

**Exploring drug delivery systems tailored for
[Pt(phen)(acylthiourea)]⁺ complexes**

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG



Faculty of Science

School of Chemistry

Nthabiseng Manama

1415255

MSc (Chemistry)

Supervisor: Dr Izak Kotze

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree Master of Science (MSc) in Chemistry.

Declaration:

I, the undersigned, hereby declare that the dissertation “Exploring drug delivery systems tailored for [Pt(phen)(acylthiourea)]⁺ complexes” is my own unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. I have not previously submitted this work in its entirety or in part at any other university for a qualification.



Nthabiseng P. Manama

On this __11th__ day of __November__ 2022__

Abstract

Three [Pt(phen)(Lⁿ-O,S)]Cl complexes (PtL_n), where phen represents 1,10-phenanthroline and Lⁿ-O,S various *N,N*-di(alkyl)-*N*'-acylthiourea ligands were successfully synthesized and characterized with yields ranging from 66% to 75%. Generation 4 (G4), acetamide functionalized generation 4 (G4-Ac) and generation 5 (G5-Ac) PAMAM and folic acid functionalized generation 4 (G4-FA) PAMAM dendrimers were successfully synthesized with yields ranging from 80% to 94% and characterized using ¹H NMR, ¹³C NMR, FT-IR and mass spectrometry (ESI and MALDI-TOF) where applicable.

Host-guest adduct formation between G4 and PtL_n complexes were challenging since G4 was found to exist as a polycation even near-neutral pH. The positively charged amine surface resulted in a small amount of complex degradation and inhibition of encapsulation due to electrostatic repulsion between the dendrimer and the cationic complex. To mitigate this, we acetylated the amine surface of the dendrimer and studied the host-guest interaction of this variant. The solvent was found to play an important role in the encapsulation process with DMSO-d₆ showing the most promising results. Host-guest interactions of G4-Ac and PtL1 in DMSO-d₆ were investigated in detail. Upon addition to G4-Ac, shifting of the aliphatic and aromatic peaks that belong to PtL1 were observed in the ¹H NMR spectrum with a significant shift of 0.25 ppm and 0.26 ppm for H_d and H_d' of PtL1. At 300K, DOSY data showed the association between the complex and dendrimer with significant slower diffusion in the mixture (PtL1: 0.3 x10⁻¹⁰ m²/s, G4-Ac: 0.2 x10⁻¹⁰ m²/s) as compared to the free species (PtL1: 1.0 x10⁻¹⁰ m²/s, G4-Ac: 0.4 x10⁻¹⁰ m²/s). The observed ¹H NMR chemical shifts and diffusion coefficient was a molar fraction-weighted average between the "bound" and "free" state of PtL1 which indicated that the dendrimer-complex interaction was in fast-exchange relative to the NMR time-scale. In efforts to further ascertain the extent of the interaction, viscosity measurements were conducted in order to calculate each respective hydrodynamic radii (R_H) and volumes (V_H) which will give a clear indication of host-guest association. The increase in viscosity for the mixture was attributed to an increase in concentration and increase in ionic strength since the complex is cationic. An 84% and 49% increase in V_H was observed in PtL1 and G4-Ac respectively which indicates the formation of the host-guest complex. Dendrimer cavity size played an important role in encapsulation as NOE crosspeaks were observed between the aromatic protons of PtL3 and internal G5-Ac protons whereas none were observed between the complexes and G4-Ac. The mixture of [TBA]Cl and G4-Ac was briefly studied to investigate the effect of ionic strength on the dendrimers diffusion behaviour. An increase in viscosity was observed as a result of increased concentration and ionic strength. A 31% increase in G4-Ac V_H was observed while a 40% decrease in [TBA]Cl V_H was observed which provided evidence of dendrimer aggregation in the presence of a salt. We

also investigated the host-guest interaction of well-known 5-Fluorouracil (5FU) as a positive control. Host-guest interactions of G5-5 Fluorouracil (5FU) indicated an electrostatic interaction as seen by the shifting of surface G5 protons (g' and f') upon addition of 5FU as a result of proton abstraction from the weakly acidic 5FU by the basic surface amine groups of G5.

The free complexes, 5FU (positive control), free dendrimers and dendrimer-drug mixtures were screened against A549 lung cell line to evaluate their cytotoxic activity at 50 μ M and 5 μ M in DMSO- d_6 /D $_2$ O (6:1). The solvent displayed minimal cytotoxic activity which was attributed to the presence of deuterium as deuterated solvents were found to be more cytotoxic compared to their non-deuterated counterparts in literature. The free complexes and dendrimer-complex mixtures were highly active with less than 1% cell viability at 50 μ M. Free 5FU displayed less activity with a cell viability of 62.3% while G5+5FU displayed improved activity with a cell viability of 19.2% at 50 μ M. G4-Ac +PtL1 and G4-Ac +PtL2 displayed higher cytotoxicity compared to their free counterparts at 5 μ M which could be indicative of effective drug delivery although the results are preliminary. PAMAM dendrimers were found to be biocompatible with a cell viability of 53.30%, 58.78% and 64.53% for G4-Ac, G5 and G5-Ac respectively at 50 μ M and acetamide functionalized dendrimers displayed less cytotoxicity compared to the amine terminated dendrimers.

I lift up my eyes to the mountains- where does my help come from?

My help comes from the Lord, the Maker of heaven and earth.

Psalms 121 vs 1-2

Acknowledgements

I would firstly like to thank my Lord and Saviour for all His help and for carrying me through this journey and throughout my life. Thank you for Your divine presence, love, kindness, mercy and thank you for being in control. My rock of all ages.

To my parents, thank you for being my biggest supporters. Thank you, dad, for bringing me to school everyday without fail and for encouraging me to take on tasks that I found to be quite daunting. Thank you, mom, for tending to my every need, the prayers and the jokes. You two are the absolute best!

To My supervisor, Dr Izak Kotze, thank you for being hilarious, patient, kind and teaching me everything I know. You made this experience very enjoyable; you are the best thing sliced bread! Thank you for analysing all my DOSY experiments.

I am extremely grateful to Sasol for the financial support and making sure that I am fed.

Thank you, Dr Leonie Harmse for the use of your lab and equipment. Ms Tebogo Peega thank you for your time, effort and work that you have done in screening my samples.

Thank you to Prof Orde Munro for the use of your lab and viscometer; and Dr Cathryn Slabber for obtaining the viscometry data of my samples.

Thank you, Dr Juanita Van Wyk for the use of your equipment and chemicals.

Thank you, Dr Johan Jordaan, Ms Hestelle Stoppel, Ms Tamryn and North West University for the MALDI-TOF analysis.

Lastly, thank you to my friends for the memes and endless laughter. Thank you to my lab mates for making me feel welcome, all the advice and knowledge you shared with me and making lab 122 an amazing environment.

Table of Contents

Declaration.....	ii
Abstract.....	iii
Acknowledgements.....	vi
Table of contents.....	vii
List of Figures.....	ix
List of Schemes.....	xii
List of Tables	xiii
List of Abbreviations.....	xiv
Chapter 1.....	1
<i>Literature review on metallodrugs and potential drug delivery systems</i>	
1.1 Background.....	2
1.1.1 What is cancer?	2
1.1.2 Causes of cancer.....	3
1.1.3 Current anti-cancer treatment options.....	3
1.2 Metallodrugs	5
1.3 Drug delivery systems (DDS)	6
1.3.1 Nano-sized drug delivery systems	7
1.3.2 Types of nano-sized drug delivery systems	7
1.3.3 Dendrimers as a drug delivery system	9
1.4 Targeted delivery	12
1.4.1 Types of targeting.....	13
1.5 Folate receptor (FAR)	13
1.6 Aims and Objectives	14
1.7 References.....	16
Chapter 2.....	21
<i>Synthesis of generation 4 Polyamido amine (PAMAM) dendrimer and Pt(phen)(acylthiourea)]⁺ complexes</i>	
2.1 Introduction to platinum complexes	22
2.2 Introduction to Polyamido amines.....	23
2.3 Results and discussion.....	25
2.3.1 Synthesis of Pt(phen)Cl ₂	25
2.3.2 Synthesis of N,N-di(butyl)-N'-benzoylthiourea ligand.....	26

2.3.3 Synthesis of $[Pt(phen)(acylthiourea)]^+$ complexes	29
2.4 Synthesis of Polyamido amine (PAMAM) dendrimer	32
2.4.1 Dendrimer purification.....	40
2.4.2 Synthesis of Folic acid functionalized G4 PAMAM (G4-Fa).....	41
2.4.3 Synthesis of acetamide terminated PAMAM (G_n -Ac).....	43
2.5 Experimental section.....	48
2.5.1 Instrumentation and reagents	48
2.6 References.....	63
Chapter 3.....	66
<i>Host-guest interactions of $[Pt(phen)(acylthiourea)]^+$ complexes and G4 and G5 PAMAM dendrimers</i>	
3.1 Introduction	67
3.2 PAMAM host-guest interactions	68
3.3 Diffusion ordered spectroscopy (DOSY): a significant characterization technique	69
3.4 Results and discussion.....	71
3.4.1 ^1H NMR studies of potential host-guest molecules.....	71
3.5 Conclusion	85
3.6 References.....	87
Chapter 4.....	90
<i>Preliminary cytotoxicity screening of PAMAM encapsulated $[Pt(phen)(acylthiourea)]^+$ complexes</i>	
4.1 Introduction	91
4.2 Results and discussion.....	92
4.2.1 MTT methodology.....	92
4.2.2 Preliminary cytotoxicity screening via MTT assays	93
4.3 Conclusion	95
4.4 Experimental.....	96
4.5 References.....	100
Chapter 5.....	102
<i>Summary and conclusion</i>	
5.1 Summary.....	103

Appendix.....	107
----------------------	------------

List of Figures

- Figure 1.1:** Structure of a conventional liposome.
- Figure 1.2:** A) Chemical structure of a general cyclodextrin and B) the 3-dimensional truncated cone shape of a cyclodextrin.
- Figure 1.3:** Labelled schematic of types of polymeric nanocarriers.
- Figure 1.4:** General structure of a dendrimer with labels highlighting main structural components.
- Figure 1.5:** A) Divergent synthesis schematic and B) convergent approach schematic.
- Figure 1.6:** Synthesis of a polypropylene imine (PPI) dendrimer.
- Figure 1.7:** Image of EPR effect and active targeting.
- Figure 1.8:** Structure of folic acid showing the amino acid sub-components.
- Figure 2.1:** $[Pt(phen)(L^n-O,S)]Cl$ complexes synthesized labelled with the respective abbreviations.
- Figure 2.2:** ^1H NMR of $Pt(1,10\text{-phenanthroline})Cl_2$ in $DMSO-d_6$ at 300K.
- Figure 2.3:** ^1H NMR of $N,N\text{-di}(\text{butyl})\text{-}N'\text{-benzoylthiourea}$ in $CDCl_3$ at 300K.
- Figure 2.4:** ^{13}C NMR of ligand $N,N\text{-dibutyl-}N\text{-benzoylthiourea}$ in $CDCl_3$ at 300K.
- Figure 2.5:** FT-IR spectrum of $N,N\text{-di}(\text{butyl})\text{-}N'\text{-benzoylthiourea}$.
- Figure 2.6:** ^1H NMR of $[Pt(phen)(N'\text{-pivaloyl-}N,N\text{-diethylthiourea})]^+$ in $CDCl_3$ at 300K.
- Figure 2.7:** ^{13}C NMR spectrum of $[Pt(phen)(N'\text{-pivaloyl-}N,N\text{-diethylthiourea})]^+$ in $CDCl_3$ at 300K.
- Figure 2.8:** Aromatic region zoomed and assigned HSCQ NMR spectrum of $[Pt(phen)(N'\text{-pivaloyl-}N,N\text{-diethylthiourea})]^+$ in $CDCl_3$ at 300K.
- Figure 2.9:** Structure of G-0.5 PAMAM and tri-ester side product.
- Figure 2.10:** ^1H NMR of G -0.5 PAMAM in D_2O at 300K.
- Figure 2.11:** ^{13}C NMR of G -0.5 PAMAM in D_2O at 300K.
- Figure 2.12:** FT-IR spectrum of G-0.5 PAMAM dendrimer.

- Figure 2.13:** Mass spectrum of G-0.5 PAMAM dendrimer.
- Figure 2.14:** ^1H NMR of G 0 PAMAM in D_2O at 300K.
- Figure 2.15:** COSY spectrum of G0 PAMAM dendrimer in D_2O at 300K.
- Figure 2.16:** ^{13}C NMR of G0 PAMAM in D_2O at 300K.
- Figure 2.17:** FT-IR spectrum of G0 PAMAM dendrimer.
- Figure 2.18:** Mass spectrum of G0 PAMAM.
- Figure 2.19:** General dendrimer dialysis setup.
- Figure 2.20:** A) Illustration of folic acid covalently attached to the surface of a dendrimer where * refers to exchangeable protons. B) Stacked ^1H NMR spectra of FA in D_2O , DMSO-d_6 and G4-FA in D_2O respectively.
- Figure 2.21:** Stacked IR spectra of free folic acid and folic acid functionalized G4-PAMAM (G4-FA).
- Figure 2.22:** Stacked ^1H NMR spectra of amine terminated G4 PAMAM (G4-NH_2) and acetamide terminated G4 PAMAM (G4-Ac) in D_2O at 300K.
- Figure 2.23:** ^{13}C NMR of G4-Ac PAMAM in D_2O at 300K.
- Figure 2.24:** MALDI-TOF mass spectra of G4 (left) and G4-Ac (right) in DHB and sodium triflate matrix.
- Figure 3.1:** A) Graphical representation of a simple gradient field pulse experiment in diffusion NMR spectroscopy. B) Representation of the translational motion of molecules A and B in solution.
- Figure 3.2:** ^1H NMR spectrum of a mixture of $[\text{Pt}(\text{phen})(\text{L}^1\text{-O,S})]^+$ and G4 PAMAM in deuterated methanol (MeOD-d_4) with increasing gradient field strength (G) in a typical DOSY experiment.
- Figure 3.3:** ^1H NMR spectrum of mixture of G4 PAMAM and $[\text{Pt}(\text{phen})(\text{N-benzoyl-N,N-dibutylthiourea})]^+$ in deuterated methanol (MeOD-d_4) at 300K.
- Figure 3.4:** DOSY spectrum of a mixture of $[\text{Pt}(\text{phen})(\text{N-benzoyl-N,N-dibutylthiourea})]^+$ and G4 PAMAM in deuterated methanol (MeOD-d_4) showing the diffusion coefficients at 300K.
- Figure 3.5:** Mixture of G4 PAMAM and PtL1 after 24 hours in D_2O .

- Figure 3.6:** *¹H NMR spectrum of the concentrated water layer taken from G4 and PtL1 mixture DCM/H₂O encapsulation attempt.*
- Figure 3.7:** *¹H NMR of precursor and G4 in DMSO-d₆.*
- Figure 3.8:** *NOESY spectrum of precursor and G4 PAMAM in DMSO-d₆.*
- Figure 3.9:** *NOESY spectrum of ligand and zoomed-in aromatic region of L1 and G4 PAMAM in DMSO-d₆.*
- Figure 3.10:** *¹H NMR of PtL2 and G4 mixture in H₂O (D₂O spike) with solvent suppression.*
- Figure 3.11:** *Stacked ¹H NMR spectra of 5FU in D₂O (top), G5 PAMAM and 5FU in D₂O (middle) and G5 PAMAM in D₂O (bottom).*
- Figure 3.12:** *Stacked ¹H NMR spectra of PtL1 in DMSO-d₆ and G4-Ac and PtL1 encapsulation attempt in DMSO-d₆ at 300K.*
- Figure 3.13:** *Stacked DOSY spectra of G4-Ac +PtL1 mixture, G4-Ac and PtL1 in DMSO-d₆ at 300K.*
- Figure 3.14:** *Example of manually fitted diffusion coefficients of PtL1 (aliphatic region) in DMSO-d₆ from Mestrenova software.*
- Figure 3.15:** *Stacked ¹H NMR spectra of [TBA]Cl in DMSO and G4-Ac and [TBA]Cl mixture in DMSO-d₆ at 300K.*
- Figure 3.16:** *NOESY spectrum of PtL3 and G5-Ac mixture (left) and PtL2 and G4-Ac mixture (right) in DMSO-d₆ and D₂O (6:1) at 300K.*
- Figure 4.1:** *Structures of free the dendrimers, compounds and description of dendrimer encapsulated compounds screened against A549 at 50 μM and 5 μM.*
- Figure 4.2:** *Labelled 96 well plates of the free dendrimers, free compounds and dendrimer encapsulated compounds screened at 50 μM and 5 μM. Refer to table 4.1 and 4.2 in the experimental section.*
- Figure 4.3:** *% Cell viability of A549 cancer cell line at a concentration of A) 50 μM and B) 5 μM.*

List of Schemes

- Scheme 2.1:** *Depiction of the delocalization of electrons throughout the N,N-dialkyl-N'-acylthiourea ligand after deprotonation to afford an anionic bidentate ligand.*
- Scheme 2.2:** *A) Resonance structure of methyl acrylate (MA) displaying the delta positive and delta negative charges. B) General reaction mechanism of a general 1,4-addition.*
- Scheme 2.3:** *simplified reaction scheme of a half generation and full generation PAMAM dendrimer.*
- Scheme 2.4:** *Ideal and non-ideal dendrimer growth during amidation step of a half generation PAMAM dendrimer*

List of Tables

- Table 3.1:** Comparison of chemical shift differences of PtL1 and PtL1-G4-Ac mixture in DMSO- d_6 .
- Table 3.2:** Diffusion coefficients and hydrodynamic volume (V_H) of PtL1+ G4 -Ac mixture, PtL1 in DMSO- d_6 as well as G4-Ac in DMSO- d_6 at 300K and 310K.
- Table 3.3:** Viscosity, diffusion coefficients and hydrodynamic volume (V_H) of [TBA]Cl + G4-Ac mixture, [TBA]Cl as well as G4-Ac in DMSO- d_6 at 300K and 310K.
- Table 4.1:** Percentage cell viability of guest-host compounds screened against A549 cancer cell line at 50 μ M for 48 hours.
- Table 4.2:** Percentage cell viability of guest-host compounds screened against A549 cancer cell line at 5 μ M for 48 hours.

List of Abbreviations

5FU	5-Fluorouracil
Ac ₂ O	acetic anhydride
COSY	correlation spectroscopy
CDCl ₃	deuterated chloroform
DA	Dalton
DCM	dichloromethane
DDS	drug delivery system
DHB	2,5-dihydroxybenzoic acid
DHF	dihydrofolate
DMF	dimethyl formamide
DMSO-d ₆	deuterated dimethyl sulfoxide
DMSO	dimethyl sulfoxide
DOSY	diffusion ordered spectroscopy
DNA	deoxyribonucleic acid
E.coli	Escherichia coli
EDA	ethylenediamine
EDC-HCl	1-ethyl-3-carbodiimide hydrochloride
EPR	enhanced permeability and retention
ESI-HRMS	electrospray ionization high resolution mass spectroscopy
EtOH	ethanol
EtOH-d ₆	deuterated ethanol
FA	folic acid
FAR	folic acid receptor
FTIR	Fourier transform infrared spectroscopy
HMBC	heteronuclear multiple bond correlation

HSQC	heteronuclear single quantum coherence
HPV	human papillomavirus
GPI	glycosylphosphatidylinositol
G0	generation 0
G1	generation 1
G2	generation 2
G3	generation 3
G4	generation 4
G4-Ac	acetamide functionalized generation 4
G4-FA	folic acid functionalized generation 4
G5-Ac	acetamide functionalized generation 5
kDa	kilodalton
MA	methyl acrylate
MALDI-TOF	matrix assisted laser desorption ionization- time of flight
MALDI-TOF MS	matrix assisted laser desorption ionization- time of flight mass spectrometry
MeOH	methanol
MWCO	molecular weight cut off
NADPH	nicotinamide adenine dinucleotide phosphate
NMR	nuclear magnetic resonance
PAMAM	polyamido amine
RNA	ribonucleic acid
ROS	reactive oxygen species
[TBA]Cl	tetrabutylammonium chloride
THF	tetrahydrofolate
WHO	world health organization

Chapter 1

Literature review on metallodrugs and potential drug delivery systems

1.1 Background

Cancer is the second leading cause of the death after cardiovascular disease and has an average of 9 million fatalities per year as determined by the world health organization (WHO).¹ There has been an estimate of 19.3 million new cases of cancer in 2020 and 9.9 million deaths worldwide. Breast, lung, colorectal, prostate and stomach cancer are the most commonly diagnosed types of cancer. Lung and colorectal cancer are the leading cause of death followed by liver, stomach and breast cancer. An estimate of 28.4 million new cancer cases have been predicted for the year 2040 should the trend in global cases remain the same. These alarming statistics warrants worldwide research interest in the causes, prevention and treatment of cancer.²

1.1.1 What is cancer?

Cancer is a term given to a group of diseases which are characterized by abnormal cell growth.³ Cancer is a result of cells no longer responding to natural mechanisms that control cell division and are resistant to cell death (apoptosis), thus leading to rogue cells that are constantly and uncontrollably proliferating to form a mass of cells called tumours.⁴ A tumour can be classified into two categories which are: benign or malignant. Benign tumours are non-cancerous, they can be removed and typically do not return nor do they spread to other parts of the body. A cancerous tumour however, is said to be malignant. This means that cancer cells are able to detach from the tumour and are transported to other parts of the body via the bloodstream or lymphatic system to form tumours where they deposit i.e on other organs.⁵ When this process takes place, the cancer is said to have metastasized.

There are different subgroups of cancer, namely, sarcoma, carcinoma, leukemia, central nervous system cancer and lymphoma and myeloma. Sarcomas typically begin in fat, bone, fibrous tissue, muscle (smooth and skeletal), bone, cartilage and other connective tissue.^{3,6} Carcinomas arise from epithelial tissue such as skin as well as tissue that lines internal organs. Carcinomas can be further divided in to subgroups namely, squamous cell, basal cell, adenocarcinoma and transition cell.^{3,7} Leukemia is cancer of blood-forming tissue such as bone marrow. These abnormal cells proceed to replace normal bone marrow cells as well as enter the bloodstream.^{3,8} Central nervous system cancers stem from spine and brain tissue.³ Lymphomas arise from the cells of the immune system where the cancerous cells form tumours in the lymphatic nodes. In the case of myelomas, the cancerous white blood cells remain in the bone marrow.³

1.1.2 Causes of cancer

Cancer is caused by gene mutations which can be triggered by various factors such as lifestyle choices (smoking, unhealthy diet and increased body weight, alcohol intake), viruses (HPV), environmental exposure to carcinogens such as UV-rays. The lack of physical activity and an unhealthy diet leads to obesity. Obesity is linked to increased inflammation and increased leptin levels which are known to promote carcinogenesis and suppress apoptosis respectively.⁹ Cigarette smoke not only contains nicotine but also carcinogens and reactive oxygen species (ROS) which trigger inflammation and alter the pulmonary microenvironment which puts smokers at a higher risk of throat and lung cancer.^{2,10} Alcohol consumption results in the production of acetaldehyde (a carcinogen) from the metabolism of ethanol in the liver. Elevated acetaldehyde levels are also associated with inducing defects in the alcohol dehydrogenase gene and inactivate the tumour suppressor gene, BRCA, which then increases the risk for cancer. Increased alcohol intake (alcohol abuse) is also associated with altered folate metabolism, increased oestrogen and free radical production which all lead to increased disruptions of metabolic processes in the human body which further increases the risk of cancer.¹¹

Workers such as chemists, painters, petroleum workers and doctors are at a higher risk of developing cancer due to the regular exposure to carcinogens and/or ionizing radiation. UV radiation from the sun and tanning beds increase the risk for developing skin cancer while, human papilloma virus (HPV) is the main cause of cervical cancer.¹² Viruses such as hepatitis (B and C) can cause liver cancer and people who live with HIV-Aids are immunocompromised and are at risk for developing lymphomas.¹³ All of these abovementioned factors act by disrupting normal cellular functions and protective pathways (tumour suppressing mechanisms) to result in the development of cancer.

1.1.3 Current anti-cancer treatment options

There are various methods of treating cancer such as surgery, radiation therapy, chemotherapy, immunotherapy and hormone therapy.¹⁴ Treatment usually employs a combination of methods, for example, surgery (physically removing the tumour) followed by radiation therapy or chemotherapy.¹⁵ Radiation therapy uses high energy beams such as X-rays to damage the genetic material (DNA) that allows for cell division to occur hence killing cancer cells.¹⁶

Chemotherapy is the use of drugs to treat cancer systematically. There are various types of anticancer drugs such as alkylating agents, metalating agents, antimetabolites,

topoisomerase inhibitors, mitotic inhibitors and corticosteroids. Antimetabolites act as a substitute for the normal DNA and RNA bases (adenine, guanine, cytosine, thymine and uracil).¹⁷ They tend to be similar in structure to the DNA or RNA base but lack an important functional group that allows for the linking of the next DNA base pair needed during DNA replication. When substitution occurs, the DNA cannot replicate which results in cell division inhibition.¹⁸

Topoisomerase inhibitors interfere with the enzyme Topoisomerase which is responsible for unwinding and separating the DNA strands during DNA replication and this process of inhibition also brings cells division to a halt. Mitotic inhibitors typically act by binding to tubulin which inhibits the polymerization of tubulin into microtubules. Microtubules are a support structure found in cells and is responsible for pulling a cell apart as it divides. This action ultimately inhibits cell division and stops cancer growth.¹⁸ Corticosteroids are hormones or hormone-like drugs which are typically administered with other anticancer agents. They help to prevent allergic reactions to certain medication as well as to reduce inflammation and nausea which is induced by chemotherapy.¹⁸

Anticancer agents such as *cisplatin* and *mitomycin c* act by metalating (formation of a metal-DNA bond) and alkylating (formation of an alkyl-DNA bond) DNA. Other drugs such as *doxorubicin* act by intercalation i.e. inserting between the DNA bases. These agents act by distorting the shape of the DNA double helix consequently inhibiting cells from proliferating which leads to cells undergoing programmed cell death known as apoptosis. Other used drugs such as *teniposide* and *topotecan* have multiple modes of action where they also act by putting cells under oxidative stress which triggers cell death by free radical damage.^{5,19}

The standard first-line chemotherapeutic drugs consist of a combination of bleomycin, etoposide and cisplatin (referred to as BEP) and is administered over 21 days which concludes a treatment cycle. Cycles may be repeated for months depending on how the tumour responds and should the tumour metastasize. Second-line chemotherapeutic drugs include docetaxel, ramucirumab, erlotinib as well as others which are administered in combinations depending on the type of cancer. Healthy cells are able to repair damaged DNA to some extent, however, that is not usually the case.¹⁵ Ideally, treatment should damage as few healthy cells as possible but the reality is that anticancer drugs are not selective to cancerous cells. This results in toxicity and side effects such as radiation poisoning (in the case of radiation therapy), low blood cell count, kidney and liver problems, fatigue, hair loss, nausea and vomiting, which raises the need for an improved pharmacokinetic profile by developing selective drug delivery systems.

Targeted drug delivery aims to improve properties such as selectivity, bioavailability and efficacy of the drug while reducing toxicity. Overexpressed proteins, enzymes, receptors and other factors that differentiate cancer cells from normal cells are targeted as the basis of selectivity.²⁰

1.2 Metallodrugs

The antiproliferative property of cisplatin was serendipitously discovered in the 1960's by biophysicist Barnett Rosenberg during a study to investigate whether cell division could be influenced by an electric current.²¹ In this experiment, platinum electrodes were immersed in a solution comprised of an ammonium chloride buffer and *Escherichia coli* (*E. coli*) bacteria. After a current was applied to the solution, the bacteria began to elongate instead of remaining in its typical rod shape. It was determined that the change in shape was a result of the product, cisplatin $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$ being formed due to the hydrolysis of the platinum electrodes in the solution. Rosenberg saw it fit to test ten transition metal complexes that would induce the same effect as the hydrolysis product of the platinum electrode. It was found that cis diamminetetrachloroplatinate (IV), caused the same elongation in a range of bacilli (a genus of rod-shaped bacteria).²¹⁻²³

Given the interesting results observed in the *E. coli*, it was reasoned that these complexes should be tested on cancer cells. When 180 Swiss mice were subjected to cisplatin, the tumours in the mice had begun to shrink. After 6 months of treatment, the mice were cured from cancer and cisplatin was taken to clinical trials and successfully passed through all phases.^{21,23}

Cisplatin has since been used to treat testicular, ovarian, bladder and head & neck cancer.²⁴ However, cisplatin induces serious side effects and is prone to cell resistance rendering the drug ineffective with continued use. The discovery of biologically active metal complexes has led to extensive research being conducted to discover more transition metals that display anticancer activity.²⁵ It has been reported that platinum, palladium, ruthenium, copper and gold complexes display anticancer activity.^{25,26} Metallocenes such as titanocene, vanadocene, ferrocene, molybdocene and zirconocene as well as their derivatives are another example of metal complexes that have been found to have high antitumor activity.²⁶ Transition metals offer the advantage of existing in a number of oxidation states thus complexes can have various geometries which may have different modes of action.^{25,27} Platinum (II) complexes in particular have been found to be very effective against cancer cells as they are able to bind DNA effectively. Other platinum complexes such as oxaliplatin and carboplatin which have improved pharmacokinetic properties (solubility and toxicity) as compared to cis-platin have

been synthesized and are currently used clinically.²⁵

In our research group, R.Magwaza and T.Peega has synthesized and studied the mode of action of Pt[(diimine)(aroylthiourea)]Cl complexes.²⁸ The naphthoyl variation, [Pt(diimine)(*N,N*-di(butyl)-*N'*-naphthoylthiourea)]Cl, displayed fluorescence under blue light. Fluorescence microscopy was used and the complex was traced in order to determine where it acts in the cell. It was suggested that these platinum complexes act on DNA as they were found to accumulate in the nucleus. The complexes were screened against H1975 and A549 lung cancer lines and displayed high cytotoxicity of 94% at IC₅₀'s (Inhibitory concentration 50) 0.1-0.9 μ M.²⁹

R.Magwaza explored Sulphobutyl-ether- β -cyclodextrin and surfactant micelles as a potential drug delivery system to improve the water solubility of the platinum complexes. The drawback faced with micelles was cell wall damage thus leading to toxicity³⁰ which is undesirable if the drug is to reach both cancer and normal cells in the body. The research that will be conducted in this study intends to make use of a more biocompatible drug delivery system to minimize toxicity should the drug reach normal cells.

1.3 Drug delivery systems (DDS)

A drug delivery system can be described as a vehicle in which a drug is either encapsulated inside or is conjugated on the surface of the vehicle and is transported to the desired site. Drug delivery systems are utilized to improve properties such as stability, solubility and bioavailability by increasing half-life while reducing toxicity, unwanted (drug-drug and drug-tissue) interactions and control the rate at which the drug is released.³¹

Exploring drug delivery systems are a popular research topic as they can be functionalized to display selectivity which makes them a powerful tool for tackling the problem with nonselective drugs such as anticancer agents. Research has also focused on drug delivery systems to improve the manner in which drugs are administered to make them less invasive for example, exploring drug delivery systems for transdermal absorption/delivery in the form of topical creams instead of administering drugs intravenously. However, the biggest challenge they pose is bio-compatibility with tissues and cells i.e. the DDS itself should cause minimal to no toxicity.³²

1.3.1 Nano-sized drug delivery systems

A nanoparticle can be described as a particle that is between 1-100nm in size. Nanomaterials exhibit properties that are unique as compared to their bulk counterparts due to increase in surface area to size ratio when moving from the bulk material to the nanoscale. These properties can be applied across many fields of science from sensors, to imaging agents, drug delivery and many other applications. The application of nanotechnology in the medical field has received a lot of attention as nanoparticles displayed the ability to transport drugs.³³ Nanomaterials are small enough to remain in the circulatory system and tissue for long periods of time. This property can be exploited to concentrate encapsulated or surface conjugated drugs to an optimum level in the body. Nano-conjugated and nano-encapsulated drugs have been shown to display increased bioavailability, increased solubility (in the case of the hydrophobic drugs) and controlled release which then improves toxicity.^{33,34}

1.3.2 Types of nano-sized drug delivery systems

Liposomes: Lipids are molecules which consist of a fatty hydrophobic region (typically a chain referred to as a tail) and a hydrophilic region (referred to as a head). In aqueous solutions, these amphiphilic molecules begin to self-assemble to form bilayers where the hydrophilic head interacts with the aqueous solution and the lipid chain is segregated from the aqueous solution to form liposomes as shown in figure 1.1.³⁵

Liposomes have received attention in the medical field due to their amphiphilic nature. Hydrophobic drugs can be incorporated into the lipophilic tails and can be transported to specific sites as liposomes display good tissue penetration.^{36,37} They can also be used to stabilise drugs which can increase bioavailability.^{36,37}

Conventional liposome

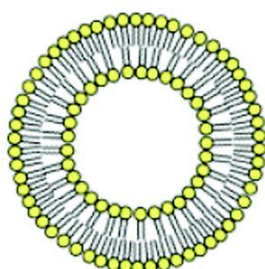


Figure 1.1: Structure of a conventional liposome.³⁸ (reproduced from reference 38)

Nanoparticles: The most common metal used for drug delivery (in particular for cancer treatment) is gold. Gold nanoparticles (AuNP's) have surface negative charges which make it possible to conjugate molecules of interest for the delivery of molecular cargo. Molecules of interest include drugs, biomolecules for targeting and genes. They also have a low inherent toxicity which makes them a promising potential DDS.³⁹

Cyclodextrins: Cyclodextrins are a group of cyclic oligosaccharides linked through 1,4-glycoside bonds.⁴⁰ The most commonly used cyclodextrins are α -, β - and γ -cyclodextrins. α -, β - and γ -cyclodextrins are differentiated by the number of linked glucopyranoside units and therefore differ in size as illustrated in figure 1.2. They have a truncated cone shaped structure and are water soluble.⁴⁰ The internal cavities are hydrophobic and can be used to host hydrophobic molecules to form inclusion complexes.⁴⁰ Cyclodextrins have been widely across various industries as mainly stabilizing, dispersing and slow-release agents.⁴⁰

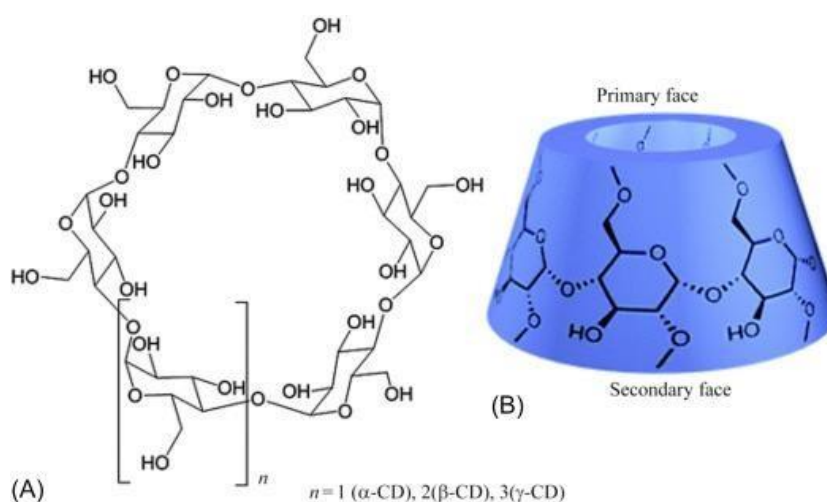


Figure 1.2: A) Chemical structure of a general cyclodextrin and B) the 3-Dimensional truncated cone shape of a cyclodextrin.⁴¹ (reproduced from reference 41)

Polymeric nanocarriers: Polymeric nanocarriers differ from conventional drug delivery systems as they are made from a variety of polymers, which can be tailored to display certain characteristics for example improved water stability which is a problem that is often faced by liposomes.⁴² Molecules of interest can be physically entrapped or encapsulated in the structure of these nanocarriers. Alternatively, they can be conjugated on the DDS. Polymeric nanocarriers have received attention due to their tuneable drug release profile which is of importance in drug delivery aimed at minimizing toxicity.^{42,43} Polymeric nanocarriers are typically synthesized by reaction two or more polymers with different properties such that

the final polymer displays a blend of properties of its parent polymers. Examples of such nanocarriers are polymeric micelles, nanogels, polymer-drug conjugates and dendrimers^{42,43} (figure 1.3).

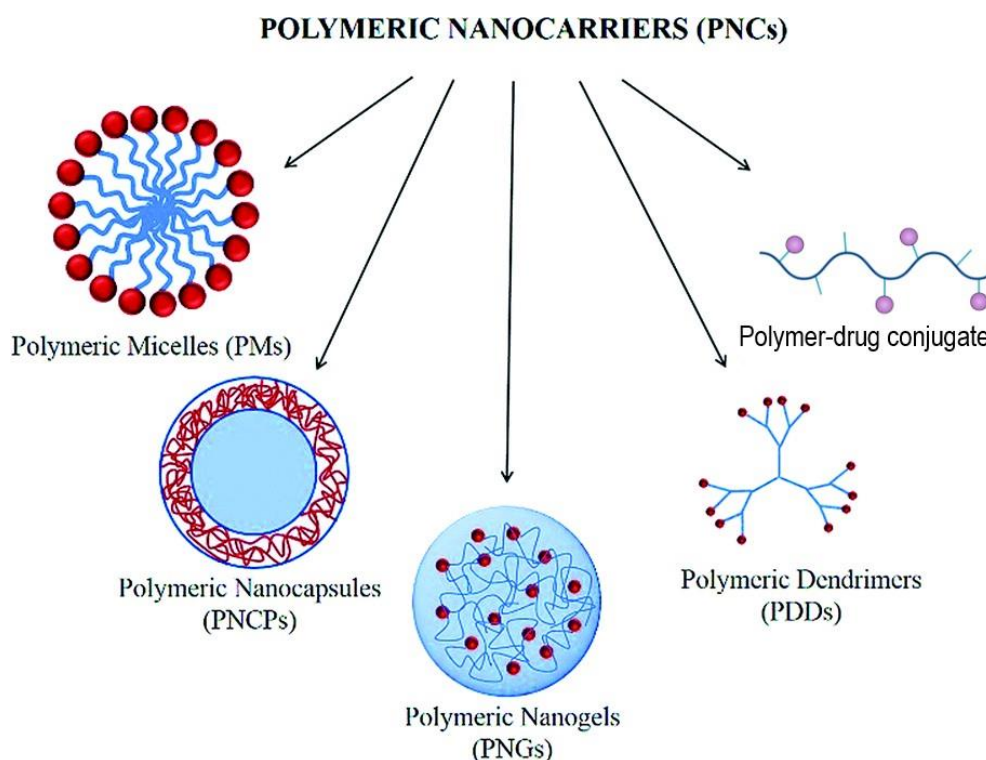


Figure 1.3: Labelled schematic of types of polymeric nanocarriers.^{42,43} (reproduced from reference 42 and 43)

1.3.3 Dendrimers as a drug delivery system

Dendrimers were thought to mimic proteins due to their globular structure and the name is derived from the word '*dendron*' meaning tree as they branch outwards in a similar manner as trees.⁴⁴ They are three-dimensional hyperbranched polymeric units typically consisting of three components: a central core, an internal structure comprised of repeating units (branches otherwise referred to as dendrons) and an exterior structure of surface functional groups as illustrated in figure 1.4. The radial layers formed by the branches are referred to as generations and the generation number (denoted as G_n) is defined as how many layers are attached to the core.^{32,45} Dendrimers have attractive properties such as a controlled uniform size, monodispersity and are available in different terminal functional groups which is important for biomedical applications.^{20,46,47} Surface functional groups can be modified to conjugate biomolecules such as antibodies or drugs which is particularly useful in targeted delivery and drug transport.²⁰ Due to the branching, dendrimers contain internal cavities which make it possible to encapsulate molecules of interest (in this case, drugs) to form a guest-host complex.²⁰ Depending on the type of dendrimer, most are soluble in aqueous solutions which result in an increase in bioavailability of lipophilic drugs and most dendrimers are able

to penetrate cell membranes with ease therefore making them effective nanocarriers and an ideal option for drug delivery.^{32,48} Dendrimers have shown to be stable in various conditions unlike micelles which are affected by conditions such pH, concentration and temperature.

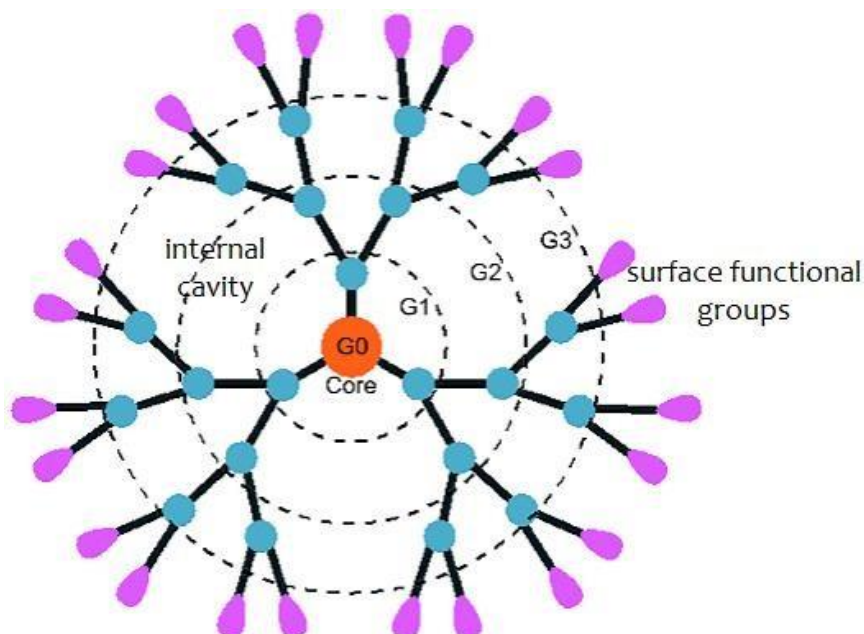


Figure 1.4: General structure of a dendrimer with labels highlighting main structural components.⁴⁴ (reproduced from reference 44)

Synthesis of dendrimers

Unlike conventional polymers which are synthesized via a “one-pot synthesis”, dendrimers are synthesized in step-wise manner where two or more reactions are repeated, one after the other, until the desired generation number is obtained. There are two methods to synthesize dendrimers namely, the convergent and divergent technique.^{44,48} In the divergent technique, the dendrimer grows outwards where the core is reacted with a monomer and the atom at the periphery (which may need to undergo activation) becomes the next reactive sites as shown in figure 1.5A. The advantage of utilizing the divergent technique is simplified purification due to the difference in molecular weights of the product and starting material, high yields as well as the ability to synthesize high generations though uneven dendrimer growth is possible and may lead to a polydisperse product.⁴⁹

The convergent method begins at the periphery, where individual dendrons (branches) are first synthesized usually by immobilizing one of the reactive sites on a template as shown in figure 1.5B. In the final step, the dendrons are coupled to a multifunctional core molecule to

produce a single dendrimer molecule.^{48,49} The advantage with this synthetic technique is monodispersity and reagent efficiency. However, the main disadvantage is difficulty with synthesizing higher generations due to the steric hindrance encountered at the final coupling step.⁴⁹

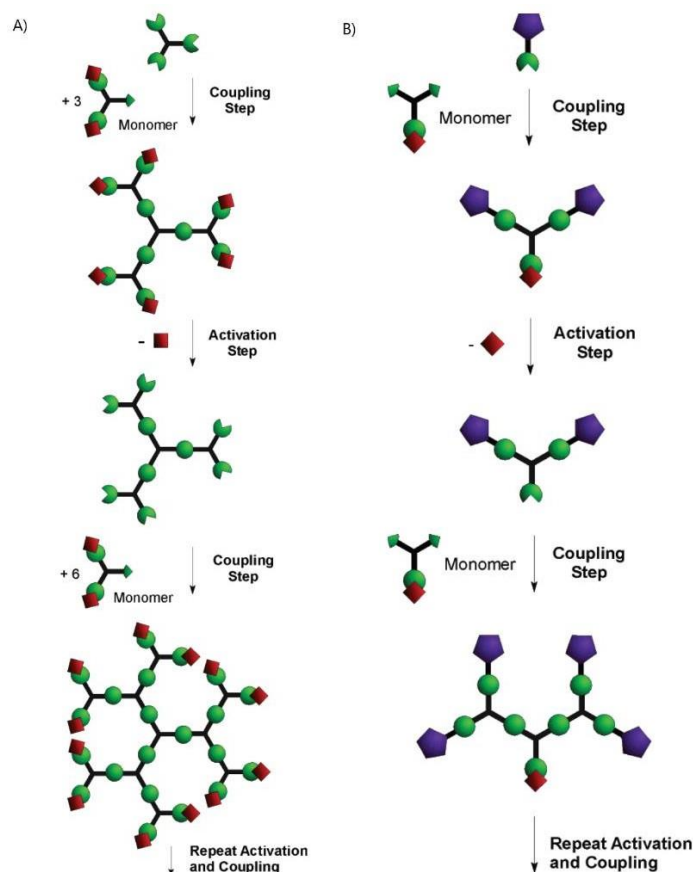


Figure 1.5: A) Divergent synthesis schematic and B) convergent approach schematic.⁵⁰ (reproduced from reference 50)

Types of dendrimers

Polypropyleimine (PPI) dendrimer: PPI dendrimers were first synthesized by Votlge and are typically synthesized using the divergent technique.^{46,51,52} The PPI dendrimer is amine terminated and also contain tertiary nitrogen atoms in the internal structure.⁵² Diaminobutane (DAB) or ethylene diamine (EDA) are used as the core molecule and acrylonitrile as the internal branching structure. The first reaction is a double Michael addition of acrylonitrile to the core molecule to produce a half generation. The next reaction is the catalytic hydrogenation (usually using Raney nickel) of the nitrile functional group to produce an amine terminated full generation (figure 1.6).⁵¹

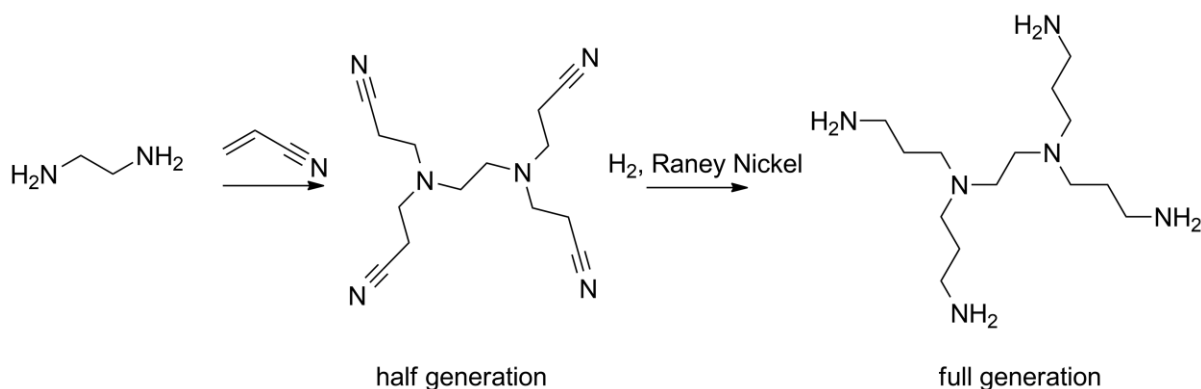


Figure 1.6: Synthesis of a polypropylene imine (PPI) dendrimer

Frechet dendrimer: These dendrimers were first synthesized by Frechet and Hawker using the convergent method.⁵³ They are comprised of poly-benzyl ether functional groups and tend to be hydrophobic.⁵³

Chiral dendrimer: These types of dendrimers are commonly synthesized via the convergent method and engineered such that the branches are chemically similar to the chiral core molecule.⁵¹ Chiral dendrimers are gaining interest in chiral molecular recognition as well as catalysis.^{51,54}

Glycodendrimer: Glycodendrimers are comprised of saccharide moieties such as glucose and galactose. The sugar moieties may be at the core or attached at the internal structure or can make up the surface functional groups.⁵¹ These dendrimers have been many used for drug delivery to lectin rich environments.⁵¹

Polyamido amine (PAMAM) dendrimer: PAMAM dendrimers were first synthesized by *Tomalia* et al using the divergent technique and are characterized by internal amide groups and amine surface functional groups.⁴⁶ EDA and methyl acrylate (MA) are the most commonly used molecules to synthesize PAMAMs. The first reaction is a Michael addition to form an ester terminated half generation and the next reaction is an amidation reaction to produce an amine terminated full generation dendrimer.⁴⁶

1.4 Targeted delivery

Traditionally, drugs are administered mainly orally and intravenously. Oral administration reduces the bioavailability of the drug due to the first pass metabolism i.e. the processes that occur in the liver to excrete the drug before the remainder of the drug is distributed to the rest of the body and site where the drug is needed.^{55,56} Intravenous administration bypasses the first pass metabolism however the drug is distributed all over the body.⁵⁵ This can be dangerous for anti-cancer agents as they can act on cells where they are not needed resulting

in toxicity. Targeted drug delivery aims to concentrate the drug at the desired site to increase bioavailability as well as decrease toxicity. In order to concentrate the drug at a specific site, drug delivery systems such as nanoparticles and molecular targets such as enzymes, proteins, receptors and antibodies are used to display a level of selectivity.^{55,56}

1.4.1 Types of targeting:

Passive targeting: this type of targeted delivery utilizes the enhanced permeability and retention (EPR) phenomenon in cancer cells. The drug is selectively accumulated and concentrated locally as cancer cells lack a lymphatic drainage system.^{4,57} As the cycling time of the drug is increased at a local site, eventually most of the drug is taken up by the cancer cells. The common delivery system used are nanoparticles.^{20,58}

Active targeting: this type of targeting delivers the drugs to the cancer cells by means of functionalizing the drug or drug delivery system with overexpressed molecular targets such as peptides, saccharides and amino acids. These moieties are used to achieve a certain level of selectivity hence reducing toxicity and side effects.²⁰

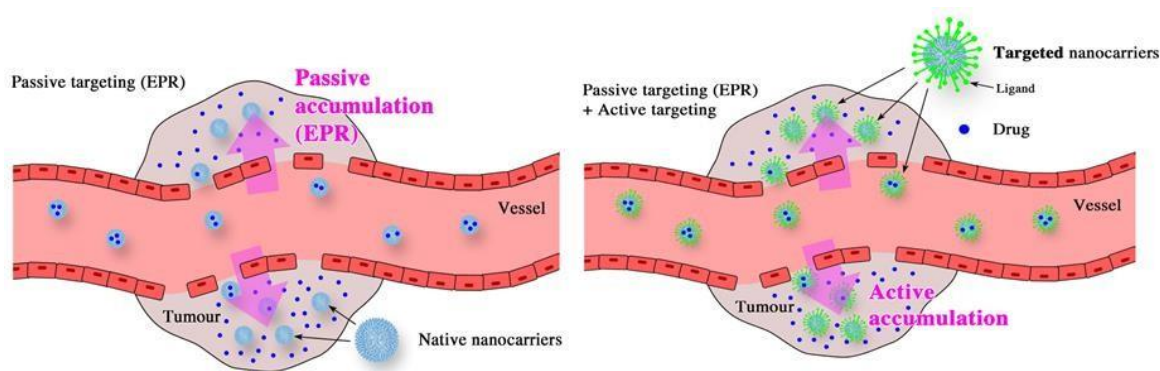


Figure 1.7: Image of EPR effect and active targeting.⁵⁹ (reproduced from reference 59)

1.5 Folate receptor (FAR): a molecular target for selectivity

Folate is an essential amino acid sourced from food such as leafy greens. Folic acid (FA), otherwise known as vitamin B9, is the synthetic form of folate which is found in fortified foods as well as a supplement. FA is a small molecule that is converted to folate in the body by reduction to dihydrofolate (DHF) and then to tetrahydrofolate (THF). The structure of FA is comprised of 3 components namely, pteridine ring, para-amino benzoic acid and glutamic acid (figure 1.7).^{60,61}

Folate plays an important role in various metabolic pathways for example the one-carbon

transfer, regulation of nicotinamide adenine dinucleotide phosphate/(NADPH/NADP) levels as well as support enzymatic reactions for the synthesis of purines.⁶⁰ The one-carbon transfer results in the output of biomolecules such as thymidine, serine and methionine - which is essential for DNA and protein synthesis. DNA replication and protein synthesis take place in proliferating cells such as fetuses and cancer cells. Given the high demand for folate, cancer cells such as ovarian, kidney, lung, colorectal and pancreatic often overexpress the folate receptor (FAR).^{60,61}

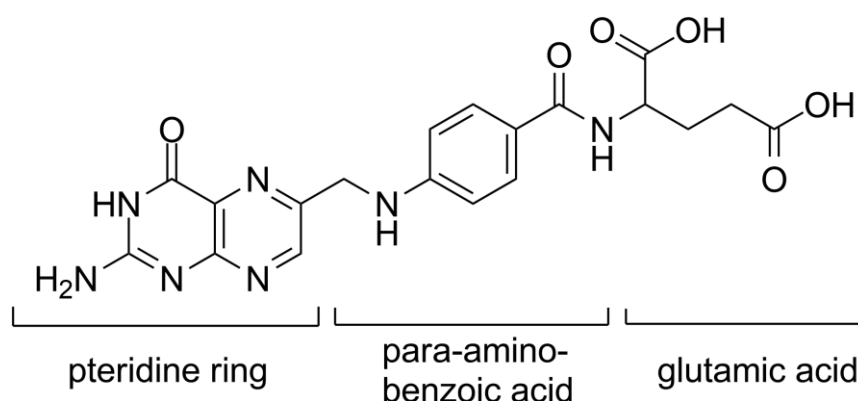


Figure 1.8: Structure of folic acid showing the amino acid sub-components.⁶⁰ (reproduced from reference 60)

FAR is a protein found on the surface of cells and is anchored by glycosylphosphatidylinositol (GPI). FARs exist in four isoforms namely, FAR α , FAR β , FAR γ and FAR δ . Folate has a high binding affinity for the receptor. Once bound to the receptor, the folate-receptor complex is transported into the cell via endocytosis.⁶² Binding studies conducted by Chen *et al* reported that when FA is bound to the FAR, the pteridine and para-amino benzoic acid are enveloped in a pocket in the receptor and the glutamic acid chain hangs out of the pocket therefore making it possible to conjugate FA to drugs and/or drug delivery systems (via the glutamic acid functional group) without affecting the binding of FA to FAR.⁶⁰ Therefore, it is rationalized that FAR is a good molecular target for cancer treatment and selectivity.

1.6 Aims and Objectives

There is a need for the development of biocompatible, efficient and selective drug delivery systems to address the lack of solubility and selectivity faced by potential drug candidates. This class of [Pt(phen)(acylthiourea)]⁺ complexes have been shown to display good anticancer activity, however, some of them are sparingly soluble in water and they do not show any selectivity towards cancer cells.³⁰ Therefore, the aims and objectives of this project are:

Aim:

To design a drug delivery system consisting of polyamido amide (PAMAM) dendrimers to address the solubility issues and lack of selectivity faced by $[\text{Pt}(\text{phen})(\text{acylthiourea})]^+$ complexes.

Objectives:

- To synthesize and characterize $[\text{Pt}(\text{phen})(\text{acylthiourea})]^+$ complexes.
- To synthesize and characterize a generation 4 (G4) PAMAM dendrimer and surface functionalized G4 dendrimers
- To encapsulate the synthesized complexes in the dendrimer variations and study the guest-host interactions in detail.
- To screen the potentially encapsulated complexes via cell viability studies (MTT assay).

