat fruits and vegetables have roughly twice the incidence of most types of cancer.

The ideal healthy diet is one that is varied and well balanced[15].

2.1.6 Day to day environment

By this we mean what is around us each day that may help to cause cancer. This could include: Tobacco smoke, the sun, natural and man made radiation, work place hazards and asbestos. Some of these are avoidable and some are not. Most are only contributing factors to cause cancers.

2.1.7 Viruses

Viruses can help to cause some cancers. But this does not mean that theses cancers can be caught like an infection. What happens is that the virus can cause genetic changes in cells that make them more likely to become cancerous.

2.1.8 The many or other causes of cancer

There are 200 different types of cancer affecting all the different body tissues. What affects one body tissue may not affect another. For example, tobacco smoke that we breathe in may help to cause lung cancer. Over-exposing our skin to the sun could give us a melanoma on our leg. But the sun won't give us lung cancer and smoking won't give us melanoma.

Apart from infections diseases, most illnesses are multifactorial. Cancer is no exception.

2.2 Cancer treatments

Cancer can be treated by surgery, radiation therapy, chemotherapy, immunotherapy, monoclonal antibody therapy or other methods.

The choice of therapy depends upon the location and the grade of the tumor and the stage of the disease, as well as the general state of the patient.

2.2.1 Surgery

Surgery is the oldest form of treating cancer consisting of removing the primary tumor and can also have an important role in diagnosing and staging of cancer[6].

In theory, cancer can be cured if entirely removed by surgery, but this is not always possible.

When cancer has metastasized to other sites in the body prior to surgery, complete surgical excision is usually impossible. In the Halstediam model of cancer progression,

tumors grow locally, then spread to the lymph nodes, then to the rest of the body. This has given rise to the popularity of local-only treatments such as surgery for small cancers. Even small localized tumors are increasingly recognized as possessing metastatic potential[1].

Surgery is done for many reasons, often to accomplish one or more of these goals: Preventative (or prophylactic) surgery, diagnostic surgery, staging surgery, curative surgery, debulking (or cytoreductive) surgery, palliative surgery, supportive surgery and restorative surgery (or reconstructive) surgery[16].

Occasionally, surgery is necessary to control symptoms, such as spinal cord compression or bowel obstruction. This is referred to as palliative treatment[1].

2.2.2 Radiation therapy

Radiation therapy, which is also called radiotherapy, X-ray therapy, or irradiation, is one of the three traditional primary forms of medical treatment consisting of ionizing radiation to kill cancer cells and shrink tumors, and for relief of symptoms. It may be used alone or in combination with surgery or chemotherapy, almost anywhere within our body. Innovative new techniques have evolved and are still evolving, enabling delivery of higher radiation doses to cancer cells and limited doses to our normal tissue[1,16].

The effects of radiation therapy are localized and confined to the region being treated. Radiation therapy injures or destroys cells in the area being treated or the target tissue, by damaging their genetic material, making it impossible for these cells to continue to grow and divide.

Although radiation damages both cancer cells and normal cells, most normal cells can recover from the effects of radiation and function properly.

The goal of radiation therapy is to damage as many cancer cells as possible, while limiting harm to near healthy tissue. Hence, it is given in many fractions, allowing healthy tissue to recover between fractions.

Radiation therapy may be used to treat almost every type of solid tumor, including cancers of the brain, breast, cervix, larynx, lung, pancreas, prostate, skin, stomach, uterus, or soft tissue sarcomas[1].

2.2.3 Targeted therapies

Targeted therapy is a type of treatment that uses drugs or other substances, such as monoclonal antibodies, to identify and attack specific cancer cells without harming normal cells. Targeted therapy may be less harmful to no0rmal cells than other types of cancer treatments.

Targeted therapy, which first became available in the late 1990s, has had a significant impact in the treatment of some types of cancer. This constitutes the use of agents specific for the deregulated proteins of cancer cells. Small molecule targeted therapy drugs are generally inhibitors of enzymatic domains on mutated, over expressed or otherwise critical proteins within the cancer cell. Prominent examples are the tyrosine kinase inhibitors *imatinib* and *gefinib*.

Monoclonal antibody therapy is another strategy in which the therapeutic agent is an antibody which specifically binds to a protein on the surface of the cancer cells.

Targeted therapy can also involve small peptides as homing devices which can bind to cell surface receptors or affected extra cellular matrix surrounding the tumor.

Radio nuclides which are attached to these peptides, such RGDs, eventually kill the cancer cell if the nuclide decays in the vicinity of the cell[1].

2.2.4 Immunotherapy

Immunotherapy, sometimes called biological therapy or biological response modifier therapy, refers to a diverse set of therapeutic strategies designed to induce either directly or indirectly the patient' own immune system to fight cancer or to lessen the side effects that may be caused by some cancer treatments [1,17].

Cancer may develop when the immune system breaks down or not functioning adequately. Biotherapy is designed to repair, stimulate, or enhance our body's own immune responses.

Biotherapy may be used to:

-stop, control, or suppress processes that permit cancer growth;

-make cancer cells more recognizable, and therefore more susceptible, to destruction by the immune system;

-boost the killing power of our system cells;

-alter cancer cells' growth patterns to promote behavior like that of healthy cells;
-block or reverse the process that changes a normal cell or a pre-cancerous cell into cancerous cell;
-enhance our body ability to repair or replace normal cells damaged or destroyed by other forms of cancer treatment, such as chemotherapy or radiation; and
-prevent cancer cells from spreading to other parts of our body[18].

2.2.5 Chemotherapeutic treatment of cancer

Chemotherapy is the treatment of cancer with drugs called anticancer drugs, which can destroy cancer cells by impeding their growth and reproduction[16].
Normal cells grow and die in a controlled way. When cancer occurs, cells in the body that are not normal keep dividing and forming more cells without control. Anticancer drugs destroy them by stopping them from growing or multiplying. Healthy cells can also be harmed, especially those that divide quickly. Harm to healthy cells is what causes side effects. These cells usually repair themselves after chemotherapy[18].
In current usage, the term chemotherapy usually refers to cytotoxic drugs which affect rapidly dividing cells in general, in contrast with targeted therapy explained billow.
Chemotherapy drugs interfere with cell division in various possible ways such as with duplication of DNA or the separation of newly formed chromosomes. Most forms of chemotherapy target all rapidly dividing cells and are not specific for cancer cells, although some degree of specificity may come from the inability of many cancer cells to repair DNA damage, while normal cells generally can. Hence, chemotherapy has the potential to harm healthy tissue, especially those tissues that have a high replacement rate, such as intestinal lining.
Because some drugs work better together than alone, two or more drugs are often given at the same time. This is called combination chemotherapy; most chemotherapy regimens are given in combination or in conjunction with radiation therapy or surgery[1].

There are several classes of antineoplastic agents that have been defined:
Alkylating agents, intercalating agent, antibiotics, antimetabolite, hormonal and plant-derived agents, of which the first four classes agents will be covered here. Although their

mechanisms of cytotoxic activity may loosely coincide in occasional instances, the members within a given classes of generally function by a mechanism peculiar to that class.

Thus *alkylating agents* are polyfunctional compounds that have the ability to substitute alkyl groups for hydrogen ions. These compounds react with phosphate, amino, hydroxyl, sulfhydryl, carbonyl, and imidazole groups, which are part of the molecular makeup of the body. In neutral or alkaline solution, alkylators ionize and produce positively charged ions that attach to susceptible nucleic acid, the most likely site of alkylation being the N-7 position of guanine. This alkylation reaction leads to abnormal base pairing, cleaving of imidazole ring of guanine, cross-linking of DNA, interference with DNA replication, transcription of RNA, and the disrupting of nucleic acid function. All this leads to an interruption in the normal cell function of both cancerous and normal tissue. This group of agents is active against a wide variety of neoplastic disease, with significant activity in the treatment of leukemias and lymphomas. However the major adverse effect of the alkylating drugs is bone marrow toxicity and suppression of myelopoiesis[19].

Intercalating agents: These act in similar way to the alkylating agents but, rather than directly forming cross-strands in the DNA molecule, they bind between the base pair molecules, e.g. binding adenine to thymine and cytosine to guanine, again preventing the DNA double strand from dividing in order to replicate and thereby stopping division[20].

Antibiotics comprise a heterogeneous group of chemotherapy agents that have proven to be extremely successful in treatment of wide variety of tumors, both epithelial and mesenchymal. *Doxorubicin*, *daunorubicin*, their analogues *epirubicin* and *idarubicin* are successful agents in the treatment of a number of neoplastic diseases. Among the numerous "non-aligned" neoplastic agents, that is, those not specifically classified, one should mention metal complex-type agents, such as platinum drugs. Many antibiotic agents bind to DNA through intercalation between specific bases and block the synthesis or interfere with cell replication or transcription. The drug inhibits DNA topoisomerase II, which belongs to this class, acts by binding DNA and nicking one of its strands, thus allowing the super coiled macromolecule to relax as the opposite strand passes through

the break. The enzyme then reattaches the broken ends together. While varying in the mechanism of drug action and frequently also in their pharmacokinetic pathways, the drugs have in common a cytotoxic effect resulting from interaction with endocytic nuclear material[19].

Antimetabolites are structural analogs of naturally occurring compounds. They interfere with the production of nucleic acids and 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[6].

A topic of fundamental importance concerns combination chemotherapy, a strategy involving multiple drug administration to produce additive and even synergistic effects without enhancing overall drug toxicity. A specific goal of combination therapy is broadening of the activity spectrum through the use of two or more agents functioning by different mechanisms and exerting different toxicity effects.
Numerous combinations of both time-proven and novel drug models have been administered clinically, and the field is steadily widening[19].

In combination with classical cancer treatment modalities, such as surgery and radiation therapy, chemotherapeutic techniques have for many decades been applied in efforts to ameliorate cancer suffering and even eradicate malignancies. However, in spite of massive research and development activities worldwide, the success rate of anticancer drugs has remained unacceptably low; only a few select types of cancer have come under complete control, whereas in the majority of malignancies, the administration of such drugs has at best caused temporary relief and frequent recurrence of cancerous growth after initial remission. The reasons for such inadequate performance of currently available anticancer agents can be traced to numerous pharmacological deficiencies, including (i) *lack of cell specificity*, with ensuing drug distribution into both normal and transformed cells. In consequence, drug application will be excessively wasteful, and

healthy tissue will be exposed to toxic side effects; (ii) *Inadequate water solubility*, hampering swift and efficacious drug distribution in the body's aqueous fluid system, with resulting enhanced exposure to macrophage activity; (iii) *Decreased serum half-life* as a consequence of catabolism, protein binding, capture by the reticuloendothelial system, or efficacious excretion mechanisms; (iv)Monophasic *salt-like* or *charged structure*, inhibiting membrane penetration and cell entry through normal passive diffusion. As a consequence, only a small fraction of the medicinal agent will successfully enter intracellular space for interaction with nuclear DNA or proteinaceous constituents; (v) *Excessive systemic toxicity*, which grossly diminishes therapeutic drug effectiveness and oftentimes necessitates premature termination of therapy; and (vi) Lack of long-term effectiveness because of *induced drug resistance* with consequent need for administration of increasingly larger doses to a maximal level, at which toxicity will outweigh therapeutic effects. It is particularly in the last-named two functions where periods of regression are limited, and relapses, rather than cures, dictate the treatment course[21].

In the light of this grossly inadequate performance spectrum of most of the anticancer drugs currently available to the clinician, it must be the foremost goal of health researchers in the cancer field worldwide to reduce the incidence of malignancies and their impact on human suffering through development of superior therapeutic treatment modalities, in particular, the design of efficacious drug delivery technologies capable of circumventing the toxicity and resistance problems, while increasing overall drug bioavailability. This work represents a contribution to that goal. In harmony with the polymer research group's principal objective, it is the project's aim to increase the bioavailability and overall therapeutic effectiveness of anticancer agents. With the aid of strategies outlined in section II.3, we have achieved this aim through reversible binding (anchoring) of selected antineoplastic drug systems to suitably designed, water-soluble macromolecules acting as biocompatible carriers throughout the major part of the drug's pharmacokinetic lifetime. The conjugates act as prodrug from which the drug proper species will be liberated in the biological environment of the target tissue to exert their antiproliferative effects. From the detailed expose in section 3, it will be seen that drug conjugation with suitably designed macromolecular carriers represents one of the most promising technologies currently under investigation aimed at counteracting the

aforementioned deficiencies of anticancer agents in present-day clinical use. It will be shown, furthermore, that polymer-drug conjugation permits increased pharmacological utilization of pharmacokinetically disadvantaged drugs, i.e. medicinal agents that would be expected to show efficaciousness yet fail to do so because of unfavorable pharmacokinetics and other factors. Lastly, it was the aim of this study to develop synthetic methods for the preparation of multi-drug conjugates, *i.e.* Structures in which two or more medicinal agents are bioreversibly bound to the same carrier polymer in an effort to achieve simultaneous drug bioavailability at the target site. It is in fact the multi-drug conjugation task that will occupy the predominant part of the overall research program.

2.2.5.1 Drug carriers

Drug carriers are substances that serve as mechanisms to improve the delivery and the effectiveness of drugs. They are used in drug delivery systems such as: Controlled-release technology to prolong in vivo drug actions; Decrease drug metabolism and reduce drug toxicity.

Carriers are also used in designs to increase the effectiveness of drug delivery to the target sites of pharmacological actions[22].

2.2.5.2 Polymer as drug carrier

The polymers chosen as drug carriers are either natural or synthetic macromolecules. They must meet certain requirements in order to maximize their potential as polymeric drug carriers by decreasing the toxicity and (or) increasing the therapeutic index of the anticancer drug.

The features required include:-*Water solubility* for rapid dissipation in the vascular (central circulation) system.-*Minimal toxicity* and *immunogenicity* to minimize carrier-induced side effects.-*Main chain biodegradability* for ease of elimination after complete drug detachment.-*Molecular mass in 25 000- 50 000 range* to manifest polymer features with minimal renal clearance and associated nephrotoxicity.-*Presence of "extra" constituents* such

as poly(ethylene oxide) segments in the backbone, for prolongation of serum half-life and other biomedical prerequisites[6].

2.2.5.2.1 Natural polymers as drug carriers

Natural polymers have the advantage of easy availability and biocompatibility, although their preparation may be restricted by the need for several purification steps, and their use is limited by their high immunogenicity. In general, these polymers biodegradable owing to their natural origin, they will be excreted from the bloodstream by natural catabolic mechanisms. However, their use as drug carriers is often limited[6].

2.2.5.2.2 Synthetic polymers as drug carriers

Synthetic polymers are advantageously used as drug carriers, since they are more susceptible to modifications than natural polymers. The advantage of the polymer synthesized is that it may contain the smallest number of bonds that are sensitive to enzymatic attack, and that scission of these bonds can be controlled by changing the structure, amount and the length of crosslink[23, 24]. More interest was developed in the use of the synthetic polymers as drug carriers since Ringsdorf[25] proposed his model of water-soluble macromolecular drug prodrugs in mid-Seventies. Ever since that time, numerous carrier types have been investigated including the poly(amino acids) and poly(amidoamines)[6].

2.3 The Polymer-drug Anchoring strategy

Recognizing the inadequate pharmacological performance of the great majority of anticancer drugs in current clinical use, pharmaceutical scientists worldwide have initiated numerous programs aiming at enhancement of cancer effectiveness. Among these, the technology of polymer-drug conjugation stands out as one of the promising approaches[26, 27, 28].

The concept of binding (conjugation) a medicinal agent to a macromolecular carrier for the purpose of enhancing the drug's overall clinical effectiveness dates back to the mid-

Seventies, and Helmut Ringsdorf must be credited with having laid the groundwork for this highly successful technology[25]. It was in Ringsdorf's laboratory where the prototype carrier-drug conjugate originated and the venerable anticancer agent, methotrexate, served in that work as the drug constituent[29]. Subsequently, Ringsdorf's strategy was creatively utilized in other laboratories, notably those of Ghose[30], Chu and Howell[31], Shen and Ryser[32], Garnett and Baldwin[33], Kanellos[34] and Deguchi[35], with coworkers, who pioneered the development of methotrexate conjugates with antibodies and other natural, proteinaceous polymers. Successful activities in this field have later been reported also by Pouton and Marriott[36], Stehle *et. al.*[37] , Boratynski *et. al.*[38] , Ghosh *et. al.*[39], and most notably, by the prolific group of Umemoto, Kato, and Hara[40].

The subject has more recently been reviewed by the last-named authors[41]. In our laboratory, man-made, *i.e.* fully synthetic macromolecules as drug carriers have been given preference over proteinaceous and other natural polymers for a number of incisive reasons elaborated previously. Most importantly, such macromolecules can be constructed precisely by design in terms of structural types, biodegradability, and frequency in the chain of the various subunits with solubilizing, drug-binding, and other facilitating functions. Polyaspartamides lend themselves particularly well to this application.

As a macromolecular species, a polymer, when administered par enter-ally in water-soluble form, can utilize intercellular and cell entry mechanisms more consistently, and often, more efficaciously, than is generally observed with nonpolymeric compounds. In particular, the EPR (enhanced permeation and retention) effect described by Maeda[42] allows macromolecular compounds uniquely to accumulate in tumorous tissue as a result of vascular leakage and lack of lymphatic drainage. The polymer-drug conjugate, as first proposed in principle by Ringsdorf[25], consists of a linear polymer chain composed of a major proportion of subunits bearing hydrosolubilizing groups, a minor proportion of subunits bearing the bioreversibly bound drug, and in the ideal case also some subunits with special affinity for the (cancerous) target tissue.

The water-soluble carrier component in the assembly functions as a vehicle transporting the medicinal agent, sterically protected from enzyme attack, protein binding, and other scavenger action, through various body compartments and membranes to, and into, the

lysosomal compartment of the affected, that is, neoplastic cells. Here the agent is enzymatically or hydrolycally released in monomeric form for biological action. This mode of drug transport, schematically depicted in Figure 2.1 for cytotoxic drug conjugate, profoundly lowers free drug concentration in the vascular system thereby reduces systemic toxicity. The conjugate's cell entry follows a pinocytotic mechanism, thus circumventing potential hindrance by drug polarity or ionicity, and increased translocation efficacy is observed in the special case of adsorptive pinocytosis as typically realized with cationic polymers. Where excessive P-glycoprotein-mediated efflux of the agent from endocytic space may have developed, the pinocytotic pathway of cell entry assumes outstanding importance as a means of neutralizing the much dreaded phenomenon of drug resistance. It is clear from the foregoing arguments that the net benefit of drug conjugation to a suitably constructed carrier polymer will be an appreciable enhancement of therapeutic effectiveness[43].

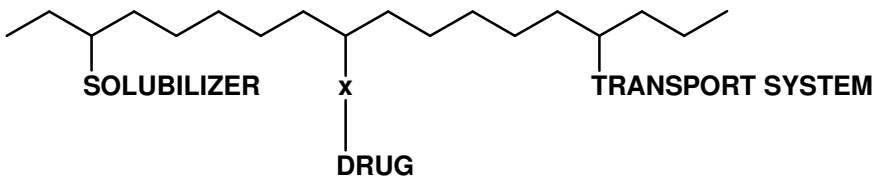
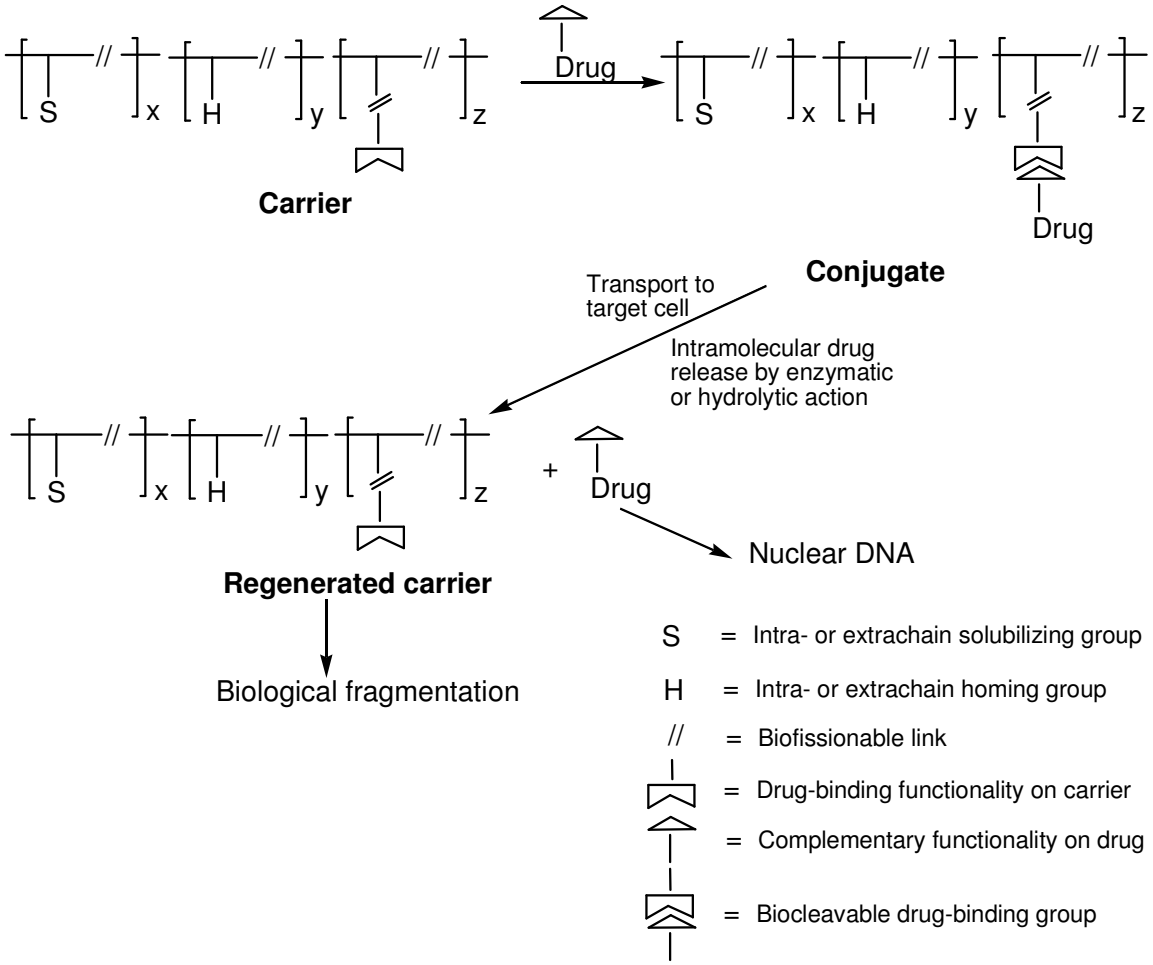


Figure2.1: General structure of a polymer carrier as proposed by Ringsdorf[25]



Scheme2.1: Polymer-drug conjugate as proposed by our group.

The ideal polymer-drug conjugate administered intravenously or intraperitoneally will show the following performance features: (i) complete and rapid dissolution, and efficient dissipation, in the body's central circulation system; (ii) constraint of free drug serum levels through inhibited drug release from the carrier while in transit; (iii) temporary (in transit) protection of attached drug species from enzymatic attack and serum binding; (iv) facilitated cell entry by a picocytotic mechanism of membrane crossing available to molecular agents with consequent alleviation of problems caused by drug polarity and resistance; (v) lysosome-mediated release of drug in intracellular space; (vi) facilitated "targeting" onto cancerous tissue trough judicious incorporation of homing devices; and (vii) preferential accumulation of the bioactive agent in macromolecular form in tumor

tissue as a consequence of enhanced vascular permeation (through vascular leakage), paired with increased retention resulting from retarded lymphatic drainage. All factors combined will result in reduced toxicity, decreased risk of drug resistance, and improved drug utilization reflected in an elevation of therapeutic index. In the polymer laboratory of this Department the afore-mentioned structural prerequisites have been reduced to practice in numerous projects involving the polymer-anchoring of organometallic and metal-free antineoplastically active agents, and use will be made of the accumulated experience in this project.

The principal drug species will be methotrexate, an antifolate agent with proven track record in cancer chemotherapy. The organoiron compound, ferrocene, an experimental drug model highly successful in as yet preclinical bioevaluation screens, is as a co-conjugation agent, in the presence or absence of additional drug or adjuvant species. The platinum compounds especially platinum(II) complexes, those containing the 1,2-diaminocyclo-hexane (DACH) ligand.
In the following, a brief background review will be presented in the areas of polymer-drug conjugation and the various drug models used in this project work.

2.4 The bioactive agents

2.4.1. Methotrexate

Methotrexate (MTX) has served as the principal drug species in this project, either alone or in combination with other agents, such as the organoiron compound, ferrocene. Both agents are key drug models in projects investigated by other students in this laboratory, although they play different parts in different chemical environments. Details follow.

Amethopterine (Methotrexate, MTX): History.

Methotrexate originated in the 1940s when Dr. Sidney Farber at Children's Hospital Boston was testing the effects of folic acid on acute leukemic (severe blood cancer) children. Inspired, he asked Dr. Y. Subbarao, then Director of the Research Division of Lederle Labs (part of American Cyanamid), to synthesize the anti-folate (methotrexate). Dr. Subbarao, who also happened to be the head of the team which had earlier

synthesized folic acid (1946) readily synthesized this anti-folate and handed it over to Dr. Farber, who in turn administered it to a small group of very ill leukemic children. The remarkable clinical improvement that was observed in these patients heralded the area of cancer chemotherapy in modern medicine. This was reported by Dr. S. Farber in June 3rd, 1948 issue of NEJM. In 1950 Dr. Farber founded in Boston the world's first Cancer Research Center. Methotrexate gained Food and Drug Administration (FDA) approval as an oncology drug in 1953[44].

Methotrexate: Uses.

Methotrexate was originally used as part of combination chemotherapy regimens to treat many kinds of cancers[44]. Folate is necessary for the production and maintenance of new cells. This is especially important during periods of rapid cell division such as cancer cell, and drugs that interfere with folate metabolism are used to treat cancer[45]. Methotrexate is a drug often used to treat cancer because it inhibits the production of the active form, tetrahydrofolate.

It represents a *classical* anticancer drug with high potency that has been in clinical use for many decades[46] and it is still the mainstay for the treatment of many neoplastic disorders such as choriocarcinoma and acute lymphocytic leukemia, in combination with platinum-type and other drugs also against osteosarcoma and carcinomas of the cervix, ovaries, lung and bladder. *Methotrexate* reacts *in vivo* as an antifolate. Folate-dependent enzymes are key performers in the generation of nucleotides required for the construction of DNA both by normal cells and transformed, *i.e.* cancerous cells. The latter pathway is particularly important if one considers the excessive rates of metabolism in the transformed cell and the consequent demand for the build-up of DNA. MTX acts as a tight-binding inhibitor of dihydrofolate reductase, the key enzyme in intracellular folate metabolism. As a result of this process, folate cofactors accumulate in their inactive dihydrofolic acid form, which in turn inhibits purine and thymidylate synthesis, leading to cell death. This process is somewhat selective, antifolate action being stronger in transformed (*i.e.* cancerous) tissues than in normal ones. Intracellular polyglutamylation of MTX (and other antifolates) contributes benefically to the drug's accumulation in the tumor cell. MTX is active against choriocarcinoma and acute lymphocytic leukemia, in combination with other drugs (including cisplatinum) also against osteosarcoma and,

most importantly in the context of this project, carcinomas of the lung, cervix, ovaries, bladder and other organs. As a typical cytotoxic agent, MTX causes highly toxic side effects and development of resistance; its uptake by the tumor cell proceeds by an active transport mechanism, which appears to be inhibited as resistance is building up. In view of these pronounced toxicity and resistance problems, which severely hamper successful clinical administration, the drug will function in this project as an excellent candidate for anchoring to suitable polymeric carriers.

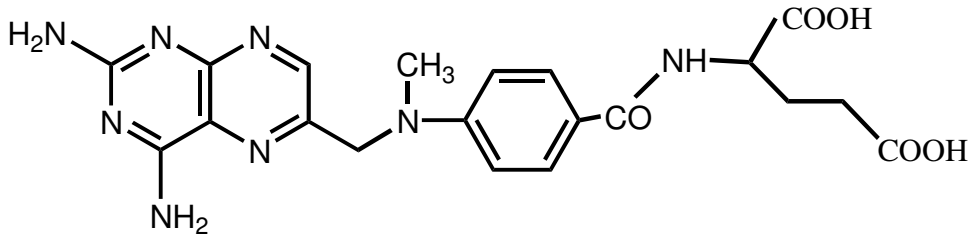


Figure2.2: Structure of Methotrexate

Mechanism of action of Methotrexate

Methotrexate (MTX) enters the cell through the reduced folate carrier (**a**) using an endocytic pathway activated by a folate receptor (**b**). After entering the cell, methotrexate is polyglutamated (Glu) by the enzyme folylpolyglutamate synthase (**c**). Methotrexate and its polyglutamates inhibit the enzyme dihydrofolate reductase (**d**), thereby blocking the conversion of dihydrofolate (FH2) to tetrahydrofolate (FH4). As tetrahydrofolate stores are depleted, thymidylate (TMP) synthesis (**e**) is reduced, which ultimately inhibits DNA synthesis (**f**). Long-chain polyglutamates of MTX have the same affinity as MTX for the target enzyme dihydrofolate reductase, but have markedly increased inhibitory effects on both thymidylate synthesis (**e**) and purine biosynthesis (**f**), which is required for RNA production[47].

