SYNTHESIS OF WATER SOLUBLE POLYMER – BOUND ANTIPROLIFERATIVE AGENTS

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A dissertation submitted to the faculty of Science, University of the Witwatersrand, in partial fulfilment of the requirements for the Degree of Masters of Science

Johannesburg, 2005

Declaration

I declare that this is my own unaided work. It is being submitted for the degree of Master of Science in the University of Witwatersrand, Johannesburg, South Africa. It has not being submitted for any degree or examination to any other University.

_____

Mark Trevor Johnson

*___**11th**___day of_____**January**_____, 2005*

ABSTRACT

Cancer is characterised by the unconstrained growth of cancerous cells, which damages the healthy cells and ultimately the tissue of the host. Chemotherapy forms an essential component in the treatment of this disease, however most anti-tumour drugs suffer from various deficiencies, e.g. increased toxicity, reduced serum half life and poor water solubility. The focus of this project was to address some of these deficiencies by conjugating selected drugs to a water-soluble polymeric carrier.

Selected water-soluble biodegradable carriers were synthesized. Copolyaspartamides, polyamidoamines and polyamides were obtained by condensation polymerisation, Michéal–type addition polymerisation and ester amine base-catalysed polymerisation. The nascent water soluble polymers were used to conjugate platinum, ferrocene and tetramethylmelamine derivative, respectively. The percentage drug in each polymer drug conjugate was determined by considering the mass of the drug in the conjugate as a percentage of the total mass of the drug-polymer conjugate.

Platinum was linked to the carrier via polymer attached amine, carboxyl and hydroxyl ligands. Platinum content of the conjugates ranged from 7 to 11 % by mass. The ferrocenylation agent, 4-ferrocenylbutanoic acid, and the tetramethylmelamine derivative, 3-(4,6-bis(N,N-dimethylamino)-1,3,5-triazacyclohexatrien-2-yl) propanoic acid was polymer-bound by amidation reactions. Iron content of the ferrocence conjugated ranged from 2 to 12 % by mass. While the drug content based on tetramethylmelamine in the 3-(4,6-bis[N,N-dimethylamino]-1,3,5-triazacyclohexatrien-2-yl) propanoic acid polymer conjugate ranged from 8.4 to 8.6 % by mass. There was a preliminary attempt to co-conjugate both, 4-ferrocenylbutanoic acid and 3-(4,6-bis[N,N-dimethylamino]-1,3,5-triazacyclohexatrien-2-yl) propanoic acid to the same polymer. This co-conjugate contained 2.9 % iron and 3.4 % tetramethylmelamine by mass.

For my Parents, for all they have done

ACKNOWLEDGEMENTS

I would like to thank the following people who made this work possible:

- Professor E.W. Neuse, my supervisor, for his most valuable guidance throughout this project. I appreciated his encouragement and constant support.

- To Richard of the Chemistry Department for the countless NMR spectra scanned for me throughout this project

- Mellon Bursary Scheme for a bursary grant (2002, 2003)

- To all my colleagues in the Polymer Laboratory. Their friendship and support made this experience worthwhile

- To my family for their endless support throughout this project

- To my wife for her love, patience and support throughout this project

- To the LORD for giving me the strength to complete this project

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LIST OF ABBREVIATIONS

AA	Atomic Absorption
AcCl	acryloyl chloride
AcCN	acrylonitrile
AIDS	Acquired Immune Deficiency Syndrome
APD	3-amino-1,2-propanediol
Asp	DL-Aspartic acid
aq	aqueous
bp	boiling point
d	days
DACH	1,2-diaminocyclohexane
DEM	diethyl malonate
Detart	Diethyl L-tartrate
DHEBA	(1,2-dihydroxyethylene)bisacrylamide
DMF	N, N-dimethylformamide
DMP	3-(dimethylamino)propylamine
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
EA	ethanolamine
EDDA	ethylenedioxy-O,O'-bis(2-ethylamine)
Et_2O	diethyl ether
Fc	ferrocenyl
η_{inh}	inherent viscosity

HMM	hexamethylmelamine
HBTU	2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
hrs	hours
M	molecular mass
MBA	N, N'-methylenebisacrylamide
mp	melting point
NEt_3	triethylamine
NMR	nuclear magnetic resonance
NaOH	sodium hydroxide
PDA	1,3-diaminopropane
ppm	parts per million
PSI	poly(DL-succinimide)
RNA	ribonucleic acid
RT	room temperature
THF	tetrahydrofuran
Tria	4,7,10-trioxa-1,13-tridecanediamine

CHAPTER 1

INTRODUCTION

> Cancer still remains one of the most dreaded diseases of the twentieth century, although in existence since the earliest time this disease still instills fear in the hearts of many. The disease is characterized by the uncontrollable cell division of cancerous cells, which progressively invade healthy tissue and ultimately kill the tissue and the host.[1]

Although most common diseases may be effectively treated, e.g. tuberculosis through appropriate medication and effective health care, cancer still remains a grave concern since no effective treatment has yet been developed and the disease still affects a large portion of the world's population Furthermore epidemiological evidence suggests that this number is increasing because of the following reasons;

1. increased urbanization resulting in environmental and dietary conditions encouraging the further spread of this disease.
2. the spread of AIDS which comprises the immune system and makes an individual more susceptible to the development of cancerous lesions and virus-associated neoplasms.

In South Africa the situation is as serious with cancer being the second highest cause of death in the white, colored and asian populations, and the third highest cause of death in the black population.[1]

Currently four approaches are used to treat cancer;

I. Surgery – the surgical, physical removal of the damaged tissue
II. Radiotherapy – the use of x-rays to destroy damaged tissue
III. Immunotherapy – activation of the patient's immune system, which then destroys the damaged tissue
IV. Chemotherapy – the use of various chemical agents to destroy the damaged tissue

Of these methods radiotherapy and chemotherapy remain the least invasive and the preferred alternative for widespread tissue damage. In addition chemotherapy has shown special promise as a long-term adjuvant therapy following surgery or radiotherapy. There has therefore been a concerted effort to develop novel chemotherapeutic agents and increase the efficiency of more established agents.

One approach to increase the efficiency of these more established agents has been to conjugate them to polymers, as an alternative drug delivery system. This has stimulated a flurry of research throughout the world, including the Polymer laboratory at the University of the Witwatersrand. This dissertation project represents a small contribution to this field.

Aims of the study

The objective of this project was to synthesize water-soluble macromolecular carriers, and subsequently conjugate them to platinum-based drug systems, a ferrocence derivative or a tetramethylmelamine derivative. Selected conjugates were then submitted for

biomedical assessment and toxicological tests. The background to this work will be discussed in *Chapter 2* where the importance of water-soluble carriers will be emphasized. Experimentally the following targets were defined;

Target 1: Synthesis of water-soluble macromolecular carriers bearing amine, hydroxyl or carboxyl groups

Target 2: Conjugation of macromolecular carriers to platinum, ferrocene or tetramethylmelamine to form carrier-drug conjugates

Target 3: Submission of selected conjugates for biomedical and toxicological tests

CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

As the area of cancer chemotherapy, polymer drug conjugation and the various drug models used in this current project have been extensively studied internationally and the publication rate in this field remains prolific, I have restricted my survey to only a small, select number of review articles and original publications in writing this review.

2.1. Introduction

Cancer remains one of the primary causes of mortality both in South Africa and throughout the world[1], consequently there has been an intensive research effort to develop various therapies. Four types of therapies currently exist;

I. Surgery – the surgical, physical removal of the damaged tissue
II. Radiotherapy – the use of x-rays to destroy damaged tissue
III. Immunotherapy – activation of the patient's immune system, which destroy the damaged tissue.[2]
IV. Chemotherapy – the use of various chemical agents to destroy the damaged tissue.[3, 4, 5]

Of these methods radiotherapy and chemotherapy remain the least invasive and the preferred alternative for widespread tissue damage. In addition chemotherapy has shown special promise as a long-term adjuvant therapy following surgery or radiotherapy. There has therefore been a concerted effort to develop novel chemotherapeutic agents and increase the efficiency of more established agents.

2.2. The Chemotherapeutic Treatment of Cancer

The use of drugs to treat human disease is not a new concept and stretches far back as the ancient Egyptian civilisation. However the modern era of chemotherapy began with the introduction of nitrogen mustard for the treatment of Hodgkins disease. Subsequently steroids were used to treat prostate cancer and the antimetabolite, aminopterin caused the remission of acute leukaemia in children.[6]

Presently there are numerous anti-tumour agents which are divided into various classes depending on their mode of action, these include; alkylating agents, antimetabolites and antibiotics. Some of these may interact directly with tumour cells while others exist as prodrugs and must be activated, either in the tumour cell or in the liver to produce its desired effect.[4, 5]

Alkylating Agents

Alkylating agents are a diverse family of compounds which share the following distinguishing characteristic; they have the ability to combine with cellular nucleophiles, primarily N- and O- nucleophilic groups on proteins and nucleic acids. This feat permits them to impair DNA replication during mitosis. A drug which functions thus is the alkylating agent nitrogen mustard; this drug contains a reactive ethylamine which binds to the N7 of the guanine molecule preventing the unwinding of the DNA double helix and its subsequent replication. In clinical practice these drugs are used to treat acute and chronic leukaemia, myeloma, non-Hodgkins lymphoma and Hodgkins disease.[7]

Antimetabolites

Antimetabolites are synthetic analogues of essential naturally occurring substances required in the metabolism of normally proliferating cells. These drugs achieve their effect by mimicking an important biological molecule and thus inhibiting an essential biological process, e.g. Methotrexate binds irreversibly to the enzyme dihydrofolate reductase, which stops the production of reduced folic acid thereby inhibiting thymidylate synthesis and the production of DNA which is necessary for cell replication.

These drugs are used in the treatment of lymphoblastic leukaemia, Burkitt's lymphoma and other lymphomas.[7]

Antibiotics

These are naturally occurring anti-tumour agents and may be obtained from bacteria or plants. Their mode of action is numerous and includes;

- bind to the enzyme topoisomerase II and stimulate strand breakage or sealing
- poison the microtubule that forms during the process of mitosis
- inhibiting RNA and thus blocking protein synthesis

This class of anti-tumour agents is used in treating myeloid, acute lymphoblastic leukaemia, small cell lung cancer, ovarian cancer and a wide variety of heamatological malignancies.[7]

2.3. The Polymer-Drug Delivery System

However, all chemotherapeutic agents, both novel and established share the following deficiencies to various degrees;

I. Poor water solubility of the agent and thus poor bio-distribution.[3, 4]
II. Poor specificity of the agent and thus increased toxicity.[4]
III. Rapid excretion of the agent thus requiring continual administration.[3,4]

A novel approach, which addresses all these problems and may be applied to both novel and established agents, recently gained momentum.[8, 9, 10] This approach uses a drug delivery system to deliver the therapeutic agent to the site of action, offering the advantage that it increases the efficiency of all drugs (both established and novel drugs).

The rationale behind the drug delivery system is as follows: It is well established that growing tumours establish their own blood supply. This neovasculature is hyper-permeable (leaky to macromolecules). In addition to this enhanced permeability, tumour tissues also have an inadequate lymphatic system so that macromolecules are not efficiently drained from the tumours and remain trapped and concentrate in these tissues. An anti-tumour agent coupled to a suitable polymeric delivery system, therefore, has the potential of dramatically increasing the concentration of an anti-tumour agent within the tumour tissue. Indeed this has been found, and in certain instances the concentration of polymer-drug conjugates in tumour cells have reached levels of 10-100 times higher than that of the free drug. This phenomenon has been described as, enhanced permeability and retention – EPR effect).

In addition, the systemic toxicity of the native drug was found to be dramatically reduced when conjugated to a carrier.[11, 12] These extremely promising effects have resulted in a rigorous research effort to develop numerous drug delivery systems. These included;

1. Hydrogels – these are water-soluble cross-linked polymers in which the drug is physically entrapped. These polymers then swell in water and release the drug at a regular rate.[13, 14, 15]
2. Liposomes – a synthetic cellular membrane consisting of a hydrophobic core which contains the drug and a hydrophilic exterior which allows the drug to be carried in the circulation to the target tissue.[16, 17]
3. Immuno-conjugates – The drug is covalently bound to an antibody which targets the drug to the affected tissue

4. Polymeric molecular conjugates – The drug is covalently bound to a synthetic polymer, which directs it to the target tissue.[18, 19, 20]

These delivery systems were investigated because they were found to meet the following criteria to varying degrees;

- *Increased water solubility*
- *Biocompatibility*
- *Increased specificity*
- *Selective release rate*
- *Decreased excretion rate*

In this project it has been decided to concentrate specifically on synthetic water-soluble polymers as an appropriate drug delivery system. This system has been chosen because of the following reasons;

i. Synthetic polymers provide a controlled delivery system in which the polymer may be tailor-made to include a drug anchoring and a water soluble portion.

ii. The water soluble portion may be regulated to ensure water solubility of the carrier, resulting in efficient delivery of the drug in the aqueous phase of the vasculature and the intraperitoneal cavity. This smooth dissipation of the drug enhances bioavailability and reduces the risk of capture by the reticulo-endothelial system.

iii. Biologically 'friendly' polymers may be synthesized which result in a reduced immune response. In addition polymer drug-conjugates have shown reduced toxicity compared to the free drug.

iv. Polymer drug conjugates may be synthesized so that they stimulate the enhanced uptake of the drug via the endocytic process irrespective of the drug present. In addition they may be tailor made so as to selectively target tumour cells e.g., the strongly cationic polymer may selectively be attracted to the negatively charged membrane present in some tumour cells.

v. The molecular-mass of synthetic polymers may be tailored so as to maintain the correct balance to ensure entrapment inside tumour cells via the EPR effect and reduced excretion by the renal system. In contrast, increased toxicity, as observed with a very high molecular-mass polymer will be impeded.

vi. A polymer-drug carrier provides temporary protection against enzymatic attack, serum binding proteins and other depletion mechanisms to which the conjugate may be exposed, while being transported in the vasculature or the intraperitoneal cavity. This protection reduces, and in certain instances prevents, the premature release of the drug from the polymer.

vii. The anchoring group within the polymer carrier may be effectively modified so that the polymer may carry more than one anti-tumour agent.

2.4. Bioactive Agents

Within the scope of this dissertation it has been decided to concentrate on three specific bioactive agents; these included the coordination compounds of the square-planar platinum (II) type, organoiron compounds of the ferrocene type and the drug, hexamethylmelamine

2.4.1. Platinum Analogues

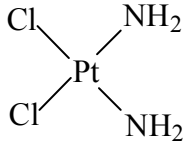
While there are numerous metal containing anti-tumour drugs,[21] only the platinum drugs have reached the level of routine clinical administration. The primary drug in this family, cisplatin, was discovered accidentally by Rosenberg in the mid sixties.[22, 23] Furthermore he found that although two forms of the complex exist, only the cis-isomer of this platinum complex was effective[22, 23].

The complex, cisplatin has been used extensively both as a single agent and in combination therapy against various tumours, including; cervical, ovarian, prostrate and testicular cancer. Consequently the clinical performance and the mechanism of cytoxicity of this complex has been extensively studied and is the subject of numerous review articles.[24, 25]

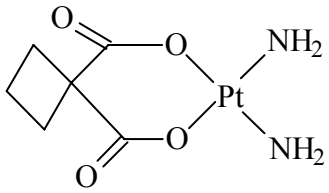
It is believed that cisplatin exerts its potent anti-tumour effect as follows; in the aqueous biological environment cisplatin is hydrolysed and forms both the monoaqua and diaqua derivative in which the chloro ligands are replaced by water molecules. These complexes subsequently form a variety of hydroxyl species through deprotonation. These modified complexes interact with various small and large molecules from phosphate groups to α-amino acids[26] and RNA. However the primary interaction occurs between the modified cisplatin complex and the N-7 portion of guanine and adenine. This interaction results in the formation of intrastrand cross-links[27] within the DNA helix, and the formation of DNA lesions which cannot be repaired by the tumour cell, resulting in its death.

Although cisplatin is efficient, it has numerous side effects e.g. nephrotoxicity and rapid excretion[28] which must be prevented. There has therefore been an intensive research effort to develop more efficient and less toxic drugs. Recently these efforts have produced some promising analogues e.g. tetraplatin and cis-diaminetetrachloro platinum(IV).[29] In addition novel second-generation platinum complexes containing carboxyl or hydroxyl ligands have also been developed, these complexes show special promise as they have reduced toxicity and are efficient against cisplatin-resistant tumour cell lines. **Fig. 1** illustrates cisplatin and other platinum analogues of clinical interest.

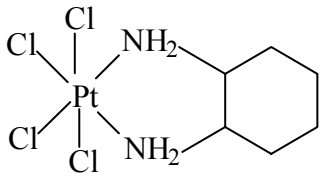
cisplatin



carboplatin



tetraplatin



DACH-platinum

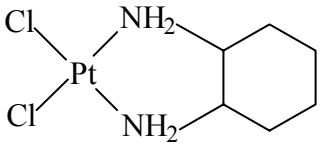


Fig. 1 Cisplatin and other platinum analogues

2.4.2. Hexamethylmelamine (HMM)

Hexamethylamine has been evaluated as an antineoplastic for more than twenty–five years, during which period this agent has shown limited activity against various tumour systems, Dunnings leukaemia, reticulum cell sarcoma and various murine sarcomas. Recently this agent has also shown special promise as a synergistic agent in combination with cisplatin and other platinum analogues.[30]

Although the precise mechanism by which HMM exerts its effect is not known, the following mechanism has been suggested.[31] Following administration the HMM is metabolically activated by cytochrome P450 to form a methylated HMM, carbinolamine and formaldehyde. Methylated HMM and carbinolamine covalently interact with proteins while the other by-product, formaldehyde, interacts directly with DNA (**fig. 2**), thus preventing the cell replication of tumour cells and their unrestricted multiplication

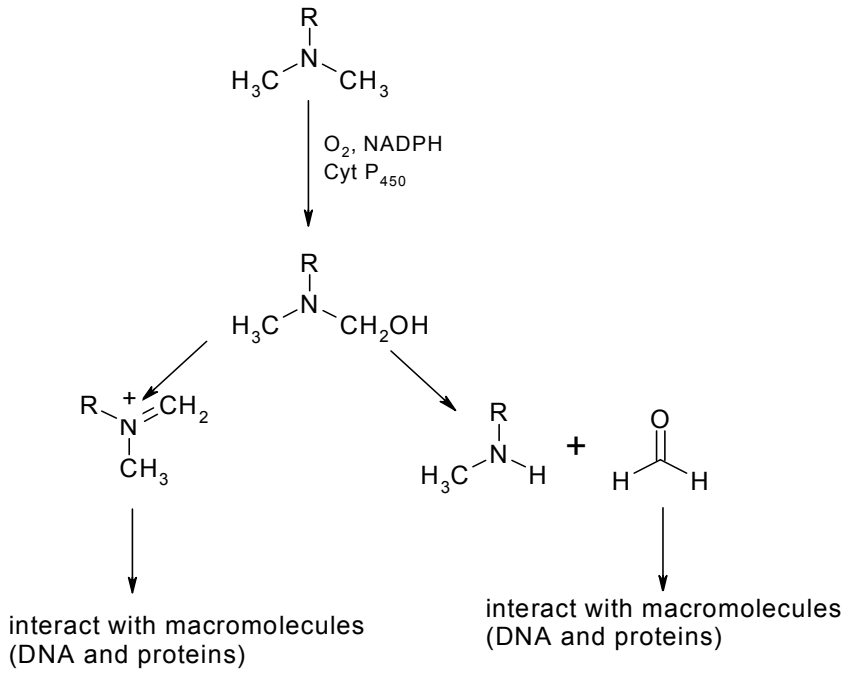


Fig. 2 Metabolism of HMM and its suggested mode of anitumour activity

2.4.3. Ferrocene

Ferrocene is a small, stable synthetic iron compound, which has numerous catalytic activity effects and a proposed anti-tumour activity.[32, 33, 34, 35] Two mechanisms have been proposed to account for the anti-tumour effect of ferrocene. The first involves an indirect mechanism whereby, ferrocene induces it's anti-tumour effect by enhancing the immune response. It achieves this by increasing the release of soluble factors, e.g. interferon γ, that are released from ferrocene-activated lymphocytes, which in turn stimulate peritoneal macrophages.[36]

The second concept also involves an indirect mechanism whereby the oxidised form of ferrocene, ferrocenium, quenches a free radical species[37], thus preventing the metastasis (recognized that free radicals are important carcinogens and their rapid neutralization provides a promising approach in terms of, the prevention of the development of tumor cells). The mechanism whereby, ferrocene neutralizes a free radical occurs as follows;

The ferricenium ion generated in a one-electron oxidation step from the neutral ferrocene complex reacts with the oxygen radical to form a dioxygen and ferrocene (**fig. 3**). The ferricenium cation may also undergo free radical combination reactions by interaction with other free radical species. The subsequent product undergoes re-arrangement from the metal substituted product to the ring substituted products, followed by deprotonation, which leads to an uncharged substituted ferrocene

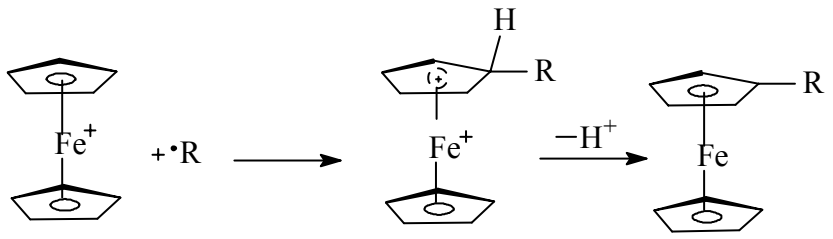


Fig. 3 Mode whereby ferrocenium ion neutralises free radicals

This anti-tumour activity has prompted numerous investigations, the most promising being a comparative study in which various fericenium salts were evaluated for the antiproliferative effects against several human tumour clonongenic cell cultures. The study showed that, although ferrocene (insoluble in water) showed no antiproliferative effects, the soluble ferricinium salts were found to be active. Furthermore, a moderate activity was also identified for ferrocenylacetic acid. These results were extremely promising in that they suggested;

i. That ferrocene could be administered in its neutral form provided the ferrocene derivative had improved water solubility

ii. That ferricinium salts, although effective, were not suitable for long term therapy as they were unstable at physiological pH 7.4 and would undergo reduction *en route* to the target tissue, thus nullifying their effect

The observation that ferrocenylacetic acid was effective as an antiproliferative agent prompted a separate investigation in this laboratory in which the effect of the carboxyl side chain length on the reduction potential of the ferrocene-ferricinium couple was investigated. It was found that the ferrocenylbutanoic acid gave the lowest positive reduction potential, thus offering optimal cation stability in biological environment.

Consequently ferrocenylbutanoic acid has been used primarily in this laboratory for the synthesis of ferrocene conjugates. Typically, the ferrocenylbutanoic acid is bound to the polymer chain via its carboxyl group to form an amide linkage (**Fig. 4**).

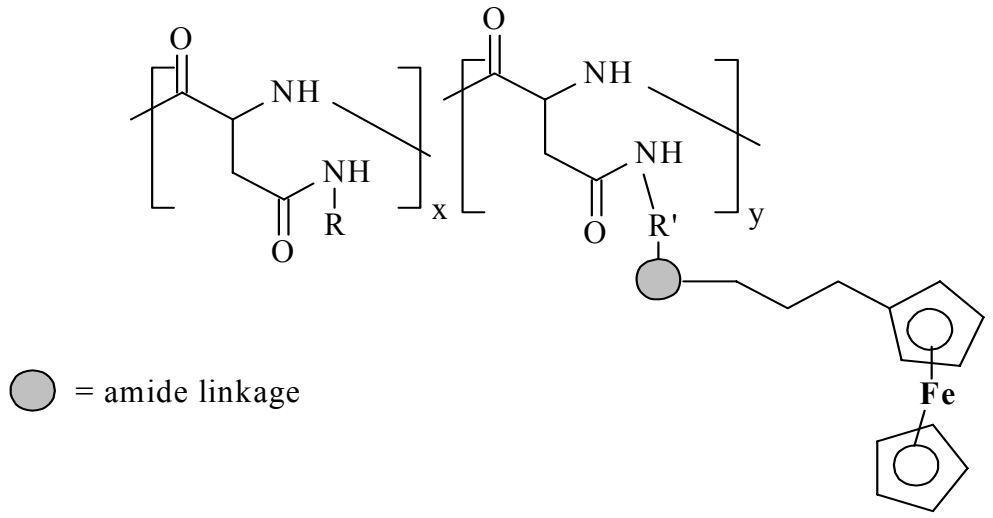


Fig. 4 General structure of an amide bound ferrocenyl butanoic acid to a water soluble carrier

Preliminary biological results of polymer-bound ferrocenylbutanoic acid have shown excellent results against the human colon cancer cell line[38] and thus have provided the impetus for the inclusion of this drug in the present dissertation.