1.7 References

- (1) Siegel, R. L.; Miller, K. D.; Jemal, A. Cancer Statistics, 2019. *CA Cancer J Clin* **2019**, *69* (1), 7–34. <https://doi.org/10.3322/caac.21551>.
- (2) Sung, H.; Ferlay, J.; Siegel, R. L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* **2021**, *71* (3), 209–249. <https://doi.org/10.3322/caac.21660>.
- (3) Mitra, S.; Ganguli, S.; Chakrabarti, J. Introduction. In *Cancer and Noncoding RNAs*; Elsevier, 2018; pp 1–23. <https://doi.org/10.1016/b978-0-12-811022-5.00001-2>.
- (4) Yu, K.; Liu, M.; Dai, H.; Huang, X. Targeted Drug Delivery Systems for Bladder Cancer Therapy. *J Drug Deliv Sci Technol* **2020**, *56* (January), 101535. <https://doi.org/10.1016/j.jddst.2020.101535>.
- (5) Aldred, E. M. Chemotherapy 39. In *Pharmacology: A Handbook for Complementary Healthcare Professionals*; 2009; pp 307–313.
- (6) Thway, K. Sarcomas. In *Brenner's Encyclopedia of Genetics: Second Edition*; Elsevier Inc., 2013; pp 327–331. <https://doi.org/10.1016/B978-0-12-374984-0.01369-3>.
- (7) Kramer, Ij. M. Prologue. In *Signal Transduction*; Elsevier, 2016; pp 1–51. <https://doi.org/10.1016/b978-0-12-394803-8.00001-2>.
- (8) Sabath, D. E. Leukemia. In *Brenner's Encyclopedia of Genetics: Second Edition*; Elsevier Inc., 2013; pp 226–227. <https://doi.org/10.1016/B978-0-12-374984-0.00862-7>.
- (9) Stone, T. W.; McPherson, M.; Gail Darlington, L. Obesity and Cancer: Existing and New Hypotheses for a Causal Connection. *EBioMedicine*. Elsevier B.V. April 1, 2018, pp 14–28. <https://doi.org/10.1016/j.ebiom.2018.02.022>.
- (10) Walser, T.; Cui, X.; Yanagawa, J.; Lee, J. M.; Heinrich, E.; Lee, G.; Sharma, S.; Dubinett, S. M. Smoking and Lung Cancer: The Role of Inflammation. In *Proceedings of the American Thoracic Society*; 2008; Vol. 5, pp 811–815. <https://doi.org/10.1513/pats.200809-100TH>.
- (11) Boffetta, P.; Hashibe, M. Alcohol and Cancer. *Lancet Oncol* **2006**, *7* (2), 149–156.
- (12) Smith-Bindman, R.; Lipson, J.; Marcus, R.; Kim, K. P.; Mahesh, M.; Gould, R.; Berrington De González, A.; Miglioretti, D. L. Radiation Dose Associated with Common Computed Tomography Examinations and the Associated Lifetime Attributable Risk of Cancer. *Arch Intern Med* **2009**, *169* (22), 2078–2086. <https://doi.org/10.1001/archinternmed.2009.427>.
- (13) Frisch, M.; Biggar, R. J.; Engels, E. A.; Goedert, J. J. Association of Cancer With AIDS-Related Immunosuppression in Adults. *Journal of the American Medicine Association* **2001**, *285* (13), 1736–1745. <https://doi.org/10.1001/jama.285.13.1736>.
- (14) Eylu, D. 9.1.1 Definition; 2018. <https://doi.org/10.1016/B978-0-12-813691-1.00009-9>.
- (15) Websites, R. Radiation Therapy. **2008**, 483–491.
- (16) Evans, S.; Young, M.; Higgins, S.; Moran, M. S. *The Breast*, Fifth Edit.; Elsevier Inc., 2018. <https://doi.org/10.1016/B978-0-323-35955-9.00046-5>.
- (17) *2-Geometrical Properties of H-Bonds and H-Bonded Organized Supramolecular Structures GEOMETRIES OF H-BONDS AT EQUILIBRIUM*; 2007. <https://doi.org/10.1016/B978-0-444-51957-3.50003-2>.
- (18) Freres, P.; Jerusalem, G.; Moonen, M. Categories of Anticancer Treatments. In *Anticancer Treatments and Cardiotoxicity: Mechanisms, Diagnostic and Therapeutic Interventions*; Elsevier Inc., 2017; pp 7–11. <https://doi.org/10.1016/B978-0-12-802509-3.00002-9>.
- (19) Conklin, K. A. Chemotherapy-Associated Oxidative Stress: Impact on Chemotherapeutic Effectiveness. *Integrative Cancer Therapies*. December 2004, pp 294–300.

<https://doi.org/10.1177/1534735404270335>.

- (20) Patri, A. K.; Kukowska-Latallo, J. F.; Baker, J. R. Targeted Drug Delivery with Dendrimers: Comparison of the Release Kinetics of Covalently Conjugated Drug and Non-Covalent Drug Inclusion Complex. *Adv Drug Deliv Rev* **2005**, 57 (15), 2203–2214. <https://doi.org/10.1016/j.addr.2005.09.014>.
- (21) Alderden, R. A.; Hall, M. D.; Hambley, T. W.; Kauffman, G. B. *Chemistry for Everyone The Discovery and Development of Cisplatin Products of Chemistry Edited By*; 2006; Vol. 83.
- (22) Percival, S. L.; Williams, D. W. Escherichia Coli. In *Microbiology of Waterborne Diseases: Microbiological Aspects and Risks: Second Edition*; Elsevier Ltd., 2013; pp 89–117. <https://doi.org/10.1016/B978-0-12-415846-7.00006-8>.
- (23) Lippert, B. *Cisplatin : Chemistry and Biochemistry of a Leading Anticancer Drug*; Verlag Helvetica Chimica Acta, 1999.
- (24) Jamieson, E. R.; Lippard, S. J. Structure, Recognition, and Processing of Cisplatin-DNA Adducts. *Chem Rev* **1999**, 99 (9), 2467–2498. <https://doi.org/10.1021/cr980421n>.
- (25) Rijt, S. H. van; Sadler, P. J. Current Applications and Future Potential for Bioinorganic Chemistry in the Development of Anticancer Drugs. **2009**, 14 (December), 1089–1097. <https://doi.org/10.1016/j.drudis.2009.09.003>.
- (26) Gómez-Ruiz, S.; Maksimović-Ivanić, D.; Mijatović, S.; Kaluderović, G. N. On the Discovery, Biological Effects, and Use of Cisplatin and Metalloenes in Anticancer Chemotherapy. *Bioinorganic Chemistry and Applications*. Hindawi Limited 2012. <https://doi.org/10.1155/2012/140284>.
- (27) Storr, T.; Thompson, H.; Orvig, C.; Thompson, K. Design of Targeting Ligands in Medicinal Inorganic Chemistry. **2006**, 534–544. <https://doi.org/10.1039/b514859f>.
- (28) Peega, T.; Magwaza, R. N.; Harmse, L.; Kotzé, I. A. Synthesis and Evaluation of the Anticancer Activity of [Pt(Diimine)(N,N-Dibutyl-N'-Acylothiourea)]⁺ Complexes. *Dalton Transactions* **2021**. <https://doi.org/10.1039/D1DT01385H>.
- (29) Peega, T.; Harmse, L.; Kotze, I. New Journal of Chemistry The Synthesis and Characterization of [Pt (Diimine)(Aroylthiourea)] Cl Complexes : Investigating Their Mode of Action as Potential Anticancer Agents Using Fluorescence Microscopy. **2017**, 1–3. <https://doi.org/10.1039/x0xx00000x>.
- (30) Magwaza, R. N.; Africa, S.; Magwaza, R. N. Synthesis and Characterization of Novel [Pt (Diimine) (Acylthiourea)] + Complexes as Potential Anticancer Agents and Exploring the Use of Sulphobutyl-Ether- β -Cyclodextrin and Surfactant Micelles as a Drug Delivery System.
- (31) Outline, C. *No Title*; 2018. <https://doi.org/10.1016/B978-0-12-813689-8.00003-3>.
- (32) Janaszewska, A.; Lazniewska, J.; Trzepinski, P.; Marcinkowska, M.; Klajnert-Maculewicz, B. Cytotoxicity of Dendrimers. *Biomolecules* **2019**. <https://doi.org/10.3390/biom9080330>.
- (33) Caminade, A. M.; Turrin, C. O. Dendrimers for Drug Delivery. *J Mater Chem B* **2014**, 2 (26), 4055–4066. <https://doi.org/10.1039/c4tb00171k>.
- (34) Howell, B. A.; Fan, D. Poly(Amidoamine) Dendrimer-Supported Organoplatinum Antitumour Agents. *Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences* **2010**, 466 (2117), 1515–1526. <https://doi.org/10.1098/rspa.2009.0359>.
- (35) Çağdaş, M.; Sezer, A. D.; Bucak, S. Liposomes as Potential Drug Carrier Systems for Drug Delivery. In *Application of Nanotechnology in Drug Delivery*; InTech, 2014. <https://doi.org/10.5772/58459>.
- (36) Torchilin, V. P. Micellar Nanocarriers: Pharmaceutical Perspectives. *Pharmaceutical Research*. January 2007, pp 1–16. <https://doi.org/10.1007/s11095-006-9132-0>.
- (37) Hanafy, N. A. N.; El-Kemary, M.; Leporatti, S. Micelles Structure Development as a Strategy to Improve Smart Cancer Therapy. *Cancers*. MDPI AG July 20, 2018. <https://doi.org/10.3390/cancers10070238>.

- (38) Liu, J.; Detrembleur, C.; Mornet, S.; Jérôme, C.; Duguet, E. Design of Hybrid Nanovehicles for Remotely Triggered Drug Release: An Overview. *Journal of Materials Chemistry B*. Royal Society of Chemistry August 14, 2015, pp 6117–6147. <https://doi.org/10.1039/c5tb00664c>.
- (39) Kong, F. Y.; Zhang, J. W.; Li, R. F.; Wang, Z. X.; Wang, W. J.; Wang, W. Unique Roles of Gold Nanoparticles in Drug Delivery, Targeting and Imaging Applications. *Molecules*. MDPI AG September 1, 2017. <https://doi.org/10.3390/molecules22091445>.
- (40) Hashidzume, A.; Takashima, Y.; Yamaguchi, H.; Harada, A. Cyclodextrin. In *Comprehensive Supramolecular Chemistry II*; Elsevier, 2017; pp 269–316. <https://doi.org/10.1016/B978-0-12-409547-2.13829-6>.
- (41) Baykal, A.; Bozkurt, A.; Jeremy, R.; Asiri, S. M. M.; Lima-Tenório, M. K.; Kaewsaneha, C.; Elaissari, A. Multistimuli-Responsive Magnetic Assemblies. In *Stimuli Responsive Polymeric Nanocarriers for Drug Delivery Applications: Volume 2: Advanced Nanocarriers for Therapeutics*; Elsevier, 2018; pp 155–193. <https://doi.org/10.1016/B978-0-08-101995-5.00006-4>.
- (42) Ekladios, I.; Colson, Y. L.; Grinstaff, M. W. Polymer–Drug Conjugate Therapeutics: Advances, Insights and Prospects. *Nature Reviews Drug Discovery*. Nature Publishing Group April 1, 2019, pp 273–294. <https://doi.org/10.1038/s41573-018-0005-0>.
- (43) Daglar, B.; Ozgur, E.; Corman, M. E.; Uzun, L.; Demirel, G. B. Polymeric Nanocarriers for Expected Nanomedicine: Current Challenges and Future Prospects. *RSC Advances*. Royal Society of Chemistry 2014, pp 48639–48659. <https://doi.org/10.1039/c4ra06406b>.
- (44) Lyu, Z.; Ding, L.; Huang, A. Y. T.; Kao, C. L.; Peng, L. Poly(Amidoamine)Dendrimers: Covalent and Supramolecular Synthesis. *Mater Today Chem* **2019**, *13*, 34–48. <https://doi.org/10.1016/j.mtchem.2019.04.004>.
- (45) Peterson, J.; Ebber, A.; Allikmaa, V.; Loop, M. Synthesis and Cze Analysis of Pamam Dendrimers With an Ethylenediamine Core. *Proceedings of the Estonian Academy of Sciences* **2001**, *50* (3), 156–166.
- (46) Tomalia, D. A. *Dendrimers and Other Dendritic Polymers Wiley Series in Polymer Science Series Editor* ; 2001; Vol. 1.
- (47) Newkome, D. G. R.; Moorefield, C. N.; Vogtle, F.; Gmbh, C. D. W. V.; Kgaa, C.; Newkome, G. R.; Moorefield, C. N.; Vogtle, F. *WILEY-VCH*.
- (48) Abbasi, E.; Aval, S. F.; Akbarzadeh, A.; Milani, M.; Nasrabadi, H. T. Dendrimers : Synthesis , Applications , and Properties. *Nanoscale Res Lett* **2014**, 1–10. <https://doi.org/https://dx.doi.org/10.1186%2F1556-276X-9-247>.
- (49) Sebestik, Jaroslav ; Reinis, Milan ; Jezek, J. *Biomedical Applications of Peptide-, Glyco- and Glycopeptide Dendrimers, and Analogous Dendrimeric Structures*; Springer, 2012.
- (50) Grayson, S. M.; Fréchet, J. M. J. Convergent Dendrons and Dendrimers: From Synthesis to Applications. *Chemical Reviews*. December 2001, pp 3819–3867. <https://doi.org/10.1021/cr990116h>.
- (51) Kesharwani, P.; Jain, K.; Jain, N. K. Dendrimer as Nanocarrier for Drug Delivery. *Progress in Polymer Science*. February 2014, pp 268–307. <https://doi.org/10.1016/j.progpolymsci.2013.07.005>.
- (52) Chaudhari, H. S.; Popat, R. R.; Adhao, V. S.; Shrikhande, V. N. DENDRIMERS: NOVEL CARRIERS FOR DRUG DELIVERY. *Journal of Applied Pharmaceutical Research* **2017**, *5* (10).
- (53) Hawker, C. J.; Fréchet, J. M. *Preparation of Polymers with Controlled Molecular Architecture. A New Convergent Approach to Dendritic Macromolecules*; 1990; Vol. 112.
- (54) Kareem, S. S.; Pathak, Y. *Clinical Applications of Artificial Neural Networks in Pharmacokinetic Modeling*; Elsevier Inc., 2016. <https://doi.org/10.1016/B978-0-12-801559-9.00020-X>.
- (55) Doogue, M. P.; Polasek, T. M. The ABCD of Clinical Pharmacokinetics. *Ther Adv Drug Saf* **2013**, *4* (1), 5–7. <https://doi.org/10.1177/2042098612469335>.

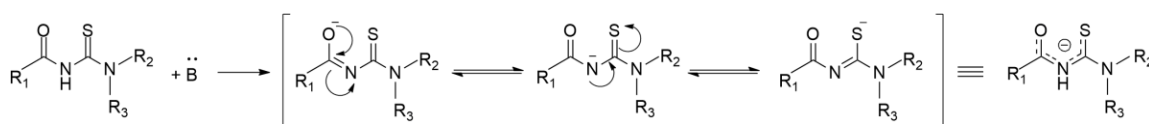
- (56) Romagnoli, B.; Hayes, W. Chiral Dendrimers - From Architecturally Interesting Hyperbranched Macromolecules to Functional Materials. *Journal of Materials Chemistry*. 2002, pp 767–799. <https://doi.org/10.1039/b110218b>.
- (57) Lu, Y.; Zhang, E.; Yang, J.; Cao, Z. Strategies to Improve Micelle Stability for Drug Delivery. **2018**.
- (58) Maeda, H.; Wu, J.; Sawa, T.; Matsumura, Y.; Hori, K. Tumor Vascular Permeability and the EPR Effect in Macromolecular Therapeutics : A Review. **2000**, 65, 271–284.
- (59) Attia, M. F.; Anton, N.; Wallyn, J.; Omran, Z.; Vandamme, T. F. An Overview of Active and Passive Targeting Strategies to Improve the Nanocarriers Efficiency to Tumour Sites. *Journal of Pharmacy and Pharmacology*. Blackwell Publishing Ltd August 1, 2019, pp 1185–1198. <https://doi.org/10.1111/jphp.13098>.
- (60) Chen, C.; Ke, J.; Edward Zhou, X.; Yi, W.; Brunzelle, J. S.; Li, J.; Yong, E. L.; Xu, H. E.; Melcher, K. Structural Basis for Molecular Recognition of Folic Acid by Folate Receptors. *Nature* **2013**, 500 (7463), 486–489. <https://doi.org/10.1038/nature12327>.
- (61) Smith, S. B.; Ganapathy, P. S.; Bozard, R. B.; Ganapathy, V. Folate Transport in Retina and Consequences on Retinal Structure and Function of Hyperhomocysteinemia. In *Handbook of Nutrition, Diet and the Eye*; Elsevier Inc., 2014; pp 349–359. <https://doi.org/10.1016/B978-0-12-401717-7.00035-6>.
- (62) Assaraf, Y. G.; Leamon, C. P.; Reddy, J. A. The Folate Receptor as a Rational Therapeutic Target for Personalized Cancer Treatment. *Drug Resistance Updates*. Churchill Livingstone 2014, pp 89–95. <https://doi.org/10.1016/j.drug.2014.10.002>.

Chapter 2

Synthesis of generation 4 Polyamido amine (G4 PAMAM) dendrimer and $\text{Pt}(\text{phen})(\text{acylthiourea})]^+$ complexes

2.1 Introduction to platinum complexes:

Platinum (II) complexes have been long discovered to display anticancer activity as shown by the widely used cisplatin. Although displaying anticancer activity, cisplatin is toxic due to the lack of selectivity and is prone to resistance.¹ Its clinically used derivatives, carboplatin and oxaliplatin, display improved toxicity but are still with their limitations due to the similarities in mode of action to that of cisplatin.^{1,2} Current research explores the synthesis of Pt (II) complexes with improved cytotoxicity while simultaneously displaying an improved pharmacokinetic profile. This can be achieved by incorporating ligands which act via non-covalent binding mechanisms or by inhibiting other cell functions that will ultimately induce apoptosis and/or paraptosis.³ We aim to achieve this by using the phenanthroline moiety due to its known biological activity that is found in diimine functional group bearing complexes.⁴ A conjugated heterocycle such as phenanthroline utilizes its pi-pi stacking ability to intercalate between the DNA.^{3,4} The *N,N*-dialkyl-*N'*-acylthiourea ligand on the other hand has been shown to display good anticancer activity when bound to certain metals to form complexes.⁵ These ligands have various modes of coordination due to the presence of multiple donor atoms (O, S and N). Coordination modes include bidentate S and N coordinated to the central metal, monodentate S bonded to the metal, bidentate O and N bonded to the metal and, lastly, bidentate S and O bonded to the metal.⁶⁻⁹ We, however, are interested in the bidentate coordination to the central metal through the oxygen and sulphur atom.^{5-7,10} This mode of coordination is facilitated by the deprotonation of the central nitrogen to produce an anionic ligand where the negative charge is delocalized to afford the resonance structures shown in scheme 2.1.^{5,11}



Scheme 2.1: Depiction of the delocalization of electrons throughout the *N,N*-dialkyl-*N'*-acylthiourea ligand after deprotonation to afford an anionic bidentate ligand.¹² (reproduced from reference 12)

There are various ways to synthesize *N,N*-dialkyl-*N'*-acylthiourea ligands which include (i) reacting *N*-acylthiourea or *N*-acyl aminoimidoyl chlorides with H₂S,¹³ (ii) acylation of *N,N*-disubstituted thioureas,¹⁴ (iii) reacting aminothiocabonylimidoyl chlorides with KSCN followed by hydrolysis,¹⁵ (iv) reaction of iminophosphorane derivatives of thioureas with carboxylic acid¹⁶ and lastly, (v) the hydrolysis of *N*-aminothiocabonylcarbodiimides with mineral acids.¹⁷ The commonly used method is that described by Douglas and Dains¹⁸ which entails reacting amines with methyl *N*-acylcarbamodithioates and acyl isothiocyanates.¹⁵

Studies conducted by Peega *et al* showed that the platinum complexes bearing the *N,N*-dibutyl-*N'*-acylthiourea variation displayed increased anticancer activity as compared to those bearing shorter alkyl chains which highlights the importance of ligand design as well as structure-activity relationships.^{10,12,19} Given that these complexes have been shown to display very low water solubility, we aim to improve this by forming guest-host complexes with the water soluble PAMAM dendrimer. By functionalizing the PAMAM surface with folic acid, we aim to improve the selectivity towards cancer cells. In this study the *N,N*-dialkyl-*N'*-acylthiourea ligand will be varied in the platinum complexes to compare the effect of the ligand on the interaction between the platinum complexes and the PAMAM dendrimers. The complexes shown in figure 2.1 were synthesised and will be referred to using the nomenclature shown from this point forward.

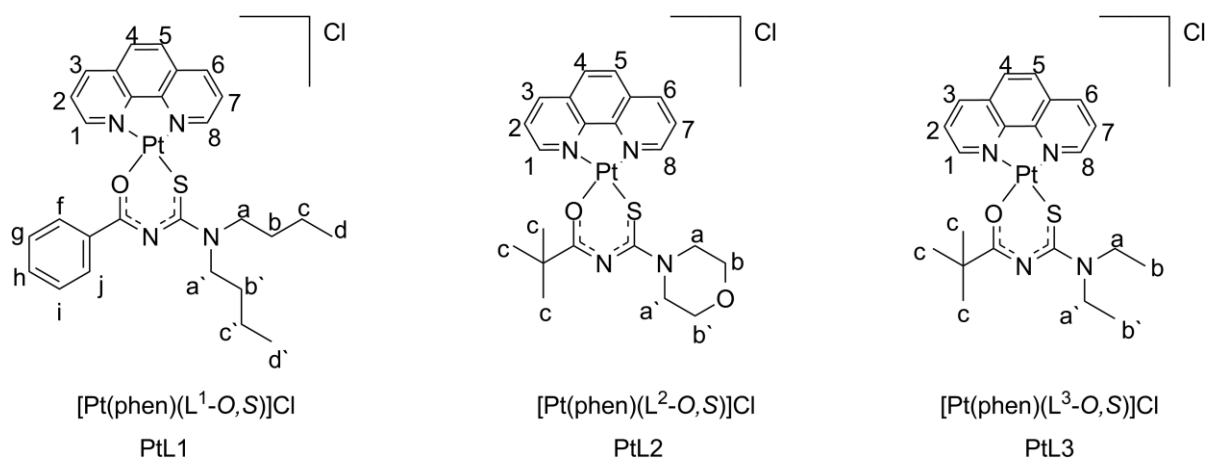


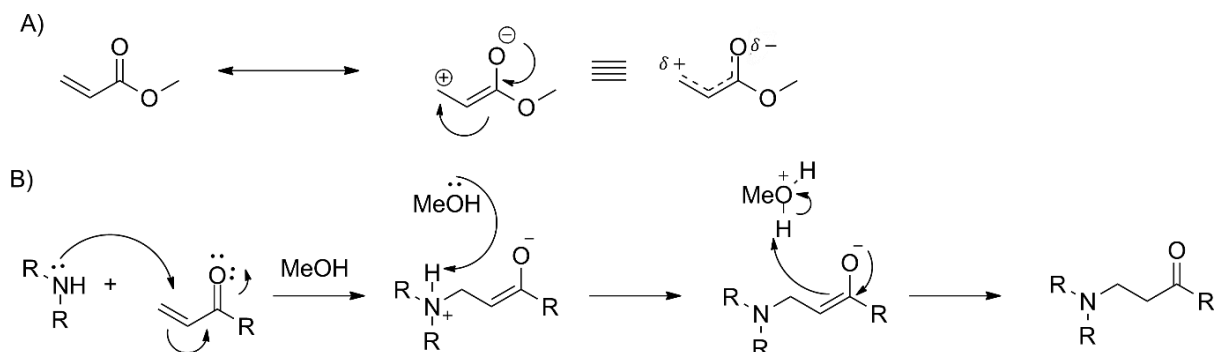
Figure 2.1: $[Pt(phen)(L^n-O,S)]Cl$ complexes synthesized labelled with the respective abbreviations.

2.2 Introduction to Polyamido amines

Polyamido amines (PAMAMs) are a family of dendrimers that are characterised by internal amide groups and surface amine functional groups and were first synthesized by Tomalia *et al* in 1985.^{20,21} PAMAM size ranges from 2nm (for G1) to approximately 13nm (for G10) and smaller generations (<G4) take on a flat shape while larger generations display a spherical structure.^{22,23}

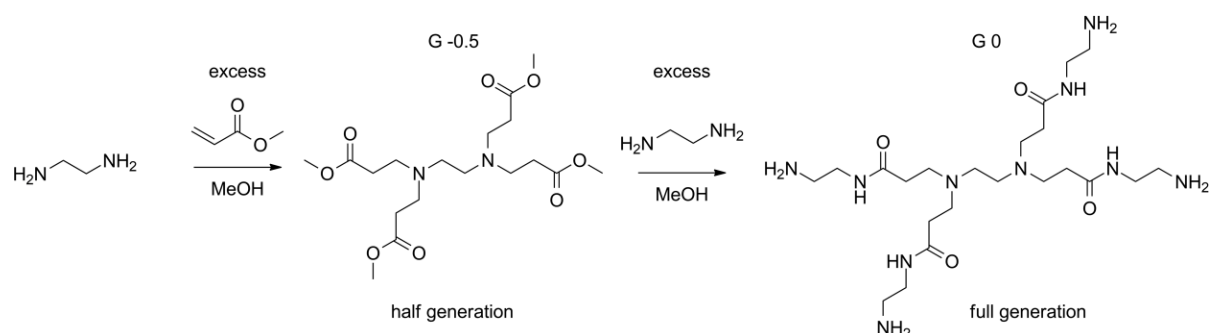
The divergent approach is the most commonly used method to synthesize PAMAM dendrimers and entails two repetitive steps namely, 1,4 - addition (Michael addition) and an amidation reaction. The 1,4 - addition reaction results in the formation of a half generation (denoted as $G_{n.5}$, where n is the generation number) with an ester being the terminal functional group which becomes the reactive site for the formation of the full generation as shown in scheme 2.2B. Dendrimer growth occurs in a stepwise manner beginning from

G-0.5 (negative 0.5). Regarding PAMAM synthesis, the Michael addition reaction makes use of methyl acrylate (MA), an α,β unsaturated carbonyl containing molecule as shown in scheme 2.2A. The carbonyl oxygen has electron withdrawing effects on the alkene and due to the resonance that is displayed in the molecule, the β -carbon becomes delta positive (δ^+) therefore allowing for nucleophilic attack by the nucleophile which is ethylene diamine (EDA) in this study.²⁴



Scheme 2.2: A) Resonance structure of methyl acrylate (MA) displaying the delta positive and delta negative charges. B) General reaction mechanism of a 1,4-addition⁵ (reproduced from reference 5).

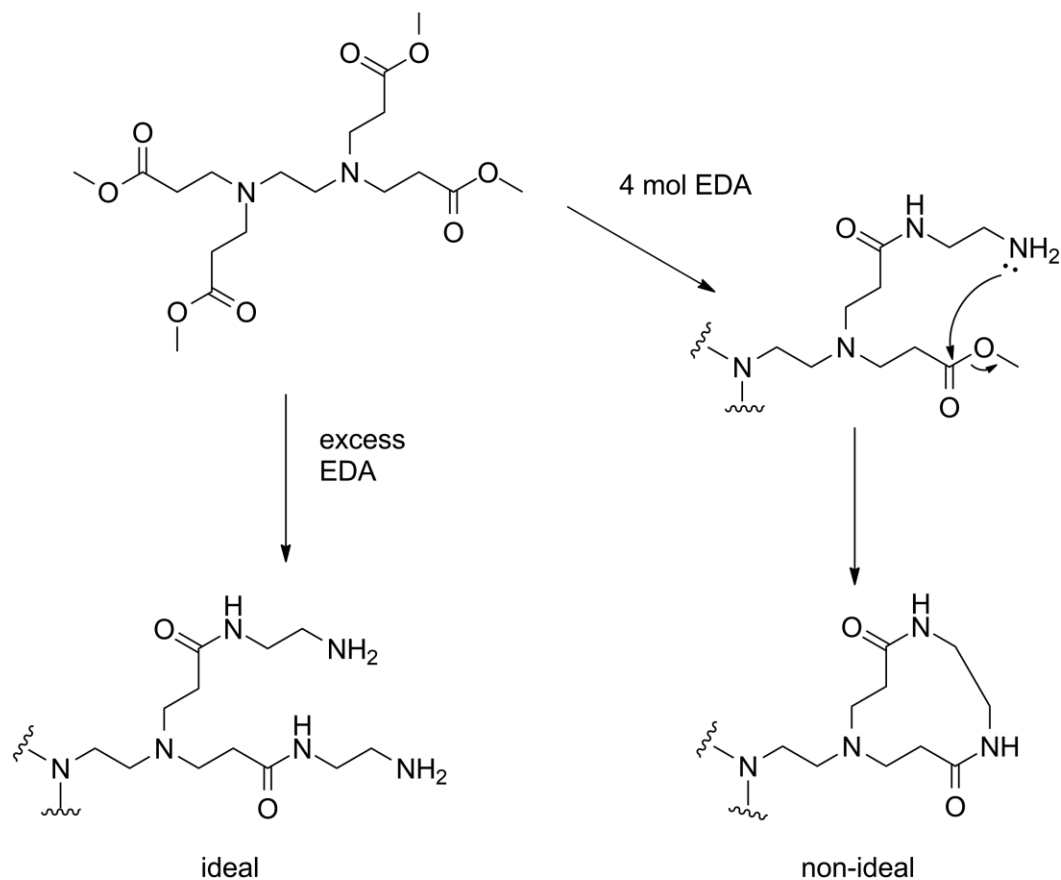
After the 1,4-addition reaction, an amidation reaction is conducted and results in the formation of a full generation (denoted as Gn, where n is the generation number) bearing a terminal amine functional group. The two reactions are repeated until the desired dendrimer generation is obtained. In this study, EDA and MA are the reagents used to synthesize a generation 4 (G4) dendrimer as shown in scheme 2.3. Other monomers such as ethyl acrylate (a Michael acceptor) and ammonia (a nucleophile) can also be used to form PAMAMs of varying internal chain lengths hence resulting in PAMAM dendrimer types of varying sizes.



Scheme 2.3: simplified reaction scheme of a half generation and full generation PAMAM dendrimer.

In the synthesis of both the half and full generation dendrimer, MA or EDA is added to the precursor dendrimer at 0°C dropwise to prevent the reverse Michael reaction (which is favoured at higher temperatures) from occurring. When the reverse reaction occurs, the dendrimer branches begin to break down which results in uneven dendrimer growth. During the synthesis of the full generation, a large excess of EDA is used to prevent cyclization.

Cyclization results in premature chain termination as well as uneven dendrimer growth which is not ideal as shown in scheme 2.4.²¹



Scheme 2.4: Ideal and non-ideal dendrimer growth during amidation step of a half generation PAMAM dendrimer (reproduced from reference 2).

2.3 Results and discussion

2.3.1 Synthesis of Pt(phen)Cl₂ precursor:

This synthetic method is taken from Morgan and Burstall.²⁵ In this method, the starting material K₂[PtCl₄] was dissolved in water and 1,10-phenanthroline was added to the reaction vessel along with a few drops of 30% HCl to slow the rate of aquation of the platinum salt. The mixture was then left to reflux for roughly 5 hours until the red-orange colour of the platinum salt was no longer visible in the reaction vessel. The reaction vessel was then allowed to cool and the yellow precipitate was filtered and washed several times with water followed by acetonitrile to remove unreacted starting material. The precursor was successfully characterized using ¹H NMR spectroscopy (figure 2.2), IR spectroscopy and mass spectrometry.

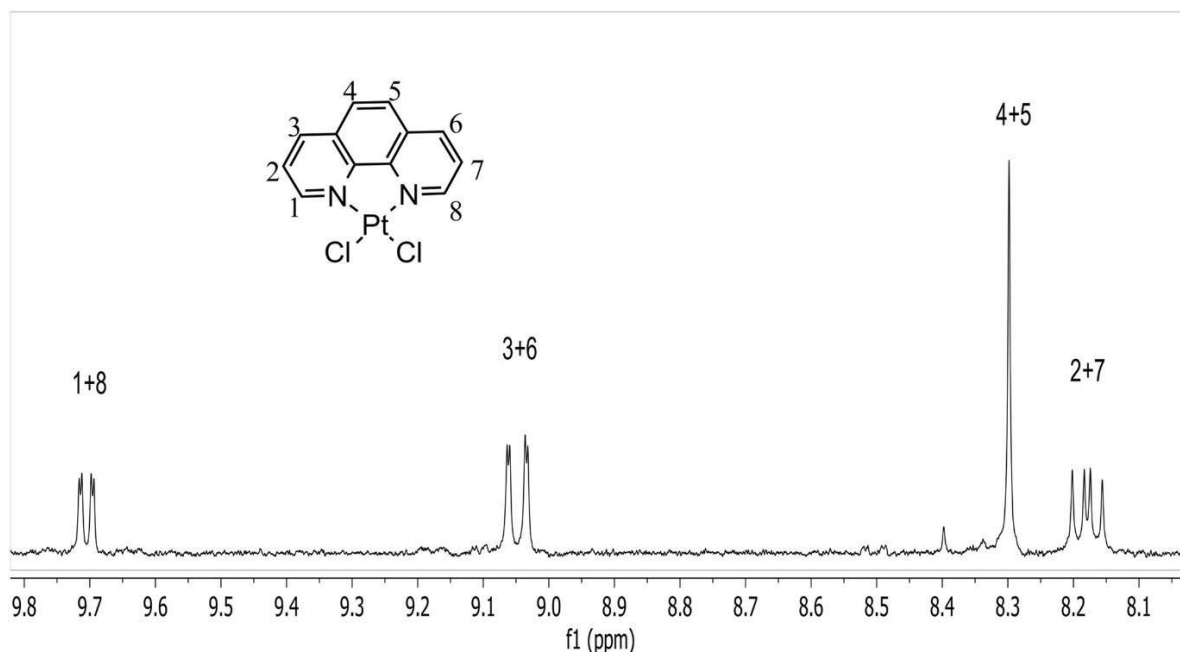


Figure 2.2: ^1H NMR of $\text{Pt}(1,10\text{-phenanthroline})\text{Cl}_2$ in $\text{DMSO}-d_6$ at 300K.

Due to symmetry in the molecule, only four sets of signals are observed in the ^1H NMR spectrum (figure 2.2). The most deshielded peak at 9.71 integrates for two protons and is assigned to protons 1 and 8 as they are closest to the electron withdrawing nitrogen groups. The next doublet of doublet at 9.05 ppm integrates for two protons and is assigned to protons 3 and 6 as they are in the para position to the nitrogen atoms. The singlet at 8.30 ppm integrates for 2 protons and is assigned to protons 4 and 5 as they have no neighbours to couple with. The doublet of doublet at 8.19 ppm integrates for two protons and is assigned to protons 2 and 7.

2.3.2 Synthesis of *N,N*-di(butyl)-*N'*-benzoylthiourea ligand:

The synthetic method utilized is a modified version of the method used by Douglas and Dains.¹⁸ In this procedure, benzoyl chloride was added to oven dried potassium thiocyanate in anhydrous acetone under an inert atmosphere and was left to reflux for 1 hour. Once cooled to room temperature, dibutyl-amine was added dropwise to the mixture and left to reflux for 3 hours. The resultant milky, white solution was left to cool, precipitate and filtered. Distilled water was added to the filtrate such that the desired product precipitated from the solution. The resultant crude ligand was then washed with a cold ethanol/water solution. The ligand was then purified by recrystallizing in isopropanol. The ligand was successfully characterized using NMR spectroscopy, IR spectroscopy and mass spectroscopy.

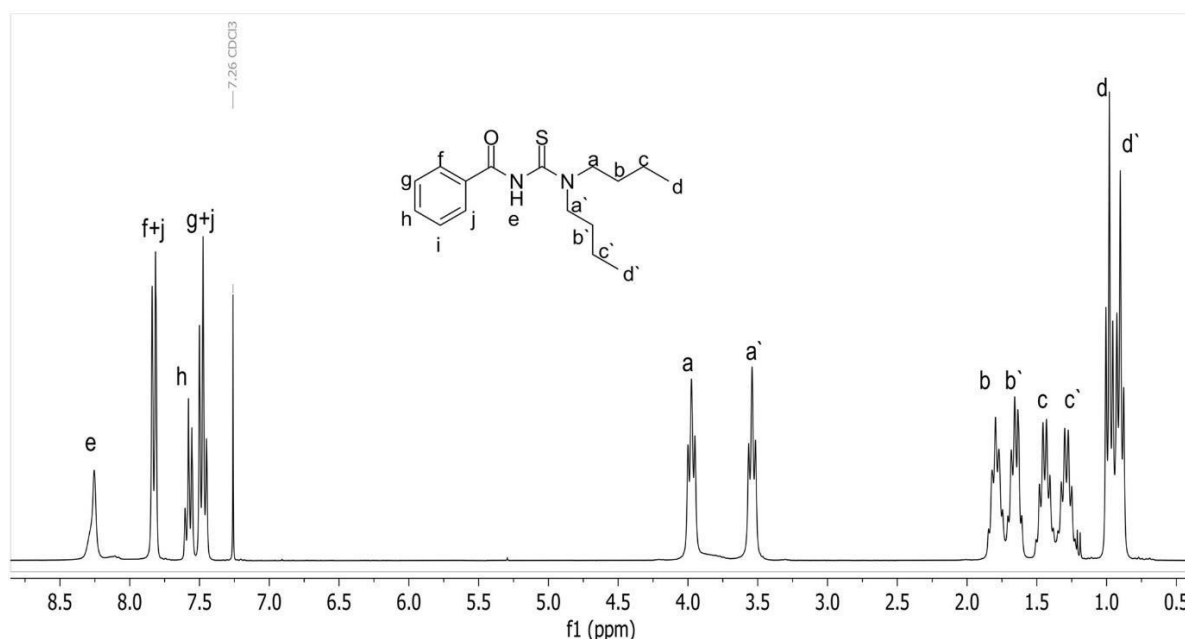


Figure 2.3: ^1H NMR of *N,N*-di(butyl)-*N'*-benzoylthiourea in CDCl_3 at 300K.

The deshielded most singlet at 8.25 ppm integrates for a single proton and is that of the NH amide group (figure 2.3). The rest of the aromatic protons were assigned with the aid of the COSY NMR spectrum (appendix figure 5.10). The doublet at 7.83 ppm integrates for two protons and belongs to protons *f* and *j*. These protons were expected to be the most deshielded protons in the aromatic region as they are closest to the electron withdrawing carbonyl group. The triplet at 7.58 ppm integrates for a single proton and is therefore assigned to proton *h*. The next triplet at 7.47 ppm integrates for two protons and is assigned to protons *g* and *i*. Proton *f* is chemically equivalent to proton *j*. Protons *g* and *i* are also chemically equivalent due to free rotation about the quaternary carbon attached to the carbonyl carbon as shown in figure 2.3.

The triplets at 3.98 and 3.54 ppm integrate for two protons each and are assigned to *a* and *a'* as they are closest to the electron withdrawing nitrogen group and experience more deshielding than the other aliphatic protons. The quintets at 1.80 and 1.66 ppm integrating for two protons each are assigned to protons *b* and *b'*. The sextets at 1.45 and 1.29 ppm integrate for two protons each are assigned to protons *c* and *c'*. Lastly, the triplets at 0.98 and 0.90 ppm are assigned to protons *d* and *d'* and integrate for three protons each. The delocalization of the lone pair on the nitrogen of the dibutyl arm and the sulphur results in the formation of a partial double bond hence the protons are not chemically equivalent as seen in the NMR spectrum (figure 2.3).

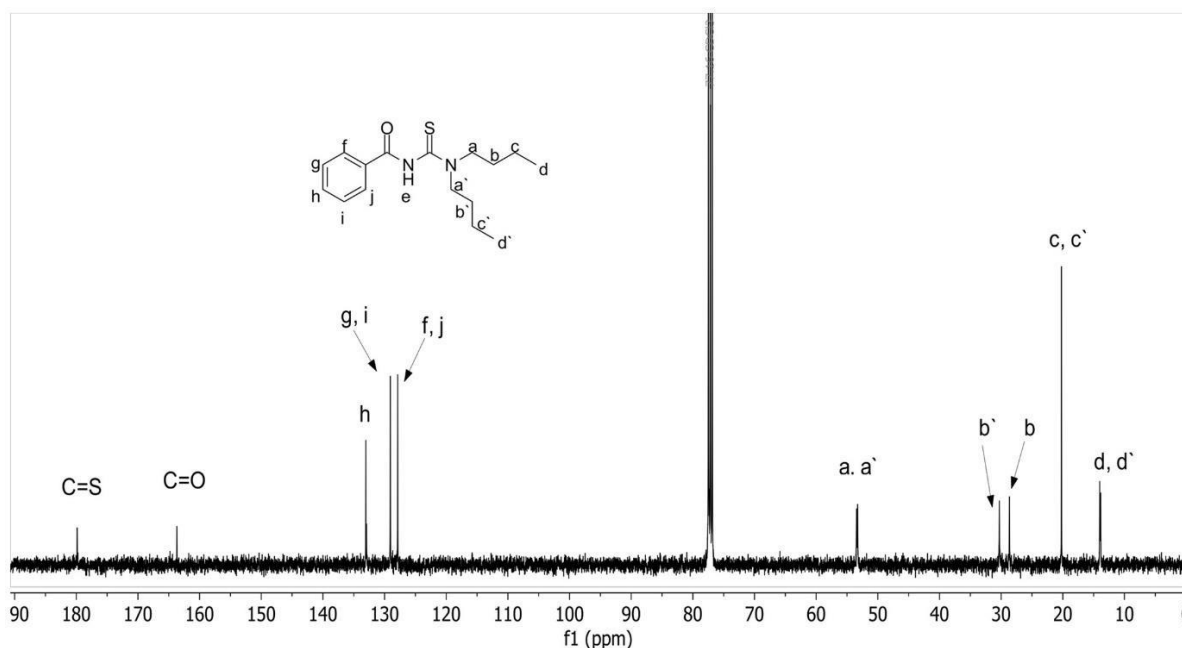


Figure 2.4: ^{13}C NMR of ligand *N,N*-dibutyl-*N*-benzoylthiourea in CDCl_3 at 300K.

The thiocarbonyl group was successfully identified from the HMBC NMR spectrum (appendix figure 5.12) and appears at 179.85 ppm, where protons a and a' couple to the C=S through long range coupling. The second most deshielded carbon appearing at 162.69 ppm is assigned to the carbonyl (C=O) group where aromatic protons f and j couple to the carbonyl through long range coupling as seen on the HMBC NMR spectrum. The rest of the carbons were successfully identified and assigned with the aid of the HSQC NMR spectrum (appendix figure 5.11). The ligand was further characterized with FT-IR spectroscopy (figure 2.5). The N-H stretch was identified at 3173 cm^{-1} , the C=O stretch was identified at 1689 cm^{-1} and the C=S stretch was identified at 1371 cm^{-1} . The identification of these functional groups confirms successful ligand synthesis.

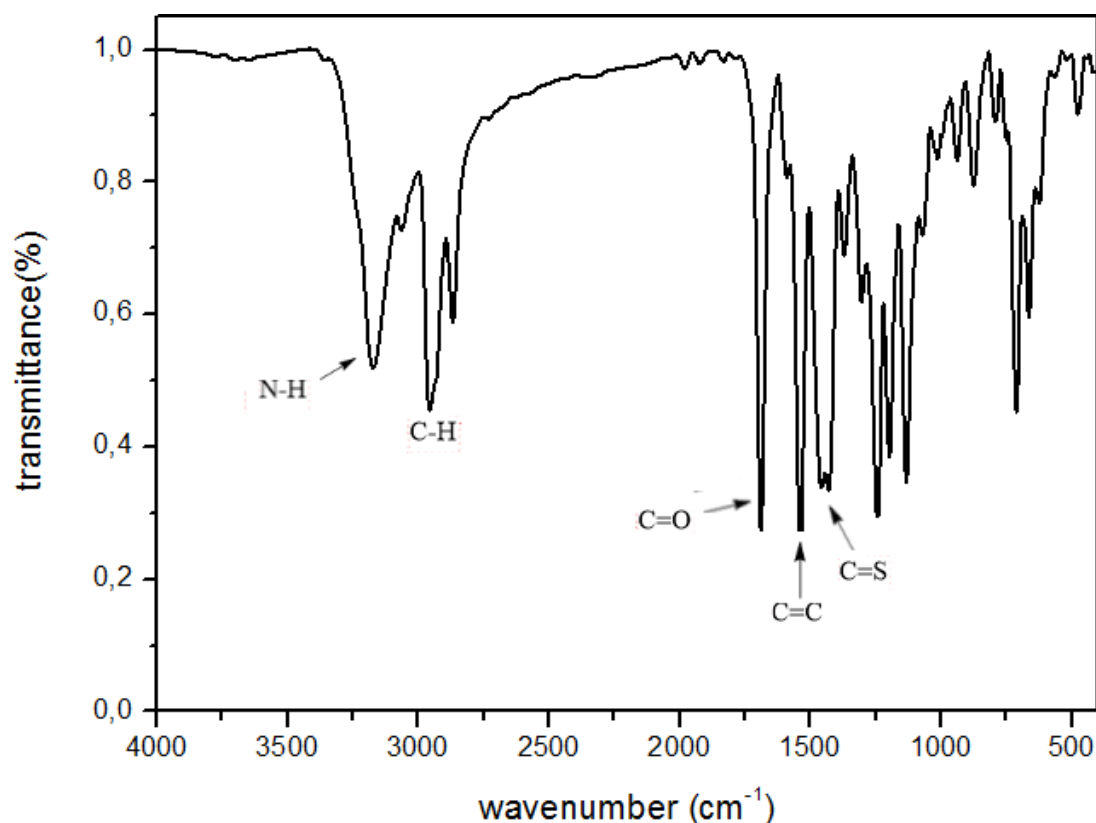


Figure 2.5: FT-IR spectrum of *N,N*-di(butyl)-*N'*-benzoylthiourea.

2.3.3 Synthesis of [Pt(phen)(acylthiourea)]⁺ complexes:

The [Pt(phen)(*N*-pivaloyl-*N,N*-diethylthiourea)]⁺ complex was synthesized by dissolving the precursor in acetonitrile and refluxing for 10 minutes. The ligand, dissolved in acetonitrile and water was added dropwise to the reaction vessel. The mixture was left to reflux for 15 minutes. Thereafter, sodium acetate dissolved in acetonitrile and water was added to reaction mixture which was then left to reflux until a colour change from cloudy yellow to pale yellow was observed which took approximately 2 hours. The sodium acetate functions to deprotonate the ligand and the addition of distilled water prevents the formation of the monodentate complex. The solvent was removed by reduced pressure to obtain a crude product. The crude product was worked up by redissolving in acetonitrile and filtered to remove the excess sodium acetate and/or sodium chloride. The resultant solid was then dissolved in a small amount of dichloromethane (DCM) and hexane was added to a centrifuge tube and the product was left to precipitate overnight. The product was then purified by washing with hexane and dried in a vacuum oven. The purified complex was then characterized using NMR spectroscopy.

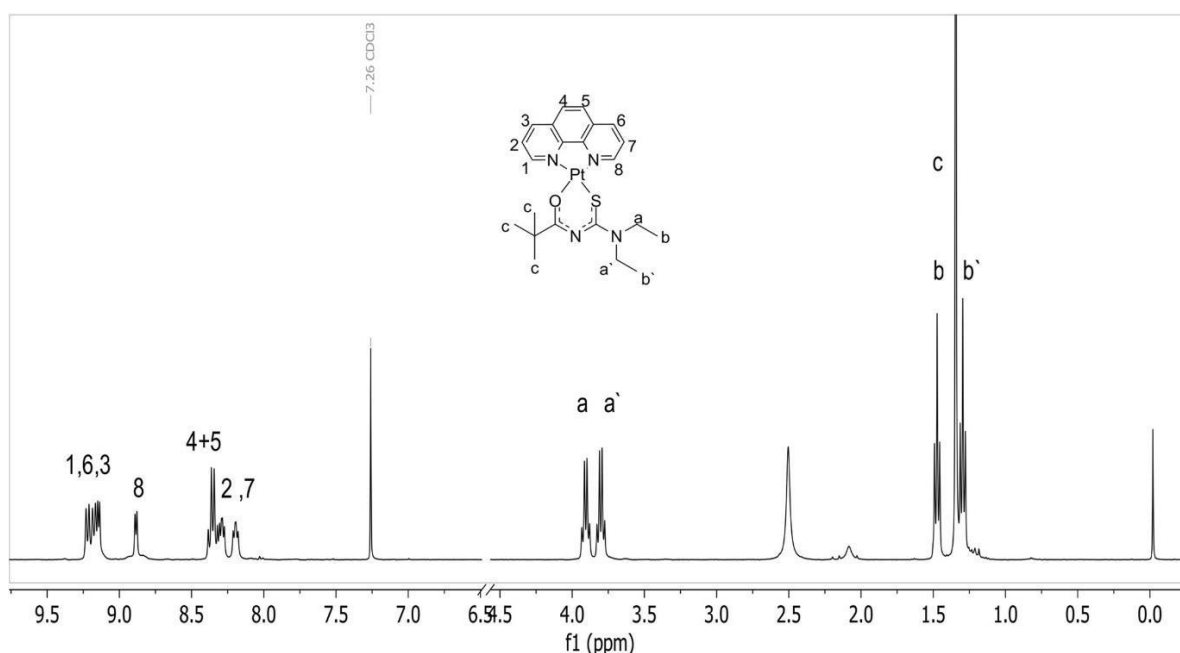


Figure 2.6: ^1H NMR of $[\text{Pt}(\text{phen})(\text{N}'\text{-pivaloyl-N,N-diethylthiourea})]^+$ in CDCl_3 at 300K.

Upon complexation, symmetry is lost in the precursor hence the appearance of additional peaks as shown in figure 2.6. Peak at 9.22 ppm integrates for one proton and is assigned to proton 1. The doublet at 9.18 ppm integrates for one proton and is that of protons 6. The doublet at 9.15 integrates for a single proton and is assigned as 3. The next doublet at 8.89 ppm integrates for a single proton and is assigned to proton 8. These protons are more deshielded than protons 2 and 7 as they experience deshielding due to the ortho-para directing nature of an aromatic group with a nitrogen substituent. The multiplet between 8.39- 8.31 ppm integrates for two protons assigned 4 and 5. The doublet of doublet at 8.29 ppm integrates for once protons and is assigned to proton 2. The doublet of doublet at 8.20 ppm integrates for one proton and is assigned as proton 7.

The quartets appearing at 3.91 and 3.80 ppm integrate for 2 protons each which are assigned to protons *a* and *a'* respectively. These protons experience deshielding as they are in close proximity to a nitrogen atom. The triplet at 1.47 integrating for three protons is assigned to *b*. The singlet at 1.35 ppm integrates for nine protons is assigned to the chemically equivalent CH_3 groups of the pivaloyl group *c*. Lastly, the triplet at 1.30 ppm which integrates for three protons is assigned to protons *b'*. The assignment of the protons was confirmed with the use of 2D NMR (COSY) (appendix figure 5.13). The broad singlet at 2.50 ppm is due to the presence of water in the sample and the broad singlet at 2.08 ppm is due to acetic acid which is a by-product that is formed as the base, sodium acetate, deprotonates the ligand. The changes in the NMR spectrum (figure 2.6) provide sufficient evidence that successful complexation has occurred.

This complex was then further characterized by ^{13}C NMR in which the HSQC was used to assign the carbons.

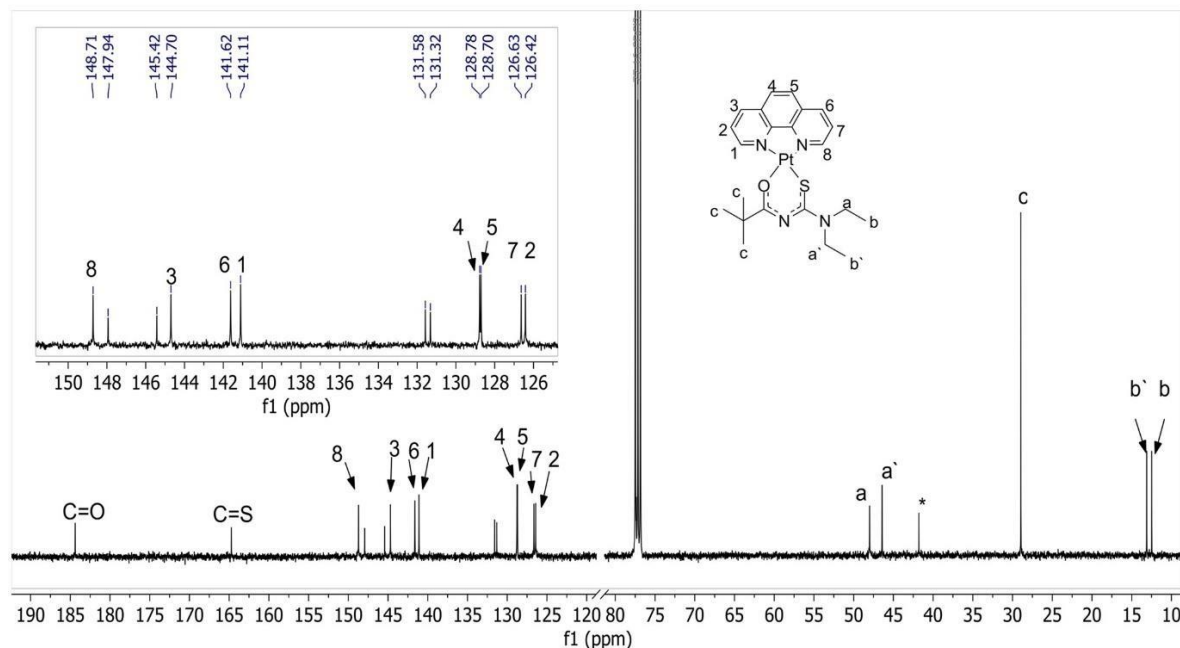


Figure 2.7: ^{13}C NMR spectrum of $[\text{Pt}(\text{phen})(\text{N}'\text{-pivaloyl-N,N-diethylthiourea})]^+$ in CDCl_3 at 300K.

The carbonyl and thiocarbonyl carbon were assigned to the most deshielded peaks at 184.38 ppm and 164.72 ppm respectively as seen in figure 2.7. Upon complexation the carbonyl carbon becomes more deshielded which is due to electron delocalization in the chelate ring.^{26,27} From the HSQC (figure 2.8), proton H^8 correlates to carbon C^8 and H^3 correlates to C^3 . The rest of the carbons were assigned in the same manner according to the HSQC which is shown in figure 2.8. Carbons C^4 , C^5 , C^7 and C^2 were distinguished and assigned with the use of HMBC NMR spectroscopy (appendix figure 5.14).