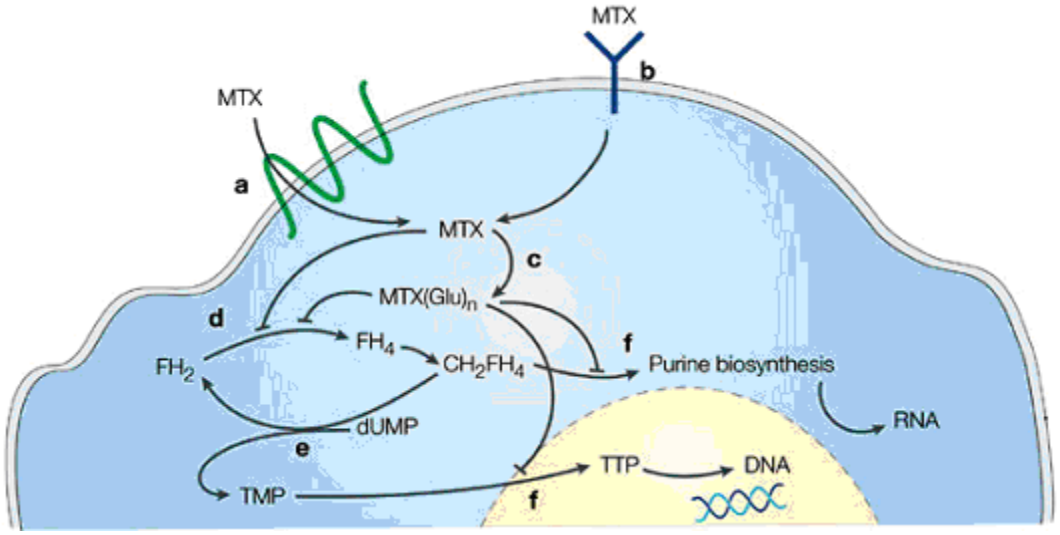


Figure2.3: Mechanism of action of Methotrexate

2.4.2 Ferrocene

Over the past thirty years platinum-based drugs, notably cisplatin and carboplatin, have dominated the treatment of various cancers by chemical agents. However, these drugs cause serious side effects, including renal impairment, neurotoxicity and ototoxicity (loss of balance/hearing). Thus, there has been considerable interest and increased research activity in developing other transition metal compounds as anticancer drugs, which are less toxic than the platinum-based drugs. Among these derivatives, metallocenes and metallocene dihalides proved to be particularly active against a number of tumors, and, despite the fact that their activity seems to follow mechanistic paths which differ from those of cisplatin; both drugs have a DNA intracellular target.

The first metallocene discovered was ferrocene, and its particular structure was brought out by Wilkinson in the early 1950s. Ever since, there has been a rapid growth in the study of ferrocene compounds for several reasons, which include their highly promising antiproliferative activity against various murine and human cancer lines, and their unusual stability owing to the sandwich structure conferred by the binding of the iron center by two Cp (cyclopentadienyl) rings.

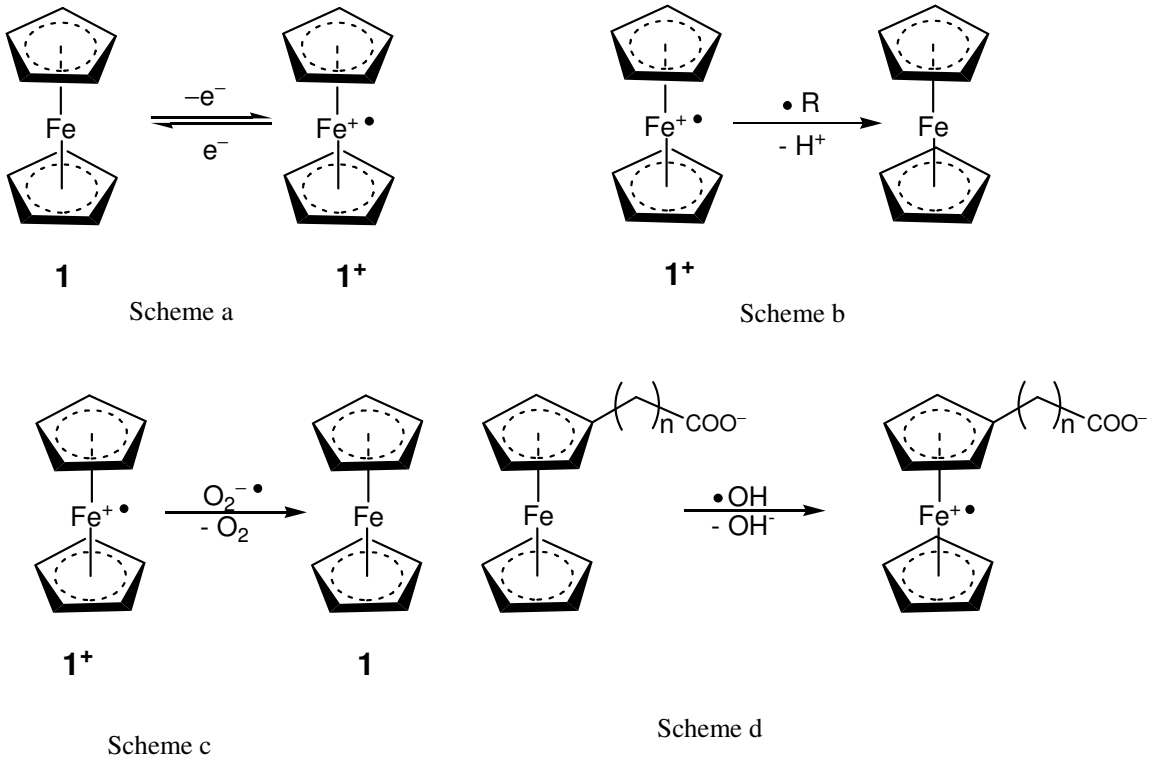
Ferrocene, (n5-Dicyclopentadienyliron(II)), contrasting with MTX, is still at an experimental stage, although both in vitro and vivo tests have shown excellent

antiproliferative and antineoplastic activities for a number of monomeric and polymeric derivatives of this compound. In addition, other biological functions unconnected with anti-tumor activities have been investigated, as will be outlined in the following text.

Behavior of Ferrocene in physiological environment: One of the important characteristics displayed by the ferrocene complex is its outstanding oxidation-reduction behavior. It readily converts to the ferricenium ion, which is a free radical of high stability. This one-electron transfer reaction is reversible (Scheme a), and has some implications in the biological realm. Electron transfer and free-radical reactions play a vital role in biological processes, and therefore the ferricenium-ferrocene system should be a topic of major interest in both biochemical and biomedical research. Many investigations dealing with the biological behavior and functioning of ferrocene compounds have indeed been reported. Thus, under enzymatic control, ferrocene is oxidized by hydrogen peroxide, whereas ferricenium ion is reduced by NADH and metalloproteins (Scheme b). Besides, ferricenium ion is reacts with the biologically important superoxide anion radical, leading to a regeneration of ferrocene and dioxygen (Scheme c). A reverse electron transfer reaction, resulting in oxidation of the ferrocene complex to its ferricenium salt, occurs with ferrocenylcarboxlates in their interaction with the highly reactive hydroxyl radical, transforming the latter to a harmless hydroxyl anion (Scheme d). Scheme 2.1 below gathers the different reactions of the ferrocene complex in the biological environment.

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 aforementioned superoxide and free radical-scanvenging 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 broad topic of ferrocene in biomedicine is covered by these selected referencies[48, 49, 50, 51, 52, 53, 54, 55, 56]



Scheme2.2: Reactions of ferrocene complex in biological environment

It was with all the above mentioned considerations in mind, the knowledge of various aspects of ferrocene chemical behavior, and the fact that ferrocene compounds tend to give high therapeutic indices and show very low toxicity when compared to other chemotherapeutic agents used, that the strategy of reversibly conjugating the inherently hydrophobic ferrocene complex to water-soluble macromolecular carriers was developed and improved in the university's laboratory.

2.4.3 Platinum Compounds

Platinum-containing drugs of cisplatin (cis-diamminedichloroplatinum(II)), type and derived structures have firmly established themselves as a class of highly potent antineoplastic agents and some of these have been in the oncologist's hand for a number of years. The activity of the parent compound, cisplatin, has been amply demonstrated against several types of tumors, such as testicular, ovarian, head and neck, and small cell lung cancer[6, 19]. Cisplatin is also involved in chemotherapy of osteogenic sarcoma, as well as prostate and advanced bladder cancers. The mode of action of platinum-

containing drugs involves the formation of aquated species in the intracellular space and subsequent intra- and interstrand DNA cross-linking with generation of lesions in the double helix, ultimately resulting in cell death. In general, the biologically effective binding to DNA requires a cis conformation of two amine ligands that represent key components of most platinum drugs. It has reported that trans- oriented diamine coordination compounds[57], display antitumor activity, although the described agents have not as yet reached clinical maturity. The mechanism of biological action generally involves hydrolytic dissociation of the leaving groups (chloride anion-deprived) in the cytoplasm fluid, and the aquated platinum complexes are regarded to be the reactive form of the compounds[58], in contrast with the behavior of *trans* isomer of cisplatin. Indeed, transplatin possesses no antineoplastic properties. The reasons are steric; The trans isomer can not bind to DNA in the same mode as established for the cis compound; It rather forms monofunctional adducts amenable to detoxification, and minor fractions of non-adjacent intra- or inter-strand crosslings may be selectively excised and repaired prior to replication, thus forestalling cell death. Because of its significant therapeutic effect in these tumors and activity against a number of other solid tumors, cisplatin became the most frequently used antitumor agent. Because of the renal and neurotoxicities of cisplatin, there were intensive efforts to develop analogs with fewer of these toxicities. This work led to the development of carboplatin, cis-diamine-1,1-cyclobutane-dicarboxylato-platinum(II) which is one of the most advanced members of the second generation of platinum drugs and which produces primarily hematopoietic toxicity and appears to have an antitumor effect similar to cisplatin[59, 60, 61]. Of the newer platinum(II) complexes, those containing the 1,2-diaminocyclohexane (DACH) ligand are of the special interest since this complex was found to be active against cisplatin-resistant tumor cell lines[62]. Figure2.4 illustrates cisplatin and other platinum analogues of clinical interest.

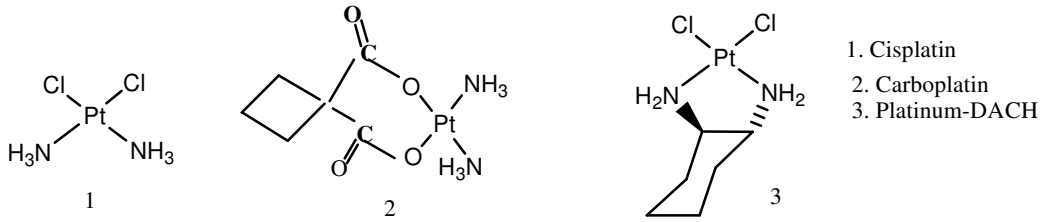


Figure2.4: Cisplatin and other platinum analogues of clinical application.

CHAPTER 3: RESULTS AND DISCUSSION

The topics in this Chapter are in accordance with the specific experimental goals outlined in Chapter 1. This includes:

Phase I: Synthesis of carrier polymers, both known and novel, preparative methods developed in previous projects and improved where necessary.

Phase II: Conjugation of the carrier polymers obtained in Phase I with selected anti-cancer drugs, namely, those of methotrexate, ferrocene and platinum.

Phase III: Submission of some conjugates for *in vitro* biomedical assessment and toxicological tests.

3.1 Synthesis of Carrier polymers

In fulfillment of the specific experimental goals indicated in Chapter I, an effort was made to afford tailor-made drug carriers through the design and derivation of several synthetic polymeric structures.

Most polymeric drug carriers described in literature have been either natural polymers or their derivatives, including proteinaceous and carbohydrate types as well as protein and polypeptide drugs which often show high rates of *in vitro* degradation by enzymatic cleavage[63, 64]. In general, all these synthetic polymers lack the important property of biodegradability. With respect to this, the polymer laboratory of the Chemistry Department of this University has placed emphasis on the design of synthetic macromolecules which, in addition to possessing water solubility and providing functional groups which serve as drug binding sites, comprising biodegradable links in the backbone.

The polymer carriers selected in this dissertation were designed with the intent to comply with the requirements outlined in Section II.3:

1. The polymeric backbone should be linear and highly flexible. This will have the benefit of increasing the positive entropy of solution and therefore favor the dissolution process.

2. The carrier should be compatible with the biological environment[65], i.e. non toxic, non-antigenic and non-thrombogenic so as not to induce any unwanted side effects such as inhibition of enzymes, or and any immune –response and blood-clotting side effects. Macromolecules must be stable and pharmacologically inactive during circulation in the blood stream.
3. The carrier macromolecule must comprise reactive functional groups suitable for drug anchoring and release. These groups should be separated from the principal chain by short side chains (5-15 atomic constituents) or spacers. The presence of spacers will serve to diminish the steric inaccessibility due to the polymeric backbone. By their nature, the spacers should allow for the hydrolytic and enzymatic cleavage of bonds arising from the drug anchoring reaction.
4. The main chain must contain both hydrolytically and enzymatically cleavable segments that facilitate biodegradation and subsequent elimination of the carrier in its "spent state" following drug release.
5. The macromolecular carrier should incorporate an interposition, between the spacer-bearing units, of subunits lacking drug-binding abilities along the principal chain .This will prevent multifunctional drug binding by reducing spacer density in the molecule.
6. Cationic functions (or precursor groups, such as tertiary amines) should be present as this will facilitate pinocytic[66] cell entry, and in so doing it should overcome problems caused by ionicity or polarity. Pinocytosis may become extremely significant once drug resistance has emerged. At the same time, cationic sites[67] may provide enhanced drug affinity for tumor cells, as many of these cells develop negative surface charge due to their typical excessive glucose metabolism[68].
7. Carrier molecular sizes should fall into the range 25000-100000, thus possessing the advantage of retarding renal excretion and avoiding renal excretion and avoiding the toxic effects frequently displayed by molecules of high molecular mass.

In compliance with the critical design requirements enumerated above the basic schematic structure shown below, which is that of polyamide-type with subunits randomly distributed along the chain, was used in this dissertation project.

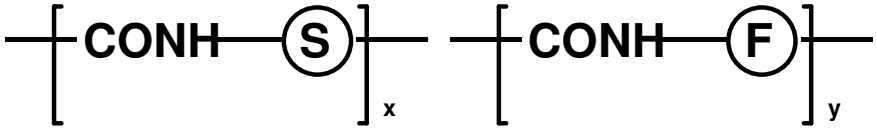


Figure 3.1: Structure of polyamide-type carrier

In this model, **S** represents an extra- or intra-chain hydrosolubilizing group required to impart water-solubility to the ultimate polymer-drug conjugate and **F** stands for an extra- or intra-chain functional group capable of reversibly binding the drug species of choice. Amide and ester groups were the functional group chosen in this project. The reason for this is the possibility of hydrolytic or enzymatic cleavage offered by both amide and ester bonds in the cytoplasmic space[55].

On the basis of 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, the option of polymeric carrier bearing extra-chain functional groups for drug species anchoring, was adopted.

Three different structural types of polyamides were selected for the project:
Polyaspartamides, polyamidoamines, and ester-amine polycondensation products. These three classes will be discussed in particular in the following subsections.

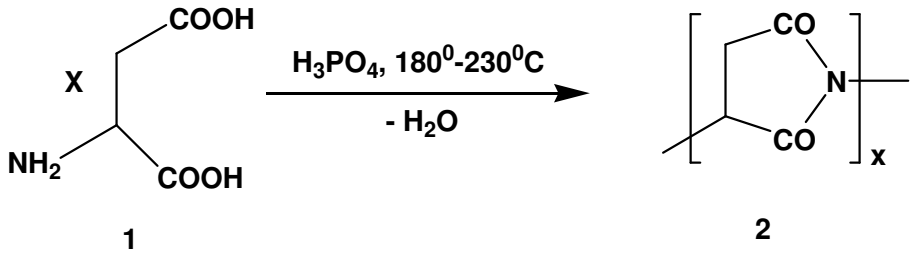
3.1.1 Polyaspartamides

The potential value of polysuccinimide-derived aspartamide polymeric carriers for biomedicinal agents was revealed by Drobnik and his co-workers[69], and for number of years the polymer research laboratory of this University has used these macromolecular

carriers for diversity of antineoplastic drug systems. Accordingly a major portion of the carrier polymers synthesized in this dissertation project were based on this class.

3.1.1.1 Poly-DL-Succinimide

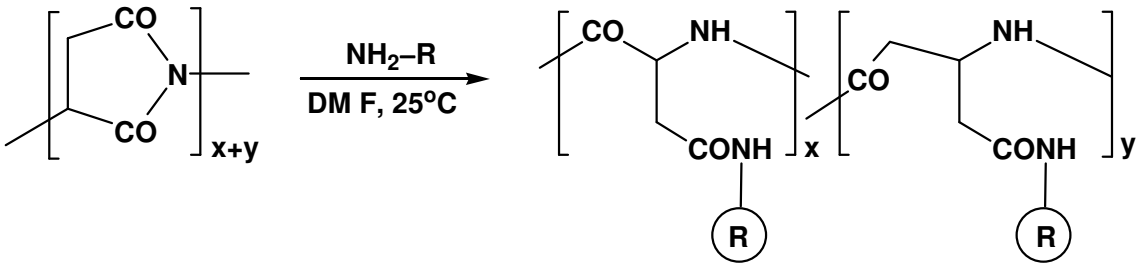
The polymerization of DL-aspartic acid[70] by condensation in orthophosphoric acid medium at high temperature (180°-230°) yielded poly-DL-succinimide **2** (PSI), (poly [2, 5-dioxopyrrolidine-1, 3-diyl]) as indicated in Scheme (1). Poly-DL-succinimide **2** was subsequently subjected to carbodiimide-mediated chain extension. This representative polymer had an inherent viscosity $52mLg^{-1}$. In the pass inherent viscosity in rang of 25 to 45mL/g has been reported by other workers of the same laboratory.



Scheme 1: Polycondensation of DL-aspartic acid.

3.1.1.2 Poly α, β-DL-aspartamides

Polyaspartamides are prepared from poly-DL-succinimide (PSI) **2** by sequential ring-opening in anhydrous, dipolar aprotic medium, such as N,N-dimethylformamide (DMF), giving rise to a mixture of D- and L-enantiomeric forms possessing both α- and β-peptidic repeat units[71]; only α peptide forms are shown for simplification, throughout this dissertation



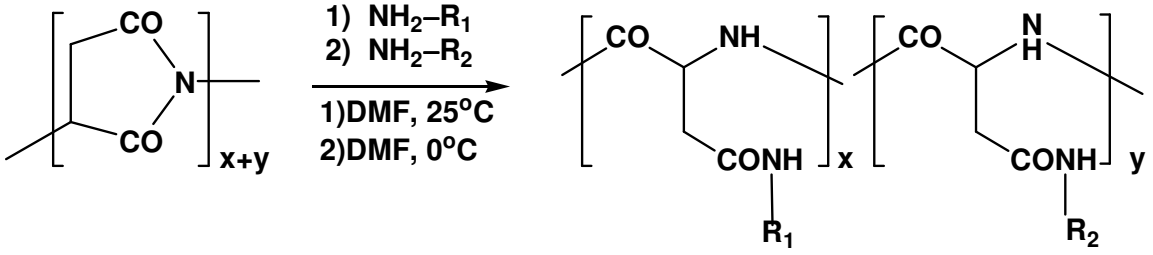
2 **3**

(α-peptide) **(β-peptide)**

Scheme 2: Synthesis of Poly-α, β-DL-aspartamide.

The ring-opening reaction of polysuccinimide was used to synthesize copolymers through a stepwise, sequential addition of two different mines NH_2-R_1 and NH_2-R_2, in given stoichiometric feed ratios. Occasionally the third amine NH_2-R_3 has been used in this operation.

Copolyaspartamides **4** are composed of randomly distributed aspartamide reaping units bearing NH_2-R_1, NH_2-R_2, etc… as shown in scheme 3.



2 **4**

Scheme 3: Copolymer, Poly-α, β-DL-aspartamide.

In general, R_1 is chosen to represent a hydrosolubilizing moiety group (e.g., hydroxyl or tertiary amine), while R_2 represent a drug-binding (e.g., carboxyl, a primary or a secondary amine) function.

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

1. The molecular mass, which on average ranges from about 25000 to 35000, is sufficiently low to suppress (chain length-dependent) inherent polymer toxicity, and still high enough to retard renal excretion.
2. The intrachain amide groups permits gradual backbone cleavage for ease of catabolic elimination of the polymer in the "spent" state. The L-type polymers are very vulnerable to enzyme attack and consequently undergo rapid degradation, while D-type polymers are more resistant to enzymatic attack. The presence of D-configurated CH groups and β-peptide units in the chain prevents the so called "unzipping" of the chain from its terminals brought about by the exopeptidases[72] class of enzymes, whose function is to cleave the terminal residues from polypeptides. It is therefore ensured that fragmentation of the polymer is retarded.
3. Polyaspartamides are essentially non toxic and are expected to have distinctly low immunogenicity compare with that of high molecular proteinaceous biopolymers. Furthermore, since the final backbone degradation product is essential amino acid, aspartic acid, one would expect this to be a "body-friendly" catabolite.
4. The S-modified subunit, i.e. the one bearing the hydrosolubilizing group R_1 can be introduce as the majority unit (x>y), as this will provide an effective insulation of F-modified (R_2) subunits from each other and, thus, will reduce the risk of intramolecular interaction of adjacent drug species.
5. Tertiary amine functions can be introduced as side group to offer the special functions of adsorptive pinocytotic cell entry and target cell affinity.
6. Polyaspartamides can be made to acquire additional solubility to a great extent in methanol, a feature that is uncommon with polymeric compounds, but useful for certain follow-on reactions that require alcoholic media, by incorporating poly (ethylene oxide) side chains[73].

The adsorptive endocytotic cell entry of a prodrug[74] is influenced deeply by its ability to undergo protonation in physiological conditions (pH~7.4); Therefore, the copolyaspartamides synthesized in this study were classified into two categories, based

on their protonation potential. Thus, the first category comprises the copolyaspartamides containing tertiary amine-terminated side chains, and the second category, those bearing both tertiary amine-terminated side chains and hydroxyl- or methoxyl- terminated side chains.

Copolyaspartamides containing tertiary amine-terminated side chain:

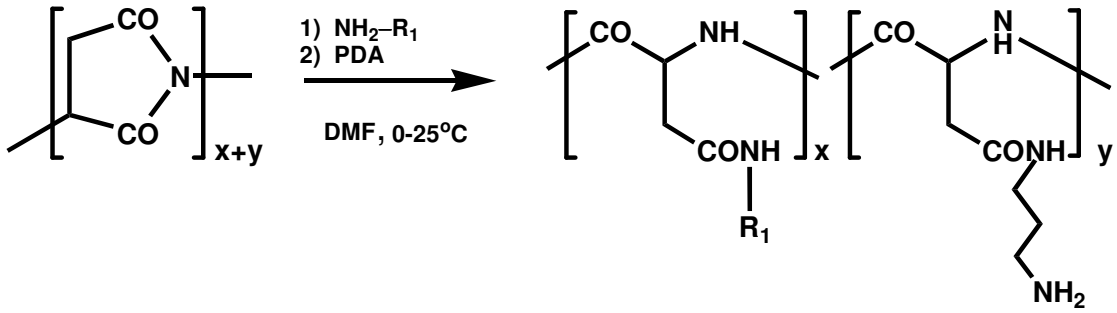
The specific polyaspartamides of general type **4, 5** and **6** listed in scheme **4,** scheme **5** and scheme **6**, were synthesized as part of this dissertation program, and some were later used for anchoring purposes.

The following series of copolymers (Schemes 4a-4e) were synthesized under experimental conditions similar to previous work performed in this laboratory, with preparative methods improved where necessary.

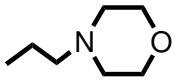
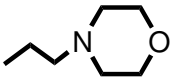
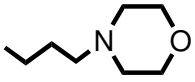
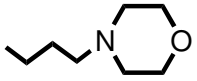
In Scheme 4, NH_2-R_2, amine nucleophile, is 1,3-propylenediamine (PDA), and NH_2-R_1 amines are exemplified by 2-(diethylamino)ethylamine (DEEA), 3-diethylamino-1-propylamine (DEP), 4-(2-aminoethyl)morpholine (AEM), 4-(3-aminopropyl)morpholine (APM), 2-(dimethylamino)ethylamine (DMEA). For the copolyaspartamides 4a-4e, various molar feed ratios were used.

The aminolysis reactions were performed by allowing in the first step, a given amount of the amine reactant NH_2-R_1 to interact with polysuccinimide (PSI) **2** for a specified time, typically 8-12 h, and in selected molar ratios (x+y)/x, whereupon, in the second step, the mixture was added to a solution with two or three times the stoichiometric amount of NH_2-R_2, and the reaction completed after a specified time, typically 12-19 h at 0ºC and additional hours at ambient temperature. These steps were carried out in DMF in the strict absence of moisture, so as to avoid undesirable hydrolytic ring opening with generation of free carboxylic acid side groups. The polymers were routinely obtained by precipitation, aqueous dialysis (in membranes 12000 molecular mass cut off limit), and ultimately isolated by freeze-drying as water soluble solids possessing inherent viscosities in the range 12-26 mLg^{-1}.

In general, higher viscosities were observed for the polymers derived from aminopropylmorpholine and aminoethylmorpholine (Table3.1), possibly because more stretched-out conformations can be expected from the bulky morpholine ring moiety as a spacer terminal than from the other groups shown.



2 **4**

Polymer	R_1	x/y
4a(90)	NEt_2	9
4a(80)	NEt_2	4
4b(90)	NEt_2	9
4b(80)	NEt_2	4
4c(90)		9
4c(80)		4
4d(90)		9
4d(80)		4
4e(90)	NMe_2	9
4e(80)	NMe_2	4

Scheme 4: Synthesis of Copolyaspartamides 4a to 4e.

Table 3.1: Summary of experimental data of Polyaspartamide carriers 4a to 4e

Polymer designation	Molar ratios [a]							Reaction temperature [c]/°c and time		Yield (%)	η_{inh} [b] (mLg^{-1})	x/y
	PSI 2	DEEA	DEP	AEM	APM	DMEA	PDA	1st Step	2nd Step			
4a(90)	1,00	0.90	-	-	-	-	0.30	RT, 7hours	0°C,15h;5h,RT	47.38	13.69	9
4a(80)	1,00	0.80	-	-	-	-	0.60	RT, 7hours	0°C,15h;5h,RT	55.05	13.73	4
4b(90)	1,00	-	0.90	-	-	-	0.30	RT, 7hours	0°C,15h;5h,RT	58.84	12.85	9
4b(80)	1,00	-	0.80	-	-	-	0.60	RT, 7hours	0°C,15h,5h,RT	57.65	12,93	4
4c(90)	1,00	-	-	0.90	-	-	0.30	RT, 7hours	0°C,15h 5h,RT	61.95	20.00	9
4c(80)	1,00	-	-	0.80	-	-	0.60	RT, 7hours	0°C,15h;5h,RT	62.19	21.04	4
4d(90)	1,00	-	-	-	0.90	-	0.30	RT, 7hours	0°C,15h;5h,RT	52.33	23.00	9
4d(80)	1,00	-	-	-	0.80	-	0.60	RT, 7hours	0°C,15h;5h,RT	59.98	25.27	4
4e(90)	1,00	-	-	-	-	0.90	0.30	RT, 7hours	0°C,15h;5h,RT	65.62	13.62	9
4e(80)	1,00	-	-	-	-	0.80	0.60	RT, 7hours	0°C,15h;5h,RT	55.91	13.64	4

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

[b] At 30,0°C±0.5°C in dist. H_2O; conc.=0.2mg/100mL

[c] RT: room temperature

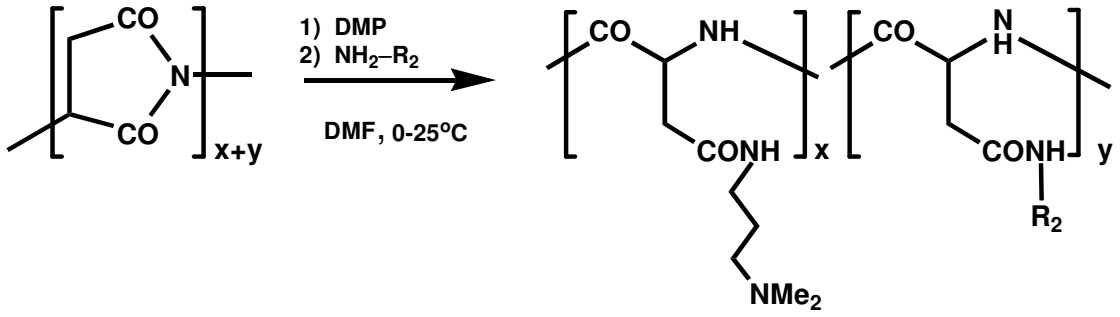
[d] DEEA:2-(diethylamino)ethylamine; DEP:3-diethylamino-1-propylamine; AEM: 4-(2-aminoethyl)morpholine; APM: 4-(3-aminopropyl)morpholine; DMEA: 2-(dimethylamino)ethylamine; PDA: 1,3-propylenediamine

Table 3.2: ^{1}H NMR data for Polyaspartamide carriers 4a to 4e.