CHAPTER 3

RESULTS AND DISCUSSION

Considering the targets highlighted in chapter one, this dissertation project was carried out within the framework of the following work phases;

Phase 1: Synthesis of water-soluble macromolecular carriers bearing amino, hydroxyl or carboxyl groups

Phase 2: Conjugation of macromolecular carriers with selected antineoplastic drug models e.g. ferrocene, tetramethylmelamine or platinum type drugs to form carrier drug conjugates

Phase 3: Submission of selected conjugates for biomedical and toxicological tests

3.1 SYNTHESIS OF MACROMOLECULAR CARRIERS

For the synthesis of the macromolecular carriers emphasis was placed on carriers which met the criteria highlighted in section 2.3. Carriers which met these requirements were all of the polyamide-type and conformed to the following general structure, indicated below;

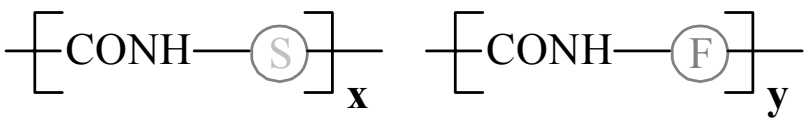


Fig. 5 Polyamide-type model

In this model, S stands for an extrachain- or intrachain-type group required to provide water solubility of the final conjugate, and F represents a functional group, inside or outside the main chain, capable of reversibly binding the drug species of choice. Amine, dicarboxyl and dihydroxyl functional groups were used as alternative drug anchors. These various functional groups offered different advantages, e.g. the dihydroxyl and dicarboxyl functional groups held the drug by chelation and could be released rapidly by hydrolytic dissociation with immediate displacement of the drug by aqua ligands or other biological nucleophiles, while the amine functionality allowed for the formation of the amide linkage, requiring enzymatic cleavage and thus providing a slow release system.

In this project fissionable groups were also directly incorporated directly into the main chain so as to allow for the regular release of the from the main chain.

3.2. POLYASPARTAMIDES

α, β-DL-Polyaspartamides were chosen as one of the primary water-soluble carriers to be used in this dissertation for the following reasons;

i. α, β-DL-Polyaspartamides (PSI) may be conveniently synthesized from readily available starting material (DL-aspartic acid).
ii. The molecular mass of the PSI on average ranges from 25000 to 35000 which is well within the desirable range to prevent inherent polymer toxicity, but still large enough to prevent premature excretion by the renal system, thus facilitating increased drug circulation time.

iii. The structure of the copolyaspartamides may be reasonably controlled by regulating the feed ratio of the two nucleophiles participating in the ring opening reaction, thus controlling the ratio of the drug solubilizing to the drug anchoring component in the polymer chain.

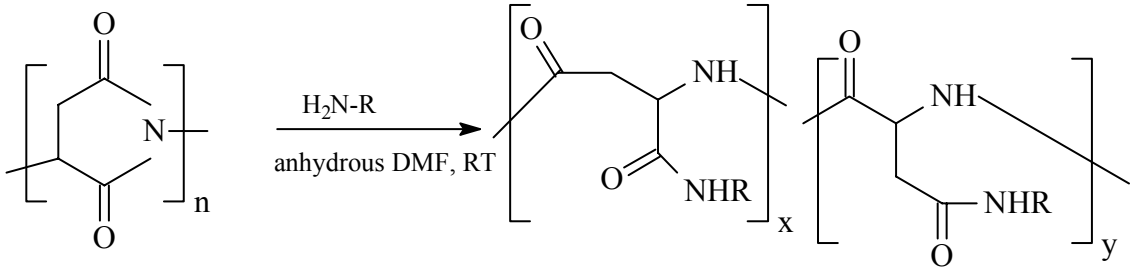
iv. The intrachain amide linkages provide for the gradual catabolism of the spent polymer chain, thus preventing excessive accumulation of the polymer in the body

v. The ability to increase the ratio of the hydrosolubilizing portion relative to the drug anchoring portion in a copolymer allows one to regulate the quantity of drug to be incorporated into the polymer chain

vi. The introduction of tertiary amines as subunits into the polymer chain enhance both the solubility of the polymer and its affinity to target cells owing to increased pinocytic cell uptake.[39]

Polyaspartamides are routinely prepared from poly-DL-succinimide by nucleophilic attack of the imide ring and its subsequent ring opening. The ring opening is mediated by a mono-functional reagent, generally a mono-functional amine, under anhydrous conditions in a dipolar aprotic solvent at room temperature (**Scheme 3.2a)**, in subsequent schemes only the alpha-peptide linkages will be illustrated for clarity. The resulting polyaspartamides consist of both alpha and beta peptide linkages. The poly-DL-succinimide precursor was synthesized by the high temperature solution polymerization of DL-aspartic acid in orthophophosphoric acid according to the method described by Neri and Antoni.[40] In this reaction the orthophosphoric acid serves as both the solvent

and the condensation agent. The isolated crude polymer is treated with dicyclohexylcarbodiimide to facilitate further chain extension.



Poly-D L-succinimide (PSI) *beta peptide linkage* *alpha peptide linkage*

Poly-D L aspartamide

Scheme 3.2a: Synthesis of the copolymer, poly-α, β-DL-aspartamide

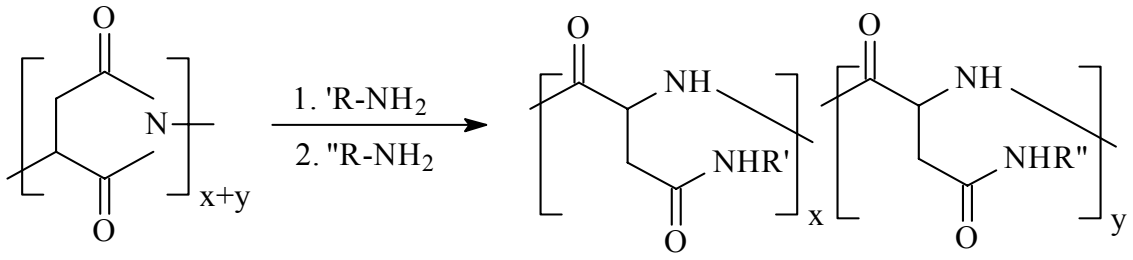
As mentioned above, the polyaspartamides are prepared by the nucleophilic ring opening of the imide ring; numerous nucleophiles may be used independently or together to open the ring, and various polyaspartamides have been thus synthesized. The polyaspartamides have therefore been divided into various classes depending on the type of side chain present on the polymer main chain;

- *homopolymer* – consists of only one type of side chain within the polymer chain
- *copolymer* – consists of two different types of side chain within the polymer chain
- *terpolymer* - consists of three different types of side chain within the polymer chain

Within the structure of this dissertation work was focused specifically on the synthesis of several copolymers. These copolymers generally consisted of two distinct subunits which were randomly distributed in a fixed ratio in the polymer chain;

- R' group which contained a hydroxyl or a tert-amino terminal group and represented the hydro-solubilizing (and in certain instances targeting) portion of the chain
- R" group which comprised a primary amino, hydroxyl or dicarboxyl terminal group and provided drug anchoring site on the chain

The copolymers were prepared according to established procedures[41, 42, 43, 44] developed in this laboratory, and involved the stepwise addition of two or more different amines in a given stochiometric feed ratio. This resulted in a copolymer in which the subunits were randomly distributed along the polymer chain in a pre-determined ratio (**scheme 3.2b**).



Poly-D, L-succinimide (PSI) *copolyaspartamide*

Scheme 3.2b: Structure of the copolymer, poly-α,β-DL-aspartamide

In this project synthetic efforts were focused specifically on two types of copolyaspartamide;

- copolyaspartamide carriers in which the anchoring subunit remains constant but the hydrosolubilizing subunit varies between a hydroxyl terminal and a tertiary amino terminal group, in order to compare the effectiveness of the hydroxyl containing polymer with that of a tertiary amino containing polymer, in terms of drug targeting and solubility

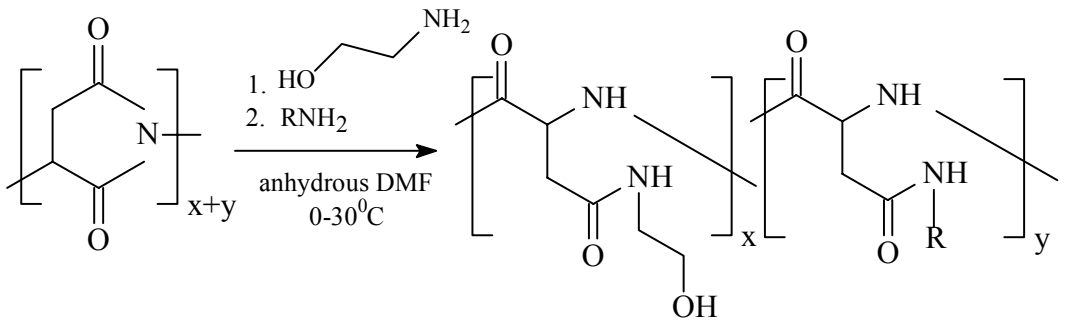
- copolyaspartamide carriers in which the anchoring subunit varies between a hydroxyl, dicarboxylato or amino terminal group, in order to bind platinum, ferrocene and tetramethylmelamine, respectively.

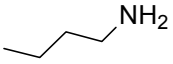
3.2.1. Copolyaspartamide carriers in which a hydroxyl is part of the solubilizing group

Within this class of copolymers, the structure of two distinct types of copolymers was considered;

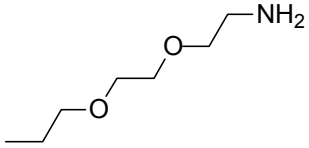
- Hydroxyl-terminated water-soluble copolyaspartamides in which a primary amino group is present in the anchoring subunit of the polymer chain. The advantage of these polymers is that they provide an anchoring site for carboxyl containing drugs, e.g. ferrocenylbutanoic acid and the β-alanine-functionalized triazine compound.
- Hydroxyl-terminated water-soluble copolyaspartamides in which a dicarboxyl group is present in the anchoring subunit of the polymer chain. The advantage of these polymers is that they provide an anchoring site for the drug, DACH-Pt aq

In each of these water-soluble copolymers the R group illustrated in **scheme 3.2b** above consisted of a 2-hydroxyethyl substituent and the reaction proceeded as illustrated in **scheme 3.2.1a and 3.2.1b**



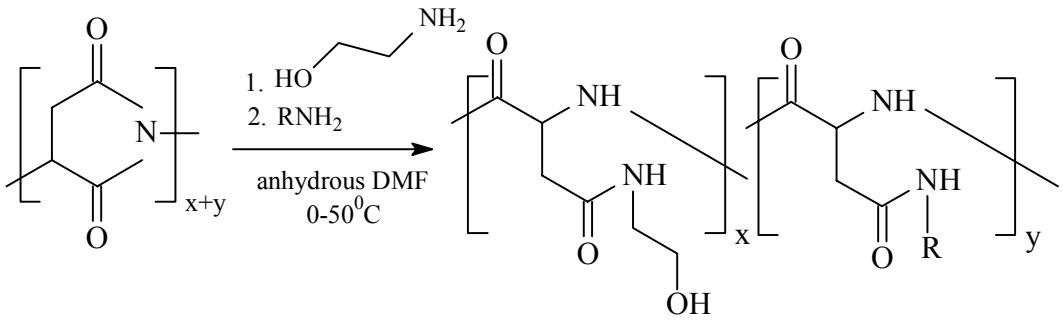
1a R = 

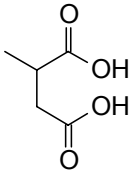
expected *x:y = 9:1*
found *x:y =9:1*

1b R = 

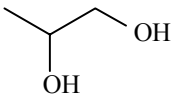
expected *x:y = 9:1*
found *x:y =9:1*

Scheme 3.2.1a: Copolyaspartamides 1a, **1b**



1c R = 

expected *x: y = 1:1*
found *x: y = 1:1*

1d R = 

expected *x: y = 9:1*
found *x: y = 9:1*

Scheme 3.2.1b: Copolyaspartamides 1c, **1d**

The formation of the copolyaspartamides occurred in a two step process. In the first step a given amount of ethanolamine (EA) was allowed to react with the PSI at room temperature for a period of 6-8 hrs. Afterwards this solution was added drop-wise to the latter reagent 1,3-diaminopropane (PDA), ethylenedioxy-O,O'-bis(2-ethylamine) (EDDA) or 3-amino-1.2-propanediol, present in a three fold excess. It was found that this order of addition provided the correct balance to ensure complete ring opening of the remaining succinimide units, while preventing cross-linking of the polymer via the introduced amino terminal groups. The reaction was conducted under strictly anhydrous conditions in order to prevent undesired hydrolytic ring opening of the succinimide units to form free carboxylic acid side groups.[45]

For the dicarboxyl containing copolyaspartamides (**scheme 3.2.1b**) the reaction proceeded slightly differently. In the first step a four fold excess of aspartic acid was allowed to react with the PSI over a period of 48 hrs at temperatures ranging from 20-50 ^{0}C, to ensure that the aspartic acid reacted with at least 50 % of the succinimide units. Afterwards, this suspension was added drop-wise to the required amount of EA needed to completely open the remaining succinimide ring, (in this instance it was found that a 10 % excess of EA was sufficient). The reaction then proceeded for a further 8 hrs at room temperature, again, under strictly anhydrous conditions in order to prevent needless hydrolytic ring opening of the succinimide units.

The polymeric products were isolated as completely water-soluble solids following a series of steps which included filtering, precipitation with an organic non-solvent, extensive washing, aqueous dialysis (finally in tubing with a 25000 molecular-mass cut-off limit), and freeze-drying. The polymers were obtained in yields ranging from 37 to 57 %**,** with inherent viscosities ranging from 6 to 9 mL g^{-1} (**table 3.2.1a**)**.** Following isolation the polymers were characterized by ^{1}H NMR spectroscopy (**table 3.2.1b**); analysis of the water-soluble copolyaspartamides yielded the following results;

In most cases the ^{1}H NMR spectra confirmed the structure of the polymer, this was indicated by the position of the various peaks in the spectra and the ratio in which they were found. Furthermore integration of the various peaks in most cases produced a proton count which matched that predicted by the ideal structure. However in certain instances there was a large discrepancy between the proton count predicted and that found, e.g. **1a**. In this instance the polymer was still deemed useful as the ratio of the solubilizing to anchoring group within the polymer was still acceptable.

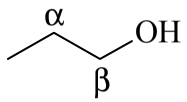
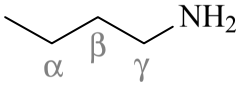
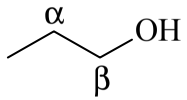
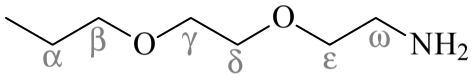
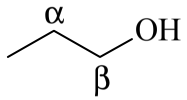
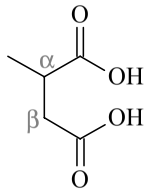
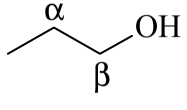
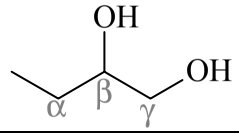
The experimental conditions and the analytical data are recorded in table **3.2.1a** and **b**.

Table 3.2.1a: Outline of the experimental conditions for the synthesis of copolyaspartamides, **1a-1d** from polysuccinimide (schemes 3.2.1a and 3.2.2a)

Concentration of reactants added (mol%)[a]			**Reaction Conditions**[b]	**Polyaspartamides**			
X	**y**	**x/y**[a]		**Polymer Design.**	**Yield**[c] **%**	η_{inh}[d] **mL.g^{-1}**	**Molar mass/ recurring unit**[e] **g/mol**
EA (90)	PDA (30)	9	**1.** 8hrs at RT, **2.** 16hrs at 0-5^{0}C and 24hrs at RT	**1a**	57	9	1614.2
EA (90)	EDDA (30)	9	**1.** 8hrs at RT, **2.** 16hrs at 0-5^{0}C and 24hrs at RT	**1b**	49	8	1650.6
EA (55)	D, L-Asp (100)	1	**1.** 48hrs at 20-60, **2.** 8hrs at RTC	**1c**	37	6	501.3
EA (90)	APD (30)	9	**1.** 8hrs at RT, **2.** 16hrs at 0-5^{0}C and 24hrs at RT	**1d**	53	8	1593.4

[a] ratio of anchoring group to solubilzing group in the polymer
[b] room temperature, 20 ± 2 ^{0}C
[c] after dialysis in 12000 and 25000 molecular mass cut off
[d] in de-ionised water, 37 ± 0.5 ^{0}C
[e] molar mass of simplest recurring unit, structures normalised to y=1

Table 3.2.1b: ^{1}H NMR data for copolyaspartamides **1a-1d**

Polymer Designation	R_1	αααR_2	^{1}H NMR[a, b]			
			Shift range δ (ppm)	Proton count		Assignment
				expected	found	
1a			3.7-3.5 3.4-3.2 2.8-2.5 1.6-1.4	18 20 22 2	18 18 12 2	β α, α Asp CH_2 and with a yield of β
1b			4.7-4.5 4.0-3.5 3.5-3.0 3.0-2.5	10 26 20 22	10 26 20 21	Asp CH β, β, γ,δ, ε α, α Asp CH_2 and ώ
1c			4.7-4.5 4.0-3.5 3.5-3.0 3.0-2.5	3 2 2 6	1 2 2 3	Asp CH and α β α Asp CH_2 and β
1d			4.0-3.5 3.5-3.0 3.0-2.5	21 20 20	21 19 21	β, β, γ α, α Asp CH_2

[a] in D_2O or in D_2O at pD 9-10

[b] Reference against sodium-3-(trimethylsilyl-2,2,3,3-d_4-propionate. Integration error limits ±15 %, protons calculated and rounded off to the nearest integer

3.2.2. Copolyaspartamide carriers in which an amino side chain is part of the solubilizing group

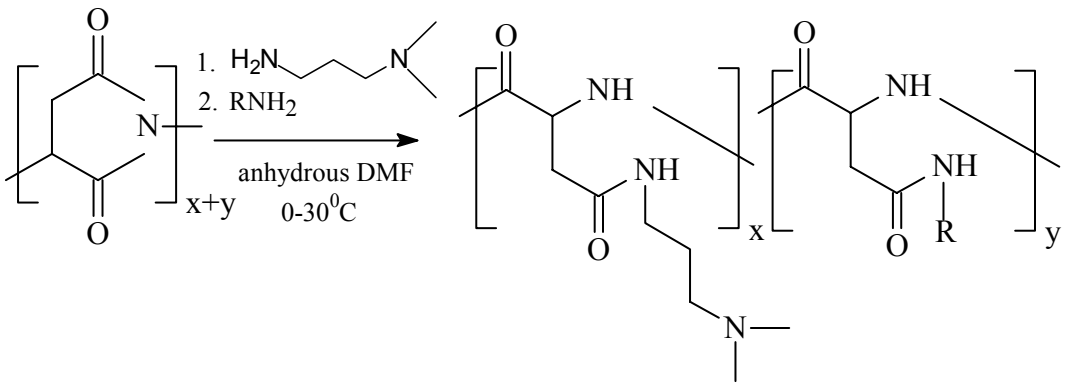
Within this class of copolymers, two distinct types of copolymers were synthesized;

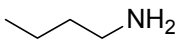
- Amine-terminated water-soluble copolyaspartamides in which a primary amino group is present in the anchoring subunit of the polymer chain.
- Amine-terminated water-soluble copolyaspartamides in which a dicarboxyl or dihydroxyl group is present in the anchoring subunit of the polymer chain.

In each of these water soluble copolymers mentioned above the R' group illustrated in ***scheme 3.2b*** consisted of 3-(N, N-dimethylamino)propylamine (DMP). This compound was favoured because it contained a tertiary amino group which is strongly basic and easily protonated under physiological conditions, thus producing a charged cationic terminal group which offer two advantages;

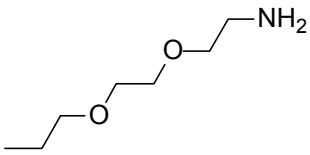
- acting as a solubilizing agent, enhancing the solubility of the polymer
- acting as a cell targeting agent, being preferentially attracted to tumour cells due to the very high negative charge present on the surface of selected tumour cells

The DMP copolyaspartamides were synthesized according to **scheme 3.2.2a** and **3.2.2b**.



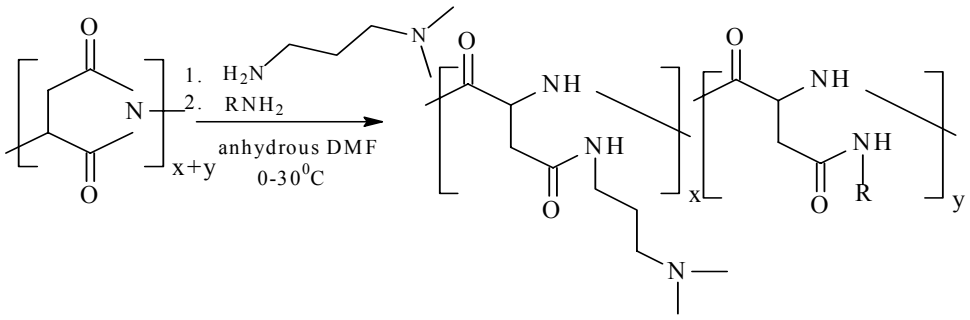
1e R =  *expected* *x:y = 3:1*

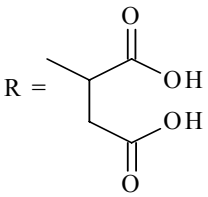
found *x:y = 3:1*

1f R =  *expected* *x:y = 9:1*

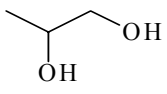
found *x:y = 9:1*

Scheme 3.2.2a: Copolyaspartamides 1e, 1f



1g R =  *expected* *x:y = 1:1*

found *x:y = 1:1*

1h R =  *expected* *x:y = 9:1*

found *x:y = 9:1*

Scheme 3.2.2b: Copolyaspartamides 1g, 1h

The synthetic approach followed was similar to that discussed in section 3.2.1 (hydroxyl-functionalized copolyaspartamide carriers). It involved the two-step aminolytic ring opening of the PSI. In the first step, DMP was allowed to react directly with the PSI for a period of 6hrs at room temperature. In the second step this solution was added drop-wise to the required amount of PDA, EDDA, 3-amino-1,2-propandiol (in all these cases a three fold excess was required to ensure complete ring opening of the succinimide subunits and prevent cross linking). Again the reaction proceeded in DMF under strictly anhydrous conditions.

For the dicarboxyl-containing copolyaspartamides (**scheme 3.2.2b**) the reaction proceeded similarly to that described in **scheme 3.2.1b**. In the first step a four fold excess of aspartic acid was reacted with the PSI over a period of 48 hrs at a temperature of 45 ^{0}C, to ensure that the aspartic acid reacted with at least 50 % of the succinimide units. Afterwards this suspension was added drop-wise to the required amount of DMP needed to completely open the remaining imide rings (in this instance it was also found that a 10 % excess of DMP was sufficient). The reaction then proceeded for a further 8 hrs at room temperature under strictly anhydrous conditions.

All the DMP-based copolyaspartamide polymers were isolated as described in Section 3.2.1, in yields ranging from 41 to 61 %, and inherent viscosities ranging from 6 to 8 mL g^{-1} (***table 3.2.2a***). Following isolation the polymers were characterized by ^{1}H NMR spectroscopy (***table 3.2.2b***), analysis of the water soluble DMP based copolyaspartamides yielded the following results;

In most cases the ^{1}H NMR spectra confirmed the structure of the polymers, e.g. polymer **1e** contained a cluster of peaks in the region 4.7-1.5 ppm. Within this cluster, peaks were found which were characteristic for DMP, PDA and the aspartamide polymer, respectively (***Table 3.2.2a***). However in other instances the ^{1}H NMR spectra did not match the predicted structure, e.g. **1g** there was a slight a deficiency of DL-aspartic acid. This may be due to;

- poor solubility of aspartic acid in the aprotic solvent resulting in a sluggish reaction
- poor nucleophilic character of the nitrogen group of aspartic acid, thus facilitating poor addition of aspartic acid to the succinimide ring.