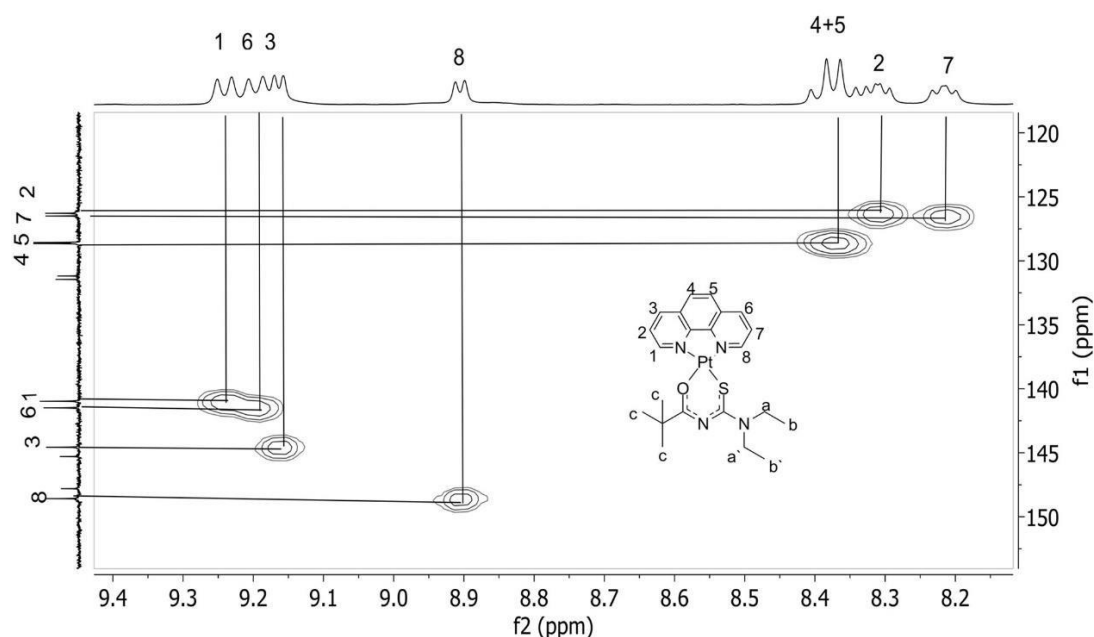
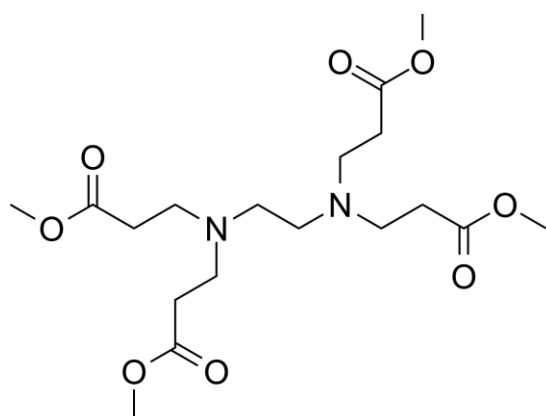


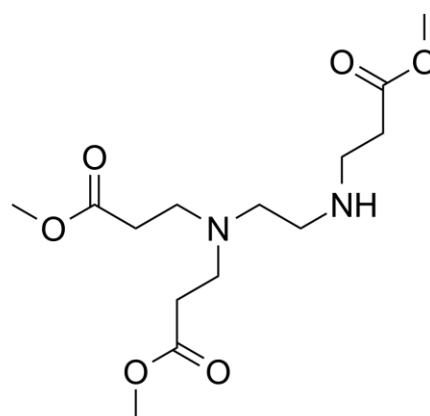
Figure 2.8: Aromatic region zoomed and assigned HSCQ NMR spectrum of $[Pt(phen)(N'\text{-pivaloyl-}N,N\text{-diethylthiourea})]^+$ in $CDCl_3$ at 300K.

2.4 Synthesis of Polyamido amine (PAMAM) dendrimer:

Every half generation dendrimer in this study (G-0.5, G0.5, G1.5, G2.5, G3.5) was synthesized according to the method described by Tomalia and Frechet.²¹ For the synthesis of G-0.5 PAMAM, a 6-molar excess of MA dissolved in methanol was added dropwise to EDA in methanol kept at 0°C using an ice bath. The mixture was kept stirring under an ice bath for 4 hours after the MA was added. The mixture was then allowed to warm up to room temperature and was kept stirring for 24 hours. The solvent and excess starting material was removed under reduced pressure. The crude G-0.5 PAMAM was an oil which contained a small amount of the tri-ester side product as shown in figure 2.9. The separation of the G-0.5 PAMAM from the tri-ester side product using column chromatography was challenging as the two compounds have very similar polarity. We then proceeded with the synthesis of the full dendrimer (generation zero) G0 without any further purification.



G-0.5 PAMAM



Tri-ester side product

Figure 2.9: Structure of G-0.5 PAMAM and tri-ester side product.

The first attempt at the synthesis of a full generation specifically, G0 PAMAM, involved a method with a shorter reaction time using the enzyme *Candida antarctica* lipase B (CALB) to catalyse the amidation.²⁸ The desired product had formed, however, purification of the desired product was difficult and resulted in low yields. The method described by Tomalia and Frechet which involved longer reaction times to synthesize the full generation was opted for which was then used to synthesize every full generation i.e. G0, G1, G2, G3 and G4.

This method entailed adding the G-0.5 PAMAM dissolved in methanol dropwise to a 40- molar excess of EDA dissolved in cold methanol at 0°C. The mixture was kept under an ice bath for 4 hours after the G -0.5 PAMAM was added and then allowed to warm up to room temperature. The mixture was kept stirring for 4 days. The amidation is a slow reaction due to the methoxy functionality of the ester being a poor leaving group as compared to the chlorine of an acyl chloride which would leave readily during a reaction where the carbonyl carbon of carboxylic acid derivatives (esters, acyl chlorides, anhydride acids, aldehydes, amides) undergoes nucleophilic attack. The solvent and excess EDA was removed under reduced pressure with the use of a 9:1 mixture of toluene/methanol to form a low boiling azeotrope with EDA. The G0 was isolated as a pale-yellow oil.

Characterization of PAMAM dendrimer:

G-0.5 was used to as an illustration to characterize the half generation dendrimer.

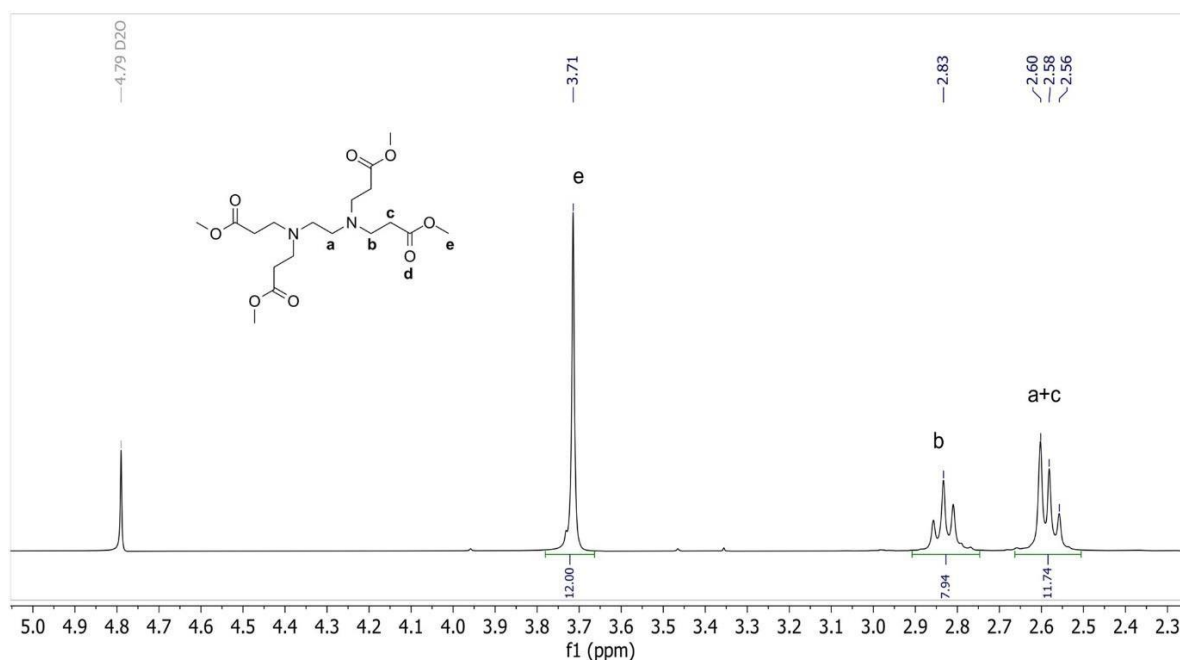


Figure 2.10: ^1H NMR of G -0.5 PAMAM in D_2O at 300K.

The singlet at 3.66 ppm integrates for 12 protons corresponding to the methyl groups labelled *e*. These protons experience deshielding due to their proximity to the electron withdrawing oxygen atom (figure 2.10). The triplet at 2.79 ppm integrates for 8 protons and is assigned as *b* as those protons are close to the electron-withdrawing nitrogen atom. Lastly, the peak appearing between 2.56 ppm and 2.52 ppm integrates for 12 protons and is a result of singlet *a* overlapping with triplet *c*. The NMR spectrum looks fairly simple and shows only four signals as the dendrons are chemically equivalent as a result of the symmetry seen in the molecule.

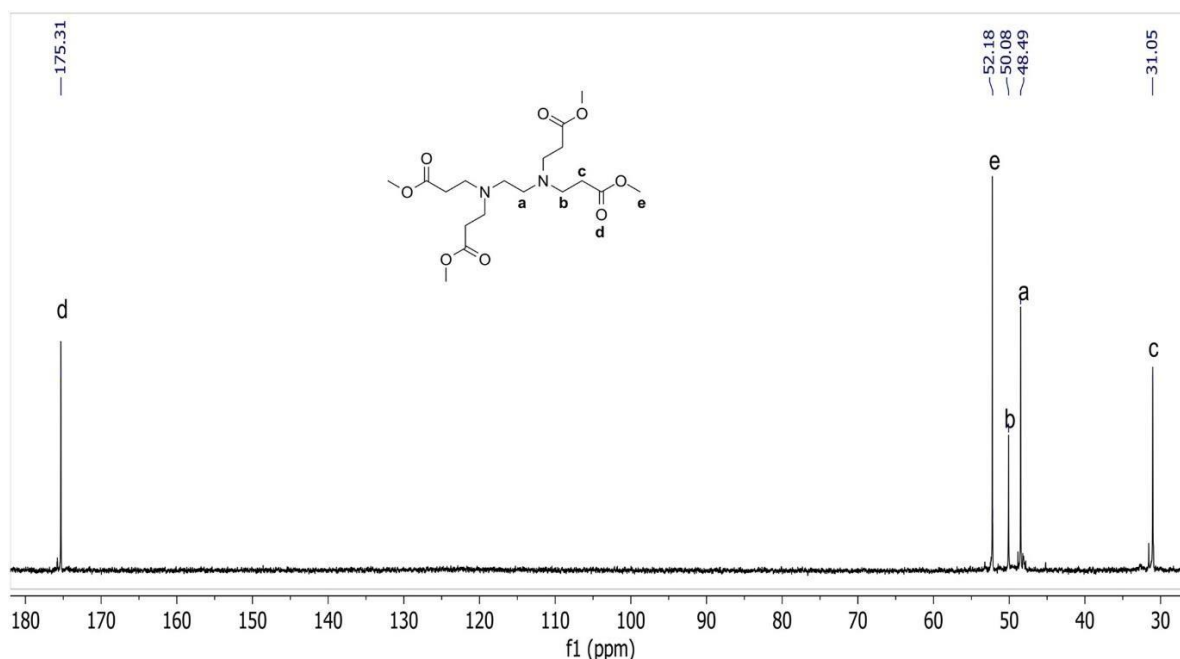


Figure 2.11: ^{13}C NMR of G-0.5 PAMAM in D_2O at 300K.

C^d was assigned using ^{13}C NMR. Signal d appeared at 175.33 ppm in the expected region of a carbonyl carbon as seen in figure 2.11. C^e was easily identified with the aid of DEPT NMR (appendix figure 5.18) as it is the only CH_3 group and is assigned to the peak at 52.23 ppm, and appears in the expected region for an ester. The remaining carbons were assigned using C-H correlation (HSQC) (appendix figure 5.19) with peaks at 50.08, 48.49 and 31.04 ppm corresponding to C^b , C^a and C^c respectively. The appearance of the tri-ester side product is more visible on the ^{13}C NMR spectrum as seen by the smaller peaks near the more intense peaks of the G-0.5.

The G-0.5 PAMAM was further characterized using infra-red (IR) spectroscopy and mass spectrometry. In figure 2.12, The weak peak at 2954 and 2830 cm^{-1} represent the C-H alkane stretch, the strong peak at 1738 cm^{-1} represents the C=O stretch of the ester. The medium signal at 1438 cm^{-1} represents the C-H alkane bend characteristic to a methyl group and the signal at 1199 cm^{-1} represents the C-O stretch. The functional groups that constitute to the make-up of the G-0.5 PAMAM have been successfully identified therefore confirming the successful synthesis of the G-0.5 PAMAM. The parent ion peak $[\text{M}+\text{H}]$ was successfully identified at 405.2216 g/mol which also confirms the successful synthesis of G-0.5 PAMAM as illustrated in figure 2.13. The characterization of every half generation is listed in section 2.5

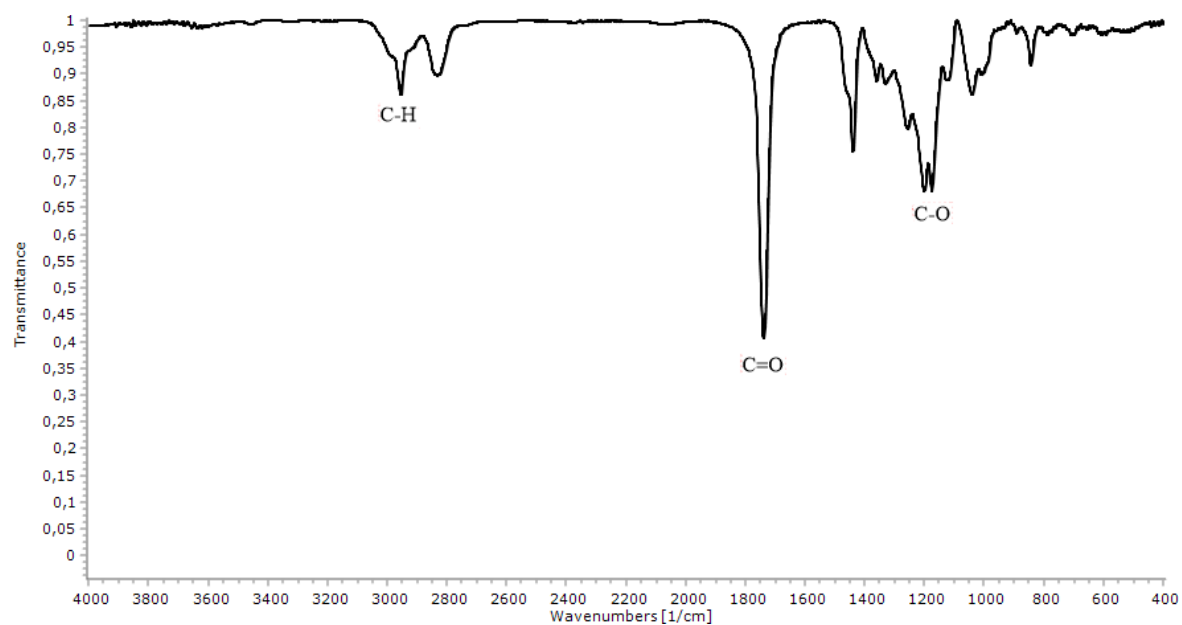


Figure 2.12: FT-IR spectrum of G-0.5 PAMAM dendrimer.

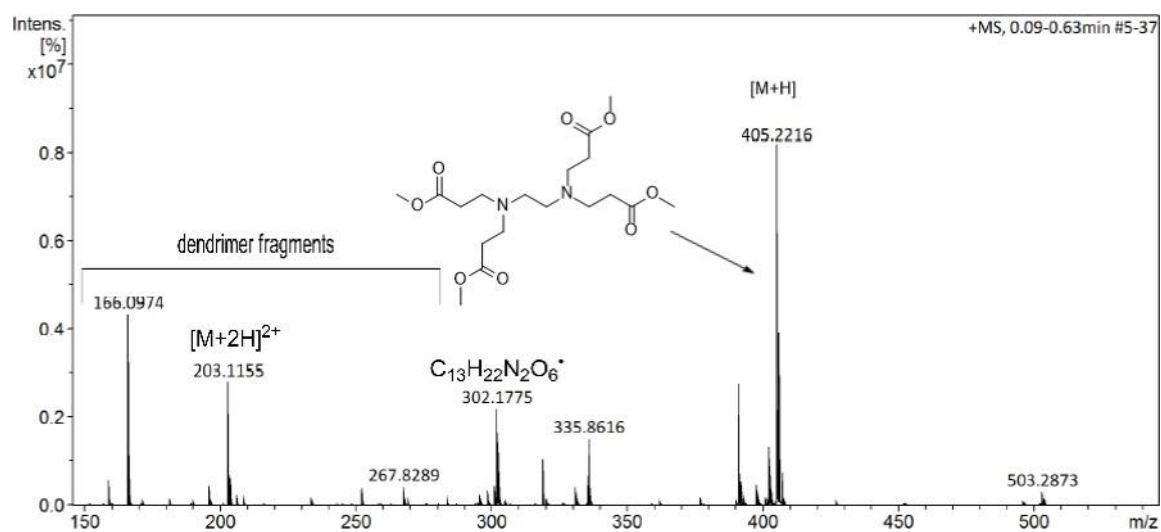


Figure 2.13: Mass spectrum of G-0.5 PAMAM dendrimer.

G0 PAMAM is used as an illustration for the characterization for a full generation.

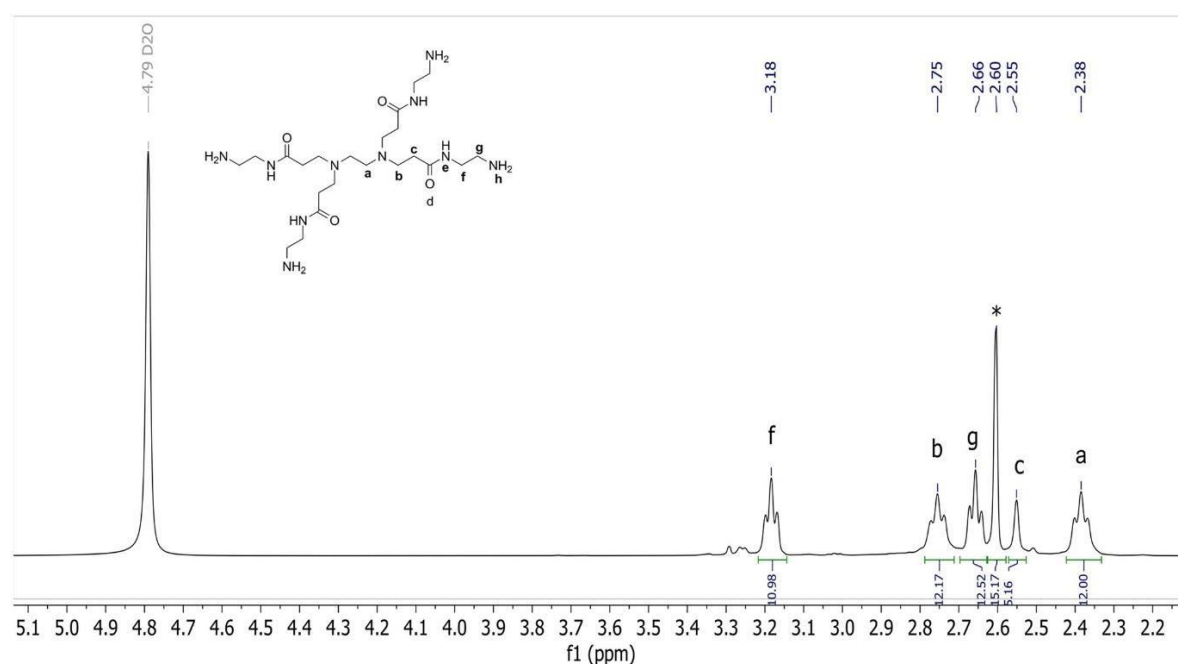


Figure 2.14: ^1H NMR of G 0 PAMAM in D_2O at 300K.

The disappearance of the singlet corresponding to the methyl of the ester group found in G-0.5 PAMAM as well as the appearance of two new peaks corresponding to CH_2 groups in figure 2.14 indicates that the desired product (G0 PAMAM) has been successfully synthesized. The triplet at 3.18 ppm integrates for 8 protons and is assigned as *f*. The triplet at 2.75 ppm integrates for 8 protons is assigned as *b*. The triplet appearing at 2.66 ppm integrates for 8 protons and is assigned as *g*. the singlet at 2.55 ppm integrates for 4 protons and is assigned to the protons that make up the core of the dendrimer i.e. protons *a*. The triplet appearing at 2.38 ppm integrates for 8 protons and is assigned as *c*. The singlet at 2.60 ppm is a result of residual EDA. The purpose of the addition toluene during the work up is to form a low boiling azeotrope with EDA which will then allow for easier removal of EDA under reduced pressure.²¹ Since a large excess of EDA is used, the free EDA is capable of participating in hydrogen bonding with the carbonyl oxygens and nitrogen atoms of the dendrimer. Methanol is then added to competitively hydrogen bond to the above-mentioned functional groups and displace the EDA molecules.²⁹ However, methanol has a lower boiling point than EDA therefore all the methanol is removed before the EDA. As seen from the spectrum above (figure 2.14), the EDA is difficult to remove even after addition of a significant amount of methanol.^{20,21}

The peaks that correspond to protons *e* and *h* are not visible on the spectrum due to the protons exchanging with the deuterium of D_2O . The assignment of the protons was confirmed using 2D COSY NMR.

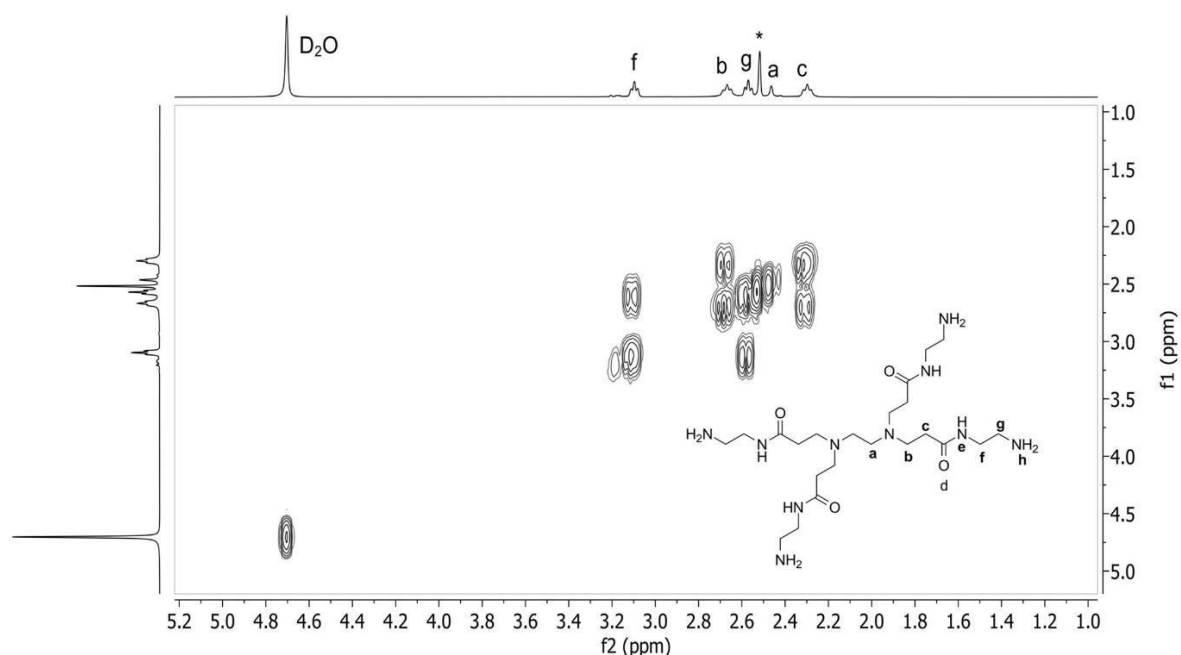


Figure 2.15: COSY spectrum of G0 PAMAM dendrimer in D₂O at 300K.

As seen in figure 2.15, the singlet at 2.25 ppm does not couple to any other protons and is therefore assigned to protons a. The most deshielded protons at 3.18 ppm is assigned to *f* and are seen to couple to neighbouring protons *g*. The singlet at 2.60 ppm does not couple to any protons and therefore it confirmed that this signal arises due to residual EDA. A sample of plain EDA was also run to confirm the chemical shift of EDA in D₂O.

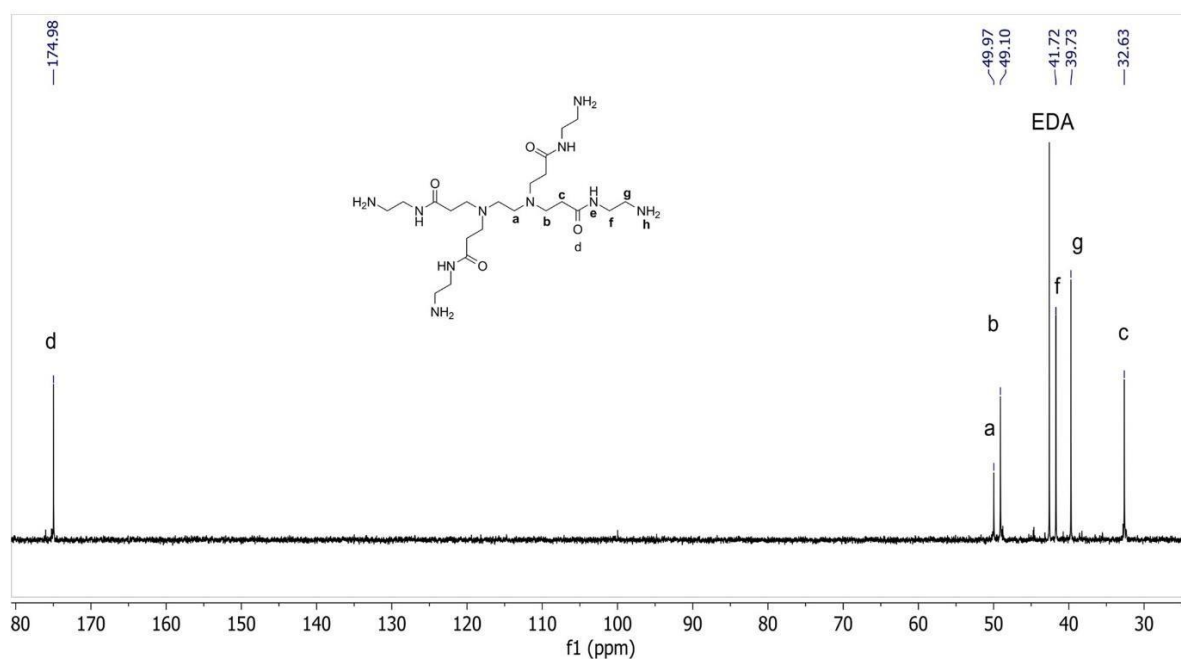


Figure 2.16: ¹³C NMR of G0 PAMAM in D₂O at 300K.

The peak at 174.9 ppm is assigned to carbon d as it is the most deshielded carbon and appears in the carbonyl region as expected (figure 2.16). This

peak does not appear in the C-H correlation (HSQC) as it has no protons associated with it. The remaining carbons were assigned using C-H correlation.

The full generation (G0) PAMAM was then characterized using FT-IR and mass spectrometry. What is immediately apparent in the FT-IR spectrum (figure 2.17) is the appearance of the band at 3280 cm^{-1} which is due to the N-H stretch of a secondary amine. The strong band at 1649 cm^{-1} represents the C=O stretch. The disappearance of the C-O functional group at 1199 cm^{-1} further indicates successful conversion from the ester functionality of the half generation to the amide functionality of a full generation. This characterization technique was essential for the confirmation of the NH and NH_2 functional groups as they are not visible on the ^1H NMR spectrum due to deuterium exchange. From the mass spectrum (figure 2.18), the parent ion peak $[\text{M}+\text{H}]^+$ was successfully identified at 517.3897 g/mol as well as the “three-armed” fragment at 403.3117 g/mol . It is worth noting that only G-0.5 and G0 were additionally characterized via ESI-MS as it is suitable for molecules of molecular weights less than 1000 g/mol . The characterization of the full generations (G1, G2, G3 and G4) are listed in section 2.5.

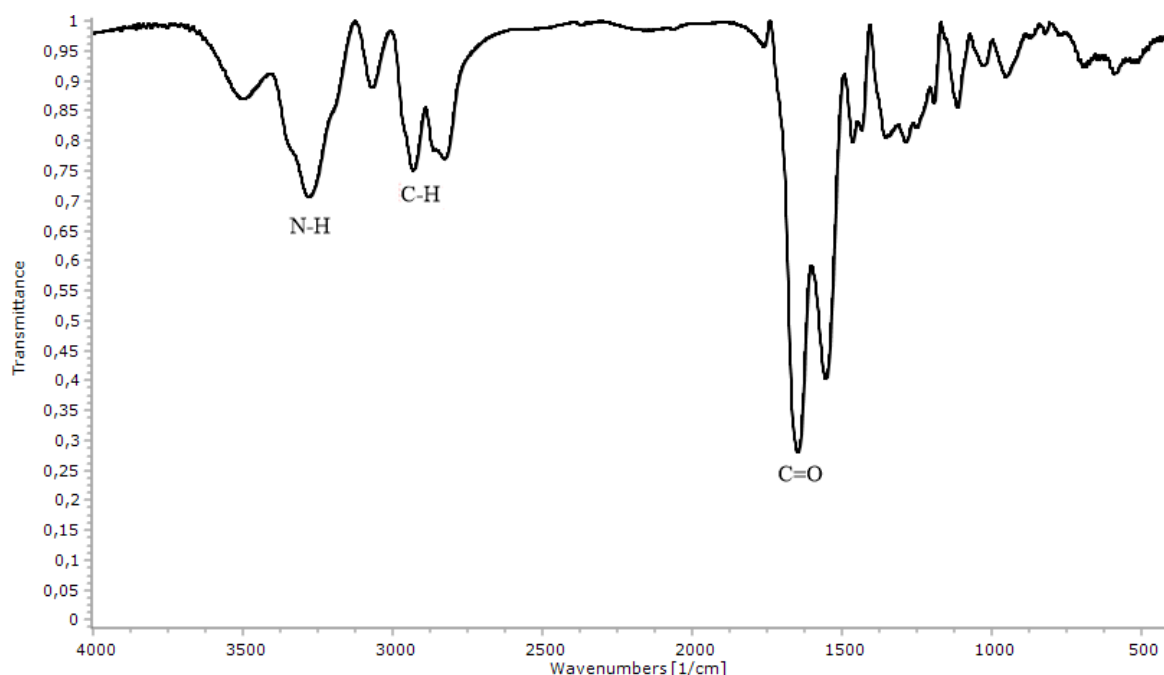


Figure 2.17: FT-IR spectrum of G0 PAMAM dendrimer.

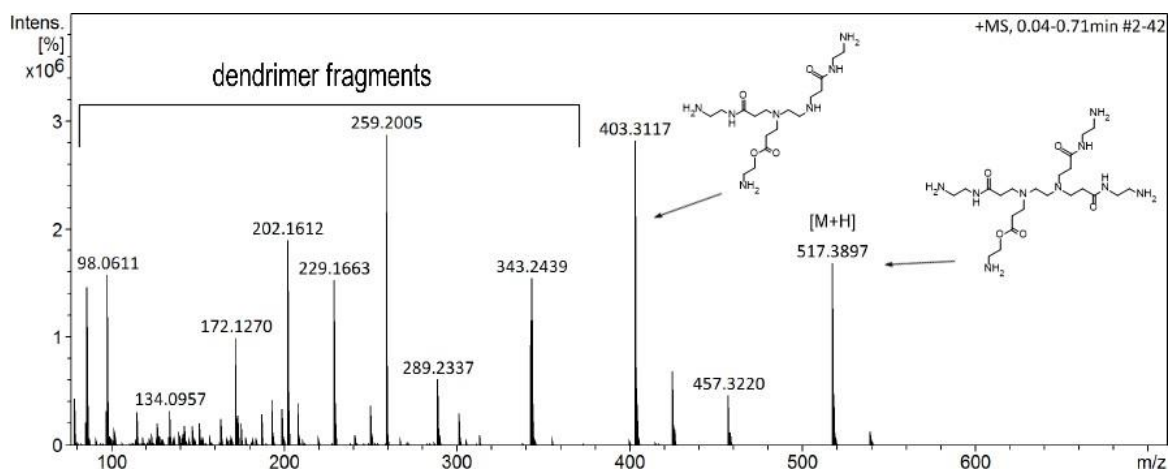


Figure 2.18: Mass spectrum of G0 PAMAM.

2.4.1 Dendrimer purification

Purification of the dendrimer is important given the application of the dendrimers since impurities can have an influence on cytotoxic activity. Purification is also important as starting material needs to be removed in order to produce a product that is as monodisperse as possible. Remaining starting material can still react with any newly added monomer added to produce lower generation PAMAM dendrimers along with the desired generation PAMAM dendrimer. Remaining EDA may react with the desired dendrimer generation to produce defective dendrimers i.e. deletion and/or cyclization. As previously mentioned, purification based on polarity (via column chromatography) proved to be difficult due to the similarities in structure. Purification based on size and molecular weight was opted for as the difference in molecular weight (and size) become significant when moving from one generation to the next in larger generations. Cellulose membranes with molecular weight cut-off (MWCO) sizes 1.5 kDa, 6 kDa and 12 kDa were used to purify the PAMAM dendrimers of the appropriate molecular weight (G1.5 to G4). After the work-up, the crude dendrimer product was dissolved in an appropriate solvent and transferred to a dialysis membrane of the appropriate MWCO. This membrane containing the dendrimer was sealed and then immersed in a beaker containing a large volume of solvent (to create a concentration gradient) along with a stirrer bar as shown in figure 2.19. The lower generation dendrimers as well as low molecular weight impurities were allowed to diffuse through the pores of the membrane and into the solvent in the beaker. The surrounding solvent (which now contained impurities) was changed as needed. The desired product left inside the membrane was then concentrated and analysed via proton NMR to see if any impurities had still remained. The method eliminated most of the impurities, however it still has its limitations such as type of solvent used during dialysis which

may have an influence on its effectivity. This purification method was sufficiently effective for the purification of PAMAM dendrimers and therefore used for every half and full generation from G1.5 to G4 PAMAM as well as any surface functionalized dendrimer.

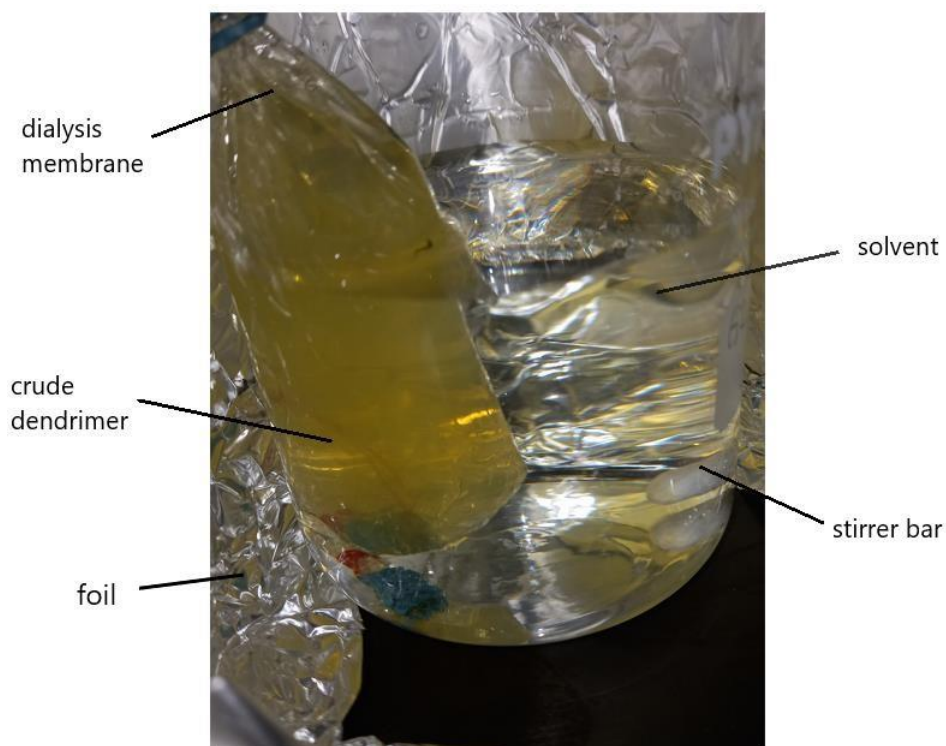


Figure 2.19: General dendrimer dialysis setup.

2.4.2 Synthesis of Folic acid functionalized G4 PAMAM (G4-FA)

Since folate is high in demand in rapidly proliferating cells, the folate receptor (FAR) has been shown to be overexpressed in certain cancer cells lines such as lung, colorectal and ovarian cancer.^{30–32} Folic acid is converted to folate in vivo and maintains its high binding affinity for the FAR while conjugated to a drug and/or drug delivery system which makes it a good molecular target for selectivity.^{33,34}

Folic acid functionalized G4 PAMAM (G4-FA) was synthesized using a modified method described by Xu *et al.*³⁵ Folic acid was allowed to react with excess 1-ethyl-3-carbodiimide hydrochloride (EDC-HCl) in a mixture of anhydrous DMF and DMSO under an inert atmosphere for three hours to form the activated o-acylisourea ester. The mixture was then added dropwise to an aqueous solution of G4 PAMAM and then left to react for 48 hours in the dark. The mixture was then dialyzed using a cellulose membrane against distilled water. The resultant solution was then concentrated to afford a viscous orange oil.

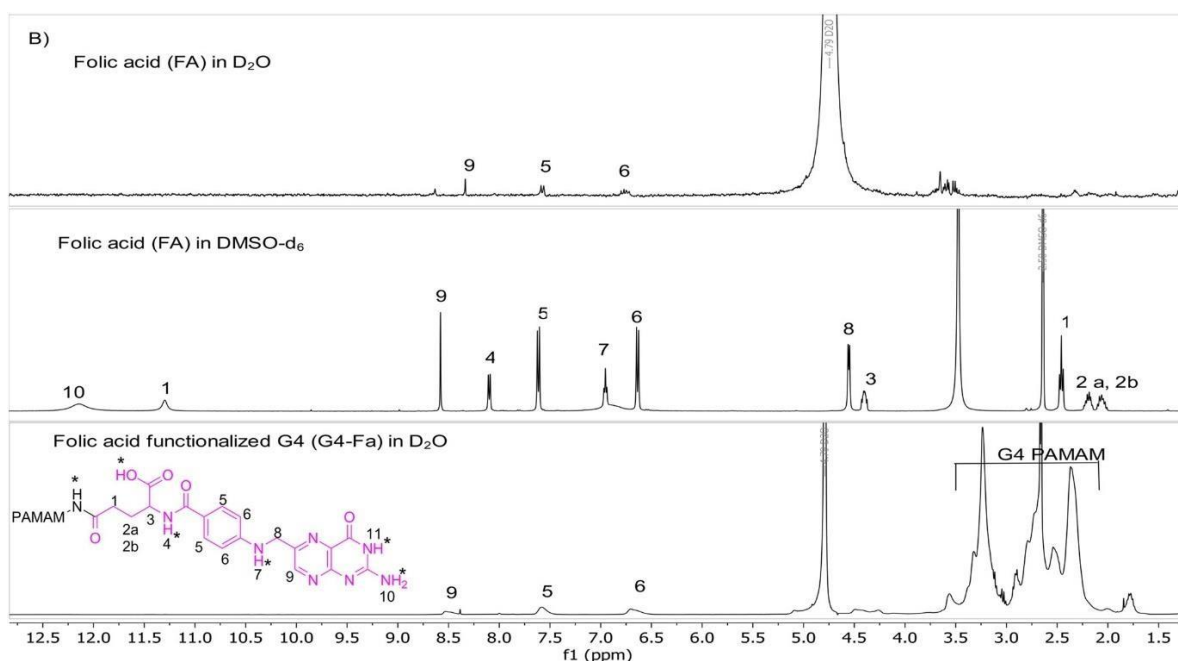
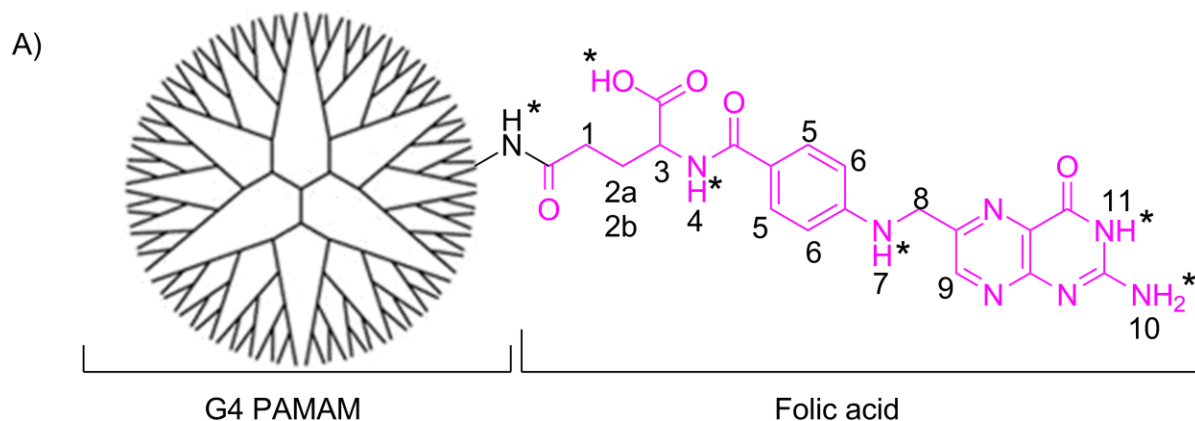


Figure 2.20: A) Illustration of folic acid covalently attached to the surface of a dendrimer where * refers to exchangeable protons. B) Stacked ¹H NMR spectra of FA in D₂O, DMSO-d₆ and G4-FA in D₂O respectively.

A ¹H NMR experiment was conducted on FA in DMSO-d₆ to assign the molecule as it is sparingly soluble in D₂O and exchangeable protons will not appear in the spectrum was seen in figure 2.20B. The FA aromatic protons assigned 9, 5 and 6 are identified in figure 2.20B and have visibly broadened which indicates successful functionalization. G4-FA is soluble in D₂O which further confirms successful functionalization as free FA is not. Integrals were employed to determine the average of folic acid molecules attached to the surface of the dendrimer. The entire dendrimer was used as reference. From this, an average of 4 folic acid molecules are attached to one dendrimer molecule. This could be due to steric hindrance caused by the proximity of the peripheral NH₂ groups to each other. The dendrimer peaks are identified between 3.25 and 2.38 ppm, the sharp peak at 2.66 is a result of residual EDC-HCl which was not sufficiently removed during dialysis.

The dendrimer was further characterized via IR spectroscopy.

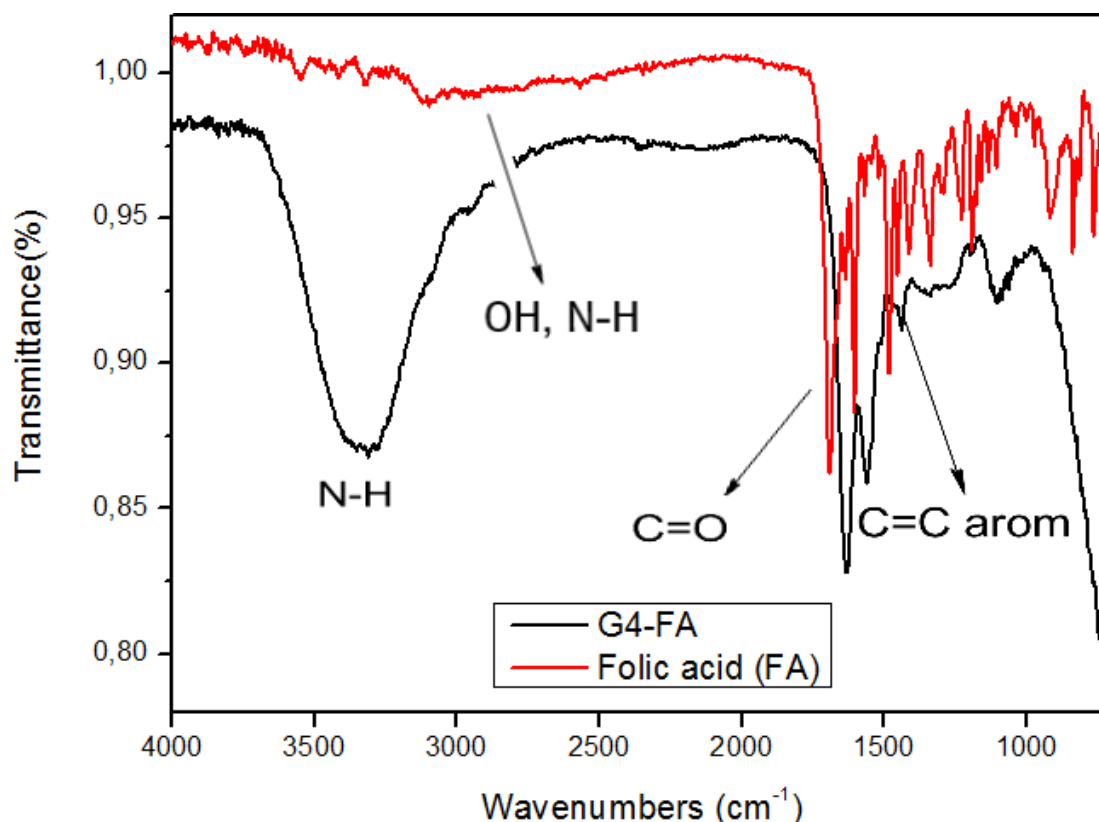


Figure 2.21: Stacked IR spectra of free folic acid and folic acid functionalized G4-PAMAM (G4-FA).

In the folic acid IR spectrum, the weak broad peak between 3507 and 2852 cm^{-1} is that of the O-H and N-H stretch in the free folic acid (FA) molecule. Upon conjugation, this peak is identified between 3649 and 2905 cm^{-1} as seen in figure 2.21. The shifting of this peak indicates conjugation of FA to the dendrimer surface through an amide bond.^{30,36} The C=O stretch in free FA appears at 1689 cm^{-1} and the aromatic C=C stretch in free FA is identified at 1494 cm^{-1} . In the conjugate these peaks are identified at 1628 and 1441 cm^{-1} respectively. The appearance of the aromatic C=C stretch as well as the shifting in the above-mentioned vibrational modes further confirms successful conjugation of FA to the dendrimer.³⁰

2.4.3 Synthesis of acetamide terminated PAMAM ($\text{G}_n\text{-Ac}$)

It has been shown that amine terminated PAMAM dendrimers become protonated at physiological pH in vivo to produce a positively charged species (R-NH_3^+) which is more toxic than neutral and anionic species as they result in cell lysis. To address this matter, the NH_2

groups were converted to non-ionisable acetamide groups (NHCOCH_3) which were proposed to result in a better pharmacological profile of the dendrimer.

Acetamide functionalized G4 PAMAM and G5 PAMAM were synthesized according to a method described by Li *et al.*³⁷ The amine terminated G4 PAMAM (denoted as G4-NH₂) was dissolved in anhydrous methanol. Acetic anhydride (Ac_2O) was added to the solution followed by the addition of triethylamine (TEA) to neutralize the acetic acid formed as a by-product. The reaction was conducted under inert atmosphere and left to stir for 24 hours to ensure complete conversion. The mixture was dialyzed in distilled water to afford the acetamide terminated G4 PAMAM dendrimer (denoted as G4-Ac). This method was conducted in the same manner for the synthesis of G5-Ac with the only difference being an increase in reaction time (24 hours to 48 hours) as the number of surface functional groups increase from 64 for G4 to 128 for G5.

G4-Ac is used as an illustration for the characterization of an acetamide terminated PAMAM dendrimer.

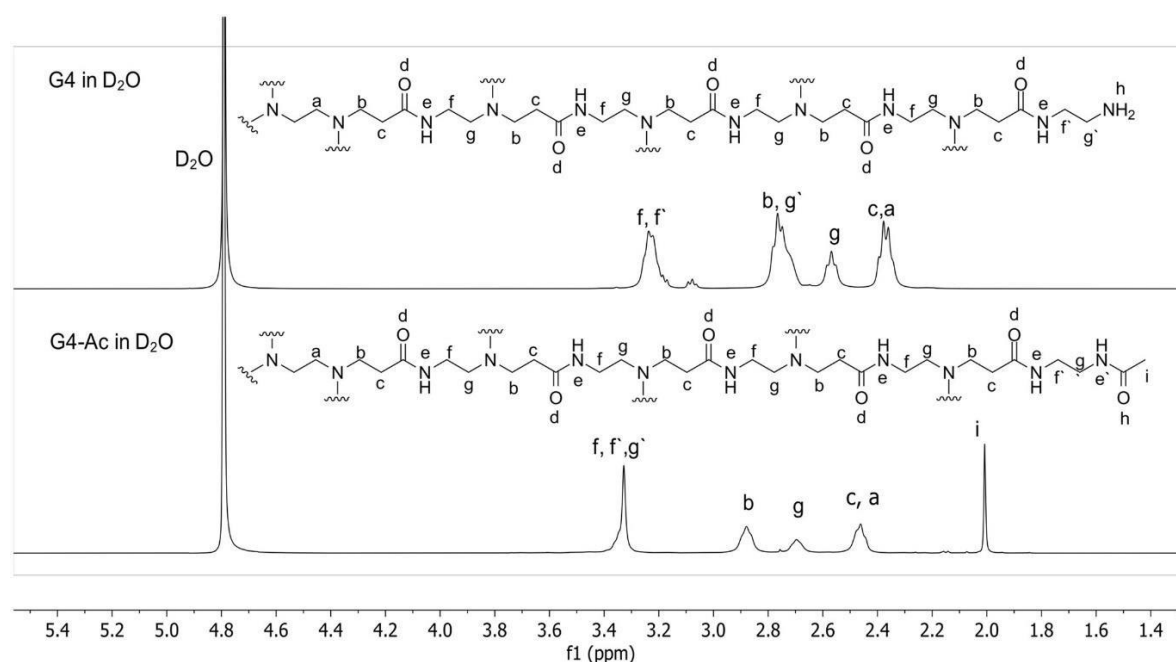


Figure 2.22: Stacked ¹H NMR spectra of amine terminated G4 PAMAM (G4-NH₂) and acetamide terminated G4 PAMAM (G4-Ac) in D₂O at 300K.

In the spectrum displaying G4-Ac (figure 2.22), the appearance of the singlet at 1.6 ppm is that of the methyl group and integrates for 192 protons which indicates the successful conversion of all amine functional groups (NH₂) to the acetamide moiety (NHCOCH₃). The multiplet between 2.44- 2.39 ppm integrates for 252 protons and is assigned to protons a and c. The broad peak at 2.67- 2.63 ppm integrates for 120 protons and is assigned to protons g. The next broad peak between 2.86-2.81 ppm integrates for 248 protons and is assigned to

protons *b*. The multiplet between 3.30-3.28 ppm integrates for 376 protons and corresponds to the most deshielded protons which are *f*, *f'* and *g'*. In the native G4 (G4- NH₂), the protons assigned *g'* appear at a similar chemical shift to protons *b*. However, with G4-Ac, protons *g'* now appear up-field due to the conjugation to the electron withdrawing carbonyl group (COCH₃) and are now in a similar chemical environment with protons *f'*. This change in chemical shift confirms successful conversion. All protons were assigned with the aid of COSY NMR on the basis that protons will interact with neighbouring protons up to three bonds away. It is also worth noting that as the dendrimer generation increases, the peaks observed in the NMR broaden due to the large number of repeating units.

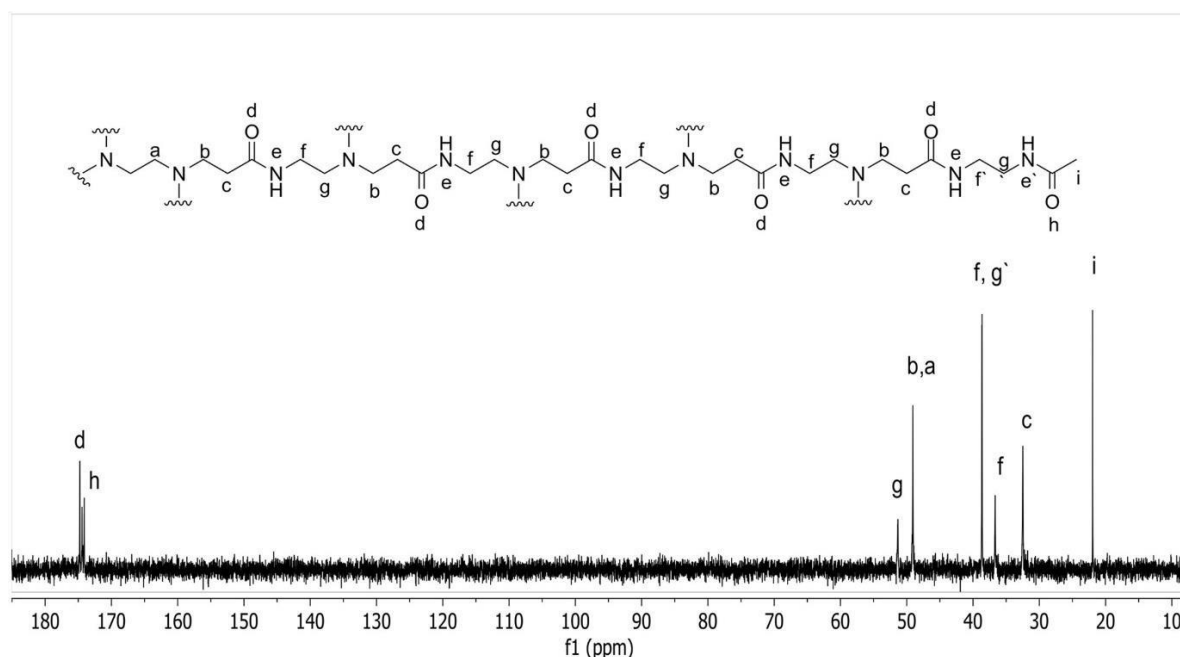


Figure 2.23: ¹³C NMR of G4-Ac PAMAM in D₂O at 300K.

A notable difference in figure 2.23 is the shielded carbon at 21.96 ppm which appears as a result of the acetamide functionalisation and is identified as the carbon of the CH₃ group of the terminal acetamide. From the HSQC the remaining carbons were successfully assigned from their corresponding protons.

The functionalised dendrimer was further characterized with MALDI-TOF mass spectrometry. The dendrimer was run in linear mode given the large molecular weights in a 2,5-dihydroxybenzoic acid (DHB) matrix to give the best resolution.