Polymer Designation	Assignment	^{1}H NMR		
		Shift range δ(ppm)	Proton count	
			Found	Expected
4a(90)	$CH_2N(CH_2\mathbf{Me})_2$ $CH_2\mathbf{CH_2}CH_2NH_2$ $\mathbf{CH_2CH_2}N(\mathbf{CH_2}Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$ $\mathbf{CH_2}CONH$	1.0-1.1 1.6-1.8 2.6-2.8 3.3-3.4	56 2 79 20	54 2 76 20
4a(80)	$CH_2N(CH_2\mathbf{Me})_2$ $CH_2\mathbf{CH_2}CH_2NH_2$ $\mathbf{CH_2CH_2}N(\mathbf{CH_2}Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$ $\mathbf{CH_2}CONH$	1.0-1.1 1.7-1.8 2.6-2.8 3.3-3.4	24.5 2 37 12	24 2 36 10
4b(90)	$CH_2N(CH_2\mathbf{Me})_2$ $CH_2\mathbf{CH_2}CH_2NEt_2$, $CH_2\mathbf{CH_2}CH_2NH_2$ $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$ $\mathbf{CH_2}CONH$	1.0-1.2 1.7-1.8 2.7-2.8 3.2-3.3	54,11 20 76.47 20	54 20 76 20
4b(80)	$CH_2N(CH_2\mathbf{Me})_2$ $CH_2\mathbf{CH_2}CH_2NEt_2$, $CH_2\mathbf{CH_2}CH_2NH_2$ $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$ $\mathbf{CH_2}CONH$	1.0-1.1 1.6-1.7 2.4-2.8 3.1-3.2	24.8 10 36 10	24 10 36 10
4c(90)	$CH_2\mathbf{CH_2}CH_2NH_2$ $\mathbf{CH_2CH_2}N(\mathbf{CH_2})_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$ $\mathbf{CH_2}CONH$ $\mathbf{CH_2OCH_2}$	1.2-1.3 2.5-2.6 3.3-3.4 3.7-3.8	2 76 21 37	2 76 20 36
4c(80)	$CH_2\mathbf{CH_2}CH_2NH_2$ $\mathbf{CH_2CH_2}N(\mathbf{CH_2})_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$ $\mathbf{CH_2}CONH$ $\mathbf{CH_2OCH_2}$	1.3-1.4 2.5-2.6 3.3-3.4 3.7-3.8	2 36 10.4 16.3	2 36 10 16
4d(90)	$CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$ $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$ $\mathbf{CH_2}CONH$ $\mathbf{CH_2OCH_2}$	1.7-1.8 2.5-2.6 3.2-3.3 3.7-3.8	20 74,59 20 34	20 76 20 36
4d(80)	$CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$ $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$ $\mathbf{CH_2}CONH$ $\mathbf{CH_2OCH_2}$	1.7-1.8 2.5-2.6 3.2-3.3 3.7-3.8	10 36.25 10 16.09	10 36 10 16
4e(90)	$CH_2\mathbf{CH_2}CH_2NH_2$ $CH_2N(\mathbf{Me})_2$ $\mathbf{CH_2CH_2}N(Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$ $\mathbf{CH_2}CONH$	1.7-1.8 2.2-2.3 2.7-2.8 3.3-3.4	2 54 40 20	2 54 40 20
4e(80)	$CH_2\mathbf{CH_2}CH_2NH_2$ $CH_2N(\mathbf{Me})_2$ $\mathbf{CH_2CH_2}N(Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$ $\mathbf{CH_2}CONH$	1.8-1.9 2.3-2.4 2.7-2.8 3.4-3.5	2 23 20 10.15	2 24 20 10

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

[b] Integration error limit ±15%



2 **5**

Polymer	R_2	x/y
5a(90)	NH_2	9
5a(80)	NH_2	4
5a(95)	NH_2	19
5b(90)	NH NH_2	9
5b(95)	NH NH_2	19
5c(90)	O O NH_2	9

Scheme 5: Synthesis of Copolyaspartamides 5a to 5c.

In Scheme (5), NH_2-R_1, is 3-dimethylamino-1-propylamine (DMP), and NH_2-R_2, amine nucleophiles are exemplified by 1,3-propylenediamine (PDA);diethylenetriamine (DET); 2,2′-(ethylenedioxy)-diethylamine (EDDA).

The same procedure as described for copolymers **4a(90), 4a(80), 4b(90), 4b(80),4c(90), 4c(80), 4d(90), 4d(80), 4e(90)** and **4e(80)** was used for the preparation of the copolyaspartamides **5a(90), 5a(80), 5a(95), 5b(90), 5b(95) and 5c(90)** for which various

molar ratios were used and their experimental details and viscosity results are presented in Table3.3, whereas ^{1}H NMR data are compiled in Table 3.4. The tertiary amine-functionalized unit derived from DMP acts as a solubilizing moiety and, in addition, it provides cationic sites that may be used for the "homing" to certain types of cancerous cells that tend to carry a negative surface charge owing to their increased rate of glycolysis.

^{1}H NMR spectra were in agreement with the structures indicated in Scheme (5).

Table 3.3 Summary of experimental data of Polyaspartamide carriers 5a to 5c

Polymer designation	Molar ratios [a]					Reaction temperature [c]/°c and time		Yield (%)	η_{inh} [b] (mLg^{-1})	x/y
	PSI 2	DMP	PDA	DET	EDDA	1st Step	2nd Step			
5a(90)	1,00	0.90	0.30	-	-	RT, 7hrs	0ºC,15h; 5h,RT	67.40	10,31	9
5a(80)	1,00	0.80	0.60	-	-	RT, 7hrs	0ºC,15h; 5h,RT	60.93	11.11	4
5a(95)	1,00	0.95	0.15	-	-	RT, 7hrs	0ºC,15h; 5h,RT	69.99	10.83	19
5b(90)	1,00	0.90	-	0.90	-	RT, 7hrs	0ºC,15h; 5h,RT	46.94	9.01	9
5b(95)	1,00	0.95	-	0.95	-	RT, 7hrs	0ºC,15h; 5h,RT	53.30	8.10	19
5c(90)	1,00	0.90	-	-	0.30	RT, 7hrs	0ºC,15h; 5h,RT	54.99	11.00	9

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

[b] At 30,0ºC±0.5ºC in dist. H_2O; conc.=0.2mg/100mL

[c] RT: room temperature

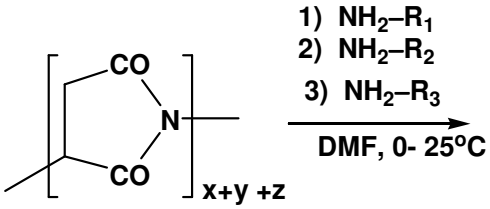
Table 3.4: ^{1}H NMR data for Polyaspartamide carriers 5a to 5c.

Polymer Designation	Assignment	^{1}H NMR		
		Shift range δ(ppm)	Proton count	
			Found	Expected
5a(90)	$CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$ $CH_2CH_2CH_2N(\mathbf{Me})_2$ $\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$ $\mathbf{CH_2}CONH$	1.6-1.7 2.1-2.2 2.3-2.8 3.1-3.2	20 53.3 40 20	20 54 40 20
5a(80)	$CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$ $CH_2CH_2CH_2N(\mathbf{Me})_2$ $\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$ $\mathbf{CH_2}CON$	1.6-1.7 2.1-2.2 2.3-2.8 3.1-3.2	10 23 20 10	10 24 20 10
5a(95)	$CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$ $CH_2CH_2CH_2N(\mathbf{Me})_2$ $\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$ $\mathbf{CH_2}CONH$	1.6-1.7 2.1-2.2 2.3-2.8 3.1-3.2	40 108 78 42	40 114 80 40
5b(90)	$CH_2\mathbf{CH_2}CH_2N(Me)_2$ $CH_2CH_2CH_2N(\mathbf{Me})_2$ $\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$, $\mathbf{CH_2CH_2}NH\mathbf{CH_2CH_2}NH_2$ $\mathbf{CH_2}CONH$	1.6-1.7 2.2-2.3 2.3-2.8 3.2-3.3	18 52 43.6 19	18 54 44 20
5b(95)	$CH_2\mathbf{CH_2}CH_2N(Me)_2$ $CH_2CH_2CH_2N(\mathbf{Me})_2$ $\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$, $\mathbf{CH_2CH_2}NH\mathbf{CH_2CH_2}NH_2$ $\mathbf{CH_2}CONH$	1.6-1.7 2.2-2.3 2.3-2.8 3.2-3.3	38 108.6 81.2 39.8	38 114 84 40
5c(90)	$CH_2\mathbf{CH_2}CH_2N(Me)_2$ $CH_2CH_2CH_2N(\mathbf{Me})_2$ $\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$,$NH\mathbf{CH_2}CH_2$,$OCH_2\mathbf{CH_2}NH_2$ $\mathbf{CH_2}CONH$ $CH_2\mathbf{CH_2}O\mathbf{CH_2CH_2}O\mathbf{CH_2}CH_2NH_2$	1.7-1.8 2.4-2.5 2.5-3.0 3.2-3.3 3.6-3.7	18 57 42 21.6 9	18 54 40 20 8

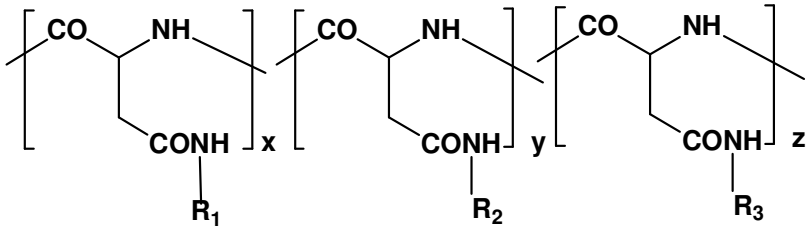
[a] In D_2O, pH 10-11. Chemical shifts, δ (ppm), referenced against internal sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate

[b] Integration error limit ±15%

The ring-opening reaction of polysuccinimide as specifed in the section 3.1.1.2 was used to synthesize copolymers through a stepwise, sequential addition of two or more different amines NH_2-R_1 and NH_2-R_2, in given stoichiometric feed ratios. Scheme (6), Scheme (7) and Scheme (8) are the cases where three different amines NH_2-R_1, NH_2-R_2 and NH_2-R_3 were used where the first is the solubilizing group, the second is the homing group and the third one is the drug-binding site.



2



6

Polymer	R_1	R_2	R_3	x/z	y/z
6a	NEt_2	NMe_2	NH_2	5	4
6b	NEt_2	NMe_2	NH_2	5	4
6c	N O	NMe_2	NH_2	5	4

Scheme 6: Synthesis of Copolyaspartamides 6a to 6c.

In Scheme (6), NH_2-R_3 amine nucleophile is 1,3-propylenediamine (PDA), NH_2-R_2 amine is 3-dimethylamino-1-propylamine (DMP), and NH_2-R_1 amine is exemplified by 3-(diethylamino)-1-propylamine (DEP), 2-(diethylamino)ethylamine (DEEA) and 4-(3-aminopropyl) morpholine (APM).

The aminolysis reactions were performed by allowing in the first step, a given amount of the amine reactant NH_2-R_1 the less reactive, to interact with polysuccinimide (PSI) **2** for a specified time, typically 4-8 h, and in selected molar ratios (x+y+z,)/x, whereupon, in the second step, NH_2-R2 (stoichiometric amount) the more reactive, was added to the first solution for a specified time, typically 8-12 h, and in selected molar ratios (x+y+z)/y; Finally, in the third step, the mixture was added to a solution with two or three times the stoichiometric amount of NH_2-R_3, and the reaction completed after a specified time, typically 12-19 h at 0ºC and additional hours at ambient temperature. These steps were carried out in DMF as solvent.

The polymers were routinely obtained by precipitation, aqueous dialysis (in membranes 12000 molecular mass cut off limit), and ultimately isolated by freeze-drying as water soluble solids possessing inherent viscosities in the range 12-21 mLg^{-1}.

Their experimental details and viscosity results are presented in Table3.5, whereas ^1H NMR data are compiled in Table 3.6.

^1H NMR spectra were in agreement with the structures indicated in Scheme (6).

Table 3.5 Summary of experimental data of Polyaspartamide carriers 6a to 6c

Polymer designation	Molar ratios [a]						Reaction temperature [c]/ºc and time			Yield (%)	η_{inh} [b] (mLg^{-1})	x/z	y/z
	PSI 2	DEP	DEEA	APM	DMP	PDA	1st Step	2nd Step	3rd Step				
6a	1.00	0.50	-	-	0.40	0.30	RT, 4hrs	RT, 8hrs	0ºC,15h;5h,RT	60.11	12.38	5	4
6b	1.00	-	0.50	-	0.40	0.30	RT, 4hrs	RT, 8hrs	0ºC,15h;5h,RT	63.63	13.04	5	4
6c	1.00	-	-	0.50	0.40	0.30	RT, 4hrs	RT, 8hrs	0ºC,15h;5h,RT	66.29	20.13	5	4

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

[b] At 30,0ºC±0.5ºC in dist. H_2O; conc.=0.2mg/100mL

[c] RT: room temperature

[d] DEEA:2-(diethylamino)ethylamine;DEP:3-(diethylamino)-1-propylamine;APM:4-(3-aminopropyl)morpholine; 3-(dimethylamino)-propylamine (DMP); PDA: 1,3-propylenediamine

Table 3.6: ^{1}H NMR data for Polyaspartamide carriers 6a to 6c.

Polymer Designation	Assignment	^{1}H NMR		
		Shift range δ(ppm)	Proton count	
			Found	Expected
6a	$CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$	1.2-1.3	30	30
	$CH_2\mathbf{CH_2}CH_2N(Me)_2$, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2Me)_2$	1.8-1.9	20	20
	$CH_2CH_2CH_2N(\mathbf{Me})_2$	2.4-2.5	24.5	24
	$\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$	2.6-3.1	60	60
	$\mathbf{CH_2}CONH$	3.2-3.3	20	20
6b	$CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$	1.0-1.1	30	30
	$CH_2\mathbf{CH_2}CH_2N(Me)_2$, $CH_2\mathbf{CH_2}CH_2NH_2$	1.6-1.7	10	10
	$CH_2CH_2CH_2N(\mathbf{Me})_2$	2.1-2.4	24	24
	$\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2CH_2}N(\mathbf{CH_2}Me)_2$	2.5-2.8	61.5	60
	$\mathbf{CH_2}CONH$	3.2-3.4	20	20
6c	$CH_2CH_2CH_2N(\mathbf{Me})_2$	1.7-1.8	24	24
	$CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$	2.3-2.4	22	20
	$\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$	2.5-2.8	62	60
	$\mathbf{CH_2}CONH$	3.2.3.3	22	20
	$\mathbf{CH_2OCH_2}$	3.7-3.8	19	20

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

[b] Integration error limit ±15%

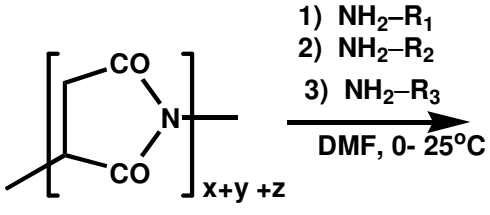
Copolyaspartamides bearing both tertiary amine and hydroxyl- or methoxyl- terminated side chains.

The following series of copolymers (Schemes7a-7c) were synthesized under experimental conditions similar to those of Scheme 6.

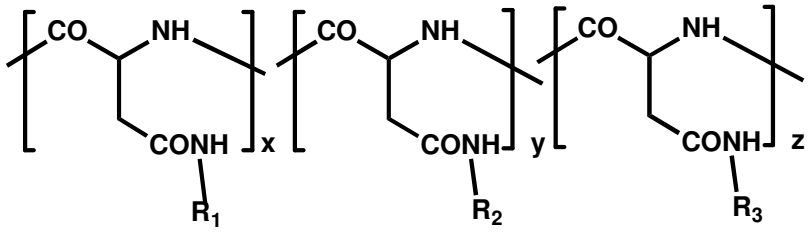
In Scheme (7), NH_2-R_3 amine nucleophile is 1,3-propylenediamine (PDA), NH_2-R_2 amine is 2-(2-Aminoethoxy)ethanol(AEE) and NH_2-R_1 amine is exemplified by 3-(diethylamino)-1-propylamine (DEP), 2-(diethylamino)ethylamine (DEEA) and 4-(3-aminopropyl) morpholine (APM).

Their experimental details and viscosity results are presented in Table3.7, whereas ^{1}H NMR data are compiled in Table 3.8.

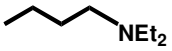
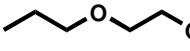
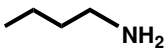
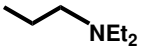
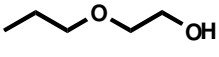
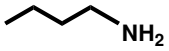
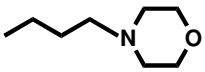
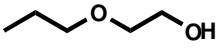
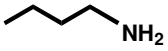
^{1}H NMR spectra were in agreement with the structures indicated in Scheme (7).



2



7

Polymer	R_1	R_2	R_3	x/z	y/z
7a				5	4
7b				5	4
7c				5	4

Scheme 7: Synthesis of Copolyaspartamide 7a to 7c.

Table 3.7 Summary of experimental data of Polyaspartamide carriers 7a to 7c

Polymer designation	Molar ratios [a]						Reaction temperature [c]/°c and time			Yield (%)	η_{inh} [b] (mLg^{-1})	x/z	y/z
	PSI 2	DEP	DEEA	APM	AEE	PDA	1st Step	2nd Step	3rd Step				
7a	1.00	0.50	-	-	0.40	0.30	RT, 4hrs	RT, 8h	0°C,15h;5h,RT	61.63	12.51	5	4
7b	1.00	-	0.50	-	0.40	0.30	RT, 4hrs	RT, 8h	0°C,15h;5h,RT	61.35	13.87	5	4
7c	1.00	-	-	0.50	0.40	0.30	RT, 4hrs	RT, 8h	0°C,15h;5h,RT	63.25	20.46	5	4

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

[b] At 30,0°C±0.5°C in dist. H_2O; conc.=0.2mg/100mL

[c] RT: room temperature

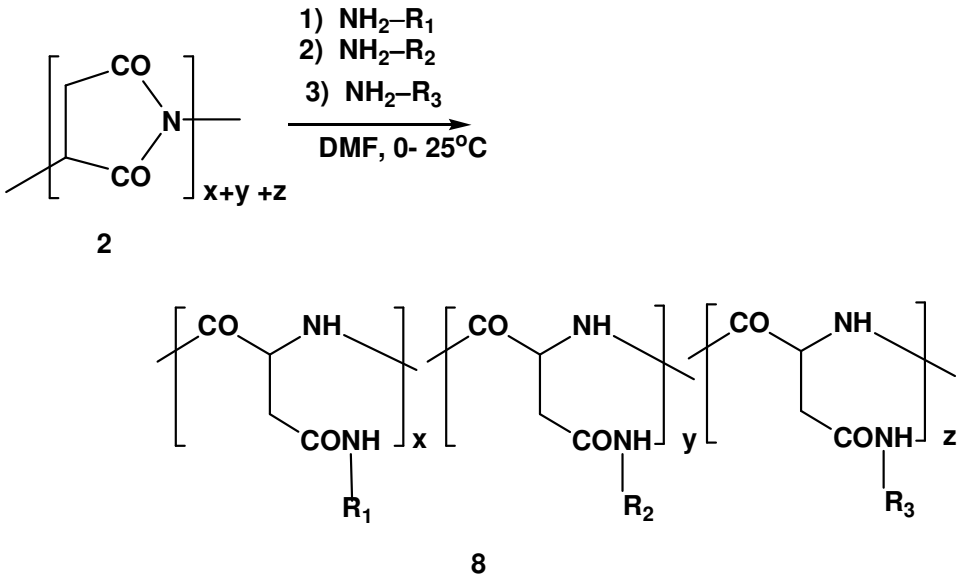
[d] DEEA:2-(diethylamino)ethylamine;DEP:3-(diethylamino)-1-propylamine;APM:4-(3-aminopropyl)morpholine;2-(2-Aminoethoxy)ethanol(AEE);PDA:1,3-propylenediamine.

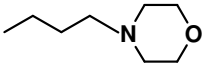
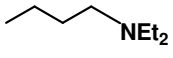
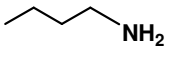
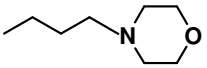
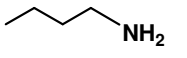
Table 3.8: ^{1}H NMR data for Polyaspartamide carriers 7a to 7c.

Polymer Designation	Assignment	^{1}H NMR		
		Shift range δ(ppm)	Proton count	
			Found	Expected
7a	$CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$	1.0-1.1	31.36	30
	$CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2Me)_2$	1.7-1.8	12	12
	$NH\mathbf{CH_2}CH_2OCH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$	2.6-2.7	53	52
	$\mathbf{CH_2}CONH$	3.2-3.5	21.68	20
	$CH_2\mathbf{CH_2}O\mathbf{CH_2}\mathbf{CH_2}OH$	3.6-3.8	26.1	24
7b	$CH_2CH_2N(CH_2\mathbf{Me})_2$	1.0-1.1	28.7	30
	$CH_2\mathbf{CH_2}CH_2NH_2$	1.6-1.7	2	2
	$NH\mathbf{CH_2}CH_2OCH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$	2.6-2.7	49.9	52
	$\mathbf{CH_2}CONH$	3.2-3.5	20.2	20
	$CH_2\mathbf{CH_2}O\mathbf{CH_2}\mathbf{CH_2}OH$	3.6-3.8	26.8	24
7c	$CH_2\mathbf{CH_2}CH_2N(CH_2)_2$,$CH_2\mathbf{CH_2}CH_2NH_2$ $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$,	1.7-1.8	12	12
	$NH\mathbf{CH_2}CH_2OCH_2$	2.5-2.6	51.6	52
	$\mathbf{CH_2}CONH$	3.2-3.5	21.8	20
	$CH_2\mathbf{CH_2}O\mathbf{CH_2}\mathbf{CH_2}OH$, $\mathbf{CH_2}O\mathbf{CH_2}$	3.6-3.8	46.5	44

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

[b] Integration error limit ±15%



Polymer	R_1	R_2	R_3	x/z	y/z
8a				5	4
8b				5	4

Scheme 8: Synthesis of Copolyaspartamides 8a to 8b.

In Scheme 8, NH_2-R_1 amine is 4-(3-aminopropyl) morpholine (APM), NH_2-R_3 amine nucleophile is 1,3-propylenediamine (PDA) and NH_2-R_2 amine is exemplified by 3-(diethylamino)-1-propylamine (DEP) and ethanolamine (EA). Different molar feed ratios of the various constituent units were used and are tabulated accordingly. Tables 3.9 and 3.10 summarize the experimental conditions and ^{1}H NMR data, which were utilized for the compositional characterization of the polymers 8a and 8b.

Table 3.9 Summary of experimental data of Polyaspartamide carriers 8a to 8b

Polymer designation	Molar ratios [a]					Reaction temperature [c]/ºc and time			Yield (%)	η_{inh} [b] (mLg^{-1})	x/z	y/z
	PSI 2	APM	DEP	EA	PDA	1st Step	2nd Step	3rd Step				
8a	1.00	0.50	0.40	-	0.30	RT, 4h	RT, 8h	0ºC,15h;5h,RT	67.76	20.33	5	4
8b	1.00	0.50	-	0.40	0.30	RT, 4h	RT, 8h	0ºC,15h;5h,RT	72.70	20.49	5	4

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

[b] At 30,0ºC±0.5ºC in dist. H_2O; conc.=0.2mg/100mL

[c] RT: room temperature

[d] Ethanolamine(EA);DEP:3-(diethylamino)-1-propylamine;APM:4-(3-aminopropyl)morpholine;PDA:1,3-propylenediamine

Table 3.10: ^{1}H NMR data for Polyaspartamide carriers 8a and 8b.

Polymer Designation	Assignment	^{1}H NMR		
		Shift range δ(ppm)	Proton count	
			Found	Expected
8a	$CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$	1.0-1.1	24	24
	$CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2Me)_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$	1.6-1.8	21.8	20
	$\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$	2.4-2.6	76.3	76
	$\mathbf{CH_2}CONH$	3.2-3.3	21.8	20
	$\mathbf{CH_2O CH_2}$	3.7-3.8	21.8	20
8b	$CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$ $NH\mathbf{CH_2}CH_2OCH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$,	1.6-1.8	12	1 2
	$\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$	2.5-2.8	48	52
	$\mathbf{CH_2}CONH$	3.2-3.4	20.2	20
	$\mathbf{CH_2O CH_2}$, $CH_2\mathbf{CH_2}OH$	3.6-3.8	27.75	28

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

[b] Integration error limit ±15%

3.1.2 Other polyamides

These polymers, prepared by ester-amine polycondensation were synthesized[75] previously as useful carrier candidates for antineoplastic drug conjugation.

As part of the project to synthesize water-soluble macromolecules as carriers of medicinal agents, aliphatic polyamides of this kind were obtained by based-catalyzed polycondensation of aliphatic diesters with diamines. The reactions are conducted in the presence of anhydrous sodium carbonate at temperatures ranging from ambient to 65ºC, initially in the undiluted state. The addition of aprotic solvent such as DMSO at a later stage serves to maintain sufficiently low viscosity for proper homogenization[66].

The diester monomer used to produce the polyamides *via* ester-amine base-catalyzed polycondensation reaction was diethyl L-tartrate (TART). The diamine monomers used

in various combinations were: 4, 7, 10-trioxa-1, 13-tridecanediamine (TRIA); 2,2′-(ethylenedioxy)-diethylamine (EDDA); diethylenetriamine (DET); 1,2-Bis(3-aminopropylamino)ethane (BAP).

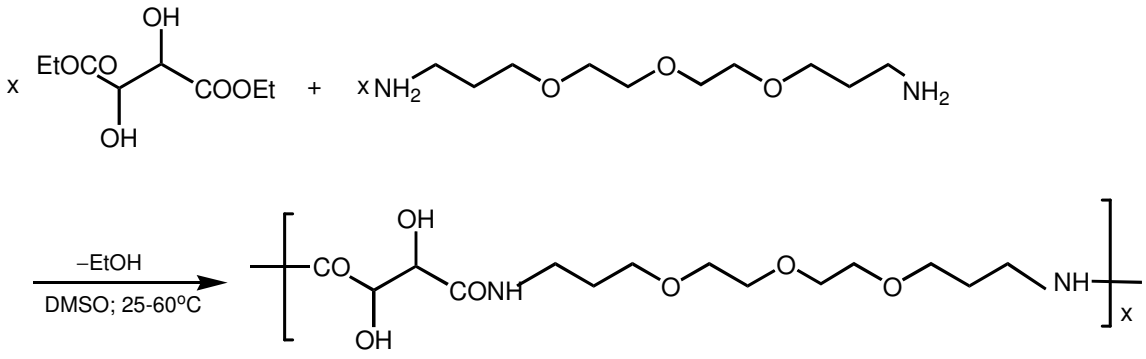
In the present dissertation, equimolar quantities of diethyl L-tartrate and 4,7,10-trioxa-1,13-tridecanediamine were copolymerized to form the polymer 9a (Scheme 9), and the combination of diethyl L-tartrate and 2,2′-(ethylenedioxy)-diethylamine (EDDA) as diamine monomer produced the straight-chain polyamide 9b (Scheme 10).

Polyamides 9c was obtained by mixing TART-EDDA-BAP with 2:1:1 as mole ratio and 9d= TART-EDDA-DET with 3:1:2 as mole ratio.

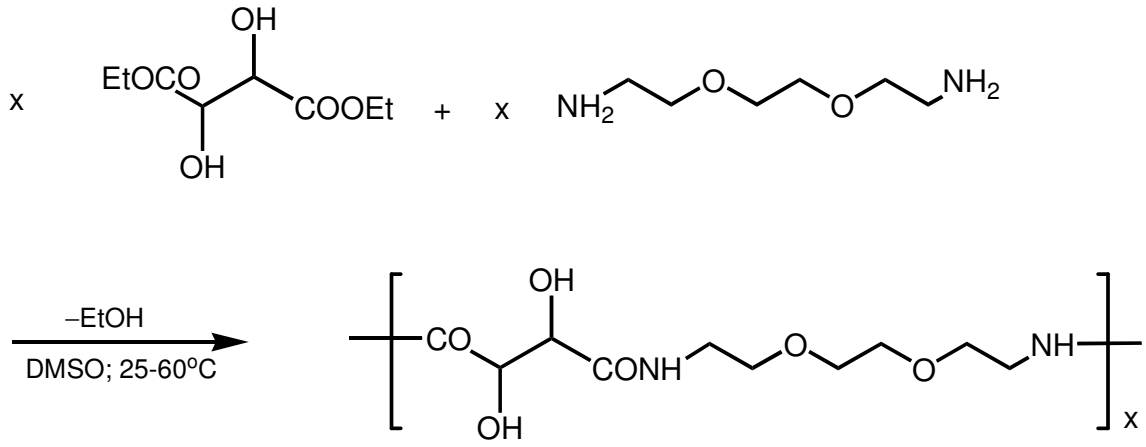
For both polyamides, initially the neat reactant mixture was stirred for 1 day at room temperature, but the mixture became too viscous for efficient stirring, at which point minor dilution with dimethylsulfoxide (DMSO) for 9-16 days at 55-65ºC was carried out.

The use of diethyl L-tartrate leads to polyamides possessing hydroxyl groups used for drug anchoring. The water-soluble polyamides were crudely fractionated by dialysis (12000 molecular mass cut off) against distilled water and collected by freeze-drying in a yield range of 42 to 71%. The **CH** proton resonance of the tartrate segment close to 4.4 ppm was important for the characterization of these polyamides. The low yield has been accepted in the investigation as the price for the realization of linear polyamide structures in accordance with the compositional expectations, a vital requirement for the proper functioning of the polymer as drug carrier.