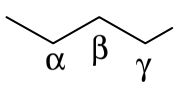
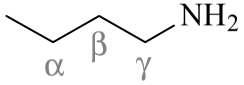
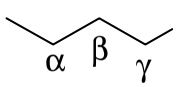
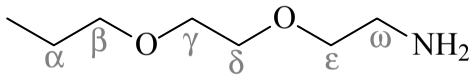
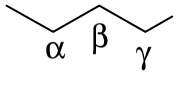
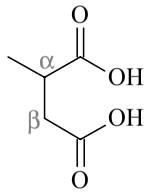
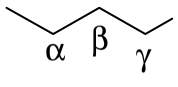
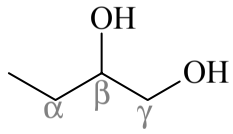
The experimental conditions and the analytical data for these copolyaspartamides were recorded in ***table 3.2.2a*** and ***b***

Table 3.2.2a: Outline of the experimental conditions under which copolyaspartamides **1e-1h** are generated from polysuccinimide (schemes 3.2.2a and 3.2.2b)

Concentration of reactants added (mol%)[a]			**Reaction Conditions**[b]	**Polyaspartamides**			
R-NH_2 x	**R-NH_2 y**	**x/y**		**Polymer Design.**	**Yield**[c] **%**	**η_{inh}**[d] **$mL.g^{-1}$**	**Molar mass/ recurring unit**[e] **g/mol**
DMP (75)	PDA (45)	3	**1.** 6hrs at RT, **2.** 16hrs at 0-5^{0}C and 24hrs at RT	**1e**	61	7	767.9
DMP (90)	EDDA (30)	9	**1.** 6hrs at RT, **2.** 16hrs at 0-5^{0}C and 24hrs at 20^{0}C	**1f**	67	8	2038.5
DMP (55)	D, L-Asp (100)	1	**1.** 48hrs at 20-60^{0}C, **2.** 8hrs at RT	**1g**	41	6	433.4
DMP(90)	APD(30)	9	**1.** 6hrs at RT, **2.** 16hrs at 0-5^{0}C and 24hrs at RT	**1h**	51	7	1981.4

[a] ratio of anchoring group to solubilzing group in the polymer
[b] room temperature, 20 ± 2 ^{0}C
[c] after dialysis 12000 and 25000 molecular mass cut off
[d] in de-ionised H_2O, 37 ± 0.5 ^{0}C
[e] molar mass of simplest recurring unit, structures normalised to y=1

Table 3.2.2b: ^{1}H NMR of copolyaspartamides **1e-1h**

Polymer Designation	R_1	R_2	^{1}H NMR			
			Shift range δ (ppm)	Proton count		Assignment
				expected	found	
1e			3.5-3.0 3.0-2.5 2.4-2.2 2.2-2.0 1.7-1.5	8 8 8 18 8	8 9 6 17 8	α, α Asp **CH₂** γ, γ $N(CH_3)_2$ β, β
1f			3.7-3.5 3.4-3.45 3.45-3.2 3.2-2.6 2.5-2.3 2.3-2.1 1.7-1.5	6 2 20 20 20 54 2	7 2 18 26 18 53 2	β, γ, δ ε α, α Asp CH_2, γ, ώ $N(CH_3)_2$ β
1g			4.5-4.0 3.0-2.55 2.55-2.45 2.45-1.8 1.7-1.5 1.6-1.4	3 2 6 2 6 2	3 2 4 3 6 2	Asp CH α Asp CH_2, β γ $N(CH_3)_2$ β
1h			4.0-3.5 3.5-3.0 3.0-2.5 2.5-2.0 1.7-1.5	3 20 20 72 18	3 20 20 73 18	β, γ α, α Asp CH_2 γ, $N(CH_3)_2$ β

[a] in D_2O or in D_2O at pD 9-10, [b] Reference against sodium-3-(trimethylsilyl-2,2,3,3-d_4-propionate. Integration error limits ±15%, protons calculated and rounded off to the nearest integer

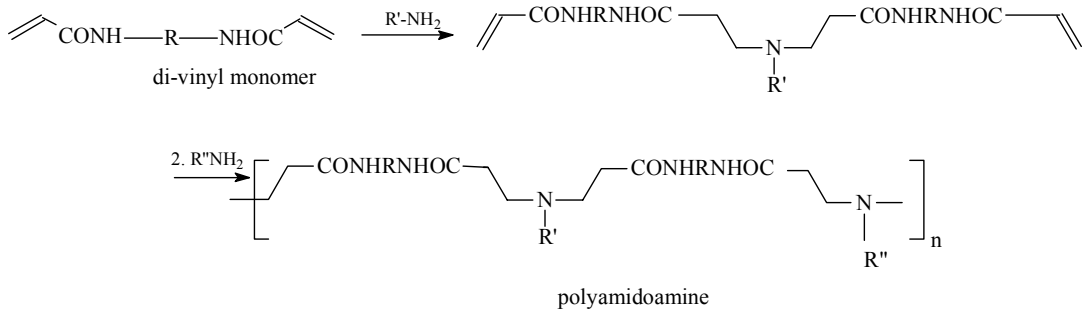
3.3 POLYAMIDOAMINES

Polyaminodamines were chosen as the second source of water-soluble carriers to be used in this dissertation program. These polymers were based on the Michéal-addition-Ferruti-type synthesis of water-soluble carriers, in which a acidic vinyl group undergoes addition with a nucleophilic amino group under basic conditions.[46, 47, 48, 49, 50] These polymers were chosen because they offer similar advantages to that of the copolyaspartamides, in addition they present a more controlled approach to introducing dicarboxyl terminal groups into the main chain and thus a more uniform polymer structure. In this dissertation, work was concentrated on the polyamidoamines containing either the dicarboxyl or the dihydroxyl terminal group. The reasons for choosing these functional groups were two fold;

- The polyamidoamines provided a more controlled method of introducing the di-carboxyl group into the polymer chain than was experienced with the dicarboxylated copolyaspartamides
- The dicarboxyl- and dihydroxyl-terminated water-soluble carries have shown special promise within this laboratory as a carrier of diaminocyclohexanediaqáuplatinum(II) salt (DACH-Pt aq), a drug system which has shown antiproliferative effectiveness against the human Hela carcinoma cell line.[51]

The copolymers were prepared according to established procedures, which involved the stepwise addition of different amines in a given stoichiometric feed ratio to a double bond-activated biacrylamido compound. This resulted in a copolymer in which the

subunits were uniformly distributed along the polymer chain in a pre-determined ratio **(scheme 3.3).**



Scheme 3.3: Structure of copolyamidoamines

As mentioned above, we synthesized polyamidoamines, falling into two classes, the dihydroxyl-functionalized and the dicarboxyl-functionalized polymers. The dicarboxyl-terminated polymer chain was characterized by the presence of R' **(scheme 3.3)** containing a dicarboxyl-terminal group, while the dihydroxyl-terminated polymer chain was characterized by the presence of an R' containing a dihydroxyl terminal group.

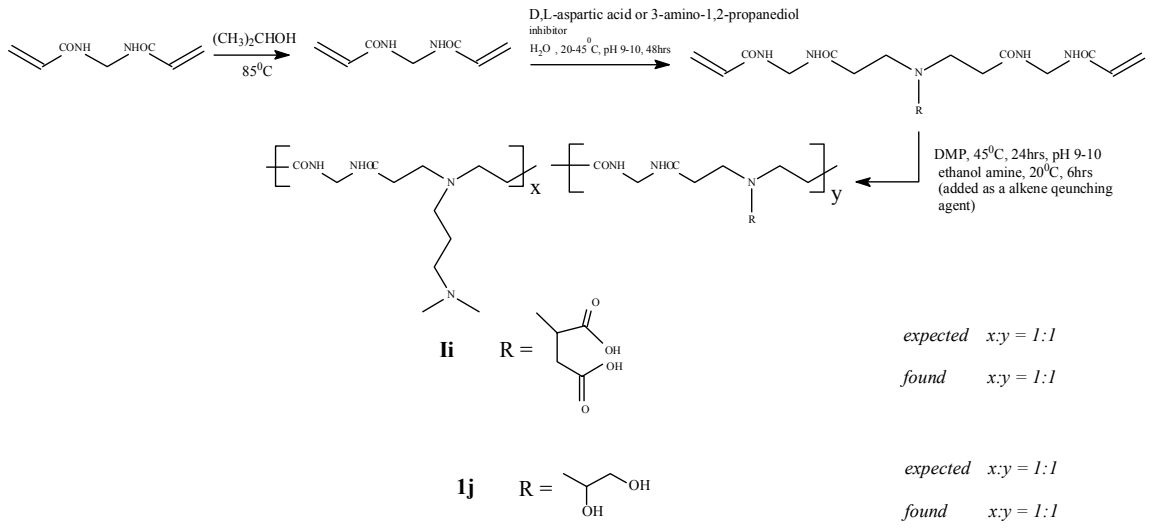
3.3.1 Polyamidoamine carriers featuring dicarboxyl and hydroxyl functionalities

In this group two synthetic approaches were followed to obtain the following carriers;

- MBA based aspartic acid polymers
- MBA based propanediol polymers
- a bis-succinic acid derived polymer
- a dihydroxyethylene(bisacrylamide) (DHEBA) derived polymer

3.3.1.1. Preparation of MBA-based DL-aspartic acid polymer and MBA-based APD polymer

These carriers was synthesized according to the reaction scheme 3.3.1.1a, illustrated below



Scheme 3.3.1.1: Copolyamidoamines 1i, 1j

It involved the dissolution of recrystallized MBA, **M1** (2:1) in water, and its reaction with the required amount of DL-aspartic acid or 3-amino-1,2-propanediol (APD). The reaction proceeded under alkaline conditions for 48 hrs, at temperatures ranging from 20-45 ^{0}C, to produce in-situ a disubstituted MBA-DL aspartic acid macro-monomer or a MBA-APD macro-monomer. These macro-monomers were allowed to react with one mole equivalent of DMP at 45 ^{0}C for a further 24 hrs. Any remaining vinyl groups were then neutralized by the addition of 5 % ethanolamine (mass:mass), and the reaction proceeded for a further 6hrs at 45^{0}C. The polymeric product was isolated as a completely

water-soluble solid following a series of steps, which included filtering, precipitation with a organic non-solvent, extensive washing, aqueous dialysis (finally in tubing with a 25000 molecular-mass cut-off limit), and freeze-drying. The polymers were obtained in yields ranging from 18 to 22 %, with a inherent viscosity ranging from 11 to 12 mL g^{-1} (***table 3.3.1.1a***). Both the purified MBA- and the resulting MBA-based polymers were characterised by ^{1}H NMR spectroscopy (***table 3.3.1.1b*** and ***c***) which confirmed both the structure of the monomer and the predicted polymers (***table 3.3.1.1b*** and ***c***)

Table 3.3.1.1a: Outline of the experimental conditions for the synthesis of the MBA-based copolyamidoamines (scheme 3.3.1.1)

Monomers (mmol)					Reaction conditions[a]	Product polymer				x/y[b]
MBA	DMP	Asp	APD	Na_2CO_3		Design.	Base molar mass[e] g/mol	Yield[c] %	η_{inh}[d] mLg^{-1}[c]	
10	5	5	-	5	**1.** 48hrs at 20-45^{0}C, **2.**24hrs at 45^{0}C	**1i**	542.60	18	11	1
10	5	-	5	2	**1.** 48hrs at 20-45^{0}C, **2.**24hrs at 45^{0}C	**1j**	501.61	22	12	1

[a] room temperature, 20 ± 2 ^{0}C
[b] ratio of anchoring group to solubilzing group in the polymer
[c] after dialysis in 12000 and 25000 molecular mass cut off tubing
[d] in de-ionised water, 37 ± 0.5 ^{0}C
[e] molar mass of simplest recurring unit, structures normalised to y=1

Table 3.3.1.1b: ^{1}H NMR of re-crystallized MBA, **M1**

Monomer designation	^{1}H NMR[a, b]			Assignment
	Shift δ (ppm)	Proton count		
		expected	found	
M1	6.3-6.1	4	4	CH=C**H$_2$**
	5.8-5.6	2	2	CONH-C**H**=CH$_2$
	4.8-4.5	2	2	CONH-C**H$_2$**-NHOC

[a] Reference against sodium-3-(trimethylsilyl-2,2,3,3-d_4-propionate. Integration error limits ±15 %, protons calculated and rounded off to the nearest integer
[b] in D_20 at pD 10

Table 3.3.1.1c: ^{1}H NMR of the polyamidoamines, **1i-1j**

Polymer designation	^{1}H NMR[a,b]			Assignment
	Shift range δ (ppm)	Proton count		
		expected	found	
1i	4.6-4.3	4	3	CONH-C**H$_2$**-NHCO
	2.7-2.5	8	8	C**H$_2$**-CONH
	2.4-2.1	14	12	N-C**H$_2$**-, Asp C**H$_2$**
	2.0-1.8	6	6	N-C**H$_3$**
	1.6-1.4	2	2	CH_2-C**H$_2$**-CH_2
1j	4.6-4.3	4	4	CONH-C**H$_2$**-NHCO
	3.8-3.6	2	2	CH(OH)-C**H$_2$**-OH
	3.4-3.4	1	1	CH_2C**H**(OH)CH_2
	2.8-2.6	8	7	C**H$_2$**-CONH
	2.6-2.4	12	13	N-C**H$_2$**-, N-C**H$_2$**-CH(OH)
	2.4-2.2	6	7	N-C**H$_3$**
	1.8-1.6	2	2	CH_2-C**H$_2$**-CH_2

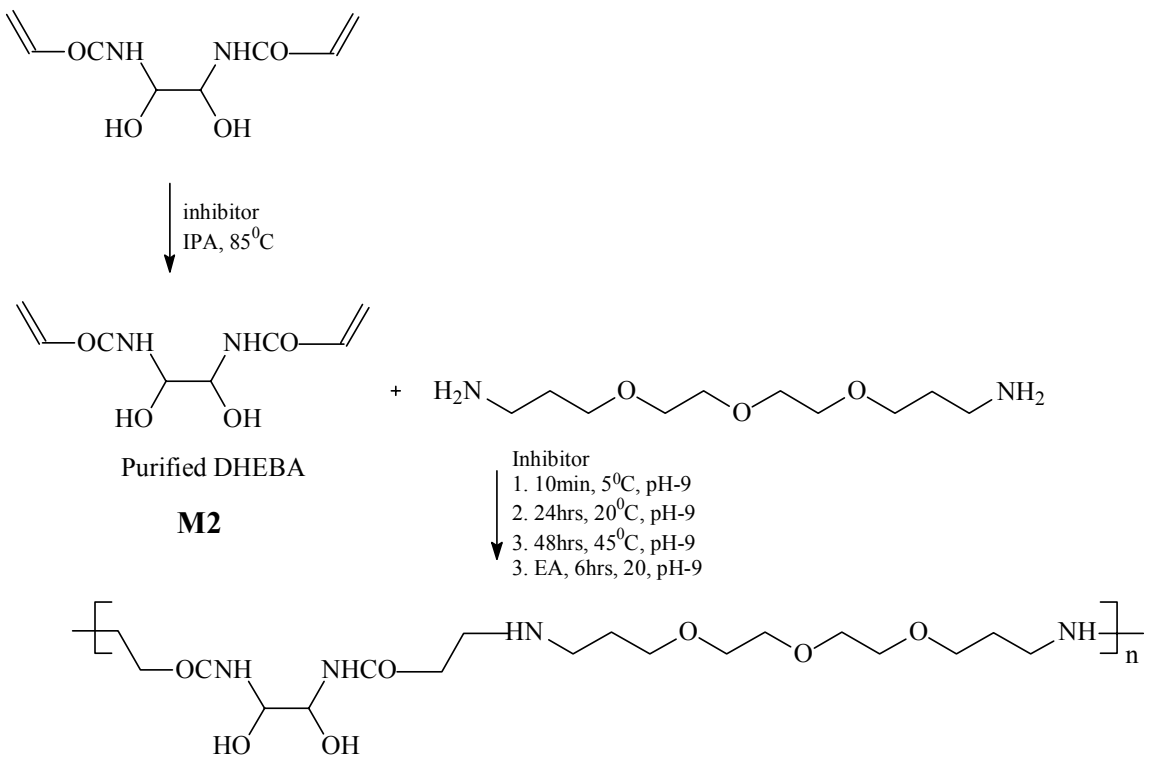
[a] in D_2O or in D_2O at pD 9-10

[b] Reference against sodium-3-(trimethylsilyl-2,2,3,3-d_4-propionate. Integration error limits ±15 %, protons calculated and rounded off to the nearest integer

3.3.1.2 Polyamidoamine carriers in which a 1,2-dihydroxyl functionality group is present within the polymer chain

Preparation of DHEBA-Tria

This carrier was synthesized according to the reaction scheme 3.3.1.2



1k

Scheme 3.3.1.2: Copolyamidoamine, 1k

DHEBA purified by recrystallization was dissolved in water and to that was added Tria in a mol ratio of 1:1 (DHEBA:Tria). The reaction proceeded for 72 hrs in alkaline medium at a temperature ranging from 20-45 ^{0}C. Afterwards 5 % (mass:mass) EA was added to the solution and the reaction continued at RT, for a further 6 hrs. This was to ensure that all remaining unreacted vinyl terminals present in the polymer were completely

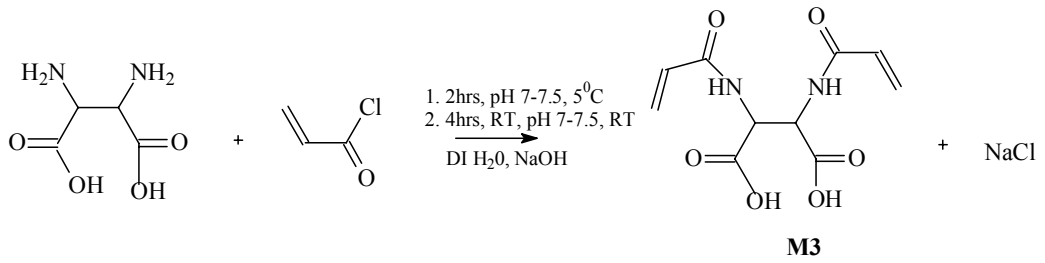
inactivated. The polymeric product was isolated as a completely water-soluble solid by the conventional series of steps. The polymer was obtained in a yield of 22 %, with an inherent viscosity of 10 mL g^{-1} (***table 3.3.1.2b***).The recrystallized DHEBA, and the polymer was characterised by ^{1}H NMR (***table 3.3.1.2a***) and both the monomer and the polymer agreed fairly well with the predicted structure. For the DHEBA mononomer this was indicative of peaks at ~5.4 ppm, (OCNH-C**H**(OH)-), ~5.8 ppm, (OCNH-C**H**=CH_2) and ~6.2 ppm, (OCNH-C**H**=C**H$_2$**). Furthermore integration of these peaks produced the correct number of protons. The polymer **1k** consisted of appropriate Tria peaks. However it appeared that the DHEBA peak for OCNH-C**H**(OH)- had shifted into the region of ~3.5 ppm, which was unusual and could not be accounted for.

3.3.1.3. Preparation of Diamido-succinic acid based tria polymer

This carrier was synthesized according to the reaction scheme 3.3.1.3a and 3.3.1.3b and involved two distinct steps.

- synthesis of a bisacryloyl substituted macro-monomer, **M3**.
- followed by the polycondensation of **M3** with the diamine, Tria.

The macro-monomer was synthesized as illustrated in **scheme 3.3.1.3a**



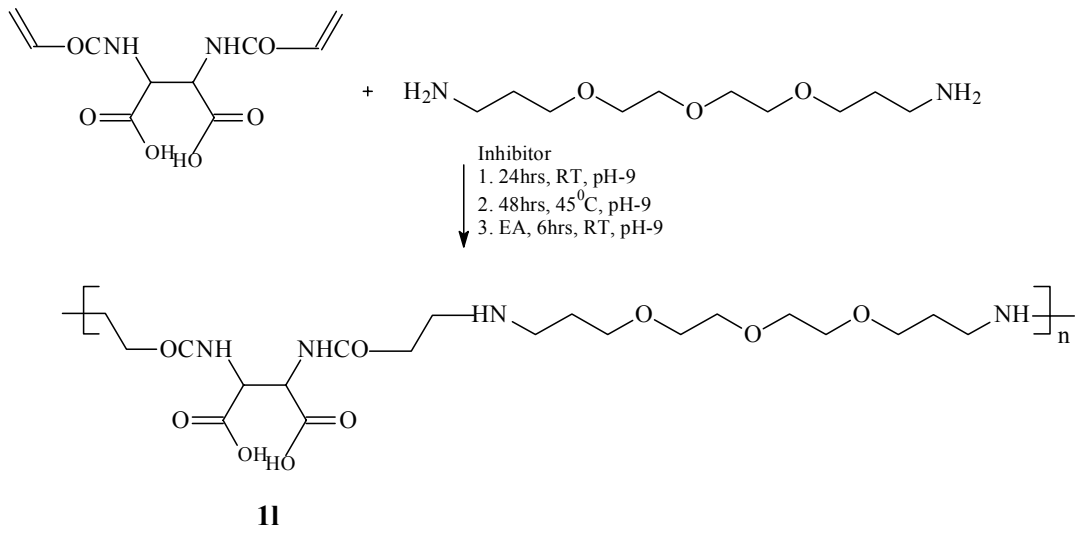
Scheme 3.3.1.3a: Synthesis of the diamidosuccinic acid precursor, M3

Two molar equivalents of acryloyl chloride were added drop-wise to an aqueous solution of diaminosuccinic acid, maintained at low temperature and slightly alkaline pH. Both the low temperature and the narrow pH range were essential to ensure a successful reaction as;

- the narrow pH range prevented the premature acid catalyzed hydrolysis of the acyl halide with formation of acrylic acid
- the low temperature reduced the risk of free radical polymerization of the precursor with formation of a cross-linked polymer, which could occur as a result of excessive heat generated during this reaction

Following the addition, the reaction proceeded for a further 4 hrs at 20 ^{0}C to ensure complete substitution of the amino groups, during which time the pH was regularly monitored and maintained at 6.5-8.0. Afterwards the solution was acidified and the product was extracted using an appropriate organic solvent, and all traces of water were removed from the extract. The pure product obtained by crystallization following reduction of the solvent volume, was isolated as a colourless solid, in a combined yield of 74 % resulting from, collected (***table .3.3.1.3a*****).** The crops were pooled and characterized by ^{1}H NMR spectroscopy and agreed with the predicted structure (***table3.3.1.3b***).

Following successful synthesis of the macro-monomer the copolymer was synthesized by the step growth addition polymerization of **M3**, and the diamine, 4,7,10-trioxa-1,13-tridecanediamine (Tria), according to the conditions described in scheme 3.3.1.3b



1l

Scheme 3.3.1.3b: Copolyamidoamine, 1l

The polymer was prepared very similarly to the step-growth polymerization of MBA, Except that this reaction involved the immediate addition of the diamine (Tria) and **M3**. Afterwards the reaction proceeded for 96 hrs in an alkaline aqueous solution at temperatures ranging from 20 to 45 ^{0}C. After this period any unreacted vinyl terminals were inactivated by the addition of 0.5 % (mass:mass) of EA, and the reaction continued for a further 6 hrs at 20 ^{0}C.

The polymeric product was isolated as a completely water-soluble solid by the conventional work-up including aqueous dialysis (finally in tubing with a 25000 molecular-mass cut off limit). The polymer was obtained in a yield of 32 %, with an inherent viscosity of 14 mL g^{-1} (***table 3.3.1.3c***). The structure of the polymer was characterised by ^{1}H NMR (***table 3.3.1.3d***), giving the following results;

Table 3.3.1.2a: ^{1}H NMR of re-crystallized DHEBA, **M2**

Monomer designation	^{1}H NMR[a, b]			Assignment
	Shift δ (ppm)	Proton count		
		expected	found	
M2	6.4-6.1	4H	4H	OCNH-CH=C**H$_2$**
	5.8-5.7	2H	2H	OCNH-C**H**=CH$_2$
	5.5-5.3	2H	2H	OCNH-C**H**(OH)

[a] in D_2O at pD 10

[b] Reference against sodium-3-(trimethylsilyl-2,2,3,3-d_4-propionate. Integration error limits ±15%, protons calculated and rounded off to the nearest integer

Table 3.3.1.3a: Outline of the experimental conditions for the synthesis of **M3**

Concentration of reactants added (mmol)[a]		Reaction Conditions	Yield (%)
AcCl	35mmol	**1.** ½hr, 0-5^{0}C, pH 6.5-8.5 **2**. 4hrs, 20^{0}C, pH 6.5-8.5	74
diamino-succinic acid	15mmol		
Na_2CO_3	30mmol		

Table 3.3.1.3b: ^{1}H NMR of the macro-monomer **M3**

Monomer designation	^{1}H NMR[a, b]			Assignment
	Shift δ (ppm)	Proton count		
		expected	found	
M3	6.3-6.1 5.8-5.6 4.7-4.5	4 2 2	4 2 2	**H**$_2$C=CH-CONH CH$_2$=C**H**-CONH OC-C**H**-CONH

[a] in D_2O

[b] Reference against sodium-3-(trimethylsilyl-2,2,3,3-d_4-propionate. Integration error limits ±15%, protons calculated and rounded off to the nearest integer

Table 3.3.1.3c: Outline of the experimental conditions for the synthesis of the copolyamidoamines **1k-1l** (schemes 3.3.1.2b and scheme 3.3.1.3)

Monomers (mmol)				Reaction conditions[b]	Product polymer				x/y[a]
M3	DHEBA	Tria	Na_2CO_3		Design.	Base molar mass[e] g/mol	Yield[c] %	η_{inh}[d] mLg^{-1}	
-	10	10	2	**1.** 24hrs at RT, **2.** 24hrs, 45^{0}C **3.** 6 hrs, RT	**1k**	418.49	22	10	1
10	-	10	10	**1.** 24hrs at RT, **2.** 24hrs, 45^{0}C **3.** 6 hrs, RT	**1l**	476.53	32	14	1

[a] ratio of anchoring group to solubilzing group in the polymer

[b] room temperature, 20 ± 2 ^{0}C

[c] after dialysis 12000 and 25000 molecular mass cut off

[d] in de-ionised H_2O, 37 ± 0.5 ^{0}C

[e] molar mass of simplest recurring unit, structures normalised to y=1

Table 3.3.1.3d: ^{1}H NMR of the copolyamidoamines **1k-1l**

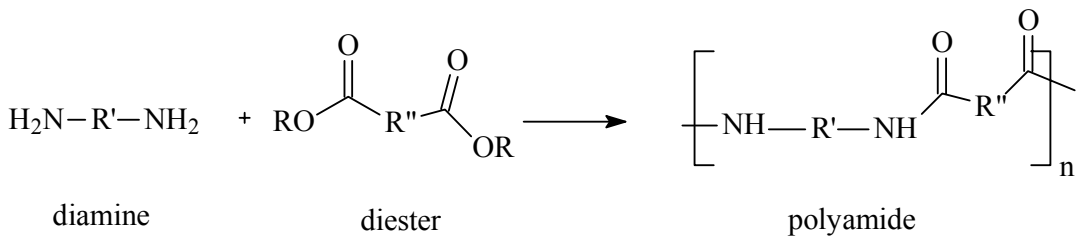
Polymer designation	^{1}H NMR[a, b]			Assignment
	Shift range δ (ppm)	Proton count		
		expected	found	
1k	3.5-3.0	14	14	CONH-C**H**(OH)-CH-, C**H$_2$**O-
	2.7-2.5	4	4	C**H$_2$**-CONH
	2.5-2.2	8	8	N-C**H$_2$**-
	1.6-1.4	4	4	CH_2-C**H$_2$**-CH_2
1l	4.5-4.3	2	1	CONH-C**H**-COOH
	3.7-3.5	12	12	C**H$_2$**O-
	2.7-2.5	4	5	C**H$_2$**-CONH
	2.4-2.1	8	1	N-C**H$_2$**-
	1.8-1.6	4	4	CH_2-C**H$_2$**-CH_2