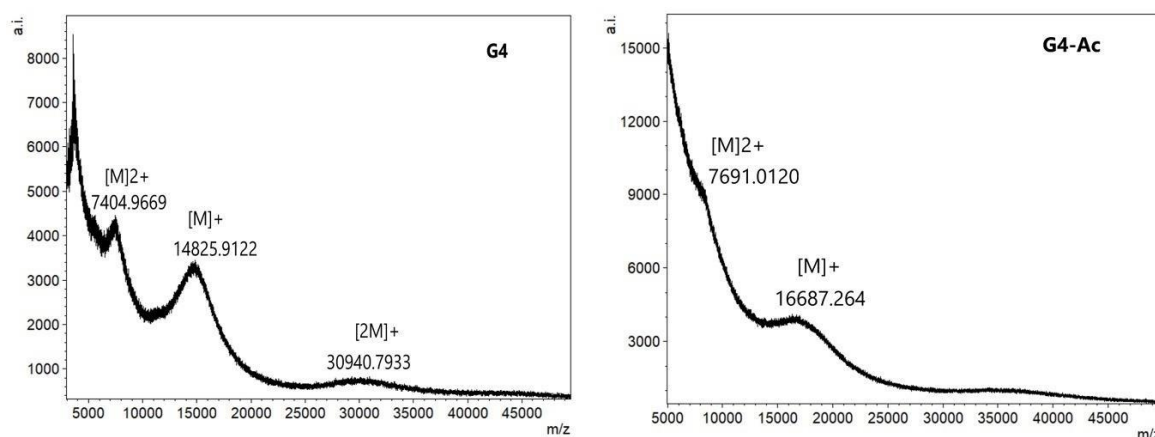


Figure 2.24: MALDI-TOF mass spectra of G4 (left) and G4-Ac (right) in DHB and sodium triflate matrix.

An increase in mass from 14 825 g/mol in the amine terminated G4 to 16 687 g/mol (G4-Ac) was observed as shown in figure 2.24. This increase in molecular weight further confirms successful acetamide functionalization. Furthermore, the $[M]^{2+}$ fragment was identified at 7691 g/mol for G4-Ac, whereas in G4 the $[M]^{2+}$ fragment appears at 7404 g/mol. It becomes challenging to identify possible structural errors in the spectrum due to line broadening observed which is a result of the large molecular weights.

Conclusion

This chapter showcases the successful synthesis of the $\text{Pt}(1,10\text{-phenanthroline})\text{Cl}_2$ precursor, the *N,N*-dibutyl-*N'*-benzoylthiourea ligand, the $[\text{Pt}(\text{phen})(\text{acylthiourea})]^+$ complexes, G4 and G5 PAMAM dendrimers as well as their folate functionalized and acylated counterparts. The $\text{Pt}(1,10\text{-phenanthroline})\text{Cl}_2$ precursor was synthesized and isolated with a yield of 88.4%. The precursor was characterized via ^1H NMR, FTIR and mass spectrometry. Only four peaks appear in the ^1H NMR due to symmetry in the molecule. The *N,N*-dibutyl-*N'*-benzoylthiourea ligand was successfully synthesized according to the method described by Douglas and Dains. The ligand was isolated with a yield of 78% and was characterized via ^1H NMR, ^{13}C NMR, FTIR and mass spectrometry. All protons and carbons were successfully assigned using 1D and 2D NMR. The delocalization of the lone pair on the nitrogen of the dibutyl arm and the sulphur results in the formation of a partial double bond which then results in chemical inequivalence of the protons of the dibutyl arm as seen in the ^1H NMR spectrum (figure 2.3). The $[\text{Pt}(\text{phen})(\text{acylthiourea})]^+$ complexes were successfully synthesized from the respective precursor and ligand. The complexes were characterized via, ^1H NMR, ^{13}C NMR, FTIR and mass spectrometry where all protons and carbons were assigned. An interesting feature in

the ^1H NMR was the loss in symmetry upon complexation (in the aromatic region) observed in the ^1H NMR spectrum. All protons and carbons were successfully assigned using 1D and 2D NMR. PAMAM dendrimers were successfully synthesized according to a method described by Tomalia *et al.* Dendrimer synthesis began with a 1,4-addition of the core (EDA) and MA to yield the first half generation dendrimer (G-0.5). The crude product contained a mixture of the G-0.5 and a small amount of the tri-ester. The G-0.5 was characterized using ^1H NMR, ^{13}C NMR, FTIR and mass spectrometry. Separation of the desired product from the mixture via column chromatography was challenging due to a similarity in polarity, therefore, the full generation (G0) was synthesized without purification of the G-0.5. The full generation (G0) dendrimer was successfully synthesized via amidation of the G-0.5. The G0 was characterized using ^1H NMR, ^{13}C NMR, FTIR and mass spectrometry where all protons and carbons were successfully assigned. These two reactions (1,4-addition and amidation) were repeated until the desired dendrimer generation was obtained. A purification based on size and molecular weight was opted for (in the form of dialysis membranes) since separation based on polarity proved to be challenging. Cellulose membranes of molecular weight cut-off (MWCO) sizes 1.5 kDa, 6 kDa and 12 kDa were used to purify the PAMAM dendrimers of the appropriate molecular weight (G1.5 to G4). This method proved to be effective but is not without its limitations as seen in a few impurities in the dendrimer ^1H NMR spectra.

The surface of the G4 PAMAM dendrimer was then modified with folic acid (FA) with the use of a coupling agent (EDC-HCl). The folate-conjugated G4 (G4-FA) was then characterized using ^1H NMR and FTIR. The solubility of G4-FA in D_2O was an indication of successful conjugation since FA is sparingly soluble in D_2O . Additionally, the broadening of the aromatic FA peaks in the ^1H NMR once conjugated to the G4 provided sufficient evidence of FA conjugation. The surface of the G4 and G5 PAMAM dendrimers were acylated in efforts to minimize the inherent cytotoxicity of the amine terminated PAMAM dendrimers. The respective dendrimers were reacted with excess acetic anhydride (Ac_2O) in anhydrous methanol and triethylamine to afford the acetylated dendrimer ($\text{G}_n\text{-Ac}$). The acetylated dendrimers were purified via dialysis and characterized using ^1H NMR, ^{13}C NMR, FTIR and mass spectrometry. A notable difference between in the ^1H NMR of the amine-terminated dendrimer (G4) and the acetylated dendrimer (G4-Ac) is the appearance of the sharp singlet at 1.6 ppm which indicates the conjugation of the CH_3 moiety. The downfield shifting of near-surface protons labelled g' towards the more deshielded protons f and f' provided additional evidence of successful acetylation.

2.5 Experimental section:

2.5.1 Instrumentation and reagents:

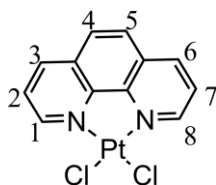
NMR experiments were recorded on a Bruker Avance 300MHz and Bruker Avance 400MHz spectrometer. Spectra were recorded in deuterated solvents (chloroform, water, methanol and dimethyl sulfoxide). Chemical shifts are reported relative to each respective solvent and is reported in parts per million (ppm) and coupling constants are reported in Hertz (Hz). Splitting patterns are described as “s”, “d”, “dd”, “t”, “q”, “qn” and “m”; these symbols indicate “singlet”, “doublet”, “doublet of doublet”, “triplet”, “quartet”, “quintet” and multiplet respectively.

ESI mass spectrometry was recorded on a Bruker compact ultimate 300 HR-MS spectrometer. MALDI-TOF mass spectrometry was recorded on a Bruker autoflex TOF/TOF spectrometer in a 2,5-dihydroxybenzoic acid (DHB) and sodium triflate matrix. FT-IR experiments were recorded on a Bruker TENSOR 27 spectrometer using both KBr and ATR.

Reagent grade chemicals obtained from Sigma Aldrich and used without further purification. With regards to the synthesis of *N,N*-di(butyl)-*N'*-benzoylthiourea and acetamide functionalized G4 and G5 PAMAM, acetone and methanol were dried using the appropriate drying agents as described in literature and distilled prior to use.

Synthesis of Pt(phen)Cl₂ precursor:

Potassium tetrachloroplatinate (204.0 mg, 0.49 mmol) was added to a round bottom flask with 30 ml deionised water and 4 drops of 32% HCl. The mixture was stirred for 10 minutes. Phenanthroline (89 mg, 0.49 mmol) was then added to the reaction vessel. The mixture was refluxed for 3 hours. The product was washed with water (x3), followed by acetonitrile (x2) and lastly diethyl ether (x1). The product was then dried under vacuum. Yield: 88.4%

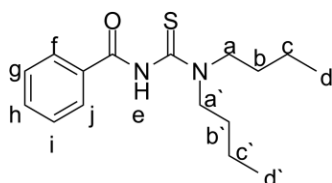


[PtCl₂(1,10-phenanthroline)] PtCl₂C₁₂H₈N₂ : Yield 88.4%, ¹H NMR (300 MHz, DMSO-d₆, 300K) δ(ppm)= 9.71 (dd, 2H, ¹J: 6Hz, ²J: 3Hz, **1, 8**), 9.05 (dd, 2H, ¹J: 6Hz, ²J: 3Hz **3, 6**), 8.30 (s, 2H, **4, 5**), 8.20 (dd, 2H, ¹J: 6Hz, ²J: 3Hz **2, 7**). FT-IR ν(cm⁻¹): 3058.24 (=C-H

aromatic), 1627 (C=N aromatic), 1578.20 (C=C aromatic). Mass spectrometry (ESI-MS) $[M]^+$: 446.9534 g/mol.

Synthesis of *N,N*-di(butyl)-*N'*-benzoylthiourea:

Potassium thiocyanate was dried in an oven for 1 hour at 150 followed by drying under vacuum for 1 hour. Benzoyl chloride (2.3 mL, 19.80 mmol) was dissolved in 20 mL of anhydrous acetone and was added to potassium thiocyanate (2.00 g, 20 mmol) dissolved in 25 mL anhydrous acetone and heated to reflux for 1 hour. The reaction mixture was then cooled to room temperature. Dibutylamine (3.8 mL, 22.55 mmol) was dissolved in 10 mL anhydrous acetone and dropped in over 30 minutes. The reaction was then heated to reflux for 2 hours. The reaction vessel was then left to cool to allow for the solid to precipitate which was then filtered. The remaining solid was washed with cold isopropanol and water in a 2:3 ratio. The product (colourless crystals) was recrystallised in isopropanol and left to dry under vacuum. Yield: 78%

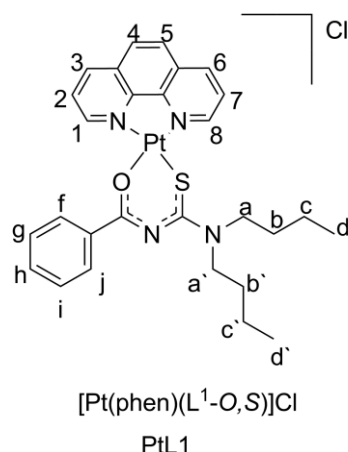


***N,N*-di(butyl)-*N'*-benzoylthiourea** $C_{16}H_{24}N_2OS$: Yield 78%, mp: 93-96 °C, ^1H NMR (300 MHz, $CDCl_3$, 300K) δ (ppm)= 8.39 (s, 1H, **e**), 7.82 (d, 2H, 9Hz, **f, j**), 7.57(t, 1H, 6Hz, **h**), 7.43 (t, 2H, 9Hz, **g, i**), 3.97 (t, 2H, 6Hz, **a**), 3.54 (t, 2H, 6Hz, **a'**), 1.79(qn, 2H, ^1J : 6Hz, ^2J : 6Hz, **b**), 1.65 (qn, 2H, ^1J : 6Hz, ^2J : 6Hz, **b'**), 1.45 (st, 2H, ^1J : 9Hz, ^2J : 6Hz, **c**), 1.30 (st, 2H, ^1J : 9Hz, ^2J : 6Hz, **c'**), 0.98 (t, 3H, 9Hz, **d**), 0.90 (t, 3H, 9Hz, **d'**). ^{13}C NMR (400 MHz, $CDCl_3$, 300K) δ (ppm)= 179.85 (C=O), 163.69 (C=S), 133.04 (C^h), 129.07 (C^{g+i}), 127.87 (C^{f+j}), 53.46 (C^a), 53.28 ($C^{a'}$), 30.29 ($C^{b'}$), 28.66 (C^b), 20.21 ($C^{c+c'}$), 14.01 (C^d), 13.86 ($C^{d'}$). FT-IR ν (cm^{-1}): 3173 (N-H), 2956 (C-H alkane stretch), 1689 (C=O), 1532 (C=C aromatic) C=S (1371). Mass spectrometry (ESI-MS) $[M+H]^+$: 293.1683 g/mol.

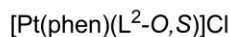
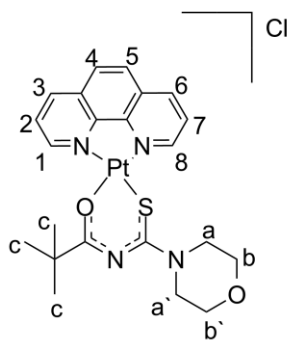
Synthesis of $[Pt(phen)(N\text{-benzoyl-}N,N\text{-dibutylthiourea})]^+$ complex

$[Pt(phen)Cl_2]$ precursor (50.0 mg) was added to a round bottom flask in 20 mL acetonitrile and was refluxed for 10 minutes. *N*-benzoyl-*N,N*-dibutylthiourea ligand (33.3 mg) dissolved in 15 mL acetonitrile and 1 mL distilled water was added dropwise to the reaction vessel and was left to reflux for 15 minutes. Sodium acetate (14.82 mg) was dissolved in 4mL acetonitrile and 4 ml distilled water. The reaction mixture was left to reflux until a colour change (from milky bright yellow to pale yellow) was observed. The product was worked up by removing the

solvent under reduced pressure. The product was then redissolved in acetonitrile and filtered through celite (to remove insoluble salts). The solvent was removed again and the crude product was dissolved in 5 mL dichloromethane (DCM) and transferred to a centrifuge tube containing 40 mL hexane. The product was left to precipitate overnight in a refrigerator. The complex was then wash via centrifuge to yield a bright yellow powder which was dried in a vacuum oven. Yield:75%

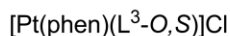
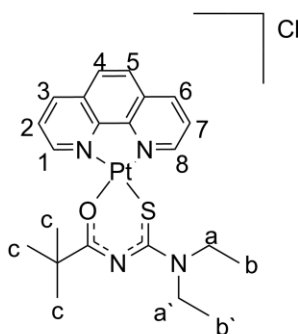


[Pt(phen)(*N*-benzoyl-*N,N*-dibutylthiourea)]Cl PtC₂₈H₃₁N₄OSCl : Yield 75%, mp: 159-162 °C, ¹H NMR (400 MHz, CDCl₃, 300K) δ(ppm)= 9.06 (d, 1H, 3 Hz, **1**), 8.98-8.91 (m, 2H, **3**, **6**), 8.61 (d, 1H, 6 Hz, **8**), 8.31 (dd, 1H, ¹J: 6Hz, ²J: 6Hz, **2**), 8.09-8.02 (m, 5H, **f**, **j**, **4**, **5**, **7**). 7.63 (t, 1H, 9 Hz, **h**), 7.52 (t, 2H, 9Hz, **g**, **i**), 3.77-3.69 (m, 4H, **a**, **a'**), 1.81 (qn, 2H, ¹J: 9 Hz, ²J: 6 Hz, **b**), 1.69 (qn, 2H, ¹J: 9 Hz, ²J: 6 Hz, **b'**), 1.52 (st, 2H, ¹J: 9 Hz, ²J: 6 Hz, **c**), 1.41 (st, 2H, ¹J: 9 Hz, ²J: 6 Hz, **c'**), 1.06 (t, 3H, 6 Hz, **d**), 0.98 (t, 3H, 6 Hz, **d'**). ¹³C NMR (400 MHz, CDCl₃, 300K) δ(ppm)= 169.09 (C=O), 164.80 (C=S), 148.29 (C⁸), 144.95 (C¹), 141.45 (C⁶), 140.90 (C³), 132.87 (C^h), 129.50 (C^{f+i}), 128.99 (C^{g+i}), 128.48, 128.41 (C⁴, C⁵), 126.78 (C⁷), 127.20 (C²), 53.73 (C^{a'}), 52.27 (C^a), 30.14 (C^{b'}), 29.40 (C^b), 20.49, 20.48 (C^c C^{c'}), 14.11 (C^d), 14.03 (C^{d'}). FT-IR ν(cm⁻¹): 2956.97 (C-H alkane stretch), 2052.97 (aromatic C-H bend), 1402.74 (C-O), 1212.91 (C-S). Mass spectrometry (ESI-MS) [M]⁺: 666.1768 g/mol.



PtL2

[Pt(phen)(*N*-pivaloyl-*N,N*-morpholynthiourea)]Cl PtC₂₂H₂₅N₄O₂SCl : Yield 68%, 158-161 °C, ¹H NMR (400 MHz, DMSO-d₆, 300K) δ(ppm)= 9.05 (d, 1H, *J*= 8Hz, **1**), 8.97 (d, 1H, *J*: 8 Hz, **6**), 8.89 (d, 1H, *J*: 8 Hz, **3**), 8.69 (d, 1H, *J*: 8 Hz **2**), 8.32-8.22 (m, 3H, **4**, **5**, **2**), 7.96 (dd, 1H, *J*₁: 8 Hz, *J*₂: 8 Hz, **7**), 4.12 (dd, 4H, *J*: 4Hz, **a**, **a'**), 3.86 (t, 2H, *J*: 4 Hz, **b**), 3.76 (t, 2H, *J*: 4 Hz, **b'**), 1.30 (s, 9H, **c**). ¹³C NMR (400 MHz, DMSO-d₆, 300K) δ(ppm)= 184.43 (C=O), 164.62 (C=S), 149.75 (C⁸) 144.89 (C³), 140.88 (C⁶), 140.37 (C¹), 128.08, 128.02 (C⁴, C⁵), 126.64 (C²), 126.30 (C⁷), 66.72 (C^{b'}), 66.49 (C^b), 49.90 (C^a), 47.30 (C^{a'}), 41.52 (C^{tBu}), 28.59 (C^c). FT-IR ν(cm⁻¹): 2967.91 (C-H alkane stretch), 2054.95 (aromatic C-H bend), 1431.57 (C-O), 1229.78 (C-S). Mass spectrometry (ESI-MS) [M]⁺: 604.1288 g/mol.



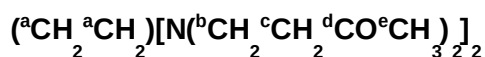
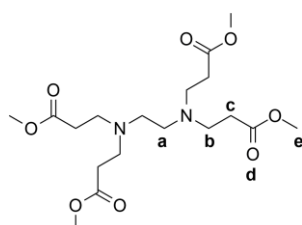
PtL3

[Pt(phen)(*N*-pivaloyl-*N,N*-diethylthiourea)]Cl PtC₂₂H₂₇N₄O₂SCl : Yield 66%, 126-131 °C, ¹H NMR (400 MHz, CDCl₃, 300K) δ(ppm)= 9.24-9.19 (3H, **1**, **3**, **6**), 8.95 (d, 1H, 4Hz, **8**), 8.43-8.38 (m, 2H, **4**, **5**), 8.29 (dd, 1H, *J*₁: 8 Hz, *J*₂: 4 Hz, **2**), 8.22 (dd, 1H, *J*₁: 8 Hz, *J*₂: 4 Hz, **7**). 3.92 (q, 2H, 8 Hz, **a**), 3.81 (q, 2H, 8 Hz, **a'**), 1.49 (t, 3H, 8 Hz, **b**), 1.36 (s, 9H, **c**), 1.30 (t, 3H, 8 Hz, **b'**). ¹³C NMR (400 MHz, CDCl₃, 300K) δ(ppm)= 184.36 (C=O), 164.72 (C=S), 148.84 (C⁸), 144.70 (C¹), 141.62 (C⁶), 141.11 (C³), 128.78 (C⁴), 128.71 (C⁵), 126.64 (C⁷), 126.42 (C²), 47.99 (C^a), 46.43 (C^{a'}), 41.79 (C^{tBu}), 28.96 (C^c), 13.11 (C^{b'}), 12.48 (C^b). FT-IR ν(cm⁻¹): 2972.95 (C-H alkane stretch), 2051.97 (aromatic C-H bend), 1432.05 (C-O), 1251.92 (C-S). Mass spectrometry (ESI-MS) [M]⁺: 590.1462 g/mol.

General synthesis of half generation (ester terminated) PAMAM dendrimers

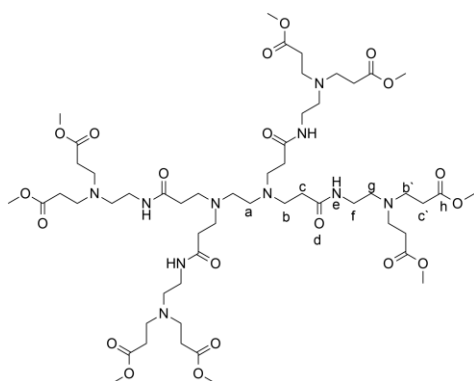
Ethylenediamine (EDA) was added to methanol and cooled down to 0 °C. A 6-molar excess of cold methyl acrylate (MA) dissolved in methanol was added dropwise to the reaction vessel while under an ice bath. The ice bath was kept for 3 hours. Afterwards, the reaction mixture was allowed to warm to room temperature and kept stirring for 48 hours. Excess MA and methanol were removed under reduced pressure to yield a pale-yellow oil. Generations 1.5 and higher were purified by dialysis against methanol using cellulose membranes (1.5 kDA, 6 kDA and 14 kDA depending on the molecular weight of each half generation).

G-0.5 PAMAM dendrimer



G -0.5 PAMAM C₁₈H₃₂N₂O₈ : Yield 98%, ¹H NMR (400MHz, D₂O, 300K), δ (ppm)= 3.66 (s, 12H, **e**), 2.79 (t, 8H, 6 Hz, **b**), 2.56-2.52 (m, 12H, **c**, **a**). ¹³C NMR (300 MHz, D₂O, 300K), δ (ppm)= 175.33 (**d**), 52.23 (**e**), 50.08 (**b**), 48.49 (**a**), 31.04 (**c**). FT-IR ν(cm⁻¹): 2958 (C-H alkane stretch), 1738 (C=O), 1199 (C-O stretch). Mass spectrometry (ESI-MS) [M+H]⁺ : 405.2216 g/mol.

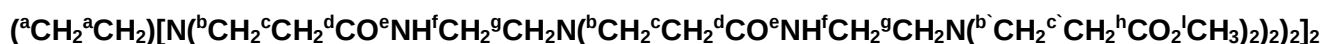
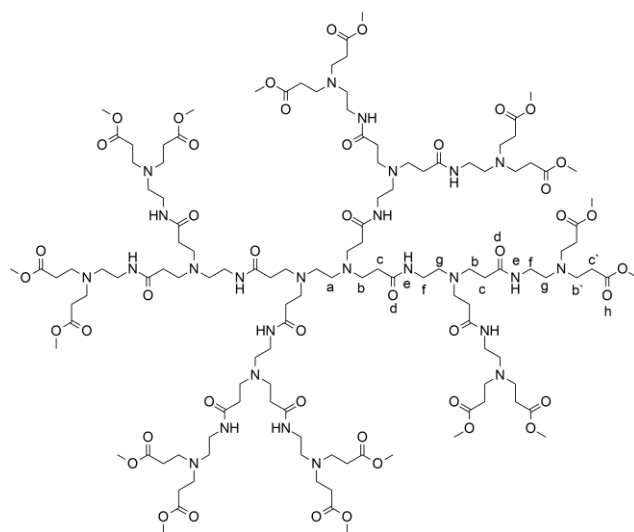
G0.5 PAMAM dendrimer





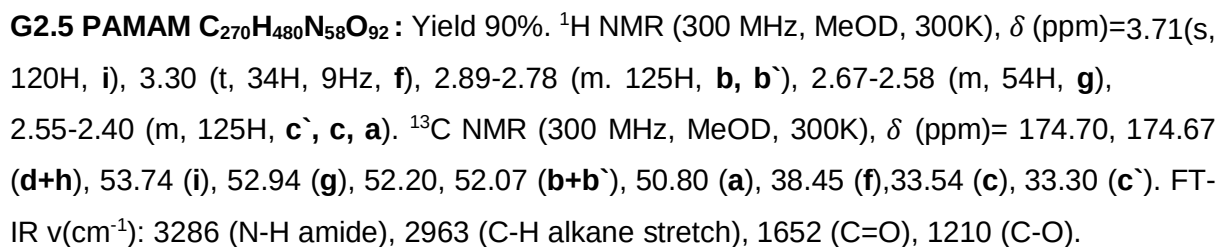
G0.5 PAMAM C₅₄H₉₆O₂₀N₁₀ : Yield 90%, ¹H NMR (400 MHz, D₂O, 300K), δ (ppm)= 3.64 (s, 29H, i), 3.24 (t, 8H, 8Hz, f), 2.83-2.76 (m, 28H, b, b'), 2.57-2.42 (m, 36H, g, c', c, a). ¹³C NMR (400 MHz, D₂O, 300K), δ (ppm)= 175.39 (h), 175.18 (d), 52.18 (i), 51.48 (g), 48.79 (a), 48.31 (b', b), 36.63 (f), 32.07 (c), 31.31 (c'). FTIR ν(cm⁻¹): 3400 (N-H amide), 2955 (C-H alkane stretch), 1729 (C=O), 1201 (C-O).

G1.5 PAMAM dendrimer

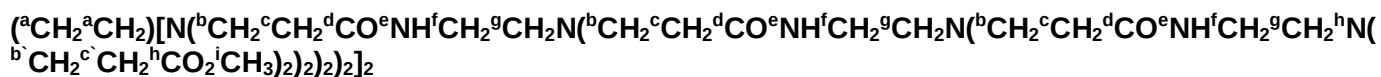
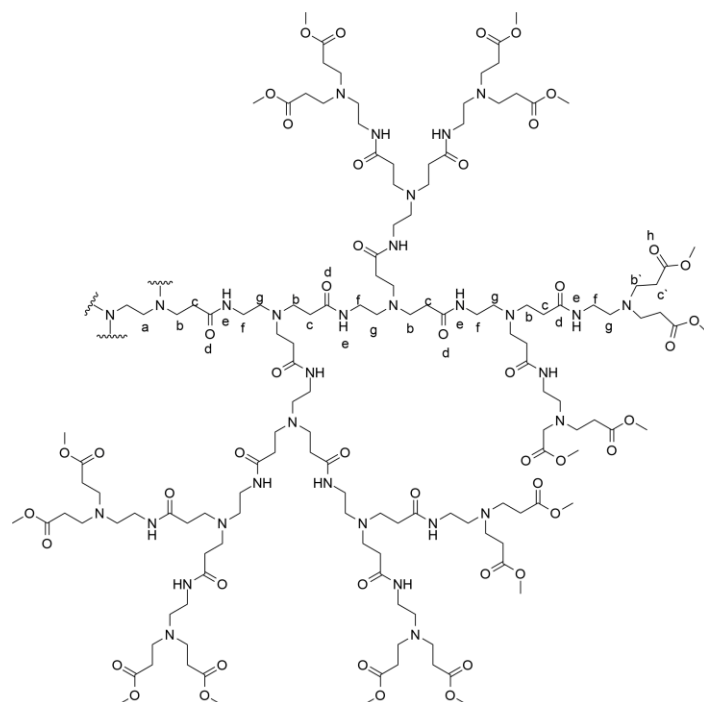


G1.5 PAMAM C₁₂₆H₂₂₄N₂₆O₄₄ : Yield 89%. ¹H NMR (300 MHz, MeOD-d₄, 300K), δ (ppm)= 3.65(s, 49H, i), 3.30-3.21 (m, 29H, f, CH₃OD-d₄), 2.80-2.71(m, 56H, b, b'), 2.47-2.34 (m, 60H, c', c, a). ¹³C NMR (300 MHz, MeOD-d₄, 300K), δ (ppm)= 174.74 (d+h), 53.75 (i), 52.96 (g), 52.22, 52.08 (b+b'), 50.83 (a), 38.47 (f), 33.57 (c), 33.31 (c'). FT-IR ν(cm⁻¹): 3295 (N-H amide), 2954 (C-H alkane stretch), 1738 (C=O), 1199 (C-O).

The image displays a complex chemical structure of a poly(amide-urethane) polymer. The main chain consists of repeating units linked by amide and urethane bonds. The structure is highly branched, featuring numerous side chains that terminate in methoxy groups (-OCH₃). A central repeating unit is highlighted with a dashed box and contains a urethane linkage. This central unit is labeled with various letters (a, b, c, d, e, f, g, h, i) indicating specific atoms or groups within the structure. The overall architecture suggests a multi-functional polymer with potential applications in materials science or biotechnology.



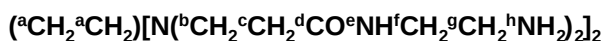
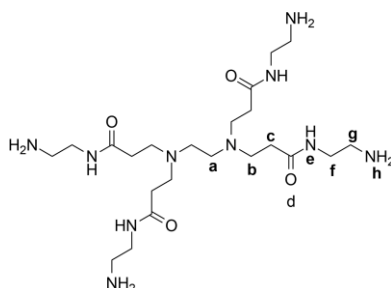
G3.5 PAMAM dendrimer



G3.5 PAMAM C₇₄H₁₃₂N₁₈O₂₄ : Yield 83%. ¹H NMR (400 MHz, D₂O, 300K) δ (ppm)= 3.65 (s, 82H, i), 3.25 (m, 241H, **f+MeOH**), 2.86-2.64 (m, 368H, **b, b', g**), 2.58-2.34 (m, 269H, **c, c', a**). ¹³C NMR (400 MHz, D₂O, 300K), δ (ppm)= 175.35, 174.63 (**d, h**), 54.88 (**i**), 52.19 (**g**), 48.81 (**b+b'**), 38.55 (**f**), 35.05 (**c**), 32.51 (**c'**). FT-IR ν(cm⁻¹): 3283 (N-H amide), 2944 (C-H alkane stretch), 1639 (C=O), 1120 (C-O).

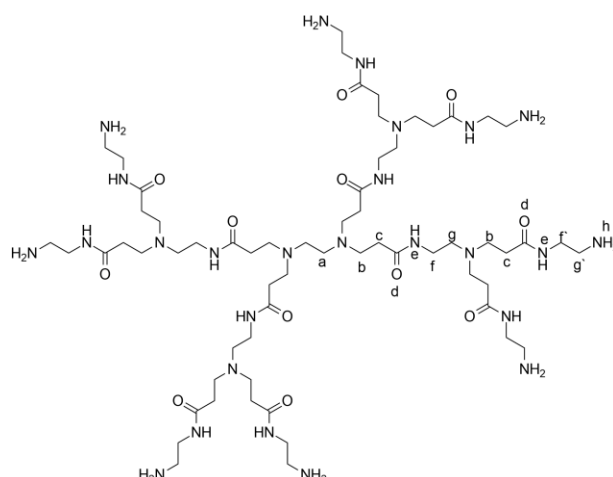
General synthesis of full generation (amine terminated) PAMAM dendrimer Half generation dendrimer dissolved in cold methanol was added dropwise to a solution of methanol at 0 °C. After addition, the ice bath was kept for 3 hours. The solution was allowed to warm to room temperature and kept stirring for 96 hours. Excess EDA was removed by the use of an azeotropic solvent comprised of toluene and methanol (9:1) and was added to the reaction mixture which was placed under high vacuum to afford a pale-yellow oil. G2 and higher were purified by dialysis against distilled water using cellulose membranes (1.5 kDA, 6 kDA and 14 kDA depending on the molecular weight of each full generation).

G0 PAMAM dendrimer



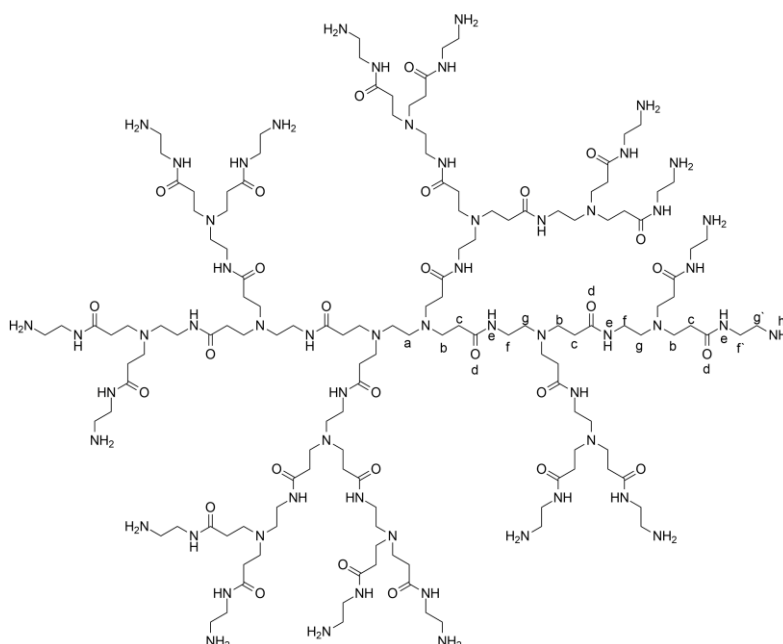
G0 PAMAM C₂₂H₄₈N₁₀O₄ : Yield 95%, ¹H NMR (400 MHz, D₂O, 300K), δ (ppm)= 3.19 (t, 8H, 8Hz, **f**), 2.76 (t, 8H, 8Hz, **b**), 2.66 (t, 8H, 8Hz, **g**), 2.25 (s, 4H, **a**), 2.39 (t, 8H, 8Hz, **c**). ¹³C NMR (400 MHz, D₂O, 300K), δ (ppm)= 174.98 (**d**), 49.97 (**a**), 49.10 (**b**), 41.72 (**f**), 39.73 (**g**), 32.63 (**c**). FT-IR IR ν(cm⁻¹): 3280 (N-H amine stretch), 2932 (C-H alkane stretch), 1649 (C=O). Mass spectrometry (ESI-MS) [M+H]⁺ : 517.3897 g/mol.

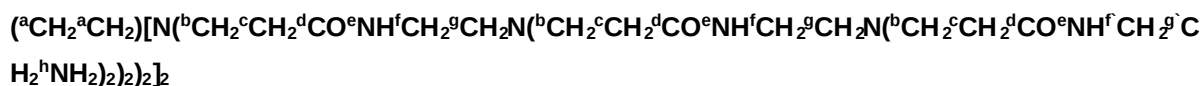
G1 PAMAM dendrimer



G1 PAMAM C₂₆H₁₂₈N₂₆O₁₂ : Yield 92%. ¹H NMR (400 MHz, D₂O, 300K), δ (ppm)= 3.35-33.31 (m, 8H, **f**), 3.32 (t, 16H, 8Hz, **f'**), 2.87-2.82 (m, 24H, **b**), 2.74 (t, 16H, 8Hz, **g'**), 2.68-2.64 (m, 8H, **g**), 2.46 (t, 28H, 8Hz, **c**, **a**). ¹³C NMR (400 MHz, D₂O, 300K), δ (ppm)=175.04 (**d**), 51.25 (**g**), 49.10 (**b**, **a**), 41.74 (**f'**), 39.81 (**g'**), 36.79 (**f**), 32.82 (**c**). FT-IR ν(cm⁻¹): 3488 (N-H amine stretch), 2937 (C-H alkane stretch), 1647 (C=O).

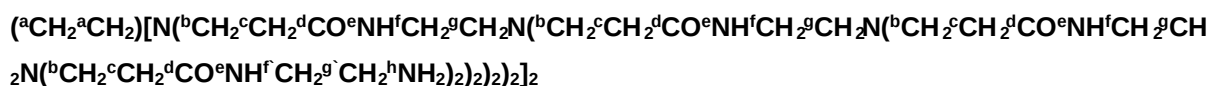
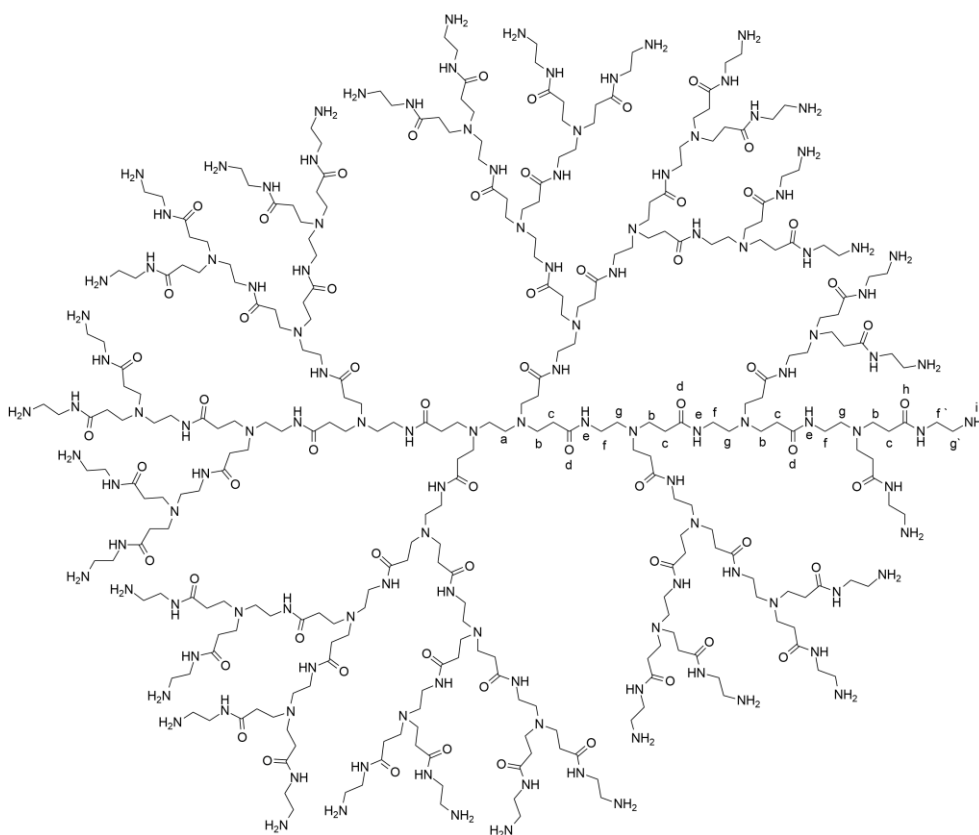
G2 PAMAM dendrimer





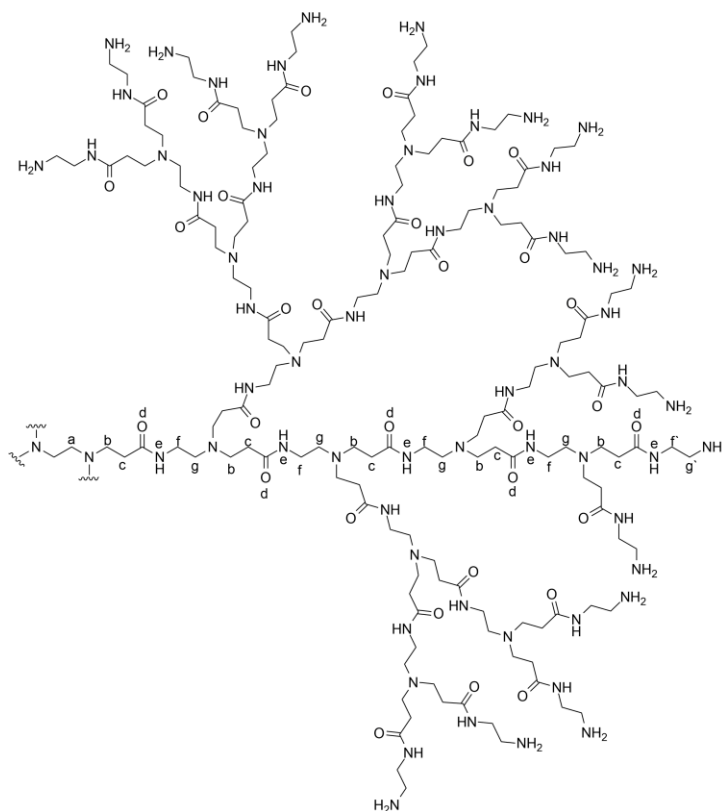
G2 PAMAM C₁₄₂H₂₈₈N₅₈O₂₈: Yield 87%. Yield: 87%. ¹H NMR (400 MHz, MeOD-d₄, 300K), δ (ppm)= 3.32-3.26 (m, 87H, **f**, **f'**, **CH₃OD**), 2.82-2.76 (m, 88H, **b**, **g'**), 2.60-2.56 (m, 29H, **g**), 2.40-2.35 (m, 60H, **c**, **a**). ¹³C NMR (400 MHz, MeOD-d₄, 300K), δ (ppm)= 175.22 (**d**), 53.25 (**g**), 50.99 (**b**, **a**), 41.67(**f'**, **g'**), 38.47 (**f**), 34.59 (**c**). FT-IR v(cm⁻¹): 3290 (N-H amine stretch), 2946 (C-H alkane stretch), 1633 (C=O).

G3 PAMAM dendrimer



G3 PAMAM C₃₀₂H₆₀₈N₁₂₂O₆₀: Yield 86%. ¹H NMR (400 MHz, D₂O, 300K), δ (ppm)= 3.33-3.17 (m, 145H, **f**, **f'**), 2.90-2.69 (m, 225H, **b**, **g'**), 2.57--2.36 (m, 180H, **g**, **c**, **a**). ¹³C NMR (400 MHz, D₂O, 300K), δ (ppm)= 174.58 (**d**), 48.97 (**g**), 47.05 (**b**, **a**), 39.37 (**f**), 38.55 (**g'**), 38.29 (**f**), 32.53 (**c**). FT-IR v(cm⁻¹): 3311 (N-H amine stretch), 2945 (C-H alkane stretch), 1631 (C=O).

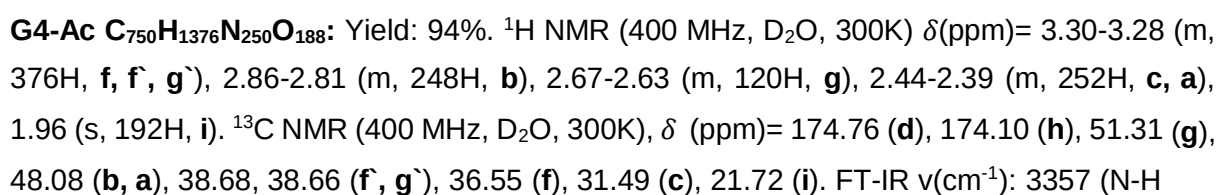
G4 PAMAM dendrimer

[illegible]

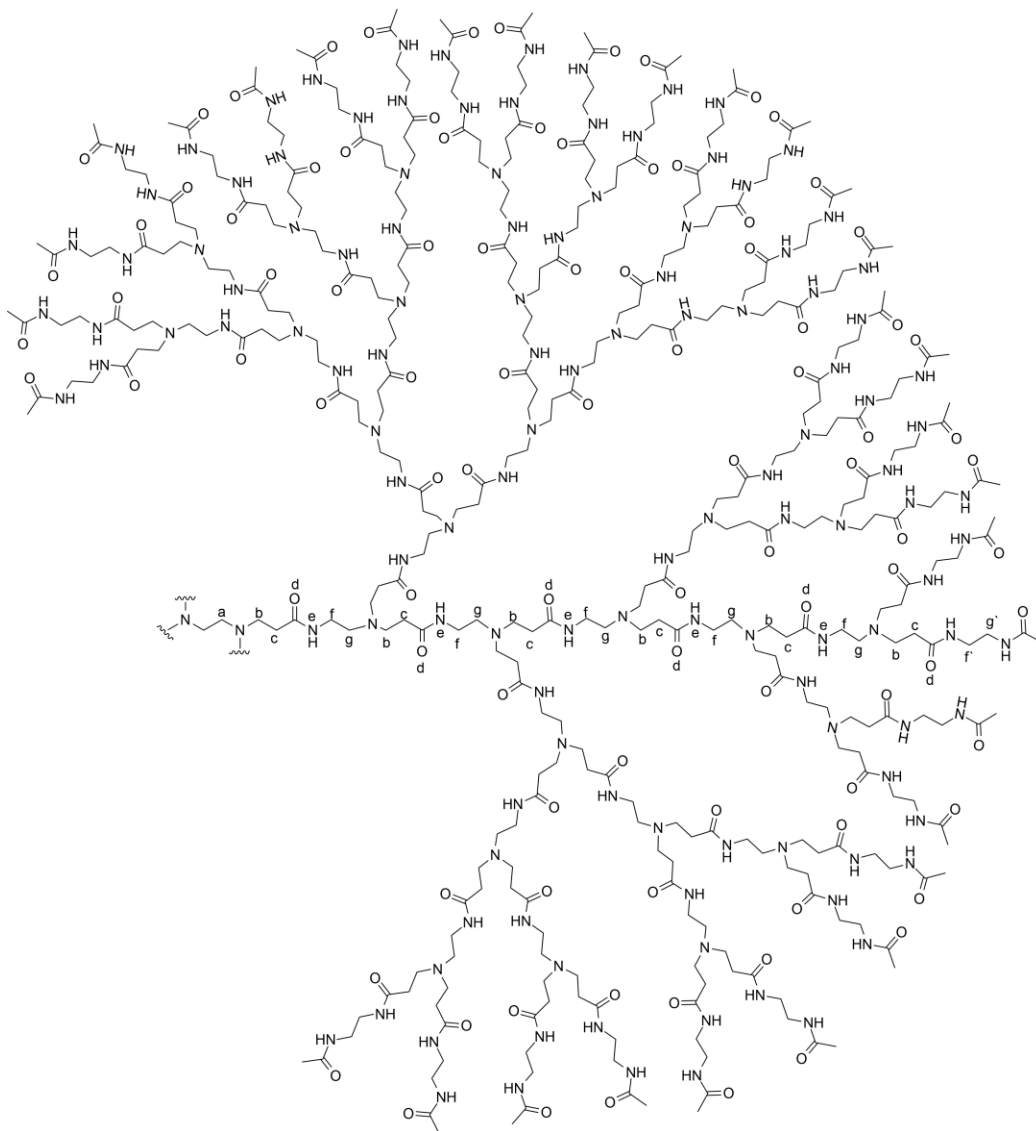
G4 PAMAM C₆₆₂H₁₂₄₈N₂₅₀O₁₂₄ : Yield 84%. ¹H NMR (400 MHz, D₂O, 300K), δ (ppm)= 3.26-3.17 (m, 248H, **f**, **f'**), 2.78-2.72 (m, 348H, **b**, **g'**), 2.59-2.55 (m, 120H, **g**), 2.40-2.34 (m, 252H, **c**, **a**). ¹³C NMR (400 MHz, D₂O, 300K), δ (ppm)= 175.03 (**d**), 51.24(**g**), 49.01(**b**, **a**), 40.33 (**f'**), 39.69 (**g'**), 36.70 (**f**), 32.73 (**c**). FT-IR v(cm⁻¹): 3281 (N-H amine stretch), 2943 (C-H alkane stretch), 1639 (C=O). Mass spectroscopy (MALDI-TOF) [M]⁺: 14 825.9122 g/mol.

The amine terminated PAMAM dendrimer was dissolved in anhydrous methanol followed by the addition of a 600 mol excess of triethylamine (TEA) to the reaction vessel while stirring. A 30 mol excess of acetic anhydride was then added to the reaction mixture while kept under inert atmosphere. The reaction was kept stirring in the dark for 24 hours for G4 PAMAM and 48 hours for G5 PAMAM. The product was purified by dialysis (MWCO 6 kDa) against distilled water. The water was then removed to obtain the acetylated PAMAM which was isolated as a colourless viscous oil.³⁵

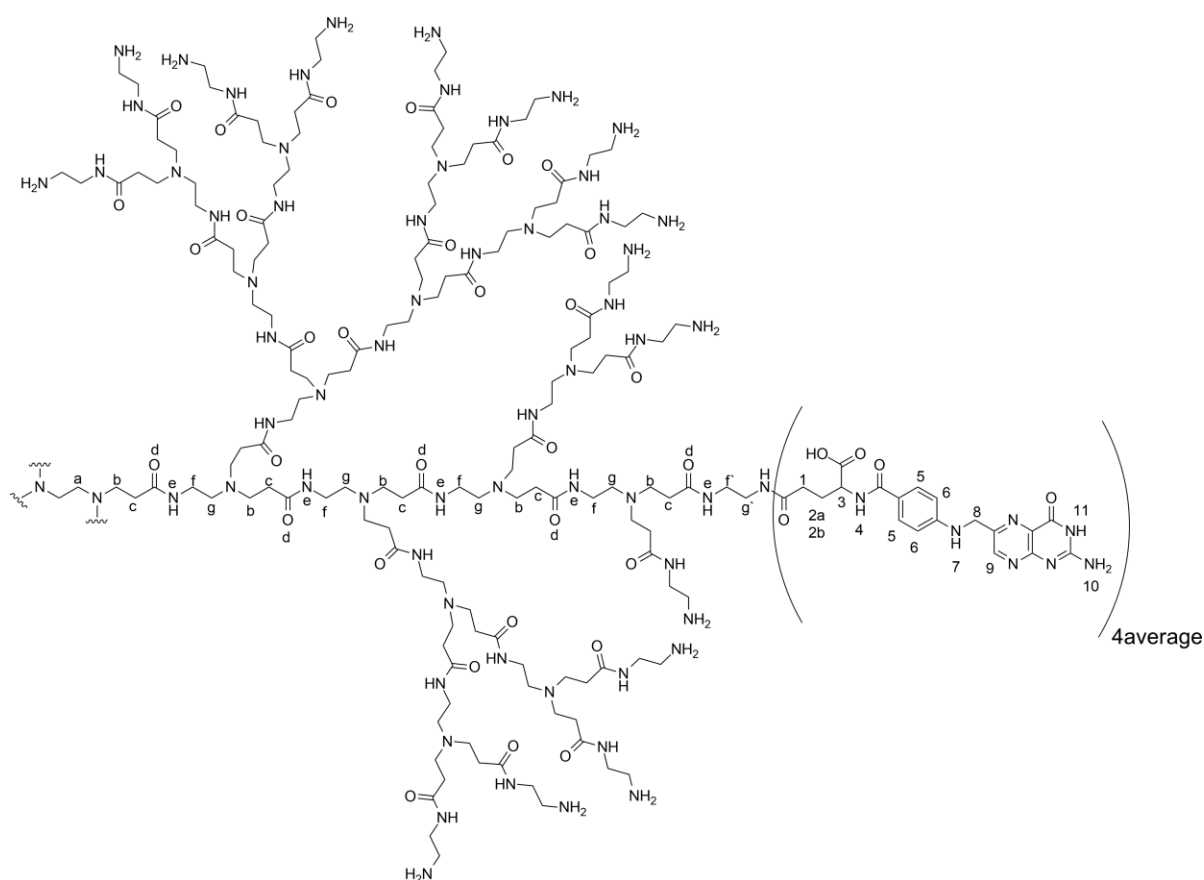
The chemical structure shows a complex, branched dendritic polymer. It features a central core with multiple terminal groups, including amide and ester functionalities. The structure is highly symmetrical and contains numerous nitrogen and oxygen atoms. The terminal groups are labeled with letters 'a' through 'g' and 'd' through 'h', indicating specific sites of interest. The overall structure is a large, multi-branching molecule with a central core and multiple terminal groups.



G5-Ac PAMAM dendrimer



1-ethyl-3-carbodiimide hydrochloride EDC-HCl (115.7 mg, 60.4 μ mol) was added to folic acid (FA) (19.0 mg, 43.0 μ mol) dissolved in dimethylformamide (DMF) (12 mL) and dimethylsulfoxide (DMSO) (4 mL) which was kept stirring under inert atmosphere for 3 hours. The mixture was added dropwise to G4 PAMAM (101.8 mg, 7.16 μ mol) dissolved in distilled water. The resultant mixture was then kept stirring for 48 hours in the dark. The solution was dialyzed against phosphate buffered saline (PBS) and then followed by distilled water to obtain the product ³². Yield: 80%



G4-FA C₉₅H₁₃₁N₂₈O₁₄ : Yield:80% . ¹H NMR (400 MHz, D₂O, 300K) δ(ppm)= 8.54 (s, 4H, **H⁹**), 7.58 (s, 8H, **H⁵**), 6.71 (s, 8H, **H⁶**), 3.25- (m, 968H, **f**, **f'**, **b**, **g'**, **g**, **c**, **a**). ¹³C NMR (400 MHz, D₂O, 300K), δ (ppm)= 55.03 (**g**), 48.99 (**b**, **a**), 42.00 (**f'**), 38.29 (**f**), 32.51 (**c**). FT-IR ν(cm⁻¹): 3649-2905 (N-H amine stretch), 2959 (C-H alkane stretch), 1628 (C=O), 1441 (aromatic C=C).

2.6 References

- (1) Galluzzi, L.; Senovilla, L.; Vitale, I.; Michels, J.; Martins, I.; Kepp, O.; Castedo, M.; Kroemer, G. Molecular Mechanisms of Cisplatin Resistance. *Oncogene*. April 12, 2012, pp 1869–1883. <https://doi.org/10.1038/onc.2011.384>.
- (2) Shen, D. W.; Pouliot, L. M.; Hall, M. D.; Gottesman, M. M. Cisplatin Resistance: A Cellular Self-Defense Mechanism Resulting from Multiple Epigenetic and Genetic Changes. *Pharmacol Rev* **2012**, 64 (3), 706–721. <https://doi.org/10.1124/pr.111.005637>.
- (3) Johnstone, T. C.; Suntharalingam, K.; Lippard, S. J. The Next Generation of Platinum Drugs: Targeted Pt(II) Agents, Nanoparticle Delivery, and Pt(IV) Prodrugs. *Chemical Reviews*. American Chemical Society March 9, 2016, pp 3436–3486. <https://doi.org/10.1021/acs.chemrev.5b00597>.
- (4) Kellett, A.; Molphy, Z.; Slator, C.; McKee, V.; Farrell, N. P. Molecular Methods for Assessment of Non-Covalent Metallodrug-DNA Interactions. *Chem Soc Rev* **2019**, 48 (4), 971–988. <https://doi.org/10.1039/c8cs00157j>.
- (5) Plutín, A. M.; Ramos, R.; Mocelo, R.; Alvarez, A.; Castellano, E. E.; Cominetti, M. R.; Oliveira, K. M.; Donizeth de Oliveira, T.; Silva, T. E. M.; Correa, R. S.; Batista, A. A. Antitumor Activity of Pd(II) Complexes with N,S or O,S Coordination Modes of Acylthiourea Ligands. *Polyhedron* **2020**, 184. <https://doi.org/10.1016/j.poly.2020.114543>.
- (6) O'Reilly, B.; Plutín, A. M.; Pérez, H.; Calderón, O.; Ramos, R.; Martínez, R.; Toscano, R. A.; Duque, J.; Rodríguez-Solla, H.; Martínez-Alvarez, R.; Suárez, M.; Martín, N. Synthesis and Structural Characterization of Cobalt(II) and Copper(II) Complexes with N,N-Disubstituted-N'-Acylthioureas. *Polyhedron* **2012**, 36 (1), 133–140. <https://doi.org/10.1016/j.poly.2012.02.008>.
- (7) Kemp, G.; Roodt, A.; Purcell, W.; Koch, K. R. Unprecedented N,S,O Co-Ordination of the Doubly Deprotonated Anion of N-Benzoyl-N'-Phenylthiourea (H₂L₂) Bridging Two Rhodium(I) Centres: Crystal Structure of the Acetone Solvate of [(PPh₃)₂(CO)Rh(μ-L₂-KN': KO,S)Rh(PPh₃)(CO)]. *Journal of the Chemical Society - Dalton Transactions* **1997**, No. 23, 4481–4483. <https://doi.org/10.1039/a705887j>.
- (8) Sheeba, M. M.; Muthu Tamizh, M.; Farrugia, L. J.; Endo, A.; Karvembu, R. Chiral (H₆-p-Cymene)Ruthenium(II) Complexes Containing Monodentate Acylthiourea Ligands for Efficient Asymmetric Transfer Hydrogenation of Ketones. *Organometallics* **2014**, 33 (2), 540–550. <https://doi.org/10.1021/om4010548>.
- (9) Koch, K. R. New Chemistry with Old Ligands: N-Alkyl-and-N,N-Dialkyl-N'-Acyl(Aroyl)Thioureas in Co-Ordination, Analytical and Process Chemistry of the Platinum Group Metals. *Coord Chem Rev* **2001**, 216–217, 473–488.
- (10) Peega, T.; Magwaza, R. N.; Harmse, L.; Kotzé, I. A. Synthesis and Evaluation of the Anticancer Activity of [Pt(Diimine)(N,N-Dibutyl-N'-Acylthiourea)]⁺ Complexes. *Dalton Transactions* **2021**. <https://doi.org/10.1039/D1DT01385H>.
- (11) Westra, A. N.; Bourne, S. A.; Esterhuysen, C.; Koch, K. R. Reactions of Halogens with Pt(II) Complexes of N-Alkyl- and N,N-Dialkyl-N'-Benzoylthioureas: Oxidative Addition and Formation of an I₂ Inclusion Compound. *Dalton Transactions* **2005**, No. 12, 2162–2172. <https://doi.org/10.1039/b503653d>.
- (12) Saeed, A.; Flörke, U.; Erben, M. F. A Review on the Chemistry, Coordination, Structure and Biological Properties of 1-(Acyl/Aroyl)-3-(Substituted) Thioureas. *Journal of Sulfur Chemistry*. Taylor and Francis Ltd. May 4, 2014, pp 318–355. <https://doi.org/10.1080/17415993.2013.834904>.
- (13) Ried, W.; Oremek, G.; Guryrn, R. Synthese Und Reaktionen von Neuen 3-Chlor- N -[Chlor(Dialkyl-amino)Methylen]Benzo[b]Thiophen-2-carboxamiden. *Chem Ber* **1980**, 113 (7), 2583–2588.