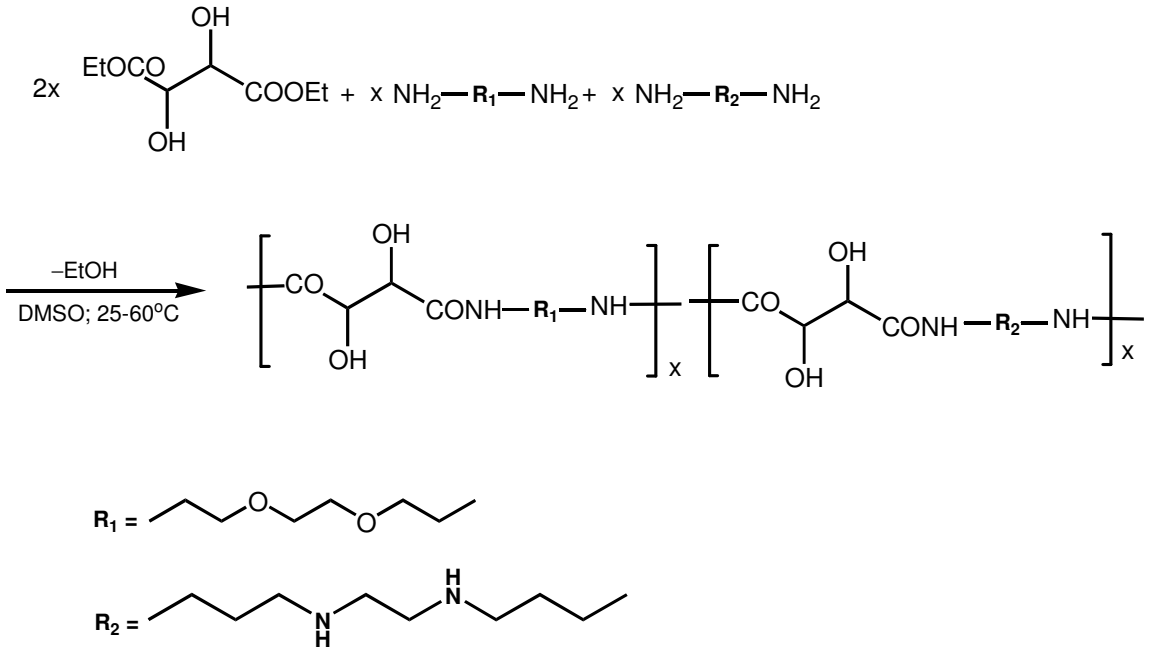
The water soluble carrier possessed inherent viscosities (H_2O) of 10.0-13.0mLg^{-1}. Individual reactant combinations, mole ratio, as well as reaction conditions and yields are shown in Table 3.11, and spectroscopic data, as well as chemical shifts are presented in the table 3.12. ^1H NMR spectroscopy confirmed the composition of polyamide structures.



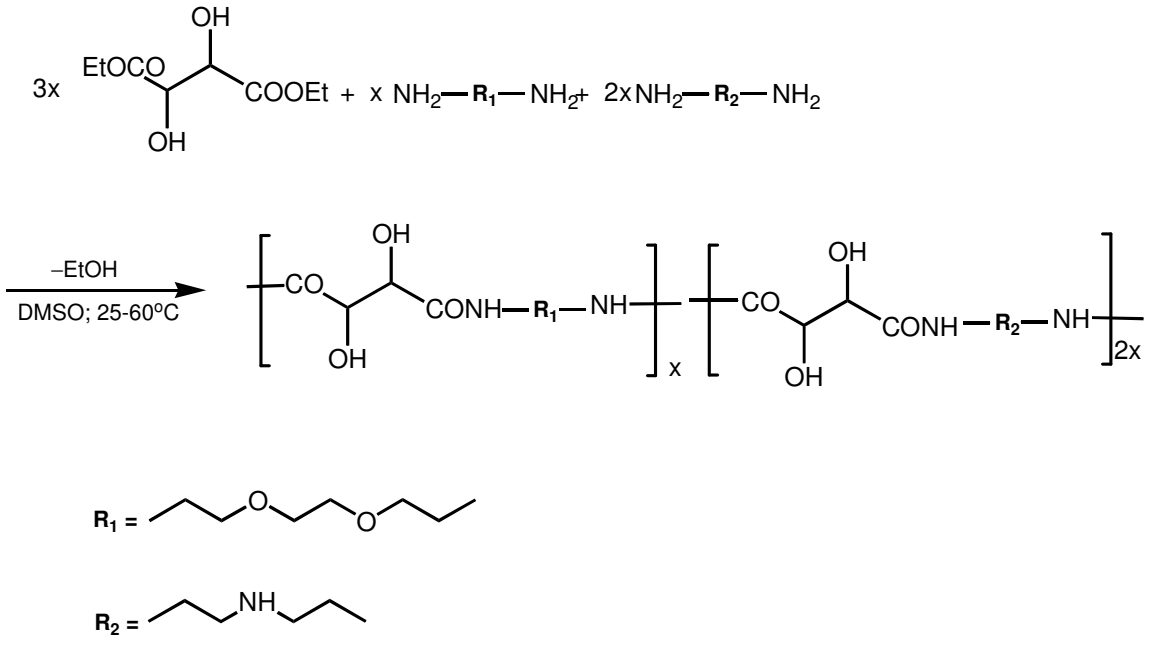
Scheme 9: Synthesis of Copolyamide 9a



Scheme 10: Synthesis of Copolyamide 9b



Scheme 11: Synthesis of Copolyamide 9c



Scheme 12: Synthesis of Copolyamide 9d

Table 3.11: Synthesis of Polyamides 9a-9d.

Reactions in Feed (Mol %)					DMSO[a] (mL)	Reaction conditions time, temperature	Polyamide	
TART	TRIA	EDDA	BAP	DET			Yield %[b]	Designation
50	50	--	--	--	6	1day,RT; 15days, 60ºC	70.28	9a
50	--	50	--	--	6	1day,RT; 15days, 60ºC	42.32	9b
50	--	25	25	--	6	1day,RT; 15days, 60ºC	58.13	9c
75	--	25	--	50	8	1day,RT; 15days, 60ºC	58.89	9d

[a] Solvent for every 10 mmoles of diester

[b] Fraction obtained after dialysis in 12000-14000 molecular mass cut-off tubing

Table 3.12: ^{1}H NMR data for Polyamides 9a-9d.

Polymer Designation	η_{inh} (mLg^{-1})[b]	Number of Protons counted[c] (expected) Chemical shifts (δ ppm)				
		δ4.6-4.4[d]	δ 3.7-3.5	δ 3.5-3.3	δ 2.7-2.5	δ 1.9-1.7
9a	10.94	1.93(2)	12.4(12)	3.95(4)	--	4(4)
9b	10.40	2(2)	8.5(8)	4(4)	--	--
9c	11.53	4(4)	9.7(8)	9.3(8)	10(8)	3(4)
9d	12.87	4.6(4)	8(6)	11.3(12)	6(6)	--

[a]In D_2O, pH 10, chemical shifts, δ/ppm, referenced against internal sodium 3-(trimethylsilyl)-2,2,3,3-d_4 propionate
[b]At 30.0°C±5°C in distilled H_2O; concentration= 0.2/100mL
[c]Integration error limits±12%.
[d]Proton assignments, 4.5-4.3 ppm: C**H**-OH; 4.1-3.9ppm: OCH_2-CO; 3.8-3.5ppm:O- CH_2- CH_2O, CH_2- CH_2- CH_2-O;3.4-3.2ppm: CONH- $\mathbf{CH_2}$; 2.8-2.5ppm: $\mathbf{CH_2}$-NH- $\mathbf{CH_2}$;
1.9-1.6ppm: CH_2- $\mathbf{CH_2}$- CH_2.

3.1.3 Polyamidoamines

Polyamidoamines (PAAs) are synthetic polymers characterized by the presence of amido and tertiary amino groups regularly arranged along the macromolecular chain. The first example of PAAs was reported in the patent dating to 1956[76], but the systematic study of PAAs as a distinct class of polymers was started in the late 1960s[77]. The potential for medical applications was discovered by Ferruti in collaboration with Marchisio in the early 1970[78, 79, 80, 81] and now widely recognized. The addition of amine nucleophiles across an activated double bond (Michael addition) was utilized by Ferruti and co-workers for the preparation of polymeric compounds possessing both amide and tertiary amine functions in the chain. Using equimolar quantities of bisacrylamides such as 1,4-diacryloylpiperazine or ethylenebisacrylamide, and secondary diamines as comonomers they prepared numerous linear macromolecules in which a pair of amide and tertiary amine functions alternate along the polymeric backbone[82, 83].

The reactions were preferably done in aqueous solutions at room temperature over several days, and the resulting, water soluble polyamidoamines possessed inherent viscosities of 10-80 mLg^{-1}. Using bisacrylamides and primary amines as comonomers, Ferruti's group obtained polymers characterized by the presence of only one tertiary amine segment for each pair of amide groups[84]. The amino group in this polyaddition process reacts dysfunctionally. The first step involves addition across one of the vinyl groups, and the resulting amino group continues to react in the subsequent step with another vinylic double bond, thereby transforming into a tertiary amine segment. Some of these polymers possessed heparin-complexing[85] or antimicrobial[86] properties, and several types showed activity as inhibitors of secondary (metastatic) growth of certain marine tumor lines[74], suggesting interesting applications in the biomedical field.

In the ongoing investigation of polymeric carrier systems possessing drug anchoring functions, this group of polymers which falls into the category of polyamidoamines, was utilized previously in the polymer research laboratory of this University[87, 88]. It is characterized by the presence of both amide links and tertiary amino groups, in the

backbone, and containing intrachain-type secondary amino groups, suitable for drug and side chain attachment.

3.1.3.1 Polyaddition reaction of methylenebisacrylamide with primary monoamines and primary diamines

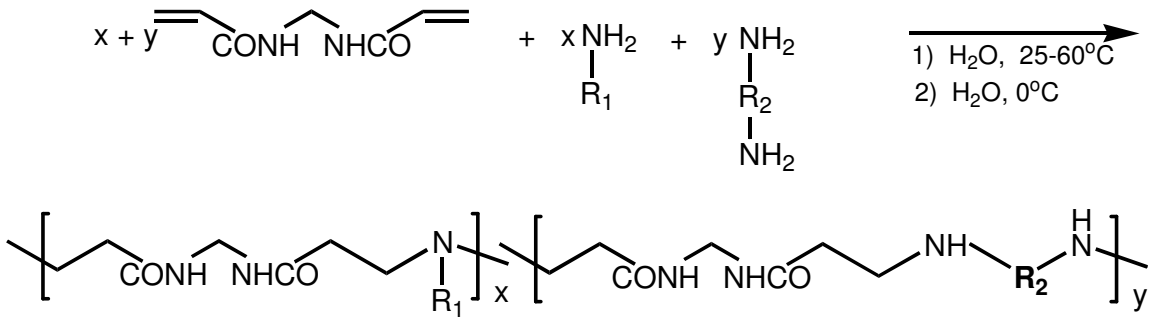
The (Michael addition) nucleophilic addition of amines across an activated double bond was utilized to prepare water-soluble polymers possessing both amide and tertiary amine functional groups in the chain. This was accomplished by allowing monomers to react in an aqueous medium under mild conditions. The synthesized polymers were purified by dialysis operations in membrane tubing with molecular-mass cut-off 12000-14000 and then isolated by freeze-drying. Polymers were then characterized by ^{1}H NMR.

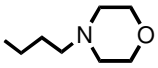
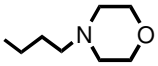
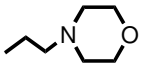
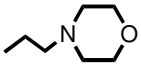
The polymers synthesized in scheme 13 were of types 10(90), 10(80), 11(90), 11(80), 12(90), 12(80), 13(90), 13(80), 14(90) and 14(80), where the comonomer responsible for drug binding purpose is 1,3-propylenediamine (PDA) and the other comonomers serving as solubilizing groups are varied. The principal monomer used was methylenebisacrylamide (MBA) and the comonomers (solubilizer) of varied ratio, included 2-(diethylamino)ethylamine (DEEA), 3-(diethylamino)-propylamine (DEP), 2-(dimethylamino)ethylamine (DMEA), 4-(2-aminoethyl)morpholine (AEM), 4-(3-aminopropyl)morpholine (APM).

Polymers synthesized in scheme 13 were strictly of the type where the solubilizing group is not incorporated in the backbone of the polymer but is pending or attached to the chain as side groups, while the drug binding group is incorporated. The chemical shifts provided by the ^{1}H NMR spectra within the experimental integration error limits (±15%), gave the correct area ratios for various protons according to the diversity of chemical environments in each polymer. However the yields obtained were low and some were drastically low and this appeared to be a general trend observed for the polyamidoamine polymers synthesized in this project. This suggests competing depolymerization reactions through hydrolysis or transamidation, probably due hydrolytic cleavage of –CONH-CH_2-

NHCO- segments of polymer backbone. This is expected because at 50°C propagation is favored, yet degradation is also favored. The washing of the product obtained is critical before dialysis is performed, in order to remove any unreacted monomer. Reaction conditions play an important role in the success of chain propagation, therefore successful polymerization of primary amines is achieved through the use of highly concentrated solutions and elevated reaction temperature (50°C). In contrast the use of diamines or tertiary amines for chain propagation, require, low temperatures and dilute conditions in order to avoid cross-linking, which would render the polymer insoluble in water or even result in premature chain termination producing polymers not suitable for drug anchoring.

Experiments were performed in aqueous solutions with MBA:Solubiliser:PDA monomer feed ratio of 10:9:1 or 10:8:1, with reaction periods of 1 day at room temperature and 2 days at 55°C and 1day in ice bath. The polyamidoamine 10(90), 10(80), 11(90), 11(80), 12(90), 12(80), 13(90), 13(80), 14(90) and 14(80) products were isolated after volume reduction, acidification and dialysis in 12000 molecular cut-off membranes, freeze-drying and routine post drying, as water soluble, hygroscopic solids in yields of 8-45% and viscosities 20-26 mLg^{-1}.



Polymer	R_1	R_2	x/y
10(90)	NEt_2		9
10(80)	NEt_2		4
11(90)	NEt_2		9
11(80)	NEt_2		4
12(90)	NMe_2		9
12(80)	NMe_2		4
13(90)			9
13(80)			4
14(90)			9
14(80)			4

Scheme 13: Synthesis of Polyamidoamines 10-14.

Table 3.13: Preparative data of Polyamidoamines 10 to 14.

Polymer designation	Molar ratios [a]							Reaction temperature/°C and time			Yield (%)	η_{inh} [b] (mLg^{-1})	x/y
	MBA[c]	DEEA	DEP	DMEA	AEM	APM	PDA	1st Step	2nd Step	3rd Step			
10(90)	1,00	-	0.90	-	-	-	0.1	RT, 1 d	60; 48h	0; 24h	18.52	20.07	9
10(80)	1,00	-	0.80	-	-	-	0.2	RT, 1d	60; 48h	0; 24h	16.65	20.21	4
11(90)	1,00	0.90	-	-	-	-	0.1	RT, 1 d	60; 48h	0; 24h	17.67	22.97	9
11(80)	1,00	0.80	-	-	-	-	0.2	RT, 1 d	60; 48h	0; 24h	20.60	22.58	4
12(90)	1,00	-	-	0.90	-	-	0.1	RT, 1 d	60; 48h	0; 24h	18.56	23.39	9
12(80)	1,00	-	-	0.80	-	-	0.2	RT, 1 d	60; 48h	0; 24h	16.41	23.81	4
13(90)	1,00	-	-	-	-	0.90	0.1	RT, 1 d	60; 48h	0; 24h	22.94	23.98	9
13(80)	1,00	-	-	-	-	0.80	0.2	RT, 1 d	60; 48h	0; 24h	19.49	24.13	4
14(90)	1,00	-	-	-	0.90	-	0.1	RT, 1 d	60; 48h	0; 24h	25.16	25.39	9
14(80)	1,00	-	-	-	0.80	-	0.2	RT, 1 d	60; 48h	0; 24h	28.25	25.53	4

[a] Base molar for **MBA**

[b] At 30,0ºC±0.5ºC in dist. H_2O; conc.=0.2mg/100mL

[c] MBA: Methylenebisacrylamide

Table 3.14: ^{1}H NMR data for Polyamidoamines 1a to 10a.

Polymer Designation	Assignment	^{1}H NMR		
		Shift range δ(ppm)	Proton count	
			Found	Expected
10(90)	$CH_2N(CH_2\mathbf{Me})_2$	1.2-1.3	54.7	54
	$CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2NEt_2$	1.7-1.8	20	20
	$CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$	2.4-2.5	40	40
	$\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$	2.7-2.8	116	116
	$CONH\mathbf{CH_2}NHCO$	4.5-4.6	20	20
10(80)	$CH_2N(CH_2\mathbf{Me})_2$	1.1-1.2	24,7	24
	$CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2NEt_2$	1.7-1.8	10	10
	$CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$	2.4-2.5	20	20
	$\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$	2.8-2.9	56	56
	$CONH\mathbf{CH_2}NHCO$	4.5-4.6	10	10
11(90)	$CH_2N(CH_2\mathbf{Me})_2$	1.2-1.3	57	54
	$CH_2\mathbf{CH_2}CH_2NH_2$	1.6-1.7	2	2
	$CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$	2.5-2.6	40	40
	$\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$, $\mathbf{CH_2}\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$	2.7-2.8	121.4	118
	$CONH\mathbf{CH_2}NHCO$	4.5-4.6	20	20
11(80)	$CH_2N(CH_2\mathbf{Me})_2$	1.2-1.3	25	24
	$CH_2\mathbf{CH_2}CH_2NH_2$	1.6-1.7	2	2
	$CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$	2.4-2.5	20	20
	$\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$, $\mathbf{CH_2}\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$	2.8-2.9	56,25	56
	$CONH\mathbf{CH_2}NHCO$	4.5-4.6	10	10
12(90)	$CH_2\mathbf{CH_2}CH_2NH_2$	1.6-1.7	2	2
	$CH_2N\mathbf{Me}_2$	2.2-2.3	54	54
	$\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$, $\mathbf{CH_2}\mathbf{CH_2}NMe_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$	2.4-2.6	78	80
	$CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$	2.8-2.9	40	40
	$CONH\mathbf{CH_2}NHCO$	4.5-4.6	20	20
12(80)	$CH_2\mathbf{CH_2}CH_2NH_2$	1.8-1.9	2	2
	$CH_2N\mathbf{Me}_2$	2.4-2.5	24	24
	$\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$, $\mathbf{CH_2}\mathbf{CH_2}NMe_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$	2.6-2.9	58.72	60
	$CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$	2.9-3.0	24	24
	$CONH\mathbf{CH_2}NHCO$	4.5-4.6	10,4	10

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

[b] Integration error limit ±15%

Table 3.14 continued

Polymer Designation	Assignment	^{1}H NMR		
		Shift range δ(ppm)	Proton count	
			Found	Expected
13(90)	$CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N$	1.8-1.9	20	20
	$\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$,			
	$\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$	2.4-2.9	120	116
	$CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$	2.9-3.0	40	40
	$\mathbf{CH_2}O\mathbf{CH_2}$	3.8-3.9	36	36
	$CONH\mathbf{CH_2}NHCO$	4.5-4.6	20	20
13(80)	$CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N$	1.7-1.8	10	10
	$\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$,			
	$\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$	2.4-2.9	52	56
	$CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$	2.9-3.0	20	20
	$\mathbf{CH_2}O\mathbf{CH_2}$	3.8-3.9	15	16
	$CONH\mathbf{CH_2}NHCO$	4.5-4.6	10	10
14(90)	$CH_2\mathbf{CH_2}CH_2NH_2$	1.7-1.8	2	2
	$\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$,			
	$\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$	2.4-2.9	120	116
	$CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$	2.9-3.0	42	40
	$\mathbf{CH_2}O\mathbf{CH_2}$	3.8-3.9	40	36
	$CONH\mathbf{CH_2}NHCO$	4.5-4.6	21.5	20
14(80)	$CH_2\mathbf{CH_2}CH_2NH_2$	1.7-1.8	2	2
	$\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$,			
	$\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$	2.4-2.9	64	56
	$CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$	2.9-3.0	21.5	20
	$\mathbf{CH_2}O\mathbf{CH_2}$	3.8-3.9	19	16
	$CONH\mathbf{CH_2}NHCO$	4.5-4.6	11	10

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

[b] Integration error limit ±15%

3.2 Polymer-drug conjugation

The polymer-drug conjugates, synthesized in accordance with defined biomedical prerequisites, are intended to increase the therapeutic index of the drug by facilitating endocytotic cell entry of the conjugate and making more drug molecules available at the diseased sites while reducing systemic drug exposure as discussed in Chapter 2, Section 2.3. Methotrexate and Ferrocenylbutanoic acid incorporated into the water-soluble and biodegradable macromolecules via the formation of biofissionable amide groups, as well as carrier bound Platinum, were investigated in this dissertation.

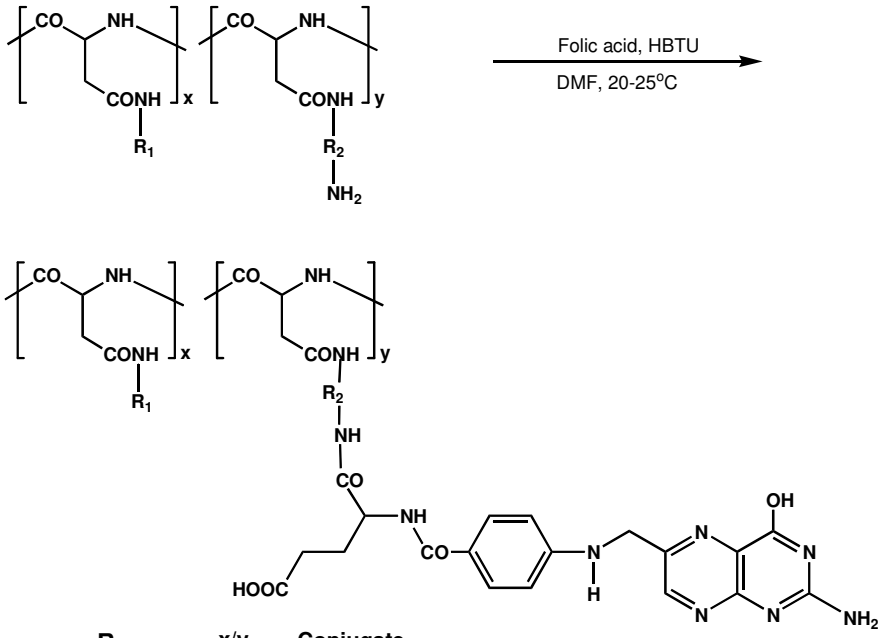
3.2.1 Polymer-Methotrexate conjugation

The literature provides numerous examples of MTX anchoring, which involves the covalent reversible attachment of the carrier through amide bond formation with one of the two carboxyl groups of the drug. Proteinaceous carriers were used in the coupling process, and these reactions were conducted in anhydrous media such as N,N-dimethylformamide (DMF). The coupling was generally brought about through the method involving the direct acid-amine coupling, mediated by HBTU coupling agent, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium fluorophosphate. In the present work, the coupling reactions were performed by treatment of the carrier with MTX and HBTU for 3.5 hours at ambient temperature in DMF solution. MTX: NH_2 molar reactant ratios typically employed were 1.2 or 1.3. Due to high cost of MTX, folic acid was first used to get optimal conditions for conjugation. The choice of folic acid was motivated by its relative cheap cost and its structural resemblance to that of MTX (See figure 3.2 below). For this reason, MTX has been attached only to few carriers in this dissertation.

3.2.1.1 Polymer-Folic acid conjugation

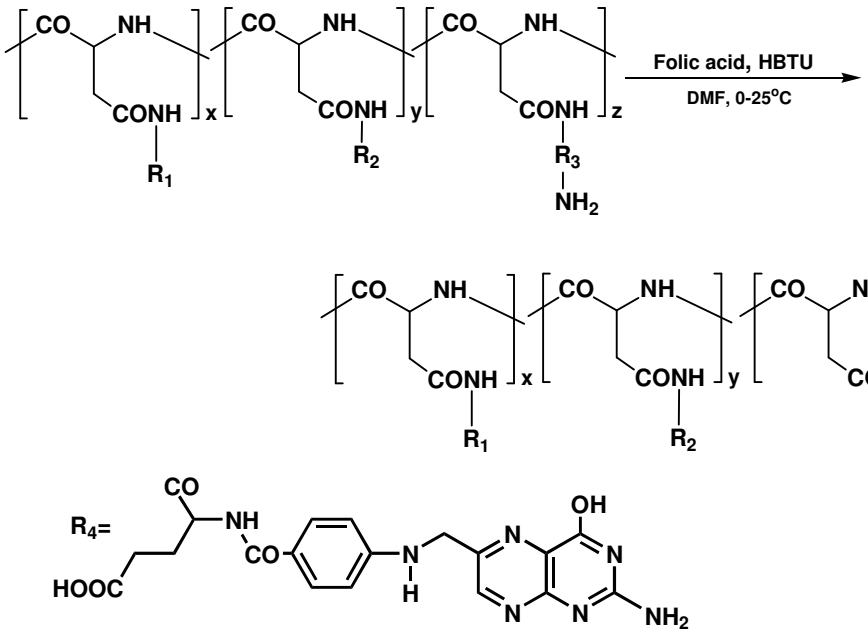
The carriers 4a(90), 4b(90), 4c(90), 4d(90), 5a(90), 6a, 6b, 6c, 7a, 7c, 8a and 8b were chosen for anchoring purpose with Folic acid. These carriers were treated with folic acid and HBTU for 3.5 hours at room temperature in DMF solution. The molar ratio, folic acid: carrier was 1.2; the polymer-folic acid conjugates, after precipitation, were isolated upon centrifugation, and subjected to size exclusion chromatography with Sephadex gel

G-25. The gel has a molecular weight cut-off limit in the 1000-5000 range. Thus, the unreacted drug and by-products were removed by this process. The resulting eluates were ultimately subjected to staged aqueous dialysis in 12000 cut-off membrane tubing under carefully controlled pH conditions. The aim was to remove lower-molecular-weight products that are inadequate for biomedical applications. The ultimate product yields typically ranged from 46 to 81%. As described above, the amidation reactions are depicted in Schemes 14 and 15. Tables 3.15 and 3.16 summarize the experimental conditions and ^{1}H NMR data, which were utilized for the compositional characterization of the conjugates 4a(90)-FA to 8b-FA.



Carrier	R_1	R_2	x/y	Conjugate
4a(90)	NEt_2		9	4a(90)-FA
4b(90)	NEt_2		9	4b(90)-FA
5a(90)	Me_2		9	5a(90)-FA
5a(95)	Me_2		19	5a(95)-FA
4c(90)	N O		9	4c(90)-FA
4d(90)	N O		9	4d(90)-FA

Scheme 14: Reaction for the synthesis of 4a(90)-FA, 4b(90)-FA, 5a(90)-FA, 5a(95)-FA, 4c(90)-FA and 4d(90)-FA.



Carrier	R_1	R_2	R_3	x/z	y/z	Conjugate
6a	NEt_2	NMe_2		5	4	6a-FA
6b	NEt_2	NMe_2		5	4	6b-FA
6c	N O	NMe_2		5	4	6c-FA
7a	NEt_2	O OH		5	4	7a-FA
7c	N O	O OH		5	4	7c-FA
8a	N O	NEt_2		5	4	8a-FA
8b	N O	OH		5	4	8b-FA

Scheme 15: Reaction for the synthesis of 6a-FA, 6b-FA, 6c-FA, 7a-FA, 7c-FA, 8a-FA and 8b-FA.

Table 3.15: Summary of experimental data for folic acid conjugates (4a(90)-FA to 8b-FA).

Reactants in feed			**Reaction time and temperature** [b]/°c	**Folic acid conjugates**	
Carrier designation	**Coupling agent**	**Polymer-drug-coupling agent ratio**(mol-%) [a]		**Yield**(%)[c]	**Designation**
4a(90)	HBTU	1:1.2:1.1	3.5h, RT	51.6	4a(90)-FA
4b(90)	HBTU	1:1.2:1.1	3.5h, RT	48.7	4b(90)-FA
4c(90)	HBTU	1:1.2:1.1	3.5h, RT	62.6	4c(90)-FA
4d(90)	HBTU	1:1.2:1.1	3.5h, RT	68.2	4d(90)-FA
5a(90)	HBTU	1:1.2:1.1	3.5h, RT	52.7	5a(90)-FA
5a(95)	HBTU	1:1.2:1.1	3.5h, RT	47.4	5a(95)-FA
6a	HBTU	1:1.2:1.1	3.5h, RT	58.3	6a-FA
6b	HBTU	1:1.2:1.1	3.5h, RT	56.3	6b-FA
6c	HBTU	1:1.2:1.1	3.5h, RT	71.6	6c-FA
7a	HBTU	1:1.2:1.1	3.5h, RT	55.8	7a-FA
7c	HBTU	1:1.2:1.1	3.5h, RT	77.9	7c-FA
8a	HBTU	1:1.2:1.1	3.5h, RT	74.7	8a-FA
8b	HBTU	1:1.2:1.1	3.5h, RT	65.3	8b-FA

[a] Molar ratio of carrier repeating unit to Folic acid to coupling agent.

[b] RT= room temperature.