[a] in D_2O or D_2O at pD 9-10

[b] Reference against sodium-3-(trimethylsilyl-2,2,3,3-d_4-propionate. Integration error limits ±15%, protons calculated and rounded off to the nearest integer

3.4. POLYAMIDES

Polyamides were chosen as the third and final type of water-soluble carrier to be used in this dissertation program. These polymers were prepared by the condensation polymerization process which occurs between a diamine and a diester (**scheme 3.4**).



diamine diester polyamide

Scheme 3.4: General reaction for the formation of a polyamide

These polymers were chosen because they offer the advantages provided by both the copolyaspartamides and the polyamidoamines. These include;

i. They may be conveniently synthesized from readily available starting material such as diethyl L-tartrate (Detart) and Tria
ii. The molecular mass of the suitably fractionated polyamides on average ranges from 25000 to 35000 provided the correct stochiometric ratio of reagents are added. This molecular mass is well within the desirable range to prevent inherent polymer toxicity, but still large enough to prevent premature excretion by the renal system.
iii. The structure of the polyamide is well defined
iv. The intrachain amide linkages provide for the gradual catabolism of the spent polymer chain, thus preventing excessive accumulation of the polymer in the body.

v. This approach provides a controlled method of introducing the dicarboxyl or di-hydroxyl functionalities into the polymer chain, thus providing a more uniform polymer.

vi. The uniform distribution of dicarboxyl and dihydroxyl functionalities within the polymer chain also provides for a more efficient carrier system as it might enhance the drug binding capacity of the polymer for certain bioactive compounds

Within this class of polymers work was focused specifically on two types;

- the carboxyl-functionalized polyamide
- the hydroxyl-functionalized polyamide

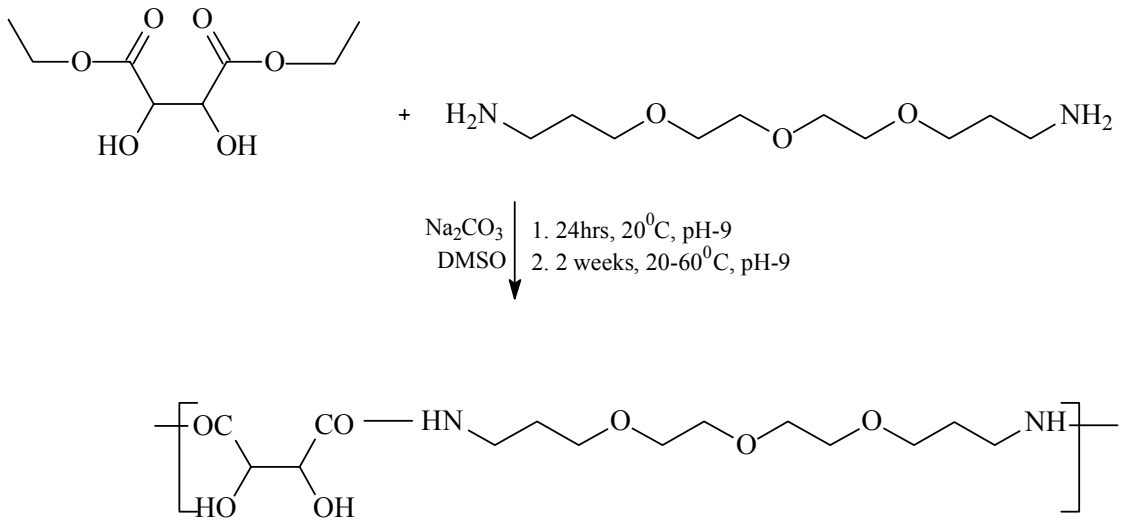
The reason for this is that these functional groups selectively bind diaminocyclohexanediaqáuplatinum(II)salt (DACH-Pt aq), a potent bioactive agent which has shown preliminary effectiveness against the Hela cell line, thus polymers with these functional groups would provide the ideal carrier for this potent drug.

3.4.1 Polyamide carriers comprising a dihydroxyl functional group within the main chain

Preparation of Poly(Detart-Tria)

The polyamide Detart-Tria was prepared as described in **scheme 3.4.1**, equimolar quantities of diethyl L-tartrate (Detart) and of 4,7,10-trioxa-1,13-tridecanediamine (Tria) were allowed to react under mild polycondensation conditions in an alkaline organic medium for a period of two weeks, at temperatures ranging from 20-65 ^{0}C. A yellow crystalline polymer was isolated by the conventional procedure as a solid in a yield of

42 % and an inherent viscosity of 12 ml g^{-1} (***table 3.4.2a***). The polymer was characterised by ^1H NMR, and the spectrum indicated the correct peaks in the required ratios (refer to ***table 3.4.2d***)



1m

Scheme 3.4.1: Polyamide, 1m

3.4.2 Polyamide carriers with a dicarboxyl functional group in the main chain

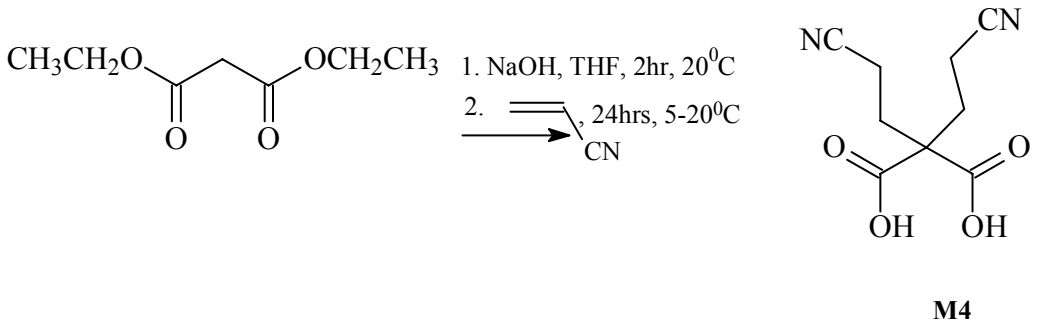
Preparation of Poly(diethylmalonic- Tria) (DEM-Tria)

The polyamide *DEM-Tria* was prepared as described in **scheme 3.4.2a and 3.4.2b**. The synthetic approach was divided into two distinct steps;

- synthesis of 2,2-bis(2-cyanoethyl)malonic acid **M4** and the ester derivative, **M5** respectively (**scheme 3.4.2a$_1$** and **3.4.2a$_2$**)
- polycondensation of **M5** with Tria to produce a dicarboxyl functionalized, water-soluble, polyamide carrier (**scheme 3.4.2b**)

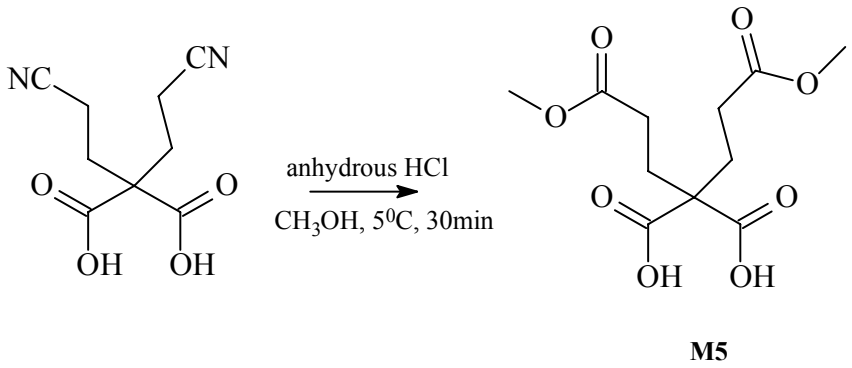
3.4.2a Synthesis of 2,2-bis(2-carbomethoxyethyl)malonic acid, M5

In order to synthesize **M5** it was necessary to prepare an appropriate precursor, 2,2-bis(2-cyanoethyl)malonic acid, **M4**. This precursor was readily synthesized by the Michéal Addition of acrylonitrile to the diethyl malonate (DEM), under slightly alkaline conditions. The precursor was subsequently converted to a diester derivative by an anhydrous acid catalyzed conversion, in which the cyano groups were converted to ester groups. It was necessary to perform this reaction under stringent anhydrous conditions as any trace of moisture resulted in destruction of the required precursor and the starting material. This occurred because the acid-catalyzed hydrolysis was a competing reaction which would readily affect the cyano- and ester functional groups.



M4

Scheme 3.4.2a$_1$: Formation of the 2,2-bis(2-cyanoethyl) malonic acid precursor, M4



M5

Scheme 3.4.2a$_2$: Conversion of M4 to the 2,2-bis(2-carbomethoxethyl) derivative, M5

The 2,2-bis(2-cyanoethyl)malonic acid precursor was prepared by dissolving diethyl malonate (DEM) in tetrahydrofuran (THF). To this solution was added 2.5 molar equivalents sodium hydroxide, and the mixture was stirred at 20 ^{0}C for 2 hrs. This was to allow sufficient time for the base to dissolve, required to catalyze the Micheal Addition. Afterwards 2.5 mol equivalents of acrylonitrile were added drop wise to the mixture at 0-5 ^{0}C, and the reaction was allowed to continue at 20 ^{0}C for a further 24 hrs. The solution was alkalified to pH 9 and any unreacted DEM was extracted with ethyl acetate. The aqueous phase was acidified to pH 3-3.5 in order to convert the product from its salt to its free acid form, which was subsequently extracted with ethyl acetate. The volume of the dried extract was reduced at room temperature under reduced pressure, and the product was allowed to crystallize. The product, **M4** was isolated as a colourless solid in a yield of 62 % and with a melting point of 145-147 ^{0}C (***table 3.4.2a***). It was characterized by ^{1}H NMR spectroscopy (***table 3.4.2b***), which produced a cluster of peaks in the region of 4.7-2.1.

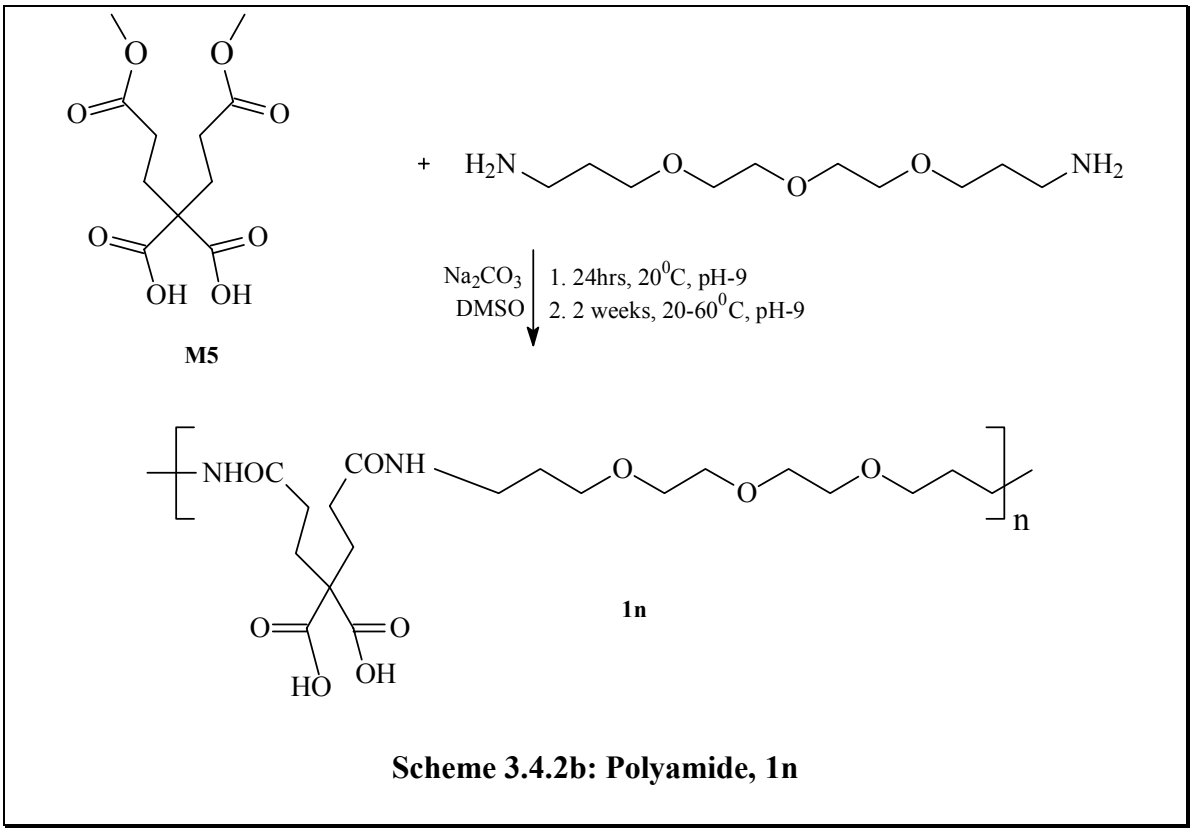
Analysis of these peaks suggests the presence of M4, indicated by a peak at ~2.7 ppm, CH_2-C**H_2**-C-$(COOH)_2$ and a peak at ~2.1 ppm, CN-C**H_2**-C-CH_2. A third peak was also present due to the impurity acetone.

Conversion of **M4** to the diester analogue **M5** was achieved by dissolving the precursor in anhydrous methanol at 0-5 ^{0}C and flushing it for 30 min at this temperature with anhydrous hydrogen chloride, produced by passing concentrated hydrochloric acid solution into 98 % (mass:mass) sulphuric acid. The solvent volume was reduced under reduced pressure at a minimum temperature, to minimize hydrolysis. The crude product was allowed to crystallize upon the addition of a non-solvent. Re- crystallization afforded a colourless solid, with a total yield of 54 %, spread over three crops, melting at 114-116 ^{0}C (***table 3.4.2a***), **M5** was structurally characterized by ^{1}H NMR which produced the required peaks at ~3.7 ppm, CH_2-COO-C**H_3**, ~2.7 ppm C**H_2**-COO-CH_3 and ~2.5 ppm, $(HOOC)_2$C-C**H_2**-CH_2. Furthermore integration of these peaks found them in the required ratio (***table 3.4.2b***).

3.4.2b Polycondensation of M5 with Tria to produce a dicarboxyl, water soluble, polyamide carrier

The water-soluble polyamide target compound was prepared under mild polycondensation conditions in which equimolar amounts of 4,7,10-trioxa-1,13-tridecanediamine (Tria) and **M5** were allowed to react in a alkaline organic medium for a period of two weeks at a temperature ranging from 20-65 ^{0}C (***table 3.4.2c***). A yellow polymer was isolated by precipitation, extensive washing, dialysis and freeze-drying. The polymer was obtained in a yield of 32 %. It was both water- and DMF-soluble and

had an inherent viscosity of 18 mL g^{-1}. The polymer was characterised by ^{1}H NMR, which indicated the required peaks in the correct ratio except for the peaks at 3.4-3.0 ppm CONH-C**H$_2$**-CH_2 $(COOH)_2$-C**H$_2$**-CH_2 which were slightly deficient (***table 3.4.2d***). This may suggest a slight excess of Tria in the polymer which may be removed by more vigorous dialysis, risking hydrolysis of the polymer.



Scheme 3.4.2b: Polyamide, 1n

In summary, the polymers synthesized thus far, were all isolated as fully water soluble compounds and retained this property even after heating under vacuum for a period of 48 hrs (suggested being equivalent to room temperature storage of the polymer for five weeks). Consequently these polymers were deemed suitable for conjugation to bioactive agents.

Table 3.4.2a: Outline of the experimental conditions for the synthesis of the macro monomers, **M4** and **M5**

Monomer Designation	Concentration of reactants added (mmol)		Reaction Conditions[a]	Macro-monomer product	
				Yield (%)	Melting point (0^C)
M4	DEM	5	**1.** 2hrs, RT **2.** 26hrs, RT	68	145-147
	AcCN	15			
	NaOH	11			
M5	dry HCl		**1.** ½ hr, 0-5^{0}C	54	114-116

Table 3.4.2b: ^{1}H NMR of the macro-monomer **M4** and **M5**

Monomer designation	^{1}H NMR			Assignment
	Shift δ (ppm)	Proton count		
		expected	found	
M4	2.8-2.6	2	2	CH_2-C**H$_2$**-C-$(COOH)_2$
	2.2-2.0	2	2	CN-C**H$_2$**-CH_2
M5	3.8-3.6	6	6	CH_2-COO-C**H$_3$**
	2.8-2.6	4	4	C**H$_2$**-COO-CH_3
	2.6-2.4	4	4	$(HOOC)_2$C-C**H$_2$**-CH_2-

[a] in D_2O

[b] Reference against sodium-3-(trimethylsilyl-2,2,3,3-d_4-propionate. Integration error limits ±15 %, protons calculated and rounded off to the nearest integer

Table 3.4.2c Outline of the experimental conditions for the synthesis of the polyamides **1m-1n**

(schemes 3.4.1a and 3.4.2b)

Monomers (mmol)				Reaction conditions[b]	Product polymer				x/y[a]
M5	**DETART**	**Tria**	**Na_2CO_3**		**Design.**	**Yield[c] %**	**η_{inh}[d] mLg^{-1}**	**Base molar mass[e] g/mol**	
-	5mmol	5mmol	4.8mmol	**1.** RT, 24hrs **2.** 13d, 45^{0}C	**1m**	38	12	349.38	1
5mmol	-	5mmol		**1.** RT, 24hrs **2.** 13d, 45^{0}C	**1n**	40	14	446.48	1

[a] ratio of anchoring group to solubilzing group in the polymer
[b] room temperature, 20 ± 2 ^{0}C
[c] after dialysis in 12000 and 25000 molecular mass cut-off tubing
[d] in de-ionised H_2O, 37 ± 0.5 ^{0}C
[e] molar mass of simplest recurring unit, structures normalised to y=1

Table 3.4.2d: ^{1}H NMR of the tria based polyamides, **1m-1n**

Carrier designation	^{1}H NMR			Assignment
	Shift δ (ppm)	Proton count		
		expected	found	
1m	4.5-4.3	2	2	CONH-C**H**(OH)
	3.7-3.5	12	13	-C**H$_2$**O
	3.4-3.2	4	4	-C**H$_2$**-CONH
	2.8-2.6	2	2	N-C**H$_2$**-
	1.8-1.6	4	4	CH_2-C**H$_2$**-CH_2
1n	3.7-3.5	12	12	CH_2-C**H$_2$**-O
	3.4-3.0	4	3	CONH-C**H$_2$**-CH_2
	2.6-2.4	4	1	NH-C**H$_2$**-CH_2 and C**H$_2$**-CONH
	2.2-1.7	4	3	$(COOH)_2$C-C**H$_2$**-CH_2
	1.7-1,5	4	4	CH_2C**H$_2$**CH_2

[a] in D_2O at pD 9-10

[b] Reference against sodium-3-(trimethylsilyl-2,2,3,3-d_4-propionate. Integration error limits ±15%, protons calculated and rounded off to the nearest integer

As mentioned previously, the bioactive agents considered for coordination and conjugation respectively, included platinum complexes, ferrocene in the form of ferrocenylbutanoic acid, and tetramethylmelamine. Fundamental coordination chemistry has been well established in this laboratory. Coordination or conjugation involving these water-soluble carriers and the selected bioactive agents is of particular interest because of the following;

- These water-soluble drug conjugates would contribute to the pool of existing conjugates which are required for biological testing
- Some of the carriers used are novel and it would be interesting to compare their biological effect with those carriers of similar structure.
- Some of the water-soluble carriers are especially noteworthy as they may be used to coordinate with the platinating agent DACH-Pt aq.
- The novel bioactive agent, tetramethylmelamine, not before polymer-bound, will also be conjugated, and it would be interesting to compare the biological effect of the conjugated drug with the unbound agent.

3.5. POLYMER PLATINUM COORDINATION

Since the advent of cisplatin as a potent antitumour agent, there has been an extensive research effort to develop more efficient analogues. This research impetus has resulted in the synthesis of platinum analogues in which the chloro ligands have been replaced with other suitable ligands, and in the preparation of novel trans-platinum complexes, and other 2^{nd} and 3^{rd} generation analogues with a high antitumour activity. However, cisplatin still remains an essential component of any therapeutic regimen. Consequently

numerous laboratories have attempted to reduce existing toxicity and resistance problems by conjugating this drug to water-soluble carriers, in this laboratory numerous methods have been developed to conjugate both cisplatin and the 2nd generation dicarboxylato-complex to a tailor-made synthetic polymer.[51, 52, 53] Within the scope of this dissertation platinum complexes have been prepared in which the metal is carrier bound via monoamine, dicarboxylato or dihydroxylato ligands.

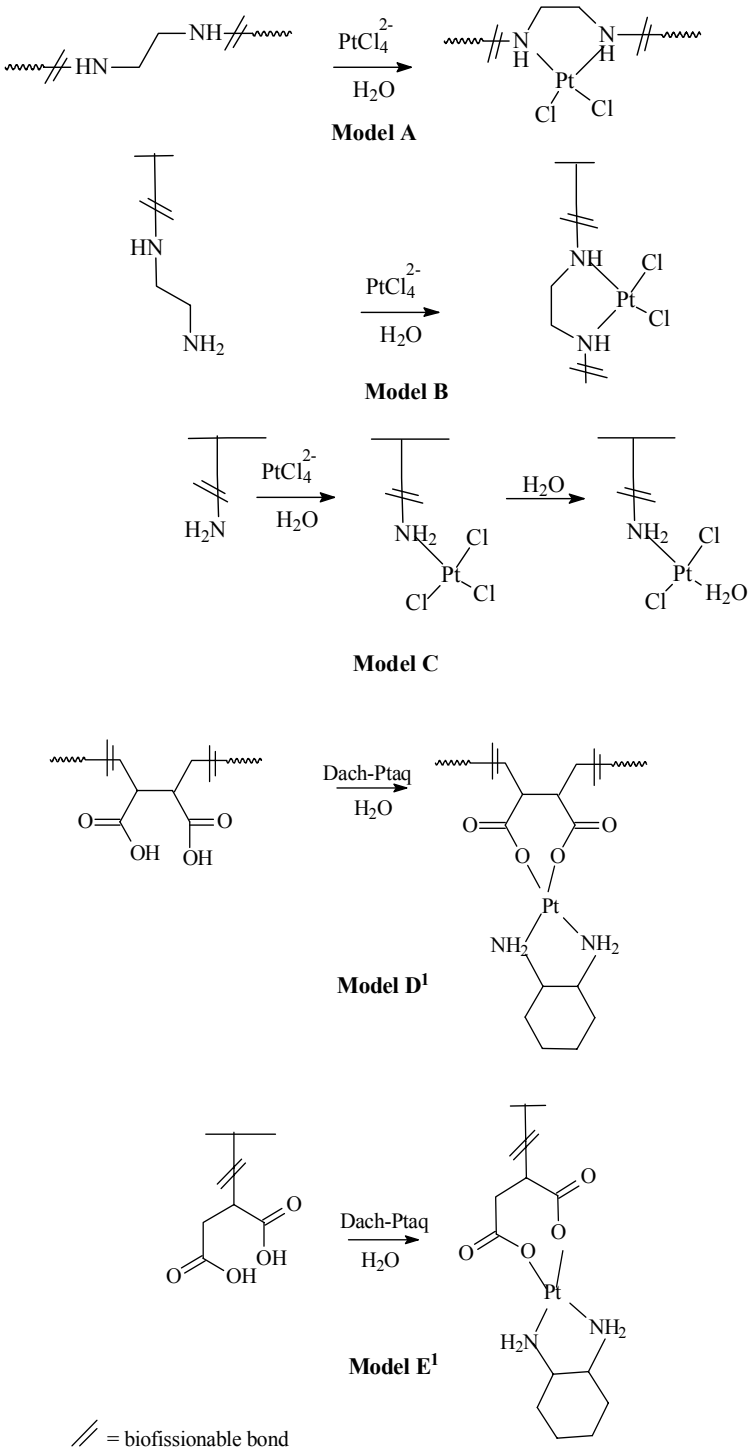
3.5.1. Modes of polymer platinum anchoring

In previous work in this laboratory numerous models for platinum chelation to the polymeric chain have been explored, depending on whether the polymer contains a terminal amino, dicarboxyl or dihydroxyl functional group. These include;

- The direct incorporation of the platinum complex into the main chain via diamine segments (**model A**)
- The incorporation of platinum complexes into the polymer via pendent diamine functionality which forms part of the side chain of the polymer (**model B**)
- The incorporation of platinum complexes into the polymer via pendent mono-functionality which forms part of the side chain of the polymer (**model C**)
- The direct incorporation of the platinum complex into the main chain via pendant difunctional carboxyl or hydroxyl groups which forms part of the main chain (**model D**)
- The incorporation of platinum complexes into the polymer via pendent difunctional hydroxyl or carboxyl groups which forms part of the side chain of the polymer (**model E**)

In models D and E, specifically for the hydroxyl functionalised polymers, coordination occurs via the oxy-group rather that than the hydroxyl group due to the presence of the platinum centre which make the hydroxyl group much more acidic, thus coordination occurs as $R\text{-}O^{-}$----Pt rather than R-O(H)----Pt

Specifically models C, D and E is described in this project, depending on the platinum complex investigated. For amine-containing platinum conjugates I concentrated specifically on model C, while for carboxyl-containing platinum conjugates polymer which corresponded to models D and E were used.