<https://doi.org/10.1002/cber.19801130724>.

- (14) Márquez, H.; Plutín, A.; Rodríguez, Y.; Perez, E.; Loupy, A. Efficient Synthesis of 1-(4'-Methylbenzoyl)-3,3-Diethylthiourea under Microwave Irradiation Using Potassium Fluoride on Alumina. *Synth Commun* **2000**, 30 (6), 1067–1073. <https://doi.org/10.1080/00397910008087124>.
- (15) Katritzky, A. R.; Kirichenko, N.; Rogovoy, B. v; Kister, J.; Tao, H.; Na, M. =. Synthesis of Mono-and N,N-Disubstituted Thioureas and N-Acylthioureas. *Synthesis (Stuttg)* **2004**, 11, 1799–1805. <https://doi.org/10.1055/s-2004-829127>.
- (16) Tamura, Y.; Kawasaki, T.; Adachi, M.; Kita, Y. Reactions of Thiocarbamoylaminophosphonium Salts Obtained from Amines and the Combined Reagent, Triphenylphosphine-Thiocyanogen (TPPT): Preparation of Thioureas, Acylthioureas and Amides. *Chem Pharm.Bull* **1979**, 27, 1636–1640.
- (17) Goerdeler, J.; Losch, R. *Darstellung Und Reaktionen von (Thiocarbamoyl)Carbodiimiden*.
- (18) Douglass, I. B.; Dains, F. B. Some Derivatives of Benzoyl and Furoyl Isothiocyanates and Their Use in Synthesizing Heterocyclic Compounds. *J Am Chem Soc* **1934**, 56 (3), 719–721. <https://doi.org/10.1021/ja01318a057>.
- (19) Burgeson, J. R.; Moore, A. L.; Boutilier, J. K.; Cerruti, N. R.; Gharaibeh, D. N.; Lovejoy, C. E.; Amberg, S. M.; Hruby, D. E.; Tyavanagimatt, S. R.; Allen, R. D.; Dai, D. SAR Analysis of a Series of Acylthiourea Derivatives Possessing Broad-Spectrum Antiviral Activity. *Bioorg Med Chem Lett* **2012**, 22 (13), 4263–4272. <https://doi.org/10.1016/j.bmcl.2012.05.035>.
- (20) Tomalia, D. A. *Dendrimers and Other Dendritic Polymers Wiley Series in Polymer Science Series Editor* ;; 2001; Vol. 1.
- (21) Fréchet, J. M. J.; Tomalia, D. A. *Dendrimers and Other Dendritic Polymers*; Wiley, 2001.
- (22) Tomalia, D. A.; Nixon, L. S.; Hedstrand, D. M. The Role of Branch Cell Symmetry and Other Critical Nanoscale Design Parameters in the Determination of Dendrimer Encapsulation Properties. *Biomolecules*. MDPI AG April 1, 2020. <https://doi.org/10.3390/biom10040642>.
- (23) Tomalia, D. A.; Naylor, A. M.; Goddard, W. A. *Starburst Dendrimers: Molecular-Level Control of Size, Shape, Surface Chemistry, Topology, and Flexibility from Atoms to Macroscopic Matter* **; 1990.
- (24) Clayden, J.; Greeves, N.; Warren, S. *Organic Chemistry*, 2nd ed.; Oxford university press: London, England, 2012.
- (25) Morgan, G.; Burstall, F. Researches on Residual Affinity and Co-Ordination. Part XXXIV. *J Chem Soc* **1934**, 965–971.
- (26) Mautjana, A. N.; Miller, J. D. S.; Gie, A.; Bourne, S. A.; Koch, K. R. Tailoring Hydrophilic N,N-Dialkyl-N'-Acylthioureas Suitable for Pt(II), Pd(II) and Rh(III) Chloride Pre-Concentration from Acid Aqueous Solutions, and Their Complex Separation by Reversed-Phase HPLC. *Dalton Transactions* **2003**, No. 10, 1952–1960. <https://doi.org/10.1039/b211885h>.
- (27) Plutín, A. M.; Alvarez, A.; Mocelo, R.; Ramos, R.; Sánchez, O. C.; Castellano, E. E.; da Silva, M. M.; Villarreal, W.; Colina-Vegas, L.; Pavan, F. R.; Batista, A. A. Structure/Activity of PtII/N,N-Disubstituted-N'-Acylthiourea Complexes: Anti-Tumor and Anti-Mycobacterium Tuberculosis Activities. *J Braz Chem Soc* **2018**, 29 (6), 1256–1267. <https://doi.org/10.21577/0103-5053.20170222>.
- (28) Monsalve, L. N.; Kaniz Fatema, M.; Nonami, H.; Erra-Balsells, R.; Baldessari, A. Lipase-Catalyzed Synthesis and Characterization of a Novel Linear Polyamidoamine Oligomer. *Polymer (Guildf)* **2010**, 51 (14), 2998–3005. <https://doi.org/10.1016/j.polymer.2010.04.071>.
- (29) Maiti, P. K.; Çağın, T.; Wang, G.; Goddard, W. A. Structure of PAMAM Dendrimers: Generations 1 through 11. *Macromolecules* **2004**, 37 (16), 6236–6254. <https://doi.org/10.1021/ma035629b>.
- (30) Chanphai, P.; Tajmir-Riahi, H. A. Thermodynamic Analysis of Biogenic and Synthetic Polyamines Conjugation with PAMAM-G4 Nanoparticles. *J Photochem Photobiol B* **2016**, 155, 13–19.

<https://doi.org/10.1016/j.jphotobiol.2015.12.006>.

- (31) Kolhatkar, R. B.; Kitchens, K. M.; Swaan, P. W.; Ghandehari, H. Surface Acetylation of Polyamidoamine (PAMAM) Dendrimers Decreases Cytotoxicity While Maintaining Membrane Permeability. *Bioconjug Chem* **2007**, *18* (6), 2054–2060. <https://doi.org/10.1021/bc0603889>.
- (32) Kesharwani, P.; Jain, K.; Jain, N. K. Dendrimer as Nanocarrier for Drug Delivery. *Progress in Polymer Science*. February 2014, pp 268–307. <https://doi.org/10.1016/j.progpolymsci.2013.07.005>.
- (33) Chen, C.; Ke, J.; Edward Zhou, X.; Yi, W.; Brunzelle, J. S.; Li, J.; Yong, E. L.; Xu, H. E.; Melcher, K. Structural Basis for Molecular Recognition of Folic Acid by Folate Receptors. *Nature* **2013**, *500* (7463), 486–489. <https://doi.org/10.1038/nature12327>.
- (34) Smith, S. B.; Ganapathy, P. S.; Bozard, R. B.; Ganapathy, V. Folate Transport in Retina and Consequences on Retinal Structure and Function of Hyperhomocysteinemia. In *Handbook of Nutrition, Diet and the Eye*; Elsevier Inc., 2014; pp 349–359. <https://doi.org/10.1016/B978-0-12-401717-7.00035-6>.
- (35) Xu, L.; Kittrell, S.; Yeudall, W. A.; Yang, H. Folic Acid-Decorated Polyamidoamine Dendrimer Mediates Selective Uptake and High Expression of Genes in Head and Neck Cancer Cells. *Nanomedicine* **2016**, *11* (22), 2959–2973. <https://doi.org/10.2217/nnm-2016-0244>.
- (36) Bourassa, P.; Hasni, I.; Tajmir-Riahi, H. A. Folic Acid Complexes with Human and Bovine Serum Albumins. *Food Chem* **2011**, *129* (3), 1148–1155. <https://doi.org/10.1016/j.foodchem.2011.05.094>.
- (37) Li, M. H.; Choi, S. K.; Thomas, T. P.; Desai, A.; Lee, K. H.; Kotlyar, A.; Banaszak Holl, M. M.; Baker, J. R. Dendrimer-Based Multivalent Methotrexates as Dual Acting Nanoconjugates for Cancer Cell Targeting. *Eur J Med Chem* **2012**, *47* (1), 560–572. <https://doi.org/10.1016/j.ejmech.2011.11.027>.

Chapter 3

Host-guest interactions of [Pt(phen)(acylthiourea)]⁺ complexes and G4 and G5 PAMAM dendrimers

3.1 Introduction

[Pt(diimine)(acylthiourea)]⁺ complexes have been shown to be highly active against certain cancer cell lines.¹ However, some of these complexes display poor water solubility which may affect their anticancer activity as well as their potential clinical use.² Poor water solubility results in low bioavailability and poor delivery of the drug to its target, therefore giving the drug a bad pharmacokinetic profile.^{3,4} Certain drug delivery systems have been shown to improve the solubility of encapsulated drugs as well as reducing the cytotoxicity of some drugs by means of controlled release.⁵

Polyamido amine (PAMAM) dendrimers have been widely studied and used as potential drug delivery systems for a variety of drugs. Studies that have been conducted show that PAMAMs can permeate across epithelial membranes via paracellular transport which is movement across the epithelium by passing through the intercellular junctions and transcellular transport (movement across the epithelium by passing through the cell) with minimal toxicity given that the dendrimer is neutral or anionic.^{6–8} Other studies show that PAMAMs can increase the bioavailability of certain encapsulated drugs by bypassing certain efflux transporters that most cancer cells develop as an adaptive advantage, therefore making PAMAM dendrimers an attractive potential drug delivery system.^{7,9}

Cationic dendrimers display higher levels of toxicity although the conjugation of molecules that neutralize the positive charges have been shown to lower the inherent toxicity of the dendrimer.^{8,10} At physiological pH, amine terminated PAMAM becomes protonated to produce a cationic species which has been shown to be damaging towards the lipid bilayer membrane that lines the cell walls of cells/living organisms resulting in cell lysis.^{7,11} An ideal drug delivery system should display minimal to no toxicity. In this study, we opted for acetylation of the peripheral amine groups located at the surface of the dendrimer to bypass this issue.⁸ This type of functionalization is easy to conduct and does not require a long reaction time. Inherent dendrimer toxicity is also linked to the size of the dendrimer whereby larger generations (G5.5 and higher) have been shown to be more toxic than smaller generation dendrimers.^{7,11} For this reason, the largest dendrimer we have used in this study is the generation 5 (G5) PAMAM.

PAMAM dendrimers have been gaining attention as potential drug delivery systems for active targeting as they can be modified with vectors to allow for potential selectivity towards cancer cells. Molecules such as folic acid, biotin, hormones, transferrin and antibodies such as Trastuzumab have been conjugated on dendrimer surfaces to target certain cancer cells.^{12–16} The folic acid receptor (FAR) is overexpressed in various cancer cell lines as

folate demand is high in rapidly proliferating cells. Folic acid (FA) has been shown to have a binding affinity for its receptor which makes it a good vector for selectivity.

The conformation in which the dendrimer is in (compact or open) is important when using PAMAM dendrimers for guest molecule encapsulation. PAMAMs are prone to backfolding (inward folding of the dendrons towards the core) since they have multiple amide bonds and the degree of backfolding increases as the generation size increases. The presence of solvent has been shown to reduce the amount of backfolding and result in dendrimer swelling which creates an open structure with more accessible voids. Solvent therefore plays a critical part in the encapsulation process.¹⁷

3.2 PAMAM host-guest interactions

PAMAM dendrimers have been widely investigated for potential use in drug delivery as discussed in section 3.1. There are two ways in which the guest molecule can interact with the dendrimer:

- a) Non-covalently:¹⁸ There are mainly two types of interactions which are classified under non-covalent. In the first scenario, the guest molecule is encapsulated in the dendrimer voids or is physically entrapped in the dendrimer structure and is held within the dendrimer by hydrophobic interactions and/or hydrogen bonding. In the second case, is an electrostatic interaction where the charged dendrimer and charged guest molecule form an ion-pair. With electrostatic interactions, the guest molecule is typically found at the surface of the dendrimer. Drug release is facilitated by conformational changes of the dendrimer which may be triggered by factors such as change in pH or a temperature change.^{18,19}
- b) Covalently:¹⁸ In this case the drug is covalently attached (usually to the surface) to the dendrimer directly or with the use of a spacer molecule. The drug is released when the covalent bond is cleaved usually by means of an enzymatic cleavage in-vivo.¹⁸

We opted for generation 4 (G4) and generation 5 (G5) PAMAM in this study as they display minimal toxicity due to their ideal size while having a globular shape and well-formed cavities. This chapter describes the attempt at encapsulating [Pt(phen)(acylthiourea)]⁺ complexes in G4 and G5 PAMAM as well as 5-fluorouracil (5FU) in G5 PAMAM as a control sample. The encapsulation was studied using ¹H NMR and DOSY NMR spectroscopy.

3.3 Diffusion ordered spectroscopy (DOSY): a significant characterization technique

Molecules in solution undergo random diffusion known as Brownian motion. Diffusion ordered spectroscopy (DOSY) aids in the identification of different molecules in a mixture by their individual diffusion coefficients.²⁰ The DOSY is a two-dimensional experiment that is used to study the translational motion of said molecules in a liquid sample.²¹ The DOSY NMR spectrum displays two dimensions, the first dimension (on the x-axis) displays the chemical shift, and the second dimension (on the y-axis) displays the diffusion coefficient of the species in solution.^{20,21}

In a typical DOSY experiment, a series of spectra are recorded with varying pulse field gradient strengths (and a fixed diffusion time).²¹ After a 90° pulse, the first gradient pulse (which is pulsed for duration δ) dephases the magnetisation and encodes the position of the molecules in the NMR tube as shown in figure 3.1A. The gradient pulse is followed by a delay (Δ), during which the molecules in the mixture diffuse a certain distance in the tube (figure 3.1B). The sample is then pulsed with a 180° rf pulse followed by a second gradient pulse which is meant to rephase the magnetisation. However, rephasing is only possible for nuclei that have not diffused (significantly) across the tube therefore, only partial rephasing is possible and an NMR signal which is decreased in intensity is attenuated.^{21,22}

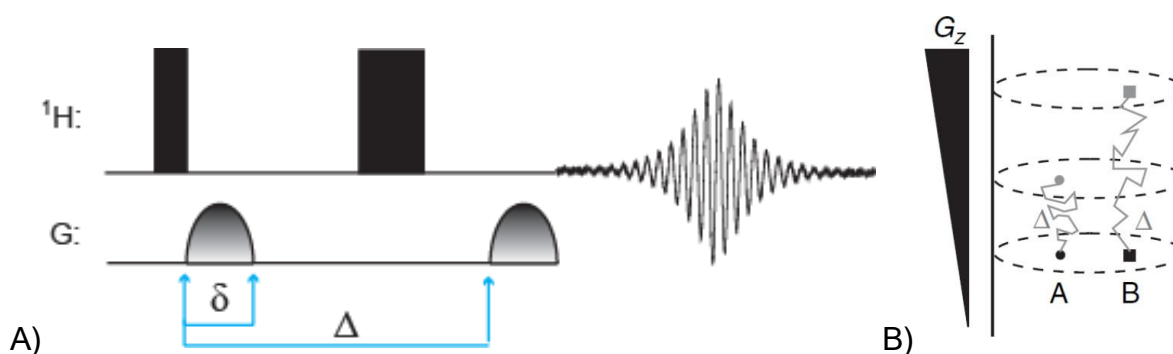


Figure 3.1: A) Graphical representation of a simple gradient field pulse experiment in diffusion NMR spectroscopy. B) Representation of the translational motion of molecules A and B in solution.

DOSY measurements may be acquired by gradients in the main magnetic field or gradients in radio frequency field. The contribution to the signal of each individual component is described by equation (1) and the intensity of the signal decreases exponentially as a function of G^2 (where G is the gradient strength). The exponential decay is described by equation (2)²⁰

$$I(i, g^2) = I_o \exp\left[-D(i)\left(\Delta - \frac{\delta}{3}\right)K^2\right] \quad (1)$$

$$K = \gamma g \delta$$

$$e^{-D\gamma\delta^2(\Delta-\delta)/3} \quad (2)$$

Where $I(i)$ is the signal amplitude of component i , $I_0(i)$ is the amplitude of the signal in the absence of an applied gradient. D is the diffusion coefficient, γ is the gyromagnetic ratio, g is the gradient strength, δ is the duration at which the gradient is pulsed and Δ is the diffusion time.

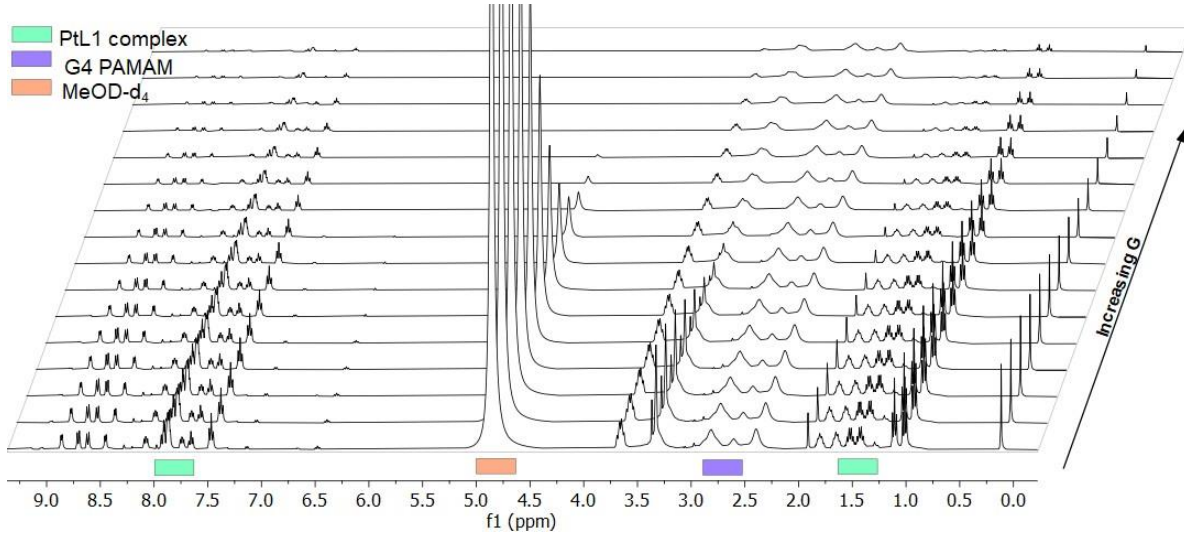


Figure 3.2: ^1H NMR spectrum of a mixture of $[\text{Pt}(\text{phen})(\text{L}^1\text{-O,S})]^+$ and G4 PAMAM in deuterated methanol (MeOD-d_4) with increasing gradient field strength (G) in a typical DOSY experiment.

Since lighter molecules diffuse faster in solution, fewer nuclei undergo refocusing as compared to the nuclei of the heavier molecules. This results in the signal intensity of the lighter molecule decreasing faster than those of the heavier molecule as the gradient field strength increases (figure 3.2). Assuming a spherical size of the molecules the individual diffusion coefficients (D) can be described by the Stokes-Einstein equation (3).²¹

$$D = \frac{kT}{6\pi\eta r_s} \quad (3)$$

Where k is the Boltzmann constant, T is the temperature, η is the viscosity of the liquid and r is the hydrodynamic radius of the molecule.

3.4 Results and discussion

3.4.1 ^1H NMR studies of potential host-guest molecules

The encapsulation of $[\text{Pt}(\text{phen})(\text{acylthiourea})]^+$ complexes in PAMAM dendrimers has never been attempted before therefore various methods were investigated. The co-precipitation method was initially attempted, where $[\text{Pt}(\text{phen})(N\text{-benzoyl-}N,N\text{-dibutylthiourea})]^+$ (referred to as PtL1 moving forward), was added to G4 PAMAM in methanol since the dendrimer is stored in a methanolic solution. The solvent plays an important role as it opens up the dendritic structure and reduces backfolding such that the cavities are available for encapsulation of the guest molecule. A 35-molar excess of the PtL1 complex was initially added to a methanol solution containing the dendrimer. The mixture was stirred for 24 hours, after which the methanol was removed under reduced pressure to yield a viscous gel-like product. The product was redissolved in deuterated methanol ($\text{MeOD-}d_4$) and ^1H NMR was recorded (figure 3.3).

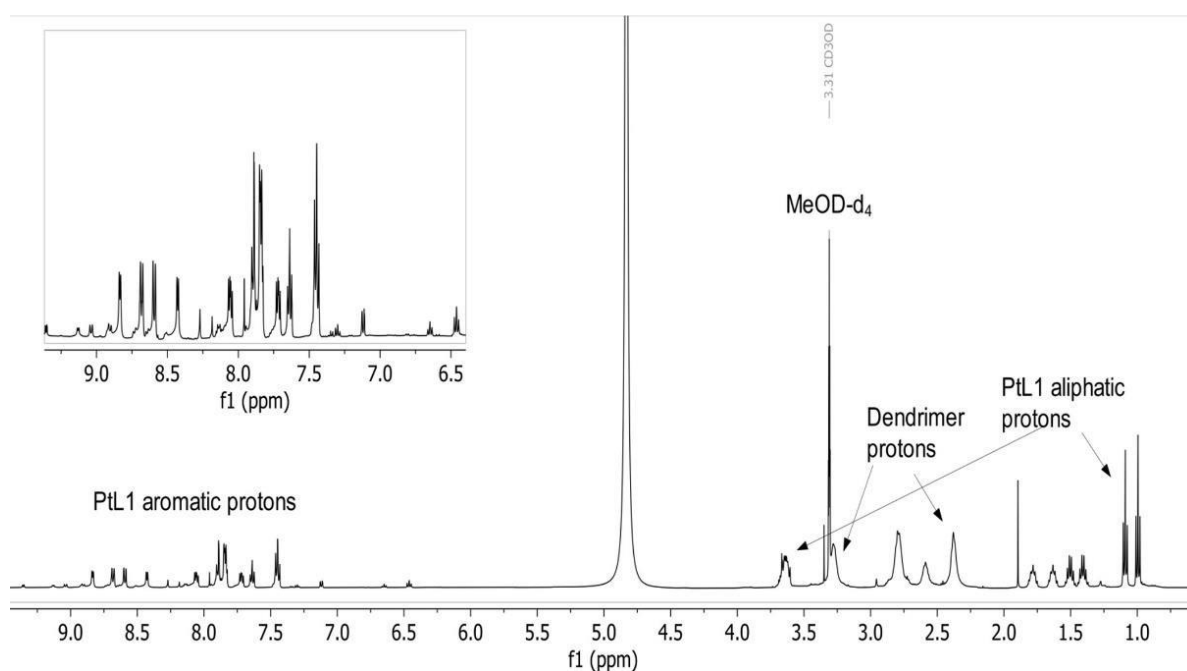


Figure 3.3: ^1H NMR spectrum of mixture of G4 PAMAM and $[\text{Pt}(\text{phen})(N\text{-benzoyl-}N,N\text{-dibutylthiourea})]^+$ in deuterated methanol ($\text{MeOD-}d_4$) at 300K.

The peaks appearing between 1.02 ppm to 1.85 ppm are successfully identified as the peaks that belong to the dibutyl arm of the complex. The broad peaks between 3.32 and 2.42 ppm are those of the dendrimer. In the aromatic region, peaks that correspond to the phenanthroline moiety are identified. However, smaller peaks which have a similar multiplicity to that of the complex are now seen in the aromatic region. This could indicate that a small quantity of the complex has been encapsulated. Therefore, this spectrum could be interpreted as a mixture of possibly successfully encapsulated complex along with a large

amount of free complex. To further understand whether encapsulation had occurred a diffusion ordered spectroscopy (DOSY) experiment was conducted on the sample (figure 3.4).

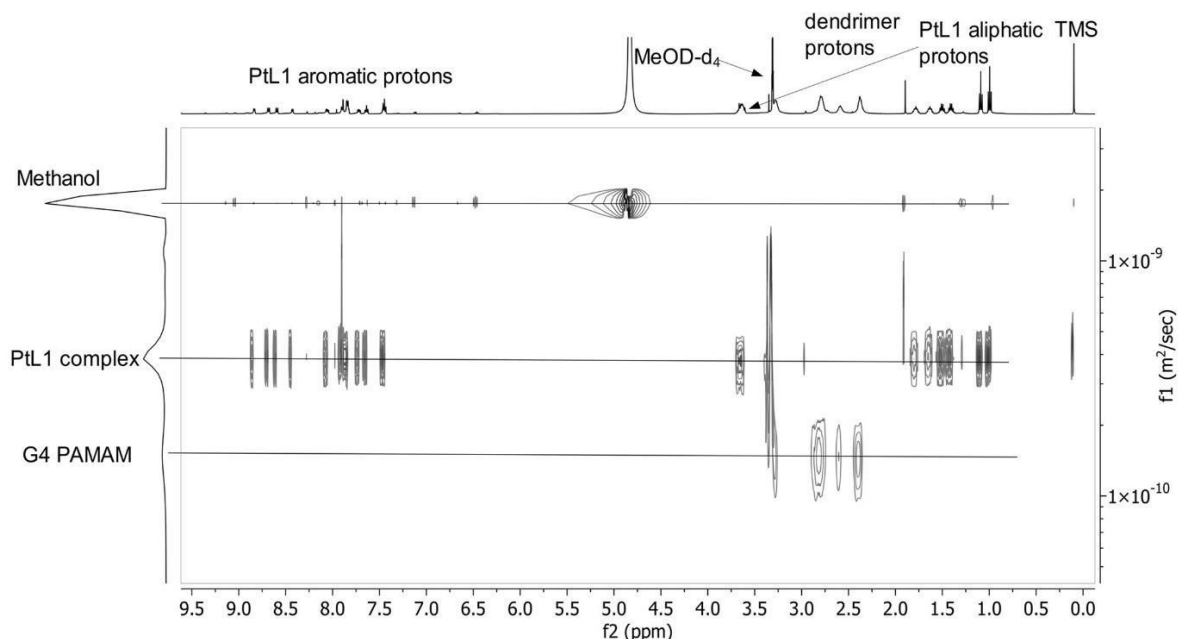


Figure 3.4: DOSY spectrum of a mixture of $[Pt(phen)(N-benzoyl-N,N-dibutylthiourea)]^+$ and G4 PAMAM in deuterated methanol ($MeOD-d_4$) showing the diffusion coefficients at 300K.

PtL1 (molecular weight 666.73 g/mol) has a diffusion coefficient of $3.80 \times 10^{-10} \text{ m}^2/\text{s}$ while G4 PAMAM (molecular weight 14 214.17 g/mol) has a diffusion coefficient of $1.40 \times 10^{-10} \text{ m}^2/\text{s}$ which is expected as G4-PAMAM is larger than the complex. The diffusion coefficient of the smaller peaks corresponds to that of methanol, which indicates decomposition of a small amount of the complex. Had encapsulation occurred, the diffusion coefficient of the encapsulated complex would be similar to that of the G4 PAMAM as the host-guest complex would be expected to diffuse slower in solution due to its large size. We suspect that during removal of the solvent, the internal dendrimer protons (pK_a 3-6)²³ are abstracted by the central nitrogen of the ligand (pK_a 7.5-10.9)²⁴ which then results in the decomposition of the complex though it happens in small amounts.

Encapsulation was then attempted using distilled water. However, PtL1 is sparingly soluble in water therefore should encapsulation be successful, the quantity of complex encapsulated would be too low to be detected and/or analysed. ^1H NMR of the sample was recorded and is shown in figure 3.5.

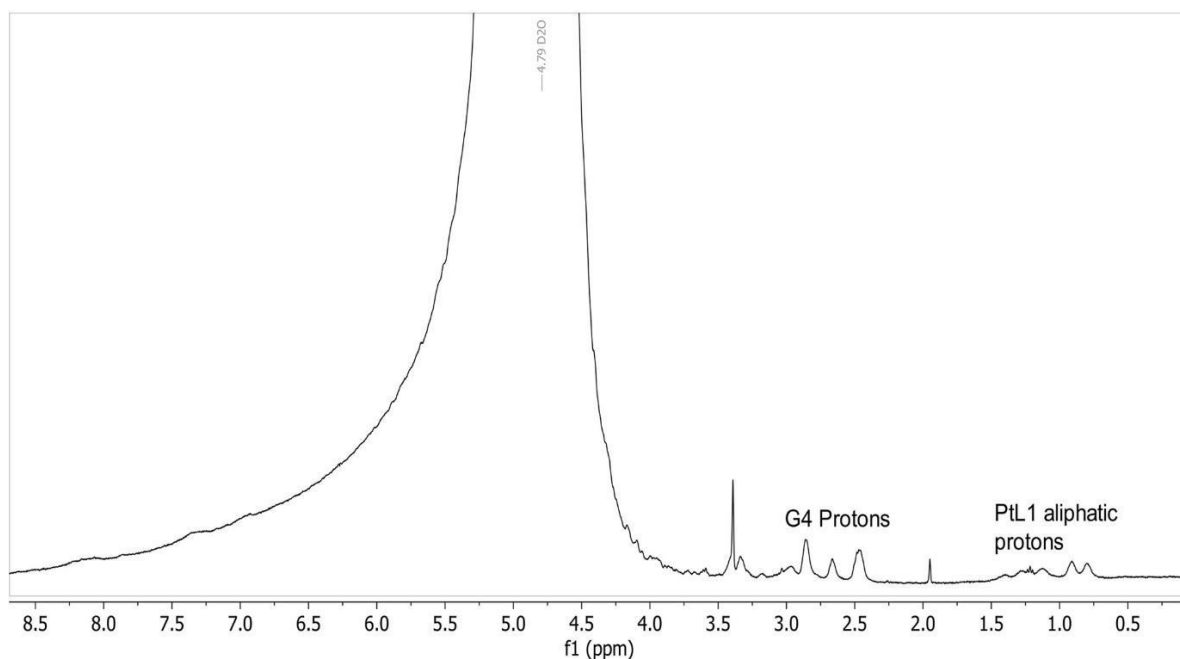


Figure 3.5: Mixture of G4 PAMAM and PtL1 after 24 hours in D₂O.

The peaks corresponding to the dibutyl arm of the complex are seen between 0.79 and 1.29 ppm. The peaks of the complex are less defined and have broadened as compared to in figure 3.3. A spectrum of plain PtL1 in D₂O where the concentration of the complex was kept constant was run for comparison. No changes in chemical shift of the complex upon the addition of the dendrimer were observed and the same peak broadening was seen. This peak broadening is a result of aggregation of the complex in aqueous solution via $\pi - \pi$ stacking of the phenanthroline moiety as shown by Kotze *et al.*²⁵

To minimize the self-association and low solubility problem we firstly dissolved the complex in an organic solvent to increase its molecular availability in solution (these complexes are readily soluble in a variety of organic solvents) followed by addition of the PAMAM dendrimer in an aqueous solution to the organic solvent. This was to make the environment outside of the cavities unfavourable for the complex to solvate in the water and rather migrate into the hydrophobic dendrimer cavities. The bi-phasic solvent method was attempted where excess PtL1 was dissolved in dichloromethane (DCM) followed by the addition of an aqueous solution of G4 PAMAM to the reaction vessel with constant stirring. We then opted for sonication as it is a more vigorous form of mixing to ensure maximum biphasic surfaces to allow for complex migration into the dendrimer cavities. A similar interface encapsulation method has been conducted in several studies and was shown to be successful.²⁶ The dendrimer containing water layer was taken and concentrated as much as possible and ¹H NMR was recorded on the sample (figure 3.6).

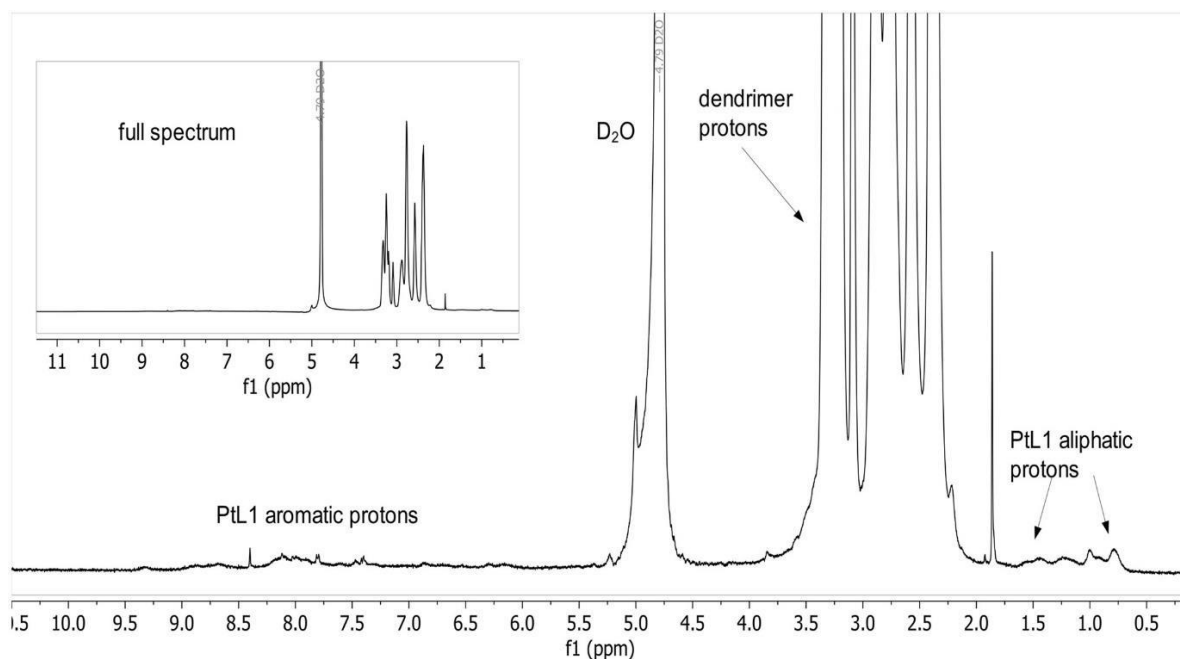


Figure 3.6: ^1H NMR spectrum of the concentrated water layer taken from G4 and PtL1 mixture DCM/ H_2O encapsulation attempt.

Once again, peak broadening is observed in the complex which is probably due to aggregation in the presence of water. A small amount of the complex was soluble in water given the low intensity as compared to the dendrimer (figure 3.6). With such low concentrations, a DOSY experiment could not be conducted on the sample. We decided to opt for a method that would result in a higher concentration of the complex in solution along with the dendrimer to improve encapsulation efficiency by using a solvent that would be miscible with water.

An attempt at encapsulating the smaller acylthiourea ligand and metal precursor $[\text{PtCl}_2(\text{phen})]$ were investigated as a model to the complex to better understand why encapsulation of the complexes have not been successful. The encapsulation of the precursor was attempted in dimethylsulfoxide (DMSO) since the platinum precursor is sparingly soluble in DMSO-d_6 . A 15 molar excess of the precursor in DMSO was added to G4 PAMAM dissolved in water and was allowed to stir for 24hrs. Upon addition, the precursor had formed a suspension which had a milky yellow colour, however, after 24 hours, a clear yellow solution had formed. The solution was then concentrated to form a bright yellow oil which was re-dissolved in deuterated DMSO (DMSO-d_6) and analysed via ^1H NMR and 2- dimensional Nuclear Overhauser Effect Spectroscopy (2D NOESY). NOESY is a technique which is used to detect interactions of protons through space up to a distance of $5\text{\AA}$ and is commonly used to study host-guest interactions.

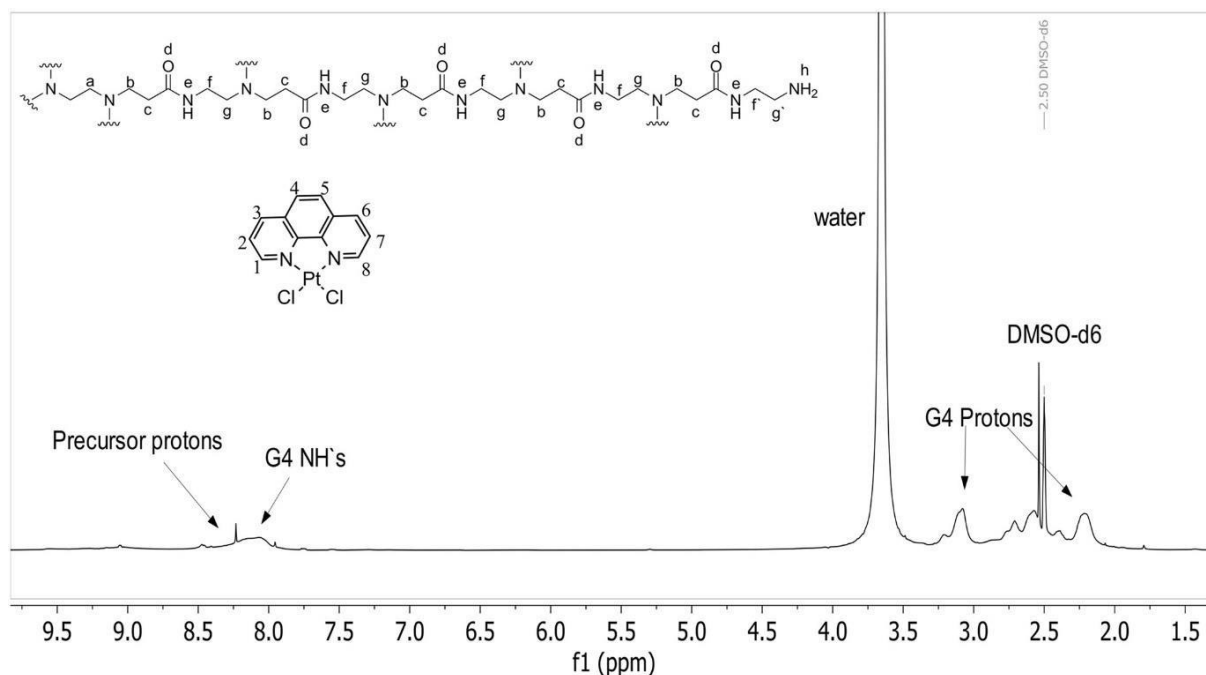


Figure 3.7: ^1H NMR of precursor and G4 in DMSO-d_6 .

The broad peak in the aromatic region is that of the dendrimer NH protons which appear to be overlapping with the protons of the precursor (figure 3.7). We were expecting to see crosspeaks that indicate an interaction between the precursor and dendrimer protons. The NOESY spectrum was symmetrized for easier viewing of the crosspeaks.

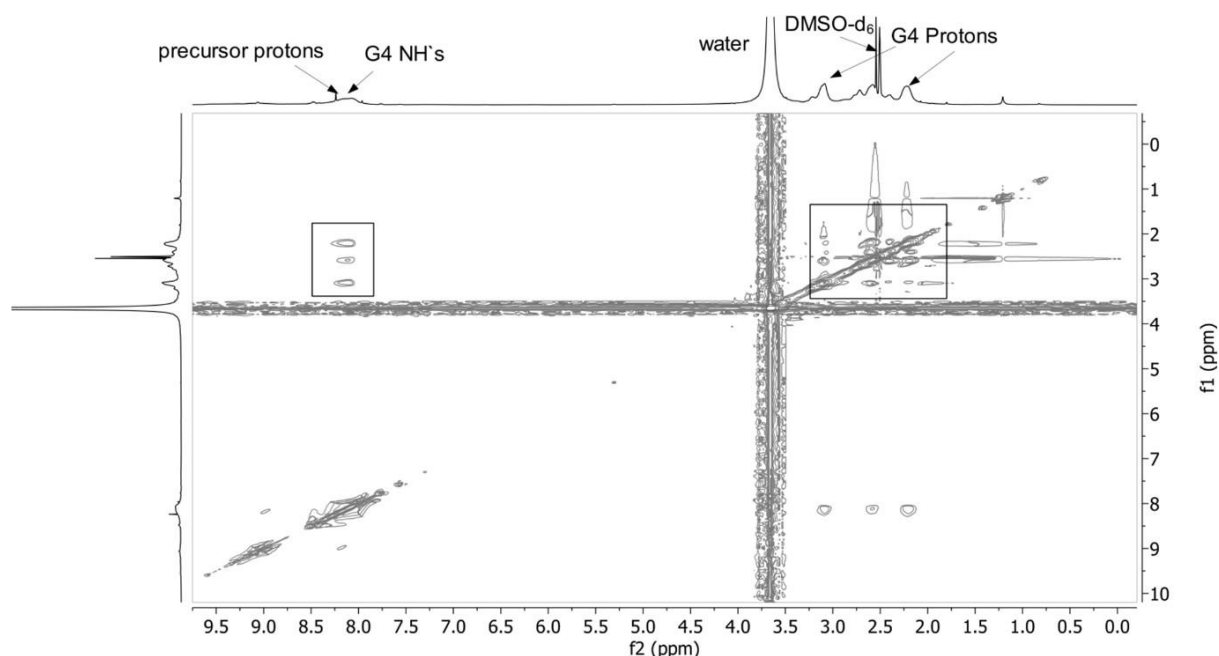


Figure 3.8: NOESY spectrum of precursor and G4 PAMAM in DMSO-d_6 .

The only visible crosspeaks are those of the dendrimer NH's interacting with the internal dendrimer protons (figure 3.8). We suspect that the dendrimer NH NOE crosspeaks might be overlapping with those of the precursor and the dendrimer internal protons. Upon the

addition of water to the sample, the precursor remained in solution which indicates successful encapsulation since the precursor on its own is completely insoluble in water. The same method was repeated with ligand L1, the resultant mixture was analysed via ^1H NMR and NOESY shown in figure 3.9.

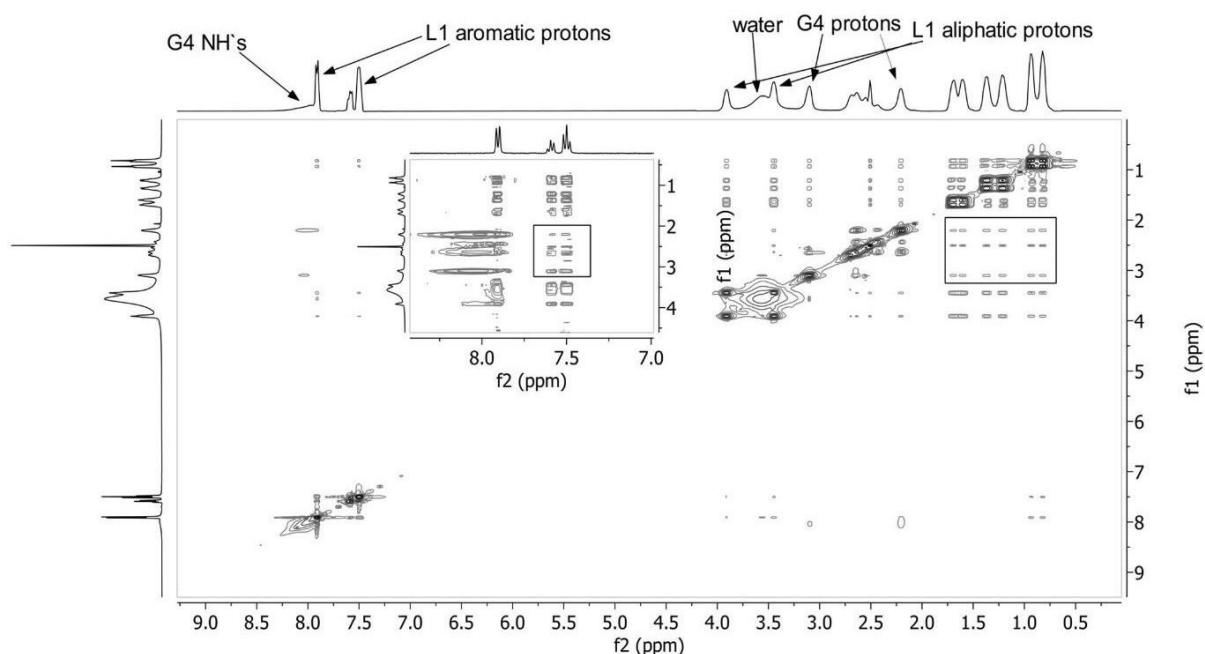


Figure 3.9: NOESY spectrum of ligand and zoomed-in aromatic region of L1 and G4 PAMAM in DMSO-d_6 .

Broadening of the ligand peaks is observed upon addition of L1 to G4 which is indicative of an interaction with the dendrimer. In figure 3.9, weak NOE crosspeaks are observed between the L1 aliphatic protons and internal dendrimer protons. Crosspeaks are also observed between the L1 aromatic protons and internal dendrimer protons when the intensity is increased as seen in the zoomed-in region of figure 3.9.

PtL2 is sufficiently soluble in water and was used to investigate if there would be a stronger interaction with the dendrimer. The complex was dissolved in distilled water and added to G4 PAMAM. The solution was stirred for 24 hours.

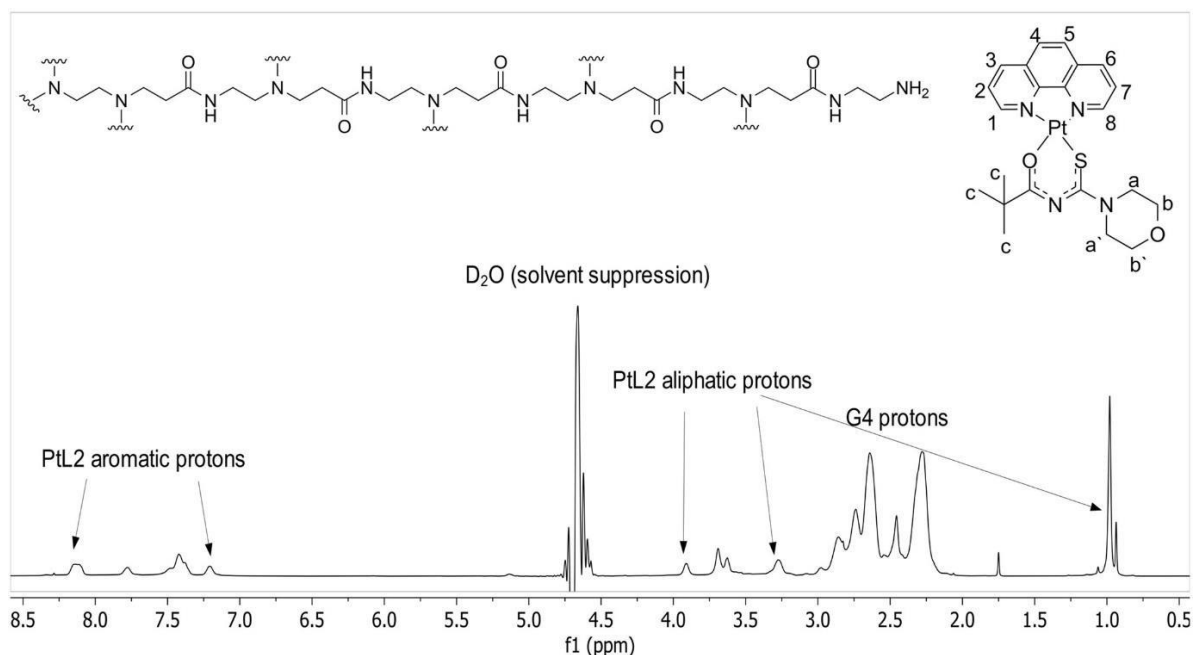


Figure 3.10: ^1H NMR of PtL2 and G4 mixture in H_2O (D_2O spike) with solvent suppression.

No changes are observed in the shape of the peaks that belong to the complex as seen in figure 3.10. This result was unexpected as theoretically, the presence of a hydrogen bond acceptor should result in a stronger interaction with the dendrimer. A study conducted by Jolly *et al*, showed that guest molecules with additional hydrogen bond donors and/or acceptors resulted in stronger interactions with the host molecule (dendrimer) as opposed to molecules that lack the ability to participate in hydrogen bonding.²⁶

This finding then led to exploring the effect of pH to trigger encapsulation. In our findings, the pH of amine terminated G4 PAMAM in solution was recorded to be basic (pH 9). As investigated by Buczkowski *et al*, amine terminated PAMAM dendrimers exist as polycations even near neutral pH due to the difference in pK_a of the internal amide protons (6.3-6.7) and surface amine protons (9-9.9). It was determined by potentiometric titration that approximately 10 cationic NH_3^+ groups are found at the surface of G4 PAMAM.¹⁹ This then explains the unsuccessful encapsulation attempts as the positive charges on the surface of the G4 repel the positively charged platinum complexes. Since G4-Fa has unprotected amine groups it also behaves as polycation. The positive surface would repel the positively charged complex, therefore, it was not used in the encapsulation of the platinum complexes. One possible solution would be to protect the amines using tert-butyloxycarbonyl (t-boc).

The host-guest interactions of 5-fluorouracil (5FU) and generation 5 (G5) PAMAM were studied briefly. An excess of 5FU was added to G5 PAMAM in D_2O , this mixture was stirred for 24 hours and the ^1H NMR spectra were recorded (figure 3.11).

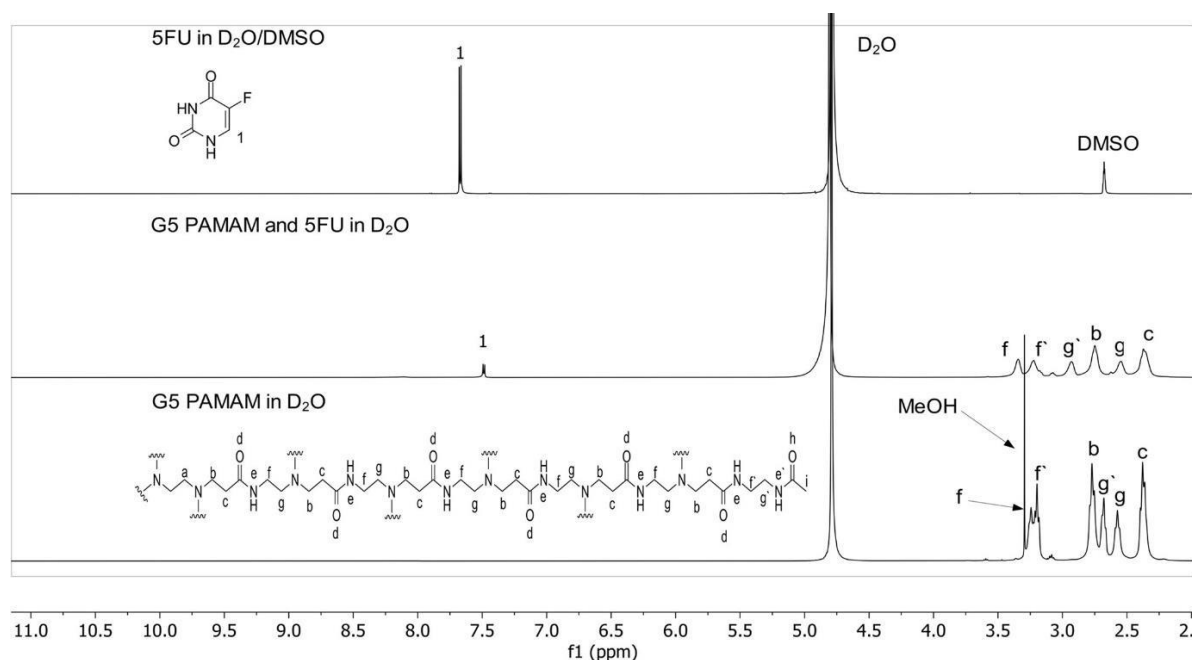


Figure 3.11: Stacked ^1H NMR spectra of 5FU in D_2O (top), G5 PAMAM and 5FU in D_2O (middle) and G5 PAMAM in D_2O (bottom).

Upon addition of 5FU to G5 PAMAM, a significant shift in dendrimer peak g' accompanied by the broadening and slight shift of peak f is seen. Since 5FU is weakly acidic and the amine functional groups of the dendrimer are basic, 5FU is bound at the surface of the dendrimer by electrostatic interactions. This is then seen in a shift of surface protons g' and f and this observation helps us to better understand the manner in which the $[\text{Pt}(\text{phen})(\text{L}-\text{O},\text{S})]^+$ complexes may interact with the dendrimer.

Now that we have established what an electrostatic interaction looks like and that amine terminated PAMAM is protonated at the surface, we decided to move onto G4-Ac which has no ionizable surface functional groups to attempt encapsulating the platinum complexes. A 15 molar excess of PtL1 dissolved in $\text{DMSO}-d_6$ was added to a solution of G4-Ac dissolved in $\text{DMSO}-d_6$ and stirred in the dark. After 24 hours, a proton NMR spectrum was recorded of the sample and is shown in figure 3.12.