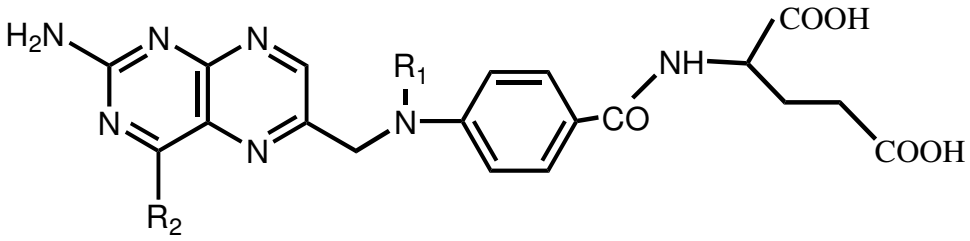
[c] Conjugate yield after size exclusion chromatography and ultimate (12000 molecular weight cut-off) dialysis.

Table 3.16: ^{1}H NMR data and viscometric results for folic acid conjugates (4a(90)-FA to 8b-FA).

Conjugate designation	η_{inh} (mLg^{-1})[a]	Base molecular weight [b]	% FA Found (NMR) [c]	% FA Calcd [d]	Protons counted (expected) Chemical shift range δ(ppm)		
					δ: 8.65-6.80[e]	**δ:** 1.70-1.50	**δ:** 1.10- 1.00
4a(90)-**FA**	16.90	2514.136	16.51	17.56	4,7(5)	2(2)	54(54)
4b(90)-**FA**	17.17	2640.370	16.10	16.72	4.8(5)	20(20)	54(54)
4c(90)-**FA**	25.94	2387.902	18.49	18.49	5(5)	2(2)	-
4d(90)-**FA**	26.32	4380.462	9.68	10.08	4.8(5)	20(20)	-
5a(90)-**FA**	14.85	2639.992	16.72	16.72	5(5)	20(20)	-
5a(95)-**FA**	13.67	2766.226	14.42	15.96	2.26(2.5)	20(20)	-
6a-FA	15.24	2528.162	16.76	17.46	4.8(5)	20(20)	30(30)
6b-FA	17.51	2458.032	18.62	17.96	5.2(5)	10(10)	30930)
6c-FA	23.43	2598.082	16.99	16.99	5(5)	20(20)	-
7a-FA	15.73	2539.986	17.38	17.38	5(5)	12(12)	30(30)
7c-FA	24.01	2609.906	16.91	16.91	5(5)	12(12)	-
8a-FA	25.96	2710.290	16.29	16.29	5(5)	20(20)	24(24)
8b-FA	25.82	2433.698	17.60	18.14	4.8(5)	12(12)	12(12)

[a] At 30,0ºC±0.5ºC in dist. H_2O; conc.=0.2mg/100mL.
[b]Molecular weight, actual (in parenthesis, calculated for 100% acylation).
[c]Derived from ^{1}H NMR spectrum (error limit ±15%).
[d]Derived from 100% acylation.
[e]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 limits ± 15%. Protons are calculated for the structural representations in Scheme14 and Scheme15.

As can be observed from Table 3.16, the inherent viscosities of the polyaspartamide-folic acid conjugates are in 13.00-26.40 mLg^{-1} range. For those determined, the average molecular weights range from 12 000 to 60 000. Theses conjugates show an increase in both molecular weights and inherent viscosities compared to their derived carriers; thus indicating incorporation of drug molecules into the polymer chain to proceed without major cleavage during the conjugation process. There is a fair match between the trend of molecular weight and inherent viscosity, the molecular weight increasing with increase in the inherent viscosity for these conjugates. Theses results should be considered cautiously since the observations were made on small numbers of conjugates.

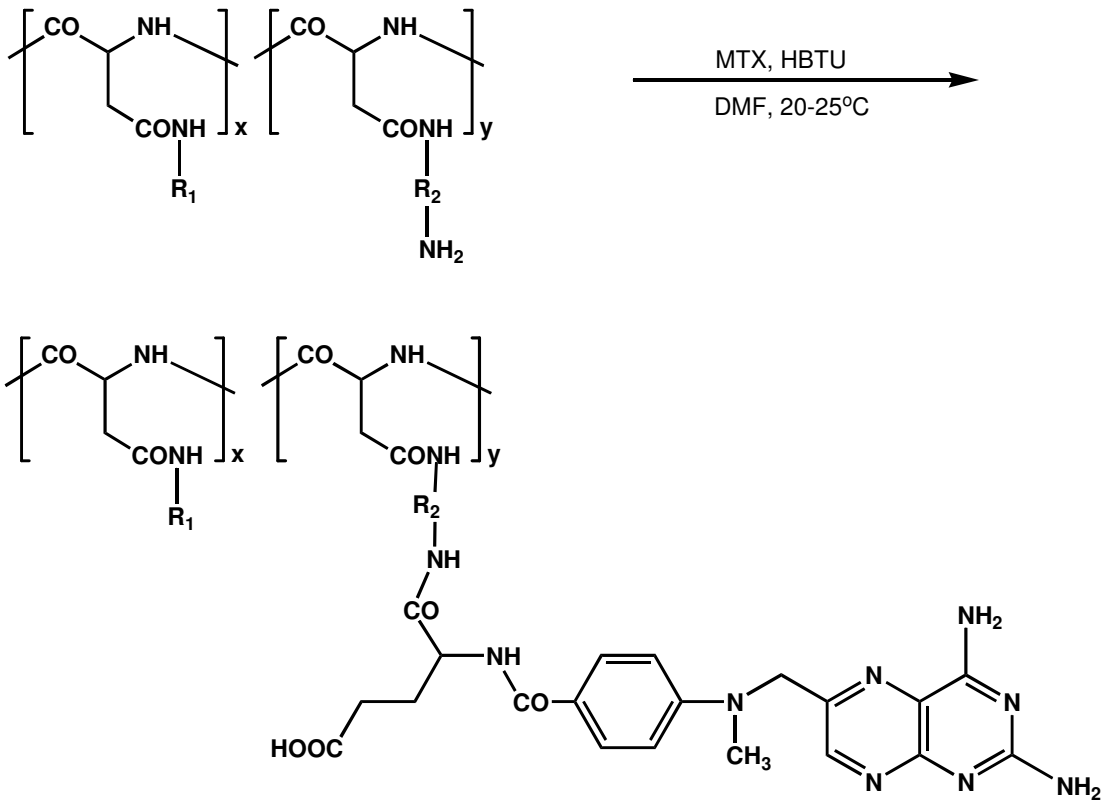


	Methotrexate	**Folic acid**
R_1	CH_3	H
R_2	NH_2	OH

Figure 3.2: Structures showing resemblance of folic acid and MTX

3.2.1.2 Polymer-MTX conjugation

As specified above in section 3.2.1.1 with respect to the high cost of MTX, only few carriers have been used for the conjugation with MTX, including 4a (90), 4b (90), 5a (90), 5b (95).



Carrier	R_1	R_2	x/y
4a(90)	NEt_2		9
4b(90)	NEt_2		9
5a(90)	NMe_2		9
5b(95)	NMe_2	NH	19

Scheme 16: Reaction for the synthesis of 4a(90)-MTX, 4b(90)-MTX, 5a(90)-MTX, 5b(95)-MTX.

Molar feed ratios of the various reactants were used and are tabulated accordingly. Tables 3.17 and 3.18 summarize the experimental conditions and ^{1}H NMR data, which were utilized for the compositional characterization of the conjugates 4a(90)-MTX, 4b(90)-MTX, 5a(90)-MTX, 5b(95)-MTX.

Similar observations were found just as in the case of folic acid conjugates concerning the viscosity results. We observed that MTX conjugates had higher viscosity values than those of the corresponding carriers.

Table 3.17: Summary of experimental data for MTX conjugates (4a(90)-MTX to 5b(95)-MTX).

Reactants in feed			**Reaction time and temperature** [b]/°c	**Folic acid conjugates**	
Carrier designation	**Coupling agent**	**Polymer-drug-coupling agent ratio**(mol-%) [a]		**Yield**(%)[c]	**Designation**
4a(90)	HBTU	1:1.2:1.1	3.5h, RT	44.7	4a(90)-MTX
4b(90)	HBTU	1:1.2:1.1	3.5h, RT	48.9	4b(90)-MTX
5a(90)	HBTU	1:1.2:1.1	3.5h, RT	41.3	5a(90)-MTX
5b(95)	HBTU	1:1.2:1.1	3.5h, RT	47.3	5b(90)-MTX

[a] Molar ratio of carrier repeating unit to Folic acid to coupling agent.
[b] RT= room temperature.
[c] Conjugate yield after size exclusion chromatography and ultimate (12000 molecular weight cut-off) dialysis.

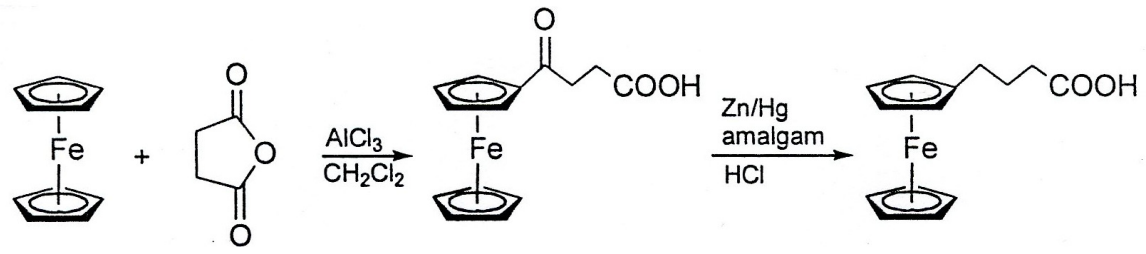
Table 3.18: ^{1}H NMR data and viscometric results for MTX conjugates (4a(90)-MTX to 8b-MTX).

Conjugate designation	η_{inh} (mLg^{-1})[a]	Base molecular weight [b]	% MTX Found (NMR) [c]	% MTX Calcd [d]	Protons counted (expected) Chemical shift range δ(ppm)		
					δ: 8.65-6.80[e]	**δ:** 1.70-1.50	**δ:** 1.10- 1.00
4a(90)-**MTX**	16,94	2527.166	16.72	17.98	4.65(5)	2(2)	54(54)
4b(90)-**MTX**	17.23	2653.4	1.47	17.13	5.1(5)	20(20)	54(54)
5a(90)-**MTX**	14.97	2400.932	18.55	18.93	4.9(5)	20(20)	-
5b(95)-**MTX**	13.81	4422.536	10.18	10.28	2.47(2.5)	38(38)	-

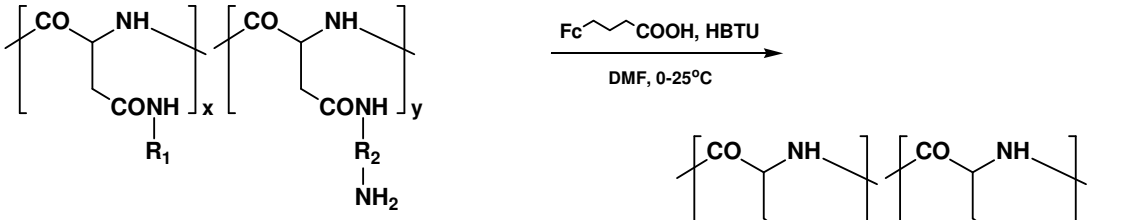
[a] At 30,0ºC±0.5ºC in dist. H_2O; conc.=0.2mg/100mL.
[b]Molecular weight, actual (in parenthesis, calculated for 100% acylation).
[c]Derived from ^{1}H NMR spectrum (error limit ±15%).
[d]Derived from 100% acylation.
[e]In D_2O, pH 10-11, chemical shifts, δ/ppm, referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limits ± 15%. Protons are calculated for the structural representations in Scheme16.

3.2.2 Polymer-Ferrocene conjugation

Numerous metallocenes, notably the parent compound ferrocene, have shown antineoplastic activities against tumor cells. In ferrocene, these activities are associated with its electronic state (see Section2.4.2 for details). Conjugation of ferrocene to a suitable carrier polymer, provides a prodrug from which the bioactive compound may be released in the intracellular space, as discussed in Section 2.3. These considerations, several years ago, prompted the initiative of an extended synthetic program in this laboratory aiming at the development and routine synthesis of such ferrocene conjugates for biomedical investigations[89, 90]. In this dissertation, several carrier types containing amine functions as drug-attachment sites were synthesized, such as amine-functionalized polyaspartamide (Scheme 3), in which R_1 stands for hydrosolubilizing group and R_2 for drug binding sites. The ferrocenylation agent used was 4-ferrocenylbutanoic acid (Scheme 17), for which a favorable electrochemical reduction potential facilitating stabilization of the ferricenium cation (Section2.4.2) had been established[91]. This acid was prepared by known literature methods[92]. Conjugation was brought about by treatment of the carrier in aprotic medium (DMF) for 3.5h at room temperature, with ferrocenylbutanoic acid, in molar feed ratios 1.2:1.5 (amine function/ ferrocene), in combination with the 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium fluorophosphate (HBTU) coupling agent. Either reaction type led to ferrocene incorporation through carboxamide formation in the linking segment, thus providing a biofissionable amide link between carrier and drug (Schemes 18&19). The carriers 4a(90), 4b(90), 4c(90), 4d(90), 5a(90), 6a, 6b, 6c, 7a, 7c, 8a and 8b were chosen for anchoring purpose with ferrocenylbutanoic acid. The conjugates 5a(90) and 4d(90) had previously been made and were repeated. The conjugates (Scheme 18 and Scheme 19), were purified by dialysis and isolated upon freeze-drying as water-soluble solid compounds, yields ranging from 35 to 41%. Degree of coupling (i.e., percentages N-acylation) was assessed from the relative intensity of the ferrocene proton signal at 4.3-4.1 in ^{1}HNMR spectra.

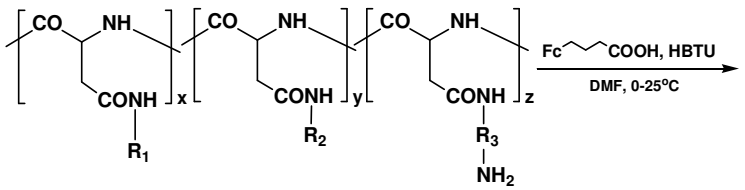


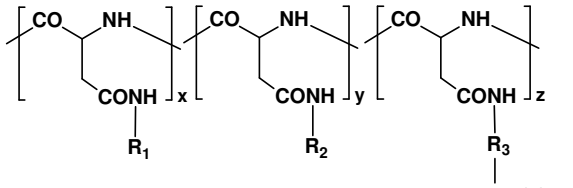
Scheme 17: Reaction for the preparation of 4-ferrocenenylbutanoic acid



Carrier	R_1	R_2	x/y	Conjugate
4a(90)	NEt_2		9	4a(90)-Fc
4b(90)	NEt_2		9	4b(90)-Fc
5a(90)	Me_2		9	5a(90)-Fc
5a(95)	Me_2		19	5a(95)-Fc
4c(90)	N O		9	4c(90)-Fc
4d(90)	N O		9	4d(90)-Fc

Scheme 18: Reaction for the synthesis of 4a(90)-Fc to 4d(90)-Fc.







Carrier	R_1	R_2	R_3	x/z	y/z	Conjugate
6a	NEt_2	NMe_2		5	4	6a-Fc
6b	NEt_2	NMe_2		5	4	6b-Fc
6c	N O	NMe_2		5	4	6c-Fc
7a	NEt_2	O OH		5	4	7a-Fc
7c	N O	O OH		5	4	7c-Fc
8a	N O	NEt_2		5	4	8a-Fc
8b	N O	OH		5	4	8b-Fc

Scheme 19: Reaction for the synthesis of 6a-Fc to 8b-Fc.

Table 3.19: Summary of experimental data for the conjugates 4a(90)-Fc to 8b-Fc

Reactants in feed			**Reaction time and temperature** [b]/°c	**Ferrocene conjugates**	
Carrier designation	**Coupling agent**	**Polymer-drug-coupling agent ratio**(mol-%) [a]		**Yield**(%)[c]	**Designation**
4a(90)	HBTU	1:1.2:1.1	3.5h, RT	58.4	4a(90)-Fc
4b(90)	HBTU	1:1.2:1.1	3.5h, RT	64.1	4b(90)-Fc
4c(90)	HBTU	1:1.2:1.1	3.5h, RT	59.7	4c(90)-Fc
4d(90)	HBTU	1:1.2:1.1	3.5h, RT	57.7	4d(90)-Fc
5a(90)	HBTU	1:1.2:1.1	3.5h, RT	53.1	5a(90)-Fc
5a(95)	HBTU	1:1.2:1.1	3.5h, RT	53.1	5a(95)-Fc
6a	HBTU	1:1.2:1.1	3.5h, RT	54.9	6a-Fc
6b	HBTU	1:1.2:1.1	3.5h, RT	52.7	6b-Fc
6c	HBTU	1:1.2:1.1	3.5h, RT	61.8	6c-Fc
7a	HBTU	1:1.2:1.1	3.5h, RT	50.4	7a-Fc
7c	HBTU	1:1.2:1.1	3.5h, RT	65.0	7c-Fc
8a	HBTU	1:1.2:1.1	3.5h, RT	68.8	8a-Fc
8b	HBTU	1:1.2:1.1	3.5h, RT	59.0	8b-Fc

[a] Molar ratio of carrier repeating unit to Folic acid to coupling agent.

[b] RT= room temperature.

[c] Conjugate yield after size exclusion chromatography and ultimate (12000 molecular weight cut-off) dialysis.

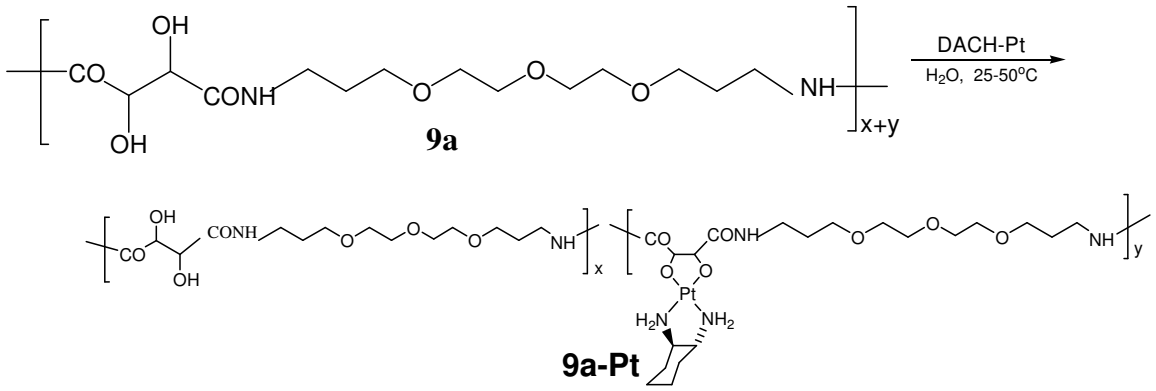
Table 3.20: ^{1}H NMR data and viscometric results for the conjugates 4a(90)-Fc to 8b-Fc.

Conjugate designation	η_{inh} (mLg^{-1})[a]	Base molecular weight [b]	% Fe Found (NMR) [c]	% Fe Calcd [d]	Protons counted (expected) Chemical shift range δ(ppm)		
					δ: 4.2-4.1[e]	**δ:** 1.70-1.50	**δ:** 1.10- 1.00
4a(90)-**Fc**	15.56	2344.84	9.9	11.60	7.67(9)	2(2)	54(54)
4b(90)-**Fc**	16.83	2471.08	10.63	11.01	8.7(9)	20(20)	54(54)
4c(90)-**Fc**	24.32	2470.70	11.42	11.01	9.3(9)	2(2)	-
4d(90)-**Fc**	25.01	2596.93	11.05	10.48	9.5(9)	20(20)	-
5a(90)-**Fc**	13.47	2218.61	11.45	12.27	8.4 (9)	20(20)	-
5a(95)-**Fc**	12.88	4211.17	6.89	6.46	9.6(9)	40(40)	-
6a-Fc	13.96	2358.87	11.40	11.54	8.9(9)	20(20)	30(30)
6b-Fc	15.39	2288.74	12.00	11.89	9.1(9)	10(10)	30930)
6c-Fc	21.07	2428.79	11.65	11.20	9.4(9)	20(20)	-
7a-Fc	13.75	2370.694	12.00	11.48	9.4(9)	12(12)	30(30)
7c-Fc	21.94	2440.614	11.15	11.15	9(9)	12(12)	-
8a-Fc	23.20	2540.998	10.83	10.71	9.1(9)	20(20)	24(24)
8b-Fc	22.97	2264.406	12.40	12.02	9.3(9)	12(12)	12(12)

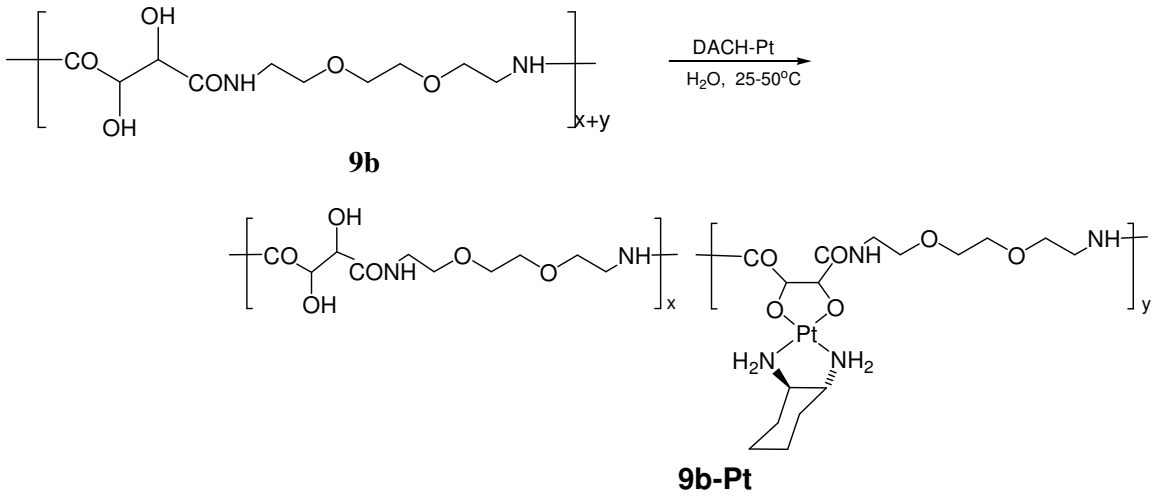
[a] At 30,0ºC±0.5ºC in dist. H_2O; conc.=0.2mg/100mL.
[b]Molecular weight, actual (in parenthesis, calculated for 100% acylation).
[c]Derived from ^{1}H NMR spectrum (error limit ±15%).
[d]Derived from 100% acylation.
[e]In D_2O, pH 10-11, chemical shifts, δ/ppm, referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate; integration error limits ± 15%. Protons are calculated for the structural representations in Scheme18 and Scheme19.

3.2.3 Polymer-Platinum conjugation

Polymer-platinum conjugation may be achieved in a number of ways, depending on the type of structure of metal ligand or ligands acting as link to the polymer. The metal may be polymer-bound either *via* amine ligands or through the leaving group ligands. In this dissertation, only cis-dihydroxylato-coordinated platinum will be examined. In this category of platinum-polymers herein investigated the metal, again in the form of 1,2-diaminocyclohexaneplatinum(II) moiety (Scheme 22), is polymer-anchored through the cis-dihydroxylato ligand system, the latter being carrier attached. Drug release in this design, hence, will proceed by hydrolytic cleavage of the hydroxyl-metal bonds. Premature hydrolytic fission of this bond would create aquated species unable to reach endocytic space, while exerting unwanted toxicity. The carriers used in this dissertation are the aliphatic polyamides 9**a** (Scheme 9) and 9**b** (Scheme 10), prepared by ester-mine polycondensation described in Section 3.1.2, containing the hydroxyl function as a side group. Platination was brought about by the procedure commonly used in this laboratory for dihydroxylatoplatinum coordination. The synthetic step (Scheme20 or Scheme21) involved the addition of polyamide 9a or 9b in water, followed by the addition of the DACH-Pt aq in a molar polyamide: DACH-Pt ratio of 1.67:1 with light-protected and ambient temperature stirring for 24h and then for10h at 50°C. The pH was maintained between 5.5-6. Purification by dialysis, essentially for the removal of nonpolymeric salts and isolation by freeze-drying provided a solid compound (9a-Pt or 9b-Pt) displaying solubility in water. Under the experimental conditions chosen, platination was not quantitative, platinum contents ranging from about 13 to 17% by mass.

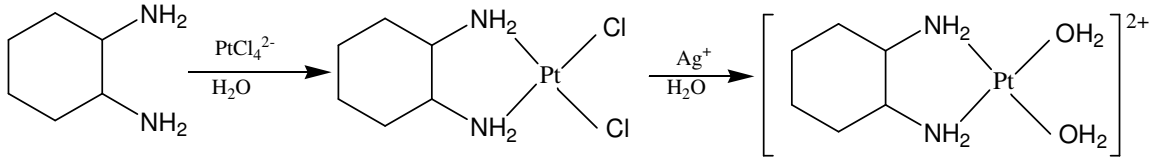


Scheme 20: Reaction for the synthesis of dihydroxylato platinum conjugate 9a-Pt.



Scheme 21: Reaction for the synthesis of dihydroxylato platinum conjugate 9b-Pt.

Experimental conditions and microanalytical data for conjugates 9a-Pt and 9b-Pt are summarized in the table3.21. The platination agent in these reactions, DACH-Pt aq., an aquated diamino-cyclohexaneplatinum derivative in which the two leaving groups are predominantly of the OH_2 type, was prepared from the corresponding *cis*-dichloro complex through chloro, aqua ligand exchange in the presence of silver nitrate and was isolated as the nitrate salt. The dichloro compound in turn was obtained by the know procedure from racemic trans-1,2-diaminocyclohexane by treatment with tetrachloroplatinate(II) salt in aqueous solution (Scheme 22, stereo arrangement neglected):



1,2-diaminocyclohexane DACH-Pt aq

Scheme 22: Preparation of platination agent DACH-Pt aq.

Table 3.21: Summary of experimental conditions and analytical data for the dihydroxylato platinum conjugates 9a-Pt and 9b-Pt

Conjugate designation	Platination agent	Carrier :Pt molar feed ration	Reaction conditions. (time, temperature)[a]		Yield (%)	η_{inh}[b] (mLg^{-1})	x/y [c]	Pt(%)		Base molar mass ($gmol^{-1}$)
			1st Step	2nd Step				Found	Calcl.[d]	
9a-Pt	DACH-Pt_{aq}	1.67: 1	24h, RT	24h, 50ºC	48.0	12.03	6.65	6.65	6.80	2867,2
9b-Pt	DACH-Pt_{aq}	1.67: 1	24h, RT	24h, 50ºC	46.0	12.79	7.50	7.50	7.68	2538.5

[a] RT= room temperature.

[b] Inherent viscosity at 30,0ºC±0.5ºC in dist. H_2O; conc.=0.2mg/100mL.

[c] x/y ratio based on % Pt found; structure normalized to y=1.

[d] % Pt calcd. for 9a-Pt and for 9b-Pt.

3.2.4 Polymer multidrug conjugation

As part of the project, we synthesized the co-conjugates from the two anticancer compounds, MTX and ferrocene which were both anchored to the same polymeric carrier backbone. The two drug systems operate by different cell killing mechanisms, and it was of interest here to establish potentiating or additive effects, if any, in subsequent bio-evaluation work. The conjugation was achieved by amide bond formation, resulting from reaction of acid group of the drugs with amine function of the carriers. Also co-conjugate folic acid-ferrocene was obtained by the same method.

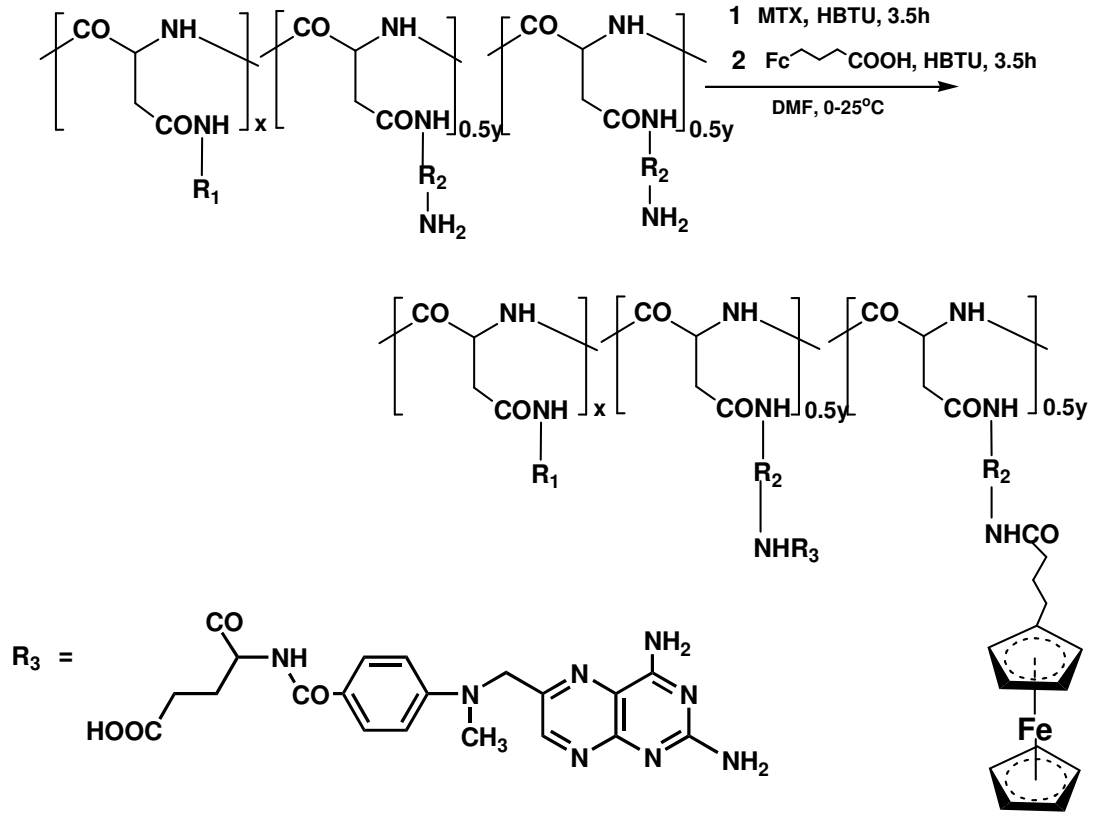
The polyaspartamides 4a(80), 4c(80) and 4d(80) featuring tertiary amine-functionalized units as hydrosolubilizing and targeting moieties, were chosen to demonstrate the co-drug binding ability of theses polymers. Each carrier was conjugated to a pair of drug systems MTX/Fc and FA/Fc in reference to methotrexate-ferrocene and folic acid-ferrocene respectively.