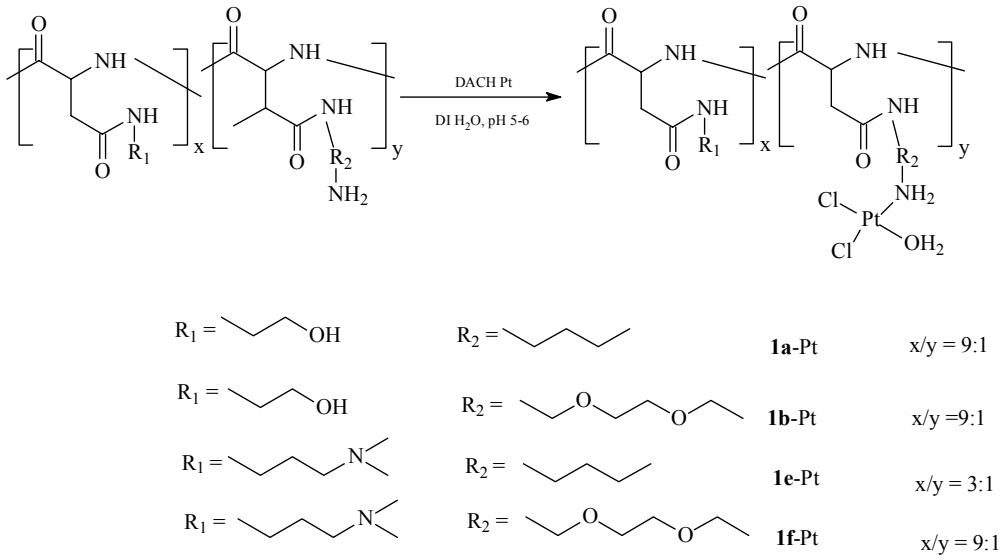


Models D' and E' apply both to dicarboxyl and dihydroxyl anchoring functionalities

Fig. 6 Models of platinum anchoring via the amino, dicarboxyl and dihydroxyl functionalities

3.5.2 Monoamine platinum complexes

These platinum complexes are characterized by the presence of an amine ligand in the complex. In this laboratory, four approaches have been used to conjugate amino group - containing platinum complexes. Of these approaches the most promising results have been found using model C. These promising effects have provided the impetus in the present project to use various copolyaspartamides (refer to **Scheme 3.4.2a**) and coordinate it to platinum via an amine terminal functionality. In the present work platination was performed in an aqueous medium at a ratio of 1:1,5 (polymer: potassium tetrachloroplatinate(II) at a pH range of 5 to 7, under meticulously controlled temperature and time conditions. Purification by aqueous dialysis under controlled pH conditions resulted in removal of the unbound platinum salts. This was followed by freeze drying which afforded the polymeric target products as water-soluble polymers in a yield ranging from 42 to 61 % and inherent viscosity from 8 to 11 mL g^{-1}. The platinum content was determined by atomic absorption, and ranged from 6 to 11 % (mass:mass). This was similar to previous results obtained in this laboratory. In addition this percentage incorporation was much higher compared to that of DACH –Pt aq and may be accounted for by the reduced steric hindrance experienced by platinum.



Scheme 3.5.2: Idealized structure of monoamine platinum(II) copolyaspartamide conjugates, of structural type C; 1a-Pt, 1b-Pt, 1e-Pt, 1f-Pt (protonation effects ignored)

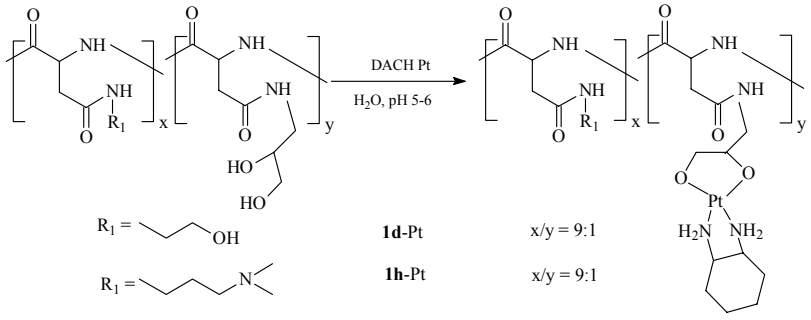
3.5.3. Polymer-Platinum Anchoring via the Dicarboxylato and the Dihydroxylato Ligands

Second-generation platinum complexes anchored to carriers via the dihydroxyl and dicarbxyl ligands have yielded very promising results as mentioned before, showing superior activity against the Hela carcinoma cell line. These promising findings have provided the drive for the synthesis of a variety of known and novel carriers for the dihydroxylato and dicarboxylato platinum complexes, so that established and new biological results could adequately be compared.

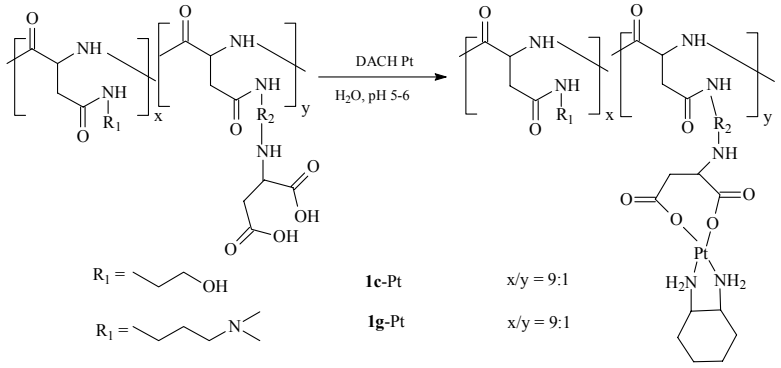
Models D and E were used to juxtapose the efficiency of the chain-integrated platinum complexation with that of the pendant platinum complexation system. Previous results have suggested the pendant complexation produces a more efficient release of the drug as the pendant group is more prone to hydrolytic cleavage. Although this argument is viable, it must be remembered that platinum complexes (especially those of the carboxyl and hydroxyl types), are particularly prone to pH changes, and thus more easily released. In this instance it may therefore be necessary to use a main chain-integrated complexation system in order to protect the complex from premature hydrolytic drug release.

Carrier conjugation reactions with both the dicarboxylato- and dihydroxylato-platinum, to produce the conjugates, were performed using copolyaspartamides, copolyamidoamines and polyamides. The process involved a two-step process in which the required amount of DACH Ptaq was pre-dissolved in an aqueous medium. This solution added drop-wise to an aqueous polymer solution, in a mole ratio of 1.5:1 (DACH-Ptaq: polymer). The reaction proceeded with stringent control of temperature, time and pH conditions. After conventional work-up, both the dihydroxylato – and dicarboxylato-platinum conjugates were afforded as colourless solids. The dihydroxylato platinum conjugates had yields ranging from 40 to 60 % and inherent viscosities ranging from 7 to 13 mL g^{-1}. For the dicarboxylato-platinum conjugates, the yields ranged from 41 to 61 %**,** and the inherent viscosities from 9 to 15 mL g^{-1}. The platinum contents for both the dihydoxylato and dicarboxylato conjugates were determined by atomic absorption and ranged from 2 to 9 % (mass:mass). Comparison of the dicarboxylato conjugates with their dihydroxylato counter-parts for a given polymer indicated a consistently higher platinum content for the

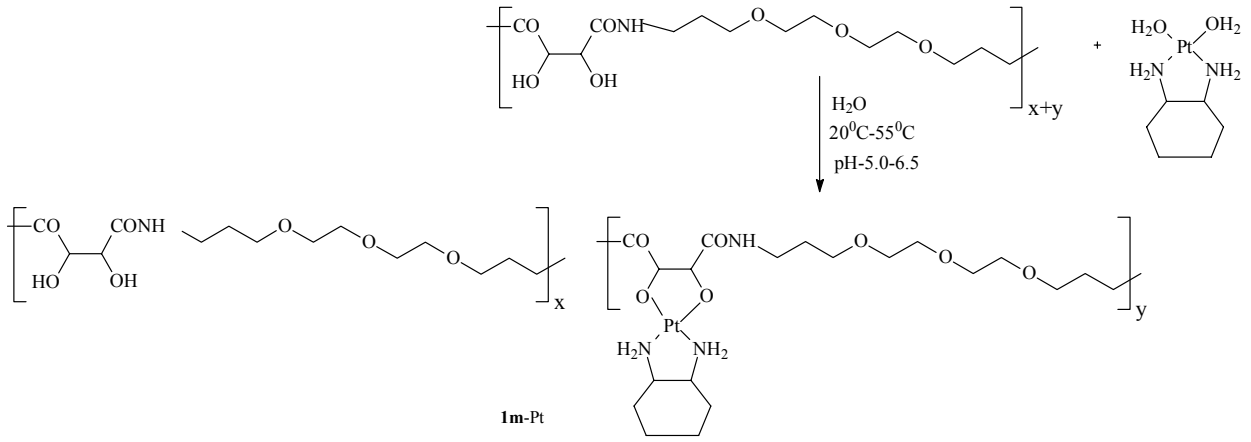
former. This was due to the more stable co-ordination complex formed by the dicarboxyl functionality which was less prone to hydrolytic release. Furthermore, on average, the maximum quantity of platinum which could be incorporated was approximately 40 % which was lower than expected, but could be accounted for by the larger idealized structure of the platinum complex, resulting in increased steric effects. Comparing the percentage DACH-Ptaq present in the various dicarboxyl-containing polymers suggest that in addition to the steric factor there appears to be additional factors inherent to the polymers which determine the quantity of platinum incorporated.



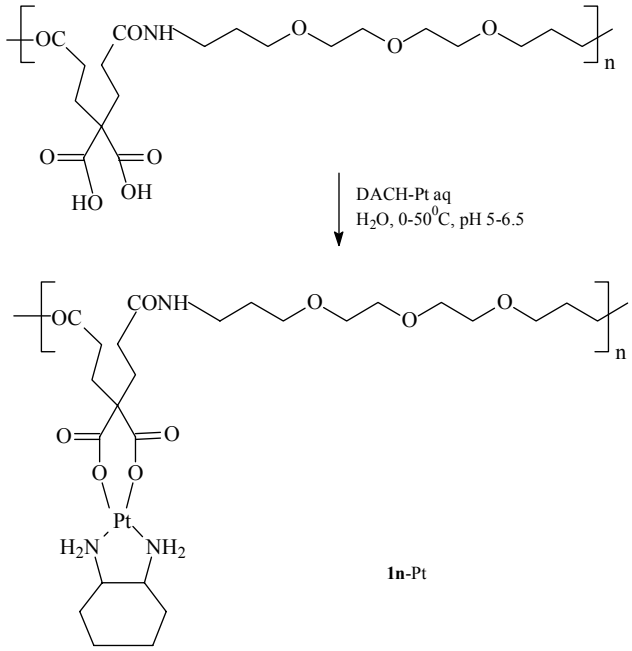
Scheme 3.5.3a: Idealized structure of dihydroxylato platinum(II) copolyaspartamide conjugates of structural type D, 1d-Pt, 1h-Pt (protonation effects ignored)



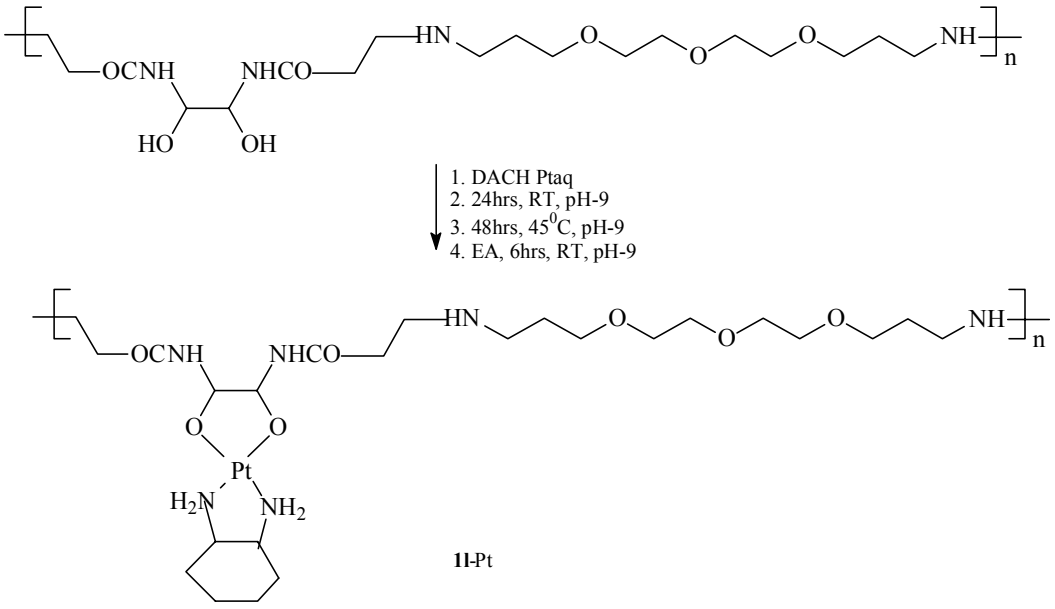
Scheme 3.5.3b: Idealized structure of dicarboxylato platinum(II) copolyaspartamide conjugates, of structural type E, 1c-Pt, 1g-Pt (protonation effects ignored)



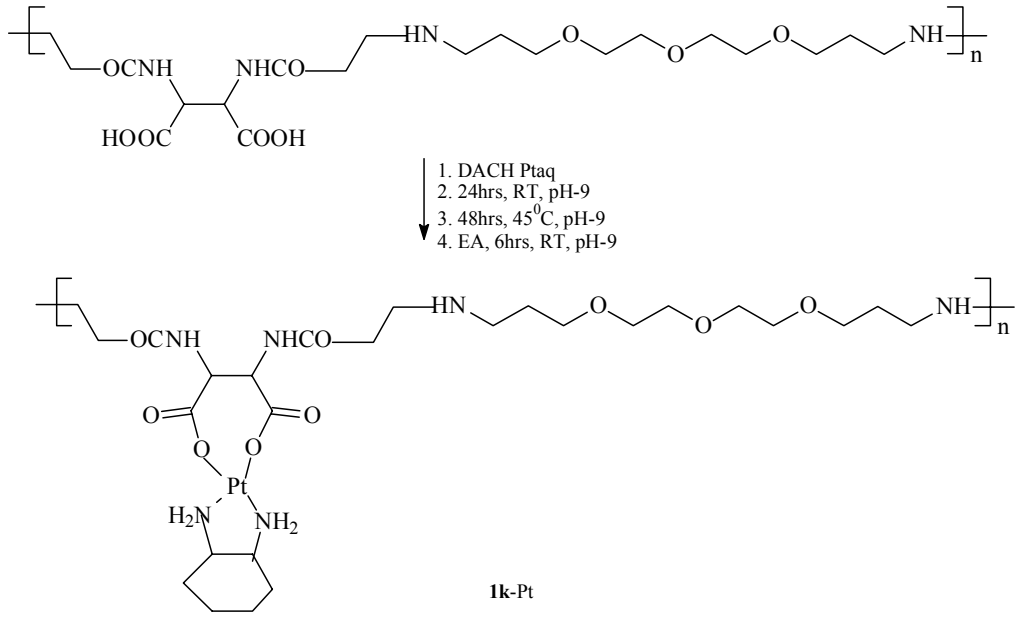
Scheme 3.5.3c: Idealized structure of dihydroxylato platinum(II) polyamide conjugates, of structural type D, 1m-Pt (protonation effects ignored)



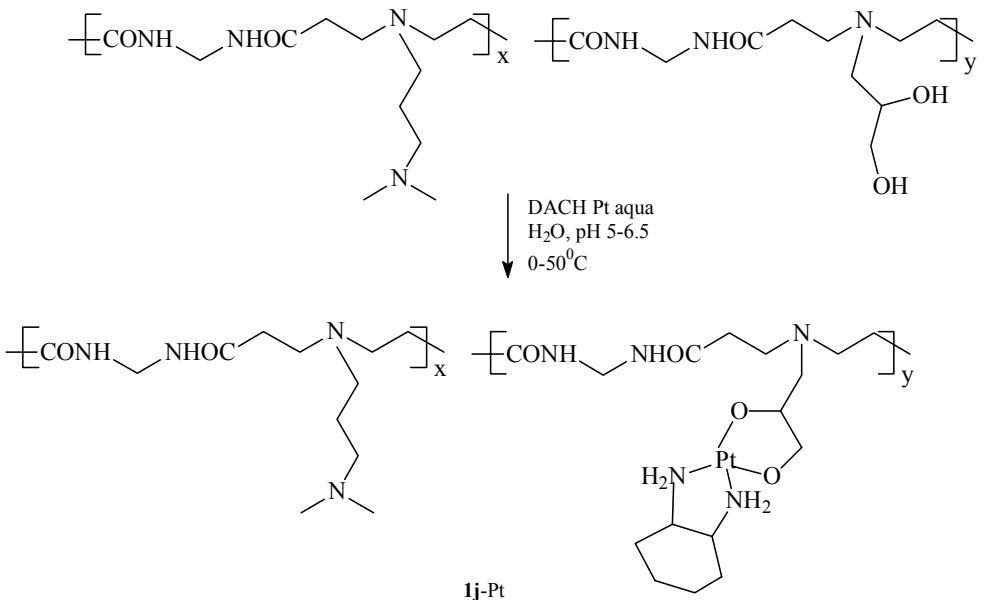
Scheme 3.5.3d: Idealized structure of dicarboxylato platinum(II)polyamides conjugates, of structural type D, 1n –Pt (protonation effects ignored)



Scheme 3.5.3e: Idealized structure of dihydroxylato platinum(II) tria-based polyamidoamine conjugates, of structural type D, 1l-Pt (protonation effects ignored)

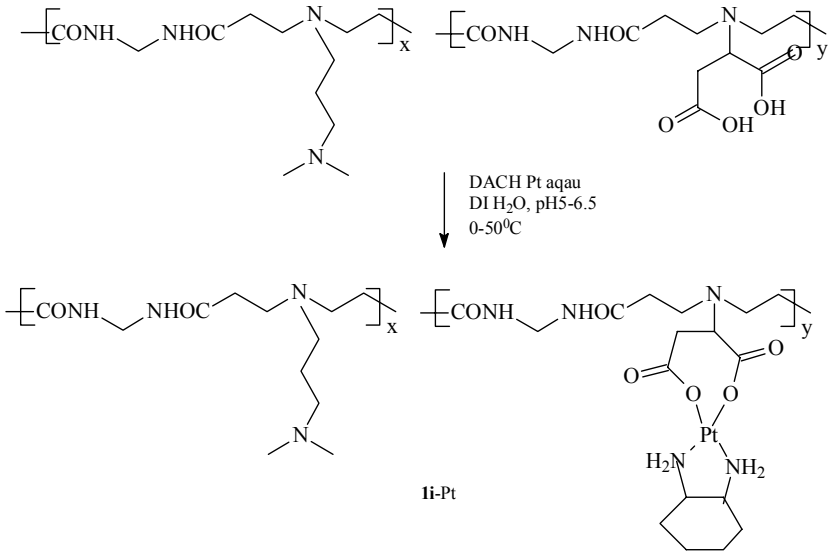


Scheme 3.5.3f: Idealized structure of dicarboxylato platinum(II) tria-based polyamidoamine conjugates, of structural type D, 1k-Pt (protonation effects ignored)



1j-Pt

Scheme 3.5.3g: Idealized structure of dihydroxylato platinum(II) MBA-based copolyamidoamine conjugates, 1j-Pt (protonation effects ignored)



1i-Pt

Scheme 3.5.3.h: Idealized structure dicarboxylato platinum(II) MBA-based copolyamidoamine conjugates, of structural type D, 1i-Pt (protonation effects ignored)

Table 3.5.2: Experimental conditions and analytical data for the monoamine platinum(11) copolyaspartamide conjugates; **1a**-Pt, **1b**-Pt, **1e**-Pt, and **1f**-Pt

Reactants in feed			Reaction conditions[a]	Platinum conjugates						
Carrier	Platination agent	Carrier:Pt ratio		Desig-nation	x/y[b]	Yield (%)[c]	η_{inh} (mLg^{-1})[d]	Base molar mass[e] g/mol	Pt%[f]	
									Calcd	Found
1a	$PtCl_4^{2-}$	1:1.2	**1.**24h,RT **2.** 48h 45^{0}C, **3.** 3h,RT	**1a**-Pt	9	55	11	1898.51	10.3	8.2
1b	$PtCl_4^{2-}$	1:1.2	**1.**24h,RT **2.** 48h 45^{0}C, **3.** 3h,RT	**1b**-Pt	9	47	9	1934.54	10	7
1e	$PtCl_4^{2-}$	1:1.2	**1.**24h,RT **2.** 48h 45^{0}C, **3.** 3h,RT	**1e**-Pt	3	61	9	1251.2	16	9
1f	$PtCl_4^{2-}$	1:1.2	**1.**24h,RT **2.** 48h 45^{0}C, **3.** 3h,RT	**1f**-Pt	9	42	7	2322.5	8.4	7.5

[a] RT – room temperature

[b] mole ratio of hydrosolubilizing group to the Pt anchoring group

[c] Yield after dialysis in 12000-14000 molecular mass cut-off tubes

[d] viscosity determined at 37±0.5 ^{0}C in de-ionised H_2O, conc = 0.2 g/100ml

[e] molar mass of ideal structure, when normalised to y = 1

[f] percentage platinum expressed in mass:mass percentage

Table 3.5.3: Experimental conditions and analytical data for the dihydroxylato and dicarboxylato platinum(II)copolyaspartamide conjugates, **1c**-Pt, **1d**-Pt, **1g**-Pt, **1h**-Pt, **1i**-Pt, **1j**-Pt, **1k**-Pt, **1l**-Pt, **1m**-Pt, **1n**-Pt

Reactants in feed			Reaction conditions[a]	Platinum conjugates						
Carrier	Platination agent	Carrier:Pt ratio		Design.	x/y[b]	Yield[c]	η_{inh}[d] (mLg^{-1})	Base molar mass[e] g/mol	Pt% mass: mass	
									Calcd.	Found
1c	DACH-Ptaq	1:1.5	**1.**24h, RT, **2.** 48h, 40-45^{0}C	**1c**-Pt	1	41	7	796.61	25	5.93
1d	DACH-Ptaq	1:1.5	**1.**24h, RT, **2.** 48h, 40-45^{0}C	**1d**-Pt	9	37	7	1988.72	9.8	2.09
1g	DACH-Ptaq	1:1.5	**1.**24h, RT, **2.** 48h, 40-45^{0}C	**1g**-Pt	1	43	8	837.67	23	9.02
1h	DACH-Ptaq	1:1.5	**1.**24h, RT, **2.** 48h, 40-45^{0}C	**1h**-Pt	9	54	9	2374.71	8.2	7.69
1i	DACH-Ptaq	1:1.5	**1.**24h, RT, **2.** 48h, 40-45^{0}C	**1i**-Pt	1	57	7	937.89	21	4.59
1j	DACH-Ptaq	1:1.5	**1.**24h, RT, **2.** 48h, 40-45^{0}C	**1j**-Pt	1	42	9	896.90	22	4.37
1k	DACH-Ptaq	1:1.5	**1.**24h, RT, **2.** 48h, 40-45^{0}C	**1k**-Pt	1	47	9	813.76	24	2.15
1l	DACH-Ptaq	1:1.5	**1.**24h, RT, **2.** 48h, 40-45^{0}C	**1l**-Pt	1	59	8	871.80	22	6.53
1m	DACH-Ptaq	1:1.5	**1.**24h, RT, **2.** 48h, 40-45^{0}C	**1m**-Pt	1	56	9	746.67	26	2.95
1n	DACH-Ptaq	1:1.5	**1.**24h, RT, **2.** 48h, 40-45^{0}C	**1n**-Pt	1	61	11	843.77	23	7.31

[a] RT – room temperature

[b] mole ratio of hydrosolubilizing group to the Pt anchoring group

[c] Yield after dialysis in 12000-14000 molecular mass cut-off tubes

[d] viscosity determined at 37±0.5 ^{0}C in de-ionised H_2O, conc = 0.2 g/100ml

[e] molar mass of ideal structure, when normalised to y = 1 or using the mass of the simplest recurring unit

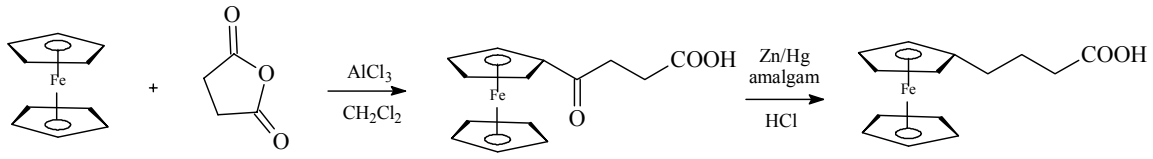
3.6. POLYMER FERROCENE CONJUGATION

It has been well established that metallocene compounds exhibit interesting biological and biomedical effects, the most interesting being the anti-proliferative effect of ferrocene, mediated by its ability to neutralize free radical species which are recognized as important protagonists of cancer. This interesting property of ferrocene complexes has resulted in an intensive research initiative within this lab aimed at optimizing the conjugation of ferrocene compounds to appropriate polymers and evaluating their biological activity.