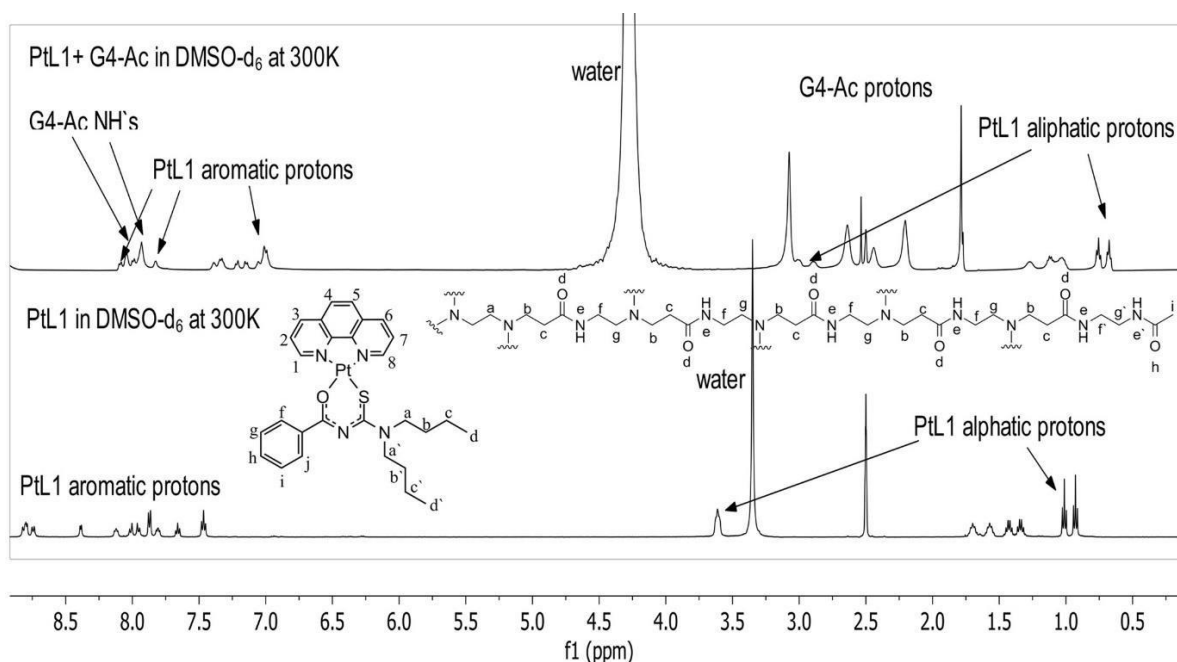


Figure 3.12: Stacked ^1H NMR spectra of PtL1 in $\text{DMSO-}d_6$ and G4-Ac and PtL1 encapsulation attempt in $\text{DMSO-}d_6$ at 300K.

Upon mixing with G4-Ac, a change in the shape of both the aliphatic and aromatic protons accompanied by a difference in chemical shift of the protons that belong to the complex is observed. These changes are indicative of an interaction between the dendrimer and complex. We can assume that no electrostatic interactions take place since no changes are observed in the dendrimer protons. The chemical shift differences of the aliphatic protons are tabulated for easy viewing (table 3.1), the aromatic protons are too complex to identify as they overlap with each other and G4-Ac NH protons in the mixture.

Table 3.1: Comparison of chemical shift differences of PtL1 and PtL1-G4-Ac mixture in $\text{DMSO-}d_6$.

PtL1 complex (ppm)	PtL1 complex (ppm)	PtL1-G4-Ac mixture (ppm)
Ha & Ha'	3.63-3.59	3.01-2.89
Hb & Hb'	1.70, 1.57	1.27-1.09
Hc & Hc'	1.43, 1.34	1.12-1.01
Hd & Hd'	1.01, 0.93	0.75, 0.68

To better understand the nature of this interaction, DOSY experiments were conducted on both the mixture as well as the free complex where the concentrations were kept constant in $\text{DMSO-}d_6$ to compare the different diffusion coefficients. The effect of temperature on the supposed host-guest complexes was also studied where the measurements were recorded

at 300K and 310K. We expect the diffusion coefficient of the complex to be similar to that of the dendrimer should the inclusion complex form. A Bayesian DOSY transformation was performed on the data for illustrative purposes and the spectra were stacked as shown in figure 3.13. The observed diffusion coefficient is a molecular fraction weighted average between a “bound” and “free” guest molecule which is represented by equation (4):

$$D_{\text{obs}} = D_{\text{bound}}(X) + D_{\text{free}}(X-1) \quad (4)$$

Where D_{obs} , D_{bound} and D_{free} represent the observed, bound, free diffusion coefficient and X , is the molecular fraction.²⁷

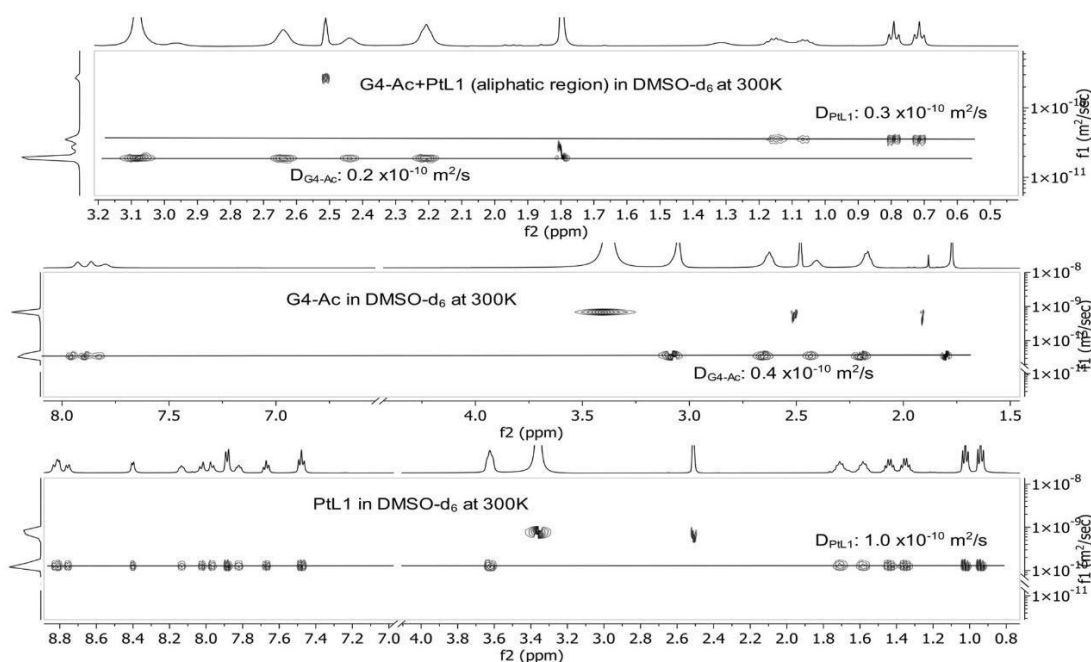


Figure 3.13: Stacked DOSY spectra of G4-Ac +PtL1 mixture, G4-Ac and PtL1 in DMSO- d_6 at 300K.

In our case, a single set of crosspeaks corresponding to the complex are seen instead of a set of signals corresponding to the “bound” complex and another for the “free” complex (figure 3.13). This means that the interaction between the dendrimer and complex is in fast exchange where the complex migrates in and out of the dendrimer cavities which indicates that it is weakly bound; this kind of interaction has been observed in large guest molecules such as surfactants.^{27–29} This dynamic interaction is faster than the NMR time scale, therefore an average between the two states are observed.

More accurate diffusion coefficients of G4-Ac, DMSO- d_6 and PtL1 were calculated by manually fitting the data using Mestrenova software and the most isolated peaks were used in order to minimize any errors associated with overlapping peaks. The integrations of the

selected peaks were used to calculate the diffusion coefficients using the monoexponential fit represented by mathematical function (5).

$$Y' = B * \exp(-x * F) \quad (5)$$

Where Y' is the integral of the selected signal, B and x are optimal parameters computed by the software and lastly, F is the diffusion coefficient in the units cm^2/s which were then converted to m^2/s as shown in figure 3.14.

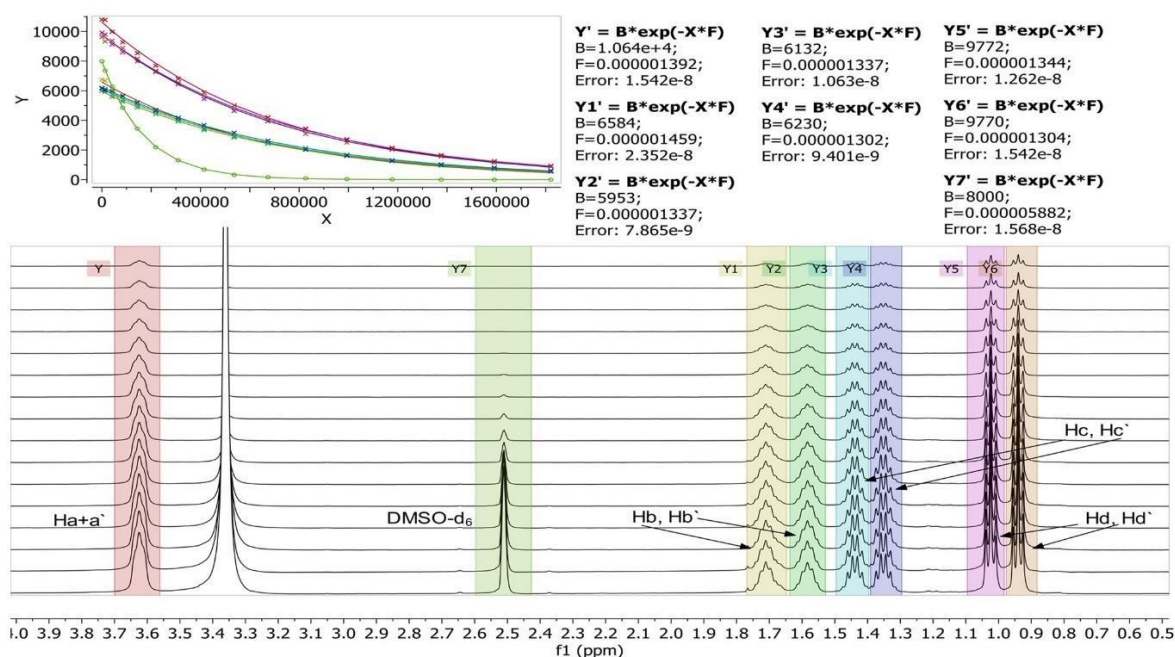


Figure 3.14: Example of manually fitted diffusion coefficients of PtL1 (aliphatic region) in DMSO-d₆ from Mestrenova software.

Only internal dendrimer protons were considered in the calculation of the diffusion coefficients for G4-Ac as the surface acetamide functional groups had a larger diffusion coefficient. The diffusion coefficient of PtL1 was calculated using the aliphatic protons as the results obtained from that region were more reliable than the aromatic region due to minimal peak overlap. Since a conclusion could not be made based on diffusion coefficients only, hydrodynamic volumes were required to ascertain the extent of this interaction. Viscosity measurements were recorded on the samples at 300K and 310K using the AntonPaar Microviscometer LOVIS2000M. The obtained viscosity values were used to calculate the hydrodynamic radii (R_H) using the Stokes-Einstein equation (3) which were then related to the hydrodynamic volume (V_H). V_H is a useful property as it can be used to model any interactions a molecule may be undergoing which is particularly important for host-guest interactions. It takes viscosity differences into account which makes it an ideal unit in which data can be compared although it assumes a spherical molecule (which brings in a certain

margin of error as not every molecule is spherical). The viscosities, diffusion coefficients and corresponding hydrodynamic volumes at 300K and 310K are summarized in table 3.2.

Table 3.2: Diffusion coefficients and hydrodynamic volume (V_H) of PtL1+ G4 -Ac mixture, PtL1 in DMSO- d_6 as well as G4-Ac in DMSO- d_6 at 300K and 310K.

Temperature	300K				310K			
	Viscosity ($\times 10^{-3}$ Pa.s)	Diffusion coefficient ($\times 10^{-10}$ m ² /s) and V_H (nm ³)			Viscosity ($\times 10^{-3}$ Pa.s)	Diffusion coefficient ($\times 10^{-10}$ m ² /s) and V_H (nm ³)		
Sample in DMSO- d_6	-	G4- Ac	PtL1	DMSO- d_6	-	G4- Ac	PtL1	DMSO- d_6
PtL1 and G4-Ac	4.1	0.2 (97.2)	0.3 (15.3)	2.3 (0.05)	3.0	0.4 (36.4)	0.7 (6.3)	3.2 (0.05)
PtL1	2.5	-	1.0 (2.5)	4.8 (0.02)	2.0	-	1.4 (2.1)	6.2 (0.02)
DMSO- d_6	2.0	-	-	6.1 (0.02)	1.7	-	-	7.7 (0.02)
G4-Ac	2.6	0.4 (49.5)	-	4.2 (0.03)	2.1	0.5 (35.2)	-	5.4 (0.03)

The viscosity of the PtL1 and G4-Ac mixture was recorded to be 4.1×10^{-3} Pa.s whereas PtL1 and G4-Ac were recorded to be 2.5×10^{-3} and 2.6×10^{-3} Pa.s respectively at 300K. At 310K a decrease in viscosity is observed in all samples which is expected as temperature and viscosity are inversely proportional. The general increase in viscosity for the mixture is not only caused by the increase in concentration but also due to an increase in the ionic strength.³⁰ The same trend was observed by Bakshi *et al* in dendrimer-surfactant systems where viscosity increased upon addition of the surfactant, accompanied by dendrimer-surfactant aggregates which were concentration dependent in their case.³¹

The hydrodynamic volumes (V_H) were calculated in order to account for the differences in viscosity. At 300K the calculated V_H of DMSO remained relatively the same throughout, except for in the mixture where an increase was observed. This is indicative of an interaction between the dendrimer and the solvent. The dendrimer cavities favour hydrophobic guest molecules, therefore minimal DMSO molecules were entrapped in the voids which was reflected in the slight increase of V_H . In the case of the complex, a decrease in the diffusion coefficient from 1.0×10^{-10} to 0.3×10^{-10} m²/s was observed once it was added to the dendrimer which was accompanied by an 84% increase in V_H . This indicates a stronger interaction with the dendrimer than what is seen between the solvent and the dendrimer. A decrease in the diffusion coefficient from 0.4×10^{-10} to 0.2×10^{-10} m²/s of the dendrimer is

seen once mixed with the complex accompanied by a 49% increase in V_H . From this, we can state that there is an interaction between the dendrimer and the complex taking place as the possible host-guest complex diffuses slower than the plain G4-Ac. At 310K, an overall decrease in V_H of both the dendrimer and complex are seen in the mixture. This means that the complex is weakly bound to the dendrimer and the host-guest molecule is easily disrupted at higher temperatures. In figure 3.12, significant peak overlap is seen in the aromatic region. It is known that conjugated aromatic compounds are easily encapsulated in the dendrimer structure and the main interaction takes place through the aromatic groups²⁶ We propose that the complex interacts with the dendrimer largely via the phenanthroline moiety although interaction via the di(alkyl) part of the ligand should not be completely ruled out.

The effect of ionic strength on dendrimer diffusion behaviour was studied since the platinum complex is a salt. Tetrabutylammonium chloride ([TBA]Cl) was added to G4-Ac in the same concentration as the platinum complex. This sample was stirred for 24 hours and then analysed. The ^1H NMR, diffusion coefficients, viscosities were obtained and the respective hydrodynamic volumes (V_H) were calculated. The results are summarised in table 3.3.

Table 3.3: Viscosity, diffusion coefficients and hydrodynamic volume (V_H) of [TBA]Cl + G4-Ac mixture, [TBA]Cl as well as G4-Ac in DMSO- d_6 at 300K and 310K.

Temperature	300K				310K			
	Viscosity ($\times 10^{-3}$ Pa.s)	Diffusion coefficient ($\times 10^{-10}$ m ² /s) and V_H (nm ³)			Viscosity ($\times 10^{-3}$ Pa.s)	Diffusion coefficient ($\times 10^{-10}$ m ² /s) and V_H (nm ³)		
Sample in DMSO- d_6	-	G4-Ac	[TBA]Cl	DMSO- d_6	-	G4-Ac	[TBA]Cl	DMSO- d_6
G4-Ac and [TBA]Cl	3.2	0.3 (71.5)	1.7 (0.3)	3.5 (0.03)	2.5	0.5 (22.4)	2.4 (0.2)	5.1 (0.02)
[TBA]Cl	1.9	-	2.4 (0.5)	6.1 (0.03)	1.6	-	3.2 (0.4)	7.8 (0.03)
DMSO- d_6	2.0	-	-	6.124 (0.02)	1.7	-	-	7.7 (0.02)
G4-Ac	2.6	0.4 (49.5)	-	4.179 (0.03)	2.1	0.5 (35.2)	-	5.4 (0.03)

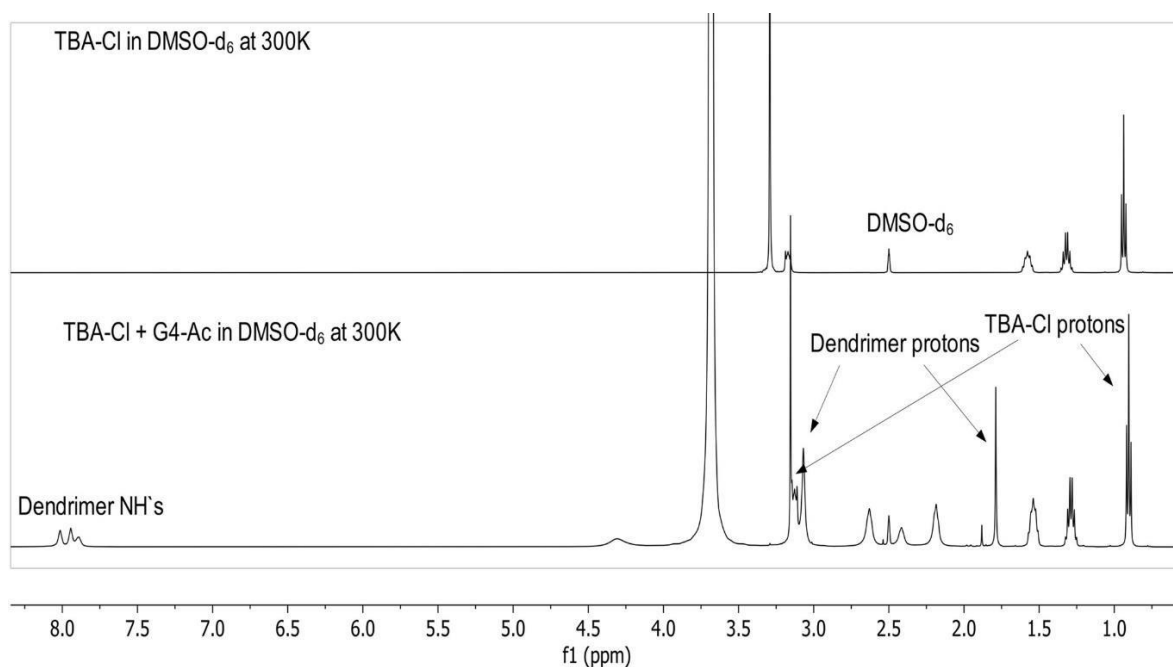


Figure 3.15: Stacked ^1H NMR spectra of [TBA]Cl in DMSO and G4-Ac and [TBA]Cl mixture in DMSO- d_6 at 300K.

No difference in chemical shift nor changes in the shape of [TBA]Cl peaks are seen in figure 3.15 upon addition to the dendrimer as opposed to what is seen with the PtL1 complex (figure 3.12). Once again, an increase in viscosity was observed in the mixture which is due to an increase in ionic strength. At 300K, the V_{H} of plain DMSO was calculated to be 0.02 nm^3 where in G4-Ac, TBA[Cl] and G4-Ac [TBA]Cl mixture V_{H} was calculated to be 0.03 nm^3 (table 3.3). This increase in V_{H} indicates an interaction between the DMSO and G4-Ac and [TBA]Cl respectively. What is interesting is the 40% decrease in the V_{H} of [TBA]Cl while a 31% increase in the V_{H} of G4-Ac is observed while accompanied by a decrease in diffusion of both species in solution (table 3.3). This suggests dendrimer aggregation instead of [TBA]Cl encapsulation and increased ionic strength has been shown to cause aggregation in certain polymeric systems.^{30–32} This further confirms the formation of a host-guest molecule between the platinum complex and the dendrimer as V_{H} of the complex increases when introduced to the dendrimer. Furthermore, this finding is in agreement with the proposition that the platinum complex interacts with the dendrimer mainly through the aromatic region.

At 310K, a decrease in V_{H} in all samples was observed with the most significant decrease being G4-Ac in the mixture. A 69% decrease was observed when the temperature was increased which means that there was disruption of the dendrimer aggregates at higher temperatures.

Dendrimer size was varied to G5-Ac to compare to the findings obtained with G4-Ac. An excess of PtL3 in DMSO- d_6 and D_2O (6:1) was added to G5-Ac and an excess of PtL2 was added to G4-Ac. The D_2O was used to assist in the dissolution of the dendrimers. Both

individual mixtures were left to stir in the dark for 24 hrs and the ^1H NMR and NOESY spectra of the resultant solutions were recorded (figure 3.16)

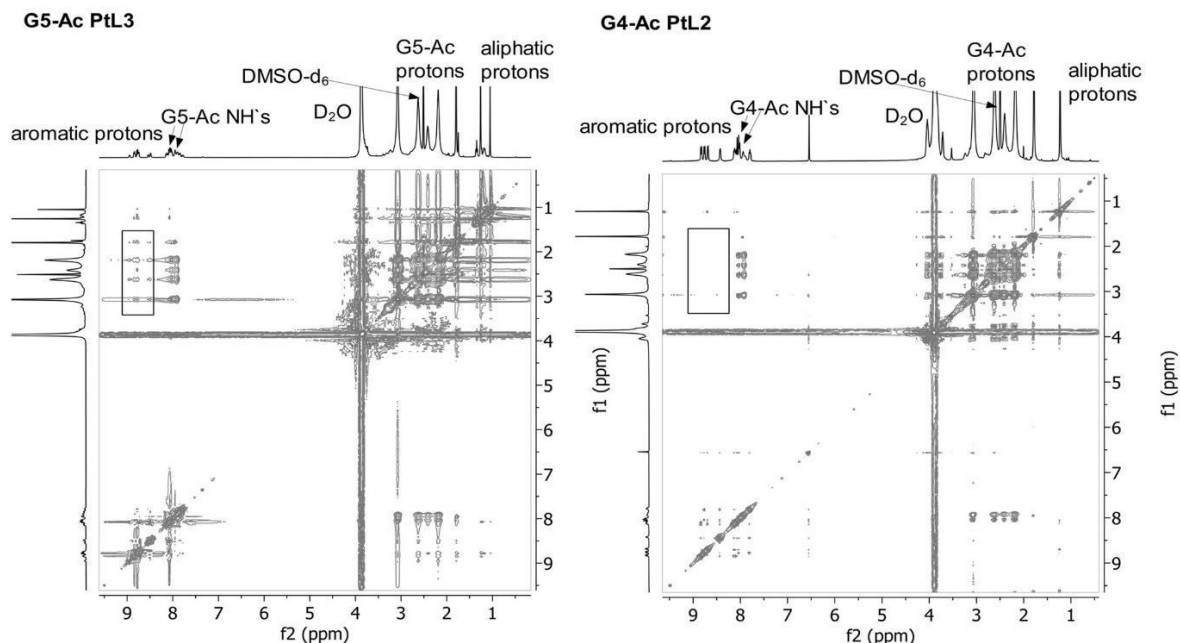


Figure 3.16: NOESY spectrum of PtL3 and G5-Ac mixture (left) and PtL2 and G4-Ac mixture (right) in DMSO- d_6 and D_2O (6:1) at 300K.

In G5-Ac and PtL3 (left), broadening of the aromatic protons is seen which is due to the combined effect of the presence of water (D_2O) as well as an interaction with the dendrimer. NOE crosspeaks between the complex's aromatic protons and internal dendrimer protons are seen which indicate a stronger interaction with the G5-Ac as compared to G4-Ac because G5-Ac has larger cavities. Fang *et al* obtained a similar result where NOE's were observed between the guest molecule and G5 and G5-Ac but none for G4 and G4-Ac.³³

3.5 Conclusion

The encapsulation of platinum complexes in G4 PAMAM was not successful as the dendrimer is found to be polycationic even at near neutral pH. For this reason, encapsulation in G4-FA was not attempted. Solvent has proven to play an important part in the encapsulation process. Between the used solvents (MeOD- d_4 , EtOD- d_6 , D_2O , DCM and DMSO- d_6) DMSO- d_6 showed the most promising results with G4-Ac. Significant chemical shift differences were observed in PtL1 upon addition to G4-Ac in DMSO- d_6 where a 0.26 ppm and 0.25 ppm shift was seen in H_d and H_d' of PtL1 upon addition to G4-Ac. The listed chemical shift differences were indicative of an interaction between the PtL1 complex and G4-Ac dendrimer. DOSY NMR data revealed further proof of association as the diffusion coefficient of the components in the

mixture (PtL1: $0.3 \times 10^{-10} \text{ m}^2/\text{s}$, G4-Ac: $0.2 \times 10^{-10} \text{ m}^2/\text{s}$) was smaller than that of the free species (PtL1: $1.0 \times 10^{-10} \text{ m}^2/\text{s}$, G4-Ac: $0.4 \times 10^{-10} \text{ m}^2/\text{s}$) at 300K. The DOSY data further revealed that the interaction of PtL1 and G4-Ac were in fast exchange relative to the NMR time-scale where the complex migrates in and out of the dendrimer cavities.

Since a conclusion could not be made based on diffusion coefficients only, viscosity experiments were conducted in order to calculate the hydrodynamic radii (R_H) and hydrodynamic volumes (V_H). G4-Ac, DMSO- d_6 , PtL1 and the G4-Ac and PtL1 mixture were recorded to have viscosities of $2.6 \times 10^{-3} \text{ Pa.s}$, $2.0 \times 10^{-3} \text{ Pa.s}$, $2.5 \times 10^{-3} \text{ Pa.s}$ and $4.1 \times 10^{-3} \text{ Pa.s}$ respectively at 300K. This major increase in viscosity in the mixture was not only attributed to an increase in concentration but also to an increase in ionic strength as PtL1 is cationic. An 84% and 49% increase in V_H was observed in PtL1 and G4-Ac respectively in the mixture which further indicated an interaction between PtL1 and G4-Ac. At 310K, an overall decrease in V_H was observed which indicated that PtL1 was weakly bound to G4-Ac as the host-guest complex is disrupted at higher temperatures.

The effect of ionic strength was briefly studied since the platinum complexes are positively charged and have a counterion. An increase in viscosity was observed upon addition of [TBA]Cl to G4-Ac ($3.2 \times 10^{-3} \text{ Pa.s}$) whereas the viscosity of G4-Ac and [TBA]Cl were recorded to be $2.6 \times 10^{-3} \text{ Pa.s}$ and $1.9 \times 10^{-3} \text{ Pa.s}$ respectively. This increase in viscosity was also attributed to an increase in ionic strength as similarly observed in the PtL1 and G4-Ac mixture. Interestingly, a 31% increase in G4-Ac V_H was observed while a 40% decrease in [TBA]Cl V_H was observed at 300K. This finding was indicative of dendrimer aggregation instead of [TBA]Cl encapsulation. At 310K a 69% decrease in V_H of G4-Ac was observed which indicates the disassembling of the aggregates.

Dendrimer size was found to have an effect on encapsulation. G5-Ac PAMAM with larger cavities displayed improved host-guest interactions with the platinum complexes which was seen in the presence of NOE cross peaks between the PtL3 aromatic protons and internal G5-Ac protons. In G4-Ac, no NOE crosspeaks between PtL2 and the dendrimer were observed. From literature, it was found that conjugated aromatic compounds are encapsulated readily in PAMAMs dendrimers. We therefore proposed that the interaction occurs largely through the aromatic moiety although interaction through the aliphatic parts of the ligand should not be ruled out. The presence of the NOE crosspeaks between the aromatic region of PtL3 and G5-Ac support this hypothesis.

Future studies are aimed at exploring anionic PAMAM dendrimers to investigate potential electrostatic interactions with the positively charged complexes as well as an amine-protected folic acid functionalized dendrimer.

3.6 References

- (1) Peega, T.; Magwaza, R. N.; Harmse, L.; Kotzé, I. A. Synthesis and Evaluation of the Anticancer Activity of [Pt(Diimine)(N,N-Dibutyl-N'-Acylthiourea)]⁺ Complexes. *Dalton Transactions* **2021**. <https://doi.org/10.1039/D1DT01385H>.
- (2) Magwaza, R. N.; Africa, S.; Magwaza, R. N. Synthesis and Characterization of Novel [Pt (Diimine) (Acylthiourea)] + Complexes as Potential Anticancer Agents and Exploring the Use of Sulphobutyl-Ether- β -Cyclodextrin and Surfactant Micelles as a Drug Delivery System.
- (3) Loftsson, T.; Brewster, M. E. Pharmaceutical Applications of Cyclodextrins : **2010**, 1607–1621. <https://doi.org/10.1111/j.2042-7158.2010.01030.x>.
- (4) Kalepu, S.; Nekkanti, V. Insoluble Drug Delivery Strategies : Review of Recent Advances and Business Prospects. *Acta Pharm Sin B* **2015**, 5 (5), 442–453. <https://doi.org/10.1016/j.apsb.2015.07.003>.
- (5) Caminade, A. M.; Turrin, C. O. Dendrimers for Drug Delivery. *J Mater Chem B* **2014**, 2 (26), 4055–4066. <https://doi.org/10.1039/c4tb00171k>.
- (6) Kolhatkar, R. B.; Kitchens, K. M.; Swaan, P. W.; Ghandehari, H. Surface Acetylation of Polyamidoamine (PAMAM) Dendrimers Decreases Cytotoxicity While Maintaining Membrane Permeability. *Bioconjug Chem* **2007**, 18 (6), 2054–2060. <https://doi.org/10.1021/bc0603889>.
- (7) Taghavi Pourianazar, N.; Mutlu, P.; Gunduz, U. Bioapplications of Poly(Amidoamine) (PAMAM) Dendrimers in Nanomedicine. *Journal of Nanoparticle Research*. Kluwer Academic Publishers 2014. <https://doi.org/10.1007/s11051-014-2342-1>.
- (8) Janaszewska, A.; Lazniewska, J.; Trzepinski, P.; Marcinkowska, M.; Klajnert-Maculewicz, B. Cytotoxicity of Dendrimers. *Biomolecules* **2019**. <https://doi.org/10.3390/biom9080330>.
- (9) D'Emanuele, A.; Jevprasesphant, R.; Penny, J.; Attwood, D. The Use of a Dendrimer-Propranolol Prodrug to Bypass Efflux Transporters and Enhance Oral Bioavailability. *Journal of Controlled Release* **2004**, 95 (3), 447–453. <https://doi.org/10.1016/j.jconrel.2003.12.006>.
- (10) Kesharwani, P.; Jain, K.; Jain, N. K. Dendrimer as Nanocarrier for Drug Delivery. *Progress in Polymer Science*. February 2014, pp 268–307. <https://doi.org/10.1016/j.progpolymsci.2013.07.005>.
- (11) Santos, S. D.; Xavier, M.; Leite, D. M.; Moreira, D. A.; Custódio, B.; Torrado, M.; Castro, R.; Leiro, V.; Rodrigues, J.; Tomás, H.; Pêgo, A. P. PAMAM Dendrimers: Blood-Brain Barrier Transport and Neuronal Uptake after Focal Brain Ischemia. *Journal of Controlled Release* **2018**, 291, 65–79. <https://doi.org/10.1016/j.jconrel.2018.10.006>.
- (12) Hu, Q.; Wang, Y.; Xu, L.; Chen, D.; Cheng, L. Transferrin Conjugated PH- and Redox-Responsive Poly(Amidoamine) Dendrimer Conjugate as an Efficient Drug Delivery Carrier for Cancer Therapy. *Int J Nanomedicine* **2020**, Volume 15, 2751–2764. <https://doi.org/10.2147/IJN.S238536>.
- (13) Kulhari, H.; Pooja, D.; Shrivastava, S.; Kuncha, M.; Naidu, V. G. M.; Bansal, V.; Sistla, R.; Adams, D. J. Trastuzumab-Grafted PAMAM Dendrimers for the Selective Delivery of Anticancer Drugs to HER2-Positive Breast Cancer. *Sci Rep* **2016**, 6 (1), 23179. <https://doi.org/10.1038/srep23179>.
- (14) Low, P. S.; Kularatne, S. A. Folate-Targeted Therapeutic and Imaging Agents for Cancer. *Curr Opin Chem Biol* **2009**, 13 (3), 256–262. <https://doi.org/10.1016/j.cbpa.2009.03.022>.
- (15) Rompicharla, S. V. K.; Kumari, P.; Bhatt, H.; Ghosh, B.; Biswas, S. Biotin Functionalized PEGylated Poly(Amidoamine) Dendrimer Conjugate for Active Targeting of Paclitaxel in Cancer.

- Int J Pharm* **2019**, 557, 329–341. <https://doi.org/10.1016/j.ijpharm.2018.12.069>.
- (16) Abedi-Gaballu, F.; Dehghan, G.; Ghaffari, M.; Yekta, R.; Abbaspour-Ravasjani, S.; Baradaran, B.; Ezzati Nazhad Dolatabadi, J.; Hamblin, M. R. PAMAM Dendrimers as Efficient Drug and Gene Delivery Nanosystems for Cancer Therapy. *Appl Mater Today* **2018**, 12, 177–190. <https://doi.org/10.1016/j.apmt.2018.05.002>.
 - (17) Maiti, P. K.; Çağın, T.; Lin, S. T.; Goddard, W. A. Effect of Solvent and PH on the Structure of PAMAM Dendrimers. *Macromolecules* **2005**, 38 (3), 979–991. <https://doi.org/10.1021/ma049168l>.
 - (18) Patri, A. K.; Kukowska-Latallo, J. F.; Baker, J. R. Targeted Drug Delivery with Dendrimers: Comparison of the Release Kinetics of Covalently Conjugated Drug and Non-Covalent Drug Inclusion Complex. *Adv Drug Deliv Rev* **2005**, 57 (15), 2203–2214. <https://doi.org/10.1016/j.addr.2005.09.014>.
 - (19) Buczkowski, A.; Sekowski, S.; Grala, A.; Palecz, D.; Milowska, K.; Urbaniak, P.; Gabryelak, T.; Piekarski, H.; Palecz, B. Interaction between PAMAM-NH₂ G4 Dendrimer and 5-Fluorouracil in Aqueous Solution. *Int J Pharm* **2011**, 408 (1–2), 266–270. <https://doi.org/10.1016/j.ijpharm.2011.02.014>.
 - (20) Huo, R.; Wehrens, R.; van Duynhoven, J.; Buydens, L. M. C. Assessment of Techniques for DOSY NMR Data Processing. In *Analytica Chimica Acta*; Elsevier, 2003; Vol. 490, pp 231–251. [https://doi.org/10.1016/S0003-2670\(03\)00752-9](https://doi.org/10.1016/S0003-2670(03)00752-9).
 - (21) Sebastião, R. C. O.; Pacheco, C. N.; Braga, J. P.; Piló-Veloso, D. Diffusion Coefficient Distribution from NMR-DOSY Experiments Using Hopfield Neural Network. *Journal of Magnetic Resonance* **2006**, 182 (1), 22–28. <https://doi.org/10.1016/j.jmr.2006.06.005>.
 - (22) Johnson, C. S. Diffusion Ordered Nuclear Magnetic Resonance Spectroscopy: Principles and Applications. *Prog Nucl Magn Reson Spectrosc* **1999**, 34, 203–256.
 - (23) Barra, P. A.; Barraza, L. F.; Jiménez, V. A.; Gavin, J. A.; Alderete, J. B. Drug-Dendrimer Supramolecular Complexation Studied from Molecular Dynamics Simulations and NMR Spectroscopy. *Struct Chem* **2014**, 25 (5), 1443–1455. <https://doi.org/10.1007/s11224-014-0424-4>.
 - (24) Plutín, A. M.; Alvarez, A.; Mocelo, R.; Ramos, R.; Sánchez, O. C.; Castellano, E. E.; da Silva, M. M.; Villarreal, W.; Colina-Vegas, L.; Pavan, F. R.; Batista, A. A. Structure/Activity of PtII/N,N-Disubstituted-N'-Acylthiourea Complexes: Anti-Tumor and Anti-Mycobacterium Tuberculosis Activities. *J Braz Chem Soc* **2018**, 29 (6), 1256–1267. <https://doi.org/10.21577/0103-5053.20170222>.
 - (25) Kotzé, I. A. Cation- π Induced Association and Nano-Structured Aggregate Formation of Water-Soluble [Pt II (Diimine)(L n - S , O)] + Complexes Examined with High-Resolution 1 H and DOSY NMR : Towards Understanding Their Potential Antimalarial Activity By. **2013**, No. December.
 - (26) Jolly, A. M.; Bonizzoni, M. Intermolecular Forces Driving Encapsulation of Small Molecules by Pamam Dendrimers in Water. *Macromolecules* **2014**, 47 (18), 6281–6288. <https://doi.org/10.1021/ma5014868>.
 - (27) Posadas, I.; Romero-Castillo, L.; el Brahmi, N.; Manzanares, D.; Mignani, S.; Majoral, J. P.; Ceña, V. Neutral High-Generation Phosphorus Dendrimers Inhibit Macrophage-Mediated Inflammatory Response in Vitro and in Vivo. *Proc Natl Acad Sci U S A* **2017**, 114 (37), E7660–E7669. <https://doi.org/10.1073/pnas.1704858114>.
 - (28) Posadas, I.; Romero-Castillo, L.; el Brahmi, N.; Manzanares, D.; Mignani, S.; Majoral, J. P.; Ceña,

- V. Neutral High-Generation Phosphorus Dendrimers Inhibit Macrophage-Mediated Inflammatory Response in Vitro and in Vivo. *Proc Natl Acad Sci U S A* **2017**, *114* (37), E7660–E7669. <https://doi.org/10.1073/pnas.1704858114>.
- (29) Fang, M.; Cheng, Y.; Zhang, J.; Wu, Q.; Hu, J.; Zhao, L.; Xu, T. New Insights into Interactions between Dendrimers and Surfactants. 4. Fast-Exchange/Slow-Exchange Transitions in the Structure of Dendrimer-Surfactant Aggregates. *Journal of Physical Chemistry B* **2010**, *114* (18), 6048–6055. <https://doi.org/10.1021/jp100805u>.
- (30) Amorim, L. V.; Barbosa, M. I. R.; Lira, H. de L.; Ferreira, H. C. Influence of Ionic Strength on the Viscosities and Water Loss of Bentonite Suspensions Containing Polymers. *Materials Research* **2007**, *10* (1), 53–56. <https://doi.org/10.1590/S1516-14392007000100012>.
- (31) Bakshi, M. S.; Kaura, A. Poly (Amidoamine) Dendrimer Interactions with Sodium Dodecyl Sulfate Studied by Viscosity Measurements. *Colloids Surf A Physicochem Eng Asp* **2004**, *244* (1–3), 45–51. <https://doi.org/10.1016/j.colsurfa.2004.06.018>.
- (32) Vasquez, E.; Masuelli, M. A.; Vega, E. D.; Vásquez, E.; Diaz, J. R. A.; Masuelli, M. A. Influence of the Ionic Strength in the Intrinsic Viscosity of Xanthan Gum. An Experimental Review. *Journal of Polymer and Biopolymer Physics Chemistry* **2015**, *3* (1), 12–18. <https://doi.org/10.12691/jpbpc-3-1-3>.
- (33) Fang, M.; Zhang, J.; Wu, Q.; Xu, T.; Cheng, Y. Host-Guest Chemistry of Dendrimer-Drug Complexes: 7. Formation of Stable Inclusions between Acetylated Dendrimers and Drugs Bearing Multiple Charges. *Journal of Physical Chemistry B* **2012**, *116* (10), 3075–3082. <https://doi.org/10.1021/jp211384p>.

Chapter 4

Preliminary cytotoxicity screening of PAMAM encapsulated [Pt(phen)(acylthiourea)]⁺ complexes

4.1 Introduction

The need for the development of selective and biocompatible drug delivery systems (DDS's) is ever increasing as most, if not all, clinically used cancer drugs are non-selective which results in side effects and increased toxicity. Selectivity may be achieved through active targeting where vectors can be covalently attached to a drug or drug delivery system. Vectors may be covalently bound to drugs through careful design of a bond that can be cleaved upon entry to the target cell through a stimulus.¹ A less complicated and more popular alternative is active targeting through the introduction of a DDS. Drugs can be non-covalently bound to a vector-conjugated DDS which eliminates issues faced with cleavable bonds.^{1,2} Furthermore, it is important that the DDS itself displays minimal cytotoxicity and is able to release the drug efficiently upon entry of the target cell. Other potential drug delivery systems such as carbon nanotubes and surfactants have been explored, however, they have been found to be less biocompatible (across various concentrations).^{3,4} PAMAM dendrimers have surface functional groups that allow for functionalization and cavities that allow for drug encapsulation⁵ as discussed in section 3.2. PAMAM dendrimers have also gained interest as a potential drug delivery system due to their biocompatibility and ability to improve water solubility of encapsulated molecules⁶ therefore, the PAMAM encapsulated platinum complexes require a dose-dependent cell viability screening on cancer cells to investigate the anti-proliferative activity of free and dendrimer encapsulated platinum complexes.

The MTT assay is a commonly used method to assess cytotoxicity due to its relative simplicity and high sensitivity.⁷ This colorimetric assay relies on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to the water insoluble formazan by mitochondrial succinate hydrogenase found in the cells.^{7,8} When introduced to cells, MTT is taken in via endocytosis and transported to the mitochondria where it is reduced to the purple-coloured water insoluble formazan crystals. The crystals are then transported out of the cells to the surface through exocytosis where they are solubilized by the addition of an organic solvent (DMSO) and measured spectrophotometrically.⁹ Since the reduction of MTT to formazan only occurs in metabolically active cells, the intensity of the colour is correlated to the number of viable cells.^{8,9} This chapter describes the 2-dose cytotoxicity screening of PAMAM encapsulated complexes and 5-Fluorouracil (5FU) against lung cancer cell line A549 (figure 4.1) via the MTT assay.

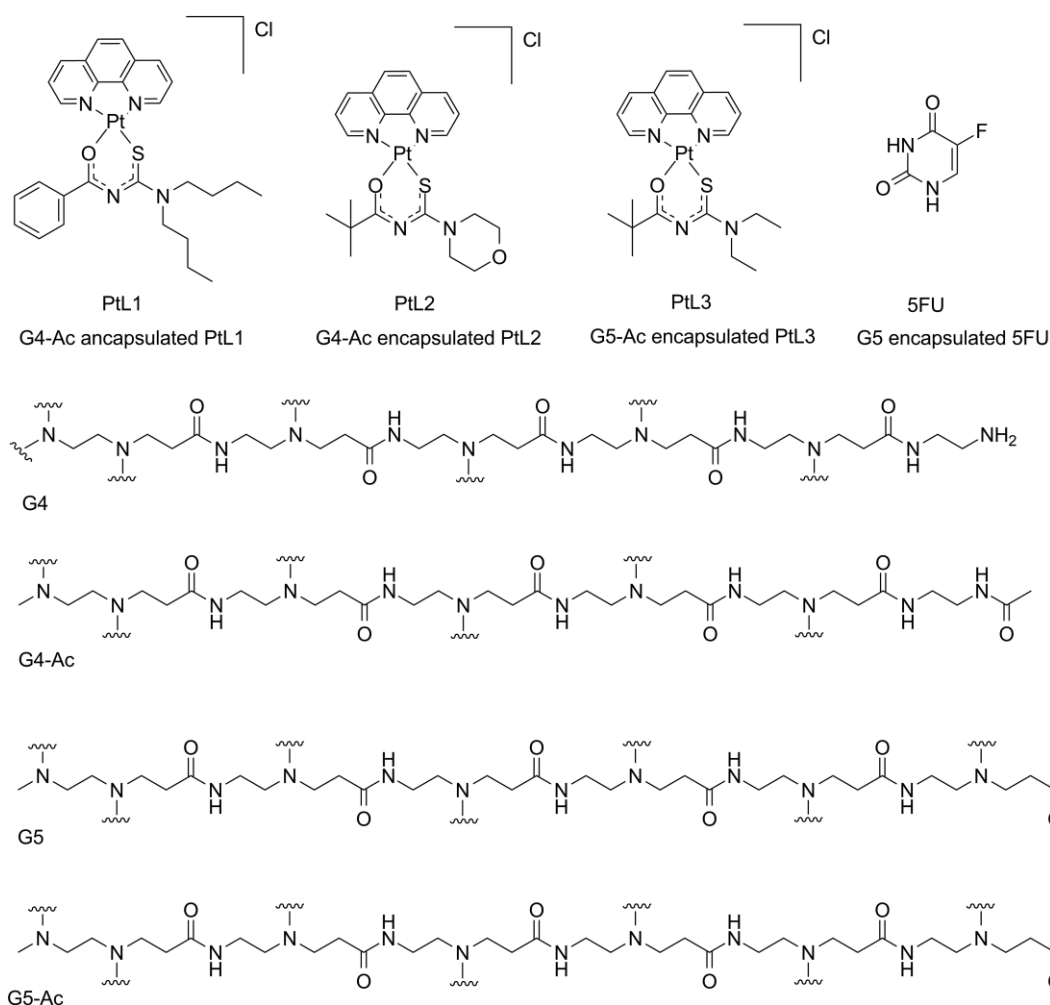


Figure 4.1: Structures of free the dendrimers, compounds and description of dendrimer encapsulated compounds screened against A549 at 50 μ M and 5 μ M.

4.2 Results and discussion

4.2.1 MTT Methodology

For this assay, 8000 cells were seeded in a 96 well plate along with cell media and were incubated for 24 hours under 5% carbon dioxide (CO_2) at 37 $^\circ\text{C}$ to allow cell attachment. Stock solutions of the free platinum complexes, free dendrimers and dendrimer encapsulated complexes were prepared as 25 mM in DMSO- $\text{d}_6/\text{D}_2\text{O}$ (6:1). These solutions were then diluted to the final concentrations of 50 μM and 5 μM using cell culture media. Cells were treated with the test compounds followed by an incubation period of 44 hours after which 40 μL of MTT solution (5 mg/mL) was added to each well followed by another 4-hour incubation. After centrifugation and careful removal of the MTT and media, DMSO was added to the wells to dissolve the formazan crystals followed by agitation for 5 minutes. The absorbance was recorded at 540 nm and 690 nm (where 690 nm serves as the background measurement) and was used to determine the percentage of viable cells. The negative control consisted of

untreated cells (with cell media), cells treated with 5FU served as the positive control and the blank consisted of wells with cell media only. The treated well plate is shown in figure 2.4.

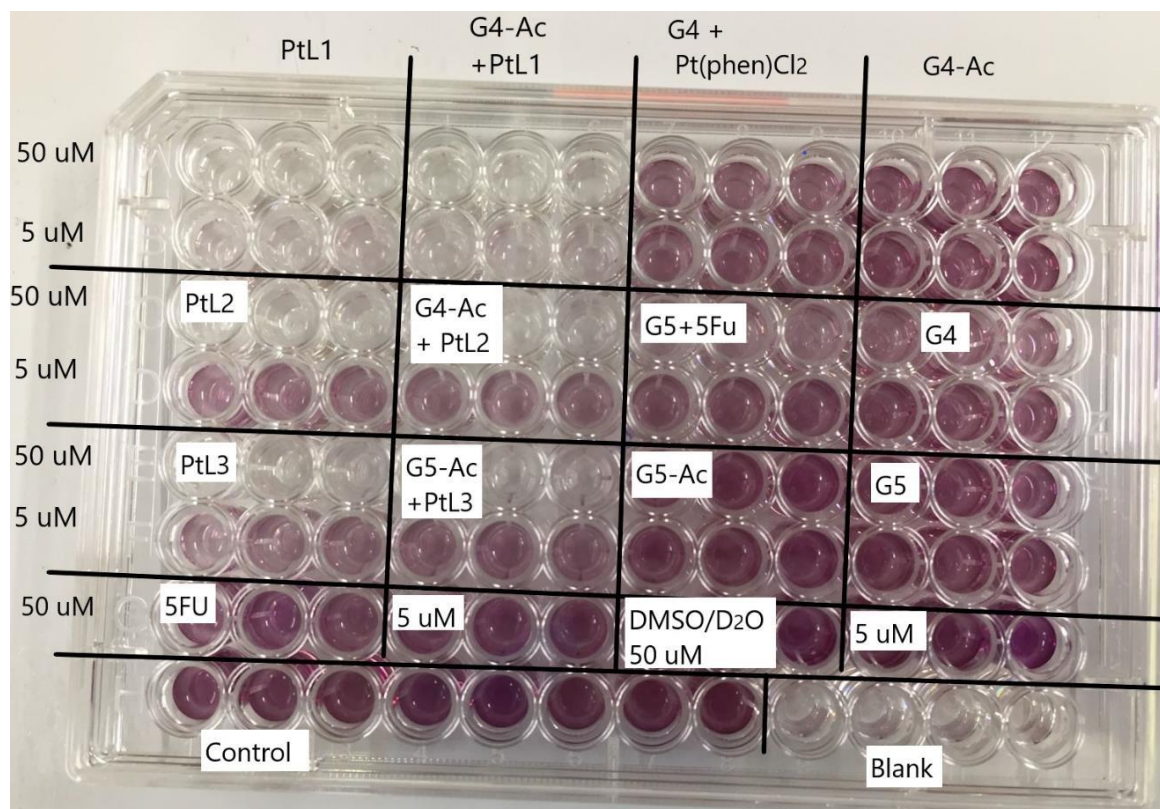
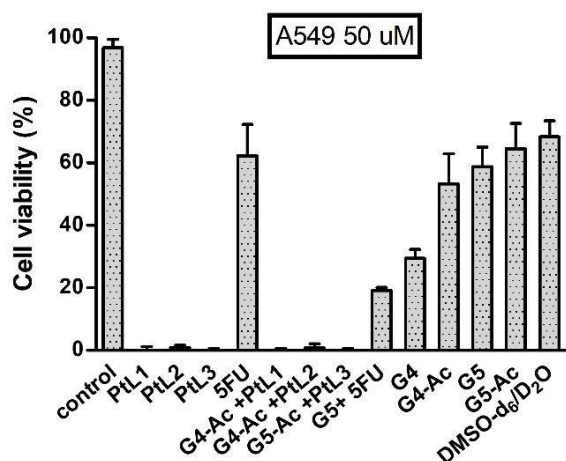


Figure 4.2: Labelled 96 well plates of the free dendrimers, free compounds and dendrimer encapsulated compounds screened at 50 μ M and 5 μ M. Refer to table 4.1 and 4.2 in the experimental section.

4.2.2 Preliminary cytotoxicity screening via MTT assays

The encapsulation of the platinum complexes was conducted in a (6:1) DMSO- d_6 /D $_2$ O mixture therefore, it was important to test the effect of the solvent on cell viability alongside the complexes at the same percentage in which the two doses (50 μ M and 5 μ M) were prepared. At high concentration (50 μ M) the cell viability was found to be 69.8% and at low concentrations (5 μ M), 76.5% as shown in figure 4.3a and 4.3b. The solvent showed the least amount of cytotoxicity compared to every sample at both concentrations, therefore, the results obtained for the other samples can be largely attributed to the active agents i.e. the complex and dendrimer although the solvent is not innocent. We suspect that the observed cytotoxicity is a result of the deuterated solvent as they are found to display higher cytotoxic activity compared to their non-deuterated counterparts as investigated by Manoharan *et al.*¹⁰

A)



B)

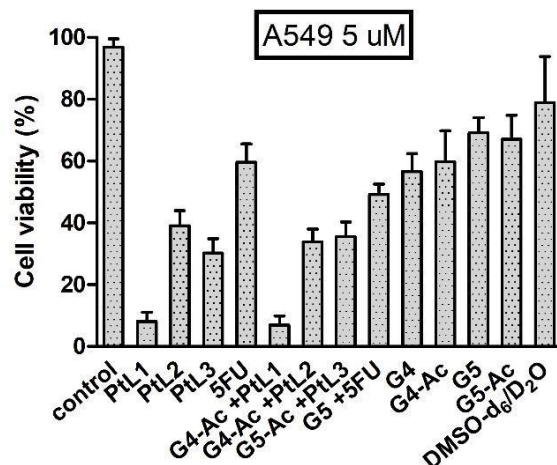


Figure 4.3: % Cell viability of A549 cancer cell line at a concentration of A) 50 uM and B) 5 uM.

At 50 μ M, all platinum complexes display cytotoxicity as the cell viability was found to be 0.18%, 0.86% and 0.0% for PtL1, PtL2 and PtL3 respectively (figure 4.3a). Interestingly, at this high concentration, the dendrimers: G4-Ac, G5 and G5-Ac still have a considerable number of viable cells at 53.30%, 58.78% and 64.53% respectively except for G4 which displays the highest cytotoxicity as seen with a cell viability of 29.42% (figure 4.3a).

Based on found evidence in literature, cytotoxicity is directly proportional to dendrimer size,^{11,12} however, the G5 and G5-Ac dendrimer unexpectedly showed less cytotoxicity as compared to G4 and G4-Ac in this cell line. Acetamide functionalization led to lower innate dendrimer toxicity by 45% for the transformation of G4 to G4-Ac and 9% for G5 to G5-Ac at 50 μ M (table 4.1) which was expected as cationic dendrimers cause cell lysis through interactions with the lipid bilayer whereas neutral (and anionic) dendrimers do not.^{13,14} At 5 μ M, PtL1 displayed the highest cytotoxic activity against A549 cancer cell line where the cell viability was found to be 8.16% whereas those of PtL2 and PtL3 were found to be 39.04% and 30.21% respectively (figure 4.3b). The dendrimers still display moderate biocompatibility as the cell viability of G4, G4-Ac, G5 and G5-Ac were found to be 54.56%, 59.81%, 69.12% and 67.03% respectively. Once again acetamide functionalization lowered dendrimer cytotoxicity for G4 as seen by a 5% increase in cell viability at 5 μ M. However, the opposite was observed for G5 where a 3% decrease in cell viability was observed upon functionalization at 5 μ M, this discrepancy warrants the need for more screening to be conducted to certify this observation. Although it is ideal to have a larger sample size from this data we can conclude that PAMAM dendrimers are biocompatible with A549 cell line at low and high concentrations.

G4-Ac + PtL1, G4-Ac + PtL2 and G5-Ac + PtL3 display high activity at 50 μ M as seen in the low cell viability values of 0.0%, 0.84% and 0.0% respectively. At lower concentration (5 μ M),

G4-Ac + PtL1 displayed the highest activity with a corresponding cell viability of 6.94% which was lower than that of free PtL1 (8.16%) and the same observation was made for G4-Ac + PtL2. This could be indicative of effective drug delivery, and not a result of the combined effect of the complex and the dendrimer since the G4-Ac is dilute in both samples (concentration of G4-Ac < 1 μ M) (table 5.1, appendix). In the case of G5-Ac + PtL3, the cell viability was found to be 35.57% where free PtL3 at the same concentration was found to be 30.21%. This could be indicative of inefficient complex release as PtL3 could be better encapsulated in the larger G5-Ac cavities as discussed in section 3.4.

5FU was used as positive control and was found to be less active than the platinum complexes and the cell viability was found to be 62.27% at 50 μ M and 59.63% at 5 μ M. At both concentrations a decrease in cell viability was observed upon dendrimer encapsulation. At the low concentration (5 μ M), the cell viability was found to be 59.63% for free 5FU whereas the host-guest complex displayed a cell viability of 49.24%. At the higher concentration, G5+5FU (19.11%) was far more toxic than free 5FU (62.27%). The increased potency in this case could possibly be attributed to prolonged bioavailability due to slow release as observed by Ly *et al.*¹⁵

Future work entails conducting more screening to further investigate the drug delivery capabilities of the PAMAM dendrimers, exploring testing against normal cells to test for selectivity using folic acid functionalized PAMAM as well as exploring more methods in which dendrimer toxicity can be lowered such as poly ethylene glycol (PEG) functionalization. Additionally, exploring ionic interactions between the positively charged platinum complexes and an anionic dendrimer would make an interesting study.