Folic acid in the pair FA/Fc was also used as an additional targeting moiety, given that the folate cellular uptake occurs by a natural endocytosis pathway mediated by folate receptor (FR), the latter being overexpressed on the surface of a variety of cancer cells including colon cancer[93]. Furthermore, since folate is an essential vitamin required by virtually all dividing cells for purine, nucleotide, and DNA synthesis, one might speculate that folic acid would be consumed totally hence required by cancer cells. Consequently, the resulting co-conjugate, owing to the presence of the two targeting moieties *i.e.* the tertiary amine and folic acid, are expected to be more efficiently cell-selective in comparison with conjugates having only the tertiary amine.

In the pair MTX/Fc, MTX is an antimetabolite and ferrocene, an agent purportedly causing oxidative DNA damage. The MTX/Fc co-conjugates could be more efficient in cancer-cell killing than the individual drugs. The advantages of synergistic effects were reported by Buzdar and co-workers[94].

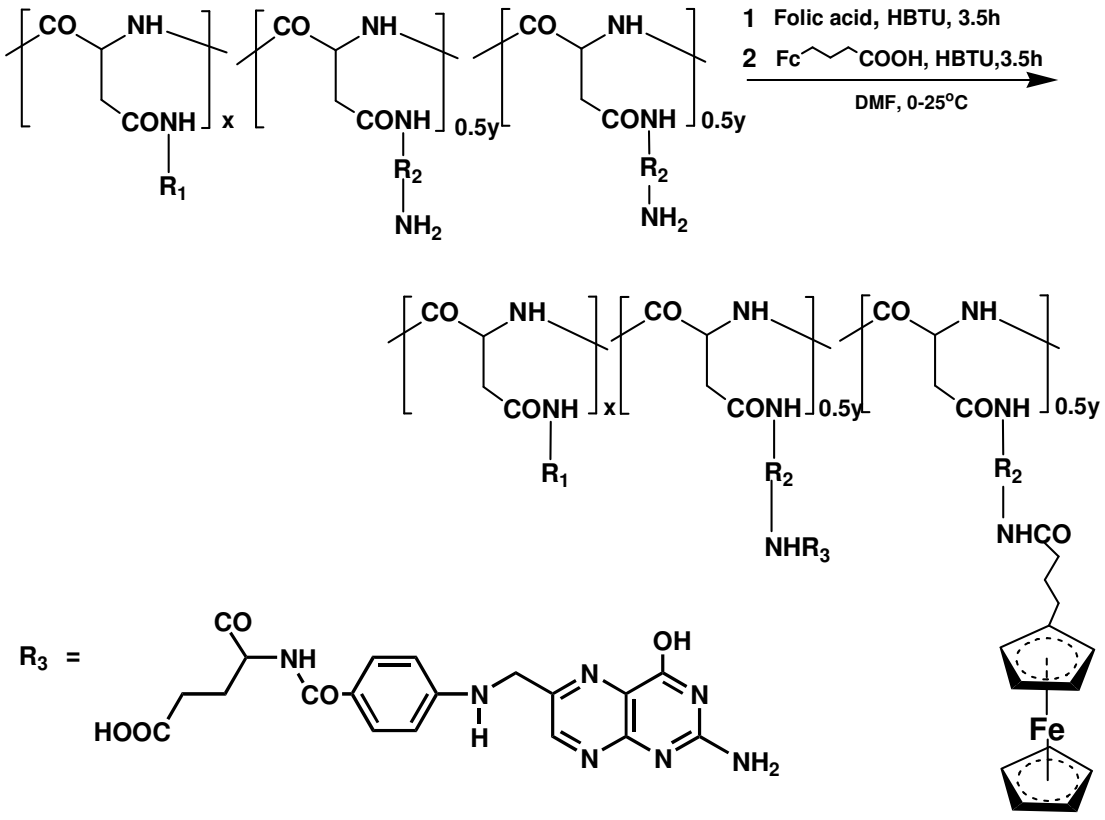
The first step leading to formation of homoconjugate with about 50% NH_2 acylation, was HBTU-mediated. It involved the treatment of the selected carriers with 1.2-1.3 equivalents of the first drug (MTX or folic acid) and 1.1 equivalent of HBTU. After conventional work-up and isolation, the drug content was determined by spectroscopic

analysis. In the second step, homoconjugate was allowed to react with the second drug (ferrocene) through HBTU coupling method. Scheme 23 and scheme 24 exemplify the preparations of MTX/Fc and FA/Fc co-conjugates. The water-soluble conjugates were precipitated from the solution and, after fractionation and purification by size exclusion chromatography and aqueous dialysis, were isolated by freeze-drying. Drug contents, in % by mass were determined by spectroscopic analysis.



Carrier	R_1	R_2	x/y	Co-conjugate
4a(80)	NEt_2		4	4a(80)-MTX-Fc
4c(80)	N O		4	4c(80)-MTX-Fc
4d(80)	N O		4	4d(80)-MTX-Fc

Scheme 23: Preparation of polyaspartamide MTX/Fc co-conjugates



Carrier	R_1	R_2	x/y	Co-conjugate
4a(80)	NEt_2		4	4a(80)-FA-Fc
4c(80)			4	4c(80)-FA-Fc
4d(80)			4	4d(80)-FA-Fc

Scheme 24 Preparation of polyaspartamide FA/Fc co-conjugates

The experimental details for the co-conjugates and reaction conditions are presented in table 3.22, whereas viscosity results and ^{1}H NMR data are compiled in Table 3.23.

Table 3.22 Summary of experimental data of Polyaspartamide co-conjugates

Reactants in feed				Reaction conditions[b]	Co-conjugates	
Carriers	**Drug/coupling agent**	**Carrier:drug:coupling agent ratio** (mol%)[a]	**Medium**		**Yield** (%)[c]	**Designation**
4a(80)	Step 1: MTX/HBTU Step 2: Fc/ HBTU	Step 1: 1:1.2:1.1 Step 2: 1:1.5:1.1	Step 1: DMF Step 2: DMF-HMP	Step 1: 3.5h, RT Step 2: 3.5h, RT	49.6	4a(80)-MTX-Fc
4c(80)	Step 1: MTX/HBTU Step 2: Fc/ HBTU	Step 1: 1:1.2:1.1 Step 2: 1:1.5:1.1	Step 1: DMF Step 2: DMF-HMP	Step 1: 3.5h, RT Step 2: 3.5h, RT	55.2	4c(80)-MTX-Fc
4d(80)	Step 1: MTX/HBTU Step 2: Fc/ HBTU	Step 1: 1:1.2:1.1 Step 2: 1:1.5:1.1	Step 1: DMF Step 2: DMF-HMP	Step 1: 3.5h, RT Step 2: 3.5h, RT	53.5	4d(80)-MTX-Fc
4a(80)	Step 1: FA/HBTU Step 2: Fc/ HBTU	Step 1: 1:1.2:1.1 Step 2: 1:1.5:1.1	Step 1: DMF Step 2: DMF-HMP	Step 1: 3.5h, RT Step 2: 3.5h, RT	56.9	4a(80)-FA-Fc
4c(80)	Step 1: FA/HBTU Step 2: Fc/ HBTU	Step 1: 1:1.2:1.1 Step 2: 1:1.5:1.1	Step 1: DMF Step 2: DMF-HMP	Step 1: 3.5h, RT Step 2: 3.5h, RT	51.6	4c(80)-FA-Fc
4d(80)	Step 1: FA/HBTU Step 2: Fc/ HBTU	Step 1: 1:1.2:1.1 Step 2: 1:1.5:1.1	Step 1: DMF Step 2: DMF-HMP	Step 1: 3.5h, RT Step 2: 3.5h, RT	61.7	4d(80)-FA-Fc

[a]Molar ratio of carrier repeating unit to drug to coupling agent in step1, and molar ratio of homoconjugate to drug to coupling agent in step 2.

[b]RT= room temperature.

[c]Co-conjugate yield (based on starting homoconjugate) after size exclusion chromatography and ultimate (12000 molecular weight cut-off) dialysis.

Table 3.23 ^{1}H NMR data and viscometric results for co-conjugates

Co-conjugates designation	η_{inh}		% [a] MTX Foud	% MTX calcl	% [a] FA found	% FA Calcd	% [a] Fe found	% Fe Calcd	NH_2 acylation (%) [b]	**Protons counted** (expected) **Chemical shift** (ppm)			
	Homoconjugate	Co-conjugate								δ 8.5-6.8 [c]	δ 4.2-4.1	δ 1.7-1.5	δ 1.1-1.0
4a(80)-**MTX-Fc**	16.85	17.05	26.50	26.50	-	-	14.10	15.87	94.4	1.3(2.5)	2.0(4.5)	2	24(24)
4c(80)-**MTX-Fc**	23.80	24.62	17.60	25.66	-	-	4.90	15.37	50.2	0.9(2.5)	0.7(4.5)	2	-
4d(80)-**MTX-Fc**	26.4	26.93	21.62	24.87	-	-	7.20	14.90	67.7	1.3(2.5)	1.0(4.5)	10	-
4a(80)-**FA-Fc**	16.70	17.83	-	-	30.0	25.94	11.40	15.99	103	1.7(2.5)	1.6(4.5)	2	24(24)
4c(80)-**FA-Fc**	23.10	23.78	-	-	13.4	25.11	8.54	15.48	54.3	0.7(2.5)	1.2(4.5)	2	-
4d(80)-**FA-Fc**	25.91	26.34	-	-	24.3	24.34	16.7	15.00	106	1.3(2.5)	2.5(4.5)	10	-

[a] Mass percentage of drug derived from ^{1}H NMR after step 2.

[b] Incorporation percentage of both drugs derived from ^{1}H NMR after step 2.

[c] Proton assignment, δ/ppm: 8.6-6.6 (aromatic and heteroaromatic CH of MTX or FA); 4.2-4.1 (CH of ferrocenyl); 1.8-1.5 ($CH_2CH_2CH_2$).

As can be observed in table 3.22, the polyaspartamide co-conjugates were obtained in yields of 49-62%. They were characterized by inherent viscosities in the range of 17-27mLg^{-1}. These were higher than those of precursor carriers (13-26 mLg^{-1}) and the derived homo-cojugates (16-26.5 mLg^{-1}). % Fe and %MTX or % FA incorported in the co-conjugate were low in some cases due to the fragmentation of the main chain in two step process involving HBTU coupling method.

3.3 Biomedical testing

In accordance with the specific experimental goals outlined in Chapter 1, this step was the last one for this project. It consisted of toxicology assessment for selected conjugates as well as carriers synthesized in Phase II. This test was performed at the department of Immunology at University of Pretoria. The following conjugates and carriers were assessed, including, 4a(90)-MTX, 4b(90)-MTX, 5b(95)-MTX, 4a(90)-Fc, 4b(90)-Fc, 6a-Fc, 7a-Fc, 8a-Fc, 4a, 4b, 5b(95), 6a, 7a and 8a. Though toxicological test results are unpublished, preliminary in vitro test revealed an acceptably low toxicity for all conjugates and carriers that lead them to be candidates for eventual in vivo tests.

CHAPTER 4: EXPERIMENTAL

4.1 GENERAL PROCEDURES

^{1}H-NMR spectra were obtained in D_2O solutions at 300 or 400MHz; chemical shifts δ, in ppm, were referenced against sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate; integration error limits ±15%. The pH of selected sample solutions was adjusted to 10-11 with sodium hydroxide, in order to eliminate potential protonation effects.

Prior to dialysis, conjugate purification was performed by size exclusion chromatography on a 2.5x 25 cm column loaded with G-25 gel, eluted with distilled water.

Dialysis of carriers and conjugates was performed against several batches of distilled water with the aid of cellulose membrane tubing (Spectrum Industries, Los Angeles, USA) of types Spectra/Por2, Spectra/Por4 and Spectra/Por6 with mass-average molecular mass cut-off limits of 6000-8000, 12000-14000, and 25000, respectively.

Aqueous polymer and conjugate solutions were freeze-dried in a VIRTIS Bench Top 3 freeze-drier (-40^{0}C, 10-15 Pa). The free-dried polymers were post-dried in a SARTORIUS Thermo Control Infrared drying apparatus and kept in a desiccator. Analytical samples of conjugates or carriers were dried for two days at 40-60^{0}C under reduced pressure in an Abderhalden apparatus, and calcium chloride was used as the drying agent.

4.2 REAGENTS, REACTANTS AND SOLVENTS

Distilled water was used for dialysis, as well as for some preparative work. The aprotic solvents dimethyl sulphoxide (DMSO) and N,N-dimethylformamide (DMF) were dried over molecular sieves 4Å. DMF was distilled under reduced pressure in a faint stream of N_2, with a fore-run of some 10% being discarded. All reactions involving its use were performed under anhydrous conditions.

The hydroxyamines and diamines, commercial grades (Fluka Chemie AG, Aldrich) were used as received. These included: 1,3-propylenediamine (PDA), 3-(dimethylamino)-1-propylamine (DMP), 2-(dimethylamino)ethylamine (DMEA), 2-(diethylamino)ethylamine (DEEA), 3-(diethylamino)-1-propylamine (DEP), ethanolamine (EA), diethylenetriamine (DET), 2,2-(ethylenedioxy)-diethylamine (EDDA), 2-(2-Aminoethoxy)ethanol(AEE), 4-(2-aminoethyl)morpholine (AEM), 4-(3-aminopropyl)morpholine (APM), N,N′-dicyclohexylcarbodiimide (DCC), methylenebisacrylamide (MBA), diethyl L-tartrate (TART), 4,7,10-trioxa-1,13-tridecanediamine (TRIA), triethylamine (TEA), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium fluorophosphate (HBTU), N-methylpyrrolidone (NMP), 1,2-Bis(3-aminopropylamino)ethane (BAP).

The following solvents, diethyl ether (Et_2O), acetone (Me_2CO), hexane (Hex.), methylene chloride and ethyl acetate (EtAc) were used as supplied.

Methotrexate, MTX (Sigma), which contains2.5 mol of water per mol of drug, and folic acid dihydrate, FA, purchased from across Organics, were pre-dried in Aberhaden tube for 48h at 40ºC before use for conjugation.

4.3 PREPARATION OF POLYMERIC CARRIERS

4.3.1 Poly-DL-succinimide (PSI) 2

Polysuccinimide(PSI) **2** was prepared by the method of Neri and Antoni[61] involving the polymerization of DL-aspartic acid in orthophosphoric acid at high temperature. N,N'-dicyclohexylcarbodiimide (DCC) was coupled to the end of carboxyl group in order to extend the chain. The products of approximately equal viscosity were pooled from several runs and thoroughly mixed to give a master batch with inherent viscosity, η_{inh}, (determined in DMF) of 38 mLg^{-1}.

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

Carrier 4a(90): The designation **4a(90)** indicates a (90:10) mole % ratio of DEEA to PDA in the polymer product. Polysuccnimide (PSI) **2**, 1 g (10.2 mmol) was dissolved in DMF (10 mL); 2-diethylaminoethylamine DEEA, 1,067 g (9,18 mmol) pre-dissolved in DMF (4 mL) were added to PSI solution. The resulting solution was saturated with

Nitrogen (N_2) gas and stirred at room temperature for seven hours. Then 1,3-propylenediamine (PDA), 0.23 g (3,11 mmol) in DMF (5 mL), was cooled in ice bath for 10 minutes. The PSI-DEEA solution was added dropwise to PDA solution. The solution was re-saturated with N_2 and stirred for 15 h in ice bath and for 5 h at room temperature.
The solution was concentrated on roti-evaporator at 60^{o}C, and allowed to cool before precipitation with solumix-hexane (2:1), 40 mL. The precipitate was washed with hot toluene four times followed by hot acetone twice and redissolved in distilled water (30 mL). The pH was adjusted between 7 and 8 with concentrated hydrochloric acid (HCl) before dialysis against distilled water in 12000 membrane tubing for 96 h.
For the last 6 h of this process, the pH in the tubing was adjusted to 9 with aqueous ammonia. After this operation, the water soluble polymer was freeze-dried, post-dried, and kept in the desiccator. The yield of the post-dried carrier was 1.01 g (47.4 %).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 56H (54H, $N(CH_2\mathbf{Me})_2$); 1.6-1.8, 2H (2H, $CONHCH_2\mathbf{CH_2}CH_2NH_2$); 2.6-2.8, 79H (76H, $CONH\mathbf{CH_2}\ \mathbf{CH_2}N(\mathbf{CH_2}CH3)_2$; $CONH\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 3.3-3.4, 20H (20H, $\mathbf{CH_2}CONH$).

Carrier 4b(90): The same procedure as used for carrier **4a** was used. 3-diethylamino-1-propylamine (DEP), 1.196 g (9.18 mmol) in DMF (4mL) was added to Polysuccinimide **2** dissolved in DMF (10 mL). The solution saturated with N_2 was stirred at room temperature for seven hours, thereafter PDA, 0.23 g (3.06 mmol) was dissolved in DMF (5 mL) and cooled in ice bath. PSI-DEP solution was added dropwise to PDA solution. The resulting solution was saturated with N_2 and stirred for 15 h in ice bath and for 5 h at room temperature. This gave **4b(90)** in yield of 1.33 g (58.8%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.2, 54.11H (54H, $N(CH_2\mathbf{Me})_2$); 1.7-1.8, 20H (20H, $CONHCH_2\mathbf{CH_2}CH_2NH_2$, $CONHCH_2\mathbf{CH_2}CH_2NEt_2$); 2.7-2.8, 76.47H (76H, $CONH\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $CONH\mathbf{CH_2}CH_2\mathbf{CH_2}NEt_2$); 3.2-3.3, 20H (20H, $\mathbf{CH_2}CONH$).
Carrier 4c(90): An analogous procedure as described in the foregoing experiment was used. To 1 g (10.2 mmol) of Polysuccnimide **2** in DMF (10 mL), was added 4-(2-

aminoethyl)morpholine (AEM), 1.2 g (9.18 mmol) in DMF (4 mL). Solution, saturated with N_2, was stirred for 7 h. The resulting solution was treated with PDA and worked up as before to get **4c(90)**, 1.4 g (62.0%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.2-2.3, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 2.5-2.6, 76 H (76H, $\mathbf{CH_2CH_2}N(\mathbf{CH_2})_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 3.3-3.4, 21H (20H, $\mathbf{CH_2}CONH$); 3.7-3.8, 37H (36H, $\mathbf{CH_2OCH_2}$).

Carrier 4d(90): Following the same procedure as above, polysuccinimide **2** (1 g, 10.2 mmol) in DMF (10 mL) was treated with 4-(3-aminopropyl)morpholine (APM), (1.32 g; 9.18 mmol) dissolved in DMF (4 mL). Solution, saturated with N_2, was stirred for 7 h. The resulting solution was treated with PDA and worked up as before to get **4d(90)**, 1.25 g (52.3%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7-1.8, 20H (20H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$); 2.5-2.6, 74.59H (76H, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 3.2-3.3, 20H (20H, $\mathbf{CH_2}CONH$); 3.7-3.8, 34H (36H, $\mathbf{CH_2OCH_2}$).

Carrier 4e(90): By similar procedure as above 2-(dimethylamino)ethylamine DMEA (0.81 g, 9.18 mmol) in DMF (4mL), was added to polysuccinimide **2** (1 g, 10.2 mmol) dissolved in DMF (10 mL). The resulting solution was treated with PDA and worked up as before to get **4e(90)**, 1.23 g (65.6%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7-1.8, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 2.2-2.3, 54H (54H, $CH_2N(\mathbf{Me})_2$); 2.7-2.8, 40H (40H, $\mathbf{CH_2CH_2}N(Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 3.3-3.4, 20H (20H, $\mathbf{CH_2}CONH$).

Carrier 4a(80): The designation **4a(80)** indicates a (80:10) mole % ratio of DEEA to PDA in the polymer product. Polysuccinimide (PSI) **2**, 1 g (10.2 mmol), was dissolved in DMF (10 mL); 2-(diethylamino)ethylamine DEEA, 0,948 g (8,16 mmol) pre-dissolved in DMF (4 mL), were added to PSI solution. The resulting solution was saturated with Nitrogen gas and stirred at room temperature for seven hours. Then 1,3-

propylenediamine (PDA), 0.64 g (6,22 mmol) in DMF (5 mL) was cooled in ice bath for 10 minutes. The PSI-DEEA solution was added dropwise to PDA solution. The solution re-saturated with N_2 and stirred for 15 h in ice bath and for 5 h at room temperature.

The solution was concentrated on roti-evaporator at 60^{o}C, and allowed to cool before precipitation with solumix-hexane (2:1), 40 mL. The precipitate was washed with hot toluene four times followed by hot acetone twice and redissolved in distilled water (30 mL). The pH was adjusted between 7 and 8 with HCl before dialysis against distilled water with 1200 membrane for 96 h.

For the last 6 h of this process, the pH in the tubing was adjusted to 9 with aqueous ammonia. After this operation, the water soluble polymer was freeze-dried, post-dried, and kept in the desiccator. The yield of the post-dried carrier was 1.15 g (55.1 %).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 24.5H (24H, $N(CH_2\mathbf{Me})_2$); 1.6-1.8, 2H (2H, $CONHCH_2\mathbf{CH_2}CH_2NH_2$); 2.6-2.8, 37H (36H, $CONH\mathbf{CH_2}\ \mathbf{CH_2}N(\mathbf{CH_2}CH3)_2$; $CONH\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 3.3-3.4, 12H (10H, $\mathbf{CH_2}CONH$).

Carrier 4b(80): The same procedure as used for carrier **4a(80)** was used. 3-diethylamino-1-propylamine (DEP), 1.063 g (8.16 mmol) in DMF (4mL) was added to Polysuccinimide **2** dissolved in DMF (10mL). The solution saturated with N_2 was stirred at room temperature for seven hours, thereafter PDA, 0.46 g (6.12 mmol) was dissolved in DMF (5 mL) and cooled in ice bath. PSI-DEP solution was added dropwise to PDA solution. The resulting solution was saturated with N_2 and stirred for 15 h in ice bath and for 5 h at room temperature. This gave **4b(80)** in yield of 1.27 g (58.0%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.2, 24.8H (24H, $N(CH_2\mathbf{Me})_2$); 1.7-1.8, 10H (10H, $CONHCH_2\mathbf{CH_2}CH_2NH_2$, $CONHCH_2\mathbf{CH_2}CH_2NEt_2$); 2.7-2.8, 36H (36H, $CONH\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $CONH\mathbf{CH_2}CH_2\mathbf{CH_2}NEt_2$); 3.2-3.3, 10H (10H, $\mathbf{CH_2}CONH$).

Carrier 4c(80): By an analogous procedure as described in the foregoing experiment was used. To 1g (10.2 mmol) of Polysuccinimide **2** in DMF (10 mL), was added 4-(2-aminoethyl)morpholine (AEM), 1.063 g (8.16 mmol) in DMF (4 mL). Solution, saturated

with N_2, was stirred for 7 h. The resulting solution was treated with PDA and worked up as before to get **4c(80)**, 1.37 g (62.2%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.2-2.3, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 2.5-2.6, 36 H (36H, $\mathbf{CH_2CH_2}N(\mathbf{CH_2})_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 3.3-3.4, 10.4H (10H, $\mathbf{CH_2}CONH$); 3.7-3.8, 16.3H (16H, $\mathbf{CH_2}O\mathbf{CH_2}$).

Carrier 4d(80): Following the same procedure as above, polysuccinimide **2** (1g, 10.2mmol) in DMF (10 mL) was treated with 4-(3-aminopropyl)morpholine (APM), (1.178g; 8.16 mmol) dissolved in DMF (4mL). Solution, saturated with N_2, was stirred for 7 h. The resulting solution was treated with PDA and worked up as before to get **4d(80)**, 1.39g (60.0%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7-1.8, 10H (10H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$); 2.5-2.6, 36.25H (36H, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 3.2-3.3, 10H (10H, $\mathbf{CH_2}CONH$); 3.7-3.8, 16.09H (16H, $\mathbf{CH_2}O\mathbf{CH_2}$).

Carrier 4e(80): By similar procedure as above 2-(dimethylamino)ethylamine DMEA (0.720 g, 8.16 mmol) in DMF (4mL), was added to polysuccinimide **2** (1 g, 10.2 mmol) dissolved in DMF (10 mL). The resulting solution was treated with PDA and worked up as before to get **4e(90)**, 1.04 g (56.0%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7-1.8, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 2.2-2.3, 23H (24H, $CH_2N(\mathbf{Me})_2$); 2.7-2.8, 20H (20H, $\mathbf{CH_2CH_2}N(Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 3.3-3.4, 10.15H (10H, $\mathbf{CH_2}CONH$).

Carrier 5a(90): The designation **5a(90)** indicate a (90:10) mole % ratio of DMP to PDA in the polymer product. Polysuccinimide (PSI) **2**, 1 g (10.2mmol) was dissolved in DMF (10 mL); 3-(dimethylamino)-1-propylamine (DMP), 0.938 g (9,18 mmol) pre-dissolved in DMF (4 mL) were added to PSI solution. The resulting solution was saturated with Nitrogen gas and stirred at room temperature for seven hours. Then 1,3-propylenediamine (PDA), 0.23 g (3,06 mmol) in DMF (5 mL), was cooled in ice bath for

10 minutes. The PSI-DMP solution was added dropwise to PDA solution. The solution re-saturated with N_2 and stirred for 15 h in ice bath and for 5 h at room temperature.

The solution was concentrated on roti-evaporator at 60^{o}C, and allowed to cool before precipitation with solumix-hexane (2:1), 40 mL. The precipitate was washed with hot toluene four times followed by hot acetone twice and redissolved in distilled water (30mL). The pH was adjusted between 7 and 8 with HCl before dialysis against distilled water with 1200 membrane for 96 h.

For the last 6 h of this process, the pH in the tubing was adjusted to 9 with aqueous ammonia. After this operation, the water soluble polymer was freeze-dried, post-dried, and kept in the desiccator. The yield of the post-dried carrier was 1.35 g (67.4 %).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.6-1.7, 20H (20H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 2.1-2.2, 53.3H (54H, $CH_2CH_2CH_2N(\mathbf{Me})_2$); 2.3-2.8, 40H (40H, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 3.1-3.2, 20H (20H, $\mathbf{CH_2}$CONH).

Carrier 5a(80): The same procedure as in carrier **5a(90)** was used. 3-(dimethylamino)-1-propylamine (DMP), 0.834 g (8.16 mmol) in DMF (4 mL) was added to Polysuccinimide **2** dissolved in DMF (10 mL). The solution saturated with N_2 was stirred at room temperature for seven hours, thereafter PDA, 0.46 g (6.12 mmol) was dissolved in DMF (5 mL) and cooled in ice bath. PSI-DMP solution was added dropwise to PDA solution. The resulting solution was saturated with N_2 and stirred for 15 h in ice bath and for 5 h at room temperature. This gave **5a(80)** in yield of 1.20 g (61.0%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.6-1.7, 10H (10H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 2.1-2.2, 23H (24H, $CH_2CH_2CH_2N(\mathbf{Me})_2$); 2.3-2.8, 20H (20H, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 3.1-3.2, 10H (10H, $\mathbf{CH_2}$CONH).

Carrier 5a(95): The designation **5a(95)** indicates a (95:5) mol % ratio of DMP to PDA in the polymer product. By similar procedure as above 3-(dimethylamino)-1-propylamine (DMP) (0.990 g, 9.69 mmol) in DMF (4 mL), was added to polysuccinimide **2** (1 g, 10.2

mmol) dissolved in DMF (10 mL). The solution saturated with N_2 was stirred at room temperature for seven hours, thereafter PDA, 0.113 g (1.53 mmol) was dissolved in DMF (5 mL) and cooled in ice bath. PSI-DMP solution was added dropwise to PDA solution. The resulting solution was saturated with N_2 and stirred for 15 h in ice bath and for 5 h at room temperature. This gave **5a(95)** in yield of 1.41 g (70.0%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.6-1.7, 40H (40H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 2.1-2.2, 108H (114H, $CH_2CH_2CH_2N(\mathbf{Me})_2$); 2.3-2.8, 78H (80H, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 3.1-3.2, 42H (40H, $\mathbf{CH_2}CONH$).