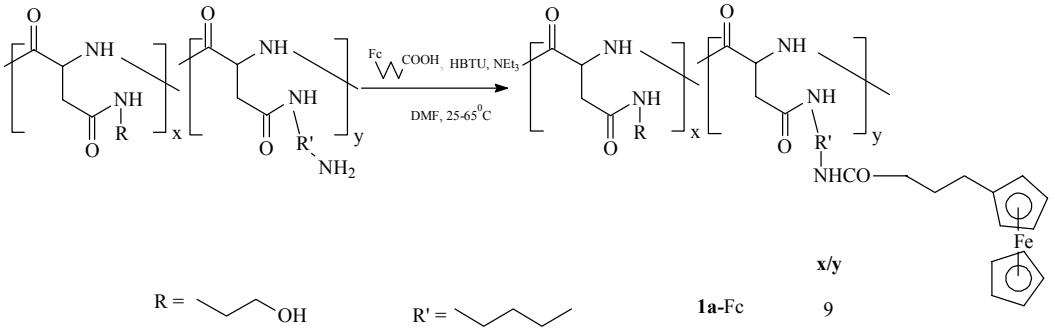
Consequently ferrocene has been chosen as one of the other drugs within this project. The approach followed involved the ferrocenylation of polyaspartamides using the ferrocene derivative, ferrocenylbutanoic acid, **M6**. The derivative was synthesised using an established laboratory procedure, and subsequently conjugated via amidation of the carriers. This linkage was felt appropriate as it was reasonably stable and would withstand premature hydrolytic release of the drug.

Ferrocene derivatisation was achieved through an established literature procedure, involving Friedel Crafts acylation of ferrocene and Clemensen reduction of the keto intermediary to ferrocenylbutanoic acid, described in **scheme 3.6a**. TLC performed in petroleum ether:acetone (1:1) signified completion of the reaction. The acid was afforded as a yellow solid in 40 % yield with a melting point of 118-120 ^{0}C. Polymer ferrocene anchoring proceeded as described in **scheme 3.6b**. It involved the dissolution of the polymer in a aprotic solvent and its subsequent conjugation to the ferrocenylbutanoic acid

in a ratio of 1: 1.2, using an appropriate coupling agent (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)). The reaction proceeded under anhydrous conditions for a period of 2-2.5 hrs at room temperature. The polymer ferrocene conjugate was isolated following a series of purification steps which included precipitation in a non-solvent, scrupulous washing, aqueous dialysis and freeze-drying. The product was obtained as a brownish, water soluble crystalline material, with various yields and inherent viscosities depending on the polymer used for binding. The iron content in the conjugate was determined using AA spectroscopy and ranged from 2 to 12 % (mass:mass).



Scheme 3.6a: Preparation of ferrocenylbutanoic acid, M6



		x/y
R = OH	R' =	**1a**-Fc 9
R = OH	R' = O O	**1b**-Fc 9
R = N	R' =	**1e**-Fc 3
R = N	R' = O O	**1f**-Fc 9

Scheme 3.6b: Ferrocenylation of copolyaspartamide carriers, 1a, 1b, 1d and 1e (protonation effects ignored)

Table 3.6a: ^{1}H NMR of ferrocenylbutanoic acid, **M6**

Carrier designation	^{1}H NMR			Assignment
	Shift δ (ppm)	Proton count		
		expected	found	
M6	4.3-4.1	9	8	ferrocenyl
	2.4-2.2	4	4	-C**H$_2$**-CH$_2$-C**H$_2$**-
	1.9-1.7	2	2	CH$_2$-**CH$_2$**-CH$_2$

[a] in D_2O, pD 9-10

[b] Reference against sodium-3-(trimethylsilyl-2,2,3,3-d_4-propionate. Integration error limits ±15 %, protons calculated and rounded off to the nearest integer

Table 3.6b: Experimental conditions and analytical data for the ferrocene conjugates; **1a**-Fc**, 1b**-Fc**, 1e**-Fc **and 1f**-Fc

Reactants in feed			Reactants & reagents			Reaction conditions	Ferrocene conjugates						
Carrier	Coupling Method	Carrier:Fc ratio	carrier	Fc	HBTU		Design.	x/y	Yield	η_{inh} mLg^{-1}	Base molar mass g/mol	%Fe mass: mass	
												Calcd.	Found
1a	HBTU	1:1.2	0.19	0.228	0.209	2-2.5hrs, RT, dark, pH 8-9	**1a**-Fc	9	58	9	1848.8	3.0	2.61
1b	HBTU	1:1.2	0.182	0.218	0.200	2-2.5hrs, RT, dark, pH 8-9	**1b**-Fc	9	67	8	1922.8	2.9	2.5
1e	HBTU	1:1.2	0.469	0.469	0.430	2-2.5hrs, RT, dark, pH 8-9	**1e**-Fc	3	54	10	1023.1	5.5	5.0
1f	HBTU	1:1.2	0.176	0.162	0.162	2-2.5hrs, RT, dark, pH 8-9	**1f**-Fc	9	61	7	2251.6	2.9	2.53

3.7. POLYMER CONJUGATION OF β-ALANINE-MODFIED TETRAMETHYLMELAMINE DERIVATIVE

A tetramethylmelamine derivative was chosen as the third medicinal agent to be included within this dissertation. The reasons for this were as follows;

i. It is a well established anti-tumour agent, whose efficiency has been illustrated against a broad range of tumours
ii. Its poor water solubility makes it a ideal agent which may be used to illustrate the benefits of a drug carrier system to provide efficient distribution in central circulation
iii. It may be synthesized using conventional methods and relatively inexpensive starting material.

However the structure of hexamethylmelamine does not lend itself to effective conjugation to the polymer carrier. To achieve suitable conjugation the following approach was followed;

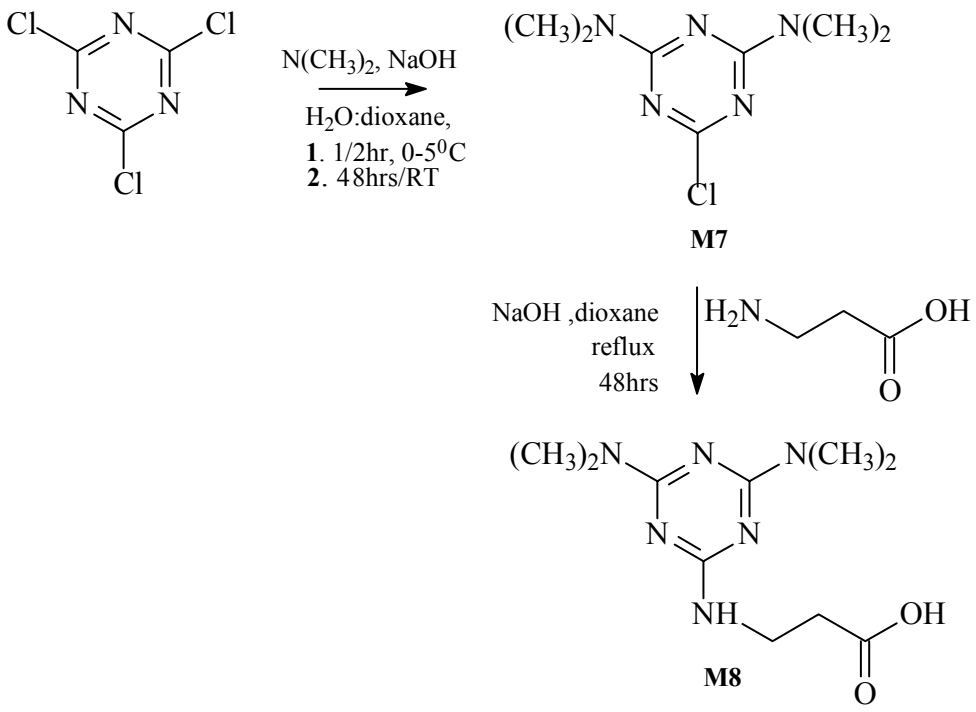
- synthesis of the tetramethylmelamine surrogate.
- conjugation of the surrogate to the carrier

3.7.1 Synthesis of the tetramethylmelamine derivative

The tetramethylmelamine derivative, **M8**, was synthesized from cyanuric chloride according to **scheme 3.7.1**, to produce an aromatic intermediate, **M7**, which reacts further to afford the tetramethylmelamine precursor, **M8**. The intermediate **M7** was prepared by a literature procedure, which involved the drop-wise addition of two equivalents of

dimethylamine in a slightly alkaline dioxane: water solution, at 0-5 ^{0}C for 30 min. The reaction proceeded for 48 hrs at RT during which the alkalinity (pH 7-7.5) was maintained by the regular addition of sodium hydroxide. This was to neutralize the hydrochloric acid which formed as a by-product during this reaction. The product, **M7**, was isolated, following alkalification, extraction with an organic solvent and recrystallisation in ethyl acetate. The product was isolated as a white crystalline solid, in yields of 74 % and with a melting point of 115-117 ^{0}C. The product was further characterized by ^{1}H NMR. The tetramethylmelamine derivative, **M8**, was prepared by refluxing **M7** with 2.5 time's equivalent β-alanine in dioxane: water, under slightly alkaline conditions, for a period of 48hrs. Afterwards the product **M8** was isolated as a white crystalline material following extraction and recrystallization from water. The product was obtained in a yield of 42 % and characterized by ^{1}H NMR spectrum (200 or 400 Mhz), measured in D_2O at slightly acidic pH (**table. 3.7.1b**)

The ^{1}H NMR indicated only a single peak for M7 in the region of ~3.0 ppm and failed to conclusively confirm, if this was the required compound. Melting point of **M7** matched that present in literature and on this basis the compound was deemed appropriate to carry forward. Analysis of **M8** indicated a cluster of peaks in the region of 3.7-2.4, identified as follows; 3.7-3.5 ppm, CH_2-C**H$_2$**-COOH, 3.1-3.0 ppm, 2.5-2.4 ppm NH-C**H$_2$**-CH_2, which match those protons present in **M8**.



Scheme 3.7.1: Synthesis of the tetramethylmelamine derivative, M8

Table 3.7.1a: Outline of the experimental conditions for the synthesis of the macro monomers, **M7** and **M8**

Monomer Designation	Concentration of reactants added (mmol)		Reaction Conditions	Macro-monomer product	
				Yield (%)	Melting point (0^C)
M7	Cyanuric chloride	15mmol	**1.** ½ hr, 0-5^{0}C, pH7-7.5 **2.** 48hrs, RT, pH 7-7,5	74	115-117
	dimethyamine	35mmol			
	NaOH	32mmol			
M8	M7	10mmol	48hrs, 80-100^{0}C, pH 7.0-8.5	42	-
	β-alanine	25mmol			
	NaOH	12mmol			

Table 3.7.1b: ^{1}H NMR of the macro-monomer **M7** and **M8**

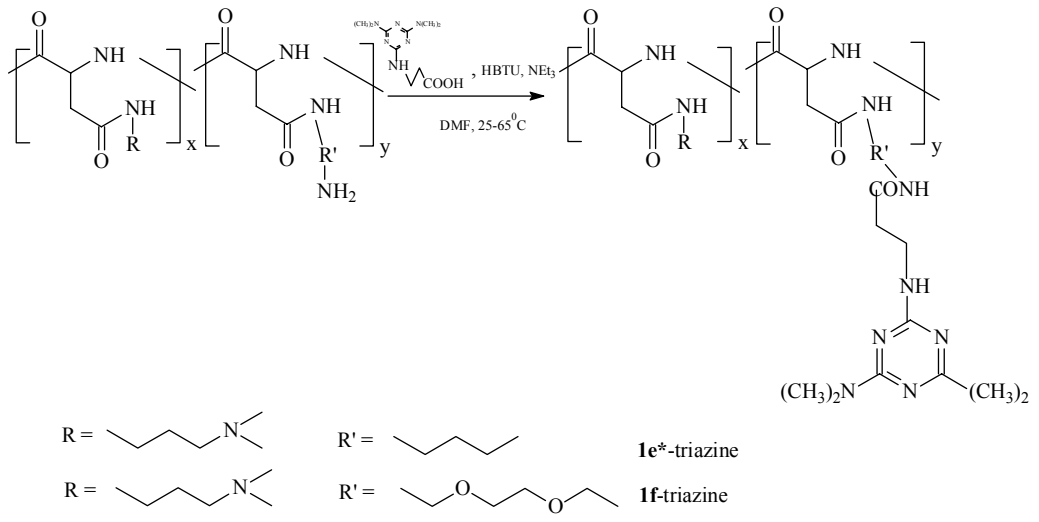
Monomer designation	^{1}H NMR			Assignment
	Shift δ (ppm)	Proton count		
		expected	found	
M7[a]	3.1-3.0	12	12	$(C\mathbf{H_3})_2$-N
M8[b]	3.7-3.5 3.1-3.0 2.5-2.4	2 12 2	2 12 2	NH-C$\mathbf{H_2}$-CH_2 $(C\mathbf{H_3})_2$-N CH_2-C$\mathbf{H_2}$-COOH

[a] – in $CDCl_3$, reference against trimethylsilane [b] – in D_2O at pD 6-7, reference against sodium-3-(trimethylsilyl-2,2,3,3-d_4-propionate. Integration error limits ± 15%, protons calculated and rounded off to the nearest integer

3.7.2 Conjugation of the precursor, M8, to a copolyaspartamide carrier

Polymer-triazine anchoring proceeded as described in **scheme 3.7.2**, It involved dissolution of **M8** in a aqueous solvent followed by pH adjustment to a slightly alkaline pH (6.5-7.5) and stirring for 30min. Afterwards **M8** was isolated by freeze-drying and conjugated to a polyaspartamide as follows;

The polymer was dissolved in an aprotic solvent and subsequently conjugated to **M8** in a ratio of 1:2.0 (polymer:M8), using a appropriate coupling agent (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)). The reaction proceeded under anhydrous conditions, in the dark for a period of 2-2.5 hrs at room temperature, and slightly alkaline pH (7-8.5). The polymer triazine conjugate was isolated following a series of purification steps which included precipitation in a non-solvent, scrupulous washing, aqueous dialysis and freeze-drying under varying pH conditions. The product was obtained as a colourless, water soluble crystalline material, with various yields (47-61 %) and inherent viscosities (7-9 mL g^{-1}) depending on the polymer used for conjugation. The quantity of tetramethylmelamine present was determined by ^{1}H NMR, using the methyl protons as a reference (***table 3.7.2b***), and were in the range of 8.4-8.6 %, considering the mass of the drug as a percentage of the total mass of the drug-polymer conjugate. This was similar to that obtained for the ferrocene conjugates, which is expected considering a similar conjugation method was used. A future approach may involve conjugating the derivative under acidic conditions using a alternative conjugating agent.



Scheme 3.7.2: Conjugation of M7 to copolyaspartamide carriers, 1e*, 1f

Table 3.7.2a: Experimental conditions and analytical data for the copolyaspartamide conjugates, **1e***-tr and **1f**-tr

Reactants in feed			Reaction conditions[b]	β-alanine triazine conjugates						
Carrier	Coupling Method	Carrier:M8 ratio		Desig-nation	x/y[c]	Yield[d]	η_{inh}[e] mLg^{-1}	Base molar mass[f] g/mol	Tetramethylmelamine % (mass:mass)	
									Calcd.	Found
1e*[a]	HBTU	1:2.0	**1.** 2-2,5hrs, RT, pH 7-8.5	**1e***-tr	9	61	9	1761.68	8.6	7
1f	HBTU	1.2.0		**1f**-tr	9	47	7	2153.7	8.4	8.5

[a] **1e*** is poly-α,β-DL-[N-(3-dimethyamino)propyl-aspartamide-co-N-(3-aminopropyl)aspartamide, with the ratio of solubilizing to drug anchoring portion being, 9 to 1

[b] RT – room temperature

[c] mole ratio of hydrosolubilizing group to the anchoring group

[d] Yield after dialysis in 12000-14000 molecular mass cut-off tubes

[e] viscosity determined at 37±0.5 ^{0}C in de-ionised H_2O, conc = 0.2 g/100ml

[f] molar mass of ideal structure, when normalised to y = 1 or using the mass of the simplest recurring unit

Table 3.7.2b: ^{1}H NMR of the copolyaspartamide-M7 conjugates, **1e***-tr and **1f**-tr

Carrier designation	^{1}H NMR[b]			Assignment
	Shift δ (ppm)	Proton count		
		expected	found	
1e*-tr	4.7-4.5	10	10	Asp C**H**
	3.2-3.0	22	24	-C**H$_2$**-NHCO
	3.0-2.9	12	10	(C**H$_3$**)$_2$-N-triazine
	2.9-2.5	22	22	Asp CH$_2$, CO-C**H$_2$**-CH$_2$
	2.5-2.2	20	24	N-C**H$_2$**CH$_2$
	2.2-1.9	54	55	CH$_2$-N-C**H$_3$**
	1.7-1.5	20	20	CH$_2$C**H$_2$**CH$_2$
1f-tr	4.7-4.5	10	8	Asp C**H**
	3.7-3.5	8	7	-C**H$_2$**O
	3.2-3.0	22	20	-C**H$_2$**-NHCO
	3.0-2.9	12	8	(C**H$_3$**)$_2$-N-triazine
	2.9-2.5	22	21	Asp CH$_2$, CO-C**H$_2$**-CH$_2$
	2.5-2.2	20	22	N-C**H$_2$**
	2.2-1.9	54	54	CH$_2$-N-C**H$_3$**
	1.7-1.5	18	18	CH$_2$-C**H$_2$**-CH$_2$

[a] **1e*** is poly-α,β-DL-[N-(3-dimethyamino)propyl-aspartamide-co-N-(3-aminopropyl)aspartamide, with the ratio of solubilizing to drug anchoring portion being 9 to 1

[b] in D_2O at pD 9-10 with NaOH, reference against sodium-3-(trimethylsilyl-2,2,3,3-d_4-propionate. Integration error limits ±15 %, protons calculated and rounded off to the nearest integer

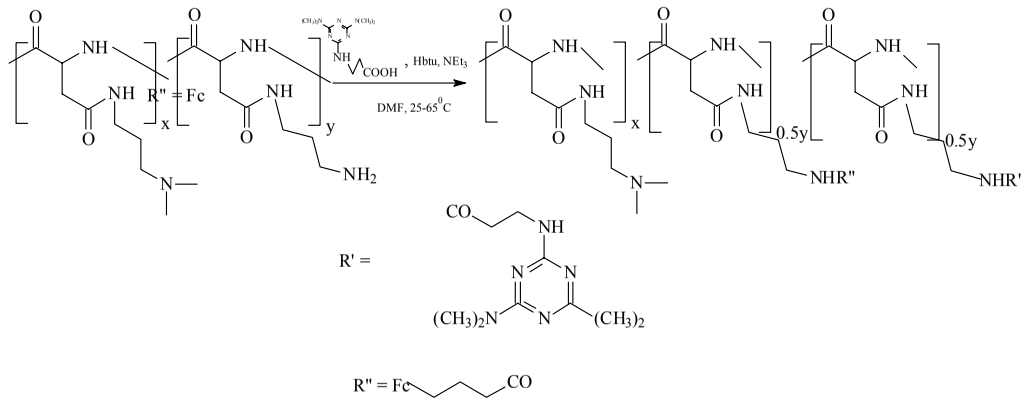
3.8. CO-CONJUGATION OF FERROCENE AND β-ALANINE-MODFIED TETRAMETHYLMELAMINE

It has been well established that certain drugs when administered together have a synergistic effect, which increases the overall efficiency compared to when they are administered independently. Furthermore the direct delivery of two anti-cancer agents with various modalities to the same site should theoretically dramatically increase the potency of the drugs and their overall effect.
It is this rational that has motivated me to attempt to co-conjugate β-alanine triazine and ferrocene to a copolyaspartamide polymer. Although literature has indicated no synergistic effect following the co-administration of these two drugs, their different modalities at a localized site should dramatically increase the potency.

Co-conjugation of the ferrocene and triazine drugs proceeded as described in 3.8 and proceeded similar to that described in section 3.7, except that a single carrier was used, 1e*, and the ratio of drug to polymer in the feed was 1:2 (ferrocene drug) and 1:1 (tetramethylmelamine derivative). Co-conjugation was completed in-situ with the ferrocene drug being added first. Anchoring involved the dissolution of the polymer in a aprotic solvent and its subsequent conjugation to the ferrocencylbutanoic acid in a ratio of 2:1 (polymer:ferrocenylbutanoic acid), using a appropriate coupling agent (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)). The reaction proceeded under anhydrous conditions for a period of 2-2.5 hrs in the dark at room

temperature. Afterwards M8 was added in a ratio of 1:1 (M8: polymer) and coupled to the remaining anchoring sites by further addition of the coupling agent (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)). The reaction continued under anhydrous conditions for a period of 2-2.5 hrs at room temperature in the dark The polymer ferrocene-triazine co-conjugate was isolated following a series of purification steps which included precipitation in a non-solvent, scrupulous washing, aqueous dialysis and freeze-drying under varying pH conditions. The product was obtained as a light brownish, water soluble material, with a yield of 69 % (mass:mass), and inherent viscosity of 9 mL g^{-1}. The co-conjugate was analysed by ^{1}H NMR, and analysis yielded a ferrocene content of 2.9 % and a triazine content of 3.4 %, both obtained by considering the mass of the drug in the conjugate as a percentage of the total mass of the drug-polymer conjugate.

Most of the conjugates synthesised thus far were submitted for biological analysis to the Department of Pathology, University of Pretoria and the results obtained, so far incomplete, fell outside the scope of this dissertation.



Scheme 3.8 Co-conjugation of ferrocene and M8 to a copolyaspartamide carrier, 1e*

Table 3.8a: Experimental conditions and analytical data for the copolyaspartamide co-conjugate, **1e***-Fc/tr

Reactants in feed			Reactants & reagents (mmol)			Reaction conditions	Ferrocene-M8 co-conjugate						
												Fe and tetramethylmelamine	
Carrier	Coupling Method	Carrier:Fc:M8 ratio (mmol)	1e*	M8	Fc		Design.	x/y	Yield %	η_{inh} mLg^{-1}	Base molar mass g/mol	Calcd. % (mass:mass)	Found % (mass:mass)
1e*[a]	HBTU	1:0.5:1	0.15	0.15	0.075	**1.** 2.5hr, RT, 0.1mmol HBTU **2.** 2.5hr, RT, 0.1mmol HBTU	1e*	9	69	9	2176.455	Fe – 4.3 tr[b] – 4.1	2.9 3.4

[a] **1e*** is poly-α,β-DL-[N-(3-dimethyamino)propyl-aspartamide-co-N-(3-aminopropyl)aspartamide, with the ratio of solubilizing to drug anchoring portion being 9 to 1

[b] **tr** refers to the mass percentage of tetramethylmelamine present within the 3-(4,6-bis(N,N-dimethylamino)-1,3,5-triazacyclohexatrien-2-yl) propanoic acid polymer conjugate

Table 3.8b: ^{1}H NMR for the copolyaspartamide co-conjugate, **1e***-Fc/tr

Carrier designation	^{1}H NMR			Assignment
	Shift δ (ppm)	Proton count		
		expected	found	
1e*-Fc/triazine	4.2-4.2	4.5	3	ferrocenyl
	3.2-3.1	22	23	-C**H**$_2$-NHCO
	3.1-2.9	6	5	(C**H**$_3$)$_2$-N-triazine
	2.9-2.5	22	21	Asp CH_2, CO-C**H**$_2$-CH_2
	2.5-2.2	19	20	N-C**H**$_2$
	2.2-1.9	54	57	CH_2-N-C**H**$_3$
	1.7-1.5	21	21	CH_2-C**H**$_2$-CH_2

[a] in D_2O at pD 9-10 with NaOH, reference against sodium-3-(trimethylsilyl-2,2,3,3-d_4-propionate. Integration error limits ±15%, protons calculated and rounded off to the nearest integer

CHAPTER 4

EXPERIMENTAL

4.1 GENERAL PROCEDURES

Melting points were determined in sealed capillary tubes. ^{1}H NMR spectra was recorded on a 200 or 400 MHz instrument, in either D_2O or $CDCl_3$ solutions. Chemical shifts, δ in ppm, were recorded relative to the internal standard sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate and tetramethylsilane, respectively. The pD of selected samples were adjusted to ~ 10, with NaOH. All spectra have an intergration error limit of 10-15 %.

Inherent viscosities (η_{inh}) were determined in a Cannon-Fenske viscometer tube at a temperature of 37 ± 0.5 ^{0}C, in deionized water (c = 2 g/100 ml), and the results were given as mL g^{-1}. Dialyses was performed using cellulose membranes (Spectrum Industries Inc., Los Angeles, USA) of the type Spectra/Por 4 (12,000 – 14,000 molecular-mass cut off limit) and Spectr/Por 6 (25,000 molecular mass cut off limit). Dialysis was performed over various periods and pH conditions against large volumes of distilled water.

A Virtis Bench Top 3 freeze drier (-40 ^{0}C, 10-15 Pa) was used to freeze-dry all the polymer samples (both carrier and conjugate samples). The samples were further dried for 2 days at 60-65 ^{0}C, under reduced pressure in an Abderhalden apparatus, with $CaCl_2$ as the drying agent. Iron and platinum content of the conjugates were determined by Anglo American Laboratories, Crown Mines, Johannesburg.