4.3 Conclusion

Since the encapsulation of the platinum complexes was conducted in a (6:1) DMSO- d_6 /D₂O mixture, it was important to test the effect of the solvent on cell viability. It was initially suspected that the observed cytotoxicity was a result of the deuteration since deuterated solvents were found to display higher cytotoxicity as compared to their non-deuterated counterparts as investigated by Manoharan *et al.*¹⁰ In our findings, the solvent displayed the least amount of cytotoxicity against the A549 cell line compared to every sample screened therefore the observed cytotoxicity can be largely attributed to the complex and dendrimers although the solvent is not completely innocent. All platinum complexes displayed high activity showing a cell viability of less than 1% for all three complexes at 50 μ M. PtL1 displayed the highest activity with a cell viability of 8.16% at 5 μ M. At 50 μ M all dendrimer encapsulated complexes were highly active as seen in a cell viability of less than 1% for all three host-guest

molecules. PtL1+ G4-Ac was the most active at 5 μ M with a cell viability of 6.94% which is lower than that of free PtL1 which could possibly indicate effective drug delivery. G4-Ac, G5 and G5-Ac displayed moderate biocompatibility as seen with a cell survival of over 50% for all three dendrimers with G4 being the most toxic with a cell viability of 29.42% at 50 μ M. At low concentration, all dendrimers display a cell survival of more than 50%. Acetamide functionalization decreased dendrimer toxicity as both G4-Ac and G5-Ac displayed lower toxicity as compared to G4 and G5 at 50 μ M, however, the opposite was observed for G5/G5-Ac. This discrepancy warrants the need for more tests to be conducted in the future to thoroughly investigate the effect of acetamide functionalization, however, from this data we can conclude that dendrimers are biocompatible with the A549 cell line.

A549 cells displayed less sensitivity to the positive control (5FU) as compared to the platinum complexes with a cell survival of 62.27% at 50 μ M and 59.63% at 5 μ M. G5-5FU on the other hand displayed improved activity as seen in the cell viability of 19.11% at 50 μ M and 49.24% at 5 μ M. The increase in anticancer activity in this case could possibly be a result of prolonged bioavailability as observed by Ly *et al.*¹⁵

Future work entails conducting more screening to further investigate the drug delivery capabilities of the PAMAM dendrimers, as well as against normal cells to test for selectivity using folic acid once encapsulation in folic acid functionalized PAMAM has been achieved. Methods to lower inherent dendrimer toxicity should be explored. Additionally, exploring ionic interactions between the positively charged platinum complexes and an anionic dendrimer as an alternative non-covalent transportation method should be explored.

4.4 Experimental

Cell culture

Aseptic techniques were employed in a cell culture lab while growing, maintaining and setting up experiments. All experiments were conducted in sterile laminar flow and 75% ethanol was used to sterilize surfaces; other utensils and pieces of equipment were sterilized by autoclaving. Solutions such as buffers and culture media were prepared with ultrapure MilliQ water. To monitor cell growth and microbial or fungal contamination, cells were observed daily with an Olympus DP72 inverted phase contrast microscope while growing in their respective cell culture flasks. A549 cells used for this study were purchased from the Fox Chase Cancer Institute.

Cell culture media preparation

A549 cells were grown using a 1:1(v/v) mixture of Hams F-12 culture media: Dulbecco's Modified Eagle's Medium (DMEM). This culture media was supplemented with 10 % fetal bovine serum (FBS), 1% sodium pyruvate and 1% L-glutamine and was sterile filtered before use. The media was stored at 4 °C warmed to 37 °C prior to use in culture. Culture media and FBS were obtained from ThermoFisher, Johannesburg.

Trypan blue exclusion assay (cell counting)

Trypan blue exclusion assay along with a haemocytometer was used to determine the number of viable cells from a cell suspension. Trypan blue allows us to distinguish between live and dead cells on the basis of colour. The dye is able to pass through the cell membranes of dead cells thereby staining them blue while live cells remain clear.¹⁶ This assay is conducted before cell plating for any experiment. The assay was conducted by pipetting 20 µL of 0.2 % trypan blue (Sigma-Aldrich) into 20 µL of the cell suspension in a small Eppendorf tube under sterile conditions. 20 µL of this 1:1 trypan blue and cell suspension mixture was added to a haemocytometer and cells were counted. Cells were only used in experiments if their viability exceeded 95 %.

Phosphate buffered saline

Phosphate buffered saline (PBS) solution was used to wash/rinse cells in culture flasks and to remove residual media that would inhibit the trypsinization process. A PBS solution was prepared by measuring the following concentrations of reagents (1.37 mM NaCl, 27 mM KCl, 100 mM Na₂HPO₄ and 18 mM KH₂PO₄ (pH 7.2-7.4). The solution was then autoclaved and stored at 4 °C, however warmed to room temperature upon usage.

Trypsin

Trypsinization is a commonly used detachment technique which not only assists in detachment of adherent cells from the culture flask but also disrupts cell clusters to form single-cell suspensions. Once cells reach a confluency of at least 75%, they are detached from the culture flask by addition to a solution of the enzyme trypsin.¹⁷ Trypsin ethylenediaminetetraacetate (trypsin-EDTA) (Sigma-Aldrich) was used in this study.

MTT solution preparation

The yellow MTT powder was dissolved in 1X PBS solution at a concentration of 5mg/ml by stirring on a magnetic stirrer while covered in foil. The solution was then sterile filtered into a 50 ml centrifuge tube using 0.2 µm syringe filter and stored at 4 °C. MTT was covered with foil

upon storage since it light sensitive.

MTT Methodology

A lung cancer cell line, A549 was used to investigate the antiproliferative activity of free and dendrimer encapsulated $[\text{Pt}(\text{phen})(\text{L}^n\text{-}\kappa\text{O},\text{S})]^+$ complexes at two concentrations, 5 μM and 50 μM . Cells were grown in 75 cm^2 cell culture flasks and maintained with cell media; Dulbecco's Modified Eagle's Medium (DMEM) enriched with 10% (v/v) fetal bovine serum, 1% (v/v) sodium pyruvate and 1% (v/v) L-glutamine in a humidified incubator with 5% CO_2 at 37 $^\circ\text{C}$. An MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to determine the cell viability of cancer cells after exposure to the complexes. In this assay, 8 000 cells were seeded in each well of a 96 well plate and incubated for 24 hours to allow cells to adhere on plate surface. Stock solutions of platinum complexes were prepared as 25 mM concentration in DMSO: D_2O solvent mixture (6:1) for complete dissolution. These were diluted to final test concentrations with aqueous cell media. Cells were then treated with the test complexes; PtL1, PtL2, PtL3, free dendrimers; G4, G4, G4-Ac, G5-Ac and dendrimer encapsulated complexes; (PtL1-G4-Ac, PtL2-G4-Ac etc) and incubated for 44 hours to evaluate and compare their cytotoxicity effect against this particular cell line. Following this incubation period, using a multichannel pipette, 40 μL of MTT solution (5 mg mL^{-1}) was added to each well and the cells were further incubated for 4 hours. Viable cells were able to metabolize MTT into purple formazan crystals via an enzyme called succinate dehydrogenase in the mitochondria of viable cells. Thereafter, each 96 well plate was centrifuged at 1000 rpm for 5 minutes following a careful aspiration of cell media and MTT solution from each well. To each well, 200 μL of DMSO was added to dissolve formazan crystals. Each 96 well plate was then placed on a thermoshaker at 1000 rpm for 5 minutes (25 $^\circ\text{C}$). The absorbance was measured using a microplate Labsystems iEMS Reader at 540 nm to measure the signal and 690 nm as background. The negative control consisted of cells with media and a blank with cell media only. All measurements were conducted in triplicate for each dose of the test compound/complex and the experiment was repeated twice. The absorbances were used to determine the percentage of viable cells relative to untreated controls.

Table 4.1: Percentage cell viability of guest-host compounds screened against A549 cancer cell line at 50 μ M for 48 hours.

Compounds	Trial 1	Trial 2	Average
PtL1	0.36	0.0	0.18
PtL2	0.26	1.46	0.86
PtL3	0.0	0.0	0.0
5FU	65.64	58.89	62.27
G4-Ac +PtL1	0.0	0.0	0.0
G4-Ac + PtL2	0.31	1.36	0.84
G5-Ac +PtL3	0.0	0.0	0.0
G5+ 5FU	18.74	19.48	19.11
G4	31.84	27.00	29.42
G4-Ac	60.08	46.51	53.30
G5	58.40	59.15	58.78
G5-Ac	62.79	66.27	64.53
DMSO-d ₆ /D ₂ O	73.65	66.01	69.83

Table 4.2: Percentage cell viability of guest-host compounds screened against A549 cancer cell line at 5 μ M for 48 hours.

Compounds	Trial 1	Trial 2	Average
PtL1	6.76	9.55	8.16
PtL2	41.25	36.83	39.04
PtL3	28.49	31.92	30.21
5FU	63.48	55.77	59.63
G4-Ac +PtL1	6.07	7.81	6.94
G4-Ac + PtL2	36.18	31.61	33.90
G5-Ac +PtL3	38.10	33.04	35.57
G5+ 5FU	48.99	49.48	49.24
G4	60.82	52.44	56.63
G4-Ac	68.45	51.16	59.81
G5	69.88	68.36	69.12
G5-Ac	67.12	66.93	67.03
DMSO-d ₆ /D ₂ O	89.76	63.30	76.53

4.5 References

- (1) Patri, A. K.; Kukowska-Latallo, J. F.; Baker, J. R. Targeted Drug Delivery with Dendrimers: Comparison of the Release Kinetics of Covalently Conjugated Drug and Non-Covalent Drug Inclusion Complex. *Adv Drug Deliv Rev* **2005**, *57* (15), 2203–2214. <https://doi.org/10.1016/j.addr.2005.09.014>.
- (2) Howell, B. A.; Fan, D. Poly(Amidoamine) Dendrimer-Supported Organoplatinum Antitumour Agents. *Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences* **2010**, *466* (2117), 1515–1526. <https://doi.org/10.1098/rspa.2009.0359>.
- (3) Tan, J. M.; Karthivashan, G.; Arulselvan, P.; Fakurazi, S.; Hussein, M. Z. Sustained Release and Cytotoxicity Evaluation of Carbon Nanotube-Mediated Drug Delivery System for Betulinic Acid. *J Nanomater* **2014**, *2014*. <https://doi.org/10.1155/2014/862148>.
- (4) Dong, L.; Witkowski, C. M.; Craig, M. M.; Greenwade, M. M.; Joseph, K. L. Cytotoxicity Effects of Different Surfactant Molecules Conjugated to Carbon Nanotubes on Human Astrocytoma Cells. *Nanoscale Res Lett* **2009**, *4* (12), 1517–1523. <https://doi.org/10.1007/s11671-009-9429-0>.
- (5) Tomalia, D. A.; Nixon, L. S.; Hedstrand, D. M. The Role of Branch Cell Symmetry and Other Critical Nanoscale Design Parameters in the Determination of Dendrimer Encapsulation Properties. *Biomolecules*. MDPI AG April 1, 2020. <https://doi.org/10.3390/biom10040642>.
- (6) Attia, M. F.; Anton, N.; Wallyn, J.; Omran, Z.; Vandamme, T. F. An Overview of Active and Passive Targeting Strategies to Improve the Nanocarriers Efficiency to Tumour Sites. *Journal of Pharmacy and Pharmacology*. Blackwell Publishing Ltd August 1, 2019, pp 1185–1198. <https://doi.org/10.1111/jphp.13098>.
- (7) van Tonder, A.; Joubert, A. M.; Cromarty, A. D. Limitations of the 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyl-2H-Tetrazolium Bromide (MTT) Assay When Compared to Three Commonly Used Cell Enumeration Assays. *BMC Res Notes* **2015**, *8* (1). <https://doi.org/10.1186/s13104-015-1000-8>.
- (8) Kuete, V.; Karaosmanoğlu, O.; Sivas, H. Anticancer Activities of African Medicinal Spices and Vegetables. In *Medicinal Spices and Vegetables from Africa: Therapeutic Potential Against Metabolic, Inflammatory, Infectious and Systemic Diseases*; Elsevier Inc., 2017; pp 271–297. <https://doi.org/10.1016/B978-0-12-809286-6.00010-8>.
- (9) Lü, L.; Zhang, L.; Wai, M. S. M.; Yew, D. T. W.; Xu, J. Exocytosis of MTT Formazan Could Exacerbate Cell Injury. *Toxicology in Vitro* **2012**, *26* (4), 636–644. <https://doi.org/10.1016/j.tiv.2012.02.006>.
- (10) Manoharan, A.; Rayner, P. J.; Iali, W.; Burns, M. J.; Perry, V. H.; Duckett, S. B. Achieving Biocompatible SABRE: An in Vitro Cytotoxicity Study. *ChemMedChem* **2018**, *13* (4), 352–359. <https://doi.org/10.1002/cmdc.201700725>.
- (11) Santos, S. D.; Xavier, M.; Leite, D. M.; Moreira, D. A.; Custódio, B.; Torrado, M.; Castro, R.; Leiro, V.; Rodrigues, J.; Tomás, H.; Pêgo, A. P. PAMAM Dendrimers: Blood-Brain Barrier Transport and Neuronal Uptake after Focal Brain Ischemia. *Journal of Controlled Release* **2018**, *291*, 65–79. <https://doi.org/10.1016/j.jconrel.2018.10.006>.
- (12) Taghavi Pourianazar, N.; Mutlu, P.; Gunduz, U. Bioapplications of Poly(Amidoamine) (PAMAM) Dendrimers in Nanomedicine. *Journal of Nanoparticle Research*. Kluwer Academic Publishers 2014. <https://doi.org/10.1007/s11051-014-2342-1>.

- (13) Zeng, Y.; Kurokawa, Y.; Win-Shwe, T.-T.; Zeng, Q.; Hirano, S.; Zhang, Z.; Sone, H. Effects of PAMAM Dendrimers with Various Surface Functional Groups and Multiple Generations on Cytotoxicity and Neuronal Differentiation Using Human Neural Progenitor Cells. *J Toxicol Sci* **2016**, *41* (3), 351–370. <https://doi.org/10.2131/jts.41.351>.
- (14) Vu, M. T.; Bach, L. G.; Nguyen, D. C.; Ho, M. N.; Nguyen, N. H.; Tran, N. Q.; Nguyen, D. H.; Nguyen, C. K.; Thi, T. T. H. Modified Carboxyl-Terminated PAMAM Dendrimers as Great Cytocompatible Nano-Based Drug Delivery System. *Int J Mol Sci* **2019**, *20* (8). <https://doi.org/10.3390/ijms20082016>.
- (15) Ly, T. U.; Tran, N. Q.; Hoang, T. K. D.; Phan, K. N.; Truong, H. N.; Nguyen, C. K. Pegylated Dendrimer and Its Effect in Fluorouracil Loading and Release for Enhancing Antitumor Activity. *J Biomed Nanotechnol* **2013**, *9* (2), 213–220. <https://doi.org/10.1166/jbn.2013.1479>.
- (16) Strober, W. Trypan Blue Exclusion Test of Cell Viability. *Curr Protoc Immunol* **2015**, *111* (1), A3.B.1-A3.B.3. <https://doi.org/10.1002/0471142735.ima03bs111>.
- (17) Kurashina, Y.; Imashiro, C.; Hirano, M.; Kuribara, T.; Totani, K.; Ohnuma, K.; Friend, J.; Takemura, K. Enzyme-Free Release of Adhered Cells from Standard Culture Dishes Using Intermittent Ultrasonic Traveling Waves. *Commun Biol* **2019**, *2* (1). <https://doi.org/10.1038/s42003-019-0638-5>.

Chapter 5

Summary and conclusion

Summary

The primary aim of this project was to synthesize and characterize [Pt(phen)(L-O,S)]Cl complexes for encapsulation in native and functionalized PAMAM dendrimers for the evaluation of antiproliferative behaviour of the guest-host molecules and efficiency of PAMAM dendrimers as a drug delivery system.

The synthesis and characterization of Pt(phen)Cl₂ precursor, *N,N*-di(butyl)-*N*'-benzoylthiourea ligand and [Pt(diimine)(L-O,S)]Cl complexes are reported. [Pt(phen)Cl₂] precursor was synthesized, purified and isolated with a yield of 88%. The precursor was characterized using ¹H NMR, FT-IR spectroscopy and ESI-MS. The *N,N*-di(butyl)-*N*'-benzoylthiourea ligand was prepared via a facile one-pot synthesis with a yield of 78%. The ligand was characterized by ¹H NMR which was successfully assigned with the assistance of COSY spectroscopy. To further confirm successful ligand preparation, ¹³C NMR spectroscopy was recorded and assigned with the aid of HSQC and HMBC NMR spectroscopy. ESI-MS and FT-IR spectroscopy were used to further confirm the successful synthesis of the ligand.

Three *N,N*-di(alkyl)-*N*'-acylthiourea ligands were used to synthesize the corresponding [Pt(phen)(L-O,S)]Cl bidentate complexes with [Pt(phen)Cl₂] as the metal precursor. Synthesis involved heating of the precursor and ligand in a 1:1 mole ratio to reflux followed by *in-situ* deprotonation of the ligand by addition of sodium acetate (a base) to form the desired [Pt(phen)(L-O,S)]Cl complex. A colour change from cloudy bright yellow to clear pale yellow was observed upon complex formation. After work-up and purification, the complexes were isolated with yields ranging from 66% to 75%. Each respective complex was characterized using ¹H NMR, ¹³C NMR where 2D NMR (COSY, HSCQ and HMBC spectroscopy) was used to unambiguously assign the protons and carbons. FT-IR spectroscopy and ESI-MS were also employed to further corroborate the synthesis of the complexes.

A generation 4 (G4) polyamido amine (PAMAM) dendrimer was synthesized through a step-wise method where each subsequent reaction resulted in the formation of half or full generation PAMAM dendrimers. The half generations (G-0.5, G0.5, G1.5, G2.5 and G3.5) were synthesized by the addition of excess methyl acrylate (MA) to a methanolic solution EDA to afford G-0.5 PAMAM and from there onwards MA was added to a full generation to result in the corresponding half generation. The excess MA and solvent were removed via reduced pressure to yield a half generation PAMAM with yields ranging from 83% to 98%. Each half generation PAMAM was characterized with use of ¹H NMR, ¹³C NMR and FT-IR spectroscopy where 2D NMR (COSY, HSQC and HMBC) was employed to conclusively assign the recorded ¹H and ¹³C spectra. G-0.5 was additionally characterized with ESI-MS because of its low

molecular weight. The full generation dendrimers (G0, G1, G2, G3 and G4) were synthesized by the amidation of the half generations where excess of EDA was added to a methanolic solution of the half generation to afford a full generation with yields ranging from 84% to 95%. The full generation dendrimers were characterized using ^1H NMR, ^{13}C NMR and FT-IR spectroscopy with the assistance of COSY, HSQC and HMBC spectroscopy to unambiguously assign the recorded ^1H and ^{13}C spectra. G0 PAMAM was additionally assigned using ESI MS and G4 with MALDI-TOF mass spectrometry to confirm successful dendrimer synthesis.

Host-guest interactions of the synthesized platinum complexes and dendrimers were investigated. Encapsulation of the platinum complexes (PtL1, PtL2 and PtL3) in amine terminated G4 PAMAM was unsuccessful as G4 PAMAM was found to be polycationic even near neutral pH which resulted in complex degradation and electrostatic repulsion of the positively charged platinum complexes, however, from these attempts, we found that solvent played an important role in the encapsulation process with DMSO- d_6 showing the most promising results. Acetamide functionalized G4 (G4-Ac) and G5 (G5-Ac) PAMAM were synthesized to mitigate issues faced with polycationic dendrimers as they have a neutral surface. They were synthesized from G4 and G5 PAMAM by addition of excess of triethyl amine (TEA) and acetic anhydride (Ac_2O) to the desired amine terminated dendrimer. After dialysis and concentration, the resultant oils were characterized using ^1H NMR, ^{13}C NMR (including 2D NMR), FT-IR and MALDI-TOF MS to confirm successful functionalization.

Folic acid serves as a good molecular target to investigate potential selective drug delivery since the folate receptor (FAR) has been shown to be overexpressed in various cancers. Folic acid functionalized G4 PAMAM (G4-FA) was synthesized through carbodiimide assisted coupling with 1-ethyl-3-carbodiimide hydrochloride (EDC-HCl) as the coupling agent. FA was reacted with EDC-HCl followed by the dropwise addition to G4 PAMAM. After dialysis and concentration, the ^1H NMR and ^{13}C NMR spectra were recorded and assigned with the use of 2D NMR spectroscopy. FT-IR was additionally used to observe the shifts in vibrations upon FA conjugation. G4-FA was not used in the host-guest interactions as it also exists as a polycation and results in complex degradation as discussed in chapter 3.

The host-guest interactions of neutral G4-Ac were investigated in detail. Shifting and broadening of aromatic and aliphatic protons of PtL1 was observed upon addition to G4-Ac in DMSO- d_6 . Further analysis with the use of diffusion ordered spectroscopy (DOSY) revealed an interaction as the diffusion coefficient of PtL1 in the mixture was smaller than that of free PtL1 and the same was observed for G4-Ac at 300K. DOSY data further revealed the interaction to be in fast exchange. Hydrodynamic volumes were required to further ascertain the extent of the interaction since a conclusion could not be made based on diffusion

coefficients only. Viscosity measurements were then recorded at 300K and used to calculate the hydrodynamic radii (R_H) and hydrodynamic volumes (V_H). G4-Ac +PtL1 had a recorded viscosity of 4.1×10^{-3} Pa.s while free G4-Ac, free PtL1 and DMSO- d_6 had viscosities of 2.6×10^{-3} , 2.5×10^{-3} and 2.0×10^{-3} Pa.s respectively. The increase in viscosity was attributed to an increase in concentration as well as an increase in ionic strength as PtL1 is cationic. The hydrodynamic volumes can be calculated using the measured diffusion coefficient and viscosity to reflect the real extent of host-guest association. An 84% and 49% increase in V_H was observed in PtL1 and G4-Ac respectively in the mixture which further indicated the formation of a host-guest molecule. At 310K, an overall decrease in V_H of PtL1 and G4-Ac was observed which indicated the dissociation of the host-guest complex at higher temperatures. Dendrimer size was found to have an effect on encapsulation as G5-Ac with subsequently larger cavities displayed better host-guest interactions which was seen by the presence of weak NOE crosspeaks between the aromatic region of PtL3 and internal G5-Ac protons. On the other hand, no NOE crosspeaks between PtL2 and G4-Ac were observed.

The effect of ionic strength on chemical shift and diffusion behaviours of the dendrimers were briefly investigated using tetrabutylammonium chloride ([TBA]Cl) and G4-Ac. An increase in viscosity was observed when [TBA]Cl was added to G4-Ac which was attributed to the increase in concentration (of molecules in solution) and ionic strength which was also observed in G4-Ac +PtL1. A 31% increase in the V_H of G4-Ac was observed while a 40% decrease was observed in the V_H of [TBA]Cl at 300K. This finding was indicative of dendrimer aggregation instead of [TBA]Cl encapsulation. At 310K, a 69% decrease in the V_H of G4-Ac was observed which indicated the disassembling of the dendrimer aggregates.

The host-guest interactions of 5-Fluorouracil (5FU) with G5 were briefly investigated as reference. A shift in surface dendrimer protons (g' and f') was observed which indicated an electrostatic interaction with 5FU at the surface. It was found that the weakly acidic 5FU protons were abstracted by the ionisable G5 surface amines to form an ion-pair.

The Free platinum complexes (PtL1, PtL2 and PtL3), 5FU, free dendrimers and dendrimer-drug mixtures were screened against A549 lung cancer line at 50 μ M and 5 μ M. Free G4-Ac, G5 and G5-Ac displayed moderate biocompatibility with a cell viability of over 50% at both concentrations. Acetamide functionalization lowered dendrimer toxicity as expected. The free and dendrimer encapsulated complexes were highly active and displayed a cell viability of less than 1% at 50 μ M. Free PtL1 displayed the highest activity among the platinum complexes with a cell viability of 8.2% while G4-Ac +PtL1 displayed highest activity among the dendrimer encapsulated complexes with a cell viability of 6.9% at 5 μ M. G4-Ac +PtL1 and G4-Ac +PtL2 displayed higher cytotoxic activity than the free complexes which could be indicative of

effective drug delivery as both native and acetamide functionalized PAMAM dendrimers were found to be biocompatible and the solvent (DMSO- d_6 /D $_2$ O) displayed the least cytotoxic activity although it was not completely innocent. 5FU was used as a positive control and was found to be less active compared to the platinum complexes with a cell viability of 62.3% at 50 μ M and 59.6% at 5 μ M. G5-5FU displayed improved cytotoxic activity with a cell viability of 19.2% at 50 μ M and 49.2% at 5 μ M which could be due to prolonged availability as seen in literature.

Future studies are aimed at the synthesis of amine-protected folic acid functionalized PAMAM, exploring anionic PAMAM dendrimers to investigate potential electrostatic interactions with the positively charged platinum complexes as well as exploring methods to lower inherent dendrimer toxicity. Once the platinum complexes are encapsulated in these dendrimers, screening against cancerous and normal cells should be conducted to test for selectivity.

Appendix

Mass spectrometry data

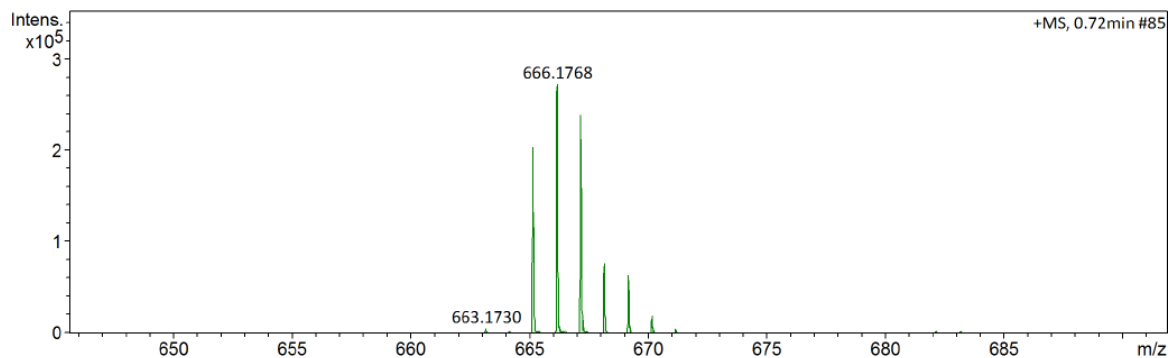


Figure 5.1: ESI mass spectrum of PtL1.

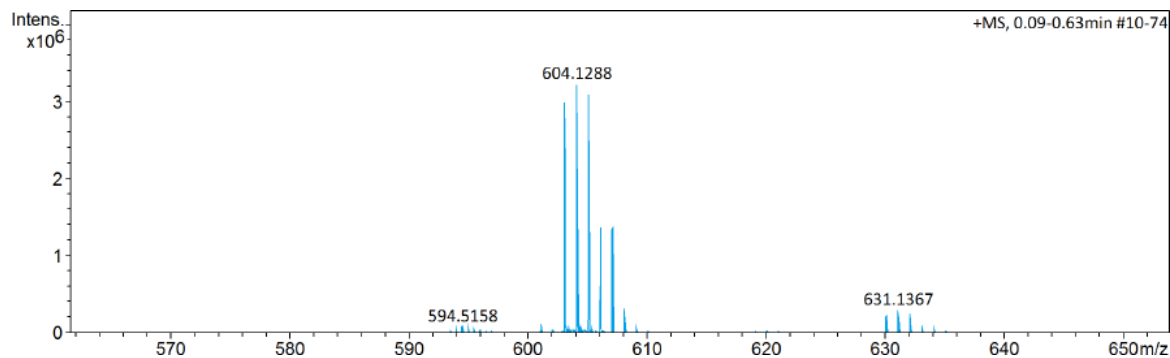


Figure 5.2: ESI mass spectrum of PtL2.

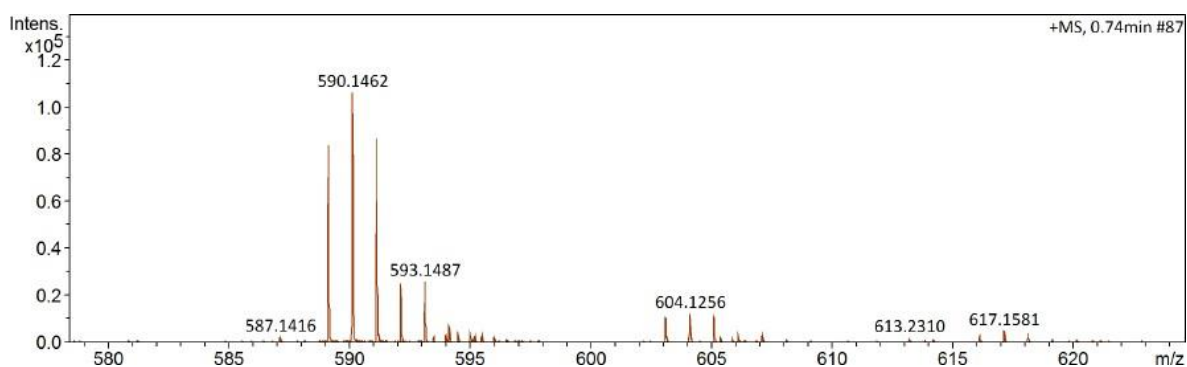


Figure 5.3: ESI mass spectrum of PtL3.

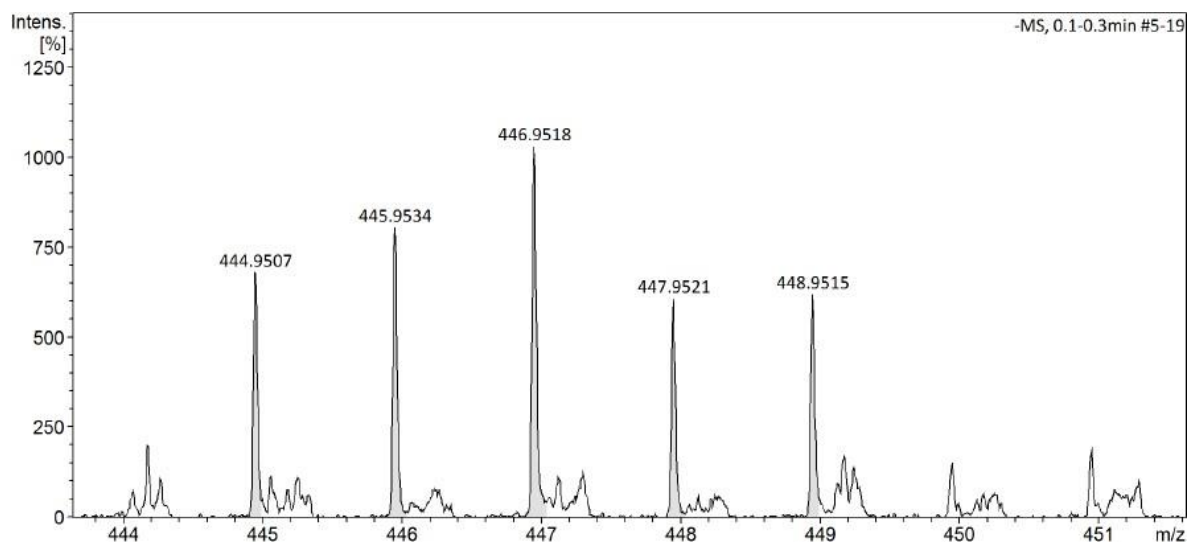


Figure 5.4: ESI mass spectrum of [Pt(phen)Cl₂].

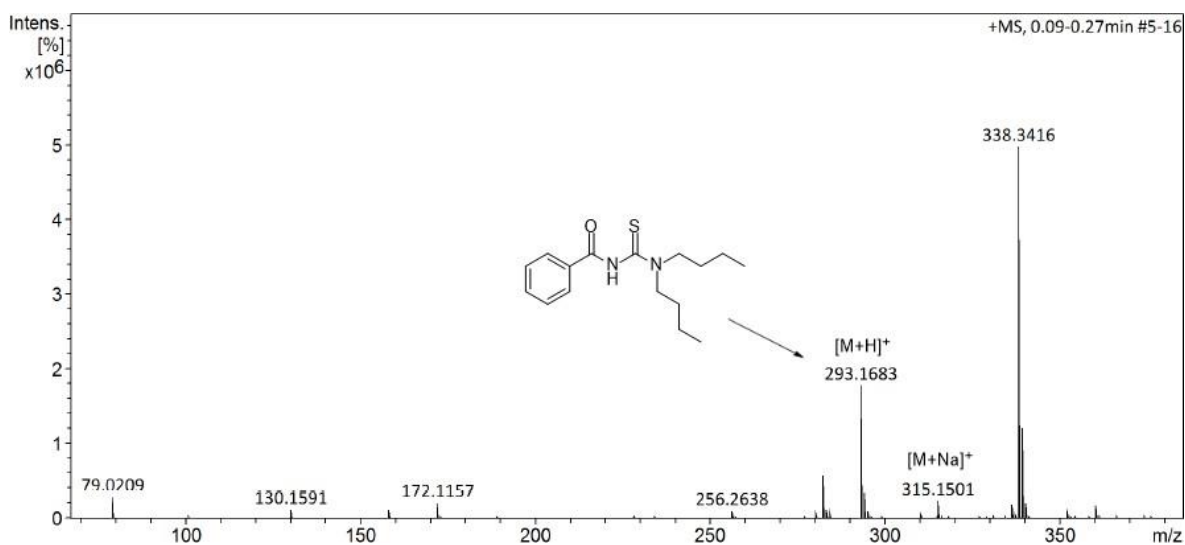


Figure 5.5: ESI mass spectrum of *N,N*-di(butyl)-*N'*-benzoylthiourea.

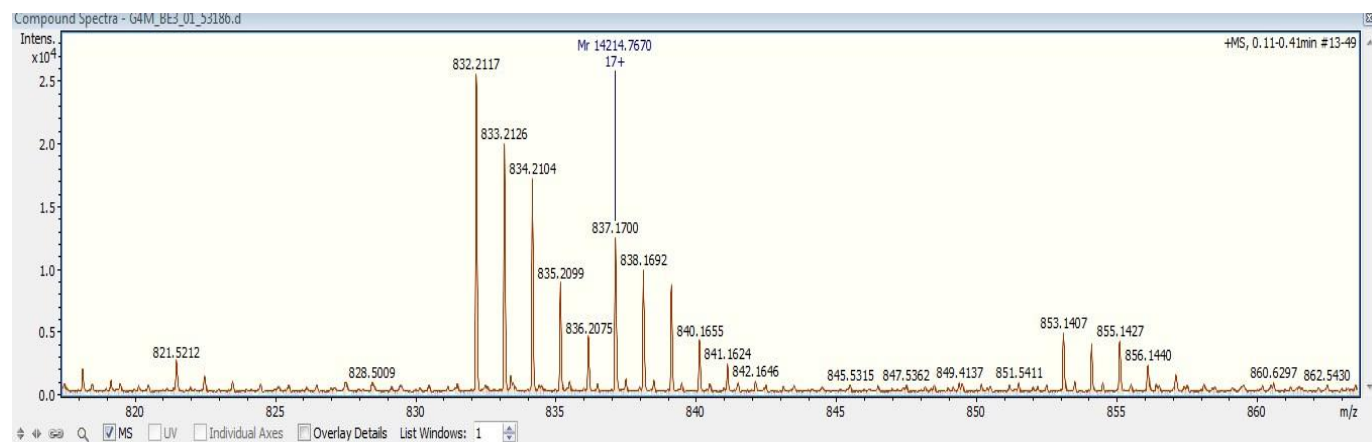


Figure 5.6: ESI mass spectrum of G4 PAMAM.

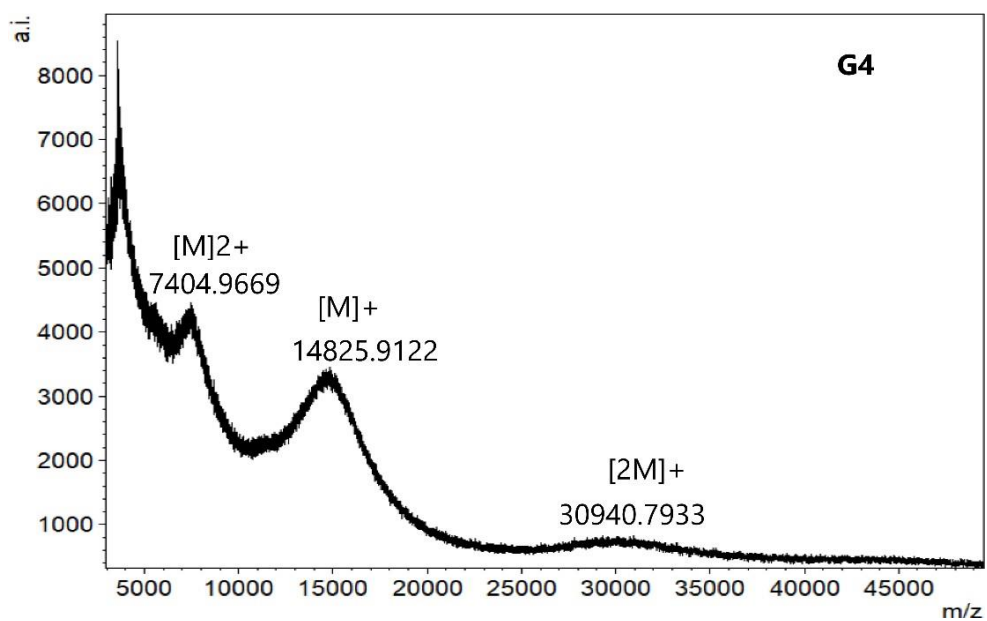


Figure 5.7: MALDI-TOF spectrum of G4 PAMAM.

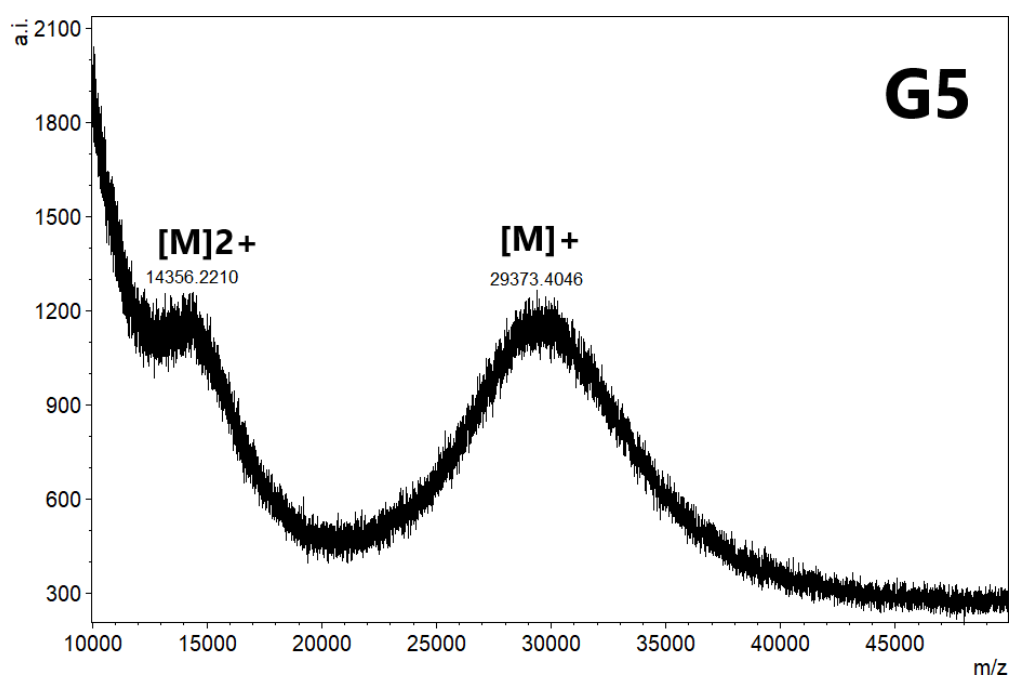


Figure 5.8: MALDI-TOF mass spectrum of G5 PAMAM.

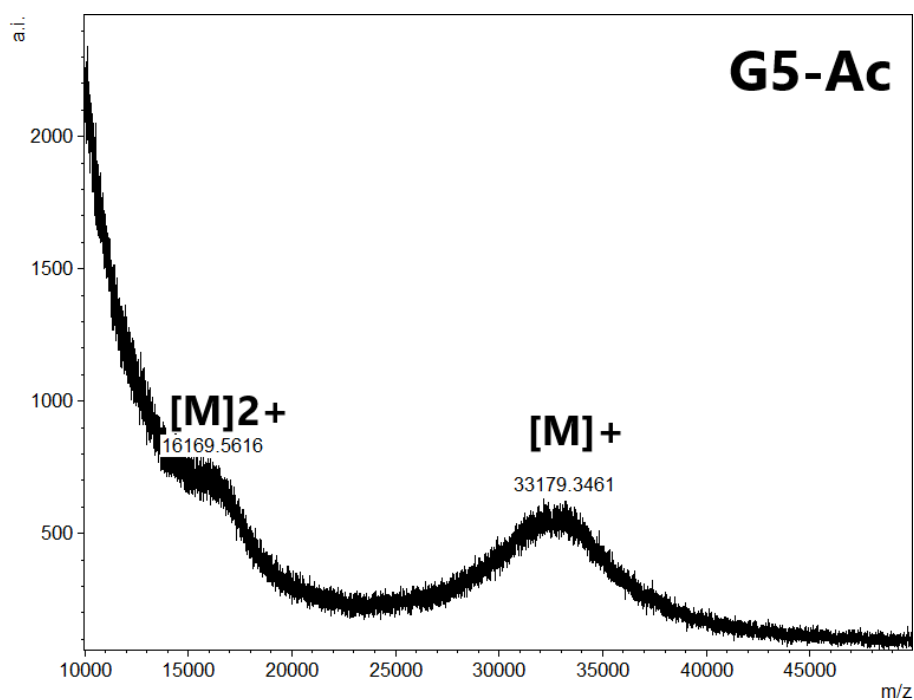


Figure 5.9: MALDI-TOF mass spectrum of G5-Ac PAMAM

NMR data

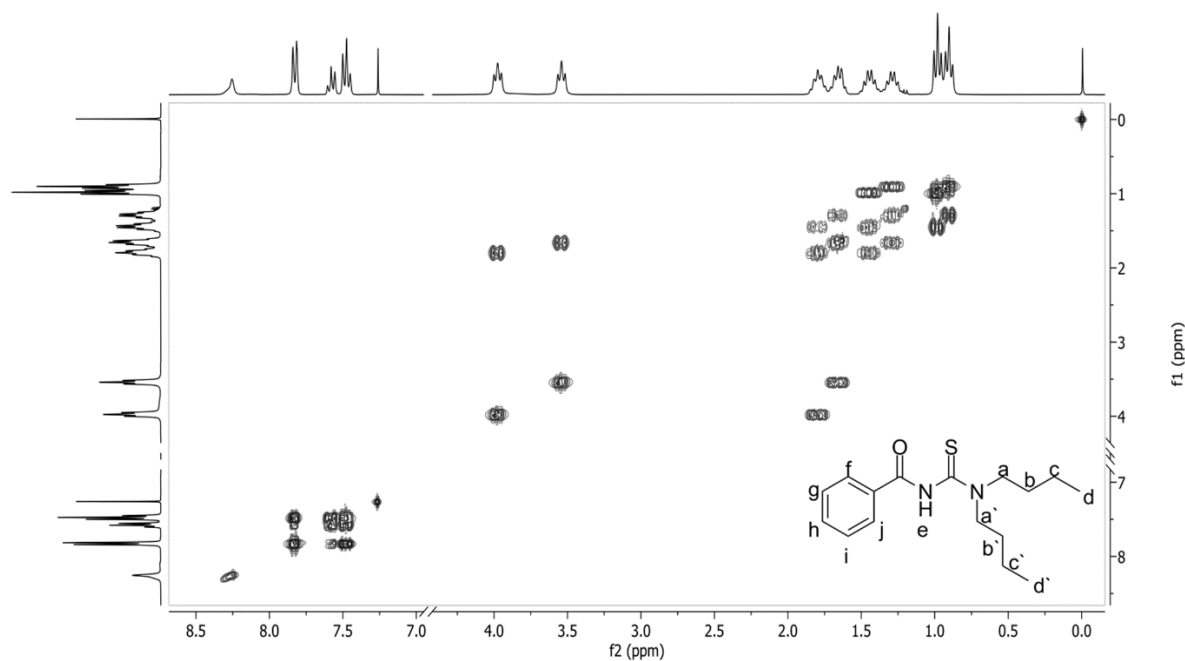


Figure 5.10: COSY NMR spectrum of N,N-di(butyl)-N'-benzoylthiourea in CDCl₃ at 300K.

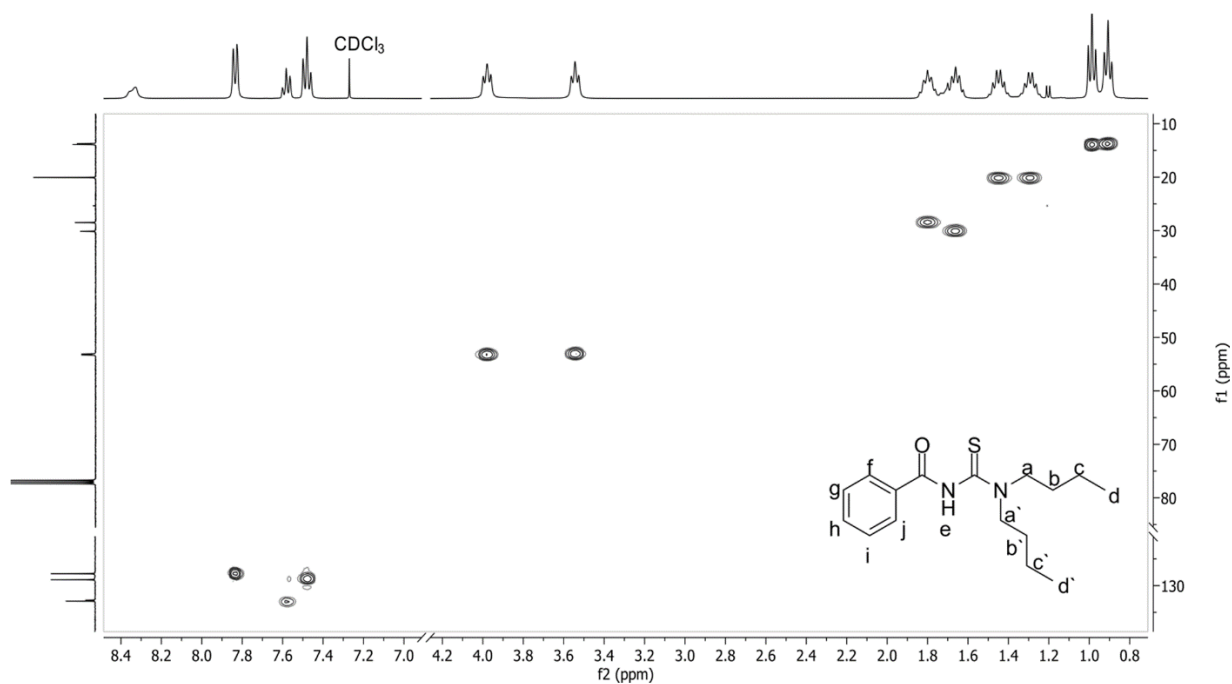


Figure 5.11: HSQC NMR spectrum of N,N-di(butyl)-N'-benzoylthiourea in CDCl₃ at 300K.

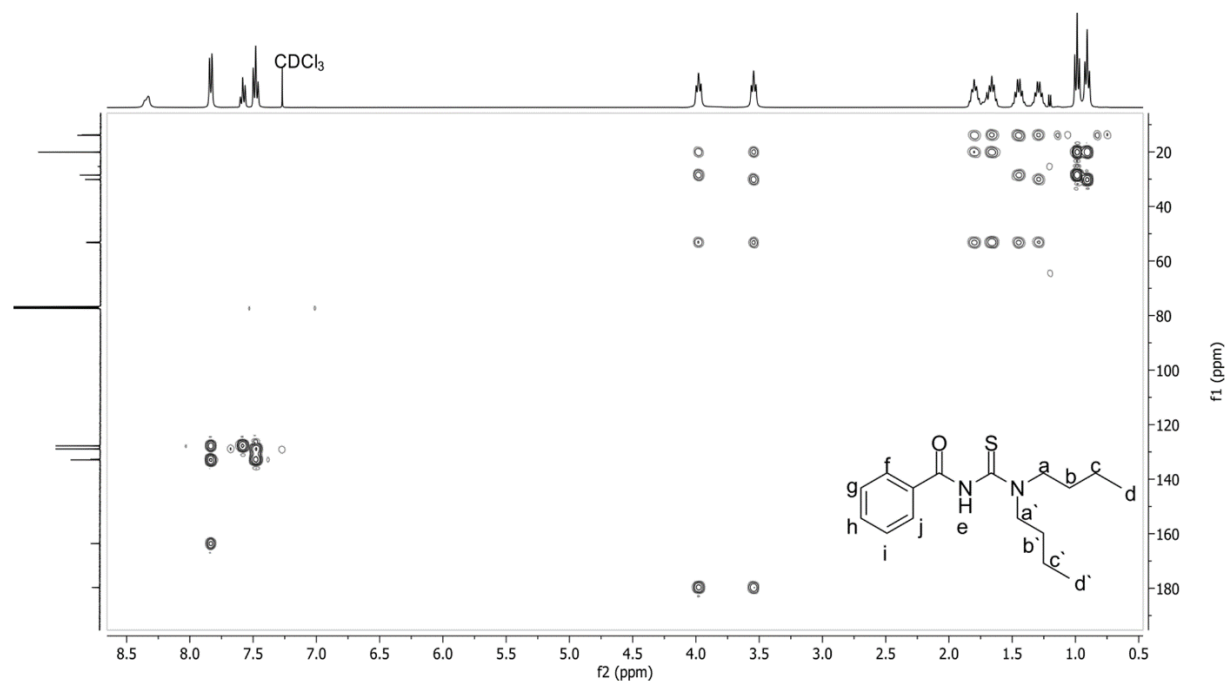


Figure 5.12: HMBC ligand NMR spectrum of N,N-di(butyl)-N'-benzoylthiourea in CDCl₃ at 300K.

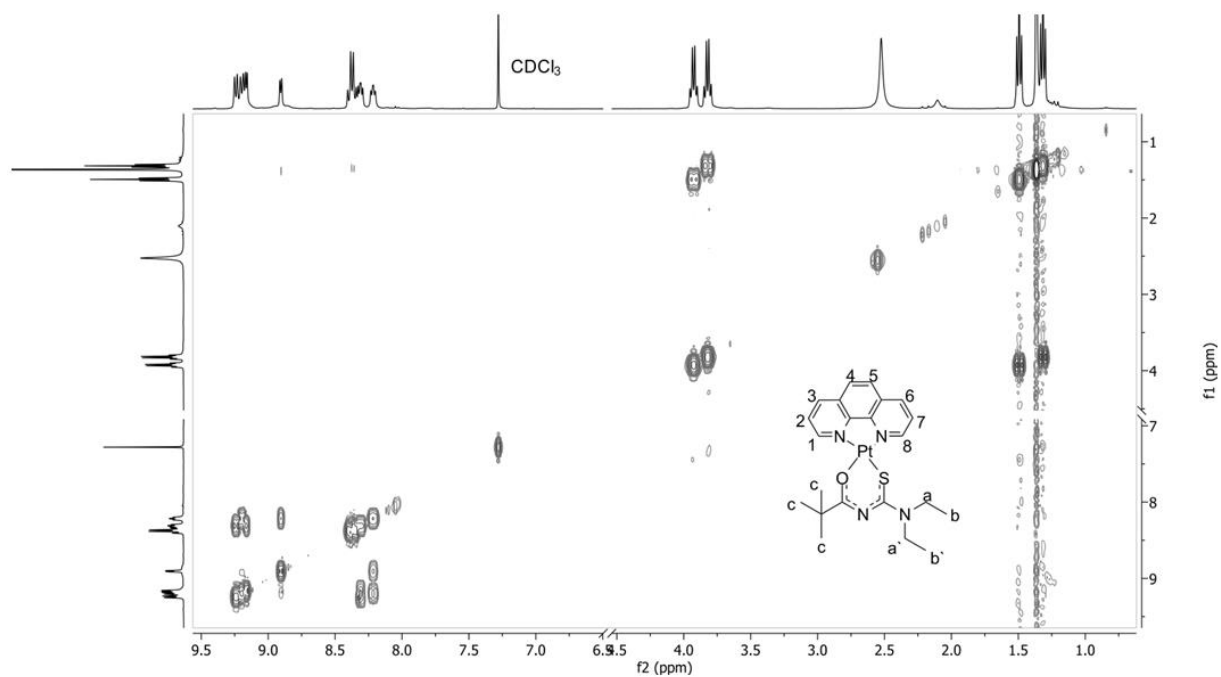


Figure 5.13: COSY NMR spectrum of $[\text{Pt}(\text{phen})(\text{N}'\text{-pivaloyl-N,N-diethylthiourea})]^+$ in CDCl_3 at 300K.

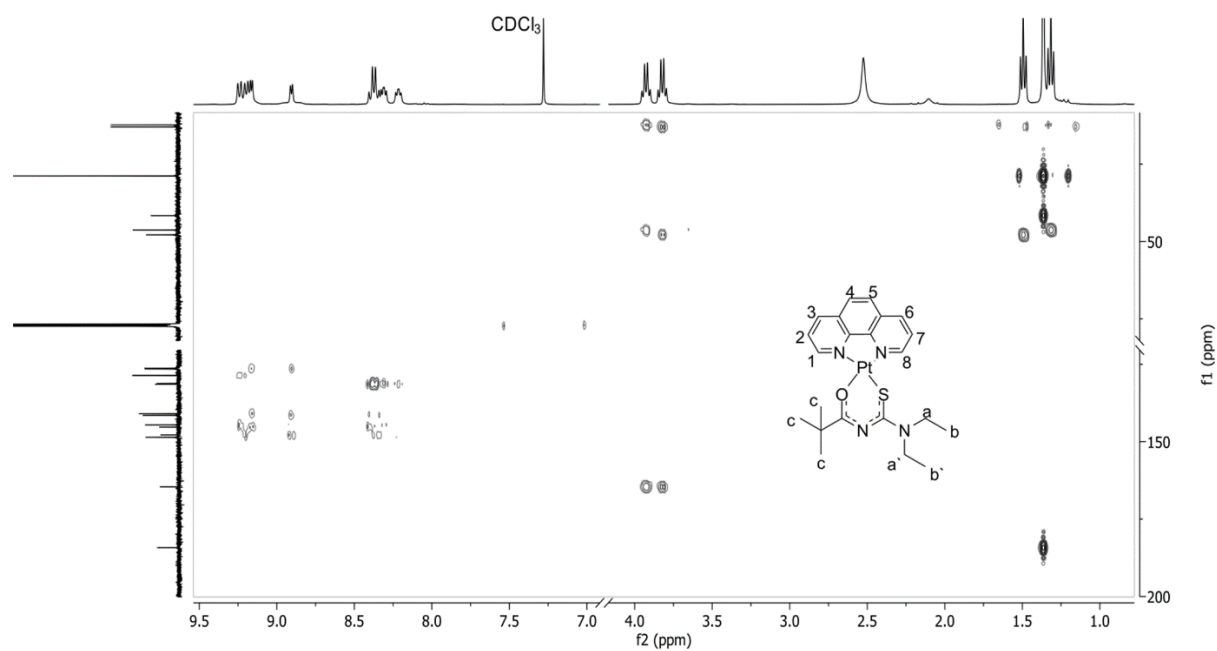


Figure 5.14: HMBC spectrum of $[\text{Pt}(\text{phen})(\text{N}'\text{-pivaloyl-N,N-diethylthiourea})]^+$ in CDCl_3 at 300K.