Carrier 5b(90): Following the same procedure as above, polysuccinimide **2** (1 g, 10.2 mmol) in DMF (10 mL) was treated with 3-(dimethylamino)-1-propylamine (DMP), 0.938 g (9,18 mmol) dissolved in DMF (4 mL). Solution, saturated with N_2, was stirred for 7 h; thereafter diethylenetriamine (DET) 0.316 g (3.06 mmol) was dissolved in DMF (5 mL) and cooled in ice bath. PSI-DMP solution was added dropwise to DET solution. The resulting solution was saturated with N_2 and stirred for 15 h in ice bath and for 5 h at room temperature. This gave **5b(90)** in yield of 1.0 g (47.0%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.6-1.7, 18H (18H, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 2.2-2.3, 52H (54H, $CH_2CH_2CH_2N(\mathbf{Me})_2$); 2.3-2.8, 43.6H (44H, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$, $\mathbf{CH_2CH_2}NH\mathbf{CH_2CH_2}NH_2$); 3.2-3.3, 19H (20H, $\mathbf{CH_2}CONH$).

Carrier 5b(95): By similar procedure as above, to 1 g (10.2 mmol) of Polysuccinimide **2** in DMF (10 mL), was added 3-(dimethylamino)-1-propylamine (DMP) (0.990 g, 9.69 mmol) in DMF (4 mL). Solution, saturated with N_2, was stirred for 7 h. The resulting solution was treated with DET and worked up as before to get **5b(95)**, 1.1 g (53.3%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.6-1.7, 38H (38H, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 2.2-2.3, 108.6H (114H, $CH_2CH_2CH_2N(\mathbf{Me})_2$); 2.3-2.8, 81.2H

(84H, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$, $\mathbf{CH_2CH_2}NH\mathbf{CH_2CH_2}NH_2$); 3.2-3.3, 39.8H (40H, $\mathbf{CH_2}CONH$).

Carrier 5c(90): An analogous procedure as described in the foregoing experiment was used. To 1g (10.2 mmol) of Polysuccinimide **2** in DMF (10mL), was added 3-(dimethylamino)-1-propylamine (DMP), 0.938 g (9,18 mmol) in DMF (4 mL). Solution, saturated with N_2, was stirred for 7 h. The resulting solution was treated with 2,2′-(ethylenedioxy)-diethylamine (EDDA) 0.454 g (3.06 mmol) was dissolved in DMF (5 mL) and cooled in ice bath. PSI-DMP solution was added dropwise to EDDA solution. The resulting solution was saturated with N_2 and stirred for 15 h in ice bath and for 5 h at room temperature. This was worked up as before to get **5c(90)**, 1.14 g (55.0%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7-1.8, 18H (18H, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 2.4-2.5, 57H (54H, $CH_2CH_2CH_2N(\mathbf{Me})_2$); 2.5-3.0, 42H (40H, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$,$NH\mathbf{CH_2}CH_2$,$OCH_2\mathbf{CH_2}NH_2$); 3.2-3.3, 21.6H (20H, $\mathbf{CH_2}CONH$); 3.6-3.7, 9H (8H, $CH_2\mathbf{CH_2}O\mathbf{CH_2CH_2}O\mathbf{CH_2}CH_2NH_2$).

Carrier 6a: Polysuccnimide (PSI) **2**, 1 g (10.2 mmol) was dissolved in DMF (10 mL); 3-(diethylamino)-1-propylamine (DEP) 0.664 g (5.1 mmol) dissolved in DMF (4 mL) were added first and stirred at room temperature, for four hours after flushing with Nitrogen gas. DMP, 0,417 g (4,08 mmol) in DMF (5 mL) were added later to the PSI-DEP solution and stirring continued at room temperature for 8 h after flushing with nitrogen gas. Then 1,3-propylenediamine (PDA), 0.23 g (3,06 mmol) in DMF (5 mL) was cooled in ice bath for 10 minutes. The PSI-DEP-DMP solution was added dropwise to PDA solution. The solution re-saturated with N_2 and stirred for 15 hours in ice bath and for 5 h at room temperature.

The solution was concentrated on roti-evaporator at 60^oC, and allowed to cool before precipitation with solumix-hexane (2:1), 40 mL. The precipitate was washed with hot toluene four times followed by hot acetone twice and redissolved in distilled water (30 mL). The pH was adjusted between 7 and 8 with HCl before dialysis against distilled water with 1200 membrane for 96 h.

For the last 6 h of this process, the pH in the tubing was adjusted to 9 with aqueous ammonia. After this operation, the water soluble polymer was freeze-dried, post-dried, and kept in the desiccator. The yield of the post-dried carrier was 1.29 g (60.1 %).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.2-1.3, 30H (30H, $CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$); 1.8-1.9, 20H (20H, $CH_2\mathbf{CH_2}CH_2N(Me)_2$, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2Me)_2$); 2.4-2.5, 24.5H (24H, $CH_2CH_2CH_2N(\mathbf{Me})_2$); 2.6-3.1, 60H (60H, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$); 3.2-3.3, 20H (20H, $\mathbf{CH_2}CONH$).

Carrier 6b: This polymer was prepared similarly to the procedure that leading to polymer **6a** with the exception that DEP was replaced by DEE in the same molar equivalent (5.1mmol, 0.593 g). Carrier 6b was obtained as fully water soluble solid, in a yield of 1.32 g (64.0%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.1-1.2, 30H (30H, $CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$); 1.6-1.7, 10H (10H, $CH_2\mathbf{CH_2}CH_2N(Me)_2$, $CH_2\mathbf{CH_2}CH_2NH_2$); 2.1-2.4, 24H (24H, $CH_2CH_2CH_2N(\mathbf{Me})_2$); 2.5-2.8, 61.5H (60H, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2CH_2}N(\mathbf{CH_2}Me)_2$); 3.2-3.3, 20H (20H, $\mathbf{CH_2}CONH$).

Carrier 6c: This polymer was prepared analogously to **6a** with the exception that DEP was replaced by AEM with the same molar equivalent (5.1 mmol, 0.664 g). Carrier 6c was obtained as fully water soluble solid, in a yield of 1.50 g (66.3%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7-1.8, 24H (24H, $CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$); 2.3-2.4, 22H (20H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$); 2.5-2.8, 62H (60H, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$); 3.2-3.3, 22H (20H, $\mathbf{CH_2}CONH$); 3.7-3.8, 19H (20H, $\mathbf{CH_2OCH_2}$)

Carriers 7a, 7b and 7c: The polyaspartamides 7a, 7b and 7c were obtained using the same procedure as that leading to polymers 6a, 6b and 6c. A minor modification was

introduced by replacing 3-(dimethylamino)-1-propylamine (DMP) by 2-(2-aminoethoxy)ethanol(AEE). Molar ratio of the reactants remained the same during the preparation (0.429 g; 4.08 mmol).

Carrier 7a was afforded in a yield of 1.33 g (62.0%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 31.36H (30H, $CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$); 1.7-1.8, 12H, (12H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2Me)_2$); 2.6-2.7, 53H (52H, $NH\mathbf{CH_2}CH_2OCH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$); 3.2-3.5, 21.68H (20H, $\mathbf{CH_2}CONH$); 3.6-3.8, 26.1H (24H, $CH_2\mathbf{CH_2}O\mathbf{CH_2CH_2}OH$).

Carrier 7b was afforded in a yield of 1.30 g (61.4%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 28.7H (30H, $CH_2CH_2N(CH_2\mathbf{Me})_2$); 1.6-1.7, 2H, (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 2.6-2.7, 49.9H (52H, $NH\mathbf{CH_2}CH_2OCH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2CH_2}N(\mathbf{CH_2}Me)_2$); 3.2-3.5, 20.2H (20H, $\mathbf{CH_2}CONH$); 3.6-3.8, 26.8H (24H, $CH_2\mathbf{CH_2}O\mathbf{CH_2CH_2}OH$).

Carrier 7c was afforded in a yield of 1,41 g (63.3%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7-1.8, 12H (12H, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$,$CH_2\mathbf{CH_2}CH_2NH_2$); 2.5-2.6, 51.6H (52H, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$, $NH\mathbf{CH_2}CH_2OCH_2$); 3.2-3.5, 21.8H (20H, $\mathbf{CH_2}CONH$); 3.6-3.8, 46.5H (44H, $CH_2\mathbf{CH_2}O\mathbf{CH_2CH_2}OH$, $\mathbf{CH_2}O\mathbf{CH_2}$).

Carrier 8a: Polysuccnimide (PSI) **2**, 1g (10.2 mmol) was dissolved in N,N-dimethylformamide DMF (10mL); 3-(diethylamino)-1-propylamine (DEP) 0.531 g (4.08 mmol) dissolved in DMF (4 mL) were added first and stirred at room temperature, for four hours after flushing with Nitrogen gas. 0.736 g (5.1 mmol) in DMF (5 mL) were added later to the PSI-DEP solution and stirring continued at room temperature for 8 h after flushing with nitrogen gas. Then 1,3-propylenediamine (PDA), 0.23 g (3,06 mmol)

in DMF (5 mL) was cooled in ice bath for 10 minutes. The PSI-DEP-APM solution was added dropwise to PDA solution. The solution re-saturated with N_2 and stirred for 15 h in ice bath and for 5 h at room temperature.

The solution was concentrated on roti-evaporator at 60^{o}C, and allowed to cool before precipitation with solumix-hexane (2:1), 40mL. The precipitate was washed with hot toluene four times followed by hot acetone twice and redissolved in distilled water (30 mL). The pH was adjusted between 7 and 8 with HCl before dialysis against distilled water with 1200 membrane for 96 hours.

For the last 6 h of this process, the pH in the tubing was adjusted to 9 with aqueous ammonia. After this operation, the water soluble polymer was freeze-dried, post-dried, and kept in the desiccator. The yield of the post-dried carrier was 1.60 g (68.0%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 24H (24H, $CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$); 1.6-1.8, 21.8H (20H, $CH_2\mathbf{CH_2}CH_2NH_2$,$CH_2\mathbf{CH_2}CH_2N(CH_2Me)_2$,$CH_2\mathbf{CH_2}CH_2N(CH_2)_2$); 2.4-2.6, 76.3H (76H, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$); 3.2-3.3, 21,8H (20H, $\mathbf{CH_2}CONH$); 3.7-3.8, 21.8H (20H, $\mathbf{CH_2OCH_2}$).

Carrier 8b: the polyaspartamide 8b was obtained using the procedure analogous to that leading to polymer 8a. A minor modification was introduce by replacing DEP by ethanolamine (EA) 0.249 g (4.08mmol). Polymer 8b was afforded in yield of 1.50 g (73.0%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.6-1.8, 12H (12H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$); 2.5-2.8, 48H (52H, $NH\mathbf{CH_2}CH_2OCH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$); 3.2-3.4, 20.2H (20H, $\mathbf{CH_2}CONH$); 3.6-3.8, 27.75H, (28H, $\mathbf{CH_2OCH_2}$, $CH_2\mathbf{CH_2}OH$).

4.3.3 Other polyamides

Carrier 9a: Polymer 9a was prepared by mixing diethyl L-tartrate (TART) 2.062 g (10 mmol), 4,7,10-trioxa-1,13-tridecanediamine (TRIA) 2.240 g (10 mmol) and anhydrous Na_2CO_3 (1.0 g ≈ 20% of total mass of reactants). The mixture was saturated with N_2 gas and stirred for 24 h at room temperature in stoppered flask. Upon dilution with 5 mL of dimethyl sulphoxide (DMSO) and resaturation with N_2, stirring was continued for 15 days at 60ºC in an incubator. Intermittently, reduced pressure was applied in a rotating evaporator for 45 min at 60ºC bath temperature for removal of eliminated EtOH, and the contents were resaturated with N_2. Suspended carbonate was removed from the mixture by centrifugation, and supernatant, diluted with 10 mL of distilled water, was dialyzed in 12000 molecular mass cut off membrane tubing for 48 h against distilled H_2O at pH 7. The tube contents were freeze-dried, giving 2.35 g (70.3%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.6-1.9, 4H (4H, CH_2- **CH_2**- CH_2); 3.3-3.5, 3.95H (4H, CONH- **CH_2**); 3.5-3.7, 12.4H (12H, O- **CH_2**- **CH_2**-O, CH_2- CH_2- **CH_2**-O); 4.4-4.6, 1.93H (2H, C**H**-OH).

Carrier 9b: The same experimental procedure leading to carrier 9a was applied. However, TRIA was replaced by 2,2-(ethylenedioxy)-diethylamine (EDDA) 1.63 g (11mmol) . Polymer 9b was obtained in a yield of 1.11 g (42.32%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 3.3-3.5, 4H (4H, CONH-**CH_2**); 3.5-3.7, 8.5H (8H, O- **CH_2**- **CH_2**-O, CH_2- CH_2- **CH_2**-O); 4.4-4.6, 2H (2H, C**H**-OH).

Carrier 9c: This carrier was prepared in analogous fashion to carrier 9a with a minor modification. This concerns the replacement of half moles of TRIA by 1,2-bis(3-aminopropylamino)ethane (BAP) 0.872 g (5mmol) and the another half by EDDA 0.815 g (5.5 mmol). Polymer 9c was obtained in a yield of 3.20 g (58.1%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.6-1.9, 3H (4H, CH_2-**CH_2**- CH_2); 2.5-2.7, 10H (8H, **CH_2**-NH- **CH_2**); 3.3-3.5, 9.3H (8H, CONH- **CH_2**); 3.5-3.7, 9.7H (8H, O- **CH_2**- **CH_2**-O, CH_2- CH_2- **CH_2**-O); 4.4-4.6, 4H (4H, C**H**-OH).

Carrier 9d: This polymer was prepared similarly to the procedure that leading to polymer 9a by mixing diethyl L-tartrate (TART) 2.062 g (10 mmol), EDDA 0.543 g (3.67 mmol), diethylenetriamine (DET) 0.688 g (6.67 mmol) and anhydrous Na_2CO_3 (1.0 g ≈ 20% of total mass of reactants). Polymer 9d was afforded in yield of 4.103 g (59.0%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 2.5-2.7, 6H (6H, **CH_2**-NH- **CH_2**); 3.3-3.5, 11.3H (12H, CONH- **CH_2**); 3.5-3.7, 8H (6H, O- **CH_2**- **CH_2**-O, CH_2-CH_2- **CH_2**-O); 4.4-4.6, 4.6H (4H, C**H**-OH).

4.3.4 Polyamidoamines

Carrier 10(90): The designation **10(90)** indicate a (90:10) mol % ratio of DEP to PDA in the polymer product. Methylenebisacrylamide (MBA), 2 g (12.97 mmol) was dissolved in 12 mL of distilled warm water; 3-(diethylamino)-1-propylamine (DEP), 1.5 g (11.7 mmol) was added. The solution was flushed with N_2 gas and stirred at 20-25ºC for 12 h, and 2 days at 60ºC in the incubator. The resulting solution was diluted with 28 mL of distilled water. PDA, 0.096 g (1.297 mmol) was dissolved in distilled water (15 mL) and cooled in an ice bath for 20 minutes. MBA-DEP solution was added dropwise to PDA solution; after N_2 gas flushing, stirring continued for 24 h in an ice bath, and further at 20-25ºC for 2 days. The solvent was removed on a rotary evaporator. The concentrated compound was then washed twice with hot acetone to remove the unreacted reagents, and dissolved in 20 mL of distilled H_2O. The pH was adjusted to 7 (HCl), after which dialysis was performed for 48 h employing 12000 cut-off membranes. The compound was then isolated by freeze drying to afford a yield of 0.67 g (18.52%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.2-1.3, 54.7H (54H, $CH_2N(CH_2\mathbf{Me})_2$); 1.7-1.8, 20H (20H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2NEt_2$); 2.4-2.5, 40H (40H, $CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$); 2.7-2.8, 116H (116H, $\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 4.5-4.6, 20H (20H, $CONH\mathbf{CH_2}NHCO$).

Carrier 11(90): This polymer was prepared analogously to **10(90)** with the exception that DEP was replaced by 2-(diethylamino)ethylamine DEEA, 1.357 g (11.7 mmol). Carrier 11(90) was obtained as fully water soluble solid, in a yield of 0.61 g (17.7%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.2-1.3, 57H (54H, $CH_2N(CH_2\mathbf{Me})_2$); 1.6-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 2.5-2.6, 40H (40H, $CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$); 2.7-2.8, 121.4H (118H, $\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$, $\mathbf{CH_2}\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 4.5-4.6, 20H (20H, $CONH\mathbf{CH_2}NHCO$).

Carrier 12(90): The same procedure as in carrier **10(90)** was used. To 2 g (12.973 mmol) of MBA dissolved in hot distilled water (12 mL), was added 2-(dimethylamino)ethylamine (DMEA) 1.03 g (11.675 mmol). Solution, saturated with N_2, was stirred for 12 h at room temperature and 2days at 60ºC. The resulting solution was treated with PDA and worked up as before to get **12(90)**, 0.58g (18.56%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.6-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 2.2-2.3, 54H (54H, $CH_2N\mathbf{Me}_2$); 2.4-2.6, 78H (80H, $\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$, $\mathbf{CH_2}\mathbf{CH_2}NMe_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 2.8-2.9, 40H (40H, $CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$); 4.5-4.6, 20H (20H, $CONH\mathbf{CH_2}NHCO$).

Carrier 13(90): This polymer was prepared similarly to the procedure that leading to polymer 10(90). MBA, 2 g (12.973mmol) was treated with 4-(3-aminopropyl)morpholine (APM), 1.684 g (11.675 mmol). The resulting solution was treated with PDA and worked up as before to get **13(90)**, 0.867 g (22.94%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.8-1.9, 20H (20H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N$); 2.4-2.9, 120H, (116H, $\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$); 2.9-3.0, 40H, (40H, $CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$); 3.8-3.9, 36H (36H, $\mathbf{CH_2}O\mathbf{CH_2}$); 4.5-4.6, 20H, (20H, $CONH\mathbf{CH_2}NHCO$).

Carrier 14(90): the polymer was made following the same procedure as above. MBA, 2 g (12.973 mmol) was treated with 4-(2-aminoethyl)morpholine (AEM), 1.520 g (11.675 mmol). The resulting solution was treated with PDA and worked up as before to get **14(90)**, 0.910 g (25.16%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7-1.8, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 2.4-2.9, 120H (116H, $\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$); 2.9-3.0, 42H (40H, $CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$); 3.8-3.9, 40H (36H, $\mathbf{CH_2}O\mathbf{CH_2}$); 4.5-4.6, 21.5H (20H, $CONH\mathbf{CH_2}NHCO$).

Carrier 10(80): The designation **10(80)** indicate a (80:20) mole % ratio of DEP to PDA in the polymer product. Methylenebisacrylamide (MBA), 2 g (12.97 mmol) was dissolved in 12mL of distilled warm water; 3-(diethylamino)-1-propylamine (DEP), 1.352 g (10.378 mmol) was added. The solution was flushed with N_2 gas and stirred at 20-25ºC for 12 h, and 2 days at 60ºC in the incubator. The resulting solution was diluted with 28 mL of distilled water. PDA, 0.192 g (2.595 g) was dissolved in distilled water (15 mL) and cooled in an ice bath for 20 minutes. MBA-DEP solution was added dropwise to PDA solution; after N_2 gas flushing, stirring continue for 24 h in an ice bath, and further at 20-25ºC for 2 days. The solvent was removed on a rotary evaporator. The concentrated compound was then washed twice with hot acetone to remove the unreacted reagents, and dissolved in 20mL of distilled H_2O. The pH was adjusted to 7, (HCl), after which dialysis was performed for 48 hours employing 12000 cut-off membranes. The compound was then isolated by freeze drying to afford a yield of 0.59 g (16.65%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.1-1.2, 24.7H (24H, $CH_2N(CH_2\mathbf{Me})_2$); 1.7-1.8, 10H (10H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2NEt_2$); 2.4-2.5, 20H (20H, $CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$); 2.7-2.8, 56H (56H, $\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 4.5-4.6, 10H (10H, $CONH\mathbf{CH_2}NHCO$).

Carrier 11(80): This polymer was prepared analogously to **10(80)** with the exception that DEP was replaced by 2-(diethylamino)ethylamine DEEA, 1.357 g (11.675 mmol). Carrier 11(80) was obtained as fully water soluble solid, in a yield of 0.70 g (20.60%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.2-1.3, 25H (24H, $CH_2N(CH_2\mathbf{Me})_2$); 1.6-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 2.5-2.6, 20H (20H, $CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$); 2.7-2.8, 56.25H (56H, $\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$, $\mathbf{CH_2}\mathbf{CH_2}N(\mathbf{CH_2}Me)_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 4.5-4.6, 10H (10H, $CONH\mathbf{CH_2}NHCO$).

Carrier 12(80): The same procedure as in carrier **10(80)** was used. To 2 g (12.973 mmol) of MBA dissolved in hot distilled water (12 mL), was added 2-(dimethylamino)ethylamine (DMEA) 1.03 g (11.675 mmol). Solution, saturated with N_2, was stirred for 12 h at room temperature and 2 days at 60ºC. The resulting solution was treated with PDA and worked up as before to get **12(80)**, 0.51 g (16.41%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.6-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 2.4-2.5, 24H (24H, $CH_2N\mathbf{Me}_2$); 2.6-2.9, 58.72H (60H, $\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$, $\mathbf{CH_2}\mathbf{CH_2}NMe_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$); 2.9-3.0, 24H (24H, $CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$); 4.5-4.6, 10.4H (10H, $CONH\mathbf{CH_2}NHCO$).

Carrier 13(80): This polymer was prepared similarly to the procedure that leading to polymer 10(80). MBA, 2 g (12.973 mmol) was treated with 4-(3-aminopropyl)morpholine (APM), 1.684 g (11.675 mmol). The resulting solution was treated with PDA and worked up as before to get **13(80)**, 0.719 g (19.49%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7-1.8, 10H (10H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N$); 2.4-2.9, 52H, (56H, $\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$); 2.9-3.0, 20H, (20H, $CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$); 3.8-3.9, 15H (16H, $\mathbf{CH_2}O\mathbf{CH_2}$); 4.5-4.6, 10H (10H, $CONH\mathbf{CH_2}NHCO$).

Carrier 14(80): the polymer was made following the same procedure as above. MBA, 2 g (12.973 mmol) was treated with 4-(2-aminoethyl)morpholine (AEM), 1.520 g (11.675 mmol). The resulting solution was treated with PDA and worked up as before to get **14(80)**, 1,001 g (28.25%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7-1.8, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 2.4-2.9, 64H (56H, $\mathbf{CH_2}CH_2CONH$, $COCH_2\mathbf{CH_2}N$, $\mathbf{CH_2}CH_2\mathbf{CH_2}NH_2$, $\mathbf{CH_2}CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$); 2.9-3.0, 21,5H (20H, $CH_2\mathbf{CH_2}CONH$, $NHCO\mathbf{CH_2}$); 3.8-3.9, 19H (16H, $\mathbf{CH_2}O\mathbf{CH_2}$); 4.5-4.6, 11H (10H, $CONH\mathbf{CH_2}NHCO$).

4.4 PREPARATION OF POLYMERIC CONJUGATES

4.4.1 Polymer-Folic acid conjugates

Conjugate 4a(90)-FA: Carrier **4a(90)** (200 mg, 0.100 mmol) was dissolved in 4 mL of DMF. Folic acid (FA) (54 mg, 0.122 mmol) predissolved in 2ml of N-methylpyrrolidone (NMP), was added to the carrier solution with stirring. Upon rapid stirring, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium fluorophosphate (HBTU) (43 mg, 0.112 mmol) predissolved in 1 mL of DMF, was added dropwise over aperiod of 10 min to the mixture, followed by the addition of triethylamine (TEA) (28 μL, 0.204 mmol). The resulting solution was stirred at room temperature for 3.5 h. The conjugate was precipitated with 10 mL of Solumix-acetone (2:1), then cooled in an ice bath for 30 min. The polymeric conjugate was isolated upon centrifugation and dissolved in 2mL of distilled water. The pH was adjusted to 10 using NaOH, followed by size exclusion chromatography on a column packed with Sephadex G25 and eluted with distilled water. The pH of eluate was readjusted to 7 using glacial acetic acid to prevent hydrolysis, and the solution dialyzed for 48 h in 12000-14000 cut-off membrane tubing. The pH of retentate was adjusted to 4 with HCl to regenerate the unconjugated carboxyl group of

FA from its salt. The aqueous solution was stirred at room temperature for 5 min, then the pH re-adjusted to 6 with aqueous ammonia, and the dialysis process was continued for 6 h in the same tubing by changing water after every 2 h for complete removal of inorganic salts. The retentate was freeze-dried to afford 124 mg (51.6%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 52H (54H, $N(CH_2\mathbf{Me})_2$); 1.5-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 8.7-6.8, 5H (5H, aromatic and heteroaromatic CH of FA). These data indicate 100% of FA incorporation, corresponding to 17.56% by mass.

Conjugate 4b(90)-FA: This conjugate was prepared in a similar fashion to 4a(90)-FA, except that carrier 4a(90) was replaced by carrier 4b(90). Thus, the reaction of polymer 4b(90) (200 mg, 0.090 mmol), FA (54 mg, 0.122 mmol), HBTU (43 mg, 0.112 mmol), (TEA) (28 μL, 0.204 mmol) afforded 116mg (48.7%) of 4b(90)-FA as yellow water soluble solid.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 52H (54H, $N(CH_2\mathbf{Me})_2$); 1.5-1.7, 20H (20H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2NEt_2$); 8.7-6.8, 4.8H (5H, aromatic and heteroaromatic CH of FA). These data indicate 96% incorporation, corresponding to 16.16% by mass.

Conjugate 4c(90)-FA: The same procedure as used for conjugate **4a(90)-FA** was used. Thus, the reaction of polymer 4c(90) (200 mg, 0.090 mmol), FA (54 mg, 0.122 mmol), HBTU (43 mg, 0.112 mmol), (TEA) (28 μL, 0.204 mmol) afforded 149 mg (62.6%) of 4c(90)-FA as yellow water soluble solid.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 8.7-6.8, 5H (5H, aromatic and heteroaromatic CH of FA). These data indicate 100% of FA incorporation, corresponding to 18.49% by mass.

Conjugate 4d(90)-FA: An analogous procedure as described in the foregoing experiment was used. Thus, the reaction of polymer 4d(90) (200 mg, 0.085 mmol), FA (54 mg, 0.122 mmol), HBTU (43 mg, 0.112 mmol), (TEA) (28 μL, 0.204 mmol) afforded 161 mg (68.2%) of 4d(90)-FA as yellow water soluble solid.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 20H (20H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$); 8.7-6.8, 4.9H (5H, aromatic and heteroaromatic CH of FA). These data indicate 98% of FA incorporation, corresponding to 9.70% by mass.

Conjugate 5a(90)-FA: This conjugate was obtained using the procedure analogous to that leading to conjugate 4a(90)-FA. A minor modification was introduce by replacing polymer 4a(90) by polymer 5a(90). Thus, the reaction of polymer 5a(90) (200 mg, 0.102 mmol), FA (54 mg, 0.12 2mmol), HBTU (43 mg, 0.112 mmol), (TEA) (28 μL, 0.204 mmol) afforded 128 mg (52.7%) of 5a(90)-FA as yellow water soluble solid.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 20H (20H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 8.7-6.8, 5H (5H, aromatic and heteroaromatic CH of FA). These data indicate 100% of FA incorporation, corresponding to 16.72% by mass.

Conjugate 5a(95)-FA: The same experimental procedure leading to conjugate 5a(90)-FA was applied. However, polymer 5a(90) was replaced by 5a(95). Polymer 5a(95) (200 mg, 0.051 mmol) was treated by FA (27 mg, 0.061 mmol), HBTU (21.5 mg, 0.056 mmol), (TEA) (14 μL, 0.102 mmol) to afford 105 mg (47.4%) of 5a(95)-FA as yellow water soluble solid.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 20H (20H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 8.7-6.8, 2.26H (2.5H, aromatic and heteroaromatic CH of FA). These data indicate 90.32% of FA incorporation, corresponding to 14.42% by mass.

Conjugate 6a-FA, 6b-FA and 6c-FA: The conjugates 6a-FA, 6b-FA and 6c-FA were obtained using the procedure similar to that leading to conjugate 4a(90)-FA. A minor modification was introduce by replacing polymer 4a(90) by polymer 6a, 6b and 6c respectively. The carriers 6a(200 mg, 0.095 mmol) , 6b(200 mg, 0.098 mmol) and 6c(200 mg, 0.092 mmol) were treated individually with FA (54 mg, 0.122 mmol), HBTU (43 mg, 0.112 mmol), (TEA) (28 μL, 0.204 mmol).

Conjugate 6a-FA was afforded in a yield of 140 mg (58.3%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.2-1.3, 30H (30H, $CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$); 1.8-1.9, 20H (20H, $CH_2\mathbf{CH_2}CH_2N(CH_2Me)_2$, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 8.7-6.8, 4.8H (5H, aromatic and heteroaromatic CH of FA). These data indicate 96% of FA incorporation, corresponding to 16.76% by mass.

Conjugate 6b-FA was afforded in a yield of 136 mg (56.3%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.2-1.3, 30H (30H, $CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$); 1.8-1.9, 10H (10H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 8.7-6.8, 5.15H (5H, aromatic and heteroaromatic CH of FA). These data indicate 103.7% of FA incorporation, corresponding to 18.62% by mass.

Conjugate 6c-FA was afforded in a yield of 171 mg (71.6%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.2-1.3, 24H (24H, $CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$); 1.8-1.9, 22H (20H, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 8.7-6.8, 5H (5H, aromatic and heteroaromatic CH of FA). These data indicate 100% of FA incorporation, corresponding to 16.99% by mass.