4.2 REAGENTS AND SOLVENTS

Deionized water was used for dialysis and viscometric operations, including any preparative work. Methanol and THF were distilled in a faint stream of nitrogen, prior to use. Dimethylformamide (DMF) was distilled under reduced pressure in a faint stream of nitrogen, with a fore-run of 15 % being discarded, once distilled the DMF was further dried over molecular sieves 4 Å. The aprotic solvent DMSO was also dried over molecular sieves 4 Å.

The following reagents (Fluka AG and/or Aldrich GmbH) were used as received: acryloyl chloride, 3-amino-1,2-propanediol (APD), DL-aspartic acid, orthophosphoric acid (85 %), diethyl malonate (DEM), N,N'-dicyclohexylcarbodiimide (DCC), N,N'-(1,2-dihydroxyethylene)bisacrylamide (DHEBA), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), ferrocene, ethanolamine (EA), 3-(dimethylamino)propylamine (DMP), 1,3-diaminopropane (PDA), Diethyl L-tartrate (Detart), ethylenedioxy-O,O'-bis(2-ethylamine) (EDDA), diethyl ether, cyanuric chloride, 4,7,10-trioxa-1,13-tridecanediamine (Tria), triethylamine (NEt_3)

Potassium tetrachloroplatinate (K_2PtCl_4) was donated by the Western Platinum Refinery.

A technical grade mixture of isopropanol and propanol (propylol) was used in certain recrystallization and precipitation procedures.

N,N-methylenebisacrylamide and (1,2-dihydroxyethylene)bisacrylamide were recrystallized from isopropanol in the presence of the inhibitor hydroquinone (0.5 % by mass). Throughout this dissertation the amount of polymeric material is given as base moles.

4.3 PREPARATION OF MACROMOLECULAR CARRIERS

4.3.1 Poly(DL-succinimide) 1

Polysuccinimde was prepared according to the literature method of Neri and Antoni.[40] This involved the high-temperature solution polymerisation of DL-aspartic acid in orthophosphoric acid and its subsequent treatment with dicyclohexylcabodiimide at a reduced temperature. The product of three successive runs were pooled together to produce a polymer with an average inherent viscosity (DMF) of 30 mL g^{-1}.

4.3.2 Poly α,β-DL- aspartamides from polysuccinimide 1 by aminolytic ring opening

*Copolyaspartamide **1a***

Poly-α,β-DL-[N-(2-hydroxyethyl)aspartamide (90)-co-N-(3-aminopropyl)aspartamide (10)]

0.97 g (10 mmol) of **1** was dissolved in 20 ml DMF and flushed with nitrogen. To this solution was added 0.543g (9 mmol) EA drop wise, under nitrogen. The reaction proceeded for 8 hrs at RT. This solution was added drop wise to 0.222 g (3 mmol) PDA in 5 ml DMF, under nitrogen at 0-5 ^{0}C. The solution was stirred at this temperature for a

further 16 hrs, and for 24 hrs at RT. The volume of DMF was reduced to 10 ml and the polymer was precipitated with 15 ml ether: hexane (2:1) (volume:volume). The precipitate was washed copiously with cold acetone and re-dissolved in 20 ml water. The polymer solution was dialysed successively for 48 hrs in Spectra/Por 4 tubing and 48 hrs in Spectra/Por 6 tubing. The retentate was freeze-dried, and a beige coloured solid was obtained, which was post-dried for 2 days at 60-65 ^{0}C, under reduced pressure in an Abderhalden apparatus. This gave copolyaspartamide **1a**, with a yield of 0.553 g (57 %); $\eta_{inh}(H_2O)$ 9 mL g^{-1}

^{1}H NMR, δ/ppm: 3.7-3.5 ppm CH_2-C**H$_2$**-OH (18H)18H, 3.4-3.0 ppm CONH-C**H$_2$**-CH_2OH and CONH-C**H$_2$**CH_2 (20H)18H, 2.8-2.5 ppm Asp C**H$_2$** and -C**H$_2$**-NH_2 (22H)24H, 1.6-1,4 ppm CH_2C**H$_2$**CH_2 (18H)18H

*Copolyaspartamide **1b***

Poly-α,β-DL-[N-(2-hydroxyethyl)aspartamide-co-N-(3,6-dioxa-9-azanonyl)aspartamide] Copolyaspartamide**1b** was prepared analogous to that of **1a**. Except that in this instance 444 mg (3 mmol) ethylenedioxy-O,O'-bis(2-ethylamine) (EDDA) in 10 ml DMF was used instead of PDA. Following analogues precipitation, dialysis, and drying conditions produced copolyaspartamide **1b**, with a yield of 0.475 g (49%); $\eta_{inh}(H_2O)$ 8 mL g^{-1}

^{1}H NMR, δ/ppm:4.7-4.5 ppm Asp C**H** 10H(10H), 4.0-3.5 ppm CH_2-C**H$_2$**-OH and CH_2C**H$_2$**-O 26H(26H), 3.5-3.0 ppm CONH-C**H$_2$**- 20H(20H), 3.0-2.5 ppm Asp C**H$_2$** and -C**H$_2$**-NH_2 21(22)

*Copolyaspartamide **1c***

10 mmol of **1** was dissolved in 20 ml DMF, under nitrogen. To this solution was added 10 mmol DL-aspartic and 10 mmol triethylamine. A white suspension formed which was stirred at 45 ^{0}C for 48 hrs. The suspension was cooled to 0-5 ^{0}C and to it was added 0.336 g (5.5 mmol) EA. The reaction continued for a further 8 hrs at RT. The suspension was filtered and the volume of the filtrate was reduced to 10 ml. The polymer was precipitated with 25 ml Et_2O:hexane (2:1) and the precipitate was washed copiously with cold acetone and re-dissolved in 30 ml distilled water. The polymer solution was dialysed successively for 48 hrs in Spectra/Por 4 tubing and 48 hrs in Spectra/Por 6 tubing. The retentate was freeze-dried and a white solid was obtained, which was post dried for 2 days at 60-65 ^{0}C, under reduced pressure in an Abderhalden apparatus. This gave copolyaspartamide **1c**, with a yield of 0.359 g (37 %); $\eta_{inh}(H_2O)$ 6 mL g^{-1}

^{1}H NMR, δ/ppm: 4.7-4.5ppm Asp C**H** 1H(3H), 4.0-3.5ppm CH_2-C**H_2**-OH 2H(2H), 3.5-3.0ppm CONH-C**H_2** 2H(2H), 3.0-2.5ppm Asp C**H_2** 3H(6H)

*Copolyaspartamide **1d***

Poly-α,β-DL-[-(2-hydroxyethyl)aspartamide-co-N-(2,3-dihydroxypropyl)aspartamide] Copolyaspartamide **1d** was prepared similar to that of **1a,** except that in this instance 0.273 g (3 mmol) 3-amino-1,2-propanediol (APD) dissolved in 10 ml DMF**,** was used instead of PDA. Following analogous precipitation, dialysis, and drying operations, this produced copolyaspartamide **1d**, with a yield of 0.514 g (53 %); $\eta_{inh}(H_2O)$ 8 mL g^{-1}

^{1}H NMR, δ/ppm: 4.0-3.5 ppm CH_2-C**H**(OH)-CH_2OH, CH(OH)-C**H_2**OH and CH_2-C**H_2**-OH 21H(21H), 3.5-3.0 ppm CONH-C**H_2**- 19H(20H), 3.0-2.5 ppm, Asp C**H_2** 21H(20H)

*Copolyaspartamide **1e***

Poly-α,β-DL-[N-(3-dimethyamino)propyl-aspartamide-co-N-(3-aminopropyl)aspartamide]

0.97 g (10 mmol) of **1** was dissolved in 20 ml DMF and flushed with nitrogen. To this solution was added 0.767 g (7.5 mmol) DMP dropwise, under nitrogen. The reaction proceeded for 6 hrs at RT. This solution was added drop wise to 0.556 g (7.5 mmol) PDA, under nitrogen at 0-5 ^{0}C. The solution was stirred at this temperature for a further 16 hrs, and for 24 hrs at RT. The volume of DMF was reduced to ~12 ml and the polymer was precipitated with 25 ml Et_2O:hexane (2:1). The precipitate was washed copiously with cold acetone and re-dissolved in 30 ml water. The polymer solution was dialysed successively for 48 hrs in Spectra/Por 4 tubing and 48 hrs in Spectra/Por 6 tubing. The retentate was freeze-dried, and a beige coloured solid was obtained, which was post-dried for 2 days at 60-65 ^{0}C, under reduced pressure in an Abderhalden apparatus. This gave copolyaspartamide **1e**, with a yield of 0.592 g (61 %); η_{inh}(H_2O) 7 mL g^{-1}

^{1}H NMR, δ/ppm: 3.5-3.0ppm CONH-C**H_2**-CH_2 8H(8H), 3.0-2.5ppm Asp-C**H_2** 9H(8H), 2.4-2.2ppm -C**H_2**-N 8H(8H), 2.2-2.0ppm N-C**H_3** 17H(18H), 1.7-1,5ppm, CH_2C**H_2**CH_2 8H(8H)

*Copolyaspartamide **1f***

Poly-α,β-DL-[N-(3-dimethyamino)propyl-aspartamide-co-N-(3,6-dioxa-9-azanonyl)aspartamide]

Copolyaspartamide **1f** was prepared similar to that of **1e**. Except that in this instance 3 mmol ethylenedioxy-O,O'-bis(2-ethylamine) (EDDA)**,** was used instead of PDA. Following analogous precipitation, dialysis, and drying conditions, produced copolyaspartamide **1f**, with a yield of 0.650 g (67 %); $\eta_{inh}(H_2O)$ 8 mL g^{-1}

^{1}H NMR, δ/ppm: 3.7-3.5 ppm CH-$_2$C**H$_2$**-O 7H(6H), 3.4-3.45 ppm CONH-CH_2-C**H$_2$**-O 2H(2H), 3.45-3.2 ppm CONH-C**H$_2$** 18H(20H), 3.2-2.6 ppm Asp-C**H$_2$**, 2.5-2.3 ppm CH_2-C**H$_2$**-N 18H(20H),2.3-2.1 ppm N-C**H$_3$** 53H(54H) 1.7-1,5 ppm , CH_2C**H$_2$**CH_2 (2H)2H

*Copolyaspartamide **1g***

Copolyaspartamide **1g** was prepared similar to that of **1c**. Except that in this instance 5 mmol DMP was used rather than EA. Following analogous precipitation, dialysis, and drying operations, this produced copolyaspartamide **1g**, 0.398 g (41 %); $\eta_{inh}(H_2O)$ 6 mL g^{-1}

^{1}H NMR, δ/ppm: 4.5-4.0 ppm Asp C**H** 2H(3H), 3.0-2.55 ppm CONH-C**H$_2$**-2H(2H), 2.55-2.45 ppm Asp C**H$_2$** 4H(6H), 2.45-1.8 ppm CH_2-C**H$_2$**-N 3H(2H), and N-C**H$_3$** 6H(6H), 1.6 -1.4 ppm CH_2-C**H$_2$**-CH_2 2H(2H)

*Copolyaspartamide **1h***

Poly-α,β-DL-[N-(3-dimethyamino)propyl-aspartamide-co-N-(2,3-dihydroxylpropyl)aspartamide]

10 mmol of **1** was dissolved in 20 ml DMF and flushed with nitrogen. To this solution was added 0.920 g (9 mmol) DMP drop wise, under nitrogen. The reaction proceeded for 8 hrs at RT. This solution was added drop wise to 0.273 g (3 mmol) APD in 15 ml DMF, under nitrogen at 0-5 ^{0}C. The solution was stirred at this temperature for a further 16 hrs, and for 24 hrs at RT. The volume of DMF was reduced to 10 ml and the polymer was precipitated with 20 ml ether:hexane (2:1) (volume:volume). The precipitate was washed copiously with cold acetone and re-dissolved in 20 ml water. The polymer solution was dialysed successively for 48 hrs in Spectra/Por 4 tubing and 48 hrs in Spectra/Por 6 tubing. The retentate was freeze-dried, and a beige coloured solid was obtained, which was post dried for 2 days at 60-65 ^{0}C, under reduced pressure in an Abderhalden apparatus. This gave copolyaspartamide **1h**, with a yield of 0.495 g (51 %); $\eta_{inh}(H_2O)$ 7 mL g^{-1}

^{1}H NMR, δ/ppm: 4.0-3.5 ppm CH_2-C**H**(OH)-CH_2OH and CH(OH)-C**H$_2$**OH 3H(3H), 3.5-3.0 ppm CONH-C**H$_2$**- 20H(20H), 3.0-2-5 ppm Asp C**H$_2$** 20H(20H), 2.5-2.0 ppm CH_2-C**H$_2$**-N 19H(18H) and N-C**H$_3$** 54H(54H), 1.7 ppm-1.5 ppm CH_2-C**H$_2$**-CH_2 18H(18H)

4.3.3 Copolyamidoamines

*Recrystallization of MBA, **M1***

7.7085 g (50 mmol) of MBA was dissolved in boiling 30 ml isopopanol, with 0.5 % (mass:mass) hydroquinone, and the solute was allowed to crystallise at RT . Three crops were collected 5.40 g (35 mmol) and characterised by ^{1}H NMR

^{1}H NMR, δ/ppm: 6.3-6.1 ppm OCNH-CH=C**H$_2$** 4H(4H), 5.9-5.7 ppm OCNH-C**H**=CH_2 2H(2H), 4.7-4.6 ppm OCNH-C**H$_2$**-CONH 2H(2H)

*Recrystallization of DHEBA, **M2***

2.002 g (10 mmol) of MBA was dissolved in boiling 20 ml isopropanol, with 0.5 % (mass:mass) hydroquinone and the solution was allowed to crystallise at RT with the addition of 5ml hexane . Two crops were collected 1.72 g (8.5 mmol) and characterised by ^{1}H NMR

^{1}H NMR, δ/ppm: 6.4-6.1 ppm OCNH-CH=C**H$_2$** 4H(4H), 5.8-5.7 ppm OCNH-C**H**=CH_2 2H(2H), 5.5-5.3 ppm OCNH-C**H**(OH)-CH(OH) 2H(2H)

*Copolyamidoamine **1i***

1.542 g (10 mmol) recrystallized MBA, **M1**, was dissolved in 10 ml warm water. To this solution was added (0.667 g) 5 mmol Asp and 0.530 g (5 mmol) Na_2CO_3, and the pH was adjusted to ~9. The solution was stirred in the dark for 24 hrs at RT and a further 24 hrs at 45 ^{0}C. Afterwards 0.510 g (5 mmol) DMP was added to the solution and the reaction continued at RT for 24hrs. During the last 6hrs of this period 0.5 % EA (mass:mass) was added to the solution, to neutralise any unreacted vinyl groups. The

volume of the polymer solution was reduced to form a viscous oil, which was copiously washed with propylol. The oil was dissolved in 30ml water and pH of the solution was reduced to 5.0. The solution was then dialysed successively for 48hrs in Spectra/Por 4 tubing and 48 hrs in Spectra/Por 6 tubing. The retentate was freeze-dried and a white solid was obtained, which was post dried for 2 days at 60-65 ^{0}C, under reduced pressure in an Abderhalden apparatus. This gave copolyamidoamine, **1i**, with a yield of 0.278 g (18 %); $\eta_{inh}(H_2O)$ 11 mL g^{-1}

^{1}H NMR δ/ppm: 4.6-4.3 ppm CONH-C**H$_2$**-NHCO 3H(4H), 2.7-2.5 ppm CONH-C**H$_2$**CH$_2$ 8H(8H), 2.4-2.1 ppm N-CH$_2$-C**H$_2$** and CHC**H$_2$**CO 14H(12H), 2.0-1.8ppm N-C**H$_3$** 6H(6H), 1.6-1,4 ppm CH$_2$C**H$_2$**CH$_2$ 2H(2H)

*Copolyamidoamine **1j***

Copolyamidoamine **1j** was prepared similar to that of **1i**, except that in this instance (0.456 g) 5 mmol 3-amino-1,2-propandiol dissolved in 5 ml de-ionised water was used rather than aspartic acid. In addition, only 0.212 g (2 mmol) Na_2CO_3 was used instead of 5 mmol. Following analogous precipitation, dialysis, and drying operations, this produced copolyamidoamine **1j**, with a yield of 0.339 g (22 %); $\eta_{inh}(H_2O)$ 12 mL g^{-1}

^{1}H NMR, δ/ppm: 4.6-4.3 ppm CONH-C**H$_2$**-NHCO 4H(4H), 3.8-3.6 ppm CH(OH)-C**H$_2$**-OH 1H(1H), 3.6-3.4 ppm CH$_2$C**H**(OH)CH$_2$ 2H(2H), 2.8-2.6 ppm CONH-C**H$_2$**CH$_2$ 7H(8H), 2.6-2.4 ppm N-CH$_2$-C**H$_2$** and N-C**H$_2$**-CH(OH) 13H(12H), 2.4-2.2 ppm N-C**H$_3$** 7H(6H) 1.8-1,6 ppm CH$_2$C**H$_2$**CH$_2$ 2H(2H)

*Copolyamidoamine **1k***

1.001 g (5 mmol) recrystallised DHEBA, **M2**, was dissolved in 20 ml warm water. To this solution was added 0.212 g (2 mmol) Na_2CO_3 and 1.102 g (5 mmol) Tria drop wise and the reaction continued for 24 hrs at RT and a further 24 hrs at 45 ^{0}C. Throughout this period the flask was light protected and the pH of the solution was maintained at ~9. Afterwards 0.5 % EA (mass:mass) was added to the solution, to neutralise any unreacted vinyl groups, and the reaction was continued in the dark for a further 6 hrs. The volume of the polymer solution was then reduced to form a viscous oil, which was copiously washed with propylol. The oil was dissolved in 25 ml water and pH of the solution was reduced to 8.0. The solution was then dialysed successively for 48 hrs in Spectra/Por 4 tubing and 48 hrs in Spectra/Por 6 tubing. The retentate was freeze-dried and a brownish solid was obtained, which was post-dried for 2 days at 60-65 ^{0}C, under reduced pressure in an Abderhalden apparatus. This gave copolyamidoamine, **1k**, with a yield of 0.220 g (22 %); $\eta_{inh}(H_2O)$ 10 mL g^{-1}

^{1}H NMR, δ/ppm: 3.5-3.0 ppm CH_2-C**H$_2$**-O and CONH-C**H**(OH)-CH 14H(14H), 2.7-2.5 ppm CONH-C**H$_2$**CH_2 4H(4H), 2.5-2.3 ppm HN-C**H$_2$**-CH_2 4H(4H), 2.4-2.2 ppm N-C**H$_3$** 7H(6H) 1.6-1,4 ppm CH_2C**H$_2$**CH_2 4H(4H)

*Macromonomer, **M3***

0.407 g (4.5 mmol) of acryloyl chloride were added drop wise to an aqueous solution of 0.297 g (2 mmol) diaminosuccinic acid, maintained at 0-5 ^{0}C and slightly alkaline pH, 6.5-8.5. Following the addition, the reaction proceeded for a further 4hrs at 20 ^{0}C to ensure complete substitution of the amino groups, during which time the pH was

regularly monitored and maintained at 6.5-8.5. Afterwards the solution was acidified to pH 3.0 and the product was extracted with five times 20 ml portions of ethyl acetate, and all traces of water were removed from the extract. The pure product obtained by crystallization following reduction of the solvent volume, was isolated as a colourless solid, in a combined yield of 0.302 g**.** The crops were pooled and characterized by ^{1}H NMR spectroscopy

^{1}H NMR, δ/ppm: 6.3-6.1 ppm **H$_2$**C=CH-CONH 4H(4H), 5.8-5.6 ppm CH_2=C**H**-CONH 2H(2H), 4.7-4.5 ppm, OC-C**H**-CONH 2H(2H)

*Copolyamidoamine **1l***

Copolyamidoamine **1l** was prepared similar to that of **1k.** Except that in this instance 2.562 g (10 mmol) **M4** was used instead of DHEBA, also 1.272 g (12 mmol) Na_2CO_3 was used instead of 2 mmol. Following analogous precipitation, dialysis (except that the polymer solution was adjusted to pH 5 prior to dialysis), and drying operations, this produced copolyamidoamine **1l**, with a yield of 0.820 g (32 %); η_{inh}(H_2O) 14mL g^{-1}

^{1}H NMR, δ/ppm: 4.5-4.3 ppm CONH-C**H**-CO 1H(2H), 3.7-3.5 ppm CH_2-C**H$_2$**-O 12H(12H), 2.7-2.5 ppm CONH-C**H$_2$**-CH2 4H(4H), 2.4-2.1 ppm NH-C**H$_2$**-CH_2 1H(4H), 1.8-1,6ppm CH_2C**H$_2$**CH_2 4H(4H)

4.3.4 Ester-amine polycondensation derived polyamides

Polyamide ***1m***

A mixture of 1.310 g (5 mmol) Detart 1.102 g (5 mmol) Tria, and anhydrous Na_2CO_3 (~0.5 g) was saturated with nitrogen and stirred at RT for 24 hrs. The mixture was then diluted with 6 ml DMSO, resaturated with nitrogen and stirring continued for 13 day at 45^0C. Subsequently the polymer mixture was copiously washed with propylol to produce a viscous mixture. The viscous mixture dissolved in 15 ml water, and the pH of the polymer solution was adjusted to 6.0. The solution was dialysed successively for 24 hrs in Spectra/Por 4 tubing and 48 hrs in Spectra/Por 6 tubing. The retentate was freeze-dried and a yellowish solid was obtained, which was post-dried for 2 days at 60-65 ^{0}C, under reduced pressure in an Abderhalden apparatus. This gave polyamide, **1m**, with a yield of 0.498 g (38 %); $\eta_{inh}(H_2O)$ 12 mL g^{-1}

^{1}H NMR, δ/ppm: 4.5-4.3ppm CONH-C**H**(OH) 2H(2H), 3.7-3.5ppm CH_2-C**H_2**-O 13H(12H), 3.4-3.2ppm CONH-C**H_2**-CH_2 4H(4H), 2.8-2.6ppm NH-C**H_2**-CH_2 1H(2H) 1.8-1,6ppm CH_2C**H_2**CH_2 4H(4H)

Macromonomer **M4**

A mixture of 11 mmol NaOH and 50 ml THF was stirred for 2 hrs at RT. To this mixture was added 0.801 g (5 mmol) of diethyl malonate (DEM) at 0-5 ^{0}C, and the mixture was stirred for a further 4hrs at RT. Acrylonitrile (AcCN), 0.811 g (15 mmol) was added drop wise to this mixture at 0-5 ^{0}C and the reaction continued for a further 24 hrs at RT. Afterwards the THF was removed under reduced pressure, and a colourless solid was

collected. The solid was dissolved in 20 ml water and to it was added three times 20 ml portions of ethyl acetate, to remove any unreacted DEM. The aqueous solution was collected and acidified with HCl to pH 3. To this aqueous solution was added five times 20 ml portions of ethyl acetate, and the ethyl acetate was collected. All traces of moisture were removed from the organic phase by the addition of ~5 g of anhydrous $MgSO_4$. The volume of the ethyl acetate, was reduced to 15 ml under reduced pressure, and hexane was added until turbidity, the product was allowed to crystallize at 0-5 ^{0}C. This gave a colourless solid, with a yield of 0.545 g and a melting point of 145-147 ^{0}C

^{1}H NMR, δ/ppm: 2.8-2.6 ppm CH_2-C**H$_2$**-C-$(COOH)_2$ 2H(2H), 2.2-2.0 ppm CN-C**H$_2$**-CH_2 2H(2H)

Macromonomer **M5**

M5, 1.051 g (5 mmol) was dissolved in anhydrous methanol at 0-5 ^{0}C and flushed for 30min at this temperature with anhydrous hydrogen chloride, produced by passing concentrated hydrochloric acid solution into a stream of 98 % (mass:mass) sulphuric acid. The solvent volume was reduced under reduced pressure at ~20 ^{0}C to minimize hydrolytic depolymerisation. The crude product was allowed to crystallize upon the addition of hexane. Re-crystallization afforded a colourless solid, with a total yield of 0.568 g, spread over three crops, melting at 114-116 ^{0}C

^{1}H NMR, δ/ppm: 3.8-3.6 ppm C**H$_3$**-OCO 5H(6H), 2.8-2.6 ppm CH_2-C**H$_2$**-C-$(COOH)_2$ 2H(2H), CN-C**H$_2$**-CH_2 2H(2H)

*Polyamide **1n***

Polyamide **1n** was synthesized similarly to that described for 1m, except in this instance, macro-monomer M5 was used instead of Detart. In addition 1.060g (10mmol) Na_2CO_3 and 5 % (mass:mass) 2-hydroxypyridine was added to the reaction mixture. After analogous isolation and purification, a colourless solid with a yield of 0.420 g (40 %), $\eta_{inh}(H_2O)$ 14mL g^{-1}, was obtained

^1H NMR, δ/ppm: 3.7-3.5 ppm CH_2-C**H_2**-O 12H(12H), 3.4-3.0 ppm CONH-C**H_2**-CH_2 3H(6H), 2.6-2.4 ppm NH-C**H_2**-CH_2 1H(2H), 2.2-1.7 ppm $(COOH)_2$-C**H_2**-CH_2 3H(4H), 1.7-1,5 ppm CH_2C**H_2**CH_2 4H(4H)