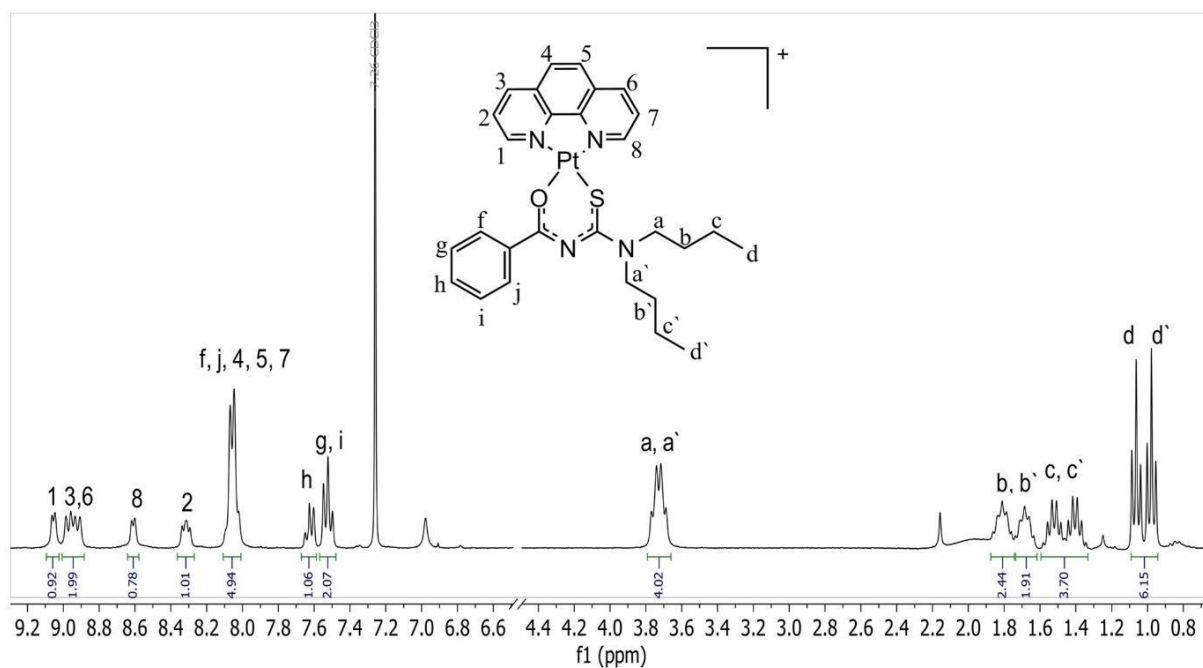


Figure 5.15: ¹H NMR of PtL1 in CDCl₃ at 300K.

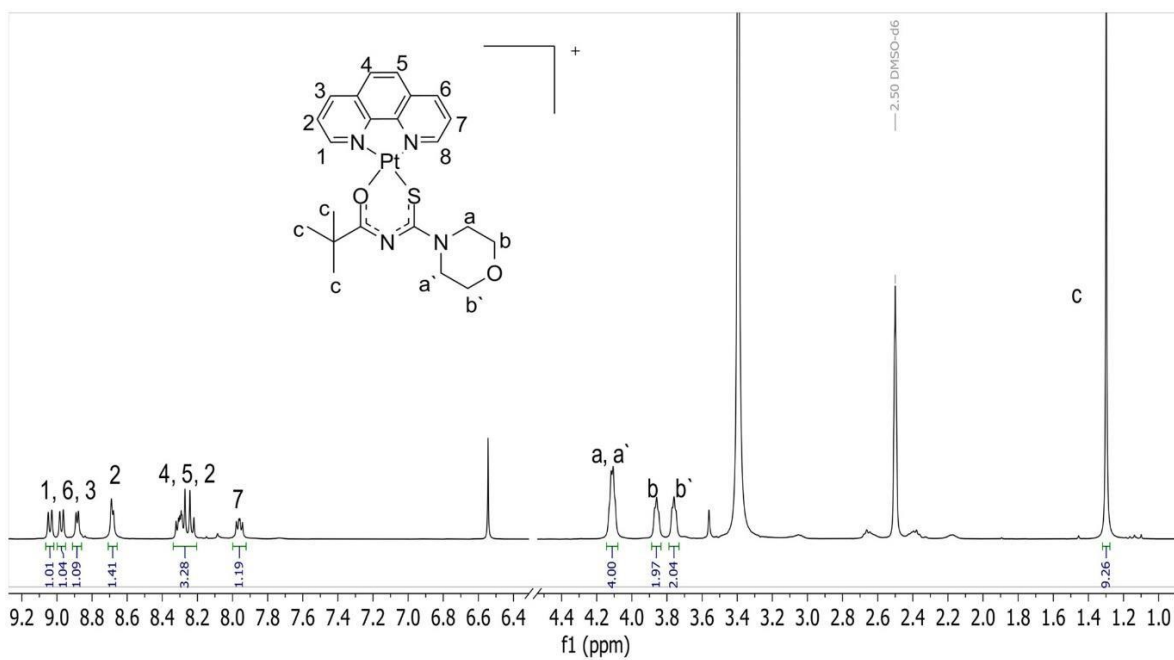


Figure 5.16: ¹H NMR of PtL2 in DMSO-d₆ at 300K.

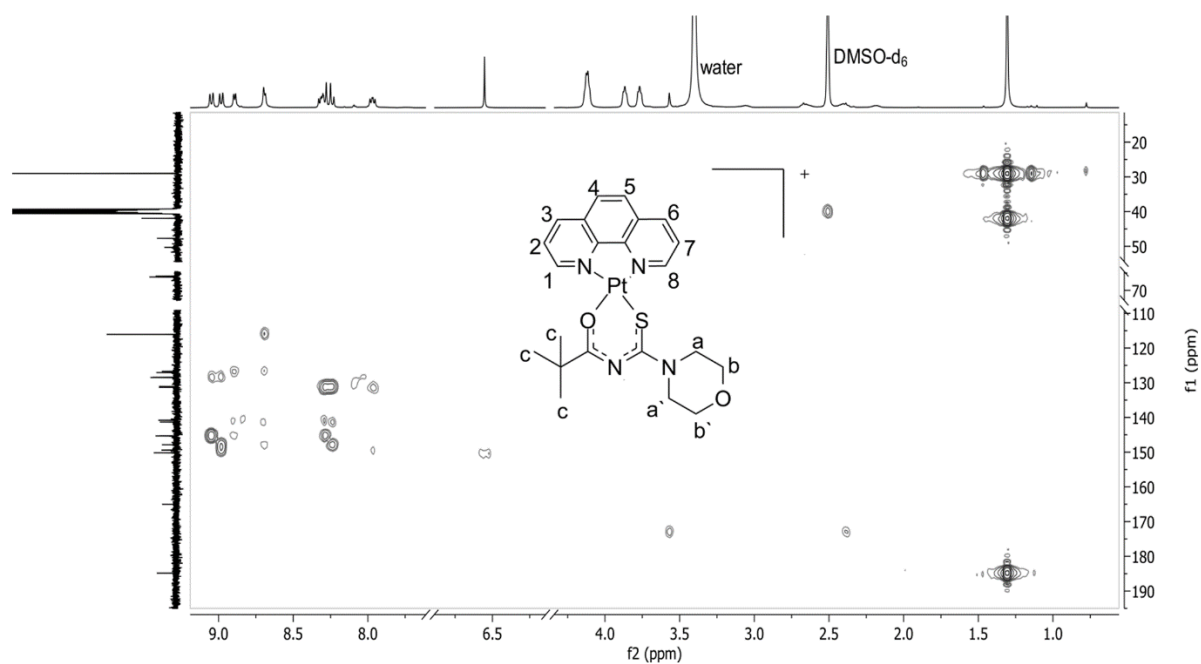


Figure 5.17: HMBC NMR of PtL2 in DMSO-d₆ at 300K.

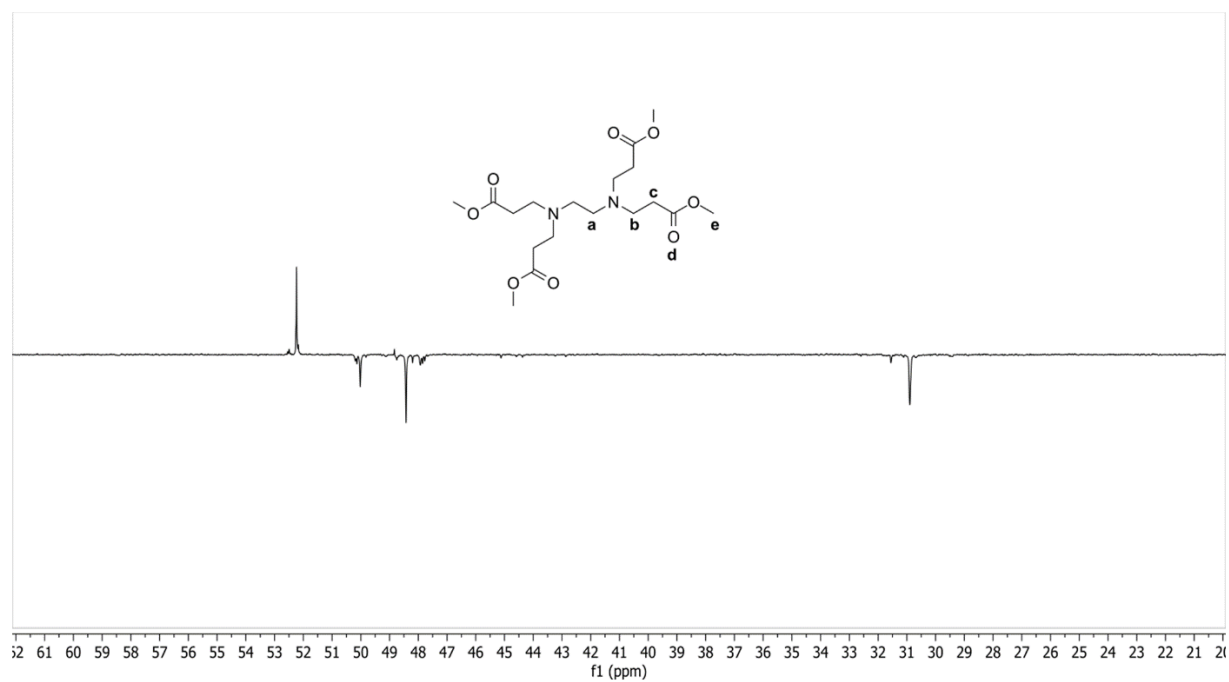


Figure 5.18; DEPT NMR of G-0.5 in D₂O.

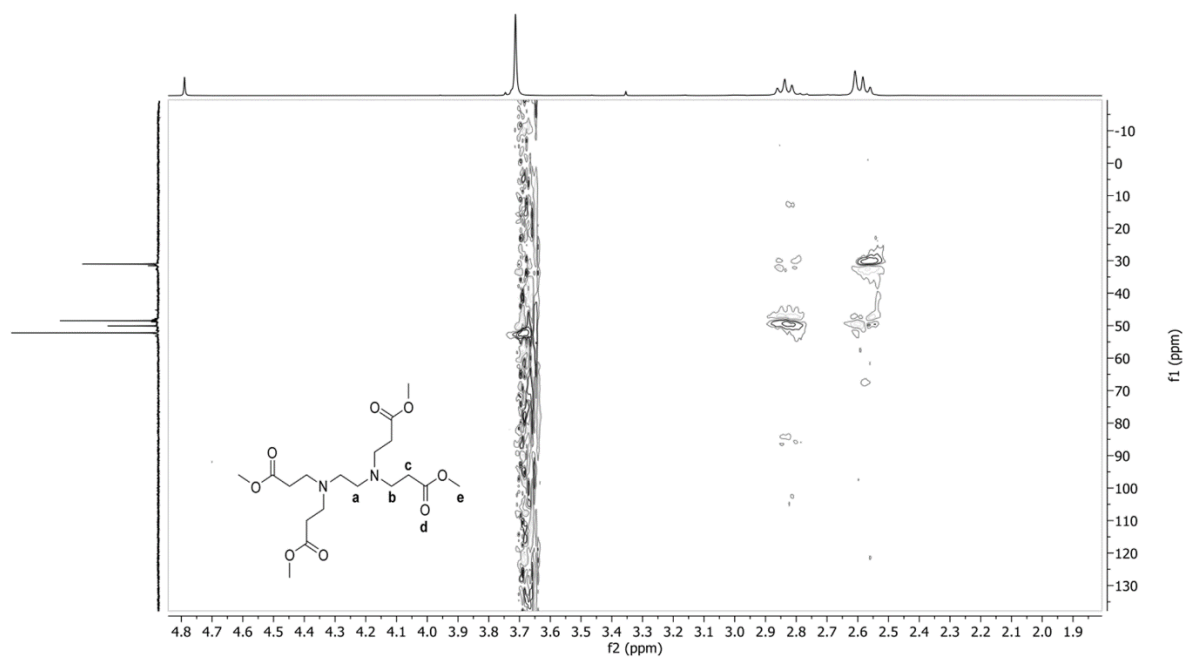


Figure 5.19: HSQC NMR of G-0.5 in D₂O.

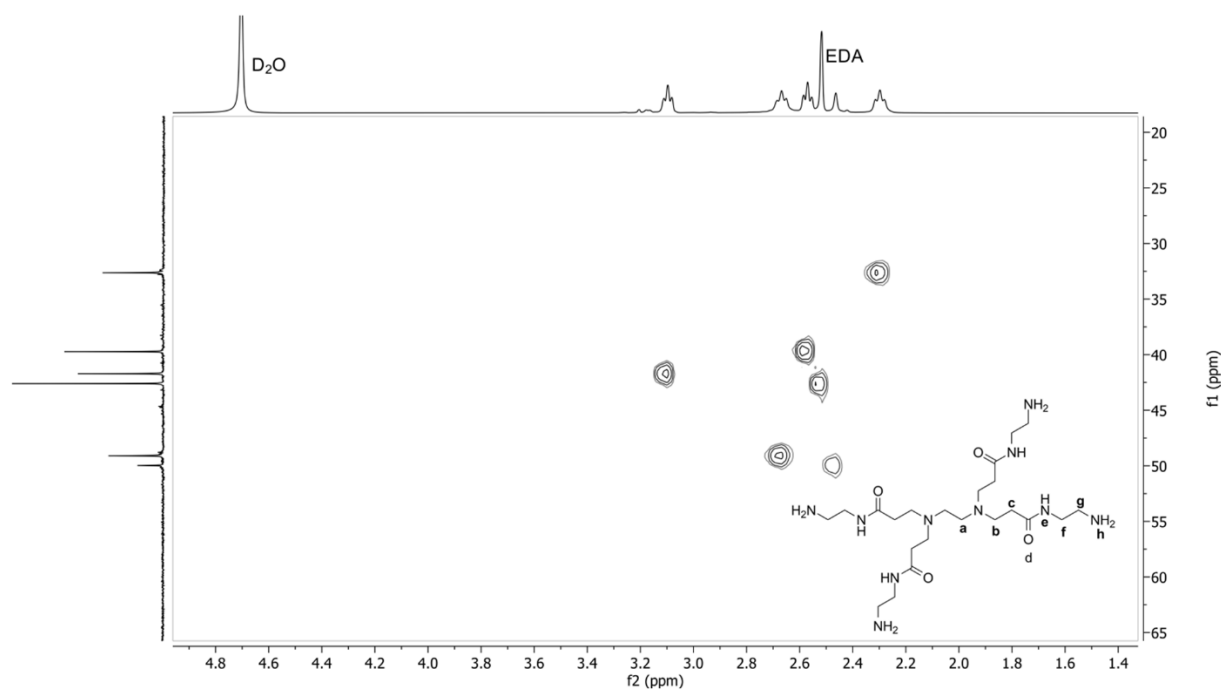


Figure 5.20: HSQC of G0 in D₂O

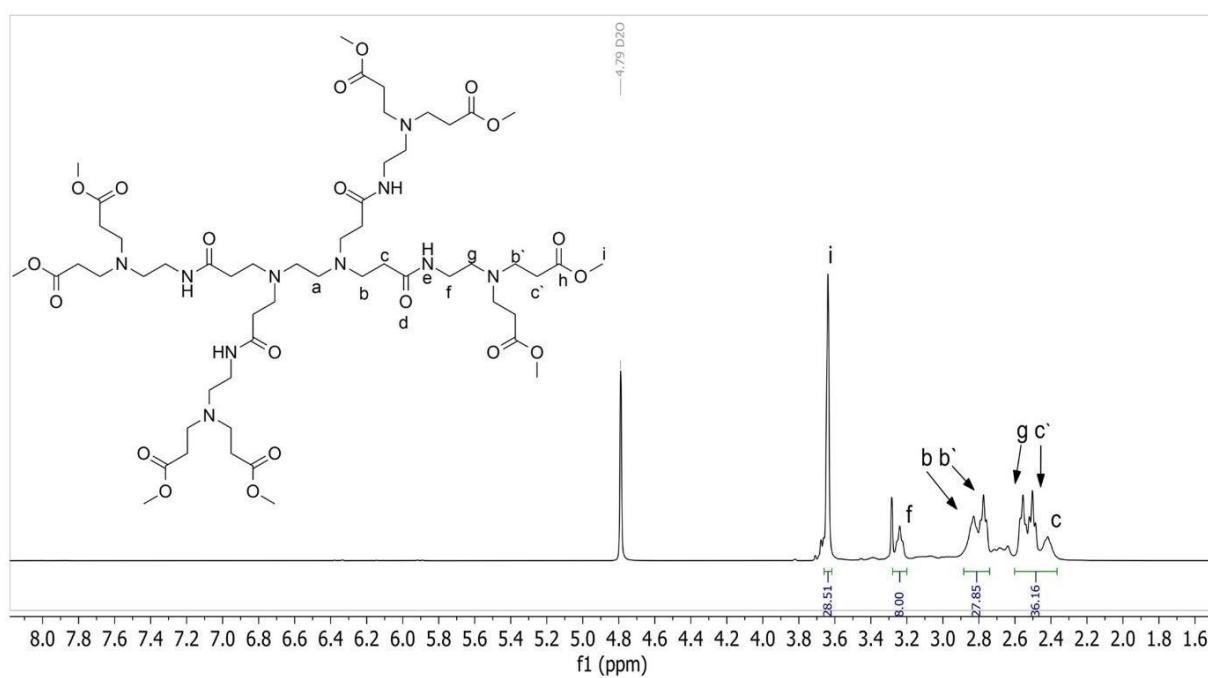


Figure 5.21: ^1H NMR of G0.5 PAMAM in D_2O at 300K.

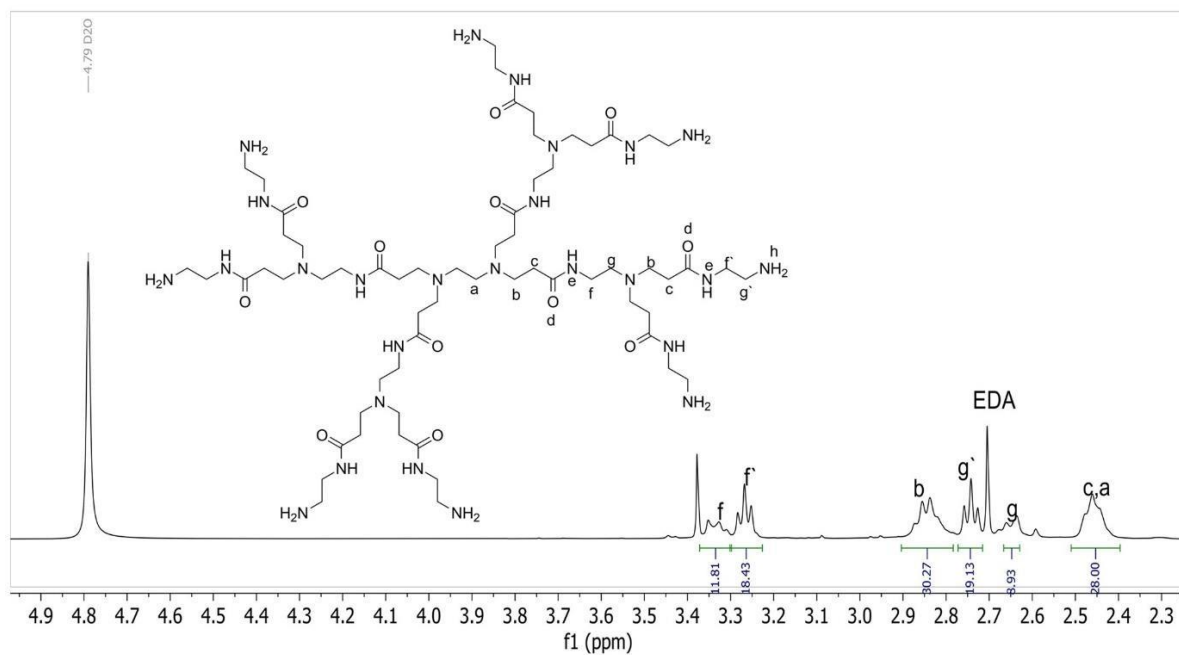


Figure 5.22: ^1H NMR of G1 PAMAM in D_2O at 300K.

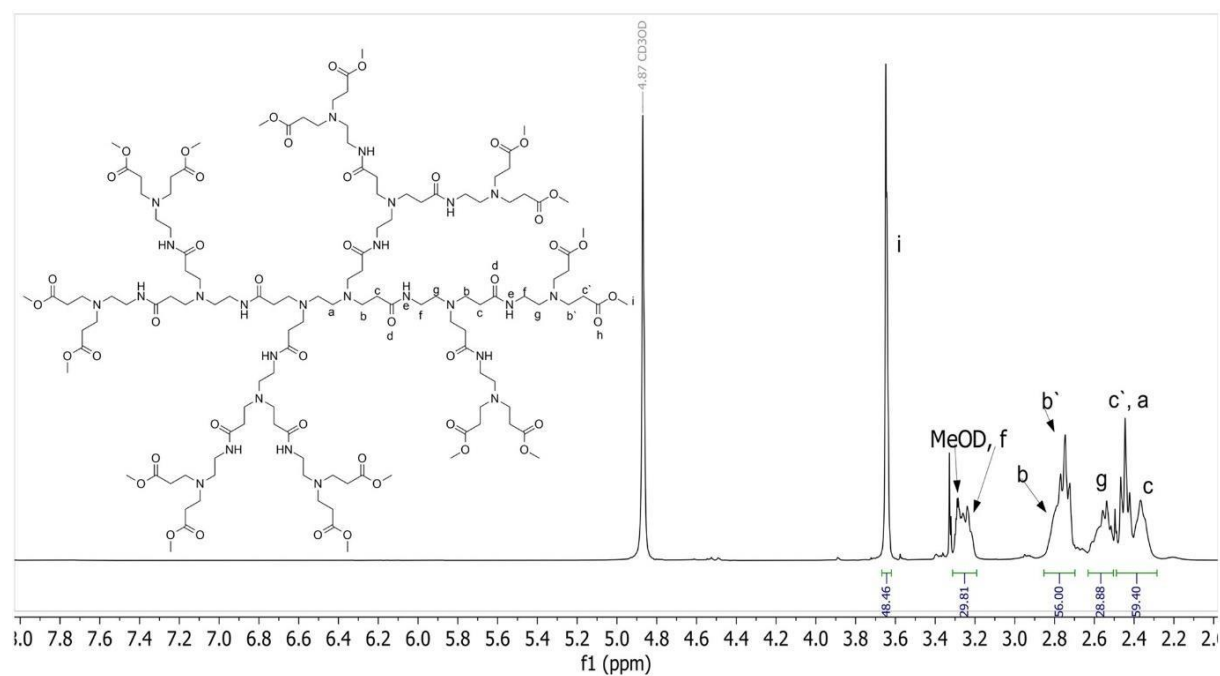


Figure 5.23: ^1H NMR of G1.5 PAMAM in MeOD-d_4 at 300K.

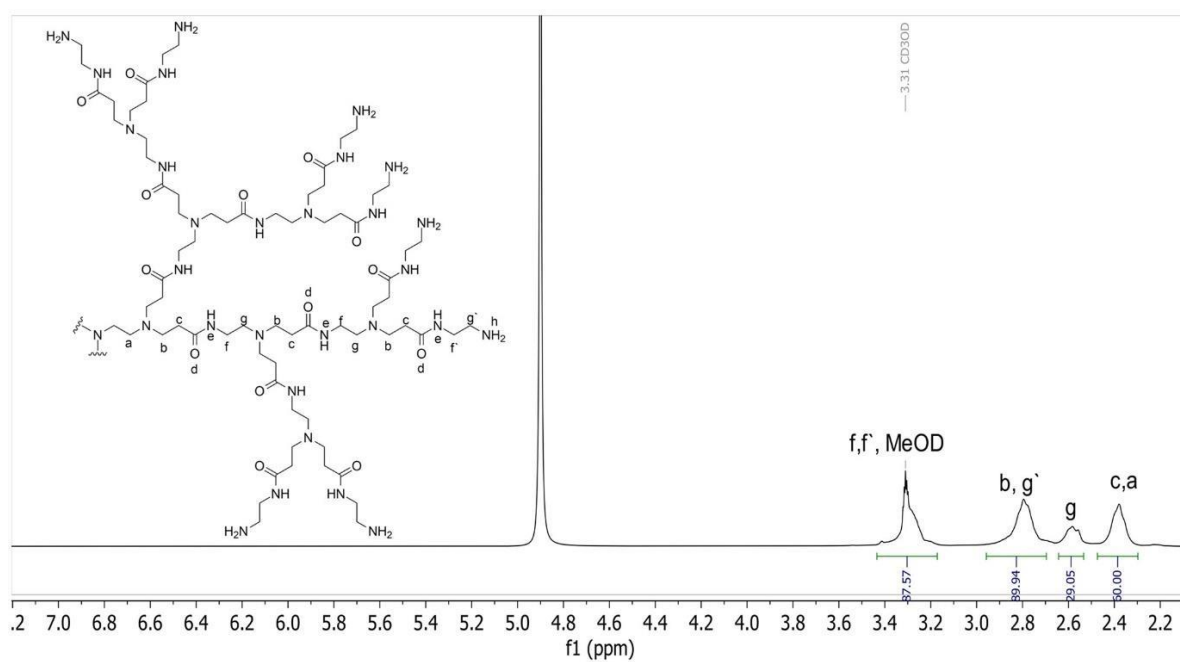


Figure 5.24: ^1H NMR of G2 PAMAM in MeOD-d_4 at 300K.

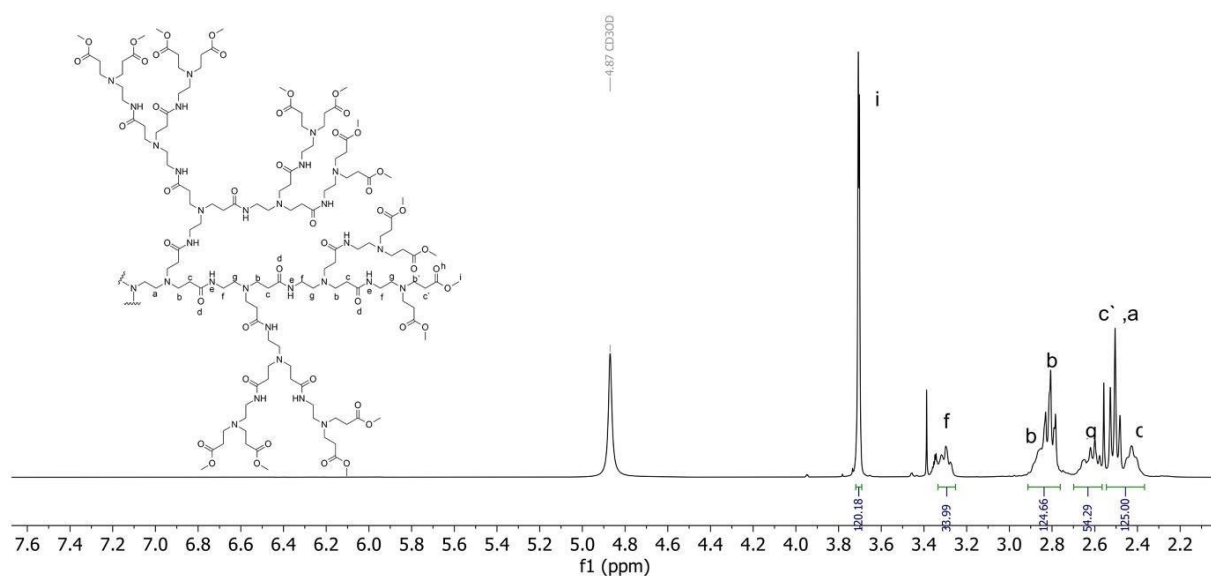


Figure 5.25: ^1H NMR of G2.5 PAMAM in MeOD-d_4 at 300K.

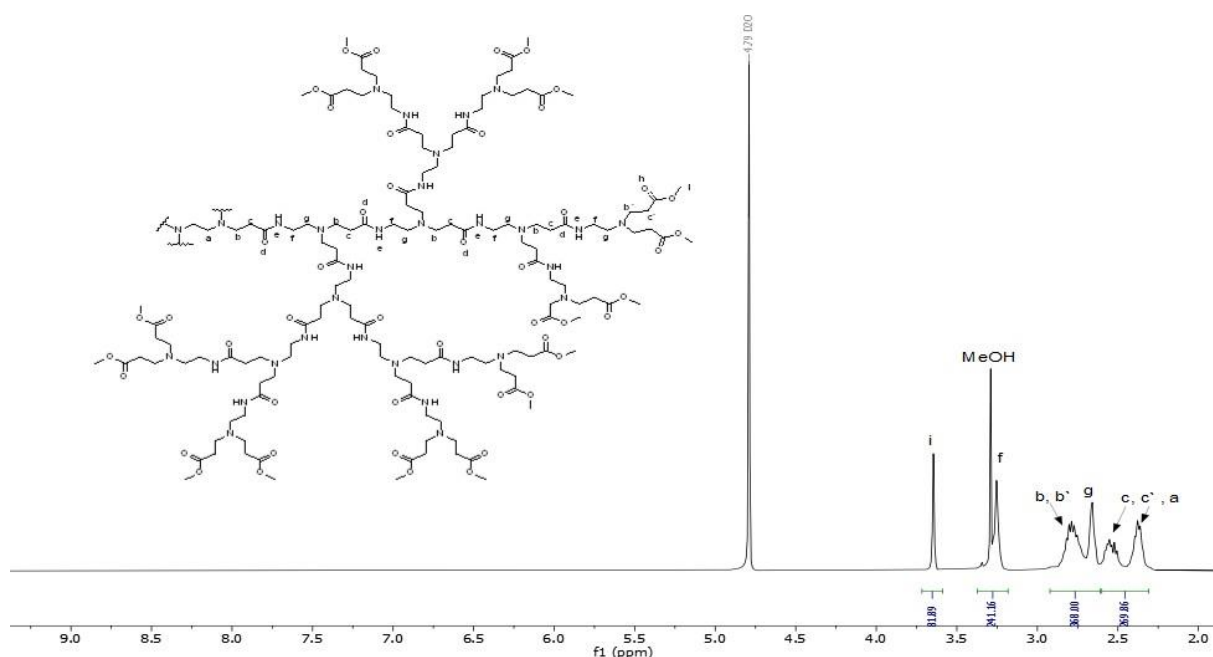


Figure 5.26: ^1H NMR of G3.5 PAMAM in MeOD-d_4 at 300K.

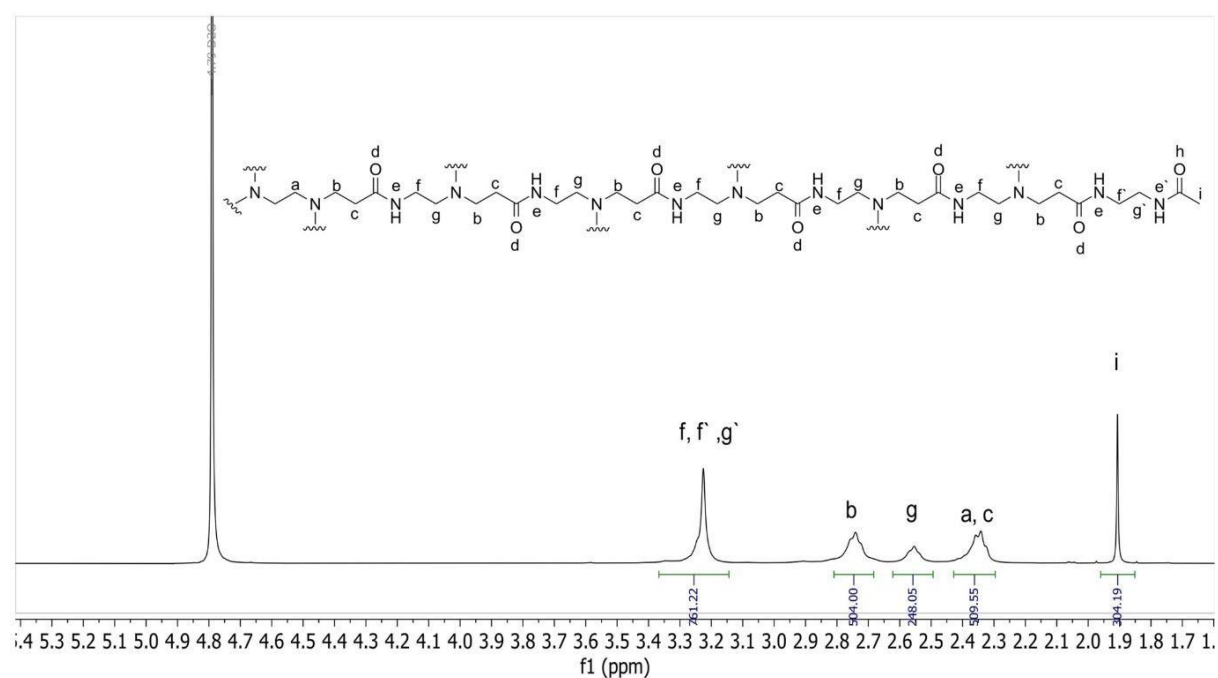


Figure 5.27: ^1H NMR of G5-Ac PAMAM in D_2O at 300K.

IR data

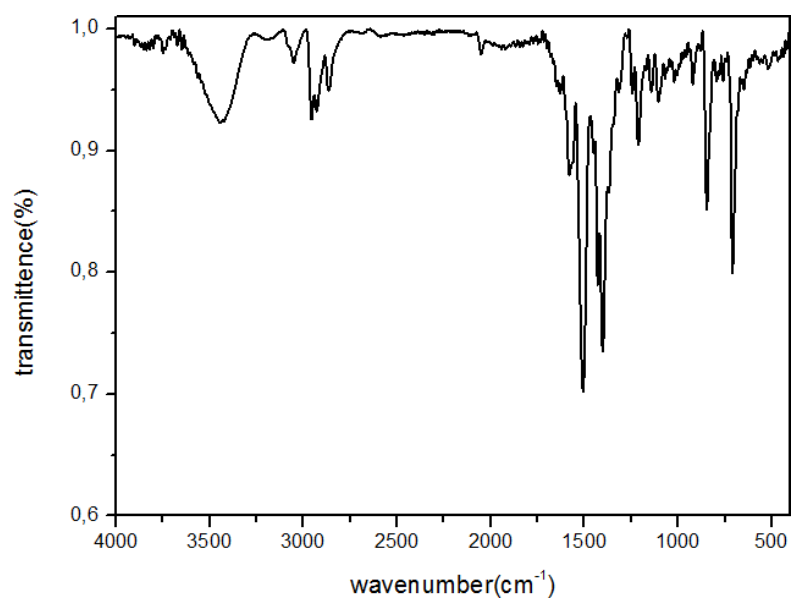


Figure 5.28: PtL1 IR spectrum.

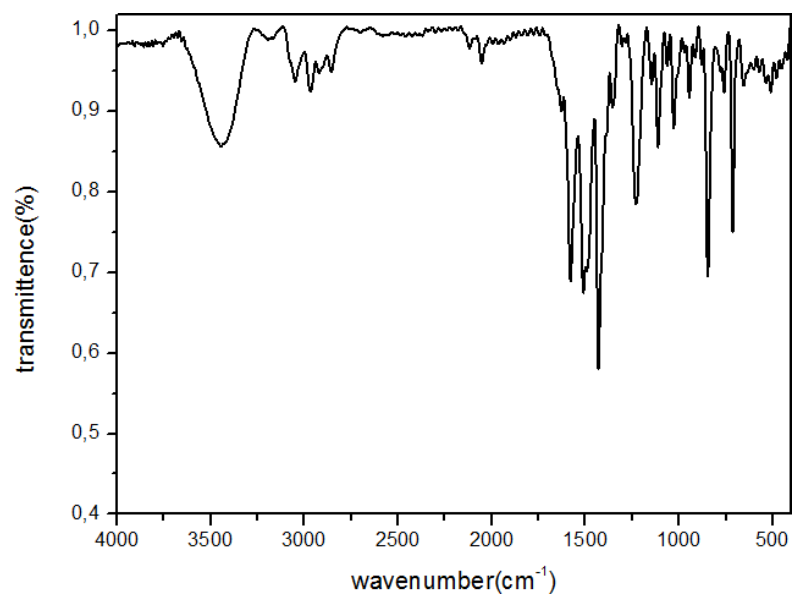


Figure 5.29: PtL2 IR spectrum.

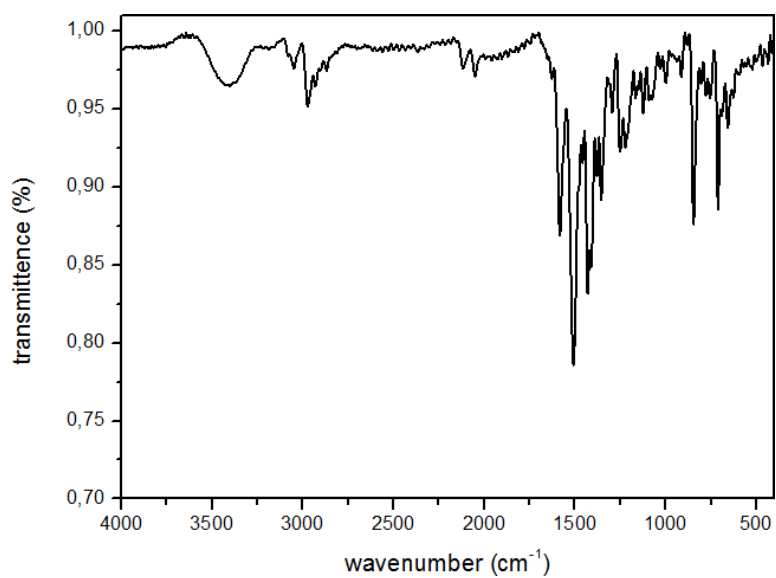


Figure 5.30: PtL3 IR spectrum.

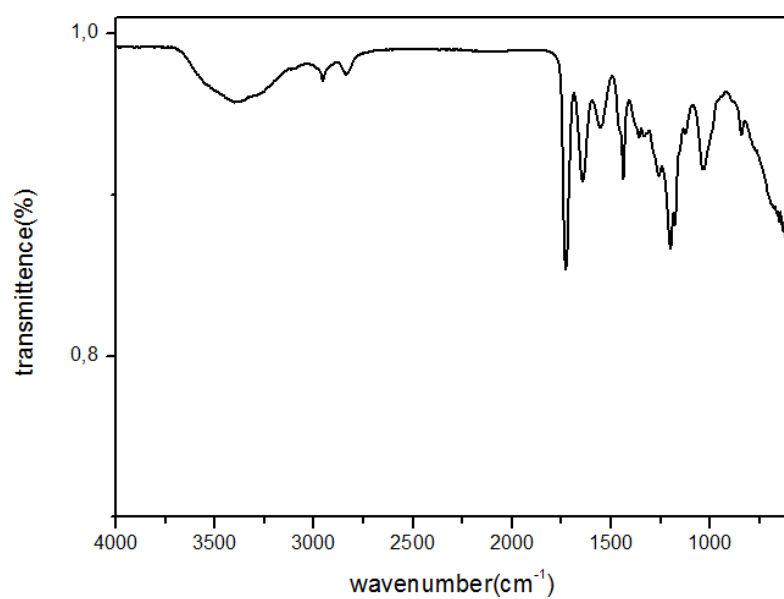


Figure 5.31: G0.5 PAMAM IR spectrum

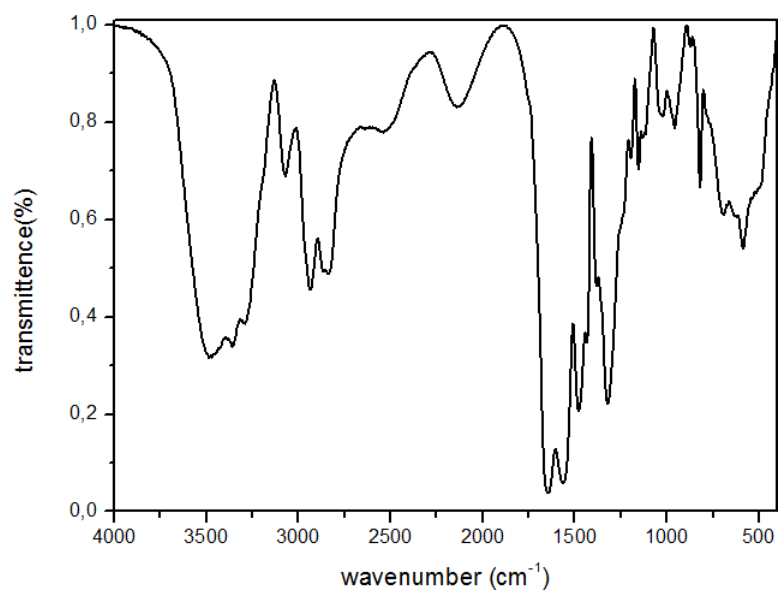


Figure 5.32: G1 PAMAM IR

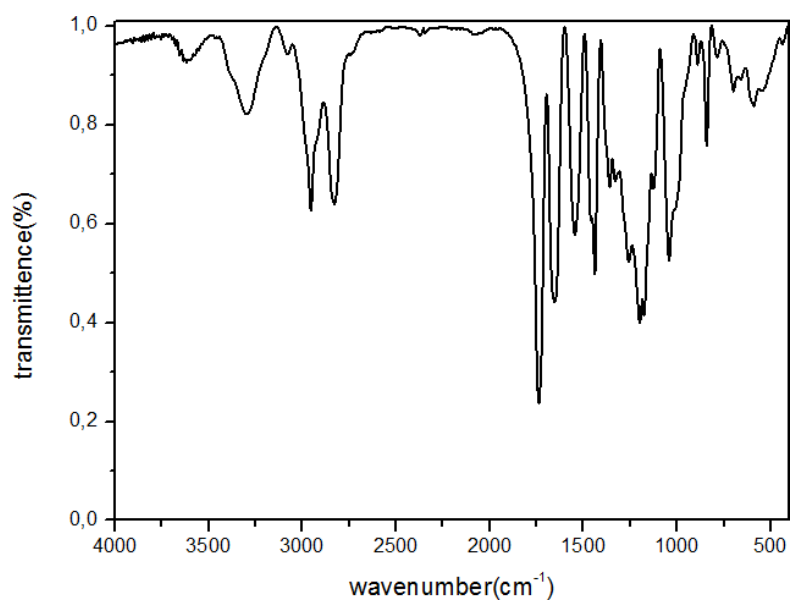


Figure 5.33: G1.5 PAMAM IR spectrum.

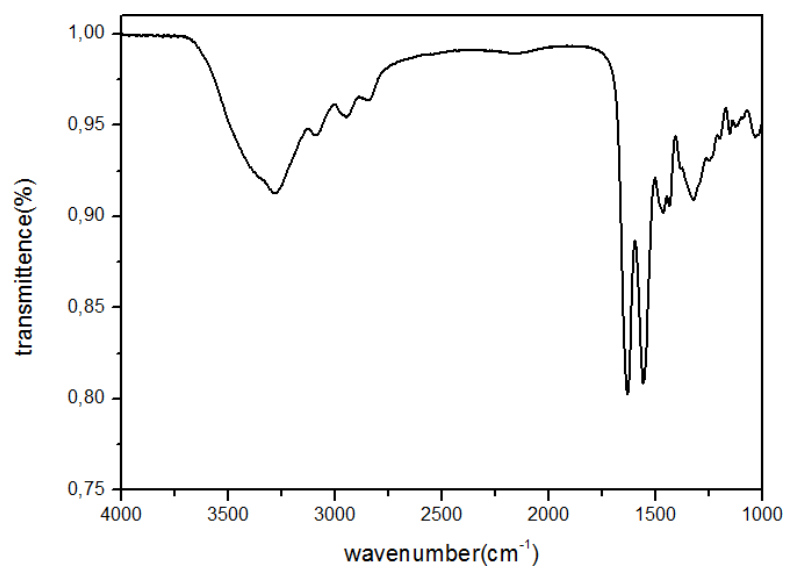


Figure 5.34: G2 PAMAM IR Spectrum.

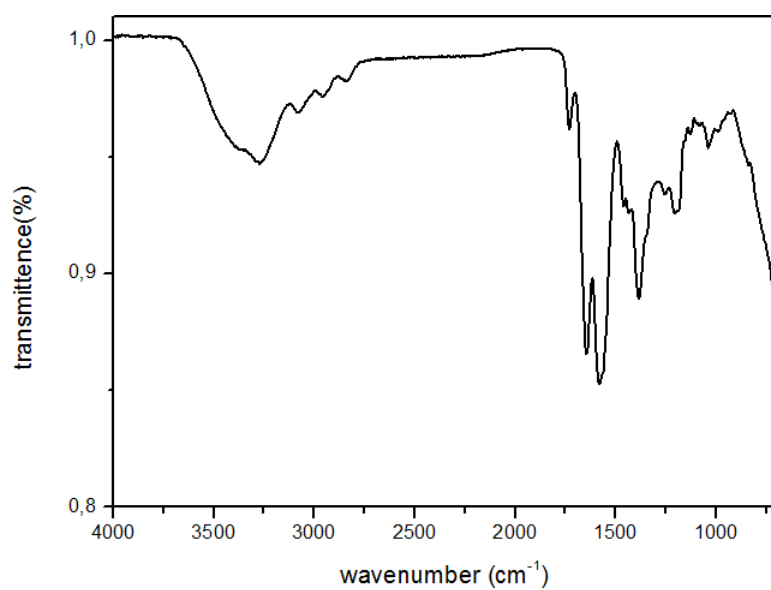


Figure 5.35: G2.5 PAMAM IR spectrum.

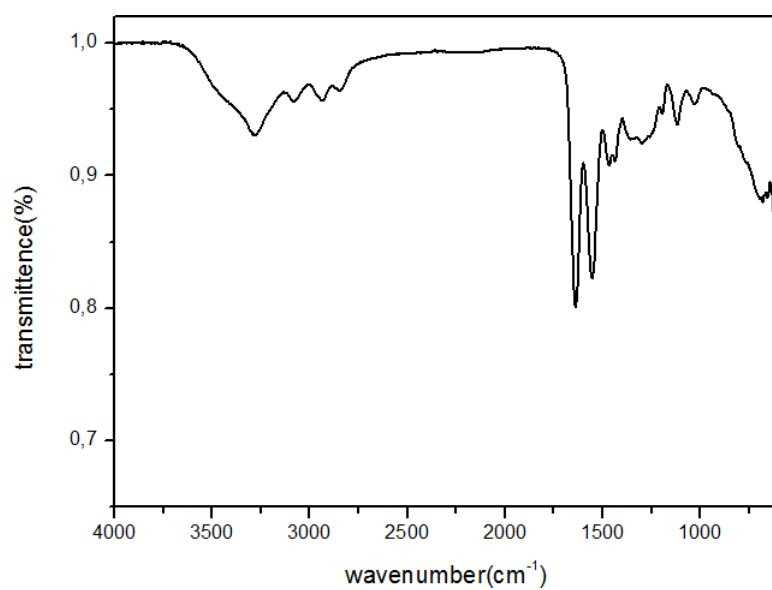


Figure 5.36: G3 PAMAM IR spectrum.

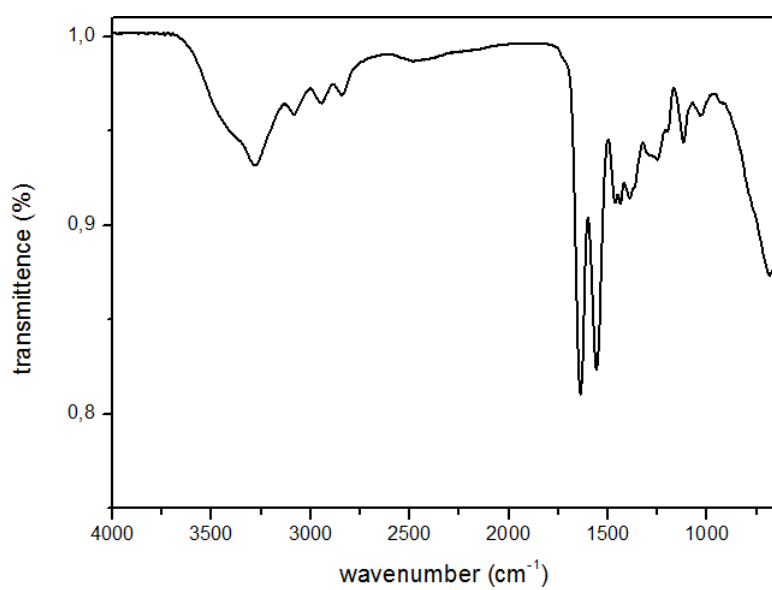


Figure 5.37: G3.5 PAMAM IR spectrum.

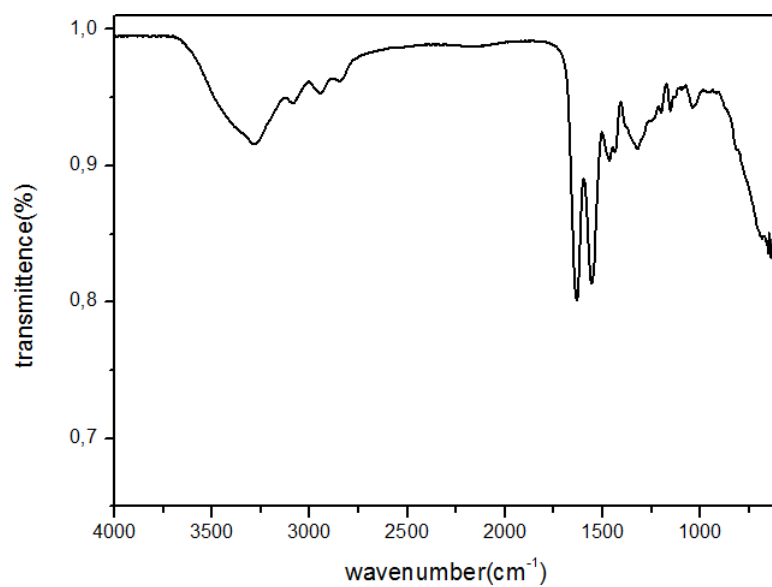


Figure 5.38: G4 PAMAM IR spectrum.

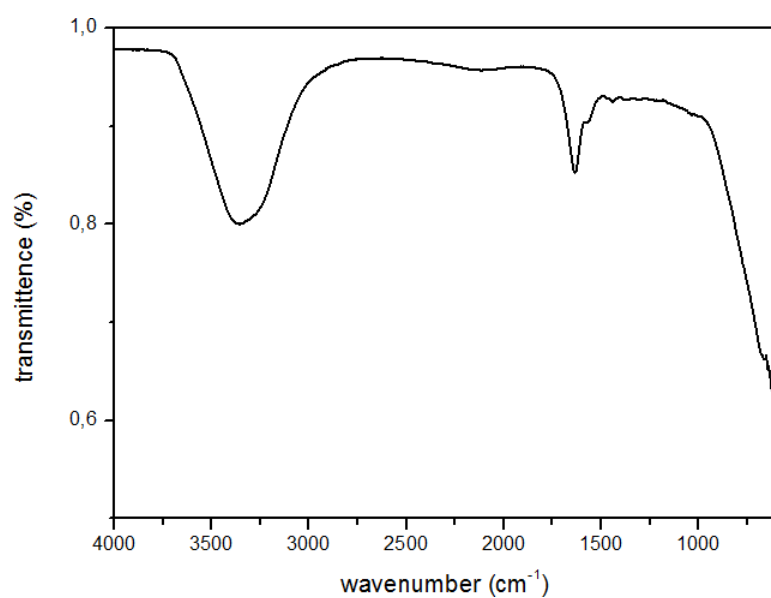


Figure 5.39: G5-Ac IR spectrum.

Cytotoxicity screening

Table 5.1: concentrations of samples used in the experiment.

Samples	Stock concentration (mM)	Prepared concentration (μM) of complex (2 mL)	Final concentration in plate (μM) (added 100 μL per well)	Prepared concentration of dendrimer (μM)	Final concentration in plate (μM)
PtL1	33.9	100 & 10	50 & 5	-	-
PtL2	25.5	100 & 10	50 & 5	-	-
PtL3	25.4	100 & 10	50 & 5	-	-
5FU	149.1	100 & 10	50 & 5	-	-
PtL1+G4Ac	33.9: 1.69	100 & 10	50 & 5	4.97 & 0.50	2.48 & 0.25
PtL2+G4Ac	25.5: 2.27	100 & 10	50 & 5	8.89 & 0.89	4.44 & 0.44
PtL3+G5Ac	25.4: 0.84	100 & 10	50 & 5	3.31 & 0.33	1.65 & 0.17
5FU+G5	149.1 :3.43	100 & 10	50 & 5	2.30 & 0.23	1.15 & 0.11
G4	1.72	-	-	4.99 & 0.50	2.49 & 0.25
G5	3.43	-	-	2.30 & 0.23	1.15 & 0.11
G4-Ac	1.69	-	-	4.97 & 0.50	2.48 & 0.25
G5-Ac	0.84	-	-	3.31 & 0.33	1.65 & 0.17
DMSO:D ₂ O	150 μL + 25 μL (6:1) 86% DMSO :14% D ₂ O	For 100 μM: 8 μL in 1992 μL media For 10 μM: Serial dilution 200 μL in 1800 μL media	50μM: 8μL/2000μL*100 = 0.4% 0.34%DMSO: 0.056%D ₂ O 5 μM: <<< less than 0.04 %	-	-