Conjugate 7a-FA and 7c-FA: The conjugates 7a-FA and 7c-FA were prepared in analogous fashion to conjugate 4a(90). Thus, the carriers 7a(200 mg, 0.094 mmol) and

7c(200 mg, 0.091 mmol) were treated individually with FA (54mg, 0.122 mmol), HBTU (43 mg, 0.112 mmol), (TEA) (28 μL, 0.204 mmol).
Conjugate 7a-FA was afforded in a yield of 134 mg (55.8%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 31H (30H, $CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$); 1.8-1.9, 12H (12H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2Me)_2$); 8.7-6.8, 5H (5H, aromatic and heteroaromatic CH of FA). These data indicate 100% of FA incorporation, corresponding to 17.38% by mass.

Conjugate 7c-FA was afforded in a yield of 186 mg (77.9%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7-1.8, 12H (12H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$); 8.7-6.8, 5H (5H, aromatic and heteroaromatic CH of FA). These data indicate 100% of FA incorporation, corresponding to 16.91% by mass.

Conjugate 8a-FA and 8b-FA: The conjugates 8a-FA and 8b-FA were obtained similarly to the procedure that leading to conjugate 4a(90)-FA. A minor modification was introduce by replacing polymer 4a(90) by polymer 8a and 8b respectively. The carriers 8a(200 mg, 0.087 mmol) and 8b(200 mg, 0.099 mmol) were treated individually with FA (54 mg, 0.122 mmol), HBTU (43 mg, 0.11 2mmol), (TEA) (28 μL, 0.204 mmol).
Conjugate 8a-FA was afforded in a yield of 177 mg (74.7%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 24H (24H, $CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$); 1.6-1.8, 20H (20H, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2Me)_2$); 8.7-6.8, 5H (5H, aromatic and heteroaromatic CH of FA). These data indicate 100% of FA incorporation, corresponding to 16.29% by mass.

Conjugate 8b-FA was afforded in a yield of 158 mg (65.3%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.6-1.8, 12H (12H, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$, $CH_2\mathbf{CH_2}CH_2NH_2$); 8.7-6.8, 4.84H (5H, aromatic and heteroaromatic CH of FA). These data indicate 97% of FA incorporation, corresponding to 16.29% by mass.

4.4.2 Polymer-Methotrexate conjugates

Conjugate 4a(90)-MTX: This conjugate was obtained by using the standard procedure leading to 4a(90)-FA except that folic acid (**FA**) was replaced by methotrexate (**MTX**). Thus, the reaction of polymer 4a(90) (200 mg, 0.100 mmol), MTX (56 mg, 0.122 mmol), HBTU (43 mg, 0.112 mmol), (TEA) (21 mg, 28 μL, 0.204 mmol), followed by the adopted work up procedure afforded 108 mg (44.7%) of 4b(90)-MTX as yellow water soluble powder.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 52H (54H, $N(CH_2\mathbf{Me})_2$); 1.5-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 8.7-6.8, 5H (5H, aromatic and heteroaromatic CH of MTX). These data indicate 100% of MTX incorporation, corresponding to 17.98% by mass.

Conjugate 4b(90)-MTX: This conjugate was prepared in a similar fashion to 4a(90)-MTX, except that carrier 4a(90) was replaced by carrier 4b(90). Thus, the reaction of polymer 4b(90) (200 mg, 0.090 mmol), MTX (56 mg, 0.122 mmol), HBTU (43 mg, 0.112 mmol), (TEA) (21 mg, 28 μL, 0.204 mmol) afforded 117 mg (48.9%) of 4b(90)-MTX as yellow water soluble solid.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 52H (54H, $N(CH_2\mathbf{Me})_2$); 1.5-1.7, 20H (20H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2NEt_2$); 8.7-6.8, 5.1H (5H, aromatic and heteroaromatic CH of MTX). These data indicate 102% of MTX incorporation, corresponding to 17.47% by mass.

Conjugate 5a(90)-MTX: This conjugate was obtained using the procedure analogous to that leading to conjugate 4a(90)-MTX. A minor modification was introduce by replacing polymer 4a(90) by polymer 5a(90). Thus, the reaction of polymer 5a(90) (200 mg, 0.102 mmol), MTX (56 mg, 0.122 mmol), HBTU (43 mg, 0.112 mmol), (TEA) (28 μL, 0.204 mmol) afforded 101 mg (41.3%) of 5a(90)-MTX as yellow water soluble solid.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 20H (20H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 8.7-6.8, 4.9H (5H, aromatic and heteroaromatic CH of FA). These data indicate 98% of MTX incorporation, corresponding to 18.55% by mass.

Conjugate 5b(95)-MTX: The same experimental procedure leading to conjugate 5a(90)-MTX was applied. However, polymer 5a(90) was replaced by 5b(95). Polymer 5b(95) (200 mg, 0.0452 mmol) was treated by MTX (28 mg, 0.061 mmol), HBTU (21.5 mg, 0.056 mmol), (TEA) (14 μL, 0.102 mmol) afforded 105 mg (47.3%) of 5b(95)-MTX as yellow water soluble solid.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 19H (19H, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 8.7-6.8, 2.47H (2.5H, aromatic and heteroaromatic CH of MTX). These data indicate 99% of MTX incorporation, corresponding to 10.18% by mass.

4.4.3 Polymer-ferrocene conjugates

4.4.3.1 ***Synthesis of ferrocenylbutanoic acid*:** This ferrocenylation agent was prepared according to the procedure described in the literature[95]. Several products of close melting points were thoroughtly mixed to give a batch of mp: 116-119ºC (Lit.[96] 119-120ºC).

4.4.3.2 *Preparation of polymer-ferrocene conjugates*

The conjugation of ferrocene to amine-functionalized polymeric carrier was achieved by HBTU coupling agent method which consists of direct free acid-primary amine coupling mediated by HBTU agent. The experimental details, which follow, illustrate the standard

procedure adopted for the preparation of all polymer-ferrocene conjugates herein investigated.

Conjugate 4a(90)-Fc: Carrier **4a(90)** (200 mg, 0.100 mmol) was dissolved in 4 mL of DMF with stirring. In separated vessel 4-ferrocenylbutanoic acid (41.3mg, 0,15 mmol) and HBTU (42 mg, 0.11 mmol), dissolved in 2 mL of DMF, were stirred for 45 min at room temperature, protected from direct light, then added in one dash to the carrier solution, followed by TEA (21 mg, 0,204 mmol). The resulting brownish solution was saturated with N_2, and stirred at ambient temperature for 3.5 h, still protected from the light. The conjugate was precipitated with 10mL of Solumix-acetone (2:1), then cooled in an ice bath for 30 min. The polymeric conjugate was isolated upon centrifugation and dissolved in 2mL of distilled water. The pH was adjusted to 10 using NaOH, followed by size exclusion chromatography on 2.5x2.5cm column packed with Sephadex G25 and eluted with distilled water. The pH of eluted was readjusted to 7 using glacial acetic acid to prevent hydrolysis, ~10 mg of ascorbic acid was added to preclude ferrocene oxidation to ferricenium salts, and solution was dialyzed for 48 h in 12000-14000 cut-off membrane tubing. The retentate was freeze-dried to afford 131 mg (58.4%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 52H (54H, $N(CH_2\mathbf{Me})_2$); 1.5-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 4.1-4.2, 9H (9H, CH of ferrocenyl). These data indicate 100% of Fc incorporation, corresponding to 11.60% by mass.

Conjugate 4b(90)-Fc: This conjugate was prepared in a similar fashion to 4a(90)-Fc, except that carrier 4a(90) was replaced by carrier 4b(90). Thus, the reaction of polymer 4b(90) (200 mg, 0.090 mmol), Fc (41.3 mg, 0.15 mmol), HBTU (42 mg, 0.11 mmol), (TEA) (28 μL, 0.204 mmol) afforded 143 mg (64.1%) of 4b(90)-Fc as water soluble solid.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 52H (54H, $N(CH_2\mathbf{Me})_2$); 1.5-1.7, 20H (20H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2NEt_2$); 4.1-4.2, 8.7H

(9H, CH of ferrocenyl). These data indicate 97% of Fc incorporation, corresponding to 10.63% by mass.

Conjugate 4c(90)-Fc: The same procedure as used for conjugate **4a(90)-Fc** was used. Thus, the reaction of polymer 4c(90) (200 mg, 0.090 mmol), Fc (41.3 mg, 0.15 mmol), HBTU (42 mg, 0.11 mmol), (TEA) (28 μL, 0.204 mmol) afforded 133 mg (59.7%) of 4c(90)-Fc as water soluble solid.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 4.1-4.2, 9.3H (9H, CH of ferrocenyl). These data indicate 100% of Fc incorporation, corresponding to 11.42% by mass.

Conjugate 4d(90)-Fc: An analogous procedure as described in the foregoing experiment was used. Thus, the reaction of polymer 4d(90) (200 mg, 0.085 mmol), Fc (41.3 mg, 0.15 mmol), HBTU (42 mg, 0.11 mmol), (TEA) (28 μL, 0.204 mmol) afforded 128 mg (57.7%) of 4d(90)-Fc as water soluble solid.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 20H (20H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$); 4.1-4.2, 9.5H (9H, CH of ferrocenyl). These data indicate 106% of Fc incorporation, corresponding to 11.05% by mass.

Conjugate 5a(90)-Fc: This conjugate was obtained using the procedure analogous to that leading to conjugate 4a(90)-Fc. A minor modification was introduce by replacing polymer 4a(90) by polymer 5a(90). Thus, the reaction of polymer 5a(90) (200 mg, 0.102 mmol), Fc (41 mg, 0.150 mmol), HBTU (42 mg, 0.11 mmol), (TEA) (28 μL, 0.204 mmol) afforded 120 mg (53.1%) of 5a(90)-Fc as water soluble solid.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 20H (20H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 4.1-4.2, 8.4H (9H, CH of ferrocenyl). These data indicate 93% of Fc incorporation, corresponding to 11.45% by mass.

Conjugate 5a(95)-Fc: The same experimental procedure leading to conjugate 5a(90)-FA was applied. However, polymer 5a(90) was replaced by 5a(95). Polymer 5a(95) (200 mg, 0.051 mmol) was treated by FA (21 mg, 0.061 mmol), HBTU (21.5 mg, 0.056 mmol), (TEA) (14 μL, 0.102 mmol) afforded 113 mg (53.1%) of 5a(95)-Fc as water soluble solid.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 20H (20H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 4.1-4.2, 9.6H (9H, CH of ferrocenyl). These data indicate 107% of Fc incorporation, corresponding to 6.89% by mass.

Conjugate 6a-Fc, 6b-Fc and 6c-Fc: The conjugates 6a-Fc, 6b-Fc and 6c-Fc were obtained using the procedure similar to that leading to conjugate 4a(90)-Fc. A minor modification was introduce by replacing polymer 4a(90) by polymer 6a, 6b and 6c respectively. The carriers 6a(200 mg, 0.095 mmol) , 6b(200 mg, 0.098 mmol) and 6c(200 mg, 0.092 mmol) were treated individually with Fc (41 mg, 0.15 mmol), HBTU (42 mg, 0.112 mmol), (TEA) (28 μL, 0.204 mmol).
Conjugate 6a-Fc was afforded in a yield of 123 mg (54.9%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.2-1.3, 30H (30H, $CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$); 1.8-1.9, 20H (20H, $CH_2\mathbf{CH_2}CH_2N(CH_2Me)_2$, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 4.1-4.2, 8.9H (9H, CH of ferrocenyl). These data indicate 99% of Fc incorporation, corresponding to 11.40% by mass.

Conjugate 6b-Fc was afforded in a yield of 119 mg (52.7%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.2-1.3, 30H (30H, $CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$); 1.8-1.9, 10H (10H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 4.1-4.2, 9.1H (9H, CH of ferrocenyl).These data indicate 103% of Fc incorporation, corresponding to 12.00% by mass.

Conjugate 6c-Fc was afforded in a yield of 138 mg (61.8%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.2-1.3, 24H (24H, $CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$); 1.8-1.9, 22H (20H, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(Me)_2$); 4.1-4.2, 9.4H (9H, CH of ferrocenyl). These data indicate 104% of Fc incorporation, corresponding to 11.65% by mass.

Conjugates 7a-Fc and 7c-Fc: The conjugates 7a-Fc and 7c-Fc were prepared in analogous fashion to conjugate 4a(90). Thus, the carriers 7a(200 mg, 0.094 mmol) and 7c(200 mg, 0.091 mmol) were treated individually with Fc (41 mg, 0.15 mmol), HBTU (42 mg, 0.11 mmol), (TEA) (28 μL, 0.204 mmol).
Conjugate 7a-Fc was afforded in a yield of 113 mg (50.4%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 31H (30H, $CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$); 1.8-1.9, 12H (12H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2Me)_2$); 4.1-4.2, 9.4H (9H, CH of ferrocenyl). These data indicate 105% of Fc incorporation, corresponding to 12.00% by mass.

Conjugate 7c-Fc was afforded in a yield of 145 mg (65.0%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.7-1.8, 12H (12H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$); 4.1-4.2, 9H (9H, CH of ferrocenyl). These data indicate 100% of Fc incorporation, corresponding to 11.15% by mass.

Conjugate 8a-Fc and 8b-Fc: The conjugates 8a-FA and 8b-Fc were obtained similarly to the procedure that leading to conjugate 4a(90)-Fc. A minor modification was introduce by replacing polymer 4a(90) by polymer 8a and 8b respectively. The carriers 8a(200 mg, 0.087 mmol) and 8b(200 mg, 0.099 mmol) were treated individually with Fc (41 mg, 0.15 mmol), HBTU (42 mg, 0.11 mmol), (TEA) (28 μL, 0.204 mmol).

Conjugate 8a-Fc was afforded in a yield of 153 mg (68.8%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 24H (24H, $CH_2CH_2CH_2N(CH_2\mathbf{Me})_2$); 1.6-1.8, 20H (20H, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2Me)_2$); 4.1-4.2, 9.1H (9H, CH of ferrocenyl). These data indicate 101% of Fc incorporation, corresponding to 10.83% by mass.

Conjugate 8b-Fc was afforded in a yield of 133 mg (59.0%).

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.6-1.8, 12H (12H, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$, $CH_2\mathbf{CH_2}CH_2NH_2$); 4.1-4.2, 9.3H (9H, CH of ferrocenyl). These data indicate 97% of Fc incorporation, corresponding to 12.40% by mass.

4.4.4 Polymer-platinum conjugates

4.4.4.1 *Preparation of DACH-Pt*

The DACH-Pt$(NO_3)_2$ platination agent, trans-1-2-diaminocyclohexanediaquaplatinum(II) dinitrate (Scheme22), was prepared by the literature method[97]. Anal. Calcd. for $C_6H_{18}N_4O_8Pt$ (469.3): Pt, 41.6%; found: Pt, 41.6%.

4.4.4.2 *Polyamides-platinum anchoring*

Conjugate 9a-Pt: To the N_2-saturated solution of carrier 9a (0.212 g, 0.634 mmol) in H_2O (5 mL), DACH-Pt (0.179 g, 0.380 mmol) in H_2O (4 mL) was added. Upon re-saturation with N_2, the solution was light protected, and stirred for 1d at ambient temperature with the pH of the solution maintained at ~5.5-6 and another 1d at 50ºC. The solution was filtered and dialyzed for 2d in Spectra/Por 4 tubing. The retentate was freeze-dried, to give 0.872 g (48.0%) of water-soluble solid **9a-Pt** . Anal. found : Pt, 6.65%.

Conjugate 9b-Pt: An analogous procedure as described in the foregoing experiment was used. A minor modification was introduced by replacing polymer 9a by polymer 9b. Thus, the reaction of polymer 9b (166 mg, 0.634 mmol) in H_2O (5 mL), DACH-Pt (0.179 g, 0.380 mmol) in H_2O (4mL) afforded 0.743 mg (46.0%) of 9b-Pt as water soluble solid. Anal. found: Pt, 7.5%.

4.4.5 Polymer multidrug conjugation

These conjugates were synthesized in a two step process. The first step consisted of binding of the first drug system to the polymeric carrier followed by the anchoring of the second drug, both steps mediated by HBTU coupling agent.

Conjugate 4a(80)-MTX-Fc: *Step 1:* Carrier 4a(80) (200 mg, 0.195 mmol) and MTX (56 mg, 0.122 mmol) were dissolved in 6mL of NMP-DMF (4:1) upon heating. To this rapidly stirred solution at RT, was added drop wise over 10 min period HBTU(43 mg, 0.112 mmol) pre-dissolved in 1mL of DMF. This was followed by the addition of TEA (21 mg, 28 μL, 0.204mmol). The resulting yellow solution stirred at RT for 3.5 h, then precipitated and cooled in ice bath for 30 min. Thereafter, the routine work-up as in the preceding experiments, gave 150 mg (52.6%) of fully water soluble homoconjugate **4a(80)-MTX**.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 24H (24H, $N(CH_2\mathbf{Me})_2$); 1.5-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 8.7-6.8, 5H (5H, aromatic and heteroaromatic CH of MTX). These data indicate 100% of MTX incorporation, corresponding to 26.5% by mass.

Step 2: The homoconjugate 4a(80)-MTX (200 mg, 0,102 mmol) was dissolved in 4mL of NMP-DMF (4:1) upon heating. In separate vessel 4-ferrocenylbutanoic acid (41.3 mg, 0,15 mmol) and HBTU (42 mg, 0.11 mmol), dissolved in 2 mL of DMF, were stirred for 45min at room temperature, protected from direct light, then added in one dash to the carrier solution, followed by TEA (21 mg, 0,204 mmol). The resulting brownish solution

was saturated with N_2, and stirred at ambient temperature for 3.5 h, still protected from the light. The conjugate was precipitated with 10mL of Solumix-acetone (2:1), and then cooled in an ice bath for 30 min. Thereafter, the routine work-up as in the preceding experiments, gave 166mg (49.6%) of fully water soluble co-conjugate **4a(80)-MTX-Fc**.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 24H (24H, $N(CH_2\mathbf{Me})_2$); 1.5-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 4.1-4.2, 8.01H (9H, CH of ferrocenyl). These data indicate 89% of Fc incorporation, corresponding to 14.1% by mass, giving 94.4% (MTX-Fc) incorporation.

Conjugate 4c(80)-MTX-Fc: This conjugate was obtained using the procedure analogous to that leading to conjugate 4a(80)-MTX-Fc. A minor modification was introduced by replacing polymer 4a(80) by polymer 4c(80). Thus, the reaction of polymer 4b(80) (200 mg, 0.185 mmol), MTX (56 mg, 0.122 mmol), HBTU(43 mg, 0.112 mmol) and TEA (21 mg, 28 μL, 0.204 mmol), afforded 163 mg (58%) of homo-conjugate 4c(80)-MTX.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 8.7-6.8, 3.45H (5H, aromatic and heteroaromatic CH of MTX). These data indicate 69% of MTX incorporation, corresponding to 17.6% by mass.

The reaction of 4c(80)-MTX, (200 mg, 0.1 mmol), 4-ferrocenylbutanoic acid (41.3mg, 0,15 mmol), HBTU (42 mg, 0.11 mmol) and TEA (21 mg, 0,204 mmol) afforded 181 mg(55.2%) of co-conjugate, giving 50.2% (MTX-Fc) incorporation.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 4.1-4.2, 2.9H (9H, CH of ferrocenyl). These data indicate 32% of Fc incorporation, corresponding to 4.90% by mass.

Conjugate 4d(80)-MTX-Fc: The same experimental procedure leading to conjugate 4a(80)-MTX-Fc was applied. However, polymer 4a(80) was replaced by 4d(80). Polymer

4d(80) (200 mg, 0.176 mmol) was treated by MTX (56 mg, 0.122 mmol), HBTU(43mg, 0.112 mmol) and TEA (21 mg, 28 μL, 0.204 mmol), to afford 153 mg (55.3%) of homo-conjugate 4d(80)-MTX.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 10H (10H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$); 8.7-6.8, 4.35H (5H, aromatic and heteroaromatic CH of MTX). These data indicate 87% of MTX incorporation, corresponding to 21.62% by mass.

The reaction of 4d(80)-MTX, (200 mg, 0.1 mmol), 4-ferrocenylbutanoic acid (41.3 mg, 0,15 mmol), HBTU (42 mg, 0.11 mmol) and TEA (21 mg, 0,204 mmol) afforded 172 mg(53.5%) of co-conjugate, giving 67.7% (MTX-Fc) incorporation.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 10H (10H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$); 4.1-4.2, 9.5H (9H, CH of ferrocenyl). These data indicate 48% of Fc incorporation, corresponding to 7.2% by mass,

Conjugate 4a(80)-FA-Fc: *Step 1:* Carrier 4a(80) (200 mg, 0.195 mmol) and FA (54 mg, 0.122 mmol) were dissolved in 6 mL of NMP-DMF (4:1) upon heating. To this rapidly stirred solution at RT, was added drop wise over 10 min period HBTU(42 mg, 0.112 mmol) pre-dissolved in 1 mL of DMF. This was followed by the addition of TEA (21mg, 28 μL, 0.204 mmol). The resulting yellow solution was stirred at RT for 3.5 h, then precipitated and cooled in ice bath for 30 min. Thereafter, the routine work-up as in the preceding experiments, gave 150 mg (53.1%) of fully water soluble homoconjugate **4a(80)-FA**.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 24H (24H, $N(CH_2\mathbf{Me})_2$); 1.5-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 8.7-6.8, 5H (5H, aromatic and heteroaromatic CH of FA). These data indicate 100% of FA incorporation, corresponding to 25.94% by mass.

Step 2: The homoconjugate 4a(80)-FA (200 mg, 0,102 mmol) was dissolved in 4mL of NMP-DMF (4:1) with heating. In separate vessel 4-ferrocenylbutanoic acid (41.3mg, 0,15mmol) and HBTU (42 mg, 0.11 mmol), dissolved in 2 mL of DMF, were stirred for 45 min at room temperature, protected from direct light, then added in one dash to the carrier solution, followed by TEA (21 mg, 0,204 mmol). The resulting brownish solution was saturated with N_2, and stirred at ambient temperature for 3.5 h, still protected from the light. The conjugate was precipitated with 10 mL of Solumix-acetone (2:1), and then cooled in an ice bath for 30 min. Thereafter, the routine work-up as in the preceding experiments, gave 189 mg (56.9%) of fully water soluble co-conjugate **4a(80)-FA-Fc**.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.0-1.1, 24H (24H, $N(CH_2\mathbf{Me})_2$); 1.5-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 4.1-4.2, 6.4H (9H, CH of ferrocenyl). These data indicate 71.2% of Fc incorporation, corresponding to 11.40% by mass.

Conjugate 4c(80)-FA-Fc: This conjugate was obtained using the procedure analogous to that leading to conjugate 4a(80)-MTX-Fc. A minor modification was introduced by replacing polymer 4a(80) by polymer 4c(80). Thus, the reaction of polymer 4b(80) (200 mg, 0.185mmol), FA (54 mg, 0.122 mmol), HBTU(42 mg, 0.112 mmol) and TEA (21 mg, 28μL, 0.204 mmol), afforded 163 mg (59.0%) of homo-conjugate 4c(80)-MTX.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 8.7-6.8, 5H (5H, aromatic and heteroaromatic CH of FA). These data indicate 53,4% of FA incorporation, corresponding to 13.4% by mass.

The reaction of 4c(80)-FA, (200 mg, 0.1 mmol), 4-ferrocenylbutanoic acid (41.3 mg, 0,15 mmol), HBTU (42 mg, 0.11mmol) and TEA (21 mg, 0,204 mmol) afforded 168 mg(51.6%) of co-conjugate, giving 67.7% (FA-Fc) incorporation.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 2H (2H, $CH_2\mathbf{CH_2}CH_2NH_2$); 4.1-4.2, 5.0H (9H, CH of ferrocenyl). These data indicate 55% of Fc incorporation, corresponding to 8.54% by mass.

Conjugate 4d(80)-FA-Fc: The same experimental procedure leading to conjugate 4a(80)-FA-Fc was applied. However, polymer 4a(80) was replaced by 4d(80). Polymer 4d(80) (200 mg, 0.176 mmol) was treated by FA (54 mg, 0.122 mmol), HBTU(43 mg, 0.112 mmol) and TEA (21 mg, 28 μL, 0.204 mmol), to afford 151 mg (55%) of homo-conjugate 4d(80)-FA.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 10H (10H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$); 8.7-6.8, 4.9H (5H, aromatic and heteroaromatic CH of FA). These data indicate 98% of FA incorporation, corresponding to 9.70% by mass.

The reaction of 4d(80)-FA, (200 mg, 0.1 mmol), 4-ferrocenylbutanoic acid (41.3 mg, 0,15 mmol), HBTU (42 mg, 0.11 mmol) and TEA (21 mg, 0,204 mmol) afforded 197 mg(61.7%) of co-conjugate, giving 106% (FA-Fc) incorporation.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.5-1.7, 10H (10H, $CH_2\mathbf{CH_2}CH_2NH_2$, $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$); 4.1-4.2, 9.5H (9H, CH of ferrocenyl). These data indicate 100% of Fc incorporation, corresponding to 16.5% by mass.

CHAPTER 5: SUMMARY AND CONCLUSIONS

The success rates of present-day chemotherapeutic approaches toward severe afflictions such as cancer have remained low in spite of continuous improvements in drug structure and administration regimens. The unsatisfactory performance of chemotherapeutic agents in cancerous and related diseases is due primarily to lacking drug efficaciousness with concomitant high systemic toxicity, poor solubility and a tendency to induce drug resistance. Among various strategies, the bio-reversible binding of a medicinal drug to water-soluble and biocompatible carrier polymers represents an advanced technology that is designed to circumvent the critical pharmacological obstacles which the drug must clear for efficacious biological action. The polymer-drug conjugate resulting from the carrier-drug anchoring step acts as prodrug which protects the active drug while in circulation and delivers it in free form at the target site. Carrier conjugation thus serves to enhance the drug's therapeutic effectiveness.

Polymer-bound drug systems were investigated. Water-soluble macromolecular carriers and their drug conjugates were synthesized generally by established but modified synthetic methods. Carefully controlled conditions of time, temperature, and pH were observed throughout, and carrier polymers were routinely fractionated by dialysis in 12000, and ultimately 25000 molecular-mass cut-off membranes and collected by freeze-drying. The polymers were carefully designed in accordance with specific biomedical requirements, to include a biodegradable, flexible backbone containing hydrosolubilizing moieties and selected functional groups to which the drug models are anchored *via* amide and ester links to allow hydrolytic and enzymatic spacer cleavage and release of the monomeric drug in the tumor cell endosomal compartment. The polymeric carriers were synthesized with suitable molecular mass (25000-35000), high enough to avoid premature renal clearance, but sufficiently low to prevent toxic effects generally associated with the accumulation of high molecular-mass molecules especially in the kidneys.

Several carrier polymers were synthesized in this project, including polyaspartamides, ester-amine condensation polymers, and polyamidoamines. Functionalized polyaspartamides were obtained in 46-73% yield by aminolytic ring-opening process resulting from the nucleophilic attack of a number of selected amines on polysuccinimide, which in turn was originally obtained by high-temperature polycondensation reaction in the presence of phosphoric acid. These polyaspartamides were characterized by inherent viscosities in the range of 8-26mLg^{-1}.

The ester-amine polymers were obtained in 42-71% yield by base catalyzed polycondensation reactions under controlled conditions of temperature, in DMSO, so as to ensure the incorporation and integrity of unprotected hydroxyl functional groups in the polymer's main chain. These polymers were characterized by inherent viscosities in the range of 10-13mLg^{-1}. Polyamidoamines were synthesized by the addition of amine nucleophiles across a double bond (Michael-type addition), under carefully controlled experimental conditions to obtain both amide and secondary amine functions in the water-soluble polymeric main chain. They were obtained in 16-29% yield and characterized by inherent viscosities in the range of 20-26mLg^{-1}.

Most of the polyaspartamide carrier polymers, were conjugated with methotrexate (MTX) and with organoiron compound (ferrocene), whereas other polyamides were conjugated with 1,2-diaminocyclohexanediaquaplatinum(II)dinitrate (DACH-Pt aq). The ferrocenylation agent throughout this project was 4-ferrocenylbutanoic acid.

MTX and 4-ferrocenylbutanoic acid as well as folic acid were conjugated to pendant amino groups of various polyaspartamide carriers, through amidation reactions, *via* the HBTU-method. A very satisfactory degree of MTX and Fe were achieved. MTX content found was in range of 10-19% by mass, and Fe content found was in range of 6-13% by mass.

The anchoring of platination agent (DACH-Pt aq) proceeded *via* well established methods involving diamine functional groups as well as dihydroxylato ligands. Pt content found was in the range of 6-8% by mass. Platinum incorporation was satisfactory.

Let us now briefly discuss the concept of drug co-conjugation. This provides a route for the simultaneous delivery of two or more anticancer compounds. The expected benefits are synergistic effects, which can potentially lead to reduction in doses and side effects caused by high dose of single drug, and the prevention of the development of multi-drug resistance. Mutidrug-binding ability of polyaspartamide-type carriers was demonstrated by the co-conjugation of ferrocene and MTX as well as the co-conjugation of ferrocene and folic acid in a two step process involving HBTU coupling method. Degree MTX found was in range of 10-19% by mass and degree Fe found was in range of 6-13% by mass. Degree MTX, Fe and FA found were in range of17-27%, 15-16% and 24-26% respectively.

A number of selected conjugates was submitted to the Department of Immunology, University of Pretoria, and to the School of Pharmacy, University of California, Los Angeles, CA, for in vitro assessment of antiproliferative activity.

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APPENDIX

(Selected number of ^{1}H NMR spectra)