4.4 POLYMER CONJUGATION

4.4.1a Polymer-platinum conjugation

Monoamineplatinum(II)--copolyaspartamide conjugates

***1a**-Pt*

Compound **1a**, 300 mg (0.190 mmol) was dissolved in 10 ml distilled water, the solution was saturated with nitrogen and to it was added 79 mg (0.228 mmol) K_2PtCl_4. The mixture was stirred in the dark at room temperature for 24 hrs and for a further 48 hrs at 45 ^{0}C, also in the dark. During this period the pH was maintained at 5-7, and in the last 30 min the pH was adjusted to 4.0 by the addition of HCl. At this point 0.5 g of NaCl was added and the solution was stirred at room temperature for a further 2 hrs. The brownish solution was dialysed in Spectra/Por 4 tubing for 48 hrs against water kept at pH 4-5. Freeze drying of the retentate afforded a water-soluble solid in the yield of 165 mg (55 %), $\eta_{inh}(H_2O)$ 11 mL $g^{-1.}$ Analysis found: Pt, 8.2 % (10.3 %)

***1b**-Pt*

Compound **1b**, 300 mg was dissolved in 15 ml distilled water, the solution was saturated with nitrogen and to it was added 91 mg (0.218 mmol) K_2PtCl_4, whereupon the pH dropped to 4.0. The pH was adjusted to 5-7 by the addition of Na_2CO_3. The mixture was stirred in the dark at room temperature for 24 hrs and for a further 48 hrs at 45 ^{0}C, also in the dark. During this period the pH was maintained, and in the last 30 min the pH was adjusted to 4.0 by the addition of HCl. At this point 0.5 g of NaCl was added and the solution was stirred at room temperature for a further 2 hrs. The reddish brown solution was dialysed in Spectra/Por 4 tubing for 48 hrs against water kept at pH 4-5. Freeze drying of the retentate afforded a water soluble solid in the yield of 141 mg (47 %), $\eta_{inh}(H_2O)$ 9 mL g^{-1}. Analysis found: Pt, 7.0 % (10 %)

***1e**-Pt*

This conjugate was prepared very similarly to that described for 1a-Pt, in this instance however 300 mg (0.391 mmol) of 1e carrier and 206 mg (0.4692 mol) K_2PtCl_4 was used. Analogous purification and isolation procedures, afforded a brown solid in the yield of 183 mg (61 %), $\eta_{inh}(H_2O)$ 9 mL g^{-1}. Analysis found: Pt, 9 % (16 %)

***1f**-Pt*

Compound **1f**, 300 mg (0.147 mmol) was dissolved in 12 ml distilled water, the solution was saturated with nitrogen and to it was added 74 mg(0.177 mmol) K_2PtCl_4, whereupon the pH dropped to 4.0, the pH was adjusted to 5-7 by the addition of Na_2CO_3. The mixture was stirred in the dark at room temperature for 24 hrs and for a further 48 hrs at

45 ^{0}C, also in the dark. During this period the pH was maintained, and in the last 30 min the pH was adjusted to 4.0 by the addition of HCl. At this point 0.5 g of NaCl was added and the solution was stirred at room temperature for a further 2 hrs. The reddish brown solution was dialysed in Spectra/Por 4 tubing for 48hrs against water kept at pH 4-5. Freeze drying of the retentate afforded a water soluble solid in the yield of 126 mg (42 %), $\eta_{inh}(H_2O)$ 7 mL g^{-1}. Analysis found: Pt, 7.5 % (8.4 %)

Dihydroxylato platinum(II) polyaspartamide conjugates

***1d**-Pt*

Compound **1d**, 300 mg (0.188 mmol) was dissolved in 10ml distilled water. To this was added 112 mg (0.282 mmol) DACH-Pt aq. and pH of the solution adjusted from 4.0 to 6.0-7.0 with Na_2CO_3. The reaction proceeded in the dark for 24 hrs and then at 45 ^{0}C for 48hrs, throughout this period the pH was maintained with either nitric acid or Na_2CO_3. Afterwards the reddish brown was dialysed in Spectra/Por 4 tubing for 48 hrs against water kept at pH 5-6. Freeze drying of the retentate afforded a water soluble brown solid in the yield of 111 mg (37 %), $\eta_{inh}(H_2O)$ 7 mL g^{-1}. Analysis found: Pt, 2.09 % (9.8 %)

***1h**-Pt*

Compound **1d**, 300 mg (0.151 mmol) of 1h was dissolved in 10 ml distilled water. To this was added 90 mg (0.227 mmol) DACH-Pt aq. and pH of the solution was 6-7.0. The solution was stirred in the dark for 24 hrs and then at 45 ^{0}C for 48 hrs, throughout this period the pH of 6-7.0 was maintained with either nitric acid or Na_2CO_3. Afterwards the reddish brown solution was dialysed in Spectra/Por 4 tubing for 48 hrs against water kept

at pH 5-6. Freeze drying of the retentate afforded a water soluble brown solid in the yield of 162 mg (54 %), $\eta_{inh}(H_2O)$ 9mL g^{-1}. Analysis found: Pt, 7.69 % (8.2 %)

Dicarboxylatoplatinum(II) copolyaspartamide conjugates

***1c**-Pt*

This conjugate was prepared very similarly to that described for **1d**-Pt, in this instance, however 300 mg (0.781 mmol) of 1c carrier and 465 mg (1.17 mmol) DACH Ptaq was used. Analogous purification and isolation procedure, afforded a brown solid in the yield of 123 mg (41 %), $\eta_{inh}(H_2O)$ 7 m Lg^{-1}. Analysis found: Pt, 5.93 % (25 %)

***1g**-Pt*

This conjugate was prepared very similarly to that described for **1g**-Pt, in this instance however 300 mg (0.702 mmol) of 1c carrier and 418 mg (0.1.053 mol) DACH Ptaq was used. Analogues purification and isolation procedure, afforded a brown solid in the yield of 129 mg (43 %), $\eta_{inh}(H_2O)$ 8mL g^{-1}. Analysis found: Pt, 9.02 % (23 %)

4.4.1b Polyamidoamine-Pt conjugates

Dihydroxylatoplatinum(II) polyamidoamine conjugates

***1j**-Pt*

Compound **1j**, 300 mg (0.599 mmol) was dissolved in 10 ml distilled water. To this was added .357 mg (0.899 mmol) DACH Ptaq. and pH of the solution adjusted from 4.0 to 6.0-7.0 with Na_2CO_3. The reaction proceeded in the dark for 24 hrs and then at 45 ^{0}C for 48 hrs, throughout this period the pH was maintained with either nitric acid or Na_2CO_3.

Afterwards the reddish brown was dialysed in Spectra/Por 4 tubing for 48 hrs against water kept at pH 5-6. Freeze drying of the retentate afforded a water soluble brown solid solid in the yield of 126 mg (42 %), $\eta_{inh}(H_2O)$ 9 mL g^{-1}. Analysis found:
Pt, 4.37 % (22 %)

***1k**-Pt*

Compound **1k**, 300 mg (0.717 mmol) was dissolved in 10 ml distilled water. To this was added 427 mg (1.075 mmol) DACH-Pt aq. and pH of the solution was 6-7.0. The solution was stirred in the dark for 24 hrs and then at 45 ^{0}C for 48 hrs, throughout this period the pH of 6-7.0 was maintained with either nitric acid or Na_2CO_3. Afterwards the reddish brown solution was dialysed in Spectra/Por 4 tubing for 48 hrs against water kept at pH 5-6. Freeze drying of the retentate afforded a water soluble brown solid solid in the yield of 121 mg (47 %), $\eta_{inh}(H_2O)$ 9 mL g^{-1}. Analysis found: Pt, 2.15 % (24 %)

Dicarboxylatoplatinum(II) copolyaspartamide conjugates

***1i**-Pt*

This conjugate was prepared very similarly to that described for 1d-Pt, in this instance however 300 mg (0.553 mmol) and 330 mg (0.830 mmol) DACH-Pt aq was used. Analogous pH regulation throughout the reaction 6-7, purification and isolation procedure, afforded a brown solid in the yield of 171 mg (57 %), $\eta_{inh}(H_2O)$ 7 mL g^{-1}. Analysis found: Pt, 4.59 % (21 %)

***1l**-Pt*

This conjugate was prepared very similarly to that described for 1g-Pt, in this instance however 300 mg (0.630 mmol) of 1l and 375 mg (0.945 mmol) DACH-Pt aq was used. Analogous pH regulation throughout the reaction 6-7, purification and isolation procedure, afforded a brown solid in the yield of 177 mg (59 %), $\eta_{inh}(H_2O)$ 8 mL g-1. Analysis found: Pt, 6.53 % (22 %)

4.1c Polyamide-Pt conjugates

Dihydroxylatoplatinum(II) polyamide conjugates

***1m**-Pt*

Compound **1m**, 300 mg (0.859 mmol) was dissolved in 10 ml distilled water. To this was added 1.289 mmol DACH-Pt aq. and the pH of the solution adjusted from 5.0 to 6.0-7.0 with Na_2CO_3. The reaction proceeded in the dark for 24 hrs and then at 45 ^{0}C for 48 hrs, throughout this period the pH was maintained with either nitric acid or Na_2CO_3. Afterwards the reddish brown solution was dialysed in Spectra/Por 4 tubing for 48 hrs against water kept at pH 5-6. Freeze drying of the retentate afforded a water soluble brown solid solid in the yield of 168 mg (56 %), $\eta_{inh}(H_2O)$ 9 mL g^{-1}. Analysis found: Pt, 2.95 % (26 %)

Dicarboxylatoplatinum(II) polyamide conjugates

***1n**-Pt*

This conjugate was prepared very similarly to that described for 1m-Pt, in this instance however 300 mg (0.672 mmol) of 1n and 400 mg (1.008 mmol) of DACH Ptaq was used.

Analogous pH maintenance throughout the reaction (pH 6-7), purification and isolation, afforded a brown solid in the yield of 183 mg (61 %), $\eta_{inh}(H_2O)$ 11 mL g^{-1}. Analysis found: Pt, 7.31 % (23 %)

4.4.2 Polymer-ferrocene conjugation

Preparation of 4-ferrocenylbutanoic acid, M6

Ferrocene derivation was achieved through an established literature procedure, involving Fiedel Crafts acylation of ferrocene and Clemensen reduction of the keto intermediary to ferrocenylbutanoic acid, TLC performed in petroleum ether:acetone (1:1) signified completion of the reaction. The acid was afforded as a yellow solid, in 0.840 g (40 %) yield with a melting point of 118-120 ^{0}C.

^{1}H NMR, δ/ppm: 4.3-4.1 ppm ferrocenyl 8H(9H), 2.4-2.2 ppm C**H$_2$**-CH_2-C**H$_2$** 4H(4H), CH_2-C**H$_2$**-CH_2 2H(2H)

Copolyaspartamide-Fc conjugates

***1a**-Fc*

Compound **1a**, 300 mg (0.190 mmol) was dissolved in 3 ml DMF and the solution was saturated with nitrogen. To this solution was added 62 mg (0.228 mmol) ferrocenylbutanoic acid and 83 mg (0.209 mmol) of the coupling agent (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)). The reaction proceeded under anhydrous conditions in the dark for a period of 2-2.5 hrs at room temperature, pH of 8-9. The polymer-ferrocene conjugate was precipitated with 10 ml Et_2O:hexane (2:1), and re-dissolved in 10ml water with 0.5 % ascorbic acid. The

solution was dialysed in Spectra/Por 4 tubing for 1 hr against water kept at pH 5-6, and subsequently for 24 hrs against de-ionised water, pH 6-7. Freeze-drying of the retentate afforded a water soluble brown solid in the yield of 174 mg (58 %), $\eta_{inh}(H_2O)$ 9 mL g^{-1}. Analysis found: %Fe, 2.61 % (3.0 %)

***1b**-Fc*

Polymer **1b**, 300 mg (0.182 mmol) was dissolved in 3 ml DMF and the solution was saturated with nitrogen. To this solution was added 59 mg (0.218 mmol) ferrocenylbutanoic acid and 80 mg (0.200 mmol) of the coupling agent (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)). The reaction proceeded under anhydrous conditions in the dark for a period of 2-2.5hrs at room temperature, pH of 8-9. The polymer ferrocene conjugate was precipitated with 10 ml Et_2O:hexane (2:1), and re-dissolved in 10 ml water with 0.5 % ascorbic acid. The solution was dialysed in Spectra/Por 4 tubing for 1hr against water kept at pH 5-6, and subsequently for 24hrs against water, pH 6-7. Freeze-drying of the retentate afforded a water soluble, brown solid in the yield of 201 mg (67 %), $\eta_{inh}(H_2O)$ 8 mL g^{-1}. Analysis found: % Fe 2.5 % (2.9 %)

***1e**-Fc*

Polymer **1e**, 300 mg (0.391 mmol) was dissolved in 3 ml DMF and the solution was saturated with nitrogen. To this solution was added 128 mg (0.469 mmol) ferrocenylbutanoic acid and 171 mg (0..4301 mmol) of the coupling agent (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)). The

reaction proceeded under anhydrous conditions, in the dark for a period of 2-2.5 hrs at room temperature, pH of 8-9. The polymer ferrocene conjugate was precipitated with 10ml Et_2O:hexane (2:1), and re-dissolved in 10 ml water with 0.5 % ascorbic acid. The solution was dialysed in Spectra/Por 4 tubing for 1hr against water kept at pH 5-6, and subsequently for 24 hrs against DI water, pH 6-7. Freeze-drying of the retentate afforded a water soluble, brown solid in the yield of 201 mg (67 %), $\eta_{inh}(H_2O)$ 8 mL g^{-1}. Analysis found: %Fe 5.0% (5.5%)

***1f**-Fc*

Polymer **1f**, 300 mg (0.147 mmol) was dissolved in 3 ml DMF and the solution was saturated with nitrogen. To this solution was added 48 mg (0.176 mmol) ferrocenylbutanoic acid and 77 mg (0.194 mmol) of the coupling agent (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)). The reaction proceeded under anhydrous conditions, in the dark for a period of 2-2.5hrs at room temperature, pH of 8-9. The polymer ferrocene conjugate was precipitated with 10ml Et_2O:hexane (2:1), and re-dissolved in 10 ml water with 0.5 % ascorbic acid. The solution was dialysed in Spectra/Por 4 tubing for 1hr against water kept at pH 5-6, and subsequently for 24hrs against DI water, pH 6-7. Freeze-drying of the retentate afforded a water soluble brown solid in the yield of 183 mg (61 %), $\eta_{inh}(H_2O)$ 8 mL g^{-1}. Analysis found: % Fe 2.53% (2.9 %)

4.4.3 Polymer conjugation with β-alanine-modified tetramethylmelamine

Synthesis of macro-monomer, M7

The intermediate **M7** was prepared by the drop-wise addition of 1.894 g (42 mmol) 95 % (mass:mass) dimethylamine to 3.688 g (20 mmol) cyanuric chloride in a slightly alkaline, pH 7-7.5, dioxane:water solution, at room temperature. The reaction proceeded for 48 hrs under these conditions, during which the alkalinity (pH 7-7.5) was maintained by the regular addition of sodium hydroxide. The product, **M7**, was isolated, following alkalification, extraction with three times 20ml portions of ethyl acetate and recrystallization from the same solvent following a reduction in volume. The product was isolated as a colourless solid, in a yield of 2.729 g (74%), and a melting point of 115 -117 ^{0}C.

^{1}H NMR, δ/ppm: 3.5-3.0 ppm –N(C**H$_3$**)$_2$ 6H(6H)

Synthesis of macro-monomer, M8

The tetramethylmelamine derivative, **M8**, was prepared by refluxing **M7,** 0.403 g (2 mmol), with 0.445 g (5 mmol) β-alanine in dioxane:distilled water (1:1), under slightly alkaline conditions, pH 7-8., by the regular addition of NaOH for a period of 48 hrs. Afterwards the product **M8**, was isolated as a white crystalline material following extraction and recrystallization from boiling water. The product was obtained in a yield of 0.169 g (42 %) and characterized by ^{1}H NMR spectrum, measured in D_2O at a slightly acidic pH.

^{1}H NMR, δ/ppm: 3.7-3.5 ppm CH$_2$-C**H$_2$**-COOH 2H(2H), 3.1-3.0 ppm (C**H$_3$**)$_2$-N 6H(6H), 2.5-2.4 ppm NH-C**H$_2$**-CH$_2$ 2H(2H)

Copolyaspartamide of β-alanine-modified tetramethylmelamine

1e*-tr

1.0 g of **M8** was added to 15ml distilled water and the pH of the mixture was adjusted to pH 6.5-7.5 and stirred for 30min. Afterwards M8 was isolated by freeze-drying and conjugated to a polyaspartamide as follows;

Compound **1e***, 300 mg (0.153 mmol) was dissolved in a 2 ml DMF, to this solution was added 77 mg (0.384 mmol) of the pre-treated M8 and 67 mg (0.168 mmol) of the coupling agent (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)). The reaction proceeded under anhydrous conditions in the dark for a period of 2-2.5 hrs at room temperature, and slightly alkaline pH (7-8.5). The conjugate was isolated following a series of purification steps which included filtering of the suspension, precipitation in a non-solvent, scrupulous washing, aqueous dialysis and freeze-drying. The product was obtained as a colourless, water soluble crystalline material, with a yield of 183 mg (61 %) and inherent viscosity of 9 mL g^{-1}. The quantity of tetramethylmelamine derivative present was determined by ^{1}H NMR, and found to be 8.4 % by mass, when considering the mass of tetramethylmelamine present in the of 3-(4,6-bis(N,N-dimethylamino)-1,3,5-triazacyclohexatrien-2-yl) propanoic acid polymer conjugate as a percentage of the total mass of the polymer-drug conjugate

^{1}H NMR, δ/ppm: 4.7-4.5 ppm Asp C**H** 10H(10H), 3.2-3.0 ppm C$\mathbf{H_2}$-NHCO 24H(22H), 3.0-2.9 ppm (C$\mathbf{H_3}$)$_2$-N-triazine 10H(12H), 2.9-2.5 ppm Asp C$\mathbf{H_2}$, CO-C$\mathbf{H_2}$-CH$_2$ 22H(22H), 2.5-2.2 ppm NH-C$\mathbf{H_2}$-CH$_2$ 24H(20H), 2.2-1.9 ppm N-C$\mathbf{H_3}$ 55H(54H), 1.7-1.5 ppm CH$_2$-C$\mathbf{H_2}$-CH$_2$ 20H(20H)

1f-tr

1.0 g of **M8** was added to 15ml de-ionised water and the pH of the mixture was adjusted to pH 6.5-7.5 and stirred for 30 min. Afterwards M8 was isolated by freeze-drying and conjugated to a polyaspartamide as follows;

Compound **1f**, 300 mg (0.147 mmol) was dissolved in a 2 ml DMF, to this solution was added 59 mg (0.294 mmol) of the pre-treated M8 and 64 mg (0.162 mmol) of the coupling agent (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)). The reaction proceeded under anhydrous conditions in the dark for a period of 2-2.5 hrs at room temperature and slightly alkaline pH (7-8.5). The conjugate was isolated following a series of purification steps which included filtering of the suspension, precipitation in a non-solvent, scrupulous washing, aqueous dialysis and freeze-drying. The product was obtained as a colourless, water soluble crystalline material, with a yield of 141 mg (47 %) and inherent viscosity of 7 mL g^{-1}. The quantity of tetramethylmelamine present in the conjugate was determined by ^{1}H NMR, and found to be 8.7 %, when considering the mass of tetramethylmelamine present in the of 3-(4,6-bis(N,N-dimethylamino)-1,3,5-triazacyclohexatrien-2-yl) propanoic acid polymer conjugate as a percentage of the total mass of the polymer-drug conjugate

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^{1}H NMR, δ/ppm: 4.7-4.5 ppm Asp C**H** 8H(10H), 3.7-3.5 ppm C$\mathbf{H_2}$-O 7H(8H), 3.2-3.0 ppm C$\mathbf{H_2}$-NHCO 20H(22H), 3.0-2.9 ppm (C$\mathbf{H_3}$)$_2$-N-triazine 8H(12H), 2.9-2.5 ppm Asp C$\mathbf{H_2}$, CO-C$\mathbf{H_2}$-CH$_2$ 21H (22H), 2.5-2.2 ppm NH-C$\mathbf{H_2}$-CH$_2$ 22H(20H), 2.2-1.9 ppm N-C$\mathbf{H_3}$ 54H(54H), 1.7-1.5 ppm CH$_2$-C$\mathbf{H_2}$-CH$_2$ 18H(18H)

4.4.4 Polymer conjugation with β-alanine-modified tetramethylmelamine and ferrocenylbutanoic acid

Copolyaspartamide of β-alanine-triazine and ferrocenylbutanoic acid

1e**-*Fc/tr***

M8, 300 mg was added to 15 ml distilled water and the pH of the mixture was adjusted to pH 6.5-7.5 and stirred for 30 min. Afterwards **M8** was isolated by freeze-drying and conjugated to a polyaspartamide as follows;

Polymer 1e*, 236 mg (0.236 mmol) was dissolved in a 1 ml DMF, to this solution was added (0.118 mmol) 32 mg of the ferrocenylbutanic acid and 52 mg (0.130 mmol) of the coupling agent (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)). The reaction proceeded under anhydrous conditions in the dark for a period of 2-2.5 hrs at room temperature and slightly alkaline pH 7-8.5. To this solution was added 47 mg (0.236 mmol) of M8 and 52 mg (0.130mmol) of the coupling agent HBTU, in situ, and the reaction continued in the dark for a further 2-2.5 hrs in the dark at a pH of 7-8.5. The conjugate was then isolated following a series of purification steps which included filtering of the suspension, precipitation in a non-solvent, scrupulous washing, aqueous dialysis and freeze-drying. The product was obtained as a colourless, water soluble crystalline material, with a yield of 207 mg (69%) and inherent viscosity of 7 mL g^{-1}. The quantity of both iron and the tetramethylmelamine present was determined by ^{1}H NMR, and found to be 2.9 % and 3.4 %, respectively, when considering the mass of iron, and the mass of the tetramethylmelamine present in the co-conjugate as a percentage of the total mass of the co-conjugate.

^{1}H NMR, δ/ppm: 4.0-4.2 ppm ferrocenyl 3H(4.5H), 3.2-3.1 ppm C**H$_2$**-NHCO 23H(22H), 3.1-2.9 ppm (C**H$_3$**)$_2$-N-triazine 5H(6H), 2.9-2.5 ppm Asp C**H$_2$**, CO-C**H$_2$**-CH$_2$ 21H (22H), 2.5-2.2 ppm NH-C**H$_2$**-CH$_2$ 20H(19H), 2.2-1.9 ppm N-C**H$_3$** 57H(54H), 1.7-1.5 ppm CH$_2$-C**H$_2$**-CH$_2$ 21H(21H)

CHAPTER 5

Conclusion and Future Work

In this project various water-soluble, biodegradable macromolecular polymer carriers of a defined structure were synthesized under meticulous conditions of time, temperature and pH. The carriers synthesized all form part of the polyamide-based family of polymers and included;

- copolyaspartamides, obtained by ring-opening reaction of poly(succinimide) in the presence of a mono-functional amine.
- polyamidoamines, obtained by the Michael-type addition polymerisation of a divinyl monomer in the presence of a mono-functional amine
- polyamides obtained by the base-catalysed ester-amine condensation polymerisation between a diester and a diamine

All the polymers were purified under meticulous dialysis conditions, in both 12000 and 25000 molecular mass cut-off membranes and freeze-dried. This afforded water-soluble solids which were used to investigate various drug models including; the square-planar platinum complexes, the organoiron compound, ferrocene and a tetramethylmelamine derivative.

The platination agents potassium tetrachloroplatinate (K_2PtCl_4) and diaminocyclohexanediaquaplatinum(II) nitrate (DACH-Pt aq) were anchored to the carriers using established methods, which involved co-ordination of the platinum to

amino, dihydroxylato or dicarboxylato- ligands present on the polymer chain, Generally the amine bound conjugates exhibited a much higher incorporation of platinum compared to the carboxyl, or hydroxyl bound counterparts. Furthermore for the carboxyl, hydroxyl-bound conjugates, the platinum content was extremely variable depending on the polymer used.

The ferrocene derivative, 4-ferrocenylbutanoic acid was synthesised using established procedures and conjugated to the carrier via pendant amino groups situated on the carrier, via amidation reaction, using the coupling agent HBTU. On average the iron contents in the conjugate were acceptably high and were similar to percentages obtained previously in this laboratory.

The tetramethylmelamine derivative was synthesised using various modified methods and conjugated to pendant amino groups present on the carrier, using an amidation reaction. Conjugation resulted in a fair degree of drug loading.

Finally there was a single attempt to incorporate both the ferrocene and the tetramethylamelamine derivative into the same carrier. Conjugation proceeded via an amidation reaction, preliminary results suggested a fair degree of loading of both the ferrocene and the tetramethylmelamine derivative.

Future research should address the following questions;

1. Investigate alternative conjugation procedures to anchor DACH-Pt aq so as to reduce the fair degree of variability in platinum content, which exists between different dicarboxyl and dihydroxyl containing polymers
2. Optimise the conjugation procedure required to anchor the tetramethymelamine derivative, to increase the drug loading within the carrier
3. Optimise the conjugation procedure required to anchor both the tetramethymelamine derivative and ferrocene on the same carrier
4. Consider other carboxyl containing drugs e.g. methotrexate (MTX), which could be co-conjugated to an appropriate carrier

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APPENDIX